Mahidol University
Faculty of Pharmacy

US-Thai
Pharmacy Consortium

ASEAN PharmNET

The Pharmacy Education Consortium of Thailand
PECT

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Proceedings

The 4th ASEAN PharmNET 2024 & the 2024 US - Thai Pharmacy Consortium Conference: The 30th Anniversary Commemoration

Global Collaboration in Pharmacy Education, Practice & Research: Bridging Borders for Health Innovation

June 12-14, 2024

Eastin Grand Hotel Phayathai

Welcome Message

from

President of Pharmacy Education Consortium of Thailand (PECT)

Dean of the Faculty of Pharmacy, Mahidol University, Thailand

On behalf of Faculty of Pharmacy Mahidol University and Pharmacy Education Consortium of Thailand (PECT), I, Assoc. Prof. Surakit Nathisuwan, extend a heartfelt welcome to the 4th ASEAN PharmNET and the 2024 US-Thai Pharmacy Consortium Conference. This combined event celebrates not only the 30th Anniversary of the US-Thai Pharmacy Consortium but also the ongoing commitment to fostering global collaboration in pharmacy education, practice, and research.

Following the challenges of the recent pandemic, this gathering holds a special significance. It's especially gratifying to see us come together once more. This conference serves as a powerful testament to the enduring spirit of collaboration that transcends borders.

For three decades, the US-Thai Pharmacy Consortium has nurtured a dynamic exchange of knowledge and best practices between our two nations. Similarly, ASEAN PharmNET has facilitated groundbreaking research and knowledge dissemination across Southeast Asia. This combined conference signifies a fantastic opportunity to leverage the collective expertise of our diverse communities.

The theme, "Global Collaboration in Pharmacy Education, Practice & Research: Bridging Borders for Health Innovation," perfectly captures the essence of this gathering. Over the next few days, we'll delve into critical topics, share cutting-edge research, and forge connections that can propel our profession forward.

The program features renowned speakers from around the globe, offering a rich tapestry of perspectives on our shared goals. But this conference is much more than just a series of lectures. It's a space for vibrant discussions, exchange of ideas, and the building of lasting collaborations that will undoubtedly contribute to advancements in global health.

We encourage you to actively participate, network with your peers, and explore the exciting possibilities that emerge when passionate minds from around the world come together.

Welcome once again, and best wishes for a productive and inspiring conference

Assoc. Prof. Surakit Nathisuwan, PharmD

Dean, Faculty of Pharmacy, Mahidol University

President of Pharmacy Education Consortium of Thailand

Welcome Message

June, 2024

On behalf of the Board of Directors of the US-Thai Pharmacy Consortium, the member US and Thai Schools, Colleges and Faculties of Pharmacy, I bring you the warmest of greetings and welcome to this historic combined meeting of the US-Thai Pharmacy Consortium and ASEAN PharmNet 2024! This meeting marks the 30th Anniversary of the US-Thai Pharmacy Consortium and the 4th meeting of ASEAN PharmNet. Your presence and participation is making history! The collaboration between American and Thai pharmacy schools has resulted in remarkable changes in pharmacy education, practice and research. This is mostly due to the hard work, dedication and creativity of our Thai colleagues, and the American partners have gained tremendously from our 30 years of collaboration. The work of the ASEAN PharmNet members is equally remarkable, supporting cutting edge research and dissemination of new knowledge throughout Southeast Asia and the world.

We are especially excited about the launch of the US-ASEAN Pharmacy Consortium at this meeting. Discussion about forming such a Consortium begin in 2016, and we are thrilled that we will have a formal mechanism for pharmacy educators, practitioners and researchers from the US and 10 ASEAN countries to work together to advance our profession and improve the lives of our patients.

The joining of the Consortium and ASEAN PharmNet conferences will allow us to share best practices in pharmacy education, practice and research. As you review the program, I'm sure you will agree that we have a program of highly relevant topics presented by world class speakers from Thailand, the US and other ASEAN countries. In addition to a great educational program and over 250 scientific posters, the meeting will allow ample time for networking, to share ideas, meet new colleagues, renew friendships and establish new relationships and collaborations.

While many people have taken part in planning this conference, I want to give special recognition to the tireless efforts of Dr. Jennis Meanwatthana and Dean Surakit Nathisuwan from Mahidol University.

Welcome to this historic conference, and thank you for your participation! I look forward to meeting you.

Michael Katz, PharmD

US-Thai Pharmacy Consortium Co-Chair

Professor and Director of International Programs.

R. Ken Coit University of Arizona College of Pharmacy

Committee

Organizing Committee

1.	Assoc. Prof. Surakit Nathisuwan	Chair
2.	Assoc. Prof. Dr. Mullika Chomnawang	Chairman for Scientific Committee
3.	Assoc. Prof. Dr. Krit Thirapanmethee	Chairman for Scientific Publication
4.	Assoc. Prof. Thanarat Suansanae	Chairman for Venue Management and Fund Raising
5.	Assoc. Prof. Dr. Montarat Thavorncharoensap	Chairman for Hospitality Management
6.	Assoc. Prof. Dr. Jiraphun Jittikoon	Chairman for Registration Management
7.	Assist. Prof. Jennis Meanwatthana	Chairman for Ceremony and Social Events
8.	Assist. Prof. Dr. Anchalee Jintapattanakit	Chairman for Treasurer and Procurement
9.	Assist. Prof. Dr. Brompoj Prutthiwanasan	Chairman for Information Technology and Digital Media
10.	Assoc. Prof. Dr. Doungdaw Chantasart	Committee
11.	Assist. Prof. Dr. Bhanubong Bongcheewin	Committee
12.	Assist. Prof. Dr. Pattamapan Lomarat	Committee
13.	Assoc. Prof. Preecha Montakantikul	Committee
14.	Assist. Prof. Dr. Luerat Anuratpanich	Committee
15.	Assist. Prof. Dr. Wichit Nosoongnoen	Committee
16.	Assist. Prof. Dr. Nattawut Charoenthai	Committee
17.	Assist. Prof. Supatat Chumnumwat	Committee
18.	Assist. Prof. Dr. Jaturong Pratuangdejkul	Committee
19.	Assoc. Prof. Dr. Pramote Tragulpiankit	Committee
20.	Assoc. Prof. Dr. Kittisak Sripha	Committee
21.	Assist. Prof. Dr. Thanika Pathomwichaiwat	Committee
22.	Assoc. Prof. Dr. Vilasinee Hirunpanich Sato	Committee
23.	Assoc. Prof. Dr. Veena Satitpatipan	Committee
24.	Assoc. Prof. Dr. Waree Limwikrant	Committee
25.	Assist. Prof. Dr. Wasu Supharattanasitthi	Committee
26.	Assoc. Prof. Dr. Savita Chewchinda	Committee
27.	Ms. Phinnaphit Saengpao	Committee
28.	Assoc. Prof. Dr. Usa Chaikledkaew	Secretariat
29.	Assist. Prof. Sayamon Sukkha	Secretariat Assistant

30.	Dr. Saowalak Turongkaravee	Secretariat Assistant
31.	Dr. Amporn Songkasiri	Secretariat Assistant
32.	Ms. Supattra Kongkaew	Secretariat Assistant

Scientific Committee

1.	Assoc. Prof. Dr. Mullika Chomnawang	Chairman for Scientific Committee
2.	Prof. Dr. Varaporn Junyaprasert	Chairman for Pharmaceutical Technology and Drug Delivery (PD)
3.	Prof. Dr. Leena Suntornsuk	Chairman for Pharmaceutical Chemistry (PC)
4.	Assoc. Prof. Preecha Montakantikul	Chairman for Pharmaceutical Education and Practice (PE)
5.	Assoc. Prof. Dr. Mullika Chomnawang	Chairman for Biopharmaceutical Sciences and Pharmaceutical Biotechnology (BB)
6.	Assoc. Prof. Dr. Pongtip Sithisarn	Chairman for Phytopharmaceuticals and Nutraceuticals (PN)
7.	Assoc. Prof. Dr. Vilasinee Hirunpanich Sato	Chairman for Pharmacology, Toxicology, and Physiology (PP)
8.	Assist. Prof. Dr. Sitaporn Youngkong	Chairman for Social and Administrative Pharmacy (SP)
9.	Assist. Prof. Supatat Chumnumwat	Chairman for Clinical Pharmacy and Personalized Medicine (CP)
10.	Assist. Prof. Dr. Krisada Sakchaisri	Scientific Committee Secretariat
11.	Dr. Teerawat Songsichan	Scientific Committee Secretariat Assistant

International Scientific Committee

1. Prof. Alan Lau

   Director of International Clinical Pharmacy Education, College of Pharmacy, University of Illinois Chicago, USA,
   Pharmaceutical Education and Practice (PE)

2. Prof. Dr. Aleth Therese L. Dacanay

   Dean, Pharmaceutical Education and Practice (PE)

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   College Secretary, Pharmaceutical Education and Practice (PE)

6. Prof. Chheang sena

   Dean, Pharmaceutical Technology and Drug Delivery (PD)

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   Former Vice President of the University of Medicine and Pharmacy at Ho Chi Minh City (UMP); Former Dean of the Faculty of Pharmacy - UMP - Ho Chi Minh City, Vietnam,
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   Director of International Professional Student Education, and Assistant Provost for Global Health Initiatives College of Pharmacy, University of Kentucky,
   Pharmaceutical Education and Practice (PE)

9. Prof. Michael Katz

   Director of International Programs, the R. Ken Coit College of Pharmacy, University of Arizona, USA / Chairman, The US-Thai Pharmacy Consortium,
   Pharmaceutical Education and Practice (PE)

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    Deputy Dean, Phytopharmaceuticals and Nutraceuticals (PN)

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    Deputy Head-Clinical Clinical, Pharmacy and Personalized Medicine (CP)

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    Head, Department of Science & Technology - Cooperation Development,
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    Head of School, Pharmaceutical Education and Practice (PE)
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    Dean, Social and Administrative Pharmacy (SP)
16. Prof. Thein May Saw
    Rector, Pharmacology, Toxicology, and Physiology (PP)

Scientific Program

The 4th ASEAN PharmNET 2024

(International Conference on Pharmacy Education and Research Network of ASEAN)

Held in Conjunction with

The US-THAI Pharmacy Consortium 2024: The 30th Anniversary Commemoration

Theme:

Global Collaboration in Pharmacy Education, Practice and Research: Bridging Borders for Health Innovation

Eastin Grand Phayathai Hotel
Bangkok, Thailand
June 12 – 14, 2024

Conference Program: June 12, 2024

DAY 1: Wednesday (June 12, 2024)	
08.00 – 09.00	Registration

09.00 – 09.45	**Opening Ceremony**
	(Phayathai Grand Ballroom 1-3)
	• Cultural Performance & ASEAN Flag Parade & Sing Along
	• Commemoration of the 30th Anniversary of the US-Thai Pharmacy Consortium Welcome Opening Remark
	  • **Assoc.Prof.Dr. Chutamanee Suthisisang, Ph.D.**
	  Founder of ASEAN Pharmnet, Acting for Director of ASEAN Institute for Health Development
	  Former Dean, Faculty of Pharmacy, Mahidol University, Thailand
	  • **Prof. Michael Katz, Pharm.D.**
	  Director of International Programs. The R. Ken Coit College of Pharmacy, University of Arizona, USA
	  Chairman, The US-Thai Pharmacy Consortium
	  • **Assoc.Prof. Surakit Nathisuwan, Pharm.D.**
	  President of Pharmacy Education Consortium of Thailand (PECT)
	  Dean, Faculty of Pharmacy, Mahidol University, Thailand
	• Group Photos

09.45 – 10.15	**Plenary Talk 1** **Advancement of Pharmacy Education Through International Collaboration: US-Thai Pharmacy Consortium** • **Prof. Michael Katz, Pharm.D**. Director of International Programs, the R. Ken Coit College of Pharmacy, University of Arizona, USA Chairman, The US-Thai Pharmacy Consortium • **Assoc.Prof. Surakit Nathisuwan, Pharm.D.** President of Pharmacy Education Consortium of Thailand (PECT) Dean, Faculty of Pharmacy, Mahidol University, Thailand
10.15 – 10.30	Coffee break & Exhibition (Pre-function area)

10.30 – 11.00	**Plenary Talk 2** **Fostering collaborative learning through integrated clinical services and pharmacy education** • **Prof. Edith A. Nutescu, Pharm.D., MS CTS, FCCP** College of Pharmacy, University of Illinois Chicago, USA					
11.00 – 11.30	**Plenary Talk 3** **Trends & Application of Disruptive Technology on Pharmaceutical Science Research** • **Prof.Dr. R. Kip Guy, Ph.D.** Dean, College of Pharmacy, University of Kentucky, USA					
11.30 – 12.00	**Plenary Talk 4** **Advancement of Pharmacy Practice: Global View** • **Prof. Alan Lau, Pharm.D.** Director of International Clinical Pharmacy Education, College of Pharmacy, University of Illinois Chicago, USA					
12.00 – 13.00	Lunch break					
Concurrent session						
13.00 – 15.00	**Symposium 1: Pharmacy Education and Practice** (Auditorium)	**Symposium 2: Biopharmaceutical Sciences and Pharmaceutical Biotechnology & Pharmacology, Toxicology, and Physiology** (Phayathai Grand Ballroom 1)	**Symposium 3: Pharmaceutical Technology and Drug Delivery** (Phayathai Grand Ballroom 2)	**Symposium 4: Pharmaceutical Chemistry** (Ari)	**Symposium 5: Phytopharmaceuticals and Nutraceuticals** (Sena)	**Symposium 6: Clinical Pharmacy and Personalized Medicine & Social Administrative Pharmacy** (Phayathai Grand Ballroom 3)

	Chair: Dr. Wannisa Dongtai Ubonratchathani University, Thailand Co-chair: Dr. Renukha Sellappans Taylor's University, Malaysia 13.00 – 13.30 ▪ Quality Assurance Measures in Pharmacy Education for ASEAN Assoc.Prof. Surakit Nathisuwan, Pharm.D. Faculty of Pharmacy, Mahidol University, Thailand Prof. Dr. Apt. Daryono Hadi Tjahjono Asian Association of Schools of Pharmacy, Indonesia	Chair: Asst.Prof. Czarina Dominique R. Delos Santos University of the Philippines Manila, Philippines Co-chair: Asst.Prof.Dr. Arnatchai Maiuthed Mahidol University, Thailand 13.00 – 13.30 ▪ Challenges and Recent Progress in Drug Discovery and Development for Tropical Diseases: The Role of Pharmacology Prof.Dr. Kesara Na-Bangchang President, Pharmacological and Therapeutic Societies of Thailand, Thailand	Chair: Prof.Dr. Yahdiana Harahap Universitas Indonesia, Indonesia Co-chair: Assoc.Prof.Dr.Jiraphong Suksiriworapong Mahidol University, Thailand 13.00 – 13.30 ▪ Innovations in Pharmaceutical Formulation Design for Aging Population Prof.Dr. Pornsak Sriamornsak Dean, Silpakorn University, Thailand	Chair: Assist.Prof.Dr. Satsawat Visansirikul Mahidol University, Thailand Co-chair: Dr. Chaiyawat Aonsri Mahidol University, Thailand 13.00 – 13.30 ▪ Exploring the Therapeutic Potential of Plant-Derived Polyphenols using Molecular Docking and Network Analysis Assoc.Prof.Dr. Pornchai Rojsitthisak Chulalongkorn University, Thailand	Chair: Prof.Dr. Triana Hertiani University Gadjah Mada, Indonesia Co-chair: Dr. Watchara Arthan Mahidol University, Thailand 13.00 – 13.30 ▪ *Eurycoma Longifolia* and *Eurycoma Harmandiana*: Phytochemical Contents, Biological Activities Evaluation and *in vitro* Culture Prof.Dr. Waraporn Putalun Khon Kaen University, Thailand	Chair: Assoc.Prof.Dr. Francis R. Capule University of the Philippines Manila, Philippines Co-chair: Dr. Mohd Shahezwan Abd Wahab Universiti Teknologi MARA, Malaysia 13.00 – 13.30 ▪ Empowering Pharmacists: The Evolving Role as Vaccinators in Thailand Asst.Prof.Dr. Supatat Chumnumwat Mahidol University, Thailand

	13.30 – 14.30 ▪ Tips & Best Practices in Designing New Curriculum: Experience from US and Thailand Prof.Dr. Paul W. Jungnickel, R.Ph. Auburn University, USA Asst. Prof. Thitima Doungngern Faculty of Pharmaceutical Science, Prince of Songkla University, Thailand	13.30 – 14.00 ▪ Network Pharmacology and Cell-Based Assessments for Potential Cancer Targets Assoc.Prof.Dr. Laddawan Senggunprai Khon Kaen University, Thailand	13.30 – 14.00 ▪ Tailoring Hydroxyappatite Scaffolds for Dual Action: Bone Regeneration and Sustained Delivery of Antibiotics Asst.Prof.Dr. Amaraporn Wongrakpanich Mahidol University, Thailand	13.30 – 14.00 ▪ Computational Drug Discovery and Development of Novel Tubulin and Phosphatidylcholine-Specific Phospholipase C Inhibitors as Potential Anticancer Drug Candidates Asst.Prof.Dr. Chatchakorn Eurtivong Mahidol University, Thailand	13.30 – 14.00 ▪ Hmong-Mien Cultural Uses of Exotic Medicinal Plants Asst.Prof.Dr. Methee Phumthum Mahidol University, Thailand	13.30 – 14.00 ▪ Roles of Pharmacist in the Emergency Department Prof.Dr. Dang Nguyen-Doan-Trang University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam
		14.00 – 14.30 ▪ Emerging Role of CAMSAP Family Proteins on Lung Cancer Metastasis Assoc.Prof.Dr. Varisa Pongrakhananon Chulalongkorn University, Thailand	14.00 – 14.30 ▪ Development of pH-sensitive Zerumbone-encapsulated Liposomes for Lung Fibrosis Assoc.Prof.Dr. Foo Jhi Biau Taylor's University, Malaysia	14.00 – 15.00 **Poster Presentation**	14.00 – 15.00 **Poster Presentation**	14.00 – 15.00 **Oral Presentation**
	14.30 – 15.00 ▪ Implementation of Interprofessional Education in Pharmacy Curriculum:	14.30 – 15.00 ▪ Toxic or Tonic? Understanding the Pharmacological Actions of Emerging	14.30 – 15.00 **Poster Presentation**			

	▪ Experience Sharing Prof.Dr. Paul W. Jungnickel, R.Ph. Auburn University, USA	New Psychoactive Substances Prof.Dr. Norazrina Azmi Universiti Kebangsaan, Malaysia				
15.00 – 15.15	Coffee break & Exhibition (Pre-function area)					
15.15 – 17.30	**Poster Presentation & Networking** (Phayathai Grand Ballroom 4)					

Conference Program: June 13, 2024

DAY 2: Thrusday (June 13, 2024)		
08.30 – 09.00	**Registration**	
09.00 – 09.45	**Country Status & Progress Report on Pharmacy Education & Practice** (Phayathai Grand Ballroom 1-2) **Moderator: Assist.Prof. Jennis Meanwatthana** Faculty of Pharmacy, Mahidol University **Pharmacy Education and Pharmacy Practice at UBD: Brunei Experience** **Dr. Nurolaini Pg Haji Muhd Kifli** PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam,Brunei	**Theme: Drug Discovery and Development** (Phayathai Grand Ballroom 3-4) **Moderator: Assist.Prof.Dr. Satsawat Visansirikul** Faculty of Pharmacy, Mahidol University **Nanoparticles for Enhanced siRNA Delivery in Cancer Models** **Prof.Dr. Shirui Mao, Ph.D.** Shenyang Pharmaceutical University, China
09.45 – 10.15	**Transforming Pharmacy Education in the Philippines: A Strategic Framework for Roadmap Development** **Assoc.Prof.Dr. Margarita M. Gutierrez, Ph.D.** College of Pharmacy, University of the Philippines Manila, The Philippines	**The Key to Success in the Cannabinoid Drug Development Journey** **Prof.Dr. Hitoshi Sato, Ph.D.** School of Pharmacy, Showa University, Japan
10.15 – 10.30	Coffee break & Exhibition (Pre-function area)	
10.30 – 11.15	**Moderator: Dr. Thongtham Suksawat** Faculty of Pharmacy, Mahidol University **Cambodia, Myanmar, Laos PDR** **Dr. Chea Sin** Faculty of Pharmacy, University of Puthisastra, Cambodia **Prof. Dr. Thein May Saw** University of Pharmacy, Mandalay, Myanmar	**Navigating Pharmaceutical Impurity: Regulation Framework and Analytical Strategies** **Prof.Dr. Leena Suntornsuk** Faculty of Pharmacy, Mahidol University

	Dr. Phoutsathaphone Sibounheuang Faculty of Pharmacy, University of health Sciences, Lao PDR	
11.15 – 12.00	**Malaysia, Indonesia, Vietnam, Singapore, and Thailand** **Prof. Dr. Mohd Makmor Bakry** Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Malaysia **Prof.Dr. Yandi Sukri** The Association of Indonesian Pharmacy Higher Education, Indonesia **Prof.Dr. Dinh Thi Thanh Hai** Hanoi University of Pharmacy, Vietnam **Prof.Dr. Paul John Gallaher** Department of Pharmacy and Pharmaceutical Sciences, National University of Singapore, Singapore **Assoc.Prof. Surakit Nathisuwan, Pharm.D.** Faculty of Pharmacy, Mahidol University, Thailand	**Exploring Metabolic Polymorphism of Antioxidant Phytochemicals in Plants** **Prof.Dr. Takayuki Tohge, Ph.D.** Nara Institute of Science and Technology, Japan
12.00 – 13.00	Lunch break	

Concurrent session						
13.00 – 15.00	**Symposium 1: Pharmacy Education and Practice** (Auditorium)	**Symposium 2: Clinical Pharmacy and Personalized Medicine & Social Administrative Pharmacy** (Phayathai Grand Ballroom 4)	**Symposium 3: Phytopharmaceuticals and Nutraceuticals** (Phayathai Grand Ballroom 3)	**Symposium 4: Pharmaceutical Technology and Drug Delivery** (Ari)	**Symposium 5: Biopharmaceutical Sciences and Pharmaceutical Biotechnology & Pharmacology, Toxicology, and Physiology** (Phayathai Grand Ballroom 2)	**Symposium 6: Pharmaceutical Chemistry** (Phayathai Grand Ballroom 1)

	Chair: Prof. Gary M. Oderda University of Utah, USA Co-chair: Prof. Dr. Li-Jiuan (Rita) Shen Associate Dean for International Affairs, College of Medicine, National Taiwan University, Taiwan	Chair: Prof. Dang Nguyen-Doan-Trang University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam Co-chair: Assoc.Prof.Dr. Montarat Thavorncharoensap Mahidol University, Thailand	Chair: Assist.Prof.Dr. Somnuk Bunsupa Mahidol University, Thailand Co-chair: Dr. Nor Khaizan Anuar Universiti Teknologi MARA, Malaysia	Chair: Assoc.Prof.Dr. Foo Jhi Biau, Taylor's University, Malaysia Co-chair: Assoc.Prof.Dr. Waree Limwikrant Mahidol University, Thailand	Chair: Assoc.Prof.Dr. Wanvisa Udomsinprasert Mahidol University, Thailand Co-chair: Dr. Teerawit Audshasai Mahidol University, Thailand	Chair: Assoc.Prof.Dr. Chutima Phechkrajang Mahidol University, Thailand Co-chair: Dr. Salinthip Jarusintanakorn Mahidol University, Thailand
	13.00 – 13.30 ▪ Tips & Best Practices in Student Assessment: Experience Sharing Prof.Dr. Paul W. Jungnickel, R.Ph. Auburn University, USA	13.00 – 13.30 ▪ Application of Health Technology Assessment on Policy Decision Making for the Development of the Universal Health Coverage: Lesson Learnt from Thailand Assoc Prof.Dr. Usa Chaikledkaew Faculty of Pharmacy, Mahidol University, Thailand	13.00 – 13.30 ▪ Projecting Plantago Major as Phytopharmaceuticals for Diabetic Wound Prof.Dr. Triana Hertiani Universitas Gadjah Mada, Indonesia	13.00 – 13.30 ▪ Potential of cannabidiol as Nasal and Pulmonary Delivery Systems Prof.Dr. Teerapol Srichana Prince of Songkla University, Thailand	13.00 – 13.30 ▪ Pharmacogenomics of Drug Induced Liver Injury Assoc.Prof.Dr. Jiraphun Jittikoon Mahidol University, Thailand	13.00 – 13.30 ▪ Tailored Paper-based Devices through Surface Modification for Point-of-Need Applications Assoc.Prof.Dr. Nantana Nuchtavorn Mahidol University, Thailand

	13.30 – 14.00 ▪ Leadership essentials to Ensure Meaningful changes Prof. Donald E. Letendre, Dean, College of Pharmacy, University of Iowa, USA 14.00 – 15.00 ▪ Strategies & Best Practices in Preceptor Development: A Tale of Two Continents US and Thailand Prof. Monica L. Miller, Pharm.D. Purdue University, USA Assoc.Prof. Weerachai Chaijamorn, BCP, FACP Chulalongkorn University, Thailand	13.30 – 14.00 ▪ Social Administrative Pharmacy and Pharmaconomics in Viet Nam: Development and Future Challenges Dr. Pham Nu Hanh Van, Hanoi University of Pharmacy, Vietnam 14.00 – 15.00 **Oral Presentation**	13.30 – 14.00 ▪ Black Rice (*Oryza Sativa* L.) and its Anthocyanins: Mechanisms, Food Applications, and Clinical Insights for Postprandial Glycemic and Lipid Regulation Prof.Dr. Sirichai Adisakwattana Chulalongkorn University, Thailand 14.00 – 15.00 **Oral Presentation**	13.30 – 14.00 ▪ Bioequivalence Studies to Ensure the Quality of Generic Product Prof.Dr. Yahdiana Harahap Universitas Indonesia, Indonesia 14.00 – 15.00 **Oral Presentation**	13.30 – 14.00 ▪ Insight Out: The Gut Microbiome Impact on Disease and Wellness Asst.Prof.Dr. Pagakrong Wanapaisan Mahidol University, Thailand 14.00 – 14.30 ▪ Study on Bioactive Secondary Metabolites from Marine-derived Fungi Assoc.Prof.Dr. Elin Julianti Bandung Institute of Technology, Indonesia 14.30 – 15.00 **Oral Presentation**	13.30 – 14.00 ▪ Transitioning From Local Wisdom to Pioneering Green Chemical/ Pharmaceutical Analysis Assoc.Prof.Dr. Chalermpong Saenjum Chiang Mai University, Thailand 14.00 – 15.00 **Oral Presentation**

15.00 – 15.15	Coffee break & Exhibition (Pre-function area)
15.15 – 16.30	**Oral Presentation** **(Phayathai Grand Ballroom 1-4, Sena, Ari, Mo Chit)**
18.30 – 20.30	**Welcome Dinner Reception & Performance from ASEAN countries** (Phayathai Grand Ballroom 2-4) Hosted by Faculty of Pharmacy, Mahidol University

June 13, 2024	**Pharmacology, Toxicology, and Physiology (Mo Chit)**	**June 13, 2024**	**Clinical Pharmacy and Personalized Medicine and Pharmacy Education and Practice (Sena)**
14.00 – 16.45	Chair: Prof.Dr. Hitoshi Sato Showa University, Japan **Oral Presentation**	13.00 – 16.45	Chair**:** Asst.Prof.Dr. Supatat Chumnumwat Co-Chair: Asst.Prof.Dr. Yingrak boondam Chuayboon Mahidol University, Thailand **Oral Presentation**

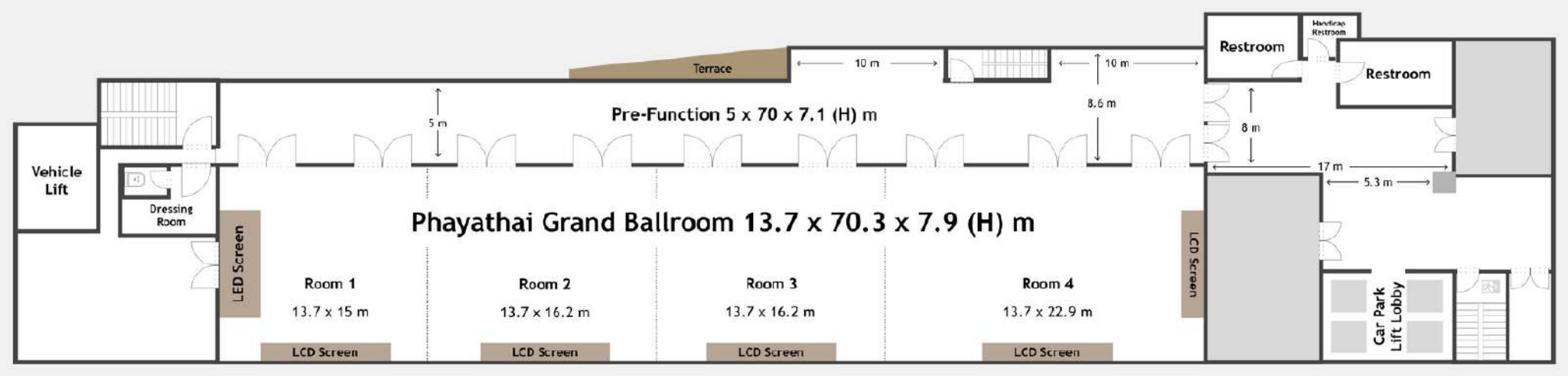
Conference Program: June 14, 2024

DAY 3: Friday (June 14, 2024)		
08.30 – 09.00	Registration	
09.00 – 09.30	**Theme: Bridging Boundaries: Optimizing Pharmacy Education for a Globalized Future** (Phayathai Grand Ballroom 1-2) **Moderator: Dr. Pemmarin Potisarach** Faculty of Pharmacy, Mahasarakham University **Integrating Teaching of Basic Science and Pharmacy Practice** **Prof. Melody Ryan, Pharm.D., MPH, BCGP, BCPS** Director of International Professional Student Education, and Assistant Provost for Global Health Initiatives College of Pharmacy, University of Kentucky, USA	**Theme: Personalized Medicine** (Phayathai Grand Ballroom 3-4) **Moderator: Dr. Pongpol Thanuphol** Faculty of Pharmacy, Mahidol University **Clinical Pharmacogenomics Implementation in Thailand: A Dream Comes True** **Prof.Dr. Chonlaphat Sukasem, Ph.D.** Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand
09.30 – 10.00	**Foresight on Pharmacy Education in the Digital Age: Where do we go from here?** **Asst.Prof.Dr. Somchai Suriyakrai, Ph.D.** Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand	**From Bench to Bedside: Translating Research into Clinical Practice** **Prof.Dr. Jeremy J. Johnson, Pharm.D., Ph.D.** College of Pharmacy, University of Illinois Chicago, USA
10.00 – 10.15	Coffee break & Exhibition (Pre-function area)	
10.15 – 10.45	**Implementation and Experiences in Entrustable Professional Activities (EPA) for Pharmacy Education** **Prof. Ellen M. Schellhase, Pharm.D., FCCP** College of Pharmacy, Purdue University, USA	**Nutrigenomics: The Next Frontier in Personalized Nutrition** **Clinical Prof. Monica L. Miller, Pharm.D.** College of Pharmacy, Purdue University, USA

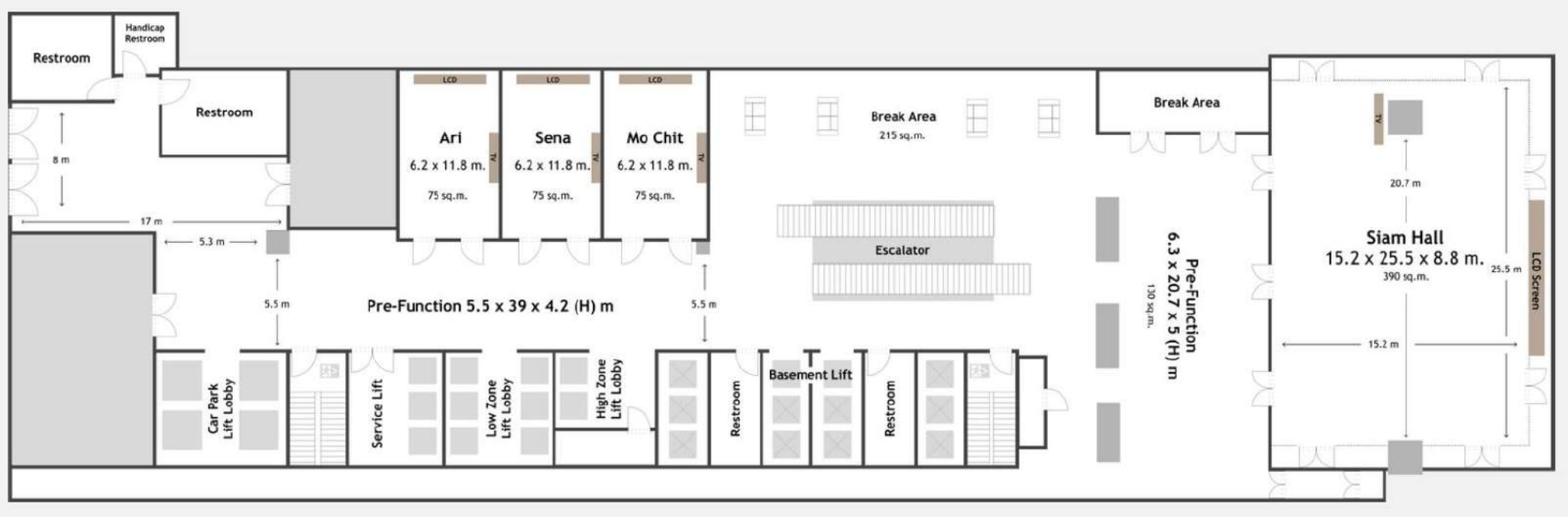
10.45 – 11.15	**Accreditation Council for Pharmacy Education (ACPE) Curriculum Standards & Quality Criteria** **Prof. Michael Katz, Pharm.D.** Director of International Programs, the R. Ken Coit College of Pharmacy, University of Arizona, USA Chairman, The US-Thai Pharmacy Consortium	**Structural and Physicochemical Evaluation of Nanomedicine** **Prof.Dr. Kunikazu Moribe, Ph.D.** Graduate School of Pharmaceutical Sciences, Chiba University, Japan
11.15 – 12.00	**Awards & Closing Ceremony** (Phayathai Grand Ballroom)	
12.00 – 14.00	Lunch break	

Conference Venue

Floor 6th: Phayathai Grand Ballroom 1-4



Floor 6th: Ari, Sena, Mo Chit Room

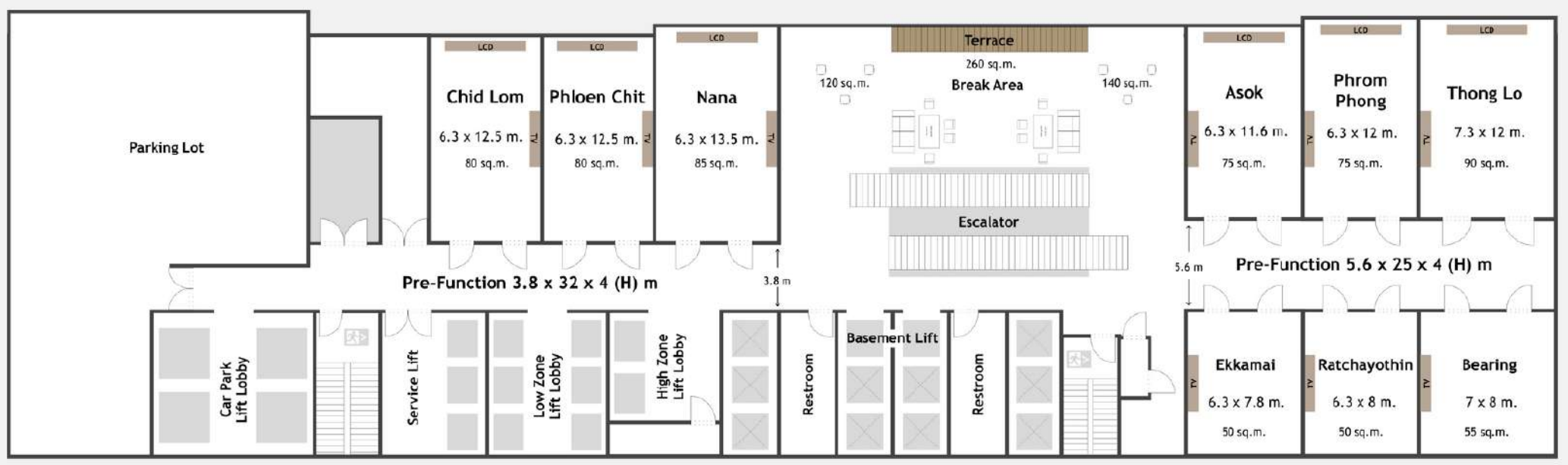


Floor 5th: The Market

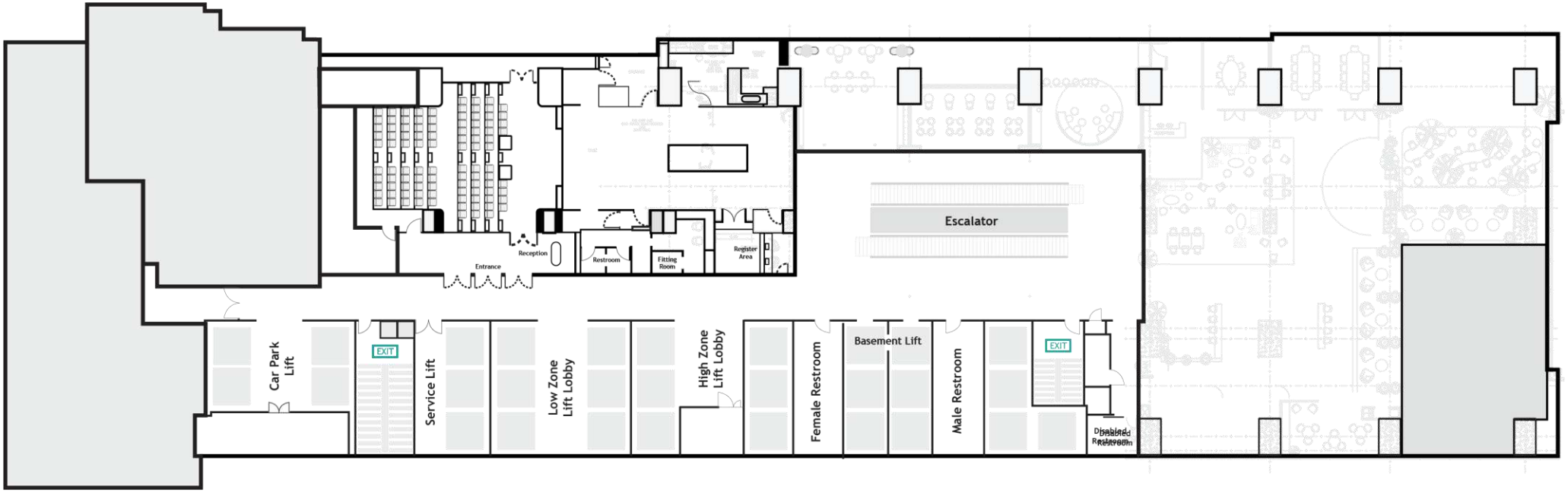
Floor 4th:

Chid Lom, Phloean Chit, Nana: Banquet

Ekkamai: Prayer room



Floor 3rd: Auditorium



List of Proceedings

No.	Presentation Code	Title
1.	CP-1501002-O	The Potential of 3 Generative AI Models in Medication Error Assessment
2.	CP-1508001-O	Characteristics of Statin-Related Myalgia Based on Statin-Associated Muscle Symptom-Clinical Index (SAMS-CI) in Indonesia Dyslipidemia Outpatients: A Preliminary Study
3.	CP-1501101-P	Impact of Tailored Pharmacy-Based Program In Improving Medication Adherence of Psychiatric Out-Patients with Schizophrenia, Bipolar, and Major Depressive Disorders
4.	CP-1501105-P	Designing a Software Program for Chemotherapy Order Processing in The Oncology Pharmacy Unit of A Tertiary Hospital in The Philippines
5.	CP-1504101-P	Patients' Adherence to Fixed-Dose Combination Medicines for Tuberculosis in the Non-National TB Program in Indonesia
6.	CP-1506109-P	Comparative Analysis of Healthcare Workers' Perceptions of Antimicrobial Resistance Management Among Private and Government Hospitals in Metro Manila
7.	CP-1508103-P	Preventability Assessment of Anticoagulant-Related Bleeding: Data from The National Pharmacovigilance Database of Vietnam
8.	CP-1511101-P	The Comparative Study on Knowledge of Emergency Contraceptive Pills Before and After Infographic Media Among Senior High School Student in Samutprakarn Province
9.	CP-1602101-P	Efficacy of Immune Checkpoint Inhibitors in Advanced Non-Small Cell Lung Cancer Who Progressed on Targeted Therapy: A Systematic Review and Meta-Analysis
10.	PN-1110001-O	Natural Product Databases in Herbal and Integrative Medicine: Bridging Traditional Knowledge to Modern Applications
11.	PN-1106101-P	Repellency of Natural Essential Oil Blends Against Adult German Cockroach, *Blattella germanica* L. (Blattaria: Blattellidae)
12.	PN-1106105-P	Exploring Free Radical Scavenging and Cardioprotective Effects via Acid-Base Extraction from *Nelumbo nucifera* Gaertn.

No.	Presentation Code	Title
13.	PN-1107102-P	*In silico* and *In vitro* Analysis of The Antihypertensive and Antioxidant Potential of Abaca (*Musa Textilis*) Ethanolic Leaf Extract
14.	PN-1108101-P	Triterpenoids and Flavonoids from *Ludwigia octovalvis* (Jacq.) P.H.Ravens and Their Bacteriostatic Effect on *Helicobacter pylori*
15.	PN-1109104-P	Investigation of The Antioxidant and Anti-Cancer Potential of *Leea indica* Leaf Extracts from Brunei Darussalam
16.	PN-1201102-P	Development of Microencapsulated Powder Containing *Lactobacillus acidophilus* and *Clitoria ternatea* L. Flower Extract
17.	SP-1701001-O	Landscape Analysis of International Facilitated Regulatory Pathway for Innovative Medicine Approval: Recommendation for Thailand
18.	SP-1703002-O	Analysis of Factors Affecting The Engagement of Pharmacists with The Organization Working in Public Health Facilities: A Quantitative Study in Vietnam
19.	SP-1705001-O	Process Evaluation of Logistics Management Information System in Myanmar: A Qualitative Approach
20.	SP-1702106-P	Knowledge, Attitudes, Practices, and Challenges in The Use of Pharmacoeconomic Evaluations among Hospital Pharmacists Involved in The Pharmacy and Therapeutics Committee in Selected Tertiary Private Hospitals in The National Capital Region
21.	SP-1702107-P	Cost-Utility Analysis of Ticagrelor Versus Clopidogrel in Patients with Acute Coronary Syndromes Undergoing Percutaneous Coronary Intervention
22.	SP-1702110-P	Analysis of Direct Medical Costs in Patients Undergoing Knee Joint Surgery at the Hospital for Traumatology and Orthopaedics in Ho Chi Minh City, Vietnam
23.	SP-1702115-P	Cost-Effectiveness Analysis of Fixed-Dose Combination Versus Free-Equivalent Combination in Hypertension Treatment for Outpatients: A Case Study at A Regional Hospital in Southern Vietnam
24.	SP-1703106-P	Correlation Analysis of Factors Influencing Customer Loyalty in Retail Pharmacy Chains: A Cross-Sectional Study in Vietnam
25.	SP-1703108-P	Level of Influence of Factors Affecting The Customer’s Decision-Making for Online Healthcare Product Purchasing in Ho Chi Minh City, Vietnam

No.	Presentation Code	Title
26.	SP-1705101-P	A Systematic Review on Telepharmacy Barriers
27.	SP-1705102-P	Self-Medication among Myanmar Migrant Workers in Thailand
28.	SP-1705107-P	The First Impression and Response to the Symptoms, Diagnosis and Treatment of Tuberculosis from the Perspective of Lay People and Healthcare Providers in Indonesia: A Qualitative Approach
29.	SP-1705108-P	Assessment of Knowledge, Attitude, and Practices of Pharmacovigilance among Hospital Pharmacists in Metro Manila, Philippines
30.	SP-1705111-P	Meta-Analysis of Observational Studies Using Propensity Score Methods for First-Line Antihypertensive Drug Classes
31.	PE-1301001-O	Factors Influencing the Score of Core Competency Examination for Pharmacist Licensure among Thai Pharmacy Students at Huachiew Chalermprakiet University
32.	PE-1401001-O	Developing International Industrial Pharmaceutical Training in Thailand
33.	PE-1303106-P	Grit Matters?: Exploring the Correlation between Grit Levels and Pharmacy License Exam Success
34.	PD-0302001-O	Comparison of Physicochemical Characteristics, Nutritional Value, and Antioxidant Activity of Collagen Extracted from Fresh and Dried White Type Jellyfish of Myanmar Marine Source
35.	PD-0102102-P	Formulation and Characterization of Cream Containing a Combination of Apigenin and Tomato Powder for Overcoming Xerosis on the Heels of the Feet
36.	PD-0102105-P	Influence of Particle Size Distribution, Thickening Agents and Tonicity Modifiers on Rheological Properties and Dissolution Profiles of Mangiferin Suspensions
37.	PD-0102106-P	Dissolution Improvement of Celecoxib by Wet Granulation
38.	PD-0102108-P	Evaluating Impacts of Formula and Processing Parameters on Mechanical Properties of Microneedles for Dermal/ Transdermal Drug Delivery

No.	Presentation Code	Title
39.	PD-0102113-P	Halal by Design Verification of Raw Materials and Evaluation of Emulgel from Clove Oil (*Syzygium Aromaticum* L)
40.	PD-0103103-P	Effects of Internal Structure on Buoyancy and Drug Release of 3D Printed Drug Delivery System Containing Levodopa and Carbidopa
41.	PD-0103104-P	Effect of Polymer Types and Concentrations on the Characteristics of Alginate-Based Microspheres Containing Vitexin and Isovitexin
42.	PD-0103106-P	Fabrication of Tailorable Controlled Release Printlets of Methylprednisolone Using A 3D Fused Deposition Modeling
43.	BB-0602001-O	Association between Shorter Telomeres and Risk of Non-Communicable Diseases
44.	BB-0602102-P	Identification of Biomarkers for Treatment Resistance in Breast and Ovarian Cancer Patients Using Transcriptome Datasets
45.	BB-0706105-P	The Constituents Potential from Melinjo Peel (*Gnetum Gnemon* L.) As Anti-Inflammatory: *In Silico* Molecular Docking and ADME-Tox Prediction
46.	BB-0706111-P	Evaluation of the Wound Healing Activity of Elephant Foot Yam (A*morphophallus Paeoniifolius* Dennst. Nicolson) Aqueous Flower Extract in Zebrafish (*Danio Rerio*) Model
47.	BB-0710103-P	*In vitro* Probiotic Potential of Lactic Acid Bacteria (Lab) Isolated from Fermented Foods with Anti – *Helicobacter pylori* Activity
48.	BB-0712102-P	Prevalence of Microbiological Contamination in Herbal Products: A Public Health Concern
49.	BB-0712104-P	Investigate The Effects of Culture Conditions on Survival, Viability and Activity of *Bacillus clausii* M31 Spores
50.	BB-0712105-P	Evaluation of Microbiological Quality in Cosmetics: A Study on Herbal and Non-Herbal Products
51.	PP-1002101-P	Growth Characteristics and Morphology of Frozen-Thawed Porcine Oviductal Epithelial Cells and Their Application in Cytotoxicity Tests of Kratom Leaf Juice Using MTT Assay
52.	PC-0502005-O	Effect of Freeze-Drying and Spray-Drying on the Physicochemical Composition of *Thunbergia Laurifolia* Leaf Extract

Contents

Clinical Pharmacy and Personalized Medicine

CP-1501002-O

The Potential of 3 Generative AI Models in Medication Error Assessment

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ABSTRACT

Medication errors represent a critical challenge in healthcare settings, leading to negative patient outcomes and increased costs. Existing error detection systems often depend on manual processes and rule-based approaches, which can be inefficient and susceptible to human error. Generative artificial intelligence (AI) has the potential to transform the way we identify medication errors. This study aims to investigate the viability of using three generative AI models, ChatGPT-4, Gemini Advanced, and Claude 3 Opus, to assess medication errors. Thirty-six simulated cases that contain different levels of medication errors were created. The generative AI models were prompted to assess these simulated cases. The models' performance was assessed in terms of their accuracy in detecting potential errors, with particular attention to their ability to identify errors that might be overlooked by conventional systems. Errors were categorized using the National Coordinating Council for Medication Error Reporting and Prevention (NCC MERP) assessment criteria. Our findings demonstrate that Claude 3 Opus had slightly higher correlation coefficients with the reference dataset in comparison to Gemini Advanced and ChatGPT-4, implying greater proficiency in spotting potential errors. This research underscores the potential of generative AI for medication error classification. The results suggest that Gemini Advanced and Claude 3 Opus may be particularly well-equipped for this application. Further studies are warranted to investigate real-world implementation of these models with the goal of improving medication safety, lessening the burden on healthcare providers, and taking proactive measures to safeguard patients from errors.

KEYWORDS: medication errors; generative AI; large language models; ChatGPT-4; Gemini Advanced; Claude 3 Opus

1. INTRODUCTION

Medication errors represent a widespread and critical problem in healthcare settings, posing significant risks to patient safety and well-being. According to the World Health Organization (WHO), these errors account for approximately 7% of hospital admissions and 20% of hospital deaths [1]. The National Coordinating Council for Medication Error Reporting and Prevention (NCC MERP) has identified 14 different types of medication errors, including the administration of the wrong drug, wrong dose, wrong route, or to the wrong patient [2]. These errors can lead to serious adverse drug events, hospitalizations, and fatalities, imposing substantial financial burdens on healthcare systems.

Traditional methods for detecting medication errors, such as manual checks and rule-based systems, can be inefficient and prone to human error, particularly when identifying complex or subtle mistakes [3]. The integration of artificial intelligence (AI) into healthcare has the potential to revolutionize the detection and prevention of medication errors. AI algorithms can analyze large volumes of data, identify patterns, and make highly accurate predictions, thereby enhancing the efficiency and effectiveness of error detection [4].

Generative AI models, such as ChatGPT-4, Gemini Advanced, and Claude 3 Opus, show particular promise in this regard. These models are capable of generating human-like text and responding to complex queries [5]. By training these models on large datasets and fine-tuning them for

specific tasks, such as identifying medication errors, it is possible to reduce the workload on healthcare providers, improve patient safety, and enhance the overall quality of care [6].

However, despite the potential benefits of generative AI models in healthcare, there is limited research on their efficacy in detecting medication errors. Previous studies have primarily focused on rule-based systems and traditional machine learning algorithms for identifying these errors [7, 8]. The capacity of generative AI models to understand and classify medication errors remains underexplored. This study aims to address this gap by evaluating the performance of three state-of-the-art generative AI models—ChatGPT-4, Gemini Advanced, and Claude 3 Opus—in assessing medication errors using simulated cases.

The primary objective of this research is to compare the accuracy of these generative AI models in detecting potential medication errors across various categories, such as wrong drug, wrong dose, wrong route, or wrong patient. By testing their performance on a wide range of simulated cases, we aim to provide insights into the applicability of generative AI models for medication error classification.

2. MATERIALS AND METHODS

2.1. Case Creation

ChatGPT-4 was employed to generate four cases for each of the nine severity levels of medication errors as defined by the NCC MERP criteria (A through I). This ensured a balanced representation of all categories. An expert in medication errors, then, reviewed the generated cases to ensure they were accurately classified into the correct severity levels.

2.2. Testing with AI Models

The validated dataset of 36 cases was then used to test the three AI models. A one-shot prompt approach was used to prompt the AI models. In this approach, a single example of a correctly classified case was provided to each model to guide their predictions.

The prompt is “Instructions: I will provide you a detailed description of the medication error incident. Based on the information provided, classify the error according to the NCC MERP Error Classification system, which ranges from Category A (circumstances that have the capacity to cause error) to Category I (error occurred that contributed to or resulted in patient death). Provide a detailed reasoning for your classification choice. Example Submission: • The prescription was for 500 mg to be taken twice daily. However, the label on the medication dispensed to the patient indicated to take two tablets twice daily, leading to a prescribed dose of 1000 mg twice daily instead of the intended 500 mg. The patient took the higher dosage for three days before the error was noticed, reporting increased drowsiness and mild nausea, which did not result in permanent harm but required monitoring and dose adjustment. Classification: • Error Category: D (Error occurred that reached the patient and required monitoring to confirm that it resulted in no harm to the patient). • Reasoning: The patient received a higher dose than prescribed, which constitutes an error that reached the patient. Since the error required monitoring to confirm that there was no harm, it is classified as Category D.”

Each AI model was given the same one-shot prompt. The description of each case was then input into the AI models one by one. The results were then recorded.

2.3. Data Analyses

The accuracy of the assessments from AI models relative to the expert's were calculated. Accuracy was defined as the proportion of cases for which the AI model's assessment matched the expert's assessment. The formula used for accuracy calculation is as follows:

Accuracy=Number of correct assessments/Total number of assessments

Confusion matrices for each AI model were generated to gain a deeper understanding of the specific discrepancies between the AI models' assessments and the expert evaluations. The confusion matrix is a tabular representation that illustrates the performance of a model by showing the actual

versus the predicted classifications. This matrix helps identify patterns of errors and areas where the AI models align or diverge from expert assessments. Cohen's Kappa coefficients were also calculated

3. RESULTS AND DISCUSSION

3.1. Overall Accuracy and Performance

The detailed examples of medication error cases, illustrating various levels of severity according to the NCC MERP criteria are shown in Table 1. Each case describes a specific scenario, the resulting patient outcomes, and the measures taken by healthcare professionals. These examples highlight the potential risks and consequences associated with medication errors, emphasizing the importance of accurate classification and appropriate intervention.

Table 1. Examples of Medication Error Cases.

Example Cases
A doctor electronically prescribes a medication that has a known interaction with another drug the patient is currently taking. The prescribing software automatically flags the drug interaction as potentially dangerous before the prescription is finalized. The alert prompts the doctor to re-evaluate the medication choices, and an alternative medication that does not interact is prescribed instead. The potential error is avoided thanks to the built-in safety features of the electronic prescribing system.
A patient reports no known drug allergies and is administered a common NSAID (non-steroidal anti-inflammatory drug) for pain relief post-surgery. After administration, the patient develops a mild rash and itching, symptoms of a mild allergic reaction. The medical team immediately discontinues the NSAID and administers an antihistamine. The patient is monitored for any further allergic reactions. The symptoms resolve quickly, and the patient suffers no lasting harm.
A pediatric patient receives an incorrect dosage of pain medication post-operatively, with the dose being significantly higher than recommended for the child's weight. The child develops symptoms of opioid toxicity, including reduced respiratory rate and sedation. Medical staff intervene with naloxone to reverse the effects of the opioid and provide respiratory support until the child's condition normalizes. The child is monitored in the pediatric intensive care unit and recovers fully, with adjustments made to future medication dosages.
A patient receives an intramuscular injection improperly administered, with the needle inadvertently striking a nerve. The patient immediately experiences severe pain and subsequently develops permanent sensory and motor deficits in the affected limb. Despite various therapeutic interventions and physical therapy, the patient suffers from lasting nerve damage that affects their ability to use the limb fully. This error results in permanent disability.
A patient's documented allergy to a specific antibiotic is overlooked during hospital admission due to incomplete transfer of electronic health records. The patient is administered the antibiotic to which they are allergic, and they quickly develop anaphylaxis. Emergency treatment, including the administration of epinephrine, fails to reverse the anaphylactic shock. The patient dies from complications related to the allergic reaction. This preventable error directly contributes to the patient's death.

Table 2. presents the classifications of 36 medication error cases, generated by GPT-4, Gemini Advanced, and Claude 3 Opus AI models, compared against expert evaluations. The cases are categorized into nine severity levels (A through I) according to the NCC MERP criteria.

The results of this study indicate that Claude 3 Opus demonstrates the highest accuracy (58.33%) in aligning with expert evaluations of medication error levels according to the NCC MERP criteria. This suggests that Claude 3 Opus is more reliable for this specific task compared to GPT-4 (41.67%) and Gemini Advanced (36.11%). Additionally, the higher Cohen's Kappa value for Claude 3 Opus (0.531) further supports its higher agreement with the expert evaluations, compared to GPT-4 (0.344) and Gemini Advanced (0.281).

Table 2. Comparison of Medication Error Levels Assessed by AI Models and Expert.

Case	GPT-4	Gemini Advanced	Claude 3 Opus	Expert
A1	C	B	B	A
A2	B	B	B	A
A3	A	A	A	A
A4	A	A	A	A
B1	A	B	B	B
B2	A	A	B	B
B3	A	B	B	B
B4	B	A	B	B
C1	D	D	C	C
C2	C	D	D	C
C3	E	D	E	C
C4	E	E	E	C
D1	F	E	E	D
D2	G	E	F	D
D3	F	F	F	D
D4	G	E	G	D
E1	F	E	F	E
E2	E	E	E	E
E3	G	G	F	E
E4	H	G	F	E
F1	F	F	F	F
F2	G	F	G	F
F3	F	F	F	F
F4	H	G	F	F
G1	I	H	I	G
G2	I	H	H	G
G3	I	H	G	G
G4	I	H	G	G
H1	H	G	H	H
H2	H	G	H	H
H3	H	G	H	H
H4	H	G	H	H
I1	I	I	I	I
I2	I	I	I	I
I3	I	I	I	I
I4	I	I	I	I

Note: The cases were created using GPT-4 to generate four instances for each level of medication errors. An expert in medication errors then validated these cases to ensure accurate

classification. The validated cases were subsequently assessed by the AI models independently to compare their performance against the expert's evaluations

The confusion matrices provide deeper insights into the specific patterns of misclassification for each AI model shown in Figure 1. For instance, all models struggled with accurately classifying category A, consistently misclassifying it as category B. This misclassification pattern might indicate a need for improved differentiation algorithms for closely related categories. Categories D and E posed significant challenges for all AI models, as they were frequently confused with each other. This suggests that the models may require additional training data or using different prompt strategy such as “few-shot” or “chain of thought” to improve the performance. The frequent misclassification of these categories could have significant implications in a clinical setting, where precise classification is crucial for patient safety.

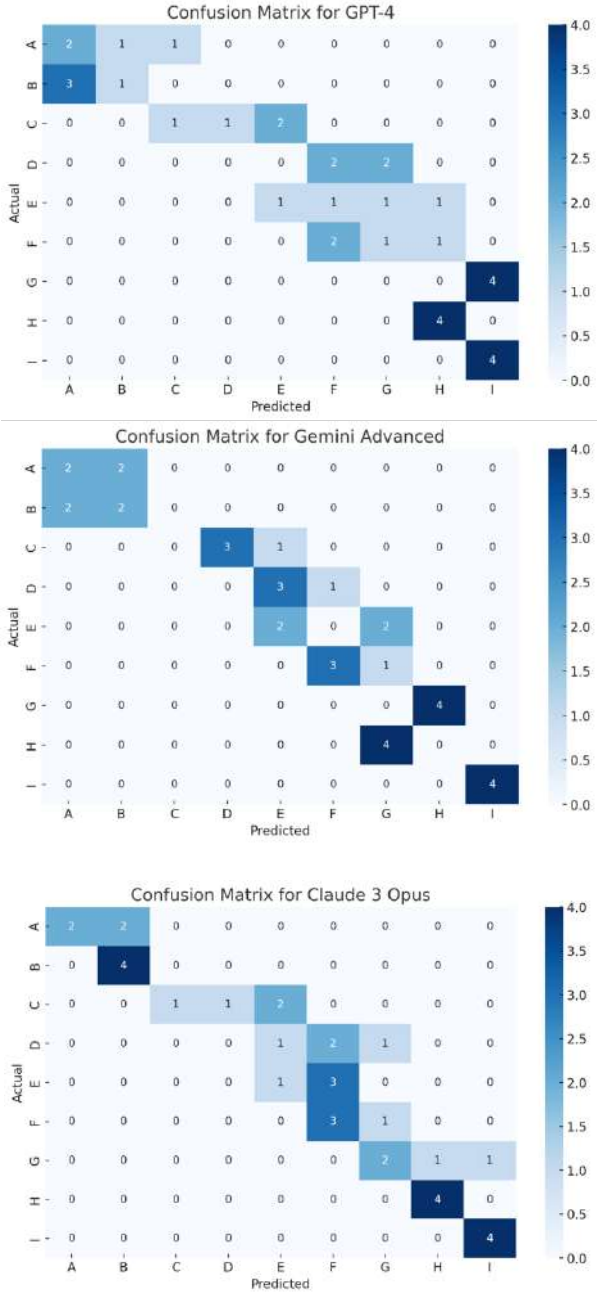

Figure 1. Confusion Matrices for GPT-4, Gemini Advanced, and Claude 3 Opus.

3.2. Model Specific Observations

While GPT-4 showed decent performance in categories B, C, and I, it faced considerable difficulties with categories A, D, E, and G. The confusion matrix suggests that GPT-4 often misclassified more severe error levels (e.g., D and E), highlighting the need for improvements in handling complex cases.

Similar to GPT-4, Gemini Advanced struggled with categories A, B, C, D, and G. Notably, it often predicted D as E, indicating potential issues in distinguishing between these error levels. The lower accuracy and Cohen's Kappa suggest that Gemini Advanced might require fine tuning before being reliably used in practical applications.

Claude 3 Opus demonstrated the best performance across most categories, particularly in B, C, F, G, H, and I, where it showed perfect agreement with the expert. Its confusion matrix indicates fewer misclassifications overall, making it a more robust choice for assessing medication error levels.

3.3. Model Specific Observations

The varying performance of the AI models underscores the importance of careful validation and testing before deployment in clinical environments. Claude 3 Opus's superior performance suggests its potential to assist healthcare professionals effectively. However, the observed misclassification patterns in categories A, D, and E highlight areas needing further refinement.

The consistent misclassification of certain categories by all models suggests that inherent complexities in these classifications are not fully captured by the current AI models. Future research should focus on understanding these complexities better and improving the models' capabilities in these areas.

3.4. Future Research Directions

Given the rapid development of AI technologies, future research should explore the following areas to further improve the accuracy and reliability of AI in assessing medication error levels:

Enhancing AI Models: Incorporate additional training data, particularly focusing on challenging categories identified in this study, to improve model accuracy and reliability.

Sophisticated Prompt: Develop more sophisticated prompt that can better differentiate between closely related error levels, reducing misclassifications and increasing overall model performance.

Continuous Learning: Implement feedback mechanisms that allow AI models to learn and adapt continuously from expert feedback, improving accuracy over time.

Broader Range of Models: Include a broader range of AI models in future studies to generalize the findings and validate the results across different technologies. Especially, the fast pace of advancements in large language models (LLMs) and AI is notable, with the latest developments such as GPT-4o and the newly updated version of Gemini promising significant improvements. Further test in these updated models is crucial.

3.4. Limitations

This study's dataset was relatively small, consisting of 36 cases. Larger datasets could provide more robust insights and validate the findings more comprehensively. Additionally, the study focused on specific AI models, and the results might vary with other models or versions. Future studies should include a broader range of models to generalize the findings. Furthermore, only one expert was used to assess the medication errors, which may introduce bias or limit the generalizability of the findings. The study also utilized only one type of prompt method, which may not capture the full range of potential errors or responses. Future research should incorporate multiple experts and various prompt methods to enhance the reliability and applicability of the results.

4. CONCLUSION

This study highlights the strengths and weaknesses of each AI model in assessing medication error levels according to the NCC MERP criteria. Claude 3 Opus demonstrated the highest accuracy and agreement with expert evaluations, suggesting its potential for practical applications in clinical settings. The advancing pace of AI and LLMs, including developments like GPT-4o and the updated Gemini model, promises further improvements in this domain. Future research should focus on enhancing AI models, developing sophisticated prompt, and implementing fine tuning to improve the accuracy and reliability of AI in medication error assessment.

5. ACKNOWLEDGMENT

None.

Conflict of interest

The authors declare that they have no conflict of interest.

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CP-1508001-O

Characteristics of Statin-Related Myalgia Based on Statin-Associated Muscle Symptom-Clinical Index (SAMS-CI) in Indonesia Dyslipidemia Outpatients: A Preliminary Study

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ABSTRACT

Statins are often associated with muscle pain (myalgia) and/or cramps in muscles that arise symmetrically in the lower extremity muscles such as hip/thigh flexor muscles and calves. The symptom is called as Statin-Associated Muscle Symptom (SAMS). Previous studies stated that 7-29% of patients taking statins experienced SAMS. There has been no research in Indonesia regarding myalgia symptoms using Statin-Associated Muscle Symptom-Clinical Index (SAMS-CI). The study was conducted to determine the location, pattern, and onset of statin-related myalgia symptoms based on SAMS-CI in dyslipidemia outpatients of Atma Jaya Hospital in June 2023. This study is cross-sectional study conducted on dyslipidemia outpatients using the SAMS-CI questionnaire. The Statistical analysis using the logistic regression method with Stata 15 software. The total respondents were 66 patients. A total of 62.1% of respondents complained of experiencing muscle pain. The majority of muscle pain /cramps were located in the calf muscles (39.4%), symmetrical pattern (54.6%), and symptoms occurred with onset 4-12 weeks after taking statin agent (40.9%). The location, pattern, and onset of statin-associated myalgia can be analyzed using the SAMS-CI questionnaire and it was found that the majority of respondents experienced myalgia located in the calf muscle, with a symmetrical pattern and the onset of myalgia was 4-12 weeks after taking statin.

KEYWORDS: Myalgia; Statin; Statin-Associated Muscle Symptom-Clinical Index; SAMS-CI

1. INTRODUCTION

Dyslipidemia is a imbalance condition in the levels of fat in the blood (cholesterol levels, Low-density Lipoprotein Cholesterol (LDL-C), triglycerides, and High-density Lipoprotein Cholesterol (HDL-C))[1]. Prevalence of dyslipidemia according to the Report on Result of National Basic Health Research (RISKESDAS) 2018, around 28.8% of Indonesian people aged 15-75 years experience dyslipidemia. The prevalence of dyslipidemia is more common in women (9.9%) compared to men (5.4%) and is more common in residents who live in urban areas[2].

Dyslipidemia is one of the main risk factors for cardiovascular disease such as Coronary Heart Disease (CHD)[3]. Dyslipidemia needs to be prevented and treated optimally to reduce the risk of death. Statins are the first-line therapy for primary and secondary prevention of cardiovascular disease acompanied by increased lipid levels, especially LDL-C[4]. However, the use of statins has side effects that are often complained of by patients who regularly consume them.

Statin-Associated Muscle Symptoms (SAMS), is a term used to describe the side effects on muscles caused by statins. SAMS is the side effect most often complained of by patients. Around 7-29% of patients taking statins are known to experienced SAMS. According to research by Saeed et al. (2021), 59 of 476 patients (12.4%) experienced SAMS[5]. SAMS common symptoms are myalgia and

cramps in the muscles, especially in the muscles symmetrically (both the left and right sides) in the lower extremities, such as the thigh and calf muscles [6].

Myalgia is a clinical symptom of the muscles that can cause the muscles to become painful, stiff, and cramped[7]. This statement is supported by research conducted by Mahwal et al. (2022) in Pontianak that 93% of respondents who took statins experienced myalgia with mild effects [8]. The side effects of myalgia that arise are generally a reason for patients to stop therapy or are the leading cause of decreased patient compliance. The side effects of myalgia can interfere with the patient's activities [9].

SAMS can be detected by an instrument called Statin-Associated Muscle Symptom-Clinical Index (SAMS-CI). SAMS-CI is a scoring questionnaire instrument that includes questions regarding the location, pattern, and onset of myalgia that arises due to statins. The research by Taylor et al. (2017), the SAMS-CI score obtained by patients who experience SAMS is higher than patients who receive a placebo [10]. More research about SAMS-CI is needed in Indonesia. For this reason, a preliminary study was carried out at Atma Jaya Hospital Jakarta regarding the effects of myalgia after taking statin agent in dyslipidemia patients.

2. MATERIALS AND METHODS

This preliminary study is cross-sectional study. It was conducted for one month, June 2023, in outpatient installation of the internal medicine polyclinic of Atma Jaya Hospital, North Jakarta. The respondents were taken using purposive sampling technique. Inclusion criteria include patients over 18 years and willing to be respondents, not currently pregnant, diagnosed dyslipidemia by a doctor, and patients who regularly comsume statin therapy for at least three weeks. Exclusion criteria are patients who cannot communicate well, have excessive physical activity in the last three days, changes in physical activity patterns in the previous three days, patient with comorbid disease as hypothyroidism, taking other medicine that interact with statins or increased side effects (such as Fibrates, Erythromycin, Itraconazole, Amiodarone, and Cyclosporine), patients with a history of renal and liver impairment, suffering congenital muscle diseases such as McArdle's disease and myasthenia gravis.

The personal data questionnaire contains the respondent's identity, such as name, age, gender, patient medical record number, and telephone number. The questionnaire for assessing myalgia side effects using the Statin-Associated Muscle Symptom Clinical Index (SAMS-CI) questionnaire created by the 2014 Statin Muscle Safety Task Force [6]. This questionnaire assess the symptoms that may occur after taking statin agent and the impact of these symptoms on muscles. The validity and reliability test of the SAMS-CI questionnaire, which has been translated into Indonesian, is carried out using the Stata 15 application.

3. RESULTS AND DISCUSSION

The total number of respondents in this study was 66 patients (Table 1). Most respondents were 46-65 years old (62.1%) and female (63.6%). Most respondents took Atorvastatin (68.2%) and had been taking Atorvastatin or Simvastatin for > 12 weeks (72.7%).

Table 2 shows the characteristics of the myalgia locations. The total respondents had experienced myalgia were 41 respondents (62.1%) after taking statin agents. Muscle pain described by patients included pain in the muscles, muscle stiffness, and cramps, which mainly occurred in the lower extremities, namely in the hip/thigh flexor muscles (22.7%) and calf muscles (39.4%). The study by Rosenson et al. (2014), which states that muscle symptoms caused by statins generally appear with pain, stiffness, and muscle cramps [6]. All respondents who complained of muscle pain took 20 mg statin dosage. Dose of 20 mg simvastatin is the standard dose recommended by FDA Drug Safety for dyslipidemia therapy [11]. The high doses of simvastatin (40-80 mg) can significantly increase the risk of muscle pain compared to other groups[10–12].

Table 1. Characteristics of Respondents

Characteristics of Respondents	Number (n)	Percentage (%)
Age (Year)		
<25	0	0
26-45	8	12.1
46-65	41	62.1
>65	17	25.8
Gender		
Male	24	36.4
Female	42	63.6
Statins		
Atorvastatin	45	68.2
Simvastatin	21	31.8
Duration of therapy		
<4 weeks	2	3.1
4 - 12 weeks	16	24.2
>12 weeks	48	72.7

Table 2. Characteristics of Myalgia Locations

Location Myalgia	Number (n)	Percentage (%)
Hip/thigh flexor muscles	15	22.7
Calf muscles	26	39.4
Proximal upper extremity muscles	0	0
Not specific to any area	0	0
No Myalgia	25	37.9

This study results also showed that most respondents who experienced muscle pain were aged 46-65 years (65.8%) and female (70.7%) (Table 3). This research is in line with Bruckert et al. (2005) study states that the average patient who experiences muscle pain is aged 47 - 69 years [12]. The results show that most muscle pain cases are experienced by women, perhaps because the majority of respondents in this study were female (Table 1).

Tabel 3. Myalgia distribution by Respondents Characteristic

Variable	Total Myalgia (41 respondents)	
	(n)	(%)
Age (year)		
26-45	4	9.8
46-65	27	65.8
>65	10	24.4
Gender		
Male	12	29.3
Female	29	70.7
Statin Agent		
Atorvastatin	31	75.6
Simvastatin	10	24.4
Total	41	100%

The characteristics of the myalgia patterns can be seen in Table 4. Most respondents stated that the symptoms of muscle pain occurred symmetrically or on both sides of the body (54.6%). This research is supported by PRIMO study, which states that symptoms of muscle pain caused by statins

generally happen on both sides of the body [12]. Stroes et al. (2015) study also stated that myalgia caused by statins generally arises symmetrically or occurs on both sides of the body [13].

Table 4. Characteristics of Myalgia Pattern

Myalgia Pattern	Number (n)	Percentage (%)
Symmetrical	36	54.5
Asymmetric	5	7.6
No Myalgia	25	37.9

The characteristics of the myalgia onset can be seen in Table 5. The data shows that 40.9% of respondents began experiencing muscle pain after taking statin agents for 4-12 weeks. This research aligns with study by Bruckert et al. (2005), which states that the average onset of myalgia symptoms was 1 (one) month after taking the statin agent [12].

Table 5. Characteristics of Myalgia Onset

Onset of Myalgia	Number (n)	Percentage (%)
<4 weeks	4	6.1
4-12 weeks	27	40.9
>12 weeks	10	15.1
No Myalgia	25	37.9

The limitations of this study is the number of respondents (sample size) too small. The most internal medicine polyclinic outpatients of Atma Jaya Hospital have dyslipidemia with diabetes mellitus (comorbid disease). Diabetes mellitus maybe influence the myalgia symptoms. This research also focus on a part of the SAMS-CI questionnaire (part A and B), not whole the questionnaire, only evaluated the part of the location, pattern, and onset of myalgia after took statin agent. Rechallange the patient with a statin regimen and myalgia symptoms after withdrawal of the statin can not be assessed.

4. CONCLUSION

In this preliminary study, it was found that the majority of respondents experienced myalgia after taking statin agents ≥ four (4) weeks. The location of myalgia is more often felt in the calf muscles than in the hip/thigh flexor muscles, with the symmetrical myalgia pattern.

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Impact of Tailored Pharmacy-Based Program In Improving Medication Adherence of Psychiatric Out-Patients with Schizophrenia, Bipolar, and Major Depressive Disorders

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ABSTRACT

Patients with mental disorder are most likely to be non-adherent due to factors such as poor reasoning and lack of understanding which can lead to symptoms relapse and reduced treatment effectiveness. To avoid these repercussions, the project developed a tailored pharmacy-based service, named *PARaMASaYA* program, that will utilize expertise of pharmacists to promote adherence among selected psychiatric out-patients at a primary hospital in Antipolo City. The purpose is to proactively involve pharmacists in the medication management of selected psychiatric patients and to address barriers to adherence that were identified in the research setting. The methodology was divided to pre-implementation, implementation, and post-implementation processes. Capacity building and planning were part of the pre-implementation. Program procedures were followed subsequently for two weeks during the trial implementation. Afterwards, program impact was evaluated in the post-implementation with the use of Morisky Medication Adherence Scale (MMAS-8). The mean score of the patients were interpreted as non-adherent if less than 7 and adherent if equal or more than 7. The scale was answered twice by the patients – the first attempt was done on the first pharmacy visit during the start of trial implementation while the second attempt was on the next visit for prescription refill. Mean scores on two attempts were compared to see if there is improvement in medication adherence. After the two-week run, MMAS-8 resulted to a mean score of 2.56 during first visit and increased to 5.00 during second visit. The scores did not reach the value of 7 to imply adherence, however, there is an increment which can still indicate that the program has positive influence on adherence. This finding represents that application of pharmacists' expertise through tailored pharmacy-based services has potential to provide patients with schizophrenia, bipolar, and major depressive disorders the appropriate pharmaceutical care they need to improve medication adherence.

KEYWORDS: Pharmacy; Medication adherence; Psychiatry; Mental disorder

1. INTRODUCTION

Mental health is an integral element of health which helps someone to reflect his own capacity to cope with stress, relate to others, and make healthy choices.[19] When mental state is characterized by clinically significant disturbance in an individual's emotional regulation or behavior, then it is labelled as mental health disorder.

It was reported in the Global Burden of Disease Study (GBD) 2017 that the Philippines has an estimated population prevalence of 1.1% (1,145,871) for Major Depressive Disorders (MDD), 0.5% (520,614) for bipolar disorder, and 0.2% (213,413) for schizophrenia which all fall under the top 3 priority mental health disorders.[18]

For patients diagnosed with mental health conditions, it is crucial that medication adherence must be observed otherwise, unwanted relapse or hospitalization may occur.[6,12] Medication taking behavior is complex and patient adherence to regimen is one of the principal determinants of treatment

success.[17] In identifying barriers to adherence, pharmacists are well-suited and knowledgeable to optimize medication therapy management, devise ways to address poor adherence, as well as to educate patients.[15] Personalized or tailored pharmacy-based interventions may address roadblocks to medication adherence by assessing factors that affect individual patient's ability to take medicines.[2]

Barriers to medication adherence of psychiatric outpatients identified in the research setting include 1) discontinuing medication use because the usual brand name is not available in the pharmacy, 2) taking doses of more than what is prescribed to induce sleep or calmness, 3) patients not motivated to take medications, 4) patients not following treatment duration plan because they already feel 'better', 5) patient's fear of adverse drug reactions that they had experienced in their past medications, 6) patient's denial of illness, 7) patients unwilling to take psychotropic drugs due to embarrassment, and 8) forgetfulness. This project developed a tailored pharmacy-based program at a primary hospital in Antipolo City specifically designed for psychiatric outpatients with Schizophrenia, Bipolar, and Major Depressive Disorders to address the adherence issues.

2. MATERIALS AND METHODS

2.1. Pre-Implementation

Preparations before the trial implementation of the program were conducted from April 24 to May 8, 2023. The processes conducted are capacity development for participant pharmacists, seeking permission from the hospital management, interview with the hospital psychiatrist to gather insights regarding concept of non-adherence and data about medication non-adherence of the psychiatric out-patients at the research setting, program name formulation, and preparation of tools and materials such as the Morisky Medication Adherence Scale (MMAS-8) (see Figure 1), Consent Form, Patient Medication Profile (PMP) sheet, Personalized Calendar and Drug Information System (DIS).

SNO	MMAS–8 Adherence Questions	Patients Response
Q1_1	Do you sometimes forget to take your prescribed medicines?	☐ Yes[0] ☐ No[1]
Q1_2	Over the past 2 weeks, were there any days when you did not take your prescribed medicines?	☐ Yes[0] ☐ No[1]
Q1_3	Have you stopped taking medications because you feel worse when you took it?	☐ Yes[0] ☐ No[1]
Q1_4	When you travel or leave home, do you sometimes forget to bring along your meds?	☐ Yes[0] ☐ No[1]
Q1_5	Did you take your prescribed medicine yesterday?	☐ Yes[0] ☐ No[1]
Q1_6	When you feel like your health is under control, do you sometime stop taking your meds?	☐ Yes[0] ☐ No[1]
Q1_7	Do you feel hassled about sticking to your prescribed treatment plan?	☐ Yes[0] ☐ No[1]
Q1_8	How often do you have difficulty remembering to take all your prescribed medicine?	☐ Never/rarely[1] ☐ Once in a while[0] ☐ Sometimes[0] ☐ Usually[0] ☐ All the time[0]
	Total Score	_____

Figure 1. MMAS-8 consists of eight (8) statements wherein the patients will answer by choosing which best describes their behavior or attitude towards their medication during the past week. It includes 8 items with a score of 0 for yes and 1 for no. The score for every item were added and interpreted as non-adherent if less than 7 and adherent if equal or more than 7.[7]

The DIS contains the following information: (1) Drug Generic Name, (2) Brand Name, (3) Dosage Strength and Form, (4) Dosage Regimen, (5) Indications and contraindications, (6) Adverse Drug Reactions, (7) Warning, (8) Pharmacist's motivational message to the patient. Selection of medications that will have its DIS was based on medicines that had dispensing record of not less than 300 tablets per month. This resulted to the inclusion of the Top 8 most commonly prescribed drugs for

Schizophrenia, Bipolar, and MDD that are dispensed in the pharmacy. These are Escitalopram 10mg tab, Olanzapine 10mg tab, Quetiapine 25mg tab, Quetiapine 100mg tab, Quetiapine 300mg tab, Risperidone 2mg tab, Clozapine 100mg tab, and Lithium carbonate 450mg tab, respectively. On the other hand, the personalized calendar tool was embedded with motivational stickers and phrases retrieved from the Community-based Drug Rehabilitation Philippines (CBDR) website.[4]

For the capacity development, three (3) participant pharmacists who have previous training about basic patient medication counseling underwent a 3-day webinar about Basic Motivational Counselling intended for specialized populations. This is for the purpose of acquiring extra skills and competencies in resolving ambivalence that leads to psychiatric patients' lack of motivation to adhere to medication regimen. The contents of the webinar are as follows: a) Implementing Motivational Interviewing (MI) in practice, b) following, directing, and guiding patients, c) MI and goal setting, d) MI and resistance, e) MI and cultural competence, f) MI and self-care, g) MI and mental health, h) MI and health promotion, i) MI and person-centered care, j) Core communication skills every healthcare provider needs, and k) health coaching practice.

2.2. Implementation

The program started its trial operation on May 9 to May 26, 2023. Due to the limited time for program implementation, the implementor decided to register only three (3) patients under the care of each participant pharmacist who had training in basic motivational counseling. Three (3) participant pharmacists were involved in the program implementation. In total, nine (9) psychiatric patients were chosen based on the following inclusion criteria: (a) 18 and above male or female, (b) diagnosed with Schizophrenia, Bipolar Disorder, and/or MDD, (c) prescribed with available psychotropic medicines in the pharmacy, and (d) with remaining two or more prescription refills, and (e) belongs to the recommendation list of the hospital psychiatrist. A consent form was distributed for voluntary participation.

2.2.1. Dispensing Process

After enrollment of patients, the dispensing process started on the assessment of the prescription (see Figure 2). Prescription of enrolled patients for their psychotropic medications was refilled a quantity that is consumable for 15 days only since the entire time period for the project trial implementation was limited. (*The first filling of prescriptions during the trial implementation was on May 9 and 10, 2023 thus, their next visit for the refill occurred around May 23 to May 25.)* After the medications were already prepared, the pharmacist printed a personalized calendar to be used in the counseling session based on the dosage regimen indicated in the prescription.

2.2.2. Patient Medication Counseling with Motivational Interviewing

Prior the start of patient medication counselling, the pharmacist clearly emphasized to the patient that the session is intended to discuss and focus first on the psychotropic medications prescribed. Inquiries regarding drugs not included in the treatment regimen for mental disorder e.g., cough and colds medicine, maintenance medicine for high blood pressure will be tackled to the patient after all the information and instructions about the prescribed psychotropic medicines have been discussed.

Patient Medication Counseling with an additional layer of motivational interviewing was conducted on the first and second visit of the selected psychiatric patients. To ensure consistency in communication to patients across the three participant pharmacists, the counselling and interviewing were done based on the learned steps and strategies in the 3-day webinar intended for specialized patient populations.

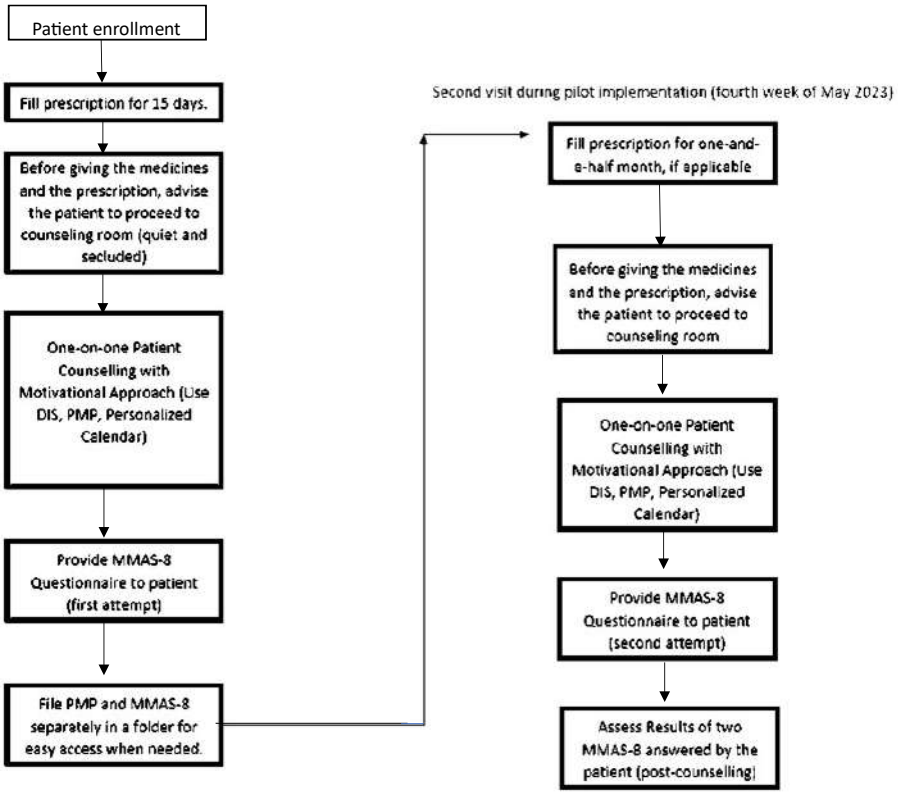

Figure 2. Overview of trial implementation process from patient enrollment to program impact evaluation

Patient Medication Profile sheet was used in the process to gather information about the patient's medicines that he or she is taking regularly, including over-the-counter and complementary medicines, to determine which medications could interact with the dispensed psychotropic drugs. If there are possible drug-drug or drug-food interactions detected, the pharmacist should explain this thoroughly according to the patient's level of understanding. Alongside this procedure, Drug Information System (DIS) for psychiatric medications and a Personalized Calendar were used as visual assistance tools while counseling patients to help them easily comprehend and remember the drug information.

2.3. Post-Implementation

After the counseling and interviewing session, the patients were asked to answer the MMAS-8 for the first time on their first pharmacy visit during the start of trial implementation. Interview questions to gather patient adherence data were acquired in the MMAS-8 questionnaire (see Figure 1). Same set of questionnaires were also answered for the second time on their next pharmacy visit for refill – scores on the first visit were compared with the scores on their second visit to assess impact of the program.

3. RESULTS AND DISCUSSION

Six (6) female and three (3) male psychiatric outpatients with age range of 18 to 52 years old were voluntarily enrolled in the program. Four (4) of these have major depressive disorder, three (3) have been diagnosed with schizophrenia, and two (2) patients have bipolar disorder. During the intervention, identified psychiatric medications administered by the selected patients include Escitalopram (mainly for MDD), Olanzapine (mainly for schizophrenia and an adjunct therapy for bipolar disorder), Quetiapine (mainly for schizophrenia and an adjunct therapy for MDD), Risperidone (mainly for schizophrenia and an adjunct therapy for bipolar disorder), Clozapine (for treatment-resistant schizophrenia), and Lithium carbonate (mainly for bipolar disorder), all in oral tablet dosage forms.

3.1. Interpretation of MMAS-8

Patients were given 1 point for every 'no/never/rarely' answer and 0 point for every 'yes/once in a while/sometimes/usually/all the time' response (see Figure 1). Nine (9) patients answered the MMAS-8 before and after the trial implementation. Based on the results after the implementation (see Table 1), there is improvement in number of patients who are confident that they do not forget to take their medicines, there is increase in number of patients who did not skip taking their medications in the past two weeks, there is an escalated number of patients who followed the pharmacists' advice of not stopping medications even after feeling worse or better. Meanwhile, to assess if the objectives of the program are met, mean scores of each patient in the scale for first and second attempt were compared (see Table 2). The results revealed that all the patients' scores increased. Although the results did not reach the score for adherence, which is equal or more than 7, the results still showed that there is improvement in mean scores in a period of two weeks during program implementation which implies medication adherence improvement.

Table 1. Average score of 9 patients in each scale item for first and second attempt

MMAS-8 SCALE QUESTIONS	MAY 9 AND 10 (FIRST ATTEMPT) ADHERENCE RESULT N = 9	MAY 22 AND 23 (SECOND ATTEMPT) ADHERENCE RESULT N = 9	ACCOMPLISHMENTS
Do you sometimes forget to take your prescribed medicines? YES (0) NO (1)	1	4	IMPROVEMENT IN NO. OF PATIENTS WHO ARE CONFIDENT THAT THEY ARE NOT FORGETTING TO TAKE THEIR MEDICINES
Over the past 2 weeks, were there any days when you did not take your prescribed medicines? YES (0) NO (1)	5	9	IMPROVEMENT IN NO. OF PATIENTS WHO DID NOT SKIP TAKING MEDICATIONS IN THE PAST 2 WEEKS
Have you stopped taking medications because you feel worse when you took it? YES (0) NO (1)	4	6	GAIN PATIENT'S TRUST; NOT STOPPING WITHOUT ADVICE FROM PROFESSIONALS
When you travel or leave home, do you sometimes forget to bring along your meds? YES (0) NO (1)	3	6	IMPROVEMENT IN NO. OF PATIENTS WHO ASSURES MEDICATION ADHERENCE
Did you take your prescribed medicines yesterday? YES (0) NO (1)	1	0	IMPROVED PATIENT ADHERENCE; NO PATIENT FORGOT TO TAKE THE MEDICATION IN SAME DAY
When you feel like your health is under control, do you sometime stop taking your meds? YES (0) NO (1)	1	8	EFFECTIVE PATIENT COUNSELLING ABOUT CONTINUOUS MEDICATION INTAKE IN SPITE OF FEELING "BETTER"
Do you feel hassled about sticking to your prescribed treatment plan? YES (0) NO (1)	5	8	IMPROVED PATIENT COMPLIANCE ON THEIR TREATMENT PLAN
How often do you have difficulty remembering to take all your prescribed medicines? Never/Rarely (1) Once in a while/Sometimes/Usually/All the time (0)	3	7	ADDRESSED FORGETFULNESS IN TAKING MEDICATIONS

Point system: No/Never/Rarely = 1; Yes/Once in a while/Sometimes/All the time = 0
Accomplishments gained in each item were analyzed every after second attempt of answering the scale.

4. CONCLUSION

Based on the outcome of this program trial implementation, pharmacy-based services have potential to provide patients the right care they need to aid in the improvement of their illness. However, the major challenge experienced by the program implementer is the lack of extensive training about dealing with psychiatric patients. In relation to this, the recommendation is to encourage pharmacy law makers to implement programs designed to produce pharmacists that are much knowledgeable in the field of psychiatry. Hence, implementation of an effective pharmacy-based programs for psychiatric patients may be achieved.

Table 2. Mean scores of each patient in the MMAS-8 scale for first and second attempt

PROGRAM ENROLEES	MMAS-8 SCORES (FIRST ATTTEMPT) MAY 9 AND 10	MMAS-8 SCORES (SECOND ATTEMPT) MAY 22 AND 23
PATIENT A	1	6
PATIENT B	5	6
PATIENT C	4	5
PATIENT D	0	4
PATIENT E	1	4
PATIENT F	5	5
PATIENT G	2	5
PATIENT H	2	5
PATIENT I	3	5
MEAN SCORES	2.56	5.00

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Conflict of interest

The authors declare that they have no conflict of interest.

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Designing a Software Program for Chemotherapy Order Processing in the Oncology Pharmacy Unit of a Tertiary Hospital in the Philippines

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ABSTRACT

Cancer chemotherapy is a complex and high-risk process, where errors may occur at any stage leading to patient harm or death when left unchecked. The World Health Organization (WHO) advocates a systems approach in improving medication safety, and stressed that a well-designed and error-proof system prevents human from committing errors. The Institute of Safe Medication Practices (ISMP) further recommends the use of technology as aid in sterile compounding, to prevent errors that are not detected by manual process. This study aimed to (1) identify errors in the manual processing of chemotherapy orders, including the root causes (2) assess the severity, detectability, and frequency of these errors through Failure Mode, Effects, and Criticality Analysis (FMECA), and (3) design a software program addressing the critical failure modes. A mixed-method developmental study design was implemented. Semi-structured interviews with employed hospital pharmacists (n=10) were conducted to identify the steps in chemotherapy order processing and the errors which may occur at each step. In the FMECA, the pharmacists were asked to evaluate the criticality of the errors identified based on severity, detectability, and frequency. The errors were ranked based on the risk priority numbers (RPN). Lastly, a focus group discussion was conducted to identify software design aimed at addressing the errors. Twenty-one (21) failure modes were identified in the manual chemotherapy order processing, rooting from high workload, demand for speedy process, multi-tasking of pharmacists, failure to follow standard checks, complacency, exhaustion, mix-up of materials, incomplete supplies, and use of substandard materials. Based on the RPNs, the most critical failure modes identified involved the pre-compounding and compounding steps. The pharmacists recommended a software design featuring computerized scheduling of patients, pharmacy access to patient information, systems interconnectivity, electronic chemotherapy ordering, automatic computation, label printing, and barcoding of materials. To reduce and mitigate medication errors, the use of a software program to automate chemotherapy order processing is recommended to increase accuracy and efficiency of pharmacy operations.

KEYWORDS: Chemotherapy, Order Processing, Oncology, Software Design, Cancer, Medication Error

1. INTRODUCTION

Cancer chemotherapy is a complex and high-risk process. High-risk processes tend to cause medication errors and unsafe medication use, consequently making chemotherapy errors prone to causing patient harm or death especially when left unchecked[1].

WHO recommends the systems approach to improve medication safety. Safeguarding patients against medication errors would require putting systems filters and counterchecks to prevent medication errors from reaching the patients[2]. The American Society of Health-System Pharmacists (ASHP) guidelines resonated the same principle in preventing medication errors in chemotherapy. The ASHP

recommends that multiple independent checks be carried out at defined points throughout the chemotherapy use process[1]. On the other hand, the Institute of Safe Medication Practices (ISMP) guidelines for the safe preparation of compounded sterile preparations recommend using technology to augment the manual preparation and verification system during chemotherapy compounding. The ISMP Medication Errors Reporting Program (ISMP MERP) indicated that manual inspection of IV admixture ingredients cannot effectively prevent preparation and dispensing errors, but incorporating technology such as barcoding, gravimetric validation, robotics, and the use of IV workflow systems, can help identify and deter errors that could not be detected by manual inspection[3,4].

Studies on the use of technology in chemotherapy compounding support that the use of technology has been proven to contribute to improved accuracy[5] and to medication error detection and prevention[6,7]. Unfortunately, there is no published literature on errors specific to chemotherapy compounding in the context of Philippine practice. Further, published articles on the use of technology in pharmaceutical compounding are also not readily available in the country.

In view of the limited data regarding the use of technology in chemotherapy order processing, this study investigated the manual processing of chemotherapy orders in the oncology pharmacy unit of the Philippine General Hospital Cancer Institute. The study setting has been providing oncology services to around 18,000 Filipino cancer patients annually. All chemotherapy preparations in the hospital are centrally ordered in the oncology pharmacy unit. At present, the services that the oncology pharmacy unit provides are limited to compounding and dispensing. There are no clinical pharmacy services, and there is no comprehensive reporting system for errors in chemotherapy order processing in the oncology pharmacy unit.

This study aimed to (1) identify errors in the manual processing of chemotherapy orders, including the root causes (2) assess the severity, detectability, and frequency of these errors through Failure Mode. Effects, and Criticality Analysis (FMECA), and (3) design a software program addressing the critical failure modes. Identifying the errors and their root causes will help determine the areas of improvement in chemotherapy order processing in the pharmacy. In addition, a project proposal for the use of technology in chemotherapy order processing may address some difficulties in the manual process, which may lead to decreased medication errors, shortened chemotherapy order processing time, decreased workload for pharmacists, and cost-savings for the hospital.

2. MATERIALS AND METHODS

2.1. Stage 1: Planning

A mixed-method two-stage developmental study design was implemented. Semi-structured face-to-face interviews of the pharmacists (n=10) of the oncology pharmacy unit were conducted. The participants were asked to outline the steps in chemotherapy order processing in the pharmacy and to identify the errors occurring in each step of the process. Subsequently, a Failure Mode, Effects, and Criticality Analysis (FMECA) of the errors identified in the face-to-face interview was accomplished. The pharmacists were asked to grade the criticality of the errors based on severity, detectability, and frequency. The description used for the severity, frequency, and detectability scoring was adapted from the Institute for Healthcare Improvement guide[8]. The risk priority number (RPN) was computed for each error using the product of the severity, frequency, and detectability scores, and then the errors were ranked based on their RPN.

2.2. Stage 2: Software Design

A focus group discussion among the oncology pharmacy pharmacists was conducted. During the focus group discussion, the results of the interviews and the FMECA were disseminated to the group. The group proceeded to discussing the creation of a software program that would aid in manual chemotherapy order processing. The discussion concentrated on the recommendations of the pharmacists about the components and use of the software program. A use case diagram was created at the end of the focus group discussion.

3. RESULTS AND DISCUSSION

3.1. Chemotherapy Order Processing and Root Cause Analysis

All pharmacists of the oncology pharmacy unit participated in the planning stage. During the interview, the pharmacists were asked to enumerate the steps in processing chemotherapy orders and to identify the errors that occur in each step. A thematic analysis of the responses showed that chemotherapy order processing in the pharmacy consists of five steps: (1) receiving the chemotherapy orders from the doctors or nurses, (2) gathering the materials needed for the preparation, (3) preparing the supplies to be used for compounding in the sterile room, (4) compounding the chemotherapy admixture, and (5) dispensing the compounded sterile preparations. Each step is performed by an individual pharmacist, which allows for counterchecks to be implemented at every step of the process (Figure 1). Twenty-one errors or failure modes were identified occurring in different steps.

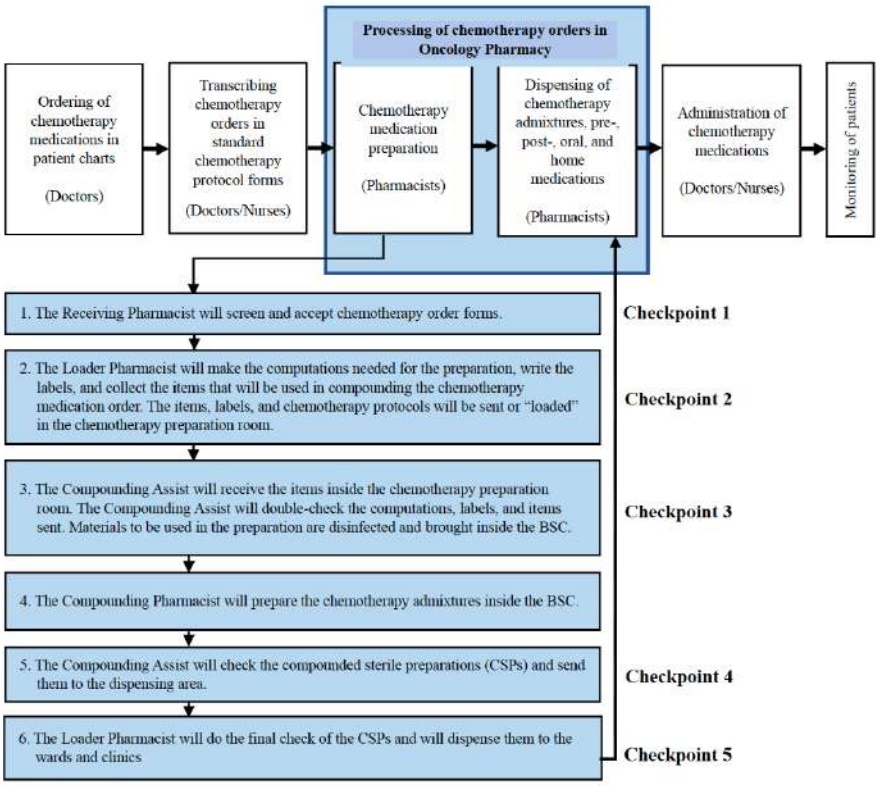

Figure 1. Schematic diagram for the chemotherapy order processing in the oncology pharmacy unit.

In addition, the interview was also able to elucidate multiple factors that can lead to errors in the chemotherapy order processing. Human factors include lack of manpower, exhaustion, complacency in performing tasks, and poor handwriting. Environmental factors such as small work areas and lack of organization contribute to the mix-up of materials in the pharmacy, and the distraction of pharmacists in performing their tasks. Furthermore, the high volume of workload, demand for speedy processes, multi-tasking, and failure to perform standard checks can also precipitate errors in the chemotherapy order processing (Figure 2).

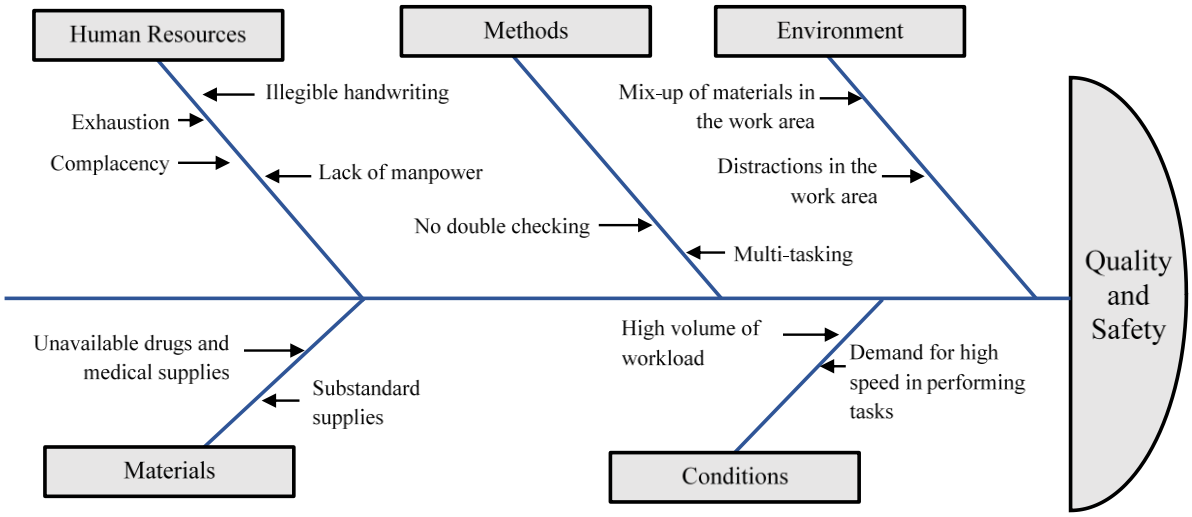

Figure 2. Root cause analysis of the errors identified during chemotherapy order processing.

3.2. Failure Mode, Effects, and Criticality Analysis

The most critical failure modes were identified to occur during the compounding and pre-compounding steps, while the least critical may occur during receiving chemotherapy orders and dispensing CSPs (Table 1). Ranking the errors helped in determining the most critical problems that the technology should address, its use in the process, and the framework for the integration of technology in the workflow. Furthermore, ISMP recommends the conduct of FMECA before the implementation of technology in the sterile compounding process, as this will enable the comparison of the current process against the modified, technology-assisted process to determine improvements[9].

3.3. Use Case Diagram for Software Design

The results of the interview and the FMECA were shared with the pharmacists in a focus group discussion. The pharmacists were asked about the software features that are needed by the pharmacy. The team suggested that the software program should allow: computerized scheduling of patients, pharmacists' access to patient electronic charts, electronic chemotherapy ordering, automatic computation, label printing, barcoding of items to be used for compounding, and electronic recording of orders. A further suggestion given was to ensure systems interconnectivity.

Online scheduling would prevent patients from missing their chemotherapy sessions because they were not scheduled by their physicians. Computerized ordering would help address problems such as incomplete details written in the order forms, wrong or incomplete pre-medications ordered, mix-ups of or losing chemotherapy orders, manual recording of orders, and illegible handwriting. Allowing pharmacists to access patients' electronic charts would allow them to verify the accuracy of the orders. Automatic computation would prevent errors in the calculation of drug dosages, while label generation would ensure that complete, correct, and legible information is printed on the admixture labels. Barcoding of supplies would ensure that all the supplies needed for compounding will be charged appropriately in the patient's bill, and the inventory of supplies is automatically monitored including the expiry of medicines. Interoperability of the new software program with other software being used in the hospital would ease the integration of the new software into the existing workflow in the pharmacy, which would improve the efficiency of the pharmacy operation. The team created the use case diagram for the software program (Figure 3).

Table 1. FMECA Criticality ranking of errors based on RPN.

Ranking	Error	Chemotherapy Order Processing Step
1	Presence of impurities.	Compounding and Pre-compounding
2	Wrong drug volume was incorporated.	
3	Wrong drug was incorporated.	
4	Illegible handwriting.	
5	Wrong compounding techniques.	
6	Wrong computation.	
	Wrong or incomplete details are written in the preparation tag and CSP label.	
7	Mix-up of medicines and supplies.	
	Wrong diluent was used.	
8	Putting expired medicines to be used in compounding.	
9	Wrong final volume was prepared.	
10	Mix up of patient's order forms.	Receiving chemotherapy orders and Dispensing chemotherapy admixtures
11	Patient has an order for chemotherapy but was not on the schedule.	
12	Lost order forms.	
13	Dispensing incorrect or incomplete chemotherapy admixtures.	
14	Wrong or incomplete chemotherapy pre-medications were ordered.	
15	Wrong packaging or proper storage conditions were not observed.	
16	Incomplete medicines and supplies were given to the compounding pharmacist.	
17	Wrong medicines/supplies were charged to the patient's account.	
18	Wrong time of dispensing	
	Incomplete order forms	

4. CONCLUSION

Despite the placement of multiple process checkpoints, errors still occur in the manual processing of chemotherapy orders in the pharmacy due to human, methodological, and environmental factors. One strategy to decrease the errors in the process is to decrease the opportunities for pharmacists to commit them in performing their tasks. The use of a software program will automate several pre-compounding tasks such as scheduling patients, order verification, dose calculation, and writing labels. The use of a software program may lead to increased accuracy in chemotherapy order processing. It may also decrease the time and the manual labor required to do the tasks. Furthermore, the decrease in workload will increase the pharmacists' spare time which may lead to the expansion of pharmacy services.

This study employed a qualitative analysis of medication errors in the manual processing of chemotherapy orders. A quantitative measurement of the number of medication errors, chemotherapy order processing time, and client satisfaction may further be carried out before and after the implementation of the software program, to establish the impact of the use of the software program in chemotherapy order processing. A Failure Mode Effects and Criticality Analysis may also be conducted after the completion of the project to determine the errors in the use of the software program.

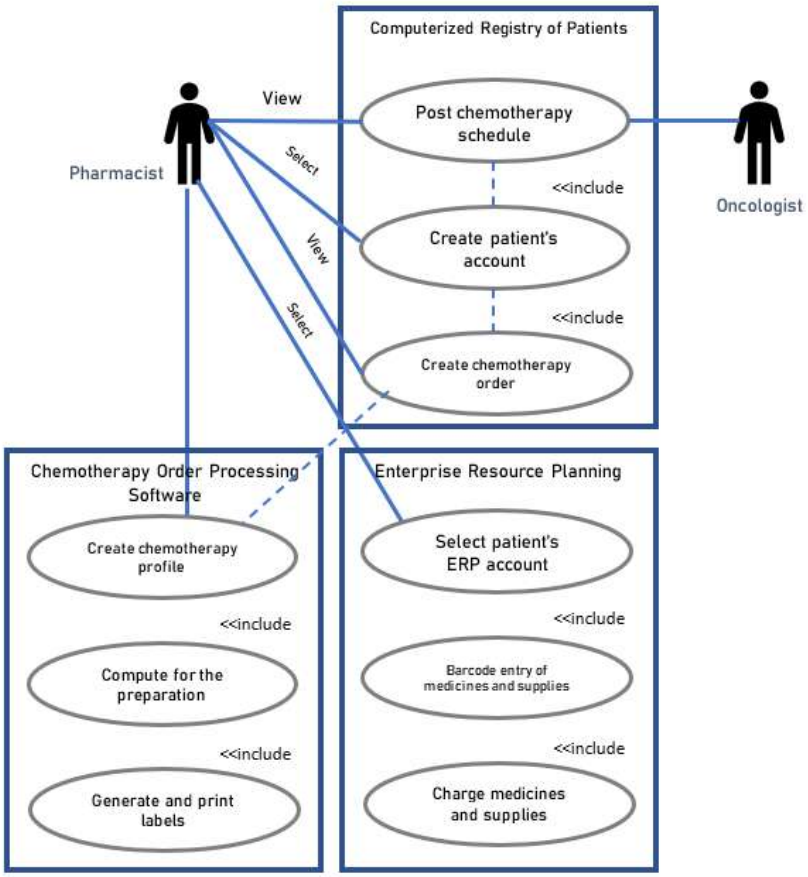

Figure 3. Use case diagram of the chemotherapy order processing software design.

5. ACKNOWLEDGMENT

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Conflict of interest

The authors declare that they have no conflict of interest

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Patients' Adherence to Fixed-Dose Combination Medicines for Tuberculosis in the Non-National TB Program in Indonesia

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ABSTRACT

In Indonesia, patients access pulmonologist clinics to receive out-of-pocket Fixed Dose Combination (FDC) drugs for pulmonary tuberculosis (TB). There were 89 pulmonary TB patients treated during 2022–2023, 27 (30.33%) completed treatment within six months, while 29 patients (32.58%) cannot continue due to cost and were referred to a public health center (puskesmas). Ten patients (11.23%) pulmonologist refer to the community health center (puskesmas). While 14 patients (15.73%) experienced lost follow-up, and 3 patients (3.37%) stopped treatment completely. Further studies are needed to identify
patients' adherence to the non-National TB Program in Indonesia.

KEYWORDS: Patient's Adherence, Fixed Dose Combination Tuberculosis Drug, Non National TB Program, Indonesia

1. INTRODUCTION

Indonesia is in the second position with the highest number of Tuberculosis (TB) cases in the world. TB cases in Indonesia are estimated at 969,000 TB cases, up 17% from 824,000 cases in 2020. The incidence of TB cases in Indonesia is 354 per 100,000 population, which means that for every 100,000 people in Indonesia there are 354 people suffering from TB. The success rate of TB treatment is still sub-optimal at 85 percent, below the global target for a treatment success rate of 90 percent [1,2] . Non-adherence to TB treatment is an important barrier and is one of the most significant barriers to TB control globally and has been a major factor in treatment failure. The high rate of TB in Indonesia is largely due to non-adherence to treatment. Adherence to TB treatment is essential to prevent transmission of the disease, achieve cure and avoid the emergence of drug resistance, relapse and death [4,5].

Factors that can affect patient compliance include TB treatment for a long time, many of the sufferers have felt cured so they stop taking drugs, the presence of other diseases, lack of knowledge of patients and families, patients are lazy to seek treatment, support factors from family, no effort from themselves or motivation and support to take medicine, education, work, being away from home, perceived and experienced stigma and discrimination, beliefs such as perceptions of health/cure, perceived risks (side effects), economic constraints to adequate food and medical expenses other than anti-TB drugs, poor health-patient provider relationships such as communication gaps, disrespect for patients, quality health care and patient satisfaction; health information and education, the amount of drugs consumed [3,6,7.] It is important that financial risk protection is a key target to achieve in end TB strategy [10].

Adherence can be divided into primary and secondary non-adherence. Primary non adherence is defined as the patient's failure to obtain prescribed medication at the first visit. This failure accounts for 5-10% of all prescriptions in primary health care [11]. Clinical pharmacy services as a single or composite intervention have the potential to improve TB outcomes [12]. We conducted preliminary study

on out-of-pocket, Non-BPJSKes Tuberculosis patients in Indonesia with the aim of determining the factors that affect drug adherence in non-BPJSkes Tuberculosis and to determine the success of therapy in non-BPJSkes Tuberculosis patients in Indonesia.

2. MATERIAL AND METHODS

Data on non-BPJSKes patients at pulmonologist clinics in Palopo City, Indonesia collected prescriptions containing tuberculosis drugs from January 2022 to December 2023. We re-examined with the patient's medical record. Doctors were not informed about this survey to prevent changes in prescribing patterns. Patient, name, gender, age, address, duration of treatment, recorded.

3. RESULT AND DISCUSSION

3.1 Result

There were 89 pulmonary TB patients treated during 2022–2023, although have national health insurance BPJSKes, patient choose to use out of pocket medicine. Twenty seven (30.33%) completed treatment within six months, while 29 patients (32.58%) cannot continue due to cost and were referred to a public health center (puskesmas). Ten patients (11.23%) pulmonologist refer to the community health center (puskesmas). While 14 patients (15.73%) experienced lost follow-up, and 3 patients (3.37%) stopped treatment completely (Table 1), we found that 6 patient data could not be completely confirmed due to a mismatch between the drug prescription and the patient's medical record. There were two brands of FDC drugs for pulmonary tuberculosis (Table 2) with the same ingredients made by two different factories in Indonesia.

Table 1 Data From Prescription and Patient Medication Record

No	Completed 6 month n = 27	Patient Request to PHC n = 29	Directly to PHC n = 10	Lost to Follow Up n = 14	Stop Theraphy n = 3	Missmatch n = 6
Male n = 36	18	19	5	9	1	3
Female n = 53	9	10	5	5	2	3

Note : PHC : Public Health Care, Missmatch: FDC A/B have differences between prescription and patient medication record.

Table 2 Data Result According to medication type , FDC-A or FDC-B

No.	Total sample (n=89)	FDC-A (n=31)	FDC-B (n=54)	OTHER (n=4)
1	Male n = 36	13	20	3
2	Female n = 53	18	34	1

Note : FDC A is a Fixed Dose Combination medication brand A, and FDC B is a Fixed Dose Combination medication brand B which available in Indonesia. Other medicine is not Fixed Dose Combination tuberculosis drug, but combination drugs only.

3.2 Discussion

The long period of tuberculosis treatment causes patients to have a high risk of forgetting or stopping the medication altogether, which increases the risk of oral anti-tuberculosis drug resistance[14]. Although TB drugs are free through public facilities in Indonesia, unless there is collaboration between public and private facilities TB patients treated at private facilities have to spend out-of-pocket money for their treatment. Collaboration between the public and private sectors, therefore, is very important in areas where the presence of private facilities is high. Patients should always be able to access free TB drugs regardless of their treatment location [15].

Finding and curing the missing TB patients in Indonesia requires a more robust understanding of how patients navigate the complex healthcare network, and how National TB Control Program can be best positioned to meet patients needed.

Understanding patient pathways can help connect patient preferences with tuberculosis-related services [16,17]. In this study we did not find any primary non-adherence, all 89 pulmonary TB patients treated during 2022–2023, taking all of their medications in the clinic, or directly refer to puskesmas.

Twenty seven (30.33%) completed treatment within six months. 29 patients (32.58%) cannot continue due to cost and were referred to a public health center (puskesmas). This patient initially sought out-of-pocket treatment but over time decided could no longer afford to pay and asked to be referred to a health center. The doctor refers to the puskesmas attaching the length of treatment according to the patient's document. Ten patients (11.23%) Pulmonologists refer patients directly to the health center, these patients know the high costs and ask to be referred directly to the puskesmas. The doctor refers to the puskesmas with a letter, according to the patient's identity. Fourteen patients (15.73%) experienced lost follow-up, this is because there is no telephone number and address that cannot be contacted due to incomplete data written by officers at the clinic and at the pharmacy. Three patients (3.37%) stopped treatment completely for unclear reasons. Staff at the clinic have been working with Tb managers at the puskesmas to follow up on this incident. Six patient data could not be completely confirmed, due to a mismatch between prescription and the patient's medical record.

Although there is a known relationship between cost and adherence in tuberculosis patients[8, 9], but this was not seen in this preliminary study. Interventions to promote adherence and retain patients in care must not neglect the early months of treatment [13]. Despite a National TB Control Program providing predominantly free-of-charge treatment services for TB, and a National Health Insurance scheme, we found there were patients use the private health sector.The limitation of the study is that there are no known factors that affect patient adherence and the success of therapy in non-BPJSkes TB patients in Indonesia.

4. CONCLUSION

There were 89 pulmonary TB patients treated during 2022–2023, 30.33% patient completed treatment within six months, 32.58% could not continue treatment and were referred to health centers, 11.23% pulmonologist direct refer to the community health center 15.73 lost to follow up and 3.37 percent patients non-adherence. Incomplete recording of patient data are difficulties to confirming patients who do not continue treatment. Further research is needed.

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Conflict of interest

Nothing to declare

Funding

Nothing to declare

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Comparative Analysis of Healthcare Workers' Perceptions of Antimicrobial Resistance Management among Private and Government Hospitals in Metro Manila

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ABSTRACT

Antibiotics have been widely used in human and veterinary medicines for decades to treat bacterial infections, significantly decreasing the percentage of deaths associated with bacterial infections and limiting the spread of disease between individuals. This, however, has led to antimicrobial resistance (AMR) becoming a major global health concern, rendering many currently available antimicrobials ineffective, resulting in severe infections, complications, unsuccessful treatment, prolonged hospital admissions, and increased mortality. To combat this, Philippine policies on the control of AMR were established, such as the National Antibiotic Guidelines and Antimicrobial Stewardship Program. The questionnaire included questions regarding the perceptions of these healthcare workers on the antimicrobial resistance management of their institution in terms of adherence and effectiveness. The collected data was compared and analyzed using descriptive statistics such as percentage, frequencies, mean and standard deviation, as well as inferential statistics, particularly ANOVA, and Chi-square test. There was a significant difference in overall perceived practices and effectiveness in the implementation of antimicrobial resistance protocols. Pharmacists were found to have the highest perception score, followed by physicians, and then nurses. The type of hospital and staff position showed a significant correlation with the perceptions of compliance and effectiveness of the antimicrobial resistance protocol. These findings provide a comprehensive overview of the healthcare workers, including physicians, pharmacists, and nurses, which is essential for understanding their perspectives on AMR management compliance and its effectiveness. The differences in their perceptions suggest that there may be varying levels of understanding and engagement with AMR management practices among different healthcare professionals. Also, considering both the organizational context and the specific roles of healthcare workers when creating strategies may further enhance AMR management in hospital settings.

KEYWORDS: Antimicrobial resistance; antimicrobial stewardship; compliance; effectiveness; healthcare workers; Philippines

1. INTRODUCTION

Antibiotics have been widely used in humans and veterinary medicines for decades to treat bacterial infections and remain essential in treating acute bronchitis, common cold, ear infections, influenza, sinusitis, skin infections, sore throat, and urinary tract infections[1,2]. The use of antibiotics and other methods for controlling infection has significantly decreased the percentage of deaths associated with bacterial infections globally[3]. Additionally, antibiotics limit the spread of disease between individuals, improving their health and well-being and providing a more considerable public health benefit in disease prevention in communities[3].

The many applications of antimicrobials have led to antimicrobial resistance becoming a major global health concern. Resistance is a natural occurrence observed in microorganisms due to their ability to render the antibiotic defective through internalized and adaptive defense mechanisms designed to eliminate or inactivate the antibiotic, modifications of intended drug receptors, and gene mutations[4]. It is a phenomenon wherein microorganisms such as bacteria, fungi, and parasites develop resistance to medications that were otherwise utilized to eliminate them[4]. For instance, antiseptic resistance genes have led to increased tolerance against common agents, such as benzalkonium chloride (BAC) and chlorhexidine digluconate (CHDG) in isolates of *Staphylococcus* spp.[5]. Prolonged exposure to these medications, particularly antivirals[6] and antibiotics[7], is at the core of the development of resistance patterns.

The main factors in the exacerbation and acceleration of antimicrobial resistance, however, are the irresponsible overuse and unnecessary misuse of antibiotics, poor hygienic practices, and lack of quality infection control[7]. The dangers of antimicrobial resistance impose grave risks to individual and public health because this compromises the use and effectiveness of antibiotics in mitigating infection and application in current medical advancements and procedures such as surgical operations, organ transplants, joint replacements, and chemotherapy[8]. As a result, the prevalence of antimicrobial resistance renders many currently available antimicrobials ineffective, resulting in severe infections, complications, unsuccessful treatment, prolonged hospital admissions, and increased mortality[9].

As a result, the DOH released Administrative Order (AO) No. 2014-0009 in 2014 with the subject 'Implementing Guidelines on the Rational Use of Medicines (RUM) Pillar of the Philippine Medicines Policy' to integrate the rational and responsible use of medicines in the healthcare system and establish a conceptual framework of the National Plan of the RUM[10]. The main objectives of AO 2014-0009 are to set guidelines that aim to institutionalize the RUM and raise awareness of RUM to achieve both the best clinical outcomes possible in the use of medicines and economic goals[10]. Following this, the National Antibiotic Guidelines (NAG) 2018 initiated the development of the national plan against AMR. Infectious disease experts and other relevant professionals streamlined guidelines and clinical pathways that hospitals must follow or integrate into their local systems[11]. These included antibiotic treatment recommendations according to the infections of different organ systems and surgical prophylaxis that hospitals must implement according to their facilities and their patients' susceptibility[11].

In the Philippines, studies on AMS policies focused on the clinicians' perceptions on the policy's acceptability, barriers, and enablers[12]. However, to further understand how these policies work in hospital settings, it is important to consider the different areas highlighted by these protocols, such as those concentrated on the medication use process, such as prescribing, dispensing, and administration, as emphasized in the NAG.

To bridge gaps in current knowledge, this study was designed to understand how hospital-based AMR protocol applied to different professions concentrated on various stages of medication use and pharmacotherapy–prescribing habits of physicians, dispensing practice in hospital pharmacies, and medication administration by the nursing staff. This provides a more specific understanding of how healthcare workers' awareness of nationally mandated policies is directly correlated to compliance, leading to a concrete description of how targeted and modern policy interventions such as interprofessional collaboration.

The research paradigm (Figure 1) outlines the steps involved in Antimicrobial Stewardship Protocol selection regarding the Guidelines on the RUM Pillar of the Philippine Medicines Policy (AO 2014-0009) and Philippine Action Plan on Antimicrobial Resistance. This involves gathering data through questionnaires, including healthcare workers' demographic profiles and perceptions, and a review of related literature. The process section involves the data collection of the healthcare workers' demographic profile and perception through an adapted questionnaire, and interpretation of data and the statistical treatment used. The output section involves the distribution of data based on the demographics of the healthcare worker, perception of the healthcare worker on the hospital's compliance, and the effectiveness of AMR protocol, identification of significant difference in perception of the healthcare workers, and correlation between demographic variables and perceptions on AMR protocol.

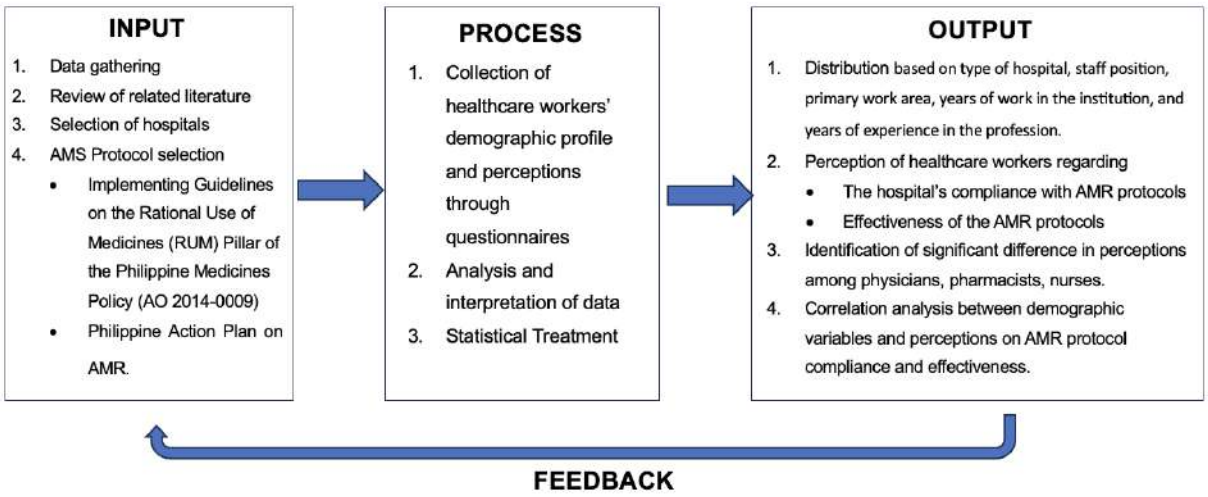

Figure 1. Conceptual framework utilizing the Input-Process-Output (IPO) Model

2. MATERIALS AND METHODS

2.1. Research Design

The researchers performed a comparative analysis to discern variations in the perceptions of healthcare workers in public and private hospitals, specifically physicians, nurses, and pharmacists, across multiple hospitals in Metro Manila concerning their hospital's adherence to the established AMR protocol and its effectiveness.

2.2. Research Instrument

In this study, the researchers systematically gathered data through a modified questionnaire with a 4-point Likert scale based on the standard AMR protocols. The first part of the questionnaire included questions regarding their demographics, the type of hospital they are working for, their staff position, and their primary work in the institution, and specific questions regarding Implementing Guidelines on the Rational Use of Medicines (RUM) Pillar of the Philippine Medicines Policy (AO 2014-0009) and the Philippine Action Plan on AMR.

2.3. Participants

The study included 129 healthcare professionals currently employed in hospitals in Metro Manila, both public and private. Participants were composed of 31 physicians, 30 pharmacists, and 68 nurses. In addition, the inclusion criteria covered a wide range of specialty fields in the healthcare context, including pediatrics, laboratory services, surgery, rehabilitation, and obstetrics. This ensured a more thorough understanding of the views and perceptions of healthcare professionals from various specializations within the Metro Manila healthcare system.

2.4. Data Gathering Procedure

Each type of healthcare worker was given different sets of questionnaires based on the nature of their work. The instrument was put through a thorough reliability testing process, including a statistician's evaluation using Cronbach's Alpha to ensure its clarity, relevancy, and efficiency in gathering the desired data. Then, the validated questionnaires were distributed to the 129 respondents in public and private facilities in Metro Manila.

2.5. Statistical Treatment

First, the research aimed to explore the demographic profile of healthcare workers, considering various factors. To explore the demographic profile of healthcare workers, descriptive statistics like frequencies and percentages were used to analyze the distribution of respondents across hospital types, staff positions, and primary work areas[13,14,15]. Similarly, the years of work at the institution and years of experience in the current profession were investigated using descriptive statistics such as weighted mean and standard deviation[16,17]. Furthermore, the study delved into the primary work areas and types of doctors, utilizing descriptive statistics for a comprehensive overview. Overall, these showed the variability in respondents' tenure at their respective institutions and experience levels.

Next, the study investigated their views on hospital compliance with AMR guidelines. Convenience sampling was done to collect data among the different healthcare workers. Descriptive statistics using mean and standard deviation was applied to summarize their perceptions. Moreover, to ascertain if there is a significant difference in perceptions among healthcare workers regarding both AMR guideline compliance and the effectiveness of the AMR protocol, a one-way Analysis of Variance (ANOVA) was conducted. This is appropriate to test for significant differences in perceptions among healthcare professionals[18]. Post-hoc tests, such as Scheffé's method, were employed since ANOVA revealed a significant difference. This allowed a more conservative interpretation of complex ANOVA analysis, such as unequal sample sizes, as seen in this study[19]. It is crucial to ensure that the assumptions of ANOVA, such as homogeneity of variances and normality, are met for accurate interpretation. Lastly, the Chi-square test of relationship was used to find the correlation between the demographics and perceptions of the respondents. These statistical treatments were executed using the IBM SPSS version 26.

3. RESULTS AND DISCUSSION

3.1. Demographic Profile of the Respondents

The demographic profile of the respondents (Table 1) in this study is diverse, encompassing professionals from various hospital types, staff positions, primary work areas, and experience levels. his diversity could provide valuable insights into the effectiveness of sustainable practices in healthcare settings, highlighting the importance of considering different healthcare professionals' unique perspectives and experiences in sustainability initiatives.

3.2. Perception of Healthcare Workers Regarding Hospital AMR Management Protocol Compliance

The physicians and pharmacists strongly agreed with the hospital's compliance with AMR management practices, while the nurses agreed. The high level of agreement among healthcare workers across different roles reflects high compliance with AMR guidelines in their respective hospitals. The results may be seen in Table 2.

Table 1. Distribution of the Respondents Based on Demographic Profile

Profile		Frequency	Percentage
Type of Hospital	Private	72	53.3
	Public	63	46.7
Staff Position	Physician	37	27.4
	Pharmacist	30	22.2
	Nurse	68	50.4
Primary Work Area/Type of Doctor	Many different units/No specific unit	16	11.9
	Pharmacy	30	22.2
	Surgery	13	9.6
	Anesthesiology	1	0.7
	Laboratory	0	0.0
	Medicine (non-surgical)	31	23.0
	Psychiatry/mental health	0	0.0
	Pediatrics	12	8.9
	Intensive care unit (any type)	1	0.7
	Obstetrics	1	0.7
	Radiology	0	0.0
	Rehabilitation	0	0.0
	Emergency department	10	7.4
	Hemodialysis Unit	2	1.5
	Medical Surgical Ward	10	7.4
	Burn Critical Surgery Unit	2	1.5
	General Ward	6	4.4
Years of Work in the Institution	Less than 1 year	42	31.1
	1 – 2 years	27	20.0
	3 – 5 years	22	16.3
	6 – 9 years	15	11.1
	10 or more years	29	21.5
Year of Experience in Current Position	Less than 1 year	17	12.6
	1 – 2 years	17	12.6
	3 – 5 years	32	23.7
	6 – 9 years	15	11.1
	10 or more years	54	40.0
Total		**135**	**100**

Table 2. Perceptions of Respondents Regarding Hospital AMR Management Compliance.

Statement	Composite Mean	SD	Interpretation
Physicians	3.33	0.78	Strongly Agree
Pharmacists	3.67	0.55	Strongly Agree
Nurses	3.14	1.01	Agree

+ The interpretations for the weighted means are as follows: 1.00 – 1.74 Strongly Disagree; 1.75 – 2.49 Disagree; 2.50 – 3.24 Agree; 3.25 – 4.00 Strongly Agree.

3.3. Perception of Healthcare Workers Regarding Hospital AMR Management Protocol Effectiveness

The perceptions of effectiveness were measured based on two questions in the questionnaire on whether patient outcomes improved due to the AMR protocol and whether the antimicrobial stewardship protocols contribute to reducing the incidence of antibiotic-resistant infections among patients. The physicians' and pharmacist's composite mean fell under strongly agree, while the nurses' composite mean score fell under agree. The results may be seen in Table 3.

Table 3. Perceptions of Respondents Regarding AMR Management Protocol Effectiveness.

Respondents	Composite Mean	SD	Interpretation
Physicians	3.30	0.74	Strongly Agree
Pharmacists	3.70	0.53	Strongly Agree
Nurses	3.19	0.92	Agree

+ The interpretations for the weighted means are as follows: 1.00 – 1.74 Strongly Disagree; 1.75 – 2.49 Disagree; 2.50 – 3.24 Agree; 3.25 – 4.00 Strongly Agree.

3.4. Difference in the Perception of Healthcare Workers Regarding Hospital AMR Management Compliance

There is a significant difference in the perception of healthcare workers based on their role. The results may be seen in Tables 4 and 5. Pharmacist perception scores are higher, while nurses have a slightly lower perception score. The F value and the significance level indicate that the differences in perception among healthcare workers are statistically significant. Therefore, the null hypothesis is rejected, suggesting that there are indeed differences in perception among physicians, pharmacists, and nurses regarding the hospital's compliance with AMR protocols. Overall, this indicates that there is general agreement among healthcare workers regarding the hospital's compliance with AMR protocols. However, the differences in perception highlight the importance of considering the inputs of different healthcare professionals when implementing and assessing AMR management protocols.

The comparison between physicians and pharmacists shows a non-significant difference in perception of the hospital's compliance with AMR protocols. The comparison between physicians and nurses also shows a non-significant difference. This indicates no significant difference in perception between physicians and nurses regarding the hospital's compliance with AMR protocols. However, the comparison between pharmacists and nurses shows a significant difference, with pharmacists having a more positive perception than nurses.

Table 4. Difference in the Perception of Respondents Regarding Hospital AMR Management Compliance

Profile	Mean	SD	f value	Sig. (p)	Decision	Interpretation
Physician	3.33	0.60	5.471	0.005	Reject Ho	Significant
Pharmacist	3.67	0.36				
Nurse	3.14	0.90				
Overall	**3.31**	**0.76**				

Table 5. Post Hoc Analysis on the Difference in Perceptions among Respondents Regarding Hospital AMR Management Compliance

Dependent Variable	(I) Staff Position	(J) Staff Position	Sig.	95% Confidence Interval	95% Confidence Interval
				Lower Bound	Upper Bound
Perception	Physician	Pharmacist	.169	-.7860	.1040
		Nurse	.453	-.1815	.5586
	Pharmacist	Physician	.169	-.1040	.7860
		Nurse	.005	.1326	.9265
	Nurse	Physician	.453	-.5586	.1815
		Pharmacist	.005	-.9265	-.1326

3.5 Difference on the Perception of Healthcare Workers Regarding Hospital AMR Management Effectiveness

There is also a significant difference in the perception of healthcare workers based on their role, as seen in Tables 6 and 7. Pharmacists have the highest perception scores, while nurses have a lower perception score, suggesting that there are differences in perception among physicians, pharmacists, and nurses regarding the effectiveness of the hospital's AMR protocol. Overall, this indicates that there is general agreement among healthcare workers regarding the effectiveness of the hospital's AMR protocol.

The comparison between physicians and pharmacists shows a non-significant difference regarding the effectiveness of the hospital's AMR protocol. The comparison between physicians and nurses also shows a non-significant difference. However, the comparison between pharmacists and nurses shows a significant difference, with pharmacists having a more positive perception compared to nurses.

Table 6. Difference on the Perception of Respondents Regarding Hospital AMR Management Effectiveness

Profile	Mean	SD	f value	Sig. (p)	Decision	Interpretation
Physician	3.30	0.69	4.555	0.012	Reject Ho	Significant
Pharmacist	3.70	0.52				
Nurse	3.19	0.90				
Overall	**3.33**	**0.79**				

Table 7. Post Hoc Analysis on the Difference in Perceptions among Respondents Regarding Hospital AMR Management Effectiveness

Dependent Variable	(I) Staff Position	(J) Staff Position	Sig.	95% Confidence Interval	95% Confidence Interval
				Lower Bound	Upper Bound
Effectiveness	Physician	Pharmacist	.110	-.8734	.0680
		Nurse	.799	-.2853	.4975
	Pharmacist	Physician	.110	-.0680	.8734
		Nurse	.013	.0889	.9287
	Nurse	Physician	.799	-.4975	.2853
		Pharmacist	.013	-.9287	-.0889

3.6 Difference on the Overall Perception of Healthcare Workers Regarding Hospital AMR Management

There is a significant difference in the overall perception of healthcare workers based on their role (Table 8). Pharmacists have the highest perception scores, while the nurses have the lowest perception score. The F value and the significance level indicate that the differences in perception among healthcare workers regarding the hospital's AMR management are statistically significant. Overall, this indicates that there is general agreement among healthcare workers regarding the hospital's AMR management.

Table 9 presents the post hoc analysis of the difference in the overall perception of healthcare workers regarding the hospital's AMR protocol. The analysis compares the perceptions of physicians, pharmacists, and nurses using pairwise comparisons.

The comparison between physicians and pharmacists shows that there is no significant difference in overall perception between physicians and pharmacists regarding the hospital's AMR management. The comparison between physicians and nurses also shows a non-significant difference. However, the comparison between pharmacists and nurses shows a significant difference. This indicates that there is a significant difference in overall perception between pharmacists and nurses regarding the hospital's AMR management, with pharmacists having a more positive perception compared to nurses.

Table 8. Difference on the Overall Perception of Healthcare Workers Regarding Hospital AMR Management

Profile	Mean	SD	f value	Sig. (p)	Decision	Interpretation
Physician	3.32	0.59	4.555	0.012	Reject Ho	Significant
Pharmacist	3.67	0.36				
Nurse	3.14	0.89				
Overall	**3.31**	**0.75**				

Table 9. Post Hoc Analysis on the Difference on the Overall Perception of Respondents Regarding Hospital AMR Management

Dependent Variable	(I) Staff Position	(J) Staff Position	Sig.	95% Confidence Interval	95% Confidence Interval
				Lower Bound	Upper Bound
Overall	Physician	Pharmacist	.154	-.7874	.0936
		Nurse	.480	-.1866	.5460
	Pharmacist	Physician	.154	-.0936	.7874
		Nurse	.005	.1336	.9196
	Nurse	Physician	.480	-.5460	.1866
		Pharmacist	.005	-.9196	-.1336

3.7 Correlation Between the Demographic Profile and Perception of Health Care Workers Regarding the Hospital's Compliance and Effectiveness of AMR protocol

The correlation analysis between the demographic profile and the perception of healthcare workers regarding the hospital's compliance with AMR compliance revealed varying findings (Table 10). Firstly, there is a significant correlation between the type of hospital and healthcare workers' perception of AMR compliance. This suggests that the type of hospital may influence how healthcare workers perceive the institution's adherence to AMR guidelines. Additionally, there is a significant correlation between staff position and perception of AMR compliance. This implies that healthcare workers in different roles may have varying perceptions of their hospital's AMR compliance. On the other hand, there is no significant correlation between primary work area/type of doctor and perception of AMR compliance. This indicates that the specific work area or type of doctor may not influence how healthcare workers perceive their hospital's adherence to AMR guidelines. Similarly, there is no significant correlation between years of work in the institution or years of experience in the current position and perception of AMR compliance. This suggests that the length of time a healthcare worker has been working in the institution or in their current position may not impact their perception of AMR compliance. These findings highlight the importance of considering the organizational context and individual roles when designing interventions to improve AMR compliance in healthcare settings.

Table 10. Correlation Between the Demographic Profile and Perception of Respondents Regarding the Hospital's Compliance with Antimicrobial Resistance Management Compliance

Profile	Sig. (p)	Decision	Interpretation
Type of Hospital	0.001	Reject Ho	Significant
Staff Position	0.000	Reject Ho	Significant
Primary Work Area/Type of Doctor	1.000	Accept Ho	Not Significant
Years of Work in the Institution	0.705	Accept Ho	Not Significant
Year of Experience in Current Position	0.959	Accept Ho	Not Significant

The correlation analysis between the demographic profile and the perception of healthcare workers regarding the effectiveness of the AMR protocol yielded insightful results (Table 11). Firstly,

there is a significant correlation between staff position and perception of AMR protocol effectiveness. This indicates that the role of healthcare workers may influence their perception of how effective the AMR protocol is in their hospital. On the other hand, there is no significant correlation between the type of hospital, primary work area/type of doctor, years of work in the institution, or year of experience in the current position and perception of AMR protocol effectiveness. This suggests that factors such as the type of hospital, specific work area/type of doctor, and length of work experience or position tenure may not have a significant impact on how healthcare workers perceive the effectiveness of the AMR protocol. Overall, these findings highlight the importance of considering the role and perspective of healthcare workers when assessing the effectiveness of AMR protocols in healthcare settings. This also underscores the need for targeted interventions and education programs tailored to healthcare workers' specific roles and responsibilities to enhance the effectiveness of AMR management protocols.

Table 11. Correlation Between the Demographic Profile and Perception of Respondents Regarding the Effectiveness of the Antimicrobial Resistance Management Protocol

Profile	Sig. (p)	Decision	Interpretation
Type of Hospital	0.371	Accept Ho	Not Significant
Staff Position	0.034	Reject Ho	Significant
Primary Work Area/Type of Doctor	0.498	Accept Ho	Not Significant
Years of Work in the Institution	0.841	Accept Ho	Not Significant
Year of Experience in Current Position	0.274	Accept Ho	Not Significant

The analysis of the overall perception of healthcare workers regarding AMR revealed significant correlations with both the type of hospital and staff position (Table 12). Firstly, there is a significant correlation between the type of hospital and overall perception of AMR management. This suggests that whether a hospital is private or public may influence how healthcare workers perceive the institution's efforts in managing AMR. Additionally, there is a significant correlation between staff position and overall perception of AMR management, indicating that different roles within the healthcare team may lead to varying perceptions of the effectiveness of AMR management efforts. In contrast, there is no significant correlation between primary work area/type of doctor, years of work in the institution, or years of experience in the current position and overall perception of AMR management. This suggests that the specific work area or type of doctor, as well as the length of work experience or position tenure, may not have a significant impact on how healthcare workers perceive the overall management of AMR in their institution. Overall, these findings highlight the importance of considering tailoring strategies to address the specific needs and perceptions of healthcare workers based on their role and the type of hospital they work in.

Table 12. Correlation Between the Demographic Profile and Overall Perception of Respondents

Profile	Sig. (p)	Decision	Interpretation
Type of Hospital	0.000	Reject Ho	Significant
Staff Position	0.000	Reject Ho	Significant
Primary Work Area/Type of Doctor	1.000	Accept Ho	Not Significant
Years of Work in the Institution	0.811	Accept Ho	Not Significant
Year of Experience in Current Position	0.911	Accept Ho	Not Significant

4. CONCLUSION

Based on the findings of the study, the demographic profile of the respondents varied in terms of the type of hospital, staff position, primary work area/type of doctor, years of work in the institution, and years of experience in the current profession. These factors provide a comprehensive overview of the healthcare workers involved in the study, which is essential for understanding their perspectives on AMR management. Furthermore, physicians, pharmacists, and nurses generally perceived their hospital's compliance and effectiveness with AMR protocols positively. They believe that the protocol contributes to improving patient outcomes and reducing the incidence of antibiotic-resistant infections, highlighting its importance in healthcare settings. Although there is an overall positive perception regarding compliance, there are still some practices that have a notably lower score.

The study's findings also revealed a significant difference in the overall perceptions of healthcare workers regarding AMR management. This difference suggests that there may be varying levels of understanding and engagement with AMR management practices among different healthcare professionals. There is a significant correlation between the type of hospital, staff position, and healthcare workers' perception of AMR protocol compliance. However, demographic factors, namely primary work area or type of doctor, years of work in the institution, and years of experience in the current position, have no significant effect on the healthcare workers' perception of AMR protocol compliance. These findings suggest that the type of hospital, whether private or public, and the different healthcare roles influence the perceptions of adherence to AMR guidelines. This highlights the significance of considering both the organizational context and the specific roles of healthcare workers when creating strategies to enhance AMR compliance in healthcare settings.

The significant correlation between specific staff positions and perceptions of AMR protocol effectiveness emphasizes the influence of experience, roles in the medication use process, and education on forming the respondents' perceptions. On the other hand, other demographic characteristics such as the type of hospital, primary work area/type of doctor, and years spent working in the institution and their current positions did not have any significant correlations to their perceptions. The results suggest that their specific roles and responsibilities are primary drivers in forming their perceptions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Ethics approval

This research is being reviewed and approved by the University of Santo Tomas Ethics Review Committee (UST ERC) as a requirement in the institution's Pharmacy Research and Thesis Writing course. No ethics approval was required by any institution from where the data was collected.

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Preventability Assessment of Anticoagulant-Related Bleeding in The National Pharmacovigilance Database of Vietnam

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ABSTRACT

Introduction: Bleeding is known as the most important adverse effect of anticoagulants. However, there is a lack of evidence of the preventability of bleeding related to those high-risk medications. This study aimed to investigate the preventability of anticoagulant-related bleeding cases reported through The Vietnamese spontaneous ADR reporting system.
Methods: We conducted a retrospective descriptive study using spontaneous ADR reports with anticoagulants as suspected drugs registered at The National Pharmacovigilance Database of Vietnam (NPDV) from 1 January 2017 to 31 December 2021. The P Method was used for the preventability assessment.
Results: Out of 144 ADR reports on anticoagulant-related bleeding, 86 (59.7%) reports were assessed as preventable ADRs (pADRs). The most reported reactions were administration site bleeding (23.6%), followed by gastrointestinal bleeding (22.9%). Being the most reported suspected drug, enoxaparin was also related to the highest number of preventable ADRs (66 cases). Five critical criteria for preventable cases were identified, all related to healthcare professionals' practices including drug-drug interaction, incorrect dose, inappropriate prescription according to patient's characteristics, inappropriate prescription according to patient's clinical condition or underlying pathology, and incorrect drug administration duration. The most common medication errors were drug-drug interactions (79 cases).
Conclusions: Our study results describe the characteristics of anticoagulant-related bleeding reported by healthcare professionals in Vietnam and the potential factors for its preventability. These findings may be useful for implementing risk management in clinical settings to reduce the burden of serious adverse outcomes of anticoagulants.

KEYWORDS: anticoagulants; bleeding; adverse drug reactions; preventability; spontaneous reporting system; pharmacovigilance

1. INTRODUCTION

Anticoagulants are classified as high-risk medications due to their narrow therapeutic range and the potential for serious adverse drug reactions (ADRs) especially bleeding (hemorrhage) if there are errors during use [1], [2]. Moreover, anticoagulants such as warfarin were found as one of the most commonly implicated in preventable events [3]. Identifying clinical situations and factors associated with preventable ADRs is a key step towards promoting proper drug use while reducing the harm related to drug use [4]. To ensure the safe, reasonable and effective use of anticoagulants, there needs to be a specific analysis of adverse reactions of these drugs recorded in clinical practice. Meanwhile, spontaneous ADR reports submitted to the National Pharmacovigilance Database of Vietnam (NPDV) have been forming a large and important source of data to help detect and evaluate drug safety in Vietnam. Therefore, we conducted this study to assess the risk factors contributing to anticoagulant-related bleeding, especially the potentially preventable adverse reactions of this group of medications.

2. MATERIALS AND METHODS

2.1 Data Source

Individual Case Safety Reports (ICSRs) with anticoagulants as suspected drugs were selected among those reported through the Vietnamese Spontaneous Reporting System from 1 January 2017 to 31 December 2021.

2.2 Data Analysis and Preventability Assessment

For descriptive purposes, information on age, gender, indications, concomitant drugs, ADR seriousness, and suspected drugs were provided for all cases. The criteria for bleeding were described as at least one of the following symptoms: Bruising/bleeding under the skin; bleeding gum; epistaxis; post-surgery heavy bleeding; intracranial hemorrhage; bleeding from the rectum; black stools; bloody urine; vomiting blood; or other cases of abnormal bleeding after taking suspected drugs; or described as "bleeding". The causality assessment was performed using the WHO scale. All reported preferred terms (PTs) related to bleeding were tabled according to their organ system class (SOC) based on MedDRA [5].

The P Method was used to assess the preventability of bleeding. This method is an algorithm developed by the World Health Organization (WHO), consisting of 20 questions to identify medication errors (MEs) [4]. According to this methodology, an ADR can be classified as preventable if it is found at least one ME as a critical criterion for preventability. The researchers used reference documents to answer all questions for each case. In this study, each researcher with experience in pharmacovigilance evaluated the preventability through a case-by-case approach. The results of pADRs were discussed and agreed upon within the team to draw conclusions as "preventable", "not preventable", and "unevaluable".

3. RESULTS AND DISCUSSION

3.1. Characteristics of Anticoagulant-Related Bleeding in ADR Reports

This is the first study conducted to evaluate anticoagulant-induced bleeding from the database of the spontaneous reporting system in Vietnam. From January 2017 to December 2021, 62452 ICSRs were submitted to the NPDV, of which 207 reported at least one anticoagulant as a suspected drug.

There were 144 cases (69.6%) out of 207 ICSRs that reported at least one adverse reaction related to bleeding making a total number of 157 ADRs. Characteristics of those cases are presented in Table 1. The mean age of patients was 68.0 years [standard deviation (SD): 13.6 years], with approximately 60% being 65 years and older. Bleeding cases classified as serious were 13.2% life-threatening, 36.8% required or prolonged hospitalization, and 1.4% of cases which were also clinically significant. Though advanced age alone does not contraindicate anticoagulant treatment, for each decade above the age of 40, the major bleeding risk increases by almost 50%, with the biggest effect seen above the age of 70 [6]. In this study, besides a majority of the patients were elders, the consequences of bleeding could be complicated and challenging considering various comorbidities such as hypertension (16.7%), renal impairment (8.3%), and common concomitant drugs in regular regimens that can increase the risk of bleeding such as platelet aggregation inhibitors (45.8%).

Among 7 anticoagulants reported as suspected drugs, 79 bleeding cases were related to enoxaparin, followed by acenocoumarol (35 cases) (Figure 1). One of the reasons for the difference in detected bleeding cases between vitamin K antagonists (VKAs) and direct oral anticoagulants (DOACs) might be that VKAs are still used more widely than DOACs in Vietnam due to their low costs, and practitioners' routines and long-term experience in VKA treatment. However, the drawbacks of VKAs are numerous and well documented such as long half-life, many drug and dietary interactions, and a narrow therapeutic window (requiring frequent laboratory monitoring and dose adjustments) that can also lead to various adverse effects, especially bleeding [1].

Table 1. Demographic characteristics of bleeding cases involving anticoagulants in the Vietnamese spontaneous reporting system from January 2017 to December 2021

Variable	Level	No. of cases (%), n = 144
Gender	Male	72 (50.0)
	Female	69 (47.9)
	Not available	3 (2.1)
Age	Mean ± SD	68.0 ± 13.6
	Elderly (≥ 65 years)	86 (59.7)
Indications	Cardiovascular diseases	102 (70.8)
	Prophylaxis (deep vein thrombosis, surgical patients, hemodialysis)	21 (14.6)
	Others (cerebral infarction, pulmonary embolism…)	7 (4.9)
	Not available	14 (9.7)
Comorbidities	Hypertension	24 (16.7)
	Impaired kidney function	12 (8.3)
	Diabetes	9 (6.3)
	Gastrointestinal diseases (gastritis, GERD…)	4 (2.8)
	Cancer	4 (2.8)
	History of bleeding	2 (1.4)
Bleeding-related ADRs	1 ADR	131 (91.0)
	2 ADRs	13 (9.0)
Seriousness	Life-threatening	19 (13.2)
	Required or prolonged hospitalization	53 (36.8)
	Other clinically significant conditions	2 (1.4)
	Unserious	70 (48.6)
Reported anticoagulants	1 drug	140 (97.2)
	2 drugs	4 (2.8)
Concomitant drugs	Cardiac medications (ACEIs, ARBs, CCBs, digoxin…)	75 (52.1)
	Platelet aggregation inhibitors (aspirin, ticagrelor, clopidogrel)	66 (45.8)
	Cholesterol lowering medications (fibrates, statins)	63 (43.8)
	Diuretics	53 (36.8)
	PPIs	48 (33.3)
	Antibiotics	25 (17.4)

GERD: Gastroesophageal reflux disease; ACEIs: Angiotensin-converting enzyme inhibitors; ARBs: Angiotensin receptor blockers; CCBs: Calcium channel blockers; PPIs: Proton pump inhibitors

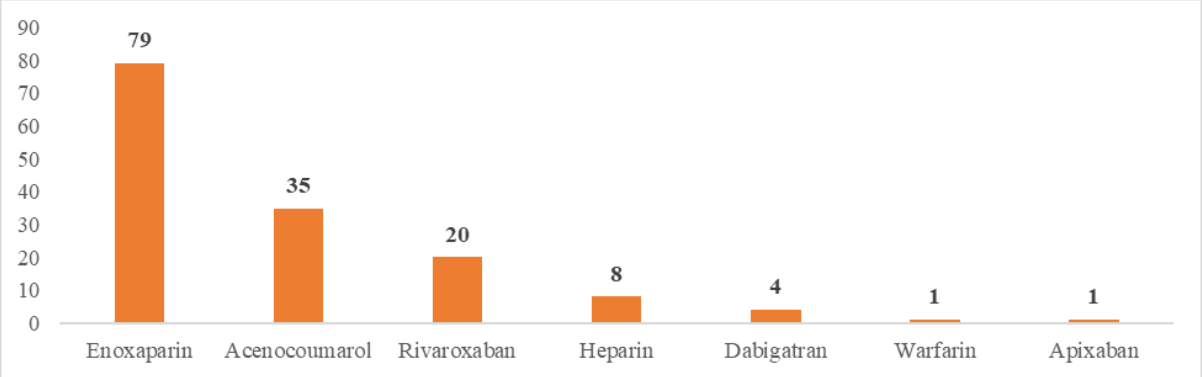

Figure 1. Number of bleeding cases related to anticoagulants

The adverse reactions categorized by System Organ Class (SOC) are presented in Table 2. Out of 144 reports, most bleeding ADRs belong to the SOC "gastrointestinal disorders" (41/144, 28.5%).

Administration site bleeding was the most common (23.6%), followed by gastrointestinal bleeding (22.9%), hemorrhage subcutaneous (17,4%), and hematuria (13.9%). These results, once again, showed that anticoagulant-related bleeding occurred in various system organs, though the event rates might be inconsistent with other studies depending on healthcare settings and practice conditions, for example, the results of a study from a regional Pharmacovigilance center in Italy where the most common bleedings in their territory were gastrointestinal bleeding and epistaxis [7].

Table 2. Adverse drug reactions (ADRs) related to bleeding categorized by System Organ Class (SOC) with anticoagulants as suspected drugs

System organ class (%)	**ADR**	**No. of cases (%), n = 144**
Blood and lymphatic system disorders	Acquired hemophilia	2 (1.4)
Eye disorders	Conjunctival hemorrhage	1 (0.7)
Gastrointestinal disorders	Gastrointestinal bleeding	33 (22.9)
	Gingival bleeding	7 (4.9)
	Mouth hemorrhage	1 (0.7)
General disorders and administration site conditions	Administration site bleeding	34 (23.6)
Injury, poisoning and procedural complications	Procedural hemorrhage	7 (4.9)
Musculoskeletal and connective tissue disorders	Muscle hemorrhage	3 (2.1)
Nervous system disorders	Cerebral hemorrhage	13 (9.0)
Renal and urinary disorders	Hematuria	20 (13.9)
	Vaginal hemorrhage	2 (1.4)
	Urinary bladder hemorrhage	1 (0.7)
Respiratory, thoracic and mediastinal disorders	Epistaxis	4 (2.8)
	Hemoptysis	2 (1.4)
	Pharyngeal hemorrhage	1 (0.7)
Skin and subcutaneous tissue disorders	Hemorrhage subcutaneous	25 (17.4)
Vascular disorders	Hemorrhage	1 (0.7)

3.2. Preventable Cases Related to the Use of Anticoagulants

By applying the P method, 86 (59.7%) cases were assessed as probably, or definitely preventable. Among those, enoxaparin had the highest number of preventable ADRs (66 cases), followed by acenocoumarol (12 cases), rivaroxaban (4 cases), heparin (5 cases), dabigatran (2 cases), and warfarin (1 case). Contributing factors of preventable cases related to each anticoagulant were reported in **Table 3**, which were identified as healthcare professionals' practices.

The most detected criteria were drug-drug interactions (79 cases). Besides a few interactions between 2 anticoagulants, most preventable cases were involved in the concomitant administration of anticoagulants with other medications, about 80% of which were aspirin, clopidogrel, and ticagrelor. While the double effect of antiplatelet and anticoagulant therapy on the bleeding risk is sometimes well accepted considering the antithrombotic effect achieved by the combined treatment, it is necessary to estimate the risk of this combination and discuss with patients to guide treatment decisions [8]. Higher doses than recommendations related to enoxaparin and rivaroxaban were the second most common (15 cases), followed by the use of anticoagulants in patients requiring precautions including elders and low body weight (9 cases), and under clinical conditions including renal impairment and bleeding history (7 cases), and longer administration duration (1 case). Those critical criteria matched with some well-known predictors for the increased risk of bleeding in patients treated with anticoagulants such as advancing age, concomitant drugs (e.g. platelet aggregation inhibitors, NSAIDs), bleeding history, and renal disease [1], [9].

Table 3. Factors associated with the use of anticoagulants in preventable bleeding cases

Drug name (no. of cases with pADRs)	Enoxaparin (n=66)	Acenocoumarol (n=12)	Rivaroxaban (n=4)	Heparin (n=5)	Dabigatran (n=2)	Warfarin (n=1)	**Total**
Drug-drug interaction[a]	62	11	2	5	2	1	**79**
Incorrect dose	13	-	2	-	-	-	**15**
Inappropriate prescription according to patient's characteristics [b]	9	-	-	-	-	-	**9**
Inappropriate prescription according to patient's clinical condition/underlying pathology [c]	5	1	1	-	-	-	**7**
Incorrect drug administration duration	1	-	-	-	-	-	**1**

[a] other concomitant drugs: aspirin, clopidogrel, ticagrelor, etoricoxib, diclofenac, fenofibrate, piracetam; [b] elderly, low body weight; [c] renal impairment, bleeding history.

In this study, we used a validated tool to assess preventable bleeding cases potentially induced by anticoagulants. However, the data source of ICSRs has its limitations such as under-reporting, unequal information quality, etc. which led to 40.3% of cases being concluded as unevaluable. Considering the little information available from ADR reports, our results may be of valuable novelty because they provide more real-world evidence of preventable bleeding related to anticoagulant treatment in Vietnam. Moreover, we have found the most reported risk factors for anticoagulant-related bleeding. Based on our study, proper risk management plans for anticoagulants should be implemented in healthcare facilities, especially in patients with high risks of bleeding such as elders and those indicated antiplatelet and anticoagulant combined therapy.

4. CONCLUSION

This study conducted on anticoagulants in the spontaneous ADR reporting system in Vietnam describes the characteristics of bleeding associated with anticoagulants, of which a significant proportion (59.7%) were preventable and safety problems with anticoagulant use leading to bleeding were all related to professional practice. The results of this study can contribute to guiding Pharmacovigilance activities in clinical practice to minimize preventable ADRs of anticoagulants and to promote the proper use of these high-risk medications.

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Conflict of interest

The authors declare that they have no conflict of interest.

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The Comparative Study on Knowledge of Emergency Contraceptive Pills Before and After Infographic Media among Senior High School Student in Samutprakarn Province

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ABSTRACT

The high rate (26%) of unwanted pregnancy among adolescents aged 15–19 years in Samutprakarn Province due to a lack of knowledge about contraception is currently concerning. The infographic media could improve emergency contraceptive information, especially emergency contraceptive pills. The aim of this study was to promote accurate information about emergency contraceptive pills among senior high school students in Samutprakarn Province. This is a quasi-experimental study. Data were collected via developed questionnaires, including the pre- and post-test scores of contraceptive knowledges before and after self-learning infographic media, respectively, education level, and other characteristics. The paired t-test, or Willcoxon signed rank test, was used to compare the data between the pre- and post-test scores. The ethic consideration was approved by the ethic committee of Huachiew Chalermprakiet University (No.HCU-EC1428/2566). This study found that 239 students, 123 (51%) female and 116 (49%) males, had the median (IQR) of pre- and post-test scores of contraceptive knowledges of 12(2) and 14(2), respectively. The post-test scores were significantly higher than the pre-test scores (p-value<0.001). The infographic media help to educate the correct use of emergency contraceptive pills in senior high school students in Samutprakarn Province. The increment of this knowledge might increase the awareness of pregnant during underage and decrease the rate of unwanted pregnant.

KEYWORDS: Emergency contraceptive pills; Infographic media; Senior high school; Knowledge; Samutprakarn province; Quasi-experimental study

1. INTRODUCTION

Samutprakarn is one of the Thai provinces with a high rate (>26%) of unwanted pregnant adolescents aged 15–19 years[1, 2], leading to unwanted births caused by a lack of and misunderstanding about the knowledge of emergency contraceptive pills (ECP)[3, 4] . The educational media, including articles, videos, and infographics, helped to improve the knowledge of ECP, whereas infographics were the most suitable for adolescents because they were more interesting, understood, and memorized than other contents by 95%, leading to active learning[5, 6]. The community pharmacists in Thailand were the key people prescribing the ECP and providing its knowledge to adolescents[5, 7]. However, the information provided might require explaining time for both pharmacists and adolescents having classes in school, and the explanatory materials were inadequate for community pharmacists[7]. Moreover, adolescents might be uncomfortable communicating with other people due to embarrassment and fear of social judgment[5]. The infographic media could be useful in solving this issue[6]. However, the

validated explanatory materials in Thailand, especially infographic media for adolescents in Samutprakarn, have not been investigated. Thus, the aims of this study were to promote accurate information about ECP via infographic media and to determine the satisfaction of ECP infographic media among senior high school students in Samutprakarn Province.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

This research was a quasi-experimental study creating infographic media that provides accurate information about ECP for senior high school students in Samutprakarn Province from June to October 2023. The eligible participants and their legal guardians, who signed informed consent to obtain study interventions, were included in this research. The interventions in this research were ECP infographic media and the developed questionnaire for the determination of satisfaction. The students who did not have smartphones and did not answer the developed questionnaire were excluded. The ethical considerations of this study protocol were determined by the Ethical Committee of Huachiew Chalermprakiet University (HCU), under the approval number HCU-EC1428/2566.

2.2. The Development of Infographic Media

The ECP infographic media (Figure 1) was developed by the authors, who were university lecturers and were trained and received certification in the topic of contraception from the Pharmacy Council of Thailand. It had applied the knowledge that taught pharmacy students to generate suitable infographic media for adolescents. During infographic development, all lecturers had the suggestion of applying ECP infographic media from the specialist, who was a pioneer member of the College of Community Pharmacy of Thailand, the Pharmacy Council of Thailand.

The finished ECP infographic media are shown in Figure 1, and it was validated by the experts, which consisted of 1) the expert qualified by the Pharmacy Council of Thailand, 2) the associate professor lecturing the topic of contraceptives in HCU, and 3) the President of the Samutprakan Province Pharmacy Association.

Figure 1. Emergency Contraceptive Pills (ECP) infographic media

2.3. The Achievement Test and Satisfaction Questionnaire of Infographic Media

The achievement test was compiled from many studies[3, 5, 8-10] to emphasize the main issue of lacking and misunderstanding knowledge of ECP. The test was a composite of 15 sentences about ECP knowledge (15 points) in a multiple-choice format, depending on the students' decision as to whether the sentences were true or false. Moreover, the achievement test also collected the students' characteristics, including gender, age, year of senior high school, and people who cohabit. For the satisfaction questionnaire of infographic media, it was developed in the Likert Rating Scale pattern, which was 5, 4, 3, 2, and 1 and meant strongly satisfied, very satisfied, neutral satisfied, dissatisfied, and most dissatisfied, respectively, for assessment feelings about ECP infographic media in every aspect.

After that, both the achievement test and satisfaction questionnaire were evaluated for validity and reliability in terms of the Index of Item Objective Congruence (IOC) and Cronbach alpha by the experts, which consisted of 1) the expert qualified by the Pharmacy Council of Thailand, 2) the associate professor lecturing the topic of contraceptives in HCU, and 3) the President of the Samutprakan Province Pharmacy Association.

2.4. Statistical Analysis

The sample size was calculated using the GPower 3.1 program in the order two-dependent groups test with a significant level and statistical power of 0.05 and 0.8, respectively. There should be at least 180 students in this study. The scores of the achievement test with characteristic questionnaire were collected from students by pre- and post-test of self-learning infographic media. Consequently, satisfaction questionnaires were also collected from students after self-learning infographic media. The categorical data were reported by frequencies and percentages. For continuous data, the normality of the data was tested by the Shapiro-Wilk test. The continuous data were reported in terms of mean ± standard deviation (SD) or median [inter-quartile range: IQR], maximum, and minimum. The scores of the achievement test were tested by the paired t-test or Wilcoxon signed rank test. The relationship between characteristics and the mean different scores of the achievement test was analyzed using the Spearman rank test.

3. RESULTS AND DISCUSSION

A total of 239 students, 123 (51%) females and 116 (49%) males, were included in this study. Age had a mean ± SD of 17 ± 0.4 years. The participants studied in Secondary 5 and 6 in the Thailand school curriculum, which had 184 (77%) and 55 (23%) students, respectively. Those staying with their parents, relatives, friends, and alone had 208 (87%), 10 (4%), 20 (8%), and 1 (<1%) students, respectively. The median [IQR], minimum, and maximum of achievement tests before self-learning infographic media were 12 [2], 4, and 15, respectively. Besides, the median [IQR], minimum, and maximum of the post-test were 14 [2], 6, and 15, respectively. The scores of the pre-test were significantly higher than the post-test (Wilcoxon signed rank test, p-value < 0.001). The scores increased by approximately 16.67%. Gender and year of senior high school did not show a correlation with the score of the achievement test (P-value = 0.926 and 0.905, respectively). The students in different years of senior high school had similar knowledge of ECP.

In parallel, most students felt strongly satisfied with ECP infographic media. The results of the satisfaction questionnaire for infographic media are demonstrated in Table 1. As a result, they agreed that ECP infographic media was intelligible, interesting, and valuable.

Table 1. The results of satisfaction of ECP infographic media among senior high school students (n = 239)

Satisfaction questions (What's your satisfaction level about these issues?)	**Strongly satisfied**	**Very satisfied**	**Neutral satisfied**	**Dis satisfied**	**Most dissatisfied**
1. Communicable and understanding language	178	55	6	0	0
2. Understanding contents and proper quantity with limited time	172	57	10	0	0
3. Colorful and noticeable	168	58	13	0	0
4. Interesting and understanding graphics	159	67	13	0	0
5. Valuable content about ECP use	201	33	5	0	0
6. Up-to-date content	189	46	4	0	0

According to the Prevention and Solution of the Adolescent Pregnancy Problem Act, B.E. 2559[8], all schools were regulated to have the subjects teaching sexuality education and life skills properly for each level of students. The junior high school students had basic ECP knowledge and increased this knowledge in senior high school. The year-level learning outcomes (YLOs) of junior high school, which consisted of the primary grades 1–3, had the sexuality adaptation, the sex attitude with the effect of sexual intercourse on education age, and the effect of pregnancy with family planning. Conversely, the YLOs of senior high school, which consisted of the Secondary 4–6, had only the problem-solving skills of sexual intercourse and family planning. There was a chance to improve the ECP knowledge through infographic media. Thus, this is the first study investing in senior high school students who were adolescents in Samutprakarn Province to promote accurate ECP information via infographic media, leading to awareness of the prevention of unwanted births. Not only the effectiveness of infographic media but also the compliance was needed to prove in the target population.

The ECP infographic media increased the ECP knowledge by approximately 17%. This was lower than in the previous study[9], which improved by 87%. Our study focused on the self-learning outcome as active learning via ECP infographic media, and there was an achievement test that assessed the ECP knowledge, whereas the previous study performed the potential improvement workshop, which was a more complicated intervention for representative students and contained the lecture sessions with activities to improve representative students' counseling ability. According to Bloom's taxonomy[10], our infographic media improved cognitive domains of educational activities in knowledge and comprehension categories because students could remember the content and recall it for explanation. If students need to apply knowledge to solve the problem or improve their affective domain, they might improve through situation-based learning and live in a proper social environment. Our study emphasized improving conceptual knowledge and understanding levels from ECP infographic media without the lecturer, but the other study improved procedural knowledge at the level for applications[9], which expected representative students to be able to advise their friends. For high baselines of ECP knowledge of participating students, the major group of students who participated in our study were living with their parents, and they already had a high level of ECP knowledge. This result was confirmed by previous studies[11, 12], which found that the family environment influenced the sexual behavior of students with contraceptive knowledge, and that the education of parents was associated with students' intellectual achievement. Moreover, living in an urban area, higher levels of educational attainment, higher socioeconomic position, and complete family dimensions were all positively associated with the knowledge improvement of ECP use[11, 12]. Although our study showed a narrow improvement in ECP knowledge by using informed ECP infographic media, the students significantly improved their achievement tests after using ECP infographic media. This result supported the concept that infographics could increase the Altmetric Attention Score more than articles and get more mentions on social media[13], which could take advantage of interest in and attraction to the original article or source of detailed information. The immediate test, after being informed by ECP infographic media, could assess short-term memory. Frequent use and application of knowledge might be required to maintain

the level of ECP knowledge[14]. The effectiveness of ECP infographic media for long-term memory was further investigated. For the satisfaction score, this study also determined the satisfaction of ECP infographic media, representing real-world use for adolescents. Several studies demonstrate the gratification of students to infographics with a positive attitude, especially the infographic poster providing the videos[15, 16].

This study might have some possible limitations. Firstly, the participating students were selected from only Secondary 5 and 6 students, representing the adolescents. The extrapolation of this study's results, applying to all adolescents, might be concerning. Secondly, students in our study were collected from three high schools in the target city. They could not represent all high school students in Samutprakarn province. Our participants came from urban areas that possibly overlooked valuable insights from rural students, which could offer differing perspectives on the studied phenomenon. Finally, our study improved only the cognitive domain; the psychomotor and affective domains required further investigation due to all components of learning being needed to influence the proper use of ECP.

4. CONCLUSION

ECP infographic media improved the ECP knowledge among adolescents studying senior high school in Samutprakarn Province, and the ECP infographic media in this study were strongly satisfied with their use.

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Conflict of interest

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CP-1602101-P

Efficacy of Immune Checkpoint Inhibitors in Advanced Non-Small Cell Lung Cancer Who Progressed on Targeted Therapy: A Systematic Review and Meta-analysis

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ABSTRACT

Non-small cell lung cancer (NSCLC) patients harboring gene mutations who progressed on targeted therapy often have limited treatment options. Immune checkpoint inhibitors have shown promise in this setting, but their overall clinical benefits remain unclear. This systematic review and meta-analysis aims to evaluate the efficacy of immune checkpoint inhibitors in managing advanced non-small cell lung cancer patients who have progressed on targeted therapy. A systematic literature search on PubMed, Scopus, abstracts, and presentations from major conference proceedings to identify relevant studies published up to January 2024. Eligible studies included randomized controlled trials (RCTs) that assessed the efficacy of immune checkpoint inhibitors (ICIs) in non-small cell lung cancer patients who had experienced disease progression on targeted therapy. Progression-free survival (PFS) was the main endpoint, assessed using hazarded ratio (HR) and 95% confidence interval. Outcome comparisons were performed using Revman 5.4 software. The study evaluated the efficacy of ICIs, including monotherapy or in combination with other regimens, compared to other standard treatments. The Cochrane risk of bias tool (RoB 2) was used to assess the risk of bias in the RCTs. A total of eight randomized controlled trials involving 1519 participants were included in the meta-analysis. The results indicate that ICIs plus chemotherapy and antiangiogenic therapy are significantly associated with prolonged PFS (HR = 0.48, 95% CI 0.38 - 0.59, $p < 0.00001$). Conversely, for ICIs monotherapy, there was no significant difference in PFS (HR = 1.88, 95% CI (1.33 - 2.66, $p = 0.0004$) compared to the control group. Immune checkpoint inhibitors and their combination therapies improve the PFS of NSCLC patients who progressed on targeted therapy. In particular, the combination of ICIs, chemotherapy, and antiangiogenic therapy may be the most effective choice for optimizing PFS.

KEYWORDS: non-small cell lung cancer; immune checkpoint inhibitors; targeted therapy; efficacy; meta-analysis, systematic review

1. INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths worldwide, accounting for the highest mortality rates among both men and women. Non-small cell Lung Cancer (NSCLC) predominates, accounting for almost 85% of lung cancer cases[1]. NSCLC is often diagnosed at advanced stages, limiting treatment options and leading to a poor prognosis.

Tyrosine kinase inhibitors (TKIs) have been developed to target specific mutations in genes such as EGFR (epidermal growth factor receptor) and ALK (anaplastic lymphoma kinase), which are commonly found in subsets of NSCLC patients. However, a significant proportion of patients eventually develop resistance to these treatments, leading to disease progression.[2] This represents a

clinical challenge, as limited effective treatment options are available for patients who have progressed on targeted therapy.

Immunotherapy, particularly immune checkpoint inhibitors, is a class of immunotherapy drugs that the FDA has approved for the treatment of NSCLC are ipilimumab, nivolumab, pembrolizumab, durvalumab, atezolizumab, and cemiplimab.[3] Immunotherapy has emerged as a promising treatment option for NSCLC and has shown significant benefits for some patients.[4] Immunotherapy can also be combined with other treatments to enhance its effectiveness. This approach has become a standard treatment for certain patients with advanced NSCLC, especially those without targetable genetic mutations (such as EGFR or ALK mutations). However, limited information was available to conclude treatment decisions for patients who experienced progression after targeted therapy.

Recent systematic reviews[5] demonstrated the beneficial effects of immune checkpoint inhibitors on overall survival and progression-free survival for first and second-line treatments compared to chemotherapy in NSCLC patients. However, the current evidence on treating patients with TKI resistance remains under investigation. Therefore, this systematic review and meta-analysis aims to investigates the efficacy of ICIs in managing advanced NSCLC patients who have progressed on targeted therapy.

2. MATERIALS AND METHODS

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. The protocol was registered with PROSPERO, the International prospective register of systematic reviews (identification number CRD42023454152)

2.1. Identification and Selection of Studies

The studies were located from MEDLINE via PubMed search engine and Scopus databases. Moreover, abstracts and presentations from major conference proceedings, the reference lists of the retrieved studies and The ClinicalTrials.gov database, were screened to identify clinical trials that had not been published. The search terms were constructed based on PICO as keywords: “non-small-cell lung cancer, immune checkpoint inhibitors, targeted therapy, previously treated. Searching was updated every three months.

Two reviewers (TM and WS) independently selected studies by screening titles and abstracts. Studies were included if they met all of the following criteria (1) studied in adult patients (aged more than 18 years old) with histologically confirmed advanced NSCLC (stages III and IV) who had disease progression on targeted therapy were defined as either assessed as having progressive disease by RECIST criteria (version 1.1) or changed treatment option from targeted therapy to subsequently use immune checkpoint inhibitors. (2) a randomized controlled trials (RCTs) study comparing the efficacy of immune checkpoint inhibitors the studies were excluded if their data were unavailable full-texts, non-English language, or insufficient data for extraction and authors did not respond after contacting two times. Any disagreements were resolved through consensus with the third author (ST)

2.2. Risk of Bias Assessment.

Two reviewers independently assessed the quality of the selected studies according to the Cochrane risk of bias tool (RoB 2) for randomized controlled trials. The included studies were then classified into one of the following three categories: low risk, high risk, and having “some concerns. Any disagreement was resolved through consensus with the third author (ST)

2.3 Statistical Analysis

Statistical analysis was performed as follows; for dichotomous outcomes, i.e., hazard ratio (HR), log HR along with its variance, and the 95% CIs will pool. The HRs was then directly pool across studies using a fixed-effect model; if heterogeneity is present, the random-effects model will apply. Heterogeneity and a degree of heterogeneity across studies were assessed before pooling by Cochrane’s

Q test and I^2 statistic. If p-value of Q test <0.1 or I^2 > 25%, the heterogeneity was considered present. A two-sided p-value < 0.05 will be considered statistically significant for all tests except for heterogeneity, where 0.10 was used. Moreover, publication bias was assessed using funnel plots. If any of these suggested asymmetry, a contour-enhanced funnel was constructed to determine whether asymmetry was caused by publication bias or heterogeneity; a p-value of less than 0.10 indicated significant asymmetry and publication bias. The analyses will be performed using Revman 5.4 software.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1 Characteristics of the Included Studies

We identified a total of 7,703 records from the databases during the preliminary literature search. After eliminating the duplicates and non-related articles through abstract screening, 18 studies were considered eligible for full-text review, and eight studies finally met our eligibility criteria[6-13]. (Figure 1). A total of 1,519 patients were enrolled to receive the following three treatments: ICIs monotherapy (n=188), ICIs plus chemotherapy (n=507), ICIs plus chemotherapy and antiangiogenic therapy (n=623) compared to standard chemotherapy treatment with or without anti-angiogenesis.

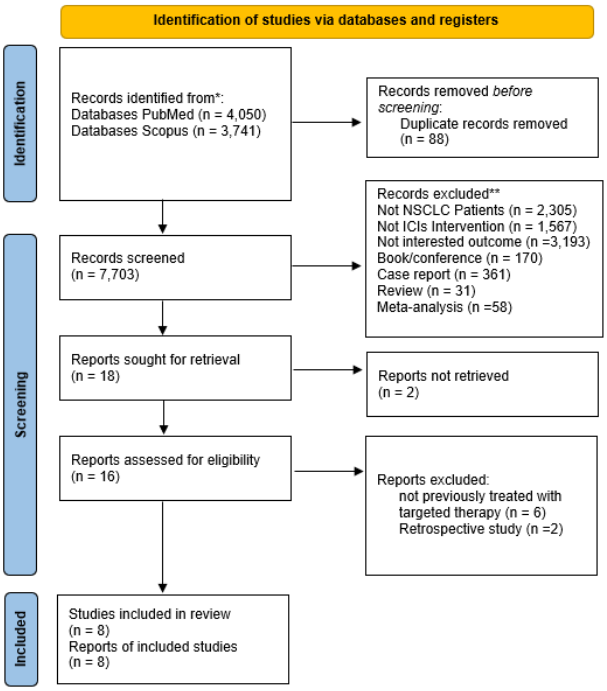


Figure 1. PRISMA flow diagram of study selection process

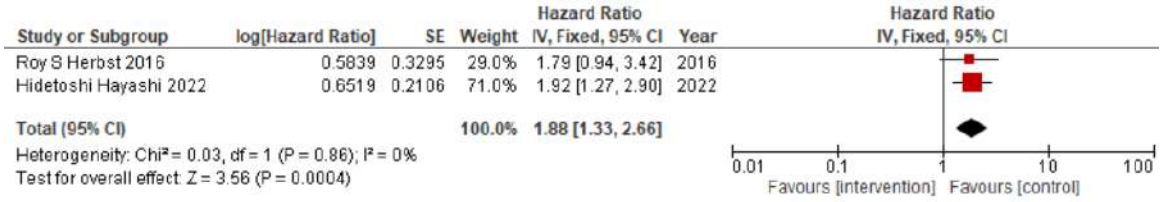
3.1.2 Pooling Results in Progression-free Survival (PFS)

Six studies investigated the PFS outcomes in ICIs alone compared to chemotherapy, the results suggested that ICIs was no improvement in progression-free survival (PFS). The treatment with chemotherapy demonstrated superior efficacy compared to ICIs (HR = 1.88, 95% CI (1.33 - 2.66, p = 0.0004) (Figure 2A). Heterogeneity was presented for HR (χ^2= 0.03, df = 1, p = 0.86, I^2 = 0%). In the ICIs based combined with chemotherapy compared to chemotherapy alone or chemotherapy combined with anti-angiogenesis, (HR = 0.75, 95% CI (0.65 - 0.85, p < 0.0001) (Figure 2B). Heterogeneity was present for HR (χ^2 = 0.20, df = 3, p = 0.98, I^2= 0%). The most significant improvement in PFS was observed with the combination of ICIs, anti-angiogenesis, and chemotherapy compared to

chemotherapy alone or chemotherapy combined with anti-angiogenesis (HR = 0.48, 95% CI 0.38-0.59, p < 0.00001) (Figure 2C). Heterogeneity was presented for HR (χ^2 = 0.69, df = 1, p = 0.41, I^2 = 0%). Publication bias was assessed by funnel plot for pooled HRs, indicating no evidence of asymmetry of funnels for all HRs

(A) Hazard Ratios in the monotherapy group.

(B) Hazard Ratios in ICI + Chemo vs Chemo / Chemo + Anti-angiogenesis.



(C) Hazard Ratios in ICIs + Anti-angiogenesis + chemo vs Chemo / Chemo + Anti-angiogenesis.

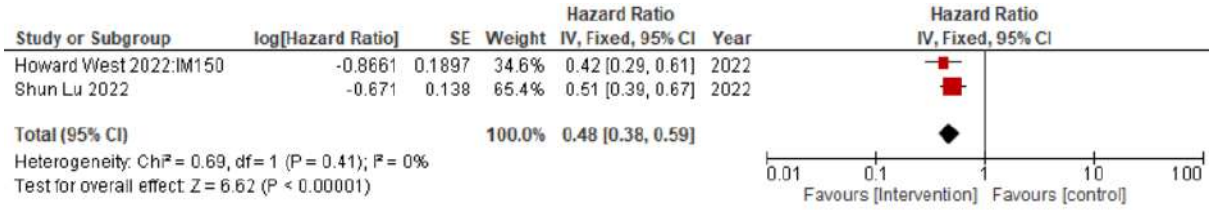


Figure 2. Forest Plot of HR Comparing PFS in Patients Who Received ICIs vs. Other treatments

3.1.3 Risk of Bias Assessment

The results obtained with the Cochrane risk-of-bias assessment tool for the eight enrolled RCTs. All studies were clearly defined for advanced NSCLC patients who have progressed on targeted therapy. Overall, the risk of bias in the included studies was low risk and some concern, primarily due to lack of inadequate allocation concealment blinding of participants and personnel in the RCTs.

3.2 Discussion

This meta-analysis investigated the efficacy of immune checkpoint inhibitors (ICIs) who progressed on targeted therapy. Our findings demonstrate that ICIs combined with chemotherapy and anti-angiogenic agents shows promise, with improved PFS compared to other treatment options. The potential mechanism for this benefit likely lies in the combined effects of each component. Anti-angiogenic drugs may enhance the effectiveness of immunotherapy by regulating tumor vasculature and decreasing vascular endothelial growth factor (VEGF) levels, thereby reducing the barriers imposed by tumor angiogenesis on immune cell infiltration. Additionally, chemotherapy can induce tumor cell apoptosis and potentially decrease immunosuppressive cell populations, further optimizing the tumor

immune microenvironment for successful immunotherapy.[14-15] This study represents the first meta-analysis to include data on patients who progressed on all targeted therapies, incorporating results from the latest relevant clinical trials. Our findings are consistent with previous meta-analyses focused on resistance to EGFR-targeted therapies[5] where a similar improvement in PFS was observed. However, our work provides additional insights by analyzing a broader patient population. Despite the promising results, limitations exist. The number of included trials is relatively small, and large clinical trials of immunotherapy often exclude patients with positive driver gene mutations. While our analysis suggests a benefit for ICI-chemotherapy combinations, further investigation is needed to refine treatment strategies and identify optimal patient selection criteria.

4. CONCLUSION

In summary, the analysis showed that compared to chemotherapy, using an immune checkpoint inhibitor (ICI) alone did not significantly improve progression-free survival in patients with non-small cell lung cancer who had progressed on targeted therapy. However, ICIs combination therapy appears more effective than monotherapy, although the efficacy of combining ICI with chemotherapy requires more investigation. ICI-based combination therapy could be considered for patients who progressed on targeted therapy, particularly with chemotherapy or antiangiogenic agents.

5. ACKNOWLEDGMENT

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Conflict of interest

The authors declare that they have no conflict of interest.

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Phytopharmaceuticals and Nutraceuticals

PN-1110001-O

Natural Product Databases in Herbal and Integrative Medicine: Bridging Traditional Knowledge to Modern Applications

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ABSTRACT

Herbal medicine, comprising traditional, complementary, and integrative approaches, utilizes plants for medicinal purposes. Natural product databases (NPDBs) have emerged alongside increasing scientific studies in herbal medicine, facilitating the transition of traditional knowledge to modern applications. While NPDBs aid drug discovery campaigns with comprehensive information, their structures require enhancement to foster scientific community engagement and prolong their lifespan. This review aims to assess NPDB usage in research, pinpoint shortcomings, and propose improvements to support herbal medicinal practices and virtual screening for drug discovery. A literature search of the relevant terms to this review was used to identify the data used in this review as well as the databases. All databases were accessed from February-April 2024. Literature searches identified 27 databases, evaluated for descriptions, search parameters, features, and accessibility. General NPDBs like COCONUT (COlleCtion of Open Natural ProdUcTs) serve referencing and screening, while smaller ones like ETM-DB (Ethiopian Traditional Medicine Database) provide relational and geographical data. Databases are well referenced and most of them have user-friendly search parameters. Neglected features including full database downloading, maintenance details, and data statistics were observed. Despite good referencing and search parameters, enhancements like navigation videos could boost user engagement. NPDBs facilitate the convergence of traditional and modern medicine by providing essential data on compound structures and disease relationships, but improvements in database structures and approaches are necessary for increased user engagement. To enhance engagement, databases could offer submission pipelines, feature requests, bug reporting systems, and user communities.

KEYWORDS: Herbal database; Herbal Medicine; Natural Product; Traditional Medicine; Virtual Screening

1. INTRODUCTION

Herbal medicine, with its rich history across various cultures, has long served as a cornerstone of traditional healthcare practices, predating current conventional medicine. This ancient practice encompasses a broad range of uses, from specific plant parts to complex formulations, documented either in written texts or passed down orally [1]. Many herbal remedies have led to the discovery of significant therapeutic agents, contributing increasingly to global healthcare [1,2].

Integrative Medicine, which combines conventional and complementary approaches, highlights the limitations of reductionism, and emphasizes the therapeutic relationship [3]. As traditional plant medicines remain prevalent in primary and preventive care, a deeper understanding of their natural product properties is essential to harness their potential and address their risks effectively.

Natural products (NPs) are bioactive chemical substances derived from plants, animals, or other natural sources. Classified into primary metabolites, secondary metabolites, and polymeric molecules, these compounds play crucial roles in plant survival and have significant pharmacological properties [4]. Secondary metabolites such as alkaloids, tannins, flavonoids, and terpenes are especially notable for their therapeutic potential [5]. The vast diversity of plants and their associated NPs, coupled with cultural variations, necessitates an efficient system for the collection, storage, and dissemination of this information. This led to the establishment of Natural Product Databases (NPDBs)

NPDBs provide comprehensive information on medicinal plants and their phytochemicals, facilitating drug discovery and development [6]. They also help in understanding the relationships between traditional medicine and modern therapeutic applications.

Previous reviews have evaluated the structure and utility of NPDBs. For instance, Xie et al. reviewed 14 NPDBs, highlighting their advantages and the need for more comprehensive drug interaction networks [7]. Sorokina and Steinbeck addressed redundancy issues by creating the COCONUT database, a non-redundant collection of natural products for easier access and use [8]. Fathifar et al. examined 25 medicinal herb databases, recommending future improvements such as multilingual support and comprehensive ontology development [9].

This review underscores the importance of NPDBs in traditional, integrative, and modern medicinal applications, aiming to enhance user engagement, promote herbal medicine and drug discovery from natural products, and offer solutions for developing more sustainable NPDBs.

2. MATERIALS AND METHODS

A comprehensive literature search on NPDBs in integrative medicine was conducted using Scopus and PubMed, focusing on articles published from January 2010 to January 2024. Articles were screened based on subject type, language, and relevance to the topic. To guide the database searches, three pivotal review articles by Xie et al., Sorokina and Steinbeck, and Fathifar et al. were utilized [7–9]. Supplementary searches were also performed using Google.

The inclusion criteria for databases were the availability of information in English or with a functional translator, and free accessibility. Databases were excluded if they had redundant information structures or provided minimal contributions to integrative medicine. The databases were accessed and evaluated between February and April 2024 to assess their status and the information they offered.

One significant challenge encountered was restricted access to certain articles and databases, which potentially contained valuable information for this review.

3. RESULTS AND DISCUSSION

3.1. Overview of Natural Product Databases use

From 2010 to 2023, numerous NPDBs were established, coinciding with the rise of modern drug design methods like virtual screening and machine learning [9]. From this study, 7 of the 27 databases have been updated with a second version within the first five years, and 3 more have had newer versions. However, nearly 63% of these databases have not received a second version. With the fast pace of technology, regular updates are needed to incorporate new software, data manipulation methods, increase data availability, and easiness of access [10].

This review primarily includes thematic databases [8]. These are further divided into taxonomy databases, such as CMAUP, DISPEL, and Phytochemica, which focus on plant-based natural products. Other thematic databases are based on traditional medicinal uses, including VIETHERB, TM-MC 2.0, TCMSP, TCMID, TCM Database@Taiwan, PharmaDB-K, IMPPAT, HERB, HerbalDB, and ETM-DB. Region-specific databases like CEMTDD, MPDB 2.0, NADI, NuBBE DB, and SANCDB do not necessarily focus on herbal applications. Other types include chemical databases like SuperNatural, biological activity databases like NPACT, ethnobotanical databases like Dr. Duke's Phytochemical and Ethnobotanical Databases, ecological databases like NP Atlas, and integrated databases like NPASS.

Regional and traditional databases significantly contribute to herbal medicine repositories, primarily benefiting local users interested in traditional applications. Localization fosters a sense of

ownership and responsibility among local researchers, encouraging engagement from local practitioners and researchers [11]. This is crucial as some traditional medicinal knowledge is transmitted orally[1].

Databases like COCONUT and SuperNatural, which compile data from other sources, serve as large repositories of natural and predicted molecules often used for virtual screening (VS) or for aiding the creation of smaller databases. These smaller databases, in turn, provide data for updating the larger repositories. They also emphasize the source organism and associated traditional medicine, aiding researchers in visualizing networks between entities and inspiring new drug development or repurposing campaigns. Most databases reviewed offer user-friendly access with keyword search and browsing options, catering to both expert researchers and general users. However, some databases have not been maintained or updated in the past three years, leading to unreliable and unstable resources [10].

Databases, such as SANCDB, offer a submission pipeline for user contributions, enhancing the database's content. Options for 'bug reports' and 'feature requests' further support maintenance and updates. NPDBs like NADI are exemplary regional databases for drug design, offering VS options. Databases like PharmaDB-K and various TCM databases provide connections to other databases, enriching data and services for medicinal drug research. All databases reviewed were highly referenced, providing users with extensive resources for additional information.

3.2. Challenges and Solutions

Several regional databases do not support database downloads or lack chemical structures, reducing engagement as virtual screening is a primary use. Many also lack images of source organisms, making it difficult for inexperienced users. Some databases lack comprehensive information, including statistics, update history, and proper introductions. Figure 1 outlines suggested solutions to enhance user engagement and prolong database lifespan, addressing these challenges alongside existing and proposed elements from previous reviews.

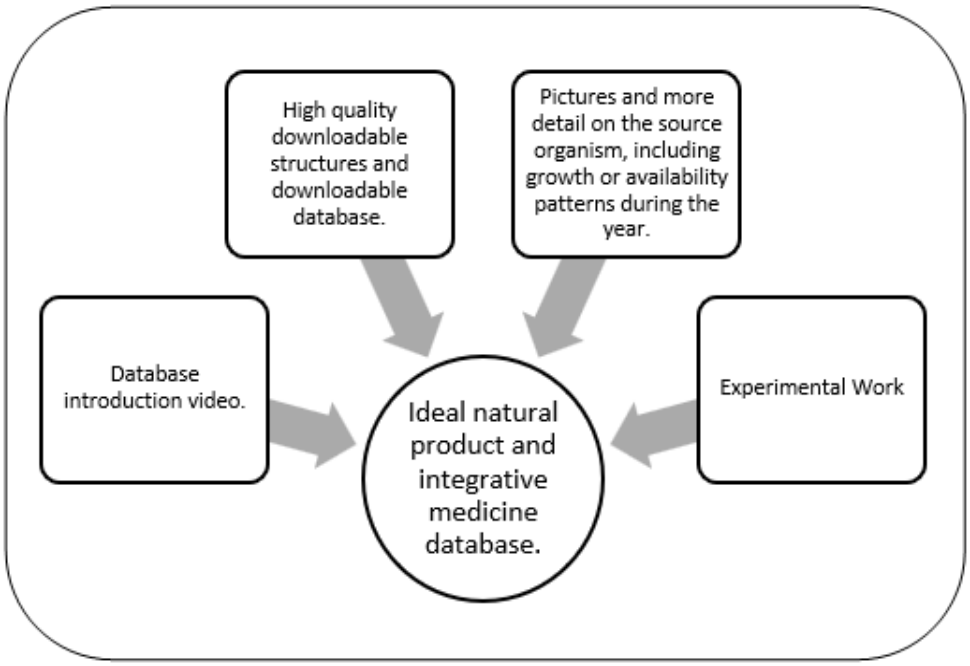

Figure 1. Suggested additional elements for an ideal natural product and integrative medicine database.

4. CONCLUSION

Natural product databases (NPDBs) are vital for bridging traditional and modern medicine, offering a structured platform to explore bioactive compounds from nature. This integration enhances the understanding of traditional remedies, validates them through scientific research, and supports the development of evidence-based healthcare solutions. The collaborative synergy between traditional and

modern medicine, guided by the comprehensive data in these databases, holds significant potential for discovering novel therapeutics. To further improve user engagement and extend the database lifespan, additional features could be incorporated. Providing detailed information on experimental work can guide researchers on potential research pathways and boost confidence among practitioners in herbal medicine. Despite the existing multitude of NPDBs, there remains a need for regional databases to engage local communities, leading to more comprehensive data collection and higher user engagement.

Conflict of interest
The authors declare that they have no conflict of interest.

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Repellency of Natural Essential Oil Blends against Adult German Cockroach, *Blattella germanica* L. (Blattaria: Blattellidae)

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ABSTRACT

The German cockroach, *Blattella germanica* has been considered as one of the most common sources of indoor allergens. Historically, cockroach control has been based on the use of chemical insecticides, which could lead to issues such as pesticide resistance, environmental contamination and human health concerns. Therefore, development of alternatives to replace chemical pesticides is essential. Several essential oils (EOs) have been reported for their repellency activities against insects including German cockroach. Furthermore, EOs have been considered generally safe and there is little concern regarding their residue in the environment. This study aimed to develop eco-friendly repellent products using EOs blends for the use in home or office areas, such as living room, kitchen and bedroom. The chosen EOs and monoterpenes, such as citronella, kaffir lime, menthol and camphor, have been widely used in food, cosmetic and pharmaceutical industries. The blends with pleasant odor were developed and tested for repellent activity against adult German cockroach at the National Institute of Health of Thailand. Repellency assays were conducted using area preference method. The chemical constituents of each EO and blend were investigated using Gas chromatography-Mass spectrometry (GC-MS). The developed blends named MU-kaffir lime and MU-Citronella exhibited repellency effects against the insect (96.7%). The MU-kaffir lime was chosen for development of repellent spray by mixing with diluents in a ratio of 30:70. It was found that the repellency rate of the diluted blend was 98.3% similar to that of the concentrated blend. The major constituents of each EO and blends were successfully identified using GC-MS. The EOs blends developed in this study could be further developed as eco-friendly cockroach repellents. The relative peak areas of the chromatograms could be used for quality control of each EO and the blends.

KEYWORDS: *Blattella germanica*; Eco-friendly; Essential oil; German cockroach; Repellent

1. INTRODUCTION

The German cockroach, *Blattella germanica* is the most common pest found in human residences. The insect has been considered as a potential vector of bacterial and fungi pathogens[1, 2], and a common source of indoor allergens causing allergic disease, such as asthma and allergic rhinitis[3]. The cockroach allergens are associated with secretions, feces, saliva, exoskeletons and dead bodies[4]. Their effects on human health have made the control of this pest critical. Several formulations of synthetic insecticides have been extensively used for the control of German cockroach. However, insecticide resistance has been a consistent barrier for the pest control[5]. Furthermore, the use of chemical insecticides could also lead to issues, such as environmental contamination and human health concerns[6]. These issues have created interest in the development of safer pest control agents.

Essential oils (EOs) are complex mixtures of volatile compounds produced as secondary metabolites and responsible for the distinctive odor of plants. Several essential oils (EOs) and their

major constituents have been reported for their potential use in the control of insect pests, including the German cockroach[7, 8]. Moreover, the plant-based EOs could be considered as good resources for development of household pest control because of their low mammalian toxicity, human and environmental safety[9].

This study aimed to develop eco-friendly repellent products using EOs blends for the use in home or office areas, such as living room, kitchen and bedroom, etc. EOs and solid terpenes that have been reported for their insecticidal, contact and/or fumigant toxicity and repellency against German cockroach were selected for development of EOs blends. The chosen EOs were citronella (*Cymbopogon winterianus*), eucalyptus (*Eucalyptus globulus*), kaffir lime (*Citrus hystrix*), lemongrass (*Cymbopogon citratus*), lemon (*Citrus limonum*), peppermint (*Mentha Piperita*)[10-12]. The solid terpenes used as minor components of the blends were borneol, camphor and menthol[13-16]. All of compounds and excipients used in this study have been widely used in food, perfume, cosmetic and pharmaceutical industries. The developed blends were investigated for their repellent activity against German cockroach. Furthermore, major and minor constituents of EOs and blends were identified using Gas chromatography-Mass spectrometry (GC-MS).

2. MATERIALS AND METHODS

2.1. Chemical

Borneol, camphor, menthol, EOs of citronella, lemon, lemongrass and peppermint were purchased from Hong Huat Co., Ltd, Bangkok, Thailand. Kaffir lime and eucalyptus oils were obtained from Thai - China Flavours and Fragrances Industry Co., Ltd, Nonthaburi, Thailand. Pesticide residue grade n-hexane was supplied by Fisher Scientific, Loughborough, UK. 95% Ethanol and isopropyl myristate were purchased from Namsiang Co., Ltd, Bangkok, Thailand. Transcutol® CG (ethoxydiglycol) was provided by P.C. Intertrade Co., Ltd, Bangkok, Thailand.

2.2. Development of EOs Blends

Since some EOs may have pungent odor which may irritate the respiratory tract, or have high prices, the use of EOs blends would help in obtaining more friendly-scents and lower formulation cost. In this study, two EOs blends were developed and named as MU-Citronella and MU-kaffir lime. The major compositions of MU-Citronella were EOs of citronella, lemongrass and eucalyptus, and minor components were borneol, camphor, menthol and lemon oil. For MU-kaffir lime, the major compositions were EOs of kaffir lime and eucalyptus, while the minor compositions were borneol, camphor, menthol and peppermint oil and lemon oil.

The MU-kaffir lime was then chosen for development of repellent conventional spray by mixing with diluents in a weight ratio of 30:70. The composition of diluents were ethoxydiglycol, isopropyl myristate and 95% ethyl alcohol.

2.3. GC-MS Analysis

The analysis was carried out on a GC system coupled with single quadrupole mass spectrometer (GCMS-QP2020NX, Shimadzu, Kyoto, Japan). The GC-MS condition was slightly modified from our previous study[10] to initially assess constituents of EOs blends which are MU-kaffir lime and MU-Citronella. The oven temperature program was optimized to achieve adequate separation and elution of the analytes, especially the major compounds, and acceptable run time. The optimal condition was then applied for analysis of other investigated compounds. Hexane was chosen as the solvent because the test substances were well soluble in it, and because of its low baseline noise and interfering peaks.

The separation was achieved on a DB-WAX capillary column (0.32 mm ID x 30 m, 0.25 μm film thickness, Agilent Technologies, USA). The carrier gas was ultra-high-purity helium (99.999%), at a flow rate of 1.54 mL/min. Sample injection (1 μL) was performed in the splitless mode. The initial oven temperature was set at 50°C with holding time of 5 min, then increased by 2°C/min to 150°C, which was maintained for 2 min, then ramped up to 220°C at a rate of 10°C/min and held for 5 min.

The total run time was 69 min. The temperature of the injection port, interface and ion source were set at 230°C. Electron impact ionization was used with an ionization energy of 70 eV. The MS was operated in the scan mode with an acquisition range of 40–400 *m/z*. The volatile constituents of EOs and blends were identified by comparing their mass spectra with WILEY 12 and NIST 2020 mass spectral library. Each investigated substance was diluted with hexane to obtain a final concentration of 100 µg/mL.

2.4. Insects

The German cockroaches were reared without exposure to any insecticide in the laboratory of the National Institute of Health (NIH) of Thailand. The insects were provided with dried mouse food and water ad libitum. The cockroaches were maintained at 30 ± 2°C and 60 ± 5% RH under a 12/12-h light-dark cycle.

2.5. Repellent Activity Assay

This study was ethical approved by the Institutional Animal Care and Use Committee, Faculty of Pharmacy, Mahidol University, Thailand (No. PYR001/2023). The repellent activity assay was performed at the NIH of Thailand using standard operation protocol for registration of cockroach repellent products in Thailand.

The internal side walls of stainless-steel boxes (50 x 50 x 10 cm) were greased with Vaseline to prevent the escape of the insects. A Whatman No.1 filter paper (50 x 50 cm) was cut in two halves and placed at the bottom of the box. One of the halves was treated with an investigated EOs blends in the amount of 10 mL/m^2, and the other one was untreated (control). Prior to experiment, ten adult male and female German cockroaches with the age of 6-8 weeks were anesthetized by exposure to CO_2 for less than 1 min. Then, the insects were gently placed at the center of each experiment box. The boxes were placed at the center of the Peet Grady Chamber (180 x 180 x 180 cm) and kept in dark and isolated area to prevent disturbances. The insects were provided with mouse food and water-soaked cotton wool in each area. The experiment conditions used were maintained at 30 ± 2°C, 60 ± 5% RH, and 24-h dark cycle. After treatment for 48 h, the number of cockroaches in the treated and controlled areas were observed. The experiments were carried out in triplicate. The repellency rate was calculated using the following equation:

$$\text{Repellency rate (\%)} = U/(T+U) \times 100$$

where U and T are the number of cockroaches in the untreated and treated areas, respectively. The tested compound is considered to exhibit repellent activity if the repellency rate is more than 80%.

3. RESULTS AND DISCUSSION

3.1. Chemical Constituents of Essential Oils and Blends

Several studies demonstrated that repellent activity of EOs have been linked to the presence of monoterpenes and sesquiterpenes. Moreover, these compounds could work synergistically resulting in improving their effectiveness[17]. As a result, the quality control of these EOs would be necessary in order to obtain consistent activity. Table 1 shows the chemical constituents of each EO and blend, and their percentage relative peak areas. The identified major compounds in MU-Citronella were geranial (14.46%), eucalyptol (12.59%), citronellal (11.50%), citral (9.31%), menthol (8.92%), geraniol (8.69%) and limonene (6.92%), as shown in Figure 1. For the chemical analysis of MU-Kaffir lime, limonene (21.64%) was identified as the most abundant compound, followed by *β*-pinene (13.49%), menthol (10.26%), *α*-terpineol (9.94%), terpinen-4-ol (8.52%) and eucalyptol (7.68%), as demonstrated in Figure 2. However, the peak of borneol was disappeared in the chromatogram of MU-kaffir lime, since it may overlap with *α*-terpineol peak or may degraded or interacted with other compounds. The most abundant compound in citronella oil was citronellal (36.58%), followed by geraniol (18.94%), *β*-citronellol (13.63%) and isopregol (7.02%). For eucalyptus oil, the most abundant compound was

eucalyptol (84.02%), followed by limonene (6.76%). The major constituents of kaffir lime oil were limonene (26.76%), *β*-pinene (18.03%), terpinen-4-ol (12.42%), and *α*-terpineol (10.94%). For lemongrass oil, the major compositions were geranial (45.28%), citral (29.04%) and geraniol (7.10%). The major constituents found in lemon oil were limonene (66.13%), *β*-pinene (13.85%) and *o*-cymene (7.56%). The identified major constituents in peppermint oil were menthol (39.93%), menthone (18.87%) and limonene (6.45%). For the solid terpenes, borneol material contained borneol (52.87%), isoborneol (30.74%) and camphor (15.15%). The peak of camphor and menthol when expressed as %relative peak areas were 93.43% and 99.87%, respectively. Although the chemical constituents of investigated EOs and solid terpenes identified in this study may differences from previous reports, however, the major compositions were the same[10-12, 18].

The chemical compositions of EOs could be considerably varied according to harvest, storage period, extraction method and climatic factors, which could affect their activities[19, 20]. Therefore, active markers of EOs should be identified and quality controlled to obtain consistent activity. GC-MS analysis has been considered as a key technique which could be used for both quantitative and qualitative analysis of EOs. In general, the quality control of an EO can be performed by comparing the chromatogram of the investigated EO with acceptance specifications. First, the appearance or disappearance of peaks were identified, and the relative percentage area would be then determined whether their differences are significant for the EO being analyzed. This technique is the most used approach, although it could give only approximated quantities of component present in the sample tested[21, 22]. In this study, the compositions in EOs, blends and solid terpenes were only identified by comparing their mass spectrum with reference mass spectra in libraries via spectrum matching and previous research. Therefore, it has limited accuracy, and the higher accuracy identification can be achieved by comparing retention time and mass spectra with that of authentic standards[22, 23]. However, chemical constituent information obtained from this study could be useful for preliminary screening of the data.

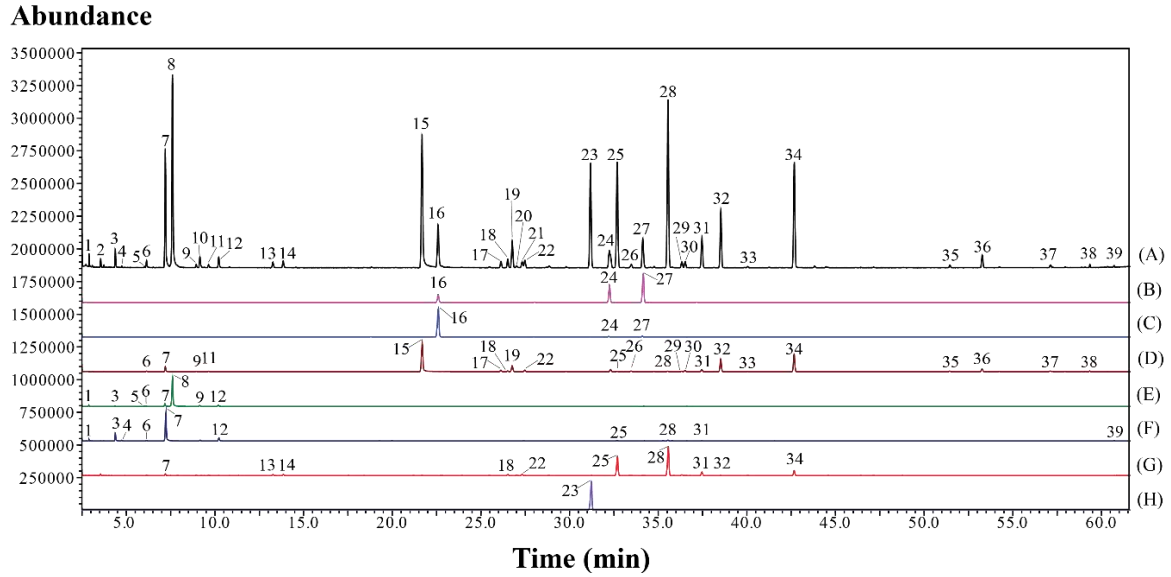

Figure 1. Representative GC-MS chromatograms of A. MU-Citronella, B. Borneol, C. Camphor, D. Citronella oil, E. Eucalyptus oil, F. Lemon oil, G. Lemongrass oil and H. Menthol; (1) *α*-Pinene, (2) Camphene, (3) *β*-Pinene, (4) Sabinene, (5) *α*-Phellandrene, (6) *β*-Myrcene, (7) *D*-Limonene, (8) Eucalyptol, (9) *α*-Ocimene, (10) *γ*-Terpinene, (11) *β*-Ocimene, (12) *o*-Cymene, (13) 4-Nonanone, (14) Sulcatone, (15) Citronellal, (16) Camphor, (17) Neoisopulegol, (18) *β*-Linalool, (19) Isopregol, (20) Isoneral, (21) *γ*-Caryophyllene, (22) *β*-Elemene, (23) Menthol, (24) Isoborneol, (25) *(Z)*-Citral, (26) (-)-Germacrene D, (27) Borneol, (28) *(E)*-Geranial, (29) *γ*-Cadinene, (30) *δ*-Cadinene, (31) Geraniol acetate, (32) *β*-Citronellol, (33) 2-Propenoic acid, (34) *cis*-Geraniol, (35) Germacrene D-4-ol, (36) Elemol, (37) Eugenol, (38) *α*-Cadinol, (39) Limonene-1,2-diol

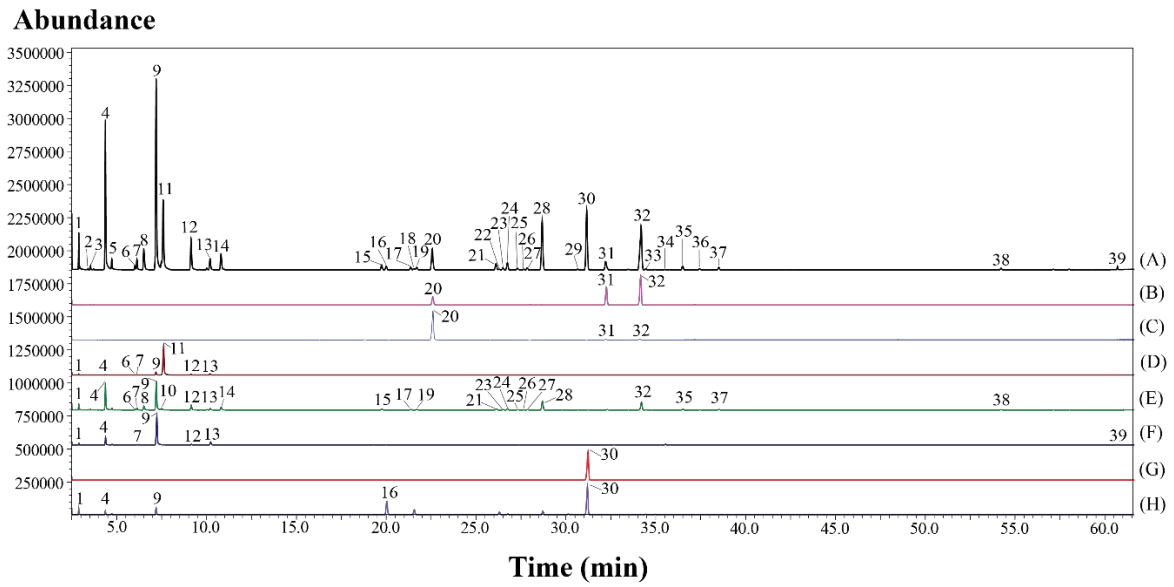

Figure 2. Representative GC-MS chromatograms of A. MU-Kaffir lime, B. Borneol, C. Camphor, D. Eucalyptus oil, E. Kaffir lime oil, F. Lemon oil, G. Menthol and H. Peppermint oil; (1) *α*-Pinene, (2) *α*-Ocimene, (3) Camphene, (4) *β*-Pinene, (5) Sabinene, (6) *α*-Phellandrene, (7) *β*-Myrcene, (8) 2-Carene, (9) *D*-Limonene, (10) *β*-Phellandrene, (11) Eucalyptol, (12) *γ*-Terpinene, (13) *o*-Cymene, (14) (+)-4-Carene, (15) *trans*-Linalool oxide, (16) *cis*-Menthone, (17) *cis*-Linalool oxide, (18) Isomenthone, (19) *α*-Copaene, (20) Camphor, (21) Neoisopulegol, (22) 1S-Neomenthyl acetate, (23) *β*-Linalool, (24) Isopregol, (25) *γ*-Caryophyllene, (26) 1-Terpinenol, (27) *α*-Fenchol, (28) Terpinen-4-ol, (29) *β*-Terpineol, (30) Menthol, (31) Isoborneol, (32) *α*-Terpineol, (33) *α*-Terpinyl acetate, (34) 2-Propenoic acid, (35) *δ*-Cadinene, (36) Geraniol acetate, (37) *β*-Citronellol, (38) *p*-Menthane-3,8-diol, (39) Limonene-1,2-diol.

According to the results, the developed method was successfully applied for analyzing major and minor components of EOs, solid terpenes and blends. Since the method was aimed to analyze all components with acceptable baseline, the lower temperature increasing rate (2°C/min) was employed with the total run time of 69 min. However, if the method focuses on analyzing major compounds, the optimized run time could be shorter than 50 min by increasing temperature increasing rate, such as 3°C/min.

3.2 Repellent Potency of EOs Blends

The results showed that the average repellency rates of MU-Citronella and MU-Kaffir lime were 96.7%, and the diluted solution of MU-Kaffir lime (30% w/w) exhibited repellent activity of 98.3%. The activity of all investigated blends was more than 80%, implying that the blends possessed repellent activity against German cockroach according to the acceptance criteria of the NIH of Thailand. The identified major components of these blends have been reported for their repellency[10-12], which would also contribute to the activity of the blends against the insect. These major components should be further used as markers for quality control. Interestingly, it was observed that the diluted MU-Kaffir lime exhibited the activity slightly higher than that of the concentrated ones. This may because the solution also contained IPM which has been used as co-solvent and fixative for many odorants[24]. As a result, evaporation rate of EOs would be reduced and thereby the efficacy of 30% MU-Kaffir lime was comparable to that of the concentrated ones. Nevertheless, their duration of action should be further investigated.

The concentrated blends, MU-Citronella and MU-Kaffir lime could be further developed as aerosol spray by mixing with inert gas or other dosage forms, while 30% MU-Kaffir lime solution could be used as conventional repellent spray against German cockroaches.

Table 1. The chemical constituents of EOs and blends identified by GC-MS and their %relative peak areas.

Constituents	RT [a] (min)	%Relative peak area [b]										
		MUCN	MUKL	CN	EU	KL	LG	LM	PP	BN	CP	MT
α-Pinene	2.89	0.37	2.24	-	1.49	2.76	0.16	1.71	3.28	-	-	-
α-Ocimene	3.41	-	0.08	-	-	0.13	-	-	-	-	-	-
Camphene	3.55	0.30	0.30	-	-	0.44	0.92	0.08	-	-	-	-
β-Pinene	4.37	0.78	13.49	-	0.52	18.03	-	13.85	2.91	-	-	-
Sabinene	4.73	0.06	0.83	-	-	1.10	-	0.96	0.91	-	-	-
α-Phellandrene	6.02	0.05	0.36	-	0.30	0.49	-	-	-	-	-	-
β-Myrcene	6.14	0.35	0.99	0.47	0.62	1.43	0.28	0.78	0.32	-	-	-
2-Carene	6.51	-	2.69	-	0.07	3.89	-	-	-	-	-	-
D-Limonene	7.20	6.92	21.64	4.02	6.76	26.76	1.56	66.13	6.45	-	-	-
β-Phellandrene	7.49	-	0.21	-	-	0.89	-	-		-	-	-
Eucalyptol	7.60	12.59	7.68	-	84.02	-	-	-	0.26	-	-	-
α-Ocimene	8.93	0.18	-	0.21	0.10	-	0.35	-	-	-	-	-
γ-Terpinene	9.15	0.69	3.91	-	2.25	4.80	-	1.56	-	-	-	-
β-Ocimene	9.64	0.17	-	0.27	-	0.05	0.29	-	-	-	-	-
o-Cymene	10.21	0.67	1.50	-	3.39	1.51	-	7.56	0.38	-	-	-
(+)-4-Carene	10.81	-	2.17	-	0.10	3.18	-	-	-	-	-	-
3-Methyl-1-cyclohexanone	12.57	-	-	-	-	-	-	-	0.19	-	-	-
4-Nonanone	13.27	0.39	-	-	-	-	1.26	-	-	-	-	-
Sulcatone	13.85	0.48	-	-	-	-	1.47	-	-	-	-	-
3-Octanol	17.77	-	-	-	-	-	-	-	0.58	-	-	-
2-Norbornanone	18.80	-	-	-	-	-	-	-	-	-	1.17	-
Limonene oxide	19.27	-	-	-	-	-	-	0.46	-	-	-	-
trans-Linalool oxide	19.75	-	0.80	-	-	1.23	-	-	-	-	-	-
trans-Limonene oxide	19.98	-	-	-	-	-	-	0.58	-	-	-	-
cis-Menthone	20.01	-	0.58	-	-	-	-	-	18.87	-	-	-
cis-Linalool oxide	21.37	-	0.40	-	-	0.63	-	-	-	-	-	-
Isomenthone	21.55	-	0.15	-	-	-	-	-	7.57	-	-	-
Citronellal	21.68	11.50	-	36.58	-	-	0.14	-	-	-	-	-
α-Copaene	21.70	-	0.38	-	-	0.71	-	-	-	-	-	-
Camphor	22.58	3.68	3.74	-	-	-	-	-	-	15.15	93.43	-
Methacrylic anhydride	23.93	-	-	-	-	-	0.08	-	-	-	-	-
3,6-Octadienal	25.49	-	-	-	-	-	0.17	-	-	-	-	-
Neoisopulegol	26.12	0.50	1.00	1.70	-	1.55	-	-	-	-	-	-
1S-Neomenthyl acetate	26.29	-	0.07		-	-	-	-	4.63	-	-	-
β-Linalool	26.51	0.67	0.30	0.75	-	0.44	1.26	0.12	0.05	-	-	-
Isopregol	26.76	2.25	1.23	7.02	-	1.77	-	-	1.55	-	-	0.13
Isoneral	27.01	0.14	-	-	-		0.50	-		-	-	-
γ-Caryophyllene	27.32	0.36	0.24	-	-	0.36	1.50	-	0.36	-	-	-
α-Bergamotene	27.40	-	-	-	-	-	-	0.49		-	-	-
β-Elemene	27.48	0.48	-	1.81	-	-	-	-	-	-	-	-
1-Terpinenol	27.65	-	0.09	-	-	-	-	-	-	-	-	-
β-Terpineol	27.68	-	-	-	-	0.15	-	-	-	-	-	-

Constituents	RT [a] (min)	%Relative peak area [b]										
		MUCN	MUKL	CN	EU	KL	LG	LM	PP	BN	CP	MT
α-Fenchol	27.86	-	0.31	-	-	0.48	-	-	-	-	-	-
2-Norbornanone	27.98	-	-	-	-	-	-	-		-	0.72	-
Neoisopulegol	28.52	-	-	-	-	-	-	-	0.64	-	-	-
Terpinen-4-ol	28.68	-	8.52	-	0.12	12.42	-	-	-	-	-	-
Neomenthol	28.72	-	-	-	-	-	-	-	5.08	-	-	-
Rosefuran epoxide	28.82	-	-	-	-	-	0.20	-		-	-	-
Isopulegol	29.80	-	-	0.28	-	-	-	-		-	-	-
cis-Isopulegone	30.14	-	-	-	-	-	-	-	1.43	-	-	-
D-Neoisomenthol	30.36	-	-	-	-	-	-	-	0.74	-	-	-
β-Terpineol	30.67	-	0.11	-	-	0.19	-	-	-	-	-	-
γ-Terpineol	31.15	-	-	-	-	-	-	-	-	-	0.36	-
Menthol	31.20	8.92	10.26	-	-	-	-	-	39.93	-	-	99.87
α-Caryophyllene	31.25	-	-	-	-	0.10	0.12	-	-	-	-	-
Isoborneol	32.23	2.20	1.65	-	-	-	-	-	-	30.74	1.72	-
β-Citronellyl acetate	32.31	-	-	2.39	-	0.38	-	-	-	-	-	-
γ-Terpineol	32.64	-	-	-	-	-	-	-	0.28	-	-	-
(Z)-Citral	32.69	9.31	-	0.35	-	-	29.04	0.87		-	-	-
1-Nonanol	32.84	-	-	-	-	-	-	-	0.13	-	-	-
Lavandulol	33.46	-	-	-	-	-	-	-	0.52	-	-	-
(-)-Germacrene D	33.48	0.31	-	0.80	-	0.13	-	-	-	-	-	-
Borneol	34.13	2.60	-	-	-	-	0.31	-	-	52.87	2.60	-
α-Terpineol	34.20	-	9.94	-	0.25	10.94	-	0.18	0.69	-	-	-
Piperitone	34.45	-	-	-	-		-	-	1.02	-	-	-
α-Terpinyl acetate	34.46	-	0.17	-	-	0.23	-	-	-	-	-	-
α-Cadinene	34.76	-	-	0.31	-	-	-	-	-	-	-	-
1-Carvone	34.97	-	-	-	-	-	-	0.12	0.81	-	-	-
β-Bisabolene	35.27	-	-	-	-	-	-	0.78	-	-	-	-
2-Propenoic acid	35.52	-	0.06	-	-	-	-	-	-	-	-	-
(*E*)-Geranial	35.55	14.46	-	0.51	-	-	45.28	1.97	-	-	-	-
Neryl Acetate	35.78	-	-		-	-	-	0.61	-	-	-	-
γ-Cadinene	36.42	0.47	-	0.44	-	-	1.11	-	-	-	-	-
δ-Cadinene	36.51	0.48	0.64	1.30	-	1.03	0.22	-	-	-	-	-
Geraniol acetate	37.46	2.48	0.18	2.42	-	0.24	5.20	0.35	-	-	-	-
1-Decanol	38.38	-	-	-	-	-	-	-	0.23	-	-	-
β-Citronellol	38.52	4.59	0.39	13.63	-	0.58	0.08	-	-	-	-	-
2-Propenoic acid	40.04	0.09	-	0.13	-	-	0.15	-	-	-	-	-
Carveol	41.56	-	-	-	-	-	-	0.20	-	-	-	-
cis-Geraniol	42.67	8.69	-	18.94	-	-	7.10	-	-	-	-	-
Geraniol butyrate	44.57	-	-	-	-	-	0.10	-	-	-	-	-
Caryophyllene oxide	47.15	-	-	-	-	-	0.20	-	-	-	-	-
Germacrene D-4-ol	51.45	0.18	-	0.66	-	-	-	-	-	-	-	-
Elemol	53.27	1.03	-	3.19	-	-	-	-	-	-	-	-
p-Menthane-3,8-diol	54.23	-	0.23	0.24	-	0.38	-	-	-	-	-	-
Eugenol	57.12	0.19	-	0.66	-	-	-	-	-	-	-	-
Eudesmol	59.08	-	-	0.13	-	-	-	-	-	-	-	-

Constituents	RT [a] (min)	%Relative peak area [b]										
		MUCN	MUKL	CN	EU	KL	LG	LM	PP	BN	CP	MT
α-Cadinol	59.34	0.14	-	0.49	-	-	-	-	-	-	-	-
Limonene-1,2-diol	60.71	0.05	0.27	-	-	-	-	0.63	-	-	-	-
cis-Isoeugenol	61.71	-	-	-	-	-	0.37	-	-	-	-	-

[a] Retention time

[b] Percentage relative peak areas of identified compounds in EOs blends, MU-citronella (MUCN) and MU-Kaffir lime (MUKL), and EOs of citronella (CN), eucalyptus (EU), kaffir lime (KL), lemongrass (LG), lemon (LM), peppermint (PP), borneol (BN), camphor (CP) and menthol (MT).

The use of essential oils along with other integrated pest management techniques can be an effective method for controlling the German cockroach in food preparation areas, storage buildings, apartments, and homes[25]. However, it is important to keep in mind that, natural products are not always safe. Some EOs could cause contact dermatitis and may irritate respiratory tract when inhaling them directly[26]. Therefore, these compounds should be cautiously applied.

4. CONCLUSION

Since the use of synthetic pest control materials raises several concerns related to environment and human health. An alternative is to use natural EOs possessing good efficacy and environmentally friendly. In this study, the EOs blends, MU-Citronella, MU-Kaffir lime and 30% MU-Kaffir lime solution possessing strong repellent activity against German cockroach were successfully developed and could be considered as eco-friendly repellents for household cockroach control. The major and minor compositions of each EO, solid terpene and blend were identified using GC-MS and their relative percentage abundance (%relative peak area) can be further applied for quality control. The concentrated blends could be further developed as repellent aerosol spray or other dosage forms. For 30% MU-Kaffir lime solution, it could be utilized as conventional repellent spray. However, these EO-based repellents tend to being short-lived in their effectiveness due to their high volatility and requirement of frequent re-application. To overcome this challenge, the use of formulation strategies, such as employing fixative materials would be further investigated. Furthermore, the application in management of other household insect pests would be further assessed.

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Conflict of interest

The authors have no conflict of interest to declare.

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Exploring Free Radical Scavenging and Cardioprotective Effects via Acid-Base Extraction from *Nelumbo nucifera* Gaertn.

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ABSTRACT

Nelumbo nucifera Gaertn. (sacred lotus) is belongs to the family Nelumbonaceae. Its plumules (P) and leaves (L) are rich in alkaloids, predominantly neferine and nuciferine, respectively. These compounds have been reported to exhibit various pharmacological activities, notably, cardiovascular effects. The objectives of study were to quantify neferine and nuciferine content in lotus plumule and leaf extracts through HPLC analysis. The extraction of these extracts was performed using acid-base extraction (ABE), a method known for its efficacy in isolating alkaloid compounds from plants. Extraction solvents of either 70% or 95% ethanol were employed in the process. Additionally, the antioxidant activity was assessed through DPPH and FRAP assays. Furthermore, the cardioprotective effect against oxidative stress induced by H_2O_2 in H9c2 cardiomyoblasts was evaluated. From our obtained results, P-ABE95 showed a higher neferine content (177.24 ± 0.79 mg/g) compared to P-ABE70 (112.46 ± 0.72 mg/g) in lotus plumule extracts. Similarly, the lotus leaf extracts revealed a higher nuciferine content in L-ABE95 (9.50 ± 0.01 mg/g) compared to L-ABE70 (8.03 ± 0.03 mg/g). In terms of antioxidant activity, P-ABE95 demonstrated the highest efficacy in both DPPH (IC_{50} of 50.416 ± 0.87 µg/ml) and FRAP assays (value of 50.416 ± 0.87 mM/100g extract). Based on the evaluation of their antioxidant activity, therefore, the most promising extract (P-ABE95) was selected for further in vitro cardioprotective testing in H9c2 cardiomyoblasts. The results indicated that P-ABE95 extract significantly increased cell viability in H9c2 cells induced by H_2O_2 ($p < 0.05$).

KEYWORDS: *Nelumbo nucifera*, acid-base extraction, neferine, nuciferine, cardioprotective effects

1. INTRODUCTION

Nelumbo nucifera Gaertn. also known as sacred lotus, is an aquatic perennial crop grown and consumed throughout Asia[1]. All parts of *N. nucifera* have been used for various medicinal purposes in various systems of medicine including folk medicines, Ayurveda, Chinese traditional medicine, and oriental medicine. Many chemical constituents have been separated from *N. nucifera* which alkaloids and flavonoids presented as major constituents[2, 3]. Leaves, plumules, and seeds are rich in alkaloids and flavonoids[3-5], with neferine being a prominent alkaloid isolated from lotus plumules, as reported in previous studies. However, it has not been reported whether neferine has been found in other parts of the lotus.

In the preliminary stage of medicinal plant product research, the extraction of plant extracts is a pivotal step. The acid-base extraction method utilizes liquid-liquid extraction techniques to separate acids and bases from complex mixtures, capitalizing on their distinct chemical properties for purification purposes. This process enables the isolation and purification of compounds such as alkaloids from crude extracts[6].

Oxidative stress is a significant contributor to tissue damage observed in cardiovascular diseases, primarily driven by the accumulation of reactive oxygen species (ROS) within cellular environments. Despite the presence of various antioxidant defense mechanisms within cells to regulate ROS levels, the production of ROS frequently exceeds the capacity of these defenses, resulting in oxidative stress[7]. This pathological condition plays a crucial role in the progression of cardiovascular diseases such as atherosclerosis, hypertension, myocardial infarction and heart failure. Moreover, oxidative stress has been implicated in myocardial stunning, infarction, apoptosis, and potentially arrhythmias. Hydrogen peroxide (H_2O_2), a metabolic byproduct, serves as a significant source of oxidative stress, exerting damage on cellular components including DNA, RNA, and proteins. To investigate mechanisms aimed at attenuating redox injury, the myoblast cell line H9c2, derived from embryonic rat heart tissue, was subjected to a challenge with H_2O_2[8].

Therefore, this study aimed to quantify the neferine and nuciferine levels in lotus plumule and leaf extracts using high-performance liquid chromatography (HPLC) analysis. These extracts were obtained utilizing the acid-base extraction method. Furthermore, the antioxidant potential was assessed through DPPH and FRAP assays, and the cardioprotective effects of lotus extracts were evaluated in H9c2 cells.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Materials

The dried lotus plumules were ground into a find powder using a powder grinder and fresh lotus leaves were washed and dried in a hot air oven at 60°C for 12h. and further ground to a fine powder.

2.2 Acid-Base Extraction (ABE)

A 20 g quantity of lotus powder was initially subjected to defatting using petroleum ether. Following defatting, the sample was treated with ammonia solution and subsequently extracted with either 70% or 95% ethanol for 8 hours over a 3-day period using a rotary shaker. The resulting solutions underwent filtration, concentration, and drying. The dried extract was then mixed with dichloromethane and treated with 2% HCl to acidify the solution within a separatory funnel. The aqueous phase was collected and made basic with ammonia solution to yield a basic solution, which was further extracted with dichloromethane. The lower organic layer was collected and subjected to centrifugation at 4500 rpm for 5 minutes using a low-speed centrifuge, after which the resulting precipitate was dried. The percentage yield of the extract was subsequently determined.

2.3 High Performance Liquid Chromatography (HPLC)

The separation process was conducted utilizing a C_{18} column (150 x 4.6 mm, i.d. 5 µm) with a C_{18} guard column (4 x 10 mm, i.d. 5 µm). A gradient system was employed, utilizing 0.1% v/v triethylamine in water (solvent A) and acetonitrile (solvent B) as mobile phases at a flow rate of 1 mL/min. The elution gradient program proceeded as follows: from 0 to 35 minutes, the solvent composition transitioned from 70:30 (A:B) to 40:60; from 35 to 40 minutes, it remained at 40:60; and from 40 to 50 minutes, it returned to 70:30 for equilibration. The total run time for the process was 50 minutes. An injection volume of 20 µl was employed, and the UV detector was set at 280 nm.

2.4 Determination of Antioxidant Activity

2.4.1 DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was freshly prepared in methanol at a final concentration of 0.1 mM. Each sample extract was diluted in methanol at varying concentrations. In a 96- well plate, 100 µL of each extract were added to each well followed by 100 µL of methanolic DPPH solution. Ascorbic acid was used as a standard and was treated under the same condition as the samples.

The mixtures were allowed to stand at room temperature in the dark for 30 min. The absorbance was recorded at 517 nm using a microplate reader.

2.4.2 Ferric reducing antioxidant power (FRAP)

Each sample extract was diluted in methanol to obtain varying concentrations. The resulting mixture was added to a 96-well plate according to the specifications outlined in Table 1.

Table 1. Parameters of each well consisted of FRAP assay

Parameters	Each well consists of
A (Test sample)	Different concentration of sample 25 µl + FRAP reagent 175 µl
B (Blank)	Different concentration of sample 25 µl + Acetate buffer 175 µl
C (Control)	Distill water 25 µl + FRAP reagent 175 µl

The experiment was conducted in triplicate and left to incubate for 30 min before recording the absorbance at 593 nm employing a microplate reader. Ferrous sulfate ($FeSO_4$) was employed to establish the standard curve.

2.5 Cell Viability of H9c2 Cells in H_2O_2-Induced Oxidative Stress

H9c2 cells were seeded overnight in 96-well plates at a density of 5×10^4 cells/well in DMEM with 1% FBS. After seeding, medium was removed, and cells were treated with P-ABE95 extract, the selected concentration of extract (0.1 µg/ml) 100 µl and incubated for 24 h. After extract pre-treatment, the media was removed and 250 µM of H_2O_2 was added to the wells (100 µl) and incubated for 24 h. Finally, the cells were substituted with MTT assay, and the insoluble formazan product were dissolved with DMSO. The absorbance value of solution was measured using a microplate reader at 570 nm. The experiments were repeated at least three times.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

The lotus extracts were obtained using the acid-base extraction (ABE) method, employing either 95% or 70% ethanol as solvents. The crude lotus extracts exhibited dark brown or green coloration, with samples extracted using 70% ethanol displaying a darker hue compared to those extracted with 95% ethanol. The yield of lotus plumule extract, P-ABE70, was higher at 0.731% than that of P-ABE95 at 0.653%. Similarly, the yield of lotus leaf extract, L-ABE70, was higher at 2.846% compared to L-ABE95, which yielded 1.401%.

3.2. Quantification of Neferine and Nuciferine in Lotus Extracts using HPLC Analysis

Neferine content was determined via HPLC analysis, with chromatographic peaks corresponding to neferine detected at a retention time (Rt) of approximately 35 minutes. Among the samples, P ABE95 exhibited a neferine content of 177.24 ± 0.79 mg/g, while P-ABE70 showed a content of 112.46 ± 0.72 mg/g. In the lotus leaf extract, nuciferine content was detected, with L-ABE95 containing 9.50 ± 0.01 mg/g and L-ABE70 exhibiting a content of 8.03 ± 0.03 mg/g, both observed at a retention time of around 36 minutes (Table 2).

Table 2. The content of neferine and nuciferine from lotus plumules and leaves extracts using HPLC analysis.

Lotus plumule extract	Neferine (mg/g)	%RSD
P-ABE95	177.24 ± 0.79	0.45
P-ABE70	112.46 ± 0.72	0.64
Lotus leaves extract	**Nuciferine (mg/g)**	**%RSD**
L-ABE95	9.50 ± 0.01	0.07
L-ABE70	8.03 ± 0.03	0.32

* Results expressed as mean ± standard deviation (SD) n=3.

3.3 Determination of Antioxidant Activity

The P-ABE95 extract derived from lotus plumules demonstrated notably potent DPPH scavenging activity, with an IC_{50} value of 50.416 ± 0.87 µg/ml, and high FRAP values, measuring 0.332 ± 0.01 mM/100g extract. These findings highlight the robust antioxidant activity of P-ABE95 from lotus plumules, which correlates with the neferine content (Table3). The P-ABE95 extract was further subjected to H9c2 cell viability testing.

Table 3. Antioxidant activity, neferine and nuciferine contents from lotus extracts.

Samples extract	DPPH IC_{50} (µg/ml)*	FRAP (mM/100g extract)*	Neferine (mg/g)*	Nuciferine (mg/g)*
P-ABE95	50.416 ± 0.87	0.332 ± 0.01	177.24 ± 0.79	-
P-ABE70	54.729 ± 1.94	0.202 ± 0.02	112.46 ± 0.72	-
L-ABE95	130.622 ± 1.78	0.250 ± 0.01	-	9.50 ± 0.01
L-ABE70	87.292 ± 2.68	0.510 ± 0.03	-	8.03 ± 0.03

* Results expressed as mean ± standard deviation (SD) n=3

3.4 Cell Viability of H9c2 Cells in H_2O_2-Induced Oxidative Stress

To investigate the cardioprotective effect of lotus extracts, cell viability on H9c2 cells was assessed using MTT assay following pretreatment with P-ABE95 extracts (0.1 µg/ml) for 24 hours, followed by induction with H_2O_2 (250 µM) for another 24 hours. H_2O_2 treatment significantly decreased cell viability to 78.59 ± 1.60%. Adding P-SE95 (0.1 µg/ml) before H_2O_2 treatment significantly increased the cell viability to 82.59 ± 0.91 ($p < 0.05$).

4. CONCLUSION

This observation highlights the effectiveness of the acid-base extraction (ABE) method, attributed to its ability to extract neferine in its free-base form, thereby optimizing extraction efficiency. Acid-base extraction is well-suited for isolating alkaloids due to their chemical properties. This process exploits the differing solubilities of alkaloids in acidic and basic solutions, allowing for the selective separation of alkaloids from other components in a mixture. Consequently, acid base extraction facilitates the efficient isolation and purification of alkaloids, making it a preferred method for extracting them from natural sources. Nevertheless, the ABE method applied to lotus leaf extracts resulted in a low quantity of nuciferine. This outcome could be ascribed to the specific solubility properties inherent to the leaf extract. Furthermore, the presence of chlorophyll content within the leaf extract may potentially impede the separation and alkaloid extraction procedures.

In terms of assessing antioxidant properties via the DPPH and FRAP assays, P-ABE95 exhibited the most significant antioxidant activity. This observation was consistent with their extracts containing the highest concentrations of neferine from lotus plumules, when normalized to equal weights of crude extract.

For evaluating cardioprotective potential, the highest antioxidant efficacy (P-ABE95) was selected. Remarkably, pretreatment with a concentration of 0.1 µg/ml of P-ABE95 elicited a significant increase in cell viability percentage in H9c2 cells exposed to oxidative stress induced by 250µM of H_2O_2 ($p < 0.05$).

Based on the results, this study has identified the acid-base extraction method as potent in extracting neferine from lotus plumules. These extracts demonstrated notable radical scavenging and cardioprotective effects. These findings have promising implications for the advancement of lotus extract in potential pharmaceutical applications in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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In silico and *In vitro* Analysis of the Antihypertensive and Antioxidant Potential of Abaca (*Musa textilis*) Ethanolic Leaf Extract

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ABSTRACT

Hypertension prevails to be one of the leading causes of cardiovascular diseases in the Philippines. Given its significant health risks and economic burden, research into management strategies has led to the exploration of antihypertensive and antioxidant properties found in abaca (*Musa textilis*), a plant endemic to the Philippines. This study aims to establish the efficacy of *M. textilis* ethanolic leaf extract at different concentrations in the treatment of hypertension and antioxidant potential. In silico analysis is used to predict the interaction of compounds present in *M. textilis* with the target protein involved in hypertension and antioxidant activity. Moreover, the *M. textilis* ethanolic leaf extract is subjected to in vitro analysis through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and angiotensin-converting enzyme (ACE) inhibition assay for the evaluation of its antioxidant and antihypertensive properties, respectively. The findings from the DPPH assay indicate that the IC50 value of the ethanolic leaf extract of *Musa textilis* (*M. textilis*) is comparable to that of the positive control, ascorbic acid. Moreover, the ACE inhibition activity exhibited by the *M. textilis* ethanolic leaf extract is comparable to that of the positive control, captopril. In the in silico analysis, bioactive compounds present in the *M. textilis* ethanolic leaf extract, demonstrated favorable interactions in addition to a limited number of unfavorable bonds characterized by negative binding affinity when bound to the angiotensin-converting enzyme (ACE), lipoxygenase, CYP2C9, NADPH-oxidase, and xanthine oxidase. These outcomes underscore the potential of the *M. textilis* ethanolic leaf extract as a probable dual antihypertensive and antioxidant for managing hypertension.

KEYWORDS: *Musa textilis*, antihypertensive, antioxidant, *in silico*, *in vitro*

1. INTRODUCTION

Hypertension remains the primary cause of cardiovascular diseases worldwide, especially in areas classified as low and middle-income countries. Despite the attempt of developed countries to reduce the elevations of blood pressure, prevalence of hypertension globally is still on the rise. In the Philippines, hypertension is not given importance due to misconceptions and its asymptomatic nature[13]. In a survey by the Philippine Heart Association, results revealed that 38.6% of the hospital-based population has cardiovascular diseases[16]. Subsequently, uncontrolled hypertension in the Philippines negatively influences productivity at work and home[14]. Natural products have made vital contributions to pharmacotherapy in managing hypertension. Moreover, the family Musaceae is characterized to have flavonoids, saponins, cardiac glycosides, phenolic acids, and alkaloids as their major components[12]. Particularly, the methanolic and acetone extractions of potassium-rich *Musa* species exhibit an antihypertensive activity by inhibiting the renin-angiotensin system[3]. In addition, *Musa* species contain phenols and flavonoids, which are known to exhibit antioxidant properties that are beneficial in suppressing heart damage and preventing the degradation of systolic functions caused by hypertension[18]. *Musa textilis*, also from the Family Musaceae and a plant endemic to the Philippines,

also exhibits different pharmacologic properties due to the presence of alkaloids, flavonoids, amino acids, triterpenoids, terpenoids, steroids, saponins, and tannins[2].

The study aims to determine the antihypertensive and antioxidant potential of abaca (*M. textilis*) ethanolic leaf extract (ELE) in managing hypertension by conducting in-silico analysis, angiotensin-converting enzyme (ACE) assay, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. In silico analysis of abaca ELE is used to predict the binding affinity between the bioactive compounds from abaca ELE and the target receptors and compare it with the positive control in terms of its antihypertensive and antioxidant activities.

2. MATERIALS AND METHODS

2.1 Standard Reagents and Chemicals

The chemical used in the extraction of abaca ELE is 70% technical-grade ethanol which was procured from Puljed Trading Laboratory and Medical Supplies. The ACE kit-WST was purchased from Everlife-Scientific Resources in Singapore and the DPPH reagent was purchased from Belman Laboratories. For the positive controls for assays, captopril and ascorbic acid were obtained from a local drugstore and the Fr. Lorenzo Rodriguez, O.P. Laboratory, respectively.

2.2 Plant Collection, Identification, and Extraction

Fresh abaca leaves were identified by Jose Vera Santos Memorial Herbarium. Once identified, seven kilograms of the plant leaves were collected from San Andres, Catanduanes. Following the collection of the leaves, subsequent washing, dust removal, and air drying were conducted. Consequently, the dried leaves underwent percolation where it was soaked in 70% ethanol. The extract collected and subjected to rotary evaporator and water bath.

2.3 Characterization of Abaca ELE using GC-MS

The bioactive constituents found in abaca ELE were analyzed and verified through Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS was carried out using the Agilent 8890 GC system coupled to a 5977B Mass Selective Detector (MSD) and the resulting data was processed with the Agilent MassHunter Qualitative Analysis 10.00 software.

2.4 Preparation of Abaca ELE and Control Concentrations

In terms of the abaca ELE concentrations 0.0375 g, and 0.075 g of the abaca leaf extract were diluted in 50 mL deionized water for ACE inhibition assay and 0.01875 g, 0.0375 g, and 0.075 g of the abaca leaf extract were diluted in 50 mL of 70% technical-grade ethanol for DPPH assay. The positive controls for each assay, captopril for ACE inhibition and ascorbic acid for DPPH assay, also followed the concentration preparation of abaca ELE. The negative control for both assays contained deionized water for ACE inhibition assay and 70% technical-grade ethanol for DPPH assay.

2.5 ACE Inhibition Assay

ACE Kit-WST is utilized to determine the antihypertensive properties of the two different concentrations of abaca ELE and Captopril. Consequently, all samples and reagent are pipetted into a 96-well plate and incubated at 37°C for 1 hour. Then, 200 µL of the indicator solution is added and the resulting mixture is incubated again at room temperature for 10 minutes. Afterwards, the microplate was read in spectrophotometer at 450 nm. The percent inhibition is calculated using the formula below. The IC50 was determined through GraphPad Prism.

$$Percent\ inhibition = \frac{A_{blank1} - A_{sample} - A_{sample\ blank}}{A_{blank1} - A_{blank2}} \text{ x } 100$$

Where:

A_{blank1}= absorbance of the positive control at 450 nm (no ACE inhibition)
A_{blank2}= absorbance of the reagent blank at 450 nm
$A_{blank\ sample}$ = absorbance of the sample and deionized water at 450 nm
A_{sample} = absorbance of sample at 450 nm

2.6 DPPH Assay

The reagent used in the assay was prepared by weighing 0.0276 g of powder DPPH reagent and incorporating 70 mL of 70% technical-grade ethanol in a dark room. Consequently, all samples and DPPH reagent were transferred to a 96-well microplate and incubated at room temperature for 10 minutes. Afterwards, the microplate was placed in a spectrophotometer to evaluated at 517 nm. The percent inhibition of the sample was computed using the formula below. The IC50 was determined through GraphPad Prism.

$$Percent\ inhibition = \frac{A_C - A_S}{A_c} \text{ x } 100$$

Where:

A_c = absorbance of the negative control at 517 nm
A_s = absorbance of the sample at 517 nm

2.7 *In Silico* Analysis using Molecular Docking

The 3D SDF file of the ligands was obtained from PubChem, while the the other proteins are from RCSB Protein Data Bank. Specifically, the reference compound for the protein Angiotensin Converting Enzyme (4C2P) is captopril, 3,4-dihydroxybenzoic acid for Lipoxygenase (1N8Q), 3,4-Hypoxanthine for Xanthine oxidase (3NRZ), Adenosine-5'-diphosphate for NADPH-oxidase (2CDU), and Warfarin for CYP2C9 (1OG5). Any irrelevant metabolic proteins in the general structure were eliminated in UCSF Chimera and both the receptor and the ligand were imported into PyRx. After verifying the accuracy of the procedure, the poses of all ligands obtained from PyRx were uploaded to Biovia which was used in docking and analyzing the results.

3. RESULTS AND DISCUSSION

3.1 Chemical Profile of Abaca (*Musa textilis*) Leaf Extract

The abaca (*Musa textilis*) ethanolic leaf extract (abaca ELE) displayed a prominent dark green coloration and an extraction yield of 7.11%. The extraction yield of abaca ELE is considered a moderate to high yield compared to other plant extracts. Particularly, the typical extraction yield of plants that utilize ethanolic solvent ranges from 2% to 18%[5].

Subsequent GC-MS analysis revealed the presence of 24 distinct compounds characterized through spectral matching against the National institute of Standards and Technology (NIST) Library. The compounds found are acetic acid 2-propanone, 1-hydroxy, N,N-dimethylaminoethanol, propanoic acid, 2-hydroxy-, methyl ester, propanoic acid, 2-oxo-, methyl ester, H-pyrrole, 2,3-dimethyl and 1H-pyrrole, 2,4-dimethyl, butanoic acid, 4-hydroxy, 2-cyclopenten-1-one, 2-hydroxy, glycerin, phenol, 2-methoxy, 2-methoxy-4-vinylphenol, 2-propenoic acid derivatives, hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, phytol and 7,10,13-eicosatrienoic acid, methyl ester. The analysis identified hexadecanoic acid, methyl ester as the compound with the highest match factor (971) while butyrolactone (908) had the lowest match factor among the ten selected compounds.

Among the ten compounds that exhibited a high match factor, 2-methoxy-4-vinylphenol (2M4VP) and acetic acid (AcOH) showed potential antihypertensive activities. The oxidative inhibition of these compounds explains the antihypertensive potential of the extract. Meanwhile, all the compounds possess hydroxyl group that serves as an antioxidant by acting as an electron donor [4,18].

3.2 Angiotensin – Converting Enzyme (ACE) Inhibition Activity

The absorbances of abaca ELE were measured at 3.233, 4.149, 0.223 and 0.446. Meanwhile, the absorbance of blank 1 was 4.267. The difference in the absorbance between blank 1 and the samples proves the antihypertensive activities of abaca ELE and captopril. The graph of percent inhibition and the IC50 values of abaca ELE and captopril is illustrated below.

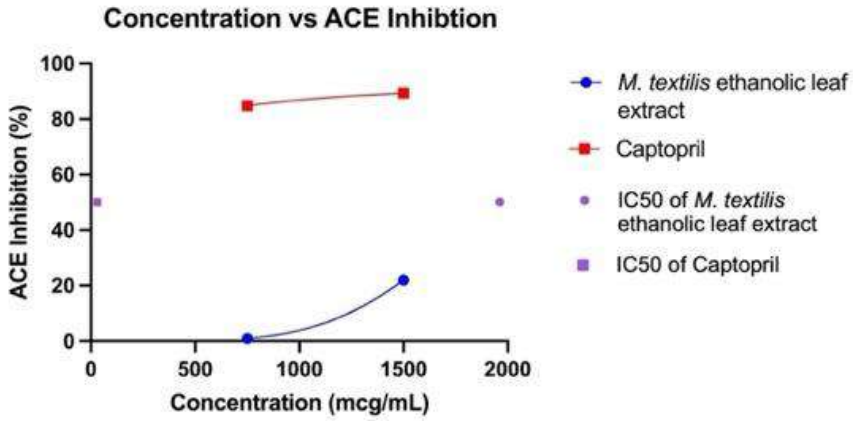

Figure 1. IC50 of abaca ELE and Captopril. The percent inhibition and IC50 values of abaca ELE and captopril are plotted on the graph.

At concentrations of 750 mcg/mL and 1500 mcg/mL, the extract exhibited percent inhibition values of 1.00 ± 12.90 and 21.97 ± 12.90, respectively. In contrast, the positive control demonstrated percent inhibition values of 89.25 ± 3.19 and 84.81 ± 3.19 at the same concentrations. Additionally, the IC50 values were also determined to be 1953 mcg/mL and 97.11 mcg/mL for the abaca ELE and Captopril, respectively. The discovery of angiotensin-converting enzyme (ACE) inhibitory activity in the abaca ELE suggests its potential role in modulating physiological processes associated with the renin-angiotensin system (RAS). This indicates the presence of bioactive compounds in the extract that can interfere with ACE function, specifically acetic acid and 2-methoxy-4-vinylphenol. Notably, while the phenol, 2-methoxy, also known as guaiacol, was found to cause vasodilation leading to hypotension, its mechanism of action is not through ACE inhibition but rather through blocking of the calcium channel[1]. In addition, the IC50 of the *M. textilis* is higher than that of the Captopril, indicating its low potency in inhibiting 50% of the ACE activity. However, the Captopril exhibited a high IC50 that is greater than other literatures, such as 3.56 µg/mL IC50[8] and the 20 nM/mL IC50[9]. These differences can be attributed to the variances in the methods utilized for the assay of the positive control.

3.3 Antioxidant Activity

The absorbance of the negative control, 70% ethanol of the assay was measured at 2.5. Furthermore, the absorbances of abaca ELE were measured at 0.588, 0.824, 1.138. Moreover, the absorbances of ascorbic acid were measured at 0.161, 0.739, 1.563. The difference in the absorbance reading confirms the presence of antioxidant properties in abaca ELE at different concentrations, making it comparable to ascorbic acid. The graph of the percent inhibition and IC50 values are presented below.

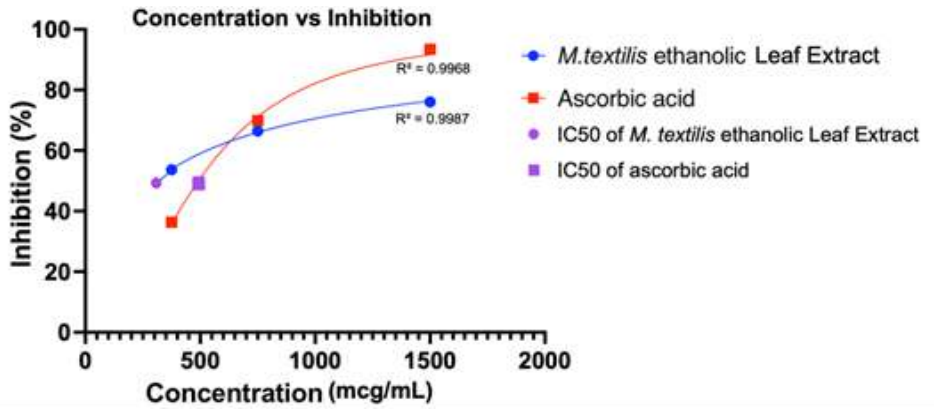

Figure 2. IC50 of abaca ELE and ascorbic acid. The percent inhibition and IC50 values of abaca ELE and ascorbic acid are plotted on the graph.

Abaca ELE has been discovered to exhibit a strong antioxidant activity. At different concentrations, the percent inhibition of abaca ELE was around 76.06 ± 11.24, 45 ± 11.24, and 66 ± 11.24. On the other hand, 93.44 ± 28.69, 69.91 ± 28.69, and 36.36 ± 28.69. The IC50 of abaca ELE and ascorbic acid was determined, having values of 303.6 mcg/mL and 492.2 mcg/mL, respectively. The abaca ELE exhibited a strong antioxidant activity that can be attributed to the identified chemical compounds found in its extract. *Musa paradisiaca*, a plant from the same family Musaceae also contains hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid, and phytol compounds which are known to elicit anti-inflammatory, antioxidant, and anti-cancer activities[11]. Additionally, compared to other plants in the family of Musaceae, *M. textilis* showed the highest DPPH radical activity. The IC50 of abaca ELE presented a comparable value, particularly a lower IC50 than ascorbic acid. A low IC50 would mean a low concentration needed in achieving antioxidant activity. Although the extract has an IC50 value that is 188.6 lower than ascorbic acid, other studies reveal that the obtained IC50 for ascorbic acid is different. Specifically, ascorbic acid has an IC50 value of 2.6 mcg/mL[10] and an IC50 value of 24.34±0.09 in another study[11]. The varying and inconsistent results can be attributed to the experimental protocols followed by researchers.

3.4 Molecular Docking

An RMSD of ≤ 2.5 Å was observed for all proteins redocked since an RMSD of 2.5 Å or less is usually considered as geometric equivalent from the two poses[7]. Furthermore, a more negative binding affinity indicates a better molecular docking prediction and a stronger ligand-receptor interaction[6]. The conventional hydrogen bonds and carbon hydrogen bonds play a significant role in ligand binding, while other hydrophobic interactions decrease the binding affinity, thus creating a better interaction[17]. In terms of the ACE inhibition activity, 2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester and 2-Methoxy-4-vinylphenol showed strong binding affinities which are presented below.

Table 1. Binding interactions of ligands with Angiotensin-converting enzyme (4C2P)

Ligand	Binding Affinity (kcal/mole)
2-Propenoic acid, 3-(4-hydroxy-3- methoxyphenyl)-, methyl ester	-5.8
2-Methoxy-4-vinylphenol	-5.8

The binding affinities of the ligands were -5.8 kcal/mole which is stronger than Captopril. Conventional hydrogen and alkyl bonds, and amino acids HIS 383 and VAL 380 are seen in these

ligands. Therefore, these amino acids and types of bonds may have contributed to their high binding affinity. The average binding affinities for the antioxidant potential of abaca ELE is presented below.

Table 2. Average binding affinity of ligands

Ligand	Average Binding Affinity
2-Propenoic acid, 3-(4-hydroxy-3- methoxyphenyl)-, methyl ester	-5.45
2-Methoxy-4-vinylphenol	-5.125
Phytol	-4.725

With regards to the antioxidant property, there are no ligands that demonstrated a stronger binding affinity than the reference compounds in lipoxygenase, CYP2C9, and NADPH oxidase; however, all ligands showed a negative binding affinity, hence there is an interaction between the protein. Moreover, 2-Propenoic acid, 3-(4-hydroxy-3- methoxyphenyl)-, methyl ester showed a stronger binding affinity than 3,4-Hypoxanthine. ALA 1079 may be a vital indicator of an interaction with Xanthine oxidase, which may contribute to its antioxidant activity since it is present in both compounds.

4. CONCLUSION

Abaca ELE exhibited antihypertensive and antioxidant potential in managing hypertension. Additional analysis and isolation of extract is needed to further analyze the potential of the extract.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Ethics approval

None to declare.

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Triterpenoids and flavonoids from *Ludwigia octovalvis* (Jacq.) P.H.Ravens and their bacteriostatic effect on *Helicobacter pylori*

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ABSTRACT

In Vietnamese folk medicine, *Ludwigia* species are used in the management of peptic ulcers caused by *Helicobacter pylori*. To search for evidence of the plant's folk use, this work investigated in the bacteriostatic effect on *H. pylori* of extracts and related phytoconstituents from *Ludwigia octovalvis* (Jacq.) P.H.Ravens. Following bioactivity-guided isolation methodology, triterpenoids and flavonoids were isolated from the methanolic extract of *L. octovalvis* (Jacq.) P.H.Ravens. The compounds were then tested for bacteriostatic effect on *H. pylori.* The plant's aerial parts (1,000 g) were percolated with methanol, yielding the crude extract (110.1 g). The crude extract was then fractioned into *n*-hexane (39.6 g), ethyl acetate (15.6 g), and *n*-butanol fraction (8.2 g). Bioactivity-guided isolation was then performed following bacteriostatic capacity on *H. pylori*, where the activity of fractions and subsequently isolated compounds were assessed by broth microdilution assay (obtaining MIC value). Potential fractions were selected for isolation of phytoconstituents by utilization of column chromatography. The compounds' structures were determined by analysis of their 1D-, 2D-NMR, and MS data. The compounds' activity was also tested. The ethyl acetate and *n*-hexane fractions revealed notable activity, with MIC < 6.25 mg/mL. Luteolin **(1)** - 20 mg was obtained from the EtOAc fraction. Five known triterpenoids were isolated from the *n*-hexane fraction, including ursolic acid **(2)** – 22 mg, oleanolic acid **(3)** – 19 mg, lupeyl myristate **(4)** – 18 mg, urs-12-ene-2α,3β,7β,16α-tetraol **(5)** – 33 mg, and daucosterol **(6)** – 980 mg. This work reported the first identification of **4** and **5** from *L. octovalvis*, and the first full-structural NMR assignment of **4**. All compounds exhibited moderate suppression of bacteria growth, whereas **1** revealed itself the most potential candidate (MIC < 0.25 mg/mL). These results suggested that luteolin could be a potential suppressor of *H. pylori* from *L. octovalvis*, whereas triterpenoids could assist in the bioactivity. This work could partially provide reasonable implementation for the use of *L. octovalvis* in folk medicine.

KEYWORDS: *Ludwigia octovalvis*; *Helicobacter pylori*; ursolic acid; oleanolic acid; lupeyl myristate; luteolin.

1. INTRODUCTION

Ludwigia species, or primrose-willows, belong to the Onagraceae family[1]. According to some Oriental medicine systems, important parts of the plant used for medicinal purposes are leaves and stems[1]. In Vietnamese folk medicine, *Ludwigia* species are used in the management of peptic ulcers caused by *Helicobacter pylori*[1]. Reasonable evidence for this tendency of folk use could be found through several research employing modern pharmacological models for such goals[2,3]. Recent works

have reported promising pharmacological impacts of these plants on stomachache or gastric ulcers, in which a hydroalcoholic leaf extract *L. octovalvis* showed significant reduction in mucosa lesion surface area and gastric content acidity in rats ($p < 0.05$)[3], or a methanolic extracts of *L. repens* showed potential inhibitory effect on *H. pylori* (MIC < 125 µg/mL)[2]. Phytochemical investigation is always required to be done simultaneously with bioactivity assays, yet the phytoconstituents accounting for bacteriostatic effect on *H. pylori* in *Ludwigia* species have not been clearly reported. To search for evidence of the plant's folk use, this work investigated in the bacteriostatic effect on *H. pylori* of extracts and related phytoconstituents from *L. octovalvis* (Jacq.) P.H.Ravens.

2. MATERIALS AND METHODS

2.1. Materials

A strain of *Helicobacter pylori* (ATCC 02649) was supplied by Nam Khoa Inc. (Vietnam). Media for bacterial culture were BacterPlate™ HP VTCN 05012 (blood agar containing X factor - hemin, V factor - nicotinamide adenine dinucleotide - NAD and antibiotics 10 µg/mL vancomycin, 5 µg/mL trimethoprim, 5 µg/mL colistin, 2.5 U/mL nystatin), Mueller Hinton Fastidious Broth 0132 (MHFB) which contains 10% horse blood and 20 mg/L NAD. The medium was purchased from LABone Scientific Co.Ltd. (Vietnam). Solvents used for extraction and isolation were purchased from Fisher Scientific Inc. (USA). Silica gel, RP-C18 silica gel, Sephadex® LH-20, and silica gel G60 immersed on aluminum sheets for thin layer chromatography were purchased from Merck® (Germany).

2.2. Plant Materials

The aerial parts of *L. octovalvis* were collected in the southwest of Vietnam in May 2021. Authentication was performed and determination of the precise plant was concluded by Dr. Nguyen Thanh Triet at Faculty of Traditional Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, where a voucher specimen (LOVL230521) was stored.

2.3. General Procedures

For incubation of bacteria, a tri-gas incubator containing 10% CO_2, 5% O_2, and 85% N_2 (ESCO, Singapore) was employed and operated at 37 ^oC.

1D- and 2D-NMR experiments were recorded on a Bruker Avance NEO 600 (Germany) operating at 600.04 MHz (^1H) and 150.81 MHz (^{13}C). NMR solvents were either $CDCl_3$, CD_3OD or DMSO-d_6 with 0.03% tetramethylsilan (TMS) used as internal standard. For low resolution LC-electrospray ionization (ESI)-MS, the HPLC system Acquity QDA, Waters (USA) was coupled with Sciex X500® quadrupole module.

2.4. Bacteria Growth Condition

The new bacteria were cultured in HP VTCN plates, then incubated in 72 hours. Approximately 5 – 10 new-formed colonies were suspended into 0.5 mL of MHFB medium and this stock suspension would be stored at -80 ^oC. For every single test, the stock suspension was diluted with NaCl 0,9% to obtain a new suspension having optical density of 0.05 to 0.08 (measured at 625 nm). This absorbance would be equal to that of McFarland 0.5 suspension and the new suspension contained 10^7 colony forming units (CFU)/mL. Each testing cell would contain a final concentration of 10^6 CFU/mL.

2.5. Determination of Minimum Inhibitory Concentration (MIC)

Independently, the extracts and pure compounds were totally dissolved in DMSO to obtain stock solutions with concentration of 50 g/mL. The stock solutions were diluted with MHFB to obtain a series of twofold diluted solutions. Broth microdilution method was used in MIC assay according to

the method of Xue Shen *et al*[4]. 50 µL serial twofold dilutions of extracts in BHI broth and 50 µL inoculum (final concentration ~10^6 CFU/mL) were added in the wells of 96-well microplates. Amoxicillin was used as positive control. The culture medium MHFB served as negative control. Plates were incubated in a microaerophilic atmosphere. MIC was expressed as the lowest concentration which inhibited *H. pylori* growth and judged by lack of turbidity in the well. The experiment was repeated three times.

2.6. Extraction, Isolation and Structural Determination of Phytoconstituents

Milled parts of *L. octovalvis* were percolated with methanol, following the solid-liquid ratio of 1:15. The solution was concentrated *in vacuo*, yielding the crude extract (110.1 g). The initial separation was performed by means of liquid-liquid partition method, chronologically employing organic solvents with increasing polarities: *n*-hexane, ethyl acetate, and *n*-butanol. Following the outcome of bioactivity assessment, potential fractions were selected for isolation by means of column chromatography and separating process was monitored by employing thin layer chromatography. The pure compounds' structures were determined by analysis of their 1D-, 2D-NMR, and MS data, and by comparison to published literature.

3. RESULTS AND DISCUSSION

3.1. Extraction

From 1,000 g milled plant, performance of percolation and liquid-liquid partition resulted in the yielding of crude extract (110.1 g) and 3 fractions of *n*-hexane (39.6 g), ethyl acetate (15.6 g), and *n*-butanol (8.2 g), respectively. The extraction yields suggested that the *n*-hexane and ethyl acetate fractions contained all most phytoconstituents.

3.2. Determination of MIC of Extracts and Compounds

Through the bioactivity assessment, the MIC values of extracts and compounds were summarized and reported in Table 1 and Table 2.

Table 1. MIC (mg/mL) values of extracts

Extract	MIC (mg/mL)
Crude extract	12.5
***n*-hexane fraction**	6.25
Ethyl acetate fraction	6.25
***n*-butanol fraction**	50

Table 2. MIC (mg/mL) values of compounds

Compounds	MIC (mg/mL)
Compound 1	0.25
Compound 2	100
Compound 3	100
Compound 4	200
Compound 5	200
Compound 6	> 200

Following results in table 1, the ethyl acetate and *n*-hexane fractions revealed notable activity, with MIC < 6.25 mg/mL. Hence, they are selected for phytochemical investigations.

3.3. Isolation

The ethyl acetate fraction (80,5 g) was subjected to silica gel column chromatography (CC) using stepwise gradient of $CHCl_3$ and MeOH (100:0 to 0:100, v/v) as mobile phase to obtain 10 subfractions (E.1 to E.10). Subfraction E.4 was purified by using reversed phase silica gel (RP C18) employing a mixture of MeOH-H_2O (60:40, v/v) for isocratic elution, consequently yielding compound **1** (20 mg).

A part of the *n*-hexane fraction (22.5 g) was subjected to silica gel CC, using stepwise gradient of *n*-hexane and EtOAc (100:0 to 0:100, v/v) and then EtOAc – MeOH (98:2, v/v) as mobile phase to obtain 10 subfractions (H.1 to H.10). Solid pattern (55 mg) aggregating from H.6 (419 mg) was subjected to Sephadex® LH-20 CC using chloroform-methanol (80:20, v/v) as mobile phase to yield compound **2** (22 mg) and compound **3** (19 mg). Subfraction H.4 was dissolved in chloroform and cooled down (5 ^{o}C) to allow crystallization, and compound **4** (18 mg) was obtained by filtrating and rinsing the crystal with acetone. Likewise, crystallization of subfraction H-10 from its methanol solution led to yielding of compound **6** (960 mg). Subfraction H-9 was purified by means of silica gel CC, using stepwise gradient of $CHCl_3$ and MeOH (100:0 to 95:5, v/v), resulting in the attainment of compound **5** (33 mg).

3.4. Analysis of Spectroscopic Data and Structure Determination

Luteolin (1): yellow crystals. **ESI-MS$^+$** *m/z* 287,3 ([M+H]$^+$, intensity 100%).**^{13}C-NMR (DMSO-d_6, 150 MHz)** δ_C 163.9 (C-2), 102.9 (C-3), 181.6 (C-4), 161.5 (C-5), 98.8 (C-6), 164.1 (C-7), 93.8 (C-8), 156.9 (C-9), 104.7 (C-10), 121.5 (C-1'), 113.4 (C-2'), 145.7 (C-3'), 149.7 (C-4'), 116.0 (C-5'), 118.9 (C-6'). **^{1}H-NMR (DMSO-d_6, 600 MHz)** δ_H 12.98 (1H, *s*, OH-12), 6.42 (1H, *s*, H-3), 5.95 (1H, *d*, *J* = 2.0 Hz, H-6), 6.21 (1H, *d*, *J* = 2.0 Hz, H-8), 7.27 (1H, *d*, *J* = 2.4 Hz, H-2'), 6.71 (1H, *d*, *J* = 8.4 Hz, H-5'), 7.29 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-6'). The data was compatible with the work of Lee *et al*[5].

Ursolic acid (2): amorphous, white powder. **ESI-MS$^+$** *m/z* 457,4 ([M+H]$^+$, intensity 2,3%). **^{13}C-NMR ($CDCl_3$, 150MHz)** δ_C 38.7 (C-1), 27.2 (C-2), 78.9 (C-3), 38.7 (C-4), 55.3 (C-5), 18.2 (C-6), 33.0 (C-7), 39.5 (C-8), 47.6 (C-9), 37.0 (C-10), 23.2 (C-11), 125.9 (C-12), 137.9 (C-13), 42.0 (C-14), 28.0 (C-15), 24.2 (C-16), 47.9 (C-17), 52.8 (C-18), 39.0 (C-19), 38.8 (C-20), 30.5 (C-21), 36.6 (C-22), 28.0 (C-23), 15.4 (C-24), 15.3 (C-25), 17.0 (C-26), 23.4 (C-27), 179.9 (C-28), 16.8 (C-29), 20.9 (C-30). **^{1}H-NMR ($CDCl_3$, 600 MHz)** δ_H 1.65 (1H, *m*, Hα-1/Hβ-1), 1.03 (1H, *m*, Hβ-1/Hα-1), 1.61 (1H, *m*, Hα-2/Hβ-2), 1.56 (1H, *m*, Hβ-2/Hα-2), 3.22 (1H, *dd*, *J* = 12.0, 5.1 Hz, H-3), 0.75 (1H, *m*, H-5), 1.36 (1H, *m*, Hα-6/Hβ-6), 1.53 (1H, *m*, Hβ-6/Hα-6), 0.49 (1H, *m*, Hα-7/Hβ-7), 1.39 (1H, *m*, Hβ-7/Hα-7), 1.54 (1H, *m*, H-9), 1.94 (2H, *m*, H-11), 5.28 (1H, *t*, *J* = 3.7 Hz, H-12), 1.13 (1H, *m*, Hα-15), 1,89 (1H, *dd*, *J* = 13.9, 4.5 Hz, Hβ-15), 1.68 (1H, *m*, Hα-16), 2.04 (1H, *dt*, 13.4, 4.5 Hz, Hβ-16), 2.22 (1H, *d*, *J* = 11.2 Hz, H-18), 1.33 (1H, *m*, H-19), 0.96 (1H, *m*, H-20), 1.71 (1H, *m*, Hα-21/Hβ-21), 1.51 (1H, *m*, Hβ-21/Hα-21), 1.76 (1H, *dt*, *J* = 13.1, 3.7 Hz, Hα-22), 1.68 (1H, *m*, Hβ-22), 1.01 (3H, *s*, H-23), 0.80 (3H, *s*, H-24), 0.95 (3H, *s*, H-25), 0.83 (3H, *s*, H-26), 1.11 (3H, *s*, H-27), 0.88 (3H, *d*, *J* = 6,4 Hz, H-29), 0.96 (3H, *d*, *J* = 6,4 Hz, H-30). The data was compatible with the work of Dais *et al*[6].

Oleanolic acid (3): amorphous, white powder. **ESI-MS$^+$** *m/z* 457,2 ([M+H]$^+$, intensity 3,1%). **^{13}C-NMR ($CDCl_3$, 150 MHz)** δ_C 38.4 (C-1), 27.2 (C-2), 79.0 (C-3), 38.7 (C-4), 55.2 (C-5), 18.3 (C-6), 32.6 (C-7), 39.3 (C-8), 47.6 (C-9), 37.1 (C-10), 23.4 (C-11), 122.6 (C-12), 143.6 (C-13), 41.6 (C-14), 27.7 (C-15), 23.0 (C-16), 46.5 (C-17), 41.0 (C-18), 45.9 (C-19), 30.6 (C-20), 33.8 (C-21), 32.4 (C-22), 28.1 (C-23), 15.5 (C-24), 15.3 (C-25), 17.1 (C-26), 25.9 (C-27), 182.2 (C-28), 33.0 (C-29), 23.6 (C-30). **^{1}H-NMR ($CDCl_3$, 600 MHz)** δ_H 1.60 (1H, *m*, Hα-1/Hβ-1), 0.97 (1H, *m*, Hβ-1/Hα-1), 1.62 (1H, *m*, Hα-2/Hβ-2), 1.56 (1H, *m*, Hβ-2/Hα-2), 3.22 (1H, *dd*, *J* = 11.4, 4.3 Hz, H-3), 0.74 (1H, *m*, H-5), 1.56 (1H, *m*, Hα-6/Hβ-6), 1.36 (1H, *m*, Hβ-6/Hα-6), 1.78 (1H, *dd*, *J* = 13.9, 4.4 Hz, Hα-7), 1.44 (1H, *m*, Hβ-7), 1.54 (1H, *m*, H-9), 1.99 (1H, *dd*, *J* = 12.4, 7.6 Hz, Hα-11), 1.89 (1H, *dd*, *J* = 12.4, 3.9 Hz, Hβ-11), 5.28 (1H, *dd*, *J* = 7.6, 3.9 Hz, H-12), 1.72 (1H, *m*, Hα-15), 1.08 (1H, *dd*, *J* = 13.7, 3.9 Hz, Hβ-15), 1.88 (1H, *m*, Hα-16), 1.99 (1H, *td*, *J* = 13.7, 3.9 Hz, Hβ-16), 2.82 (1H, *dd*, *J* = 13.7, 4.6 Hz, H-18), 1.62 (1H, *m*, Hα-19), 1,16 (1H, *dd*, *J* = 4.6, 2.1 Hz, Hβ-19), 1.40 (1H, *m*, Hα-21/Hβ-21), 1.21 (1H, *m*, Hβ-21/Hα-21), 1.78 (1H, *m*, Hα-21/Hβ-21), 1.59 (1H, *m*, Hβ-22/Hα-22), 0.99 (3H, *s*, H-23), 0.76 (3H, *s*, H-24), 0.90 (3H, *s*, H-25), 0.78 (3H, *s*, H-26), 1.14 (3H, *s*, H-27), 0.91 (3H, *s*, H-29), 0.93 (3H, *s*, H-30). The data was compatible with the work of Dais *et al*[6].

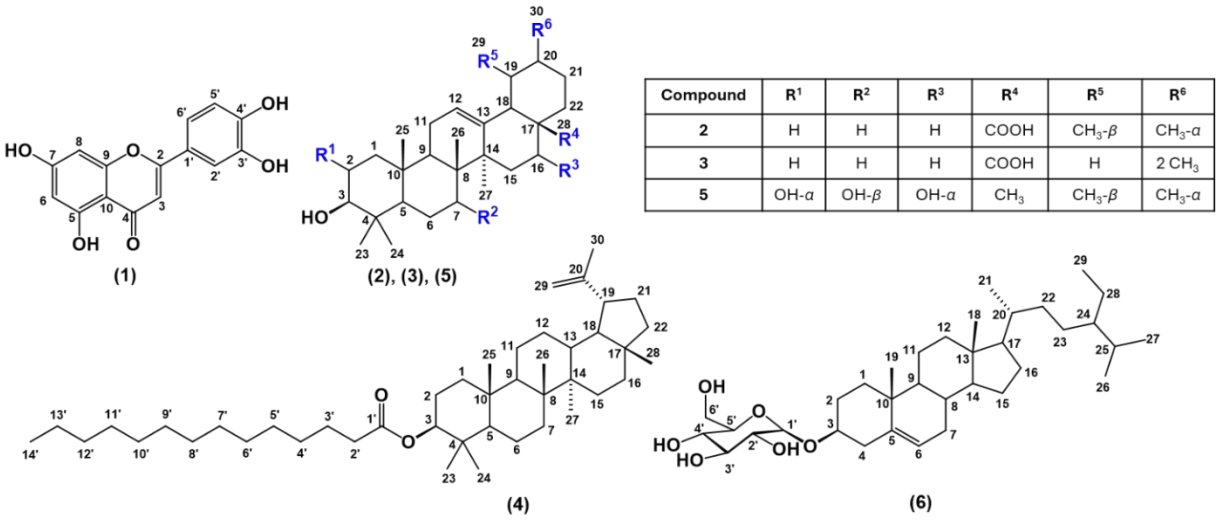
Lupeyl myristate (4): white crystals. **ESI-MS$^+$** *m/z* 430,3 ([M - $^{\bullet}C_{14}H_{28}O_2$ + Na]$^+$, intensity 5%).**^{13}C-NMR ($CDCl_3$, 150 MHz)** δ_C 38.4 (C-1), 23.7 (C-2), 80.6 (C-3), 37.8 (C-4), 55.4 (C-5), 18.2 (C-6), 34.2 (C-7), 40.9 (C-8), 50.3 (C-9), 37.1 (C-10), 20.9 (C-11), 25.1 (C-12), 38.1 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 43.0 (C-17), 48.3 (C-18), 48.0 (C-19), 150.9 (C-20), 29.8 (C-21), 40.0 (C-22), 28.0 (C-23), 16.6 (C-24), 16.1 (C-25), 16.0 (C-26), 14.5 (C-27), 18.0 (C-28), 109.3 (C-29), 19.3 (C-30), 173.7 (C-1'), 34.8 (C-2'), 25.2 (C-3'), 29.6 (C-4'), 29.7 (C-5'), 29.7 (C-6'), 29.6 (C-7'), 29.6 (C-

8'), 29.6 (C-9'), 29.2 (C-10'), 29.2 (C-11'), 31.9 (C-12'), 22.7 (C-13'), 14.1 (C-14'). **^{1}H NMR ($CDCl_3$, 600 MHz)** δ_H 1.00 (1H, *m*, H*α*-1/H*β*-1), 1.62 (1H, *m*, H*β*-1/H*α*-1), 1.26 (2H, *m*, H-2), 4.47 (1H, *dd*, *J* = 11.1, 5.3 Hz, H-3), 0.80 (1H, *m*, H-5), 1.47 (1H, *dd*, *J* = 12.6, 4.6 Hz, H*α*-6), 1.50 (1H, *m*, H*β*-6), 1.38 (2H, *m*, H-7), 1.30 (1H, *m*, H-9), 1.08 (1H, *td*, *J* = 12.9, 4.7, H*α*-11), 1.30 (1H, *m*, H*β*-11), 1.67 (2H, *m*, H-12), 1.30 (1H, *m*, H-13), 1.92 (1H, *dtd*, *J* = 13.8, 10.4, 8.3 Hz, H*α*-15), 1.59 (1H, *m*, H*β*-15), 1.38 (2H, *m*, H-16), 1.26 (1H, *m*, H-18), 2.37 (1H, *td*, *J* = 11.1, 5.8 Hz, H-19), 2.28 (2H, *m*, H-21), 1.38 (1H, *m*, H*α*-22/H*β*-22), 1,19 (1H, *m*, H*β*-22/H*α*-22), 1.03 (3H, *s*, H-23), 0.86 (3H, *s*, H-24), 0.84 (3H, *s*, H-25), 0.84 (3H, *s*, H-26), 0.94 (3H, *s*, H-27), 0.79 (3H, *s*, H-28), 4.57 (1H, *dd*, *J* = 2.3, 1.4 Hz, H-29), 4.69 (1H, *d*, *J* = 2.3 Hz, H-29), 1.68 (3H, *s*, H-30), 1.27 (2H, *d*, *J* = 7,3 Hz, H-2'), 1.25 (2H, *m*, H-3' → H-13'), 0.88 (3H, *t*, *J* = 7.0 Hz, H-14').The data was compatible with the work of Silva *et al*[7].

Urs-12-ene-2*α*,3*β*,7*β*,16*α*-tetraol (5): white crystals. **ESI-MS$^+$** *m/z* 495,2 ([M - H_2O + K]$^+$, intensity 33%).**^{13}C-NMR (CD_3OD, 150 MHz)** δ_C 49.5 (C-1), 69.5 (C-2), 84.5 (C-3), 40.4 (C-4), 56.7 (C-5), 29.2 (C-6), 73.6 (C-7), 48.2 (C-8), 49.2 (C-9), 38.1 (C-10), 24.8 (C-11), 129.3 (C-12), 140.1 (C-13), 43.1 (C-14), 33.9 (C-15), 72.2 (C-16), 40.6 (C-17), 56.8 (C-18), 40.4 (C-19), 40.5 (C-20), 31.6 (C-21), 38.1 (C-22), 29.3 (C-23), 17.2 (C-24), 17.5 (C-25), 19.5 (C-26), 24.1 (C-27), 19.5 (C-28), 17.8 (C-29), 21.5 (C-30). **^{1}H-NMR (CD_3OD, 600 MHz)** δ_H 1.96 (1H, *m*, H*α*-1/H*β*-1), 1.00 (1H, *m*, H*β*-1/H*α*-1), 3.65 (1H, *m*, H-2), 2.92 (1H, *d*, *J* = 9.1 Hz, H-3), 0.82 (1H, *m*, H-5), 1.61 (1H, *m*, H*α*-6/H*β*-6), 1.40 (1H, *m*, H*β*-6/H*α*-6), 3.53 (1H, *dd*, *J* = 11.2, 4.5 Hz, H-7), 1.46 (1H, *m*, H-9), 1.97 (2H, *m*, H-11), 5.23 (1H, *t*, *J* = 3.7 Hz, H-12), 1.64 (1H, *m*, H*α*-15/H*β*-15), 1.35 (1H, *m*, H*β*-15/H*α*-15), 3,57 (1H, *dd*, *J* = 11.4; 4.4 Hz, H-16), 2.19 (1H, *d*, *J* = 8.0 Hz, H-18), 1.44 (1H, *m*, H-19), 0.92 (1H, *m*, H-20), 1.52 (1H, *m*, H*α*-21/H*β*-21), 1.28 (1H, *m*, H*β*-21/H*α*-21), 1.61 (1H, *m*, H*α*-22/H*β*-22), 1.32 (1H, *m*, H*β*-22/H*α*-22), 1.02 (3H, *s*, H-23), 0.81 (3H, *s*, H-24), 1.01 (3H, *s*, H-25), 1.11 (3H, *s*, H-26), 1.91 (3H, *s*, H-27), 1.14 (3H, *s*, H-28), 0.86 (3H, *d*, *J* = 6.4 Hz, H-29), 0.95 (3H, *d*, *J* = 6.4 Hz, H-30). The data was compatible with the work of Tung *et al*[8].

Daucosterol (6): white crystals. **ESI-MS$^+$** *m/z* 409,0 ([M - $^{\bullet}C_5H_{12}O_6$ + H]$^+$, intensity 4%). **^{13}C-NMR (DMSO-*d*$_6$, 150 MHz)** δ_C 36.7 (C-1), 29.2 (C-2), 76.8 (C-3), 38.2 (C-4), 140.4 (C-5), 121.1 (C-6), 31.3 (C-7), 31.3 (C-8), 49.5 (C-9), 36.7 (C-10), 20.5 (C-11), 40.0 (C-12), 41.8 (C-13), 56.1 (C-14), 23.8 (C-15), 27.7 (C-16), 55.3 (C-17), 11.6 (C-18), 19.0 (C-19), 36.1 (C-20), 18.5 (C-21), 33.3 (C-22), 25.4 (C-23), 45.1 (C-24), 28.6 (C-25), 19.6 (C-26), 18.8 (C-27), 22.5 (C-28), 11.7 (C-29), 100.7 (C-1'), 73.4 (C-2'), 76.7 (C-3'), 70.0 (C-4'), 76.6 (C-5'), 61.0 (C-6'). **^{1}H-NMR (DMSO-*d*$_6$, 600 MHz)** δ_H 1.81 (1H, *m*, H*α*-1/H*β*-1), 1.01 (1H, *m*, H*β*-1/H*α*-1), 1.78 (1H, *m*, H*α*-2/H*β*-2), 1.48 (1H, *m*, H*β*-2/H*α*-2), 3.46 (1H, *tt*, *J* = 11.5, 4.6 Hz, H-3), 2.36 (1H, *m*, H*α*-4/H*β*-4), 2.14 (1H, *m*, H*β*-4/H*α*-4), 5.33 (1H, *dt*, *J* = 4.9, 2.5 Hz, H-6), 1.53 (1H, *m*, H*α*-7), 1.92 (1H, *ddt*, *J* = 13.1, 4.9, 2.5 Hz, H*β*-7), 1.41 (1H, *m*, H-8), 0.88 (1H, *m*, H-9), 1.51 (1H, *m*, H*α*-11/H*β*-11), 1.39 (1H, *m*, H*β*-11/H*α*-11), 1.96 (1H, *m*, H*α*-12/H*β*-12), 1.39 (1H, *m*, H*β*-12/H*α*-12), 0.98 (1H, *m*, H-14), 1.63 (1H, *dd*, *J* = 6.9, 4.8 Hz, H*α*-15), 1.16 (1H, *m*, H*β*-15), 1.83 (1H, *m*, H*α*-16), 1.20 (1H, *q*, *J* = 7.0 Hz, H*β*-16), 1.17 (1H, *d*, *J* = 7.0 Hz, H-17), 0.66 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 1.35 (1H, *m*, H-20), 0.91 (3H, *d*, *J* = 6.6 Hz, H-21), 1.27 (2H, *m*, H-22), 1.54 (2H, *m*, H-23), 0.77 (1H, *m*, H-25), 0.81 (3H, *d*, *J* = 6.9 Hz, H-26), 0.82 (3H, *d*, *J* = 6.9 Hz, H-27), 0.80 (2H, *m*, H-28), 0.84 (3H, *t*, *J* = 7.5 Hz, H-29), 4.22 (1H, *d*, *J* = 7.8 Hz, H-1'), 2.90 (1H, *ddd*, *J* = 8.9, 7.8, 3.6 Hz, H-2'), 3.13 (1H, *td*, *J* = 8.9, 4.8 Hz, H-3'), 3.03 (1H, *ddd*, *J* = 9.7, 8.9, 4.0 Hz, H-4'), 3.07 (1H, *ddd*, *J* = 9.7, 5.9, 2.1 Hz, H-5'), 3.65 (1H, *ddd*, 11.7, 5.9, 2.1 Hz, H-6'), 3.41 (1H, *td*, 11.7, 5.9 Hz, H-6'), 4.84 (1H, *d*, *J* = 3.6 Hz, OH-2'), 4.85 (1H, *d*, *J* = 4.8 Hz, OH-3'), 4.83 (1H, *d*, *J* = 4.0 Hz, OH-4'), 4.40 (1H, *t*, *J* = 5.9 Hz, OH-6'). The data was compatible with the work of Nitiema *et al*[9].

The structures of isolated compounds **1** to **6** were provided in Figure 1. To the best of our findings, this work reported the first identification of **4** and **5** from *L. octovalvis*, and the first full-structural NMR assignment of **4**.



Compound	R^1	R^2	R^3	R^4	R^5	R^6
2	H	H	H	COOH	CH_3-β	CH_3-α
3	H	H	H	COOH	H	2 CH_3
5	OH-α	OH-β	OH-α	CH_3	CH_3-β	CH_3-α

Figure 1. Structures of isolated compounds **1** to **6**

3.5. Bacteriostatic Effect on *Helicobacter pylori* of Isolated Triterpenoids and Flavonoids

According to results in Table 2, this work reported that all compounds exhibited moderate suppression of bacteria growth, whereas **1** revealed itself the most potential candidate (MIC < 0.25 mg/mL).

4. CONCLUSION

These results suggested that luteolin could be a potential suppressor of *H. pylori* from *L. octovalvis*, whereas triterpenoids could assist in the bioactivity. This work could partially provide reasonable implementation for the use of *L. octovalvis* in folk medicine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Investigation of the Antioxidant and Anti-Cancer Potential of *Leea indica* Leaf Extracts from Brunei Darussalam

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ABSTRACT

Conventional cancer treatment modalities are often marred by adverse side effects. Future prospects point towards using medicinal plants that are rich in phytochemicals. Previous studies have indicated that the leaves of a medicinal plant, *Leea indica*, exhibit various biological properties, including antioxidant activity and cytotoxicity in cancer cell lines. In this study, we investigate the phytochemical constituents and antioxidant activity of local *L. indica* leaf extracts and tested their cytotoxic effects against human cancer cell lines. Fresh and healthy *L. indica* leaves from Brunei Darussalam were harvested and extracted via microwave-assisted extraction using three different solvents, namely water, 50% ethanol, and 100% ethanol. The phenolic and flavonoid contents of these extracts were determined and compared using Folin-Ciocalteu and aluminium chloride colorimetric assays, respectively. The antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Cytotoxicity against two different cancer cell lines, A549 (lung) and MCF-7 (breast), at four different concentrations (5, 10, 50, and 100 µg/mL) was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Of the three tested *L. indica* leaf extracts, the 50% ethanolic extract contained the highest phenolic and flavonoid contents at 515.2 ± 121.3 mg gallic acid equivalent/g and 16.7 ± 0.8 mg quercetin equivalent/g dry sample, respectively. Both 50% and 100% ethanolic extracts demonstrated the highest antioxidant activity at 942.3 ± 10.4 and 894.2 ± 21.4 mg ascorbic acid equivalent/g dry sample, respectively. Interestingly, the water extract displayed the most potent cytotoxic effect against both A549 and MCF-7 cancer cell lines at 100 µg/mL, with IC_{50} values of 97.1 ± 54.9 µg/mL and 79.8 ± 7.1 µg/mL, respectively. Our findings suggest the presence of phytochemicals in the different *L. indica* leaf extracts with promising antioxidant properties and cytotoxic effects against cancer cell lines. Additional studies entailing comprehensive phytochemical analyses and further testing against other cell lines will further validate the potential therapeutic qualities of local *L. indica* leaves.

KEYWORDS: Cancer; Phytochemical; Antioxidant; Cytotoxicity; Plant extracts; *Leea indica*

1. INTRODUCTION

Cancer is a leading cause of morbidity and mortality globally. In 2022, the International Agency for Research on Cancer reported approximately 20 million new cancer cases and 9.7 million deaths worldwide, with lung and breast cancer being the most prevalent types[1]. Defined as a heterogenous group of diseases, cancer is characterised by the aberrant behaviour of transformed cells, infringing upon the normal cell division mechanisms via uncontrolled proliferation and exponential growth[2]. Notably, cancer cells possess the capacity to metastasise, an ability that exists due to transformation phenomenon[3]. These may include genetic mutations in predominantly proto-oncogenes and tumor

suppressor genes, which can be induced by oxidative stress, evasion of apoptosis which authorise the survival of cancer cells despite being old or damaged, and achievement of cellular immortality by bypassing the Hayflick limit[4,5].

The diverse genetic and molecular nature of this disease cause it to respond variably to treatments, necessitating a broad range of therapeutic options[6]. Current treatment modalities, such as chemotherapy and radiation, target rapidly dividing cells and affect both cancerous and non-cancerous cells. This inevitably leads to negative side effects including nausea, poor appetite, and increased vulnerability to infections. Furthermore, cancer cells can develop resistance to treatments over time, an occurrence known as acquired drug resistance, in addition to risk of cancer recurrence following cancer treatments[7]. Therefore, a continuous search for selective cancer treatment is imperative for improving survival rates, minimising side effects, and enhancing quality of life of patients.

Recently, there has been a growing interest in phytotherapy or medicinal plants, a practice of complementary and alternative medicine (CAM), highlighting its relevance in contemporary healthcare[8]. Medicinal plants have traditionally been utilised in the prevention and treatment of various diseases including cancer[910]. Phytotherapy may also complement conventional treatments, either by potentially reducing side effects of medications or enhancing drug efficacy by modulating drug metabolism pathways and reducing resistance in cancer cells, a relationship known as herb-drug synergy[11]. The different components of the plant, such as the leaves, bark, seeds, roots, fruits and flowers, have been documented to contain a plethora of phytochemicals that possess distinct therapeutic effects[12,13]. These components can be processed in various forms such as powders, decoctions, tinctures, and infusions[14]. Moreover, the benefits of these medicinal plants depend on the yield and stability of the extracted phytochemicals, which are influenced by the method of extraction, solvent used, temperature and time of extraction[13].

Phytochemicals are compounds synthesised by plants that serve various roles, for example, as defense against pests or environmental stresses[15]. These compounds include polyphenols, alkaloids, flavonoids, and saponins, which have been described to exert protective and therapeutic effects on the human body either directly, indirectly, or synergistically[13]. For instance, certain phytochemicals such as vinblastine, an alkaloid, are capable of disrupting microtubule structures of cancerous cells by binding to tubulin, thereby preventing its polymerisation and ultimately inhibiting cell proliferation[16]. The indirect action of certain phytochemicals such as flavonoids is evident by their antioxidant properties, capable of intercepting and scavenging free radicals, therefore providing protective mechanism against the damage of DNA due to oxidative stress[17]. In the context of cancer cells, antioxidants reduce reactive oxygen species (ROS) levels, which would otherwise promote a malignant phenotype and drive tumour development[18].

Among the plethora of medicinal plants being scientifically investigated for potential therapeutic benefits in accordance with its traditional beliefs, *Leea indica (L. indica)*, typically known as Bandicoot berry, or locally known as *mali-mali* or *memali* has emerged as a subject of interest. *L. indica*, a member of the Vitaceae family, manifests as a small tree or perennial shrub, ranging from 2 to 16 m in height. This plant is widely distributed and utilised in tropical regions of Asia, parts of Australia, and Southeast Asia[19]. It is characterised by its dense green compound leaves and clusters of small white or greenish flowers, which produce dark berries upon maturation[19,20]. Components of *L. indica*, predominantly in the form of decoctions has traditionally been employed for treating a variety of ailments. For example, the leaves and shoot of the plants have been reported for treating hypertension, diabetes and wound healing in Malaysia[21–23], whereas in India and Bangladesh, the roots and leaves have been utilised for treating bone fracture, hyperdipsia, diarrhoea, joint pain, ulcer, and dysentery[24–26], underscoring its significance in ethnobotanical practices.

Several studies have also noted the presence of bioactive phytochemicals in certain parts of *L. indica*, contributing towards various pharmacological properties such as antimicrobial, anti-cancer, antioxidative, and anxiolytic activities[27,28]. The methanolic and ethanolic leaf extracts of *L. indica* were shown to exhibit promising cell proliferation inhibitory effect against various cancer cell lines including liver, ovarian, breast, cervical, and colon[29]. Phytochemical analyses of fractionated *L. indica* ethyl acetate leaf extracts have indicated its rich repository of bioactive phytoconstituents, including phenolic acids, polyphenolic and flavonoids[30]. Plant phenolics have been widely studied as potential anti-cancer agents, including its antioxidant capacity, which is capable of neutralising free radicals in an attempt to

inhibit carcinogenesis. Moreover, phenolics can modulate cell signaling pathways and regulate signals that determine the promotion of cancer cell growth and apoptosis[31].

To our knowledge, there has been no reported studies investigating the biological or pharmacological activities of *L. indica* leaves from Brunei Darussalam. Thus, this study aimed to evaluate the phytochemical constituents and antioxidant activity of local *L. indica* leaf extracts. The leaf extracts were also subjected to cytotoxicity tests against two human cancer cell lines, namely lung (A549) and breast (MCF-7) cancer cells.

2. MATERIALS AND METHODS

2.1. Plant Source

Fresh and healthy *L. indica* leaves were procured from Bukit Ladan Forest Reserve, Tutong, Brunei Darussalam in December 2023. Voucher specimen of the plant (Ubrc2024-0013) was deposited at the Universiti Brunei Darussalam (UBD) herbarium.

2.2. Preparation of Plant Extracts

Leaf samples were washed with tap water, blotted dry, and oven-dried at 35°C for 2 days. Dried leaves were then ground into powdered form and subjected to microwave-assisted extraction (MAE) using a domestic microwave oven (Toshiba, Japan). Three different solvents comprising distilled water, 50% ethanol and 100% ethanol were utilised for phytochemical extraction. For each solvent, a total of 30 g of powdered leaf samples were immersed in the specified solvents at a sample: solvent ratio of 1:10 (w/v). MAE was conducted at 450 W in 15-second intervals for a total of 5 minutes whilst maintaining a desired temperature of approximately 50°C. The water crude extract was lyophilised using a freeze-dryer (Labconco Corporation, USA), whereas the 100% ethanolic crude extract was filtered and concentrated using a rotary evaporator (IKA RV10 with HB10 Bath, IKA Works, Inc., USA). The 50% ethanolic crude extract was subjected to rotary evaporation followed by freeze-drying. The prepared extracts were used for phytochemical, antioxidant and cytotoxicity screening.

2.3. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the leaf extracts was determined using the Folin-Ciocalteu colorimetric assay as previously described with minor modifications[28]. Briefly, 20 µL of leaf extract (1 mg/mL) or standard (10-100 µg/mL gallic acid) dissolved in dimethyl sulfoxide (DMSO) was mixed with 100 µL of diluted Folin-Ciocalteu reagent (10% (v/v) in distilled water) and 80 µL of sodium carbonate solution (7.5% (w/v) in distilled water). The mixture was incubated for 30 minutes at room temperature in a 96-well plate. The absorbance values were then read at 765 nm using a spectrophotometer (EPOCH 2 Microplate Spectrophotometer, BioTek, USA). The TPC of the leaf extracts were expressed as mg gallic acid equivalents (GAE)/g of dry sample and estimated from standard gallic acid calibration curve: $C = (c \times V)/m$, where C = total phenolic content (mg GAE/g of dry sample), c = concentration of gallic acid obtained from the calibration curve (mg/mL), V = volume of sample used (mL), and m = mass of leaf extract tested (g). All analyses were performed in triplicates.

2.4. Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the leaf extracts was determined using the aluminium chloride colorimetric assay as previously described with minor modifications[28]. Briefly, 20 µL of leaf extract (2 mg/mL) or standard (10-100 µg/mL quercetin) dissolved in DMSO was mixed with 60 µL methanol, 4 µL 10% aluminium chloride, 4 µL 1M potassium acetate and 112 µL distilled water. The mixture was incubated for 30 minutes at room temperature in a 96-well plate. The absorbance values were then read at 415 nm using a spectrophotometer. The TFC of the leaf extracts were expressed as mg quercetin equivalents (QE)/g of dry sample and estimated from standard quercetin calibration curve: $C = (c \times V)/m$, where C = total flavonoid content (mg QE/g of dry sample), c = concentration of

quercetin obtained from the calibration curve (mg/mL), V = volume of sample used (mL), and m = mass of leaf extract tested (g). All analyses were performed in triplicates.

2.5. Evaluation of Antioxidant Capacity

The antioxidant capacity of the leaf extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as previously described with minor modifications[28]. Briefly, 40 µL of leaf extract (100 µg/mL) or standard (10-100 µg/mL ascorbic acid) diluted in DMSO was mixed with 140 µL of 200 µM DPPH dissolved in methanol. The mixture was incubated for 30 minutes at room temperature in a 96-well plate. The absorbance values were then read at 517 nm using a spectrophotometer. The antioxidant capacity of the leaf extracts were expressed as mg ascorbic acid equivalents (AAE)/g of dry sample and estimated from standard ascorbic acid calibration curve: A = (c x V)/m, where A = antioxidant capacity (mg AAE/g of dry sample), c = concentration of ascorbic acid obtained from the calibration curve (mg/mL), V = volume of sample used (mL), and m = mass of leaf extract tested (g). All analyses were performed in triplicates.

2.6. General Cell Culture Methods

Two human cancer cell lines derived from lung (A549) and breast (MCF-7) were used. The cells were obtained from the American Type Culture Collection (ATCC), USA. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS; Gibco), 1% (v/v) penicillin-streptomycin (Gibco, Thermo Fisher Scientific, USA), and L-glutamine in a humidified incubator at 37°C with 5% CO_2.

2.7. Assessment of Cytotoxicity Against Cancer Cell Lines

The cytotoxic capacity of the leaf extracts was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described with minor adjustments[32]. Prior to treatment, the cell lines were seeded in a flat-bottomed 96-well microplate at a density of 0.5 X 10^4 cells/well in a volume of 100 µL of supplemented DMEM and incubated for 24 hours at 37°C with 5% CO_2. Subsequently, the media was aspirated from each well, followed by washing with 100 µL of phosphate-buffered saline (PBS) and treatment with 0.5% DMSO in serum-free DMEM as the negative/vehicle control (untreated) group, and varying concentrations of leaf extract dissolved in DMSO and diluted in serum-free DMEM (5, 10, 50, and 100 µg/mL). Cisplatin dissolved in distilled water and diluted with DMEM (10 µg/mL) was used as the positive control group. After 24 hours, the media was removed and 10 µL of MTT solution (5 mg/mL) dissolved in PBS and 90 µL of DMEM was added to each respective well and incubated for 2 hours at 37°C. Post incubation period, the mixtures in each well were aspirated and 100 µL of DMSO was then added to solubilise any formazan formed and placed on a microplate shaker for 15 minutes. The absorbance values were measured at 570 nm using a spectrophotometer. Percentage cell viability was calculated with the given formula: Cell viability (%) = (Mean absorbance of treated cells)/(Mean absorbance of untreated cells)x 100%. Cell viability of the treated group was compared to the untreated group which was normalised to 100% viability. All experiments were performed in triplicates.

2.8. Statistical Analysis

GraphPad Prism software version 10.2.2 (GraphPad software, Inc., San Diego, CA, USA) was employed for statistical analysis of data collected. Results from at least three independent experiments were expressed as mean ± standard deviation (SD). One-way ANOVA was used to evaluate the significant differences between means of the three types of leaf extracts, followed by Tukey's Honestly Significant Difference (HSD) test for the assessment of significant difference among pairs of extract type means. Two-way ANOVA was used evaluate the significant differences between means of the three types of leaf extracts and its concentrations, followed by Tukey's Honestly Significant Difference (HSD) test for the assessment of significant difference among pairs of extract type means. P values

from either ANOVA or Tukey's HSD test that were less than 0.05 were considered as statistically significant (*p < 0.05, **p < 0.01, and ***p < 0.001).

3. RESULTS AND DISCUSSION

3.1. Extraction Yield of *L. indica* Leaf Extracts

The *L. indica* leaves were extracted by MAE using three different solvents, namely water, 50% ethanol and 100% ethanol. The highest extraction yield was observed in 50% ethanolic extract at 15.6 ± 5.2%, followed by 100% ethanolic extract at 9.3 ± 1.8%, and water extract at 5.4 ± 1.2%.

3.2. Total Phenolic and Flavonoid Contents of *L. indica* Leaf Extracts

The TPC and TFC of *L. indica* leaf extracts using different solvents were found to be variable (Table 1). Both 50% and 100% ethanolic leaf extracts demonstrated similar TPC values at 515.2 ± 121.3 and 446.6 ± 87.3 mg GAE/g dry sample respectively, which were significantly higher compared to water extract at 281.6 ± 60.4 mg GAE/g dry sample. Similarly, the 50% ethanolic extract exhibited the highest TFC at 16.7 ± 0.8 mg QE/g dry sample, followed by 100% ethanolic extract at 13.8 ± 0.6 mg QE/g dry sample, and lastly water extract at 11.1 ± 1.4 mg QE/g dry sample. The findings of our study, in which the ethanolic extracts contained higher phenolic content compared to water extract, corresponded with a previously conducted study on *L. indica* leaf extracts[33].

Table 1. Total phenolic and flavonoid contents of *L. indica* leaf extracts.

Type of leaf extract	TPC (mg GAE/g dry sample)	TFC (mg QE/g dry sample)
Water	281.6 ± 60.4^{b}	11.1 ± 1.4^{c}
50% ethanol	515.2 ± 121.3^{a}	16.7 ± 0.8^{a}
100% ethanol	446.6 ± 87.3^{ab}	13.8 ± 0.6^{b}

+ TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent, QE: Quercetin equivalent. Values are expressed as mean ± standard deviation of at least three independent experiments. Means with different letters within the same column represent statistically significant differences ($p < 0.05$).

3.3. Antioxidant Capacity of *L. indica* Leaf Extracts

The antioxidant capacity of *L. indica* leaf extracts using different solvents were determined based on DPPH scavenging activity and expressed as ascorbic acid equivalents (Figure 1). Both 50% and 100% ethanolic extracts demonstrated the highest antioxidant activity at 942.3 ± 10.4 and 894.2 ± 21.4 mg AAE equivalent/g dry sample, respectively. The water extract displayed lower antioxidant capacity at 507.8 ± 61.2 mg AAE equivalent/g dry sample. This observation was similar to a previous study on *L. indica* leaf extracts that recorded higher antioxidant activity in ethanolic compared to water extract[33]. Further, the higher TPC and TFC observed in both ethanolic extracts corroborate its superior antioxidant capacity. Phenolics and flavonoids possess the capacity to donate hydrogen atoms or electrons, thus stabilising the free radicals that may cause oxidative stress and damage cellular components like DNA and proteins[34]. Indeed, phenolics such as gallic acid and methyl gallate, and flavonoids including kaempferol, quercitrin and myricitrin, have previously been identified in *L. indica* leaf extracts[30]. The presence of such phenolics and flavonoids are thought to contribute significantly to antioxidant activity[35,36]. However, further elucidation on the antioxidant capacity mechanisms of these extracts using other assays such as ferric reducing antioxidant power (FRAP) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) (ABTS) is essential to support our findings[37].

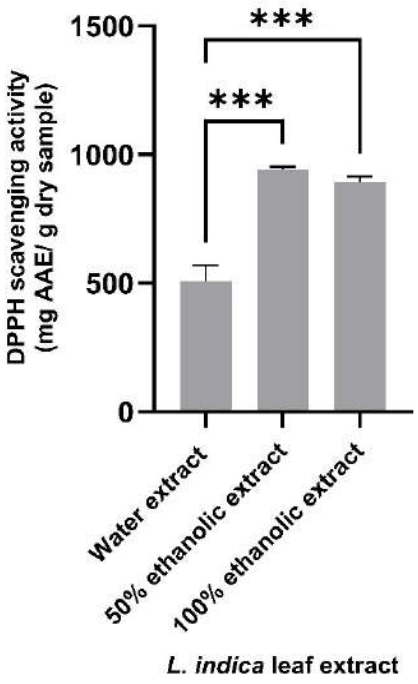
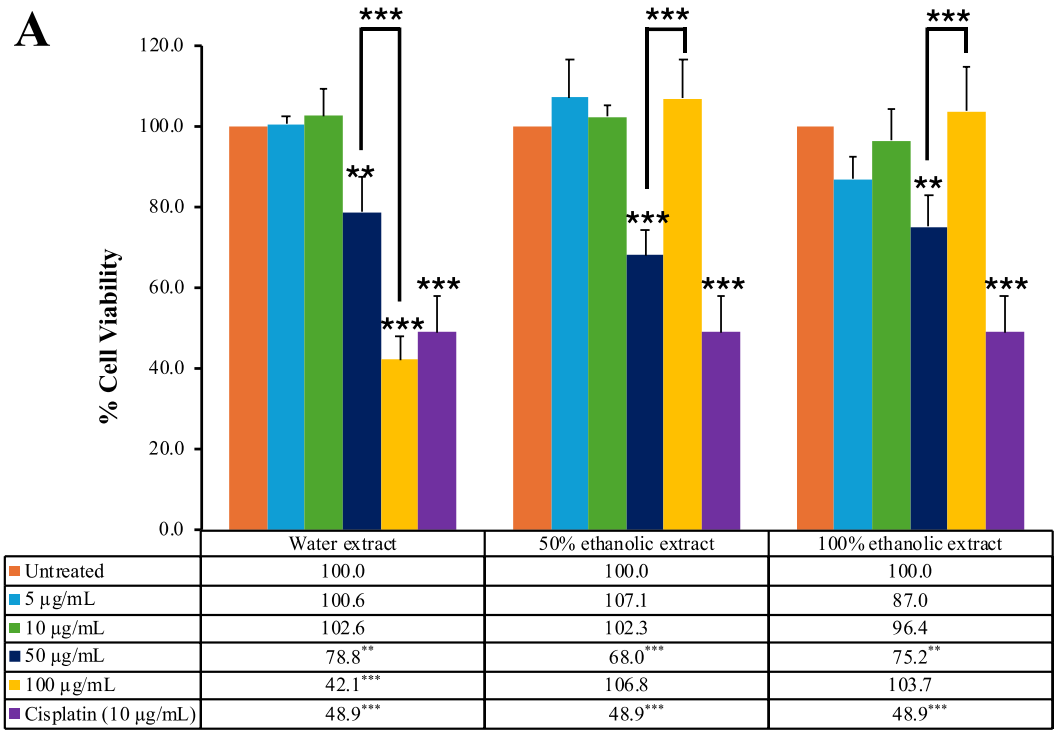


Figure 1. Antioxidant capacity of *L. indica* leaf extracts. The antioxidant capacity of the *L. indica* leaf extracts were determined based on DPPH radical scavenging activity and expressed in terms of ascorbic acid equivalent (AAE). Data are expressed as mean ± standard deviation of at least three independent experiments. The asterisk symbol (***) indicate ***$p < 0.001$.

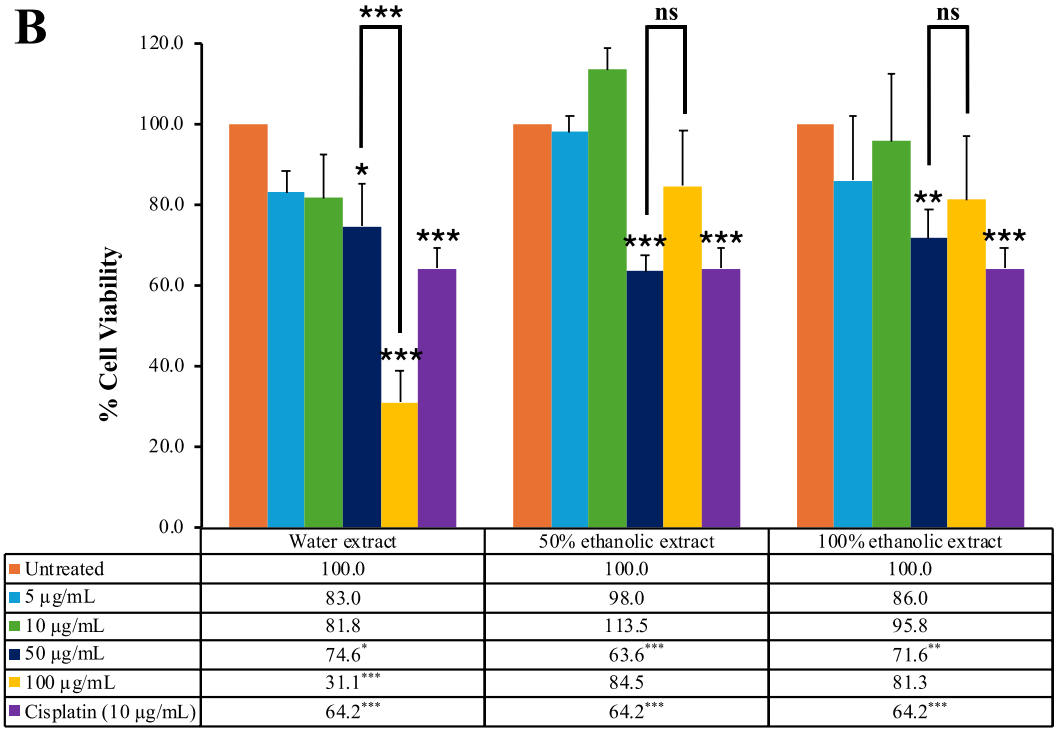
3.4. Cytotoxic Activity of *L. indica* Leaf Extracts against A549 and MCF-7 Cell Lines

Cytotoxic activity of the *L. indica* leaf extracts were tested at various concentrations against lung (A549) and breast (MCF-7) cancer cell lines at a single time point of 24 hours (Figure 2). For A549 cells, variable percentage cell viability upon treatment with water extract (100.6±2.0%, 102.6±6.5%, 78.8±8.5%** and 42.1±5.7%***), 50% ethanolic extract (107.1±9.5%, 102.3±2.7%, 68.0±6.1%*** and 106.8±9.7%) and 100% ethanolic extract (87.0±5.3%, 96.4±8.0%, 75.2±7.6%** and 103.7±11.0%) were observed at 5, 10, 50, and 100 µg/mL, respectively (Figure 2A). Similary for MCF-7 cells, percentage cell viability varied following incubation with water extract (83.0±5.1%, 81.8±10.5%, 74.6±10.4%* and 31.1±7.7%***), 50% ethanolic extract (98.0±4.1%, 113.5±5.2%, 63.6±3.8%*** and 84.5±13.9%) and 100% ethanolic extract (86.0±16.0%, 95.8±16.6%, 71.6±7.2%** and 81.3±15.7%) at 5, 10, 50, and 100 µg/mL, respectively (Fig. 2B). Cisplatin (10 µg/mL) revealed significant cytotoxicity compared to untreated group at 48.9±8.9% for A549 and 64.2 ± 5.0% for MCF-7 cells (Figure 2).

The observations and patterns of cell cytotoxicity in both A549 and MCF-7 cell lines were similar for each respective leaf extract. The water extract exhibited significant cytotoxicity at 50 and 100 µg/mL for both cancer cell lines in comparison to the negative control; with markedly higher cytotoxicity observed at 100 µg/mL compared to 50 µg/mL (Figure 2). The 50% and 100% ethanolic extracts exerted significant cytotoxicity only at 50 µg/mL concentration when compared to the untreated group (Figure 2). Interestingly, a higher percentage cell viability was observed in A549 cells upon treatment with the 50% and 100% ethanolic extracts at 100 µg/mL when compared to 50 µg/mL concentration (Figure 2A). Cell viability at 50 and 100 µg/mL were found to be similar in MCF-7 cells upon treatment with 50% or 100% ethanolic extracts, but trended towards higher cell viability with increased extract concentration (Figure 2B). Overall, the water extract exhibited the most potent cytotoxicity against the A549 and MCF-7 cell lines at 100 µg/mL, with IC_{50} values of 97.1 ± 54.9 µg/mL and 79.8 ± 7.1 µg/mL, respectively.



	Water extract	50% ethanolic extract	100% ethanolic extract
Untreated	100.0	100.0	100.0
5 µg/mL	100.6	107.1	87.0
10 µg/mL	102.6	102.3	96.4
50 µg/mL	78.8**	68.0***	75.2**
100 µg/mL	42.1***	106.8	103.7
Cisplatin (10 µg/mL)	48.9***	48.9***	48.9***



	Water extract	50% ethanolic extract	100% ethanolic extract
Untreated	100.0	100.0	100.0
5 µg/mL	83.0	98.0	86.0
10 µg/mL	81.8	113.5	95.8
50 µg/mL	74.6*	63.6***	71.6**
100 µg/mL	31.1***	84.5	81.3
Cisplatin (10 µg/mL)	64.2***	64.2***	64.2***

Figure 2. The cytotoxic effect of *L. indica* leaf extracts against A549 and MCF-7 cells. The leaf extracts were tested at 5, 10, 50, and 100 µg/mL and treated against both (A) A549 (lung) and (B) MCF-7 (breast) cancer cell lines for 24 hours. Cisplatin (10 µg/mL) was used as the positive control. Percentage cell viability was determined to evaluate the cytoxicity of these treatments. Cell viability of the treated group was compared to the untreated group (0 µg/mL) which was normalised to 100% viability. Data are expressed as mean ± standard deviation of at least three independent experiments. The asterisk symbols (*, **, and ***) indicate *p < 0.05, **p < 0.01, and ***p < 0.001, respectively; 'ns' denotes non-significant difference.

Despite demonstrating lower phenolic and flavonoid contents, the water extract of *L. indica* leaves may contain other phytochemicals that were relatively weaker in terms of antioxidant capacity yet superior in eliminating human cancer cells *in vitro*. Phytochemicals apart from phenolics and flavonoids, such as saponins, alkaloids, or terpenoids, which were not quantified in this study, have been described for their diverse pharmacological activities and may have influenced the observed cytotoxic effect[38–40]. Mechanisms for anti-cancer activity using medicinal plants includes induction of apoptosis, inhibition of cell proliferation or angiogenesis, and reprogramming of cell metabolism[41]. Mollic acid arabinoside, a cycloartane triterpenoid, was isolated from *L. indica* leaves and have been reported to induce apoptosis in Ca Ski cervical cancer cells[42]. The antioxidant property of phytochemicals, which can modulate ROS levels and cancer development, is an important aspect to consider when elucidating potential anti-cancer effects of plant extracts; however, this represents merely one potential mechanism which may affect cancer cells[17,18]. Furthermore, previous findings have indicated that plant extracts with strong antioxidant properties do not necessarily correlate with high levels of cytotoxicity against cancer cells[43]. Further studies, involving analysis of apoptotic and metabolic pathways, are required to comprehensively characterize the pathways leading to cytoxicity in cancer cells.

With regard to the reduced cytotoxicity of A549 cells when tested with 100 µg/mL compared to 50 µg/mL of both ethanolic extracts, we can only speculate a potential hormetic or biphasic dose-response[44]. Such response might be driven by the cancer cells activating stress response pathways at higher concentrations, which could lead to detoxification and a protective effect. Recently, methyl gallate, a polyphenolic compound present in *L. indica*, was reported to upregulate stress ligands in the human ovarian cancer cell line OVCAR-5[45]. Notably, further phytochemical screening and testing at concentrations higher than 100 µg/mL of the leaf extracts is necessary to further validate these observations.

4. CONCLUSION

Phytochemical extraction from medicinal plants is influenced by various factors, including the solvent and the extraction method utilised. In our study, the 50% ethanolic extract of *L. indica* leaves demonstrated the highest phenolic and flavonoid contents among the different extracts. Both 50% and 100% ethanolic extracts displayed the highest antioxidant capacity. Despite the lower TPC, TFC, and antioxidant capacity, the water leaf extract exhibited the most potent cytotoxic effect at 100 µg/mL against both A549 and MCF-7 cell lines. Overall, these findings underscore the presence of phytochemicals in the different *L. indica* leaf extracts that possess therapeutic benefits, namely antioxidant properties and cytotoxicity against cancer cell lines. Further testing is necessary for a comprehensive understanding of the potential of this plant and its extracts.

5. ACKNOWLEDGMENT

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Conflict of interest

The authors declare that they have no conflict of interest.

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Development of Microencapsulated Powder Containing *Lactobacillus acidophilus* and *Clitoria ternatea* L. Flower Extract

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ABSTRACT

Lactobacillus acidophilus, one of the most common probiotics, has been found to show health benefits to human such as anti-infective, anti-inflammatory, and gut health modulatory activities. *Clitoria ternatea* L. flower contains anthocyanins as major bioactive compound and shows several biological activities including antioxidant, antimicrobial, and anti-inflammatory activities. This study aims to develop microencapsulated powder containing *L. acidophilus* and *C. ternatea* flower extract. Spray drying technique was used for encapsulation of *L. acidophilus* and *C. ternatea* aqueous extract. The parameters were set as follow: aspirator 100%, air pressure of 0.6 mPa, pump 10%, and feed rate of 3.0 mL/min. The inlet temperatures were varied at 130, 140, 150, and 170 °C. Maltodextrin (3 and 5% w/w) was added as a carrier. *L. acidophilus* JCM1132 1 mL (1.00×10^{12} CFU/mL) and *C. ternatea* flower extract (TSS 8 °Brix) 100 mL were added. The powder was evaluated for yield, moisture content, total monomeric anthocyanin content, and total plate count. The powder produced by using the inlet temperature at 140 °C and maltodextrin at 5% w/w demonstrates the best powder properties. The encapsulation efficiency in terms of the number of *L. acidophilus* was 46.30% and the yield was found to be 34.03%. The spray-dried powder has moisture content of 4.45 ± 0.70 % and contains total monomeric anthocyanin content of 1.93 ± 0.03 mg delphinidin-3-glucoside/g powder. In conclusion, *L. acidophilus* and anthocyanins in *C. ternatea* flower extract could be preserved in the developed powder. Further studies on the variation of other types of carriers and stability of the microencapsulated powder are recommended.

KEYWORDS: Encapsulation; *Lactobacillus acidophilus*; *Clitoria ternatea* L. flower extract; Anthocyanins; Delphinidin-3-glucoside.

1. INTRODUCTION

Lactobacillus acidophilus, one of the most common probiotics, has been found to show health benefits to human such as anti-infective, anti-inflammatory, and gut health modulatory activities. *L. acidophilus* has been isolated from many fermented and dairy products. It was found that *L. acidophilus* produces the maximum amount of lactic acid, some acetic acid with no hydrogen, and no catalase[1]. This bacteriocin with suitable properties is preferred in the food industry.

Clitoria ternatea L. flower contains anthocyanins as major bioactive compound and shows several biological activities including antioxidant, antimicrobial, and anti-inflammatory activities. Previous study indicated that its free radical scavenging ability attributed to the active compounds, ternatin anthocyanins, delphinidin derivatives, and kaempferol[2]. Delphinidin is the main anthocyanin responsible for the deep blue to purple color. Anthocyanins have also received significant attention due to their prebiotic function by promoting the growth of beneficial bacteria and increasing the

concentration of short-chain fatty acid[3]. Nonetheless, similar to probiotics, anthocyanins are sensitive to high temperature at 180 ºC[8], pH variation, and presence of light and oxygen.

Microencapsulation is a widely used technique in food and pharmaceutical industries. The concept of incorporating plant extracts with probiotics has been studied. Dark sweet cherry and blueberry extracts were encapsulated with *L. rhamnosus* in the previous studies. By the addition of polymeric matrices, bioactive compounds, and probiotics were protected from degradation during processing and storage[3,6]. Spray drying is one of the most common methods that offers fast processing time, low operational cost, and product stability. Hence, this study aims to develop microencapsulated powder containing *L. acidophilus* and *C. ternatea* flower extract by using spray drying technique.

2. MATERIALS AND METHODS

2.1. Plant Preparation and Extraction

C. ternatea flower 50 g was mixed with reverse-osmosis water (RO water) 1 L and then boiled in water at 90-95 °C for 15 min. After that, the extract was filtered through white cloth and Whatman filter paper No.4. Total soluble solid (TSS) and pH of *C. ternatea* flower extract was measured by refractometer and pH meter[4]. *C. ternatea* flower extract was concentrated by using a rotary evaporator until TSS reached 8 °Brix. The volume of the extract was recorded, and stored at -20 ºC.

2.2. Probiotic Strain and Growth Conditions

The probiotic strain from *L. acidophilus* JCM1132 was activated probiotic bacteria by streak plate technique and MRS broth was incubated at 37 °C for 24 h in microaerophilic jars. After that, it was centrifuged at 3000 × g at 4 °C for 10 min. *L. acidophilus* was washed and resuspended with 0.85% sodium chloride solution (NaCl) stored in centrifuge tube at 4 °C. Serial dilution in phosphate buffer saline (PBS) was added on de Man Rogosa Sharpe (MRS) agar by spread plate technique[6,7].

2.3. Co-Encapsulation of *L. acidophilus* JCM1132 and *C. ternatea* Flower Extract by Spray Drying

The spray drying was carried out in a mini-spray dryer (Buchi B-290, Switzerland). The parameters were set as follows: aspirator 100%, air pressure of 0.6 mPa, pump 10%, and feed rate of 3.0 mL/min. The inlet temperatures were varied at 130, 140, 150, and 170 °C. Maltodextrin (3 and 5% w/w) was added as a carrier. All 8 tested conditions were described in Table 1.

Table 1. Tested conditions for the production of microencapsulated powder containing *L. acidophilus* and *C. ternatea* flower extract

Conditions (C)	C1	C2	C3	C4	C5	C6	C7	C8
Inlet temperature (°C)	130		140		150		170	
C. ternatea extract (mL)	100		100		100		100	
Maltodextrin (% w/w)	5	3	5	3	5	3	5	3
L. acidophilus JCM1132 (mL)	1	1	1	1	1	1	1	1

2.4. Determination of Yield, Moisture Content, and Total Monomeric Anthocyanin Content

The drying yield of the spray drying process was calculated from the weights of dried plant, carrier agent, and the developed powder. The moisture content of the powder (0.2 g) was determined by a moisture analyzer at 105 °C according to the International Dairy Federation Bulletin (IDF 1993). Total anthocyanins content was determined by using the pH-differential method[5]. The content of anthocyanins (A) can be calculated from the following formula: $A = (A_{520nm}\text{-}A_{700nm})_{pH1.0} - (A_{520nm}\text{-}A_{700nm})_{pH\ 4.5}$. Total monomeric anthocyanin content as delphinidin-3-glucoside equivalents was calculated as follows:

$$\text{Anthocyanin content (Delphinidin-3-Glucoside equivalents, mg/L)} = \frac{(A \times MW \times DF \times 10^3)}{\varepsilon \times 1}$$

where, MW = molecular weight (500.8 g/mol) for delphinidin-3-glucoside, DF = dilution factor, 10^3 = factor for conversion from g to mg, ε = 23,700 molar extinction coefficient of the delphinidin-3-glucoside in L × mol^{-1}× cm^{-1} for aqueous solutions, and 1 = pathlength in cm of the cuvette from Beer and Lambert's law[5].

2.5. Determination of *L. acidophilus* Number

The cell viability of probiotics bacteria in microencapsulated powder of 0.1 g or probiotic culture of 1 mL was determined[6]. The results were expressed in a colony-forming unit (log CFU/g) of the product or mL of solution.

$$\text{Cell viability (CFU/g or CFU/mL)} = \frac{\text{Colony forming units (CFU)} \times \text{Dilution count number}}{\text{Volumn in plates (mL)}}$$

Where N is the number of viable cells (log CFU/g) released from the microencapsulated powder and N_0 is the number of viable cells (log CFU/mL) in the feed solution before microencapsulation[6]. The encapsulation efficiency (EE) was calculated as follows: % encapsulation efficiency = $N/N_0 \times 100$.

3. RESULTS AND DISCUSSION

C. ternatea flower extract was obtained as a dark blue solution (TSS 8 °Brix) with pH 5.32. One gram of the extract solution contains total monomeric anthocyanin content as delphinidin-3-glucoside (D3G) equivalent equal to 0.207 mg D3G/g. After spray drying, the yield, moisture content, and total monomeric anthocyanin content of microencapsulated powder obtained from 8 tested conditions are shown in Table 2. However, the survival of *L. acidophilus* was found only in 4 conditions, C2-C5 in Table 3.

Table 2. Yield, moisture content, and total monomeric anthocyanin content of microencapsulated powder

Conditions	Inlet temperature (℃) / Maltodextrin (% w/w)		Appearance	Drying yield (%)	Moisture content (%)	Total monomeric anthocyanin content (mg D3G/g powder)
C1	130	3	Dark blue powder	36.15	6.20 ± 2.43	2.78 ± 0.05
C2		5		40.18	5.11 ± 0.40	2.13 ± 0.04
C3	140	3		32.74	4.84 ± 0.20	3.61 ± 0.02
C4		5		34.03	4.45 ± 0.70	1.93 ± 0.03
C5	150	3	Blue powder	28.36	7.12 ± 0.60	1.38 ± 0.50
C6		5		40.79	3.65 ± 0.30	2.36 ± 0.04
C7	170	3		37.69	4.61 ± 0.25	2.41 ± 0.04
C8		5		39.62	6.95 ± 0.40	2.03 ± 0.05

Note: D3G (Delphinidin-3-glucoside).

Table 3. Cell viability of *L. acidophilus* in microencapsulated powder and probiotic culture

Inlet temperature (°C)		Maltodextrin (% w/w)	*L. acidophilus* JCM1132 (mL)	Cell viability				Encapsulation efficiency (%)
				Before spray drying (CFU/mL)	After spray drying (CFU/g)	Before spray drying (log CFU/mL)	After spray drying (log CFU/g)	
C2	130	5	1	1.01×10^8	1.20×10^4	9.004	4.078	45.29
C3	140	3	1	4.50×10^{14}	8.13×10^4	14.653	4.910	33.51
C4	140	5	1	1.00×10^{12}	3.60×10^5	12.000	5.556	46.30
C5	150	3	1	1.01×10^8	3.80×10^4	9.004	4.580	50.86

Notes: C (Condition), CFU (Colony forming units), and D3G (Delphinidin-3-glucoside).

According to the results, cell viability of *L. acidophilus* (% encapsulation efficiency) and moisture content were the main factors to decide the most suitable spray drying condition for the production of microencapsulated powder containing *L. acidophilus* and *C. ternatea* flower extract. The powder produced by using the inlet temperature at 140 °C and maltodextrin at 5% w/w (C4) demonstrates the best powder properties. However, this condition led to the low drying yield and low amount of total monomeric anthocyanin. Further study using design of experiment is needed to improve these outputs. % encapsulation efficiency in terms of the number of *L. acidophilus* was 46.30% and moisture content of 4.45 ± 0.70 %. The number of *L. acidophilus* (3.60×10^5 CFU/g) is almost similar to the value indicated in the announcement by the Ministry of Public Health in which the viable probiotic bacteria must be at least 10^6 CFU/1 g of food during its shelf life[9].

4. CONCLUSION

The microencapsulated powder produced by using inlet temperature at 140 °C and maltodextrin at 5% w/w shows the best properties. *L. acidophilus* and anthocyanins in *C. ternatea* flower extract could be preserved in the developed powder. Further studies on the variation of other types of carriers and stability testing of the microencapsulated powder are recommended.

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Conflict of interest

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Landscape Analysis of International Facilitated Regulatory Pathway for Innovative Medicine Approval: Recommendation for Thailand

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ABSTRACT

Timely access to innovative medicine is the ultimate goal of pharmaceutical companies, regulators, and patients. Innovative medicines have long development and complex manufacturing processes. In addition, the regulation process is still the bottleneck that leads to prolonged marketing authorization approval. National regulatory authorities (NRAs) established facilitated regulatory pathways (FRPs) to expedite drug development and approval in the shortest possible time. The Thai Food and Drug Administration (Thai FDA) is also in the process of developing FRPs for innovative medicines This study aimed to identify the key characteristics of the FRPs by the NRAs in the United States, European Union (EU), Japan, and Australia. This was conducted by a systematic review of the guidelines and literature. Results from this study will lead to the recommendation regulatory pathway for innovative medicine approval in Thailand. The United States, the EU, Japan, and Australia are innovative initiators. They have the pathway to encourage drug development, especially unmet medical needs, and serious diseases by increasing the opportunities for regulator-sponsor interaction and scientific advice. They have the pathway to approve medicine before available confirmatory studies. Moreover, they have expedited pathways to speed up and reduce the timeline by prioritizing essential medicine. Additionally, Australia has a pathway to avoid duplication by using the reports of the listed authorities for referring the evaluation results. Adapting and providing a regulatory framework that maximizes the benefit of patients and sponsors but remains aligned with the government's strategic goals is recommended for the Thai FDA. They should support local researchers to approve innovative medicine through scientific or regulatory consultation, and rolling submission. While importing innovative medicines that have been approved by NRAs, the Thai FDA should refer to their evaluation results and focus on pharmacovigilance activity. These are consistent with good regulatory practices of WHO recommendation.

KEYWORDS: Regulatory Pathway; Facilitated Regulatory Pathway; Expedited Regulatory Pathway; Accelerated Regulatory Pathway; Accelerated Approval; Accelerated Review

1. INTRODUCTION

Innovation medicine is a new process, product, service, method, or technology, which mostly requires complex and long-duration development process. The complexity and duration of medicine development and regulatory approval process lead to prolonged marketing authorization timelines while pharmaceutical companies, patients, and other stakeholders need to access it urgently[1]. Facilitated regulatory pathways (FRP) alternative pathways besides the standard regulatory review route to provide a formalized flexible option for accelerating the process of bringing new pharmaceutical medicine to market.[2] The USFDA, EMA, PMDA, and TGA are the stringent regulatory authorities and leaders in the innovative pharmaceutical industry. They have a variety of registration pathways to encourage drug development, promote priority review or reduce the review timeline, approve a drug before confirmatory studies, and approve a drug without confirmatory studies. Some agency has the option for registration for speeding up the regulatory review process and

reducing the duplication of review. However, the agency has the consistent objective to enhance efficiency and accelerate patient access to new treatment. While, the Thai FDA, which is the agency responsible directly for regulating and monitoring pharmaceutical health products is facing a registration management problem along with an overload of work is a current obstacle within organization management. The current registration pathway is still rigid and unclear the procedure. It impacts the possibility and target timeframe for marketing authorization approval. Moreover, many researchers or enterprises are unsuccessfully pushing their research into the market in terms of the data requirement for registration, or agency support. Thai people may not access innovation or essential medicine in time. Adjusting and establishing the regulatory pathways seems to be the mechanism for expediting development and shortening approval times for therapies that conform to good regulatory practice of WHO recommendations. The variety of regulatory pathways besides the standard pathway expresses the flexibility in responding to changing environments and situations.

2. MATERIALS AND METHODS

2.1. Study Design & Search Strategy

The Health Authorities are the agencies that act directly to regulate and monitor pharmaceutical and health products along with their life cycle. The USFDA, EMA, PMDA, and TGA which are leaders and stringent regulatory authorities have a mature system covering the pre- and post-authorization phases. Moreover, most innovative medicines were developed in these countries. These agencies have experience in their innovative application management for registration. Then, these agencies were included in this study. The methods were conducted by comprehensive review of content, summary, and guidelines provided by NRAs or information published on Health Authorities' websites with the latest updated version until 31 December 2023 and context from relevant published literature from PubMed, Scopus, and Google Scholar during the year 2017-2023. The main keywords used for searching consist of the regulatory pathway, facilitating regulatory pathway, or expedited regulatory pathway and following with the name of health authorities. After that, this was conducted a landscape analysis of Facilitated Regulatory Pathways in the USFDA, EMA, PMDA, and TGA.

2.2. Literature Selection Criteria

2.2.1 Inclusion criteria

a. The regulatory pathways of the national regulatory agency will include the USFDA, EMA, PMDA, and TGA.

b. The included regulatory pathways must have the objective of designing expedited product development, accelerated submission of market authorizations, or regulatory review.

c. A regulatory pathway must be created for innovative medicine applications.

2.2.2 Exclusion criteria

a. A regulatory pathway will be excluded for generic medicine applications.

b. A regulatory pathway will be excluded for other health products, such as medical devices, cosmetics, and novel food.

3. RESULTS AND DISCUSSION

3.1. Facilitated Regulatory Pathway by USFDA[2,3,4]

US FDA has facilitated regulatory pathways covering the development and review phase. Firstly, fast-track designation is designed to facilitate during the development phase and expedite the review of drugs to treat serious conditions or life-threatening and fill an unmet medical need. Secondly, breakthrough therapy designation is designed to facilitate the drugs treat a serious or life-

threatening disease or condition and preliminary clinical evidence indicates that the drug may demonstrate substantial improvement over existing therapies. Thirdly, accelerated approval allows drugs for serious conditions that fill an unmet medical need to be approved based on a surrogate endpoint. Lastly, priority review designation is a process designed to take action by the FDA. The characterization of this designation is the shorter clock in review process for a drug that treats a serious condition and provides a significant improvement in safety or effectiveness or any application got a priority review voucher as FDA announcement. These alternative pathways act as the reward to enhance the developer for discovering innovative medicine to treat serious conditions or unmet medical needs. The developer and the reviewer need cooperation to discuss the essential studies for proving efficacy and safety. While the data is still incomplete, the risk management and post-approval study and commitment need to be conducted. Another aspect, the developer can save the cost and time to conduct only the essential study on animals and humans and they are accepted by the regulators. The reviewers have clear directions and criteria to decide whether the application conforms which the facilitated regulatory pathway. They are consistent, clear, and transparent. The patients can assess the essential medicine in time. It can improve the quality of life or prolong the lifetime of the patient. Moreover, competitive marketing occurs after the new innovative medicine is approved. That impacts positively the market price of the medicine. It can save the cost for the policymaker, national payers, insurance, and patients.

3.2. Facilitated Regulatory Pathway by EMA[2, 4]

EMA has developed programs to facilitate the development of innovative medicine, as well as expedite the review of marketing application. PRIME is a program that accelerate drug development. Accelerate assessment is a program that shortens marketing application review time. Exceptional circumstance is proposed to approve base on a limited data while the conditional marketing authorization is approved before full data set is complete. However, the benefit-risk balance concept is considered as the key. The risk minimization plan is also necessary to be enforced even in the post marketing phase. This includes things like restriction of use and condition, updating the information on the packaging, and additional efficacy or safety study. These positively and negatively impact the patients. The positive impact is the patient can access the innovative medicine rapidly. In contrast, the medicine that was approved under limited data. Thus, the agency needs to make sure that the benefit-risk outweigh is positive and need conduct closely in real world data. Renewal and reassessment need to conduct to make sure the efficacy and safety. Moreover, accelerated assessment is designed to accelerate the application evaluation or shorten the application review timeline. Mostly, they include medical products of major interest to the public, pandemic, or therapeutic innovation.

3.3. Facilitated Regulatory Pathway by PMDA[2, 4]

Flexibility of expedited regulatory pathways for innovative and regenerative medical products in Japan was established impacting the accessibility of the patient, and healthcare professional. It is not only the healthcare aspect but also affects the economic system of Japan. The essential product being firstly developed in Japan originally is the feature of SAKIGAKE designation that enhances the local developer's discovery by the government. This pathway seems to support a sustainable medical system by reducing the import of innovative medical products. PMDA developed conditional and term-limited approval for regenerative medical products for enhancing the accessibility of patients, while the efficacy and safety have tentative positive. Moreover, conditional early approval program is to expedite the drug development program intended to improve the accessibility of patients to potentially effective drugs for serious diseases or conditions without the existing effective therapy. Especially, the confirmation clinical trials are difficult to conduct due to the limited time. However, the comprehensive data at the time of approval is still incomplete, the post-marketing system must be strengthened to closely and continuously monitor the efficacy and safety data. Priority review is one of the regulatory pathway options in Japan for the orphan drug or the government considering the clinical usefulness. It speeds up the review timeline to get the marketing authorization and speed up access to new therapeutic medicines.

3.4. Facilitated Regulatory Pathway by TGA[5, 6]

TGA has experienced a comprehensive review of its regulatory system for medicine. To resolve the accessibility of essential medicine in time, medicine for serious or life-threatening conditions is included and focused on experiencing a fast-track process to make the medicine available to patients sooner than normal. TGA established the priority review to promote the essential and impact the patients or the public by reducing the review timeline. Despite the provisional approval, they approved the medicine before the available confirmatory studies. It is to enhance the patient's or the consumer's access faster. Moreover, TGA still has the COR and work sharing with the listed health agencies to speed up and reduce the duplication of the regulatory review process. Those are positive impacts gained for limiting the capacity and enhancing the consumer or public to access essential medicines.

3.5. Summary analysis of facilitated regulatory pathway from different agencies

Global agencies are adapting the regulatory pathways to legally push essential or innovative medicine into the market to meet entrepreneurs' and patients' needs. The FRPs involve drug development, marketing authorization, and the post-approval phases. The regulatory agencies must ensure that those medicines have efficacy, and safety throughout their life cycles. Different stringent regulatory agencies such as USFDA, EMA, PMDA, and TGA have proposed their FRP objectives as compared in Table 1. However, even though the named pathways are different, their objective is consistent to enhance efficiency and accelerate patient access to new treatment.

Table 1. Summary of the regulatory pathways for innovative medicine approval by USFDA, EMA, PMDA, and TGA

Purpose	USFDA	EMA	PMDA	TGA
1. To encourage drug development	Breakthrough therapy	PRIME	SAKIGAKE	-
2. To promote priority review/ reduced review timeline	Fast track/ Priority review	Accelerated assessment	Priority review	Priority review
3. To approve a drug before available confirmatory studies	Accelerated approval	Conditional Marketing Authorization	Conditional and Time-Limited Approval	Provisional approval
4. To approve a drug without confirmatory studies	-	Marketing Authorization under exceptional circumstances	Conditional approval for drugs	-
5. To speed up the regulatory review process	-	-	-	Comparable Overseas Regulator/ International Work-sharing-Access Consortium

3.6. FRP Recommendation for Thailand

The objectives of FRPs in Thailand should facilitate the development and speed up the regulatory review process. The recommendation for the potential regulatory pathway of innovation application in Thailand involves drug discovery and development till the marketing authorization approval. To expedite drug development and streamline the review process, the Thai FDA can draw

inspiration from the best practices of both the US FDA and the TGA which similar in term or process, organization, and resource as the Thai context.

To facilitate and expedite drug development, FRPs of the USFDA are role models for the Thai FDA because they have various and clear criteria for pathways, focus on the unmet medical need and medicine that treat a serious condition or the medicine' meaningful advantage over available therapy. Moreover, they can approve the medicine while comprehensive data is still not complete by using the preliminary clinical evidence or surrogate endpoint. These FRPs should be implemented for manufacturing within the country to support the Thai pharmaceutical industry.

To expedite the review process, FRPs of TGA are suitable models for the Thai FDA because they have a pathway for reducing duplication evaluation by referring to the evaluation results from other agencies. It can reduce the review timeline and save the human capacity that still limited in terms of quantity and skill.

These FRPs can involve both of local manufacturing application in term of expedite the development and importing medicine application in term of review process.

4. CONCLUSION

Global agencies are adapting the regulatory pathways to legally promote essential or innovative medicine into the market to meet entrepreneurs' and patients' needs. The main objective of FRPs is to expedite drug development and the review processes to enhance researchers/innovators competitiveness. Thus, it is essential for the Thai FDA to establish or adopt FRP from global best practices including the US FDA and TGA. FRPs of the USFDA can be used as role models for the Thai FDA to facilitate and expedite drug development since they have various and clear criteria for pathways focusing on the unmet medical need and medicine that treat a serious condition or the medicine' meaningful advantage over available therapy. Moreover, they can approve the medicine while comprehensive data is still not complete by using the preliminary clinical evidence or surrogate endpoint. These FRPs should be implemented for manufacturing within the country to support the Thai pharmaceutical industry. For the review process, FRPs of TGA are suitable models for the Thai FDA since they have a pathway to reduce duplicate evaluation by referring to the evaluation results from other agencies. It can minimize the review timeline and save the human resource, which is still limited in terms of quantity and skill. It is believed that the proposed model would greatly speed up the drug development and approval processes for the Thai FDA. However, this study focused only on the regulatory option that enhances the registration approval and patient access to the medicine in time. Thus, the commitment to risk management in the post-marketing phase needs to be carefully considered. Future work to further improve the Thai FRPs should focus on the post-authorization system strengthening and pharmacovigilance activity to ensure consumer safety.

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Conflict of interest

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Analysis of Factors Affecting the Engagement of Pharmacists with the Organization Working in Public Health Facilities: A Quantitative Study in Vietnam

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ABSTRACT

Pharmacists are among the most crucial resources, playing a pivotal role in the establishment and growth of any medical organization. The study aims to analyze the factors affecting the engagement of pharmacists working in public health facilities in the Mekong Delta with their organizations from 2023 to 2024. Interviews conducted with groups operating in public health facilities provided the data for this study. A pre-designed set of questions was used to gather information. Interviews conducted with groups operating in public health facilities provided the data for this study. A pre-designed set of questions was used to gather information. A total of 1500 participants who met the research criteria were included in the study. Data gleaned from interviews with pharmacists underwent scrutiny with the Cronbach's Alpha test, resulting in the exclusion of 3/40 variables. KMO coefficients (ranging from 0.91 to 0.972) validated the data's significance for Exploratory Factor Analysis. Meeting set criteria, results unveiled the positive impact of working conditions, development opportunities, promotion and pay-rate on retention ($p<0.05$). CFA and SEM aligned with market dynamics. According to the survey, public medical facilities consistently prioritize the well-being and needs of pharmacists, fostering conditions related to salaries, legal policies, work benefits, etc., to make them feel more integrated into their organization.

KEYWORDS: Influencing Factors; Engagement; Hospital Pharmacist; Public Health Facilities, Mekong Delta; Working Policies

1. INTRODUCTION

Engagement and job satisfaction are essential elements in any organization, especially in the medical field[1]. “Senior staff engagement is linked to high-quality services”, according to the Care Quality Commission in England. Factors associated with positive work engagement include manager, communication, and behavior[2]. Furthermore, employee satisfaction contributes to better performance and sustainability. Competent pharmacists and long-term commitment to the workplace are important factors in creating the success and sustainable development of a medical facility In addition, this

connection also contributes to optimizing drug information for medical staff, aligning with the government's policy to ensure timely access to quality drugs that are safe, effective, and reasonably priced[3]. Understanding the connection between hospital pharmacists and the organization is necessary because pharmacists play an indispensable role in both the treatment process and the business interests of the organization[4]. In other countries, the issue of medical human resources is consistently researched to identify practical solutions for strengthening existing personnel and attract future professionals, thereby preventing shortages or the phenomenon of "brain drain" [5].

According to a 2023 study evaluating employee participation in pharmaceutical care services at King Abdulaziz Medical City in the Central region (KAMC), the research results have led to conclusions that impact pharmacists' job satisfaction. Factors such as the type of pharmacy practice setting, the year of registration of pharmacists, and marital status were identified. However, there are limitations inherent in the study. For instance, the sample size is relatively small, and the survey encompasses only a limited number of hospitals[6]. Research exploring the influence of organizational support and tolerance on Chinese pharmacists in the stressful and competitive pharmacy industry in hospitals, conducted by Jing Jin and Jing Tang in 2021, showed that female pharmacists have a higher level of work engagement. The higher the professional title and position of the hospital pharmacist, the greater the organizational support they receive[7]. A study assessing job satisfaction of pharmacists working in public health facilities was conducted by Abba Khalid Abdullahi and colleagues in 2023. This study explores the determinants of satisfaction in work and turnover trends of pharmacists in public and private hospitals in Nigeria. The obtained results include many important factors that directly affect engagement such as age, religion, marital status, type of occupation, highest professional level, number of years of service, type of facility, current qualifications, pharmacy as main source of income, monthly income, number of hours worked and housing status[8]. In the Mekong Delta of Vietnam, there is a noticeable gap in research concerning pharmacists' engagement with medical facilities. Recognizing the urgency of this matter, we conducted a study to explore the connection between hospital pharmacists and organizations across multiple provinces in the Mekong Delta region from 2023 to 2024.

2. MATERIALS AND METHODS

2.1. Research Subjects

Pharmacists working in public medical facilities in provinces/cities in the Mekong Delta region.

Selection criteria

Pharmacists working in public medical facilities in provinces/cities in the Mekong Delta region are eligible for participation. Survey participants must have practical experience and a background of working in public medical facilities.

Exclusion criteria

Pharmacists who respond to the survey in a certain order and/or provide incomplete survey responses[9].

2.2. Foundation Theory

Theoretically, understanding the urgency and the particularly important role of hospital pharmacists in the organization, factors highly valued in the recruitment and retention of these professionals include the organizational conditions, opportunities for advancement, and loyalty[10,11]. Based on theoretical foundations and findings from various domestic and foreign studies, we developed a set of survey questions (Version I) and a research model with five factors affecting pharmacist retention in Figure 1. The hypotheses proposed by hospital pharmacists are as follows: H1: Working conditions (including agency’s legal policies, work environment, and work benefits), H2: Development opportunities (including career opportunities and professional development), H3: Promotion and H4: Pay-rate at work positively affects the retention of clinical pharmacists.

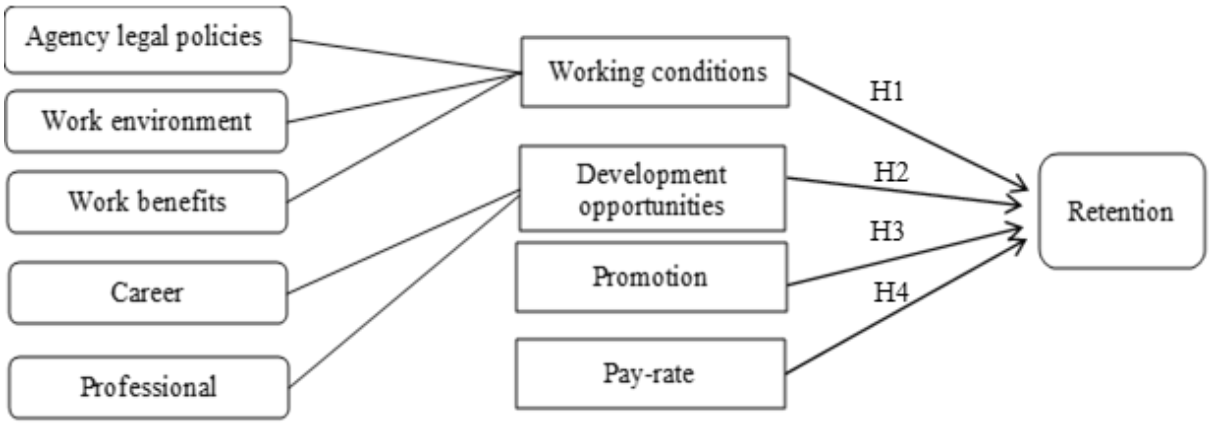

Figure 1. Model of hospital pharmacists' engagement with the organization.[12-15]

2.3. Design of Survey Questionnaires and Measurement Scales

Through cross-sectional description and data collection using the interview method with a pre-designed questionnaire, this study aims to evaluate the relationship of hospital pharmacists with organizations in 13 provinces/cities in the Mekong Delta region. We conducted research and developed a questionnaire based on the output questions[3, 16].

The questionnaire includes 2 sections:

The first part of the questionnaire comprises a summary of human-related factors, an outline of the research subjects and some questions related to object attachment[17].

Part 2 of the survey consists of in-depth questions that assess the retention of research subjects. These questions cover various aspects, including working conditions (14 questions), promotion (4 questions), development opportunities (10 questions), pay rate (5 questions), and long-term engagement with the organization (7 questions). The 5-point Likert scale is employed to evaluate these items. In this study, responses to the questions are scored as follows: 1 = 'Strongly disagree', 2 = 'Disagree', 3 = 'Neutral', 4 = 'Agree', and 5 = 'Completely agree'[18, 19].

The reliability of the second scale was assessed using Cronbach's alpha coefficient and the total variable correlation coefficient. Afterwards, we proceeded with exploratory factor analysis (EFA) to assess the convergent and discriminant values of the scale. Following that, a confirmatory factor analysis (CFA) was conducted to evaluate the appropriateness of the measurement model with real data. Subsequently, we delved into the analysis of the multidimensional relationship among various variables using Linear Structural Modeling, also known as Structural Equation Modeling (SEM). The data processing was carried out using SPSS 26.0 and AMOS software[20, 21].

2.4. How to Take Samples

Using convenient random sampling, the research team employed survey methods, such as google forms, or printed questionnaires[23].

2.5. Ethical Considerations

This study was approved by the Ethics Council for Biomedical Research of Can Tho University of Medicine and Pharmacy (Decision No. 23.051.GV/PCT-HĐĐĐ, Can Tho, dated December, 2023). The survey process strictly adhered to the principles of voluntary and anonymous participation[24, 25].

2.6. Data Analysis

In this study, Cronbach's alpha coefficient is determined under the following conditions: (1) observed variables with variable-total correlation coefficients less than 0.3 will be eliminated, and (2) a Cronbach's Alpha value of 0.6 or higher is considered satisfactory for use. Total variable correlation coefficients less than 0.3 are deemed indicative of poor-quality variables and are therefore removed from the scale. To reduce and summarize the data, an exploratory factor analysis (EFA) is conducted using the following indicators: (i) factor loading >0.3; (ii) Kaiser – Meyer – Olkin coefficient ($0.5 \leq KMO \leq 1$); (iii) Bartlett's test for statistical significance (Sig. < 0.05); and (iv) percentage of total variance (Percentage of variance > 50%)[26, 27].

3. RESULTS AND DISCUSSION

3.1. General Characteristics of Research Objects

As mentioned in Table 1, our research shows that a higher proportion of females (70.7%) participated in the survey population compared to males (29.3%). This finding is comparable to a study on factors influencing organizational support and tolerance of pharmacists in China. The similarity can be attributed to the fact that pharmacist positions in public health facilities are very stable jobs with moderate income, leading to a predominance of women in these roles[7].

Most survey participants have a university education (54.9%). According to our research, 88.3% of pharmacists would recommend the hospital where they work to other pharmacists seeking a job, and the rate of sustainable engagement with the hospital is 94.4%. This demonstrates that the local hospitals and public healthcare institutions are making an effort to meet the needs of pharmacists, providing a comfortable working environment and improving their knowledge and dedication to the organization.

Table 1. General characteristics of research subjects

Characteristics of the Study Sample		Frequency (n= 1500)	Ratio (%)
Sex	Male	440	29.3
	Female	1060	70.7
Marital status	Single	500	33.3
	Married	1000	66.7
Education Level	Intermediate college	604	40.3
	University	824	54.9
	Post-University	72	4.8
Recommend the Hospital for Work	Yes	1325	88.3
	No	175	11.7
Desire for a Lasting Relationship	Yes	1416	94.4
	No	84	5.6

3.2. Result of Cronbach's Alpha, EFA, CFA and SEM

3.2.1. Result of Cronbach's Alpha

Table 2 presents the descriptive statistics and reliability analysis of the data using SPSS. Additionally, in the *WE* component (*), factor WE2 is eliminated because the CA coefficient (0.995) is larger than the current CA coefficient; in the *CO* component (**), the CO3 factor is eliminated because the CA coefficient (0.990) is larger than the current CA coefficient and in the *PR* component (***), factor PR5 is eliminated due to the CA coefficient (0.951) surpasses the current CA coefficient.

Table 2. Descriptive statistics and reliability results (N=1500)

	Cronbach's Alpha	Mean	SD
Agency legal policies (ALP)	0.955	3.9204	0.81829
Work environment (WE)*	0.925	3.8175	0.76051
Work benefits (WB)	0.976	3.8496	0.82534
Promotion (P)	0.978	3.7670	0.79899
Career opportunities (CO)**	0.955	3.7940	0.76077
Professional development (PD)	0.982	3.7739	0.80559
Pay-rate (PR)***	0.911	3.6059	0.79267
Retention (R	0.961	3.7289	0.78052
Valid N (List wise)			

3.2.2. Confirmatory Factor Analysis Results in EFA

EFA analysis for subsection factors in independent components

The extracted variance value exceeds 90% (92.170%). Simultaneously, the KMO coefficient = 0.972 ($0.5 \leq KMO \leq 1$), and the significance level (Sig value) of the Bartlett test = 0.000 (meeting the condition of being less than 0.05). Eight groups of factors are extracted at Eigenvalue = 2.828 (> 1), all meeting the conditions. Consequently, we proceeded with the EFA analysis for the subsection factors in the independent components.

3.2.3. Exploratory Factor Analysis (EFA)

According to the research topic, observed variables exhibit KMO coefficients ranging from a minimum of 0.5 (for achievement needs analysis) to a maximum of 0.972 (for attitude analysis). These coefficients, demonstrating statistical significance, affirm the suitability of the data for exploratory factor analysis. The results also showed that the observed variables meet the requirements in terms of total variance extracted (>50%). The Eigenvalues coefficients are all greater than 1 and p-value = 0 .000.

3.2.4. Results of EFA Exploratory Analysis of Independent Variables

The extracted variance value surpasses 70% (92.170%). Additionally, the KMO coefficient is 0.972 ($0.5 \leq KMO \leq 1$), and the significance level (Sig value) of the Bartlett test is 0.000, meeting the condition of being less than 0.05. Seven groups of factors are extracted at Eigenvalue = 2.828 (> 1), all satisfying the condition. The EFA analysis was conducted for subsection factors in independent components. Table 3 presents the results of the EFA analysis. The subsections were re-divided into 7 new factors based on the correlation between the subsections and the factors. Consequently, the variables retained after EFA analysis all meet the condition that the loading factor is greater than 0.5, indicating their practical significance and meaningful role.

3.2.5. Rotated Component Matrix[a]

Table 3 presents the results of the EFA analysis. The subsections were re-divided into 7 new factors based on the correlation between the subsections and the factors. Consequently, the variables retained after EFA analysis all meet the condition that the loading factor is greater than 0.5, indicating their practical significance and meaningful role.

Table 3. Results of EFA exploratory analysis of independent variables

	Component						
	1	2	3	4	5	6	7
ALP2	0.787						
ALP4	0.777						
ALP3	0.766						
ALP5	0.766						
ALP1	0.731						
WB2		0.762					
WB1		0.759					
WB4		0.725					
WB5		0.720					
WB3		0.688					
PD2			0.730				
PD3			0.725				
PD1			0.722				
PD4			0.722				
PD5			0.712				
P3				0.764			
P1				0.764			
P4				0.764			
P2				0.758			
PR4					0.778		
PR1					0.762		
PR2					0.740		
PR3					0.700		
CO5						0.754	
CO2						0.753	
CO4						0.753	
CO1						0.747	
WE3							0.759
WE1							0.758
WE4							0.757

3.2.6. Results of EFA Exploratory Analysis of Dependent Variables

The values presented in Table 4 indicate satisfactory analysis results, with a KMO value of 0.910, a significant Bartlett's test (Sig value = 0.000), and an Eigenvalue of 5.385 (> 1), signifying the extraction of one factor. The total variance extracted = 89.742%, which satisfies the condition of greater than 50%. As a result, EFA analysis was conducted with observed variables of the dependent component "(R): Retention".

Table 4. Results of EFA exploratory analysis of the dependent variable

Component Matrix [a]

	Component
R7	0.929
R6	0.921
R1	0.916
R3	0.908
R5	0.886
R2	0.885
R4	0.862

3.2.7. Results of Confirmatory Factor Analysis CFA

The CFA results presented in Figure 2 show that the model has favorable fit indexes: TLI= 0.978, CFI= 0.981, GFI= 0.916 (> 0.9). The RMSEA= 0.046 (< 0.08) and Chi-square/df= 4.165 (< 5) is considered consistent with actual data. The research model is consistent with market data and suitable for subsequent CB-SEM analysis. The CFA results presented in Figure 2 show that the model has favorable fit indexes: TLI= 0.978, CFI= 0.981, GFI= 0.916 (> 0.9). The RMSEA= 0.046 (< 0.08) and Chi-square/df= 4.165 (< 5) is considered consistent with actual data. The research model is consistent with market data and suitable for subsequent CB-SEM analysis.

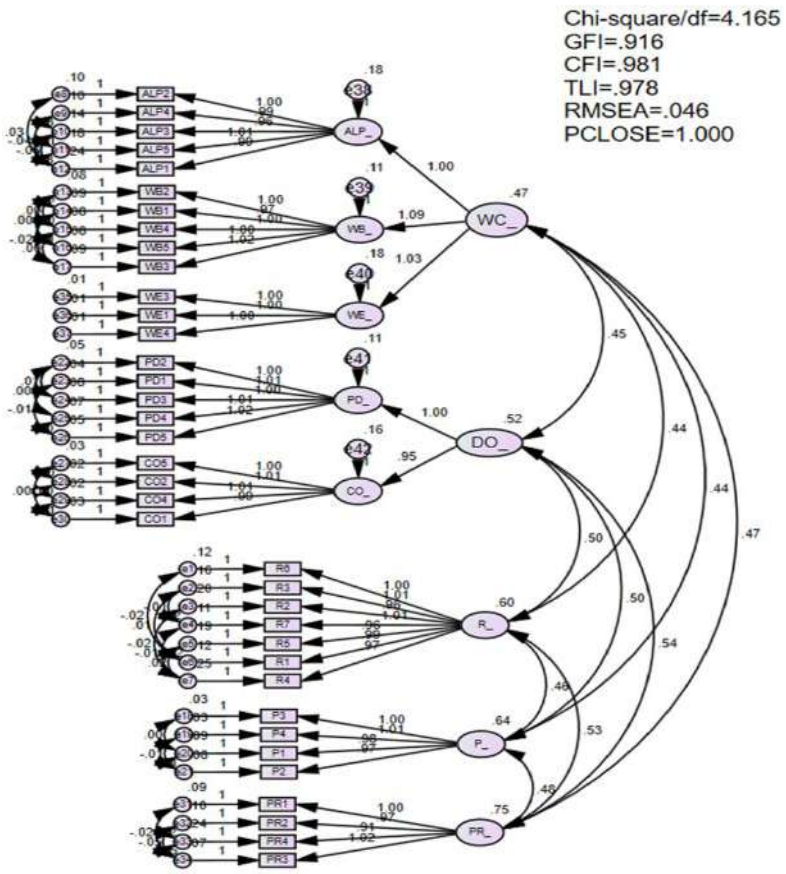

Figure 2. Description of CFA Confirmatory Factor Analysis results

3.2.8. SEM Test Results

Figure 3 provide the following including: goodness of fit index (GFI): This value ranges from 0 to 1, the GFI result for the pricing model is satisfactory at 0.916, indicating a good fit; root mean square errors of approximation RMSEA value is 0.046, which is considered satisfactory and reports for RMSEA suggest that a value below 0.08, with a 95% confidence level, is acceptable; comparative fit index (CFI): The CFI value is greater than 0.9, indicating a good fit, the higher the value, the better the fit and in this case, the model's CFI = 0.981, which is considered good; a TLI index with a value close to 1 indicates suitability, the model's TLI result is 0.978, which is considered good.

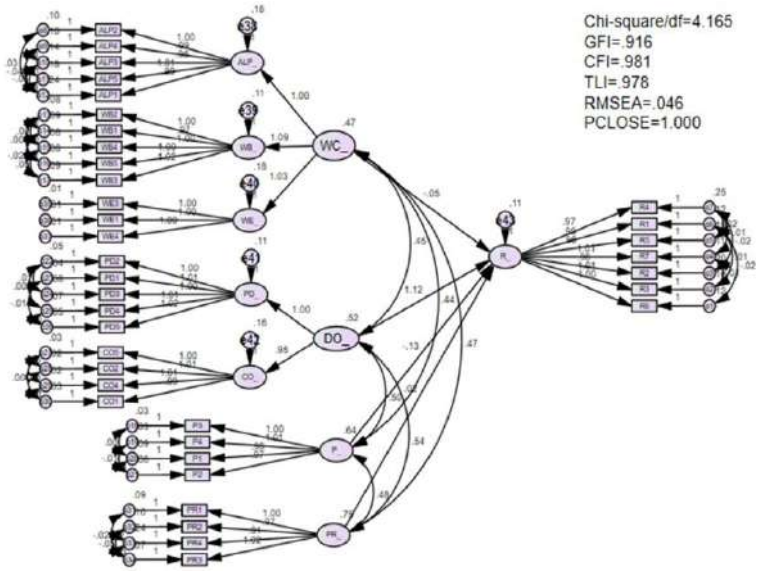

Figure 3. Description of test results (SEM)

3.2.9. Unstandardized Regression Coefficients (Estimates)

Regression Weights: (Group number 1 - Default model). The results depicted in Table 5 illustrate the impact factors as follows: Working conditions, development opportunities, promotion and pay-rate have a statistically significant impact on retention, with significance levels <0.05. *Hypothesis H1 (Working conditions) and H4 (Pay-rate)* don't have a positive impact while *hypothesis H2 (development opportunities) and H3 (promotion)* have a positive impact on retention.

3.3. Discussion of Factors Affecting Pharmacists' Engagement with the Organization at the Public Health Facilities in Mekong Delta 2023-2024

According to the survey results, we have identified four factors, including working conditions, promotion, development opportunities and pay-rate, all of which have a positive impact on retention.

The role of pharmacists in public health facilities is very important, ensuring that patients receive safe and high-quality care. As a result, they often experience high levels of pressure at work, which can affect their motivation and productivity. Consequently, working conditions, including legal policies, working environment and work benefits exert a strong influence on the pharmacist's retention within the organization[6]. However, a portion of survey participants think that there is not too much pressure at work or on patients. This perspective makes sense, given that numerous surveys indicate community pharmacists face higher levels of stress and pressure at work than pharmacists who work in hospitals[28].

Surveying pharmacists' attitudes with the factors such as salary or promotion opportunities is also very important because this information can help hospital leaders consider improving employee

satisfaction and increasing pharmacists' engagement with the organization. The motivation of pharmacists at work and their level of retention with the hospital both increase as their careers progress[29], and promotion also determines the pharmacist's work motivation. In addition, a sufficient salary is one of the main factors in retaining healthcare workers in their job[30]. Facility managers should have plans in place for paying their pharmacists so that the pharmacists can ensure the best use of their abilities and motivation to provide the best quality of service to customers[31].

Development opportunities for career advancement and professional growth are factors that highly interest pharmacists working in public health facilities. Pharmacists' work performance cannot be separated from the competencies they possess, and the skills required to meet the criteria necessary to perform their jobs well[32]. Between community pharmacists and hospital pharmacists, the latter have more opportunities to improve their knowledge and professionalism, such as participating in presentations at academic conferences, because they have access to detailed patient information, including background, medical history, and medical records[33]. Furthermore, hospital pharmacists are more likely to have contact with patients and they can also collaborate with general practitioners and other medical professionals to diagnose patients, thereby enhancing their skills and expertise[16].

According to research, retention is influenced by many factors. The relationship between pharmacists and public medical facilities will endure for a long time if hospital administrators can establish a reliable work environment. This environment should encourage pharmacists to suggest new ideas, innovate to advance the organization, and at the same time, provide the support they need to advance their careers[34].

Limitation: Firstly, the restriction to a single location may have introduced conflicts arising from personal problems and cultural prejudices. Therefore, the conclusions should not be generalized to other populations. Additionally, this study focused on factors influencing pharmacists' engagement with the organization in a specific context, specifically in relation to factors provided by public medical facilities to pharmacists. While we believe this context is representative of many other factor settings, it remains important to identify organizational service type and replicate the study in diverse contexts.

4. CONCLUSION

In Vietnam, human resource issues in the health sector, especially hospital pharmacists, consistently pose challenges for leaders. Recognizing this problem, research was conducted to provide results on factors that affect hospital pharmacists in their work, thereby breaking down each policy barrier, and strengthening the pharmacist's close relationship with the organization. The Mekong Delta is the largest delta in the country and is home to the southern key economic region. However, legal policies on hospital pharmacists within organizations are not convincing and clear, leading to a blurred connection between pharmacists and organizations, hindering the establishment of a sustainable bond. Therefore, we hope that researching this topic will bring great value to the pharmaceutical industry's economics as well as improve and enhance public health, helping organizations to establish suitable policies for pharmacists to reduce the problem of absenteeism and contribute to maintaining a stable workforce of pharmacists, preventing sudden shortages of human resources that greatly affect patient health. This section is not mandatory to summarize your main finding.

5. ACKNOWLEDGMENT

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Conflict of interest

The authors declare there are no competing interests related to this paper.

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Process Evaluation of Logistics Management Information System in Myanmar: A Qualitative Approach

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ABSTRACT

The quality of health products can be maintained through the proper implementation of the standardized logistics management information system (LMIS). This study aimed to evaluate the process of LMIS. The specific objectives were to explore the activities of logistics management and investigate the factors influencing the implementation of LMIS. In July 2023, semi-structured interviews were conducted with 14 health facilities staff who dealt with LMIS in health facilities providing services for HIV and Reproductive Health (RH) programs to the targeted communities in four regions and two states of Myanmar. The interviews' results were categorized into three themes: activities of logistics management, facilitators of the implementation, and barriers to the implementation. All staff agreed that implementing the standardized LMIS has positively impacted logistics management practices, leading to more efficient and systematic operations and better inventory management. However, the majority expressed dissatisfaction with current ordering and reporting practices, perceiving it as an unnecessary duplication of efforts. Results showed that LMIS implementation had an effect on capacity of health staff. All supply chain staff revealed that their inventory management skills noticeably improved after completion of in-person comprehensive supply chain management training, enabling them to manage products more efficiently. Most mentioned effective in-person training program, supportive supervision, and information technology as key facilitators for implementation. Barriers to implementation highlighted were online training sessions provided during COVID-19 pandemic and capacity issues, and technical-related challenges. It was found that logistics management activities such as ordering, and stock reporting were not performed as designed due to program-related barriers and the need for staff competence in some facilities and these challenges had to be rectified by central level and stakeholders together with health facilities. Therefore, some issues are needed to be invested such as capacity-building programs and software customization, to enhance the supply chain performance of health facilities.

KEYWORDS: Logistics Management Information System; LMIS; Process evaluation; Myanmar

1. INTRODUCTION

Access to quality essential health services and safe, effective, quality, and affordable essential medicines and vaccines for all is a critical benchmark for achieving universal health coverage[1]. However, according to the World Health Organization (WHO), one-third of the global population does not have regular access to medicines. The extent of the problem is even worse in some of the low-income countries in Africa and Asia, where more than half of the population has no reliable access to quality essential medicines[2]. Moreover, those countries spent an estimated 40% of their health budgets on medicines, with patients bearing the majority of the cost out of pocket. Extensive health system inefficiencies mean that up to a quarter of spending on medicines is wasted due to poor procurement and irrational use, substandard and expired medicines. There has been under-investment

in supply chain systems as well as inadequate monitoring of medicines management[3]. Therefore, the management of drugs and medical supplies is the critical to the success of any healthcare program.

Managing drug supply is a very complicated process that requires a robust organizational structure, and integrated supply chain[4]. Thus, a properly managed logistics management information system (LMIS) should be established in each health supply chain facilities to ensure an adequate supply of essential health products to the clients who need them. Logistics management (LM) is part of the supply chain management (SCM) that plans, implements, and controls the efficient, effective forward and reverse flow and storage of goods, services, and related information between the point of origin and the point of consumption in order to meet customers' requirements. It aims to achieve the six "rights" that the right products, in the right quantities, in the right condition, are delivered to the right place at the right time for the right cost. LM activities are the operational component of SCM, including functions such as quantification, procurement, inventory management, warehousing, transportation and fleet management, and data collection and reporting[5].

A data-driven LMIS is a subset of organizations' health management information systems and one of the pillars to achieving epidemic control[6]. LMIS is the system of physical- and technology-based records and reports that supply chain workers and managers use to collect, organize, present, and use logistics data gathered across all levels of the system[5]. The essential data captured are then combined to form stock reports, which are used for crucial decision-making about resupply quantities, forecasting and procurement decisions[7]. Unfortunately, LMIS is inadequate and still predominantly paper-based in many developing countries with incomplete and poor-quality data, considerable delays between data entry and availability for use, and lack of use. Moreover, a common issue with LMIS for essential medicines in developing countries is the absence of standardized forms and procedures for data collection and reporting. Therefore, as part of strengthening supply chain systems, there is an increasing focus in enhancing LMIS. Many national, international, and non-governmental organizations (NGO) have made investments in this area by providing technical assistance to developing nations[8, 9].

The Alliance Myanmar, as NGO, and its partners implemented standardized LMIS system and procedures by establishing standard operating procedures (SOPs), developing LMIS forms (Inventory Control Card, Issue and Receipt Voucher), and automating system through the use of pharmaceutical management software (mSupply) to enhance SCM in November 2021. This project aimed to enhance the inventory management, provide better visibility to essential data to make required decisions, and ensure uninterrupted service provision at health facilities. Prior to the implementation, LMIS was characterized by non-standardized data recording practices, resulting in data discrepancies and inconsistencies. Manual data entry and reliance on paper/ Excel-based records contributed to inefficiencies in tracking and monitoring inventory at all levels. In addition, this manual process often led to delayed reporting and limited visibility into supply chain operations. Moreover, the absence of digital tools for real-time stock monitoring further hindered the ability to make informed decisions. As part of the redesigned LMIS since 2021, the health staffs were trained on basic SCM, and standardized LMIS forms, procedures, and guidelines were distributed to health facilities.

Previous studies demonstrated that investing in LMIS enhanced logistics data quality and improved the logistics management practices. As a result, supply chain efficiency and the availability of commodities at health facilities have been improved, leading to better health outcomes for patients[4, 10, 11]. In Myanmar, although various LMIS initiatives have been launched in public health facilities of Ministry of Health (MoH) since 2013 with external donor support[12], the non-government sector supported by different stakeholders still needs to be tackled to enhance LMIS. Consequently, it is essential to gain a deeper understanding which logistics processes and factors affecting on SCM. Then, a process evaluation was carried out. It aimed to explore the activities of logistics management, and to investigate factors influencing the implementation of LMIS.

2. MATERIALS AND METHODS

2.1. Study Area and Setting

The study was conducted in 28 health facilities (central warehouse, health centers, and clinics) of Alliance and its partners providing HIV and RH related health services where standardized LMIS has been deployed. Alliance Myanmar is delivering health services for HIV and RH programs to the targeted communities located in Yangon, Mandalay, Tanintharyi, Sagaing Region, and Mon and Kachin State[13].

2.2. Design and Population

A qualitative method was employed. Fourteen health facilities staff were purposively included for an in-depth interview with one informant chosen from each facility, which implemented standardized LMIS. The informants who had experience of greater than six months in health logistics management and dealt with logistics management activities were purposively selected as the key informant (KI).

2.3. Instrument

An interview guide was applied to explore the current LM activities concerning product receiving, inventory management, and logistics management information system and to explore the key factors that affect the implementation of LMIS. The questions were developed in English and constructed according to relevant theories and literature review[5, 14, 15]. The guide was then conducted the content validity by three experts.

2.4. Data Collection and Analysis

An interview with health facilities staff responsible for managing LMIS was carried out using semi-structured interview guide in July 2023. The guide consisted of three parts: 1) general questions, 2) logistics management activities, such as product receiving, inventory management, and LMIS and 3) factors influencing the implementation. The informed consents were obtained prior to the interview from all key informants. The interviewer (NNH) had experience implementing a project to strengthen the supply chain system in Myanmar. Prior to the interview, the informants were encouraged to express their opinions freely, regardless of the interviewer.

Each face-to-face interview took 45-60 minutes. The interview was conducted by the researcher using local language, and the audio record was then transcribed to English language. Interview transcripts were coded, and thematic analysis was performed. This study was approved by Institutional Review Board of Mahidol University (COE.No.MU-DT/PY-IRB 2023/017.2504).

3. RESULTS AND DISCUSSION

3.1. Results

Of the 14 key informants (one warehouse staff and 13 health facilities staff) interviewed, more than half were non-health care professionals. Half of them were female. All informants have experience in logistics management between one and eighteen years (average of 5.6 years) and were ranging from 26 to 50 years old (average of 35.7 years old) (Table 1). Almost all were in charge in the facilities. The location and number of the facilities visited for interviewing 14 KIs were presented in figure 1.

In accordance with the objectives, the findings were categorized into three themes, which are 1) activities of logistic management, 2) facilitators of implementation, and 3) barriers to implementation.

Table 1. Characteristics of key informants (KI=14)

KI No	Gender	Age	Experience in health logistics (yrs)
1	Male	28	4
2	Male	27	3
3	Female	50	14
4	Female	41	18
5	Female	40	7
6	Female	30	8
7	Female	42	1
8	Female	46	2
9	Not Specify	42	1
10	Male	30	6
11	Not Specify	28	2
12	Male	33	1
13	Male	26	1
14	Female	37	10

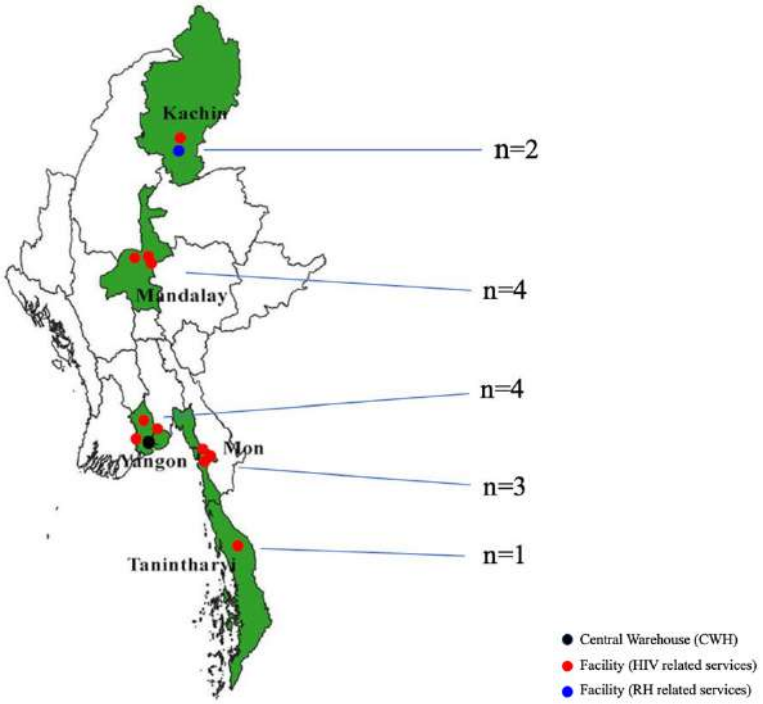

Figure 1. Location and number of the facilities (n=14)

3.1.1. Theme 1: Activities of Logistics Management

Health facilities have implemented standardized LMIS processes and tools for managing products efficiently. The three sub-themes that emerged from exploring the activities of logistics management were product receiving, inventory management (order, store and issue), and logistics management information system (data recording and reporting).

3.1.2. Product Receiving

Product receiving is the procedure that occurs when health products arrive at the storage area of receiving health facilities and were visually inspected by supply chain staff, who then verify the quantities of products received against invoice and report any discrepancies. All revealed their skills to carry out key warehousing activities of receiving significantly improved after completing basic supply chain training, enabling them to efficiently perform those activities according to standardized procedures such as unpacking and checking, recording, reporting any discrepancies and monitoring stock movement and expiry. One informant noted that the procedure for inspecting products upon arrival has been improved from a chaotic process to a thorough checking of expiry dates.

"Now, we promptly cross-check the expiration dates of the products when receiving them". [KI 10]

Moreover, the majority explained that they received the requested order quantities, which were determined based on average monthly consumption (AMC) and program targets, every quarterly or in a separate shipment within a quarter due to insufficient stock at Central Warehouse (CWH).

"Despite our requests for the required amounts, the issued quantities were based on stock availability at Central." [KI 12]

3.1.3. Inventory management

Inventory management is the core of the supply chain that included order, store, and issue activities. With regard to ordering activity, all KI mentioned that the quantification of the required quantities is now relied on the consumption patterns, remaining stock balances and program targets. However, almost all acknowledged employing diverse procedures for ordering products at their facilities after quantifying the required products. These methods included orders placed by both telephone and email, telephone orders alongside mSupply, exclusive mSupply orders, and paper-based order request form submitted through Viber. Certain health facilities opt for submitting orders via email due to the fact that they need to coordinate with Central's Project Officers (PO) to review their orders and approve their orders based on the program targets. Therefore, the red highlights in the ordering process (Figure 2) represents the procedures carried out in some health facilities that encounter program-related barrier in adhering to the implemented standardized system. However, most replied that they are attempting to submit orders either by phone or email, in addition to using mSupply to ensure that products they requested are available at CWH.

By allowing them using their preference options, all health facilities will transition to submitting orders using stock requisition function of mSupply for simplifying order processing as discussed by the warehouse focal. She highlighted that *"if facilities enter stock requests through mSupply, the workload is significantly reduced, and it becomes much easier to access and view the Stock on Hand (SOH) of the ordering facility, simplifying the resupplying process"*

Regarding storage, all responded that products were kept according to First Expiry First Out (FEFO) procedures in a separate shelf or cupboard and the expired products were stored in a discrete place. However, one informant expressed the inconvenience of using a dry ice bag, which requires adding water weekly, due to the facility's lack of a freezer for storing products like HIV test kits.

In terms of issuing products, nearly all KIs from health facilities expressed that they distributed products to peer educators (PE) and volunteers by calculating the issued quantities based on consumption, the number of clients, and the program's targets. Therefore, dispensing to clients is carried out through PE in almost all health facilities, except for three that provide treatment services only.

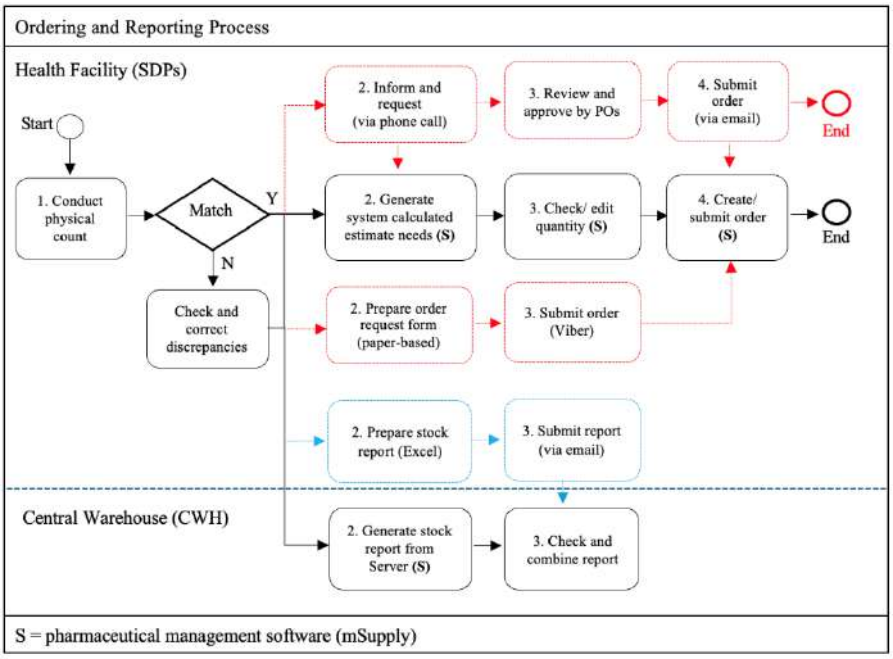

Figure 2. Current Inventory Management Process Diagram

3.1.4. Logistics Management Information System (LMIS)

LMIS is an organized system for recording, reporting, and using information for decision-making. More than half highlighted that the recording of logistics data is now seamless with inventory control cards (ICC) and mSupply which allowed them for tracking details stock information and assessing the stock levels to avoid shortage and expired. However, some emphasized the need for carefully and accurately entering logistics data to prevent any mistakes in data entry, as well as the time required to become familiar with the stock management software.

This study unveiled that nearly all, except those managing a few products, were dissatisfied with the current practice of using Excel for stock reporting, which usually takes 1-2 working days. This results in operational inefficiencies because they have been assigned to handle LMIS activities and also have responsibilities related to other program activities. As indicated in the above figure (Figure 2), warehouse staff are responsible for generating reports of all health facilities from the mSupply Server. These reports will then be checked and consolidated before being submitted to the respective donors. However, data discrepancies when checking generated stock reports have prompted CWH to request health facilities to provide monthly/quarterly stock reports in Excel format. During an interview, warehouse staff discussed that *"this approach is primarily necessitated by the fact that not all health staffs at Service Delivery Points (SPDs) possess the capability to accurately input the essential logistics data into mSupply to be able to directly generate stock report from the system's Server"*.

It was also noted that some KIs failed to record the reallocation and local procurement invoices into mSupply as instructed by the warehouse staff which leads to data discrepancies in stock reporting. On the other hand, a considerable concern raised by some staffs is the manual entry of data into Excel report format in addition to routinely entering the same data into mSupply. This redundancy in data entry processes raised concerns, as it was perceived as an unnecessary duplication of efforts. Furthermore, one KI elaborated that this practice introduced the potential for data discrepancies.

3.1.5. Theme 2: Facilitators of the Implementation

The KIs were queried regarding factors that could facilitate the LMIS implementation. The three sub themes within the facilitators of the implementation were effective in-person training program, supportive supervision, and information technology.

3.1.6. Effective In-Person Training Program

Nearly all of them are responsible for inventory management at their facilities without proper supply chain training prior to implementation. Therefore, majority highlighted an appropriate understanding of the concept of basic SCM as facilitator, which significantly enhanced their awareness of the expiry dates of the products. In addition, they explained that the deployed system provided better visibility into real-time stock information, contributing to improved inventory management and reduced wastage.

"In fact, I was responsible for stock management, but I did not receive any training specifically related to stock management prior to 2021. I learned more about the procedures and gained a better understanding after receiving the basic supply chain training". [KI 4]

3.1.7. Supportive Supervision

Almost half highlighted the effectiveness of M&E visits to their health facilities conducted by technical support team and also mentioned that customized assistance or guidance offered by them to address specific areas requiring improvement helps them feel confident in managing products.

"M&E visits have proven to be highly effective for us. Despite receiving training online or in-person, we encountered some mistakes and errors in the daily practice. However, the valuable insights gained from the explanation of the individual sites' weaknesses during these visits by the technical team have been instrumental in building our confidence and expertise in using this standardized system". [KI 8]

3.1.8. Information Technology

The regular practice of recording the essential logistics data into the system (mSupply), often on a daily or weekly basis, remains crucial in maintaining the availability of real-time stock data and tracking the detailed stock information such as stock on hand, expiration dates, and batch numbers of each product as replied by most KIs.

"We can easily access the expiry information in the system without visiting physical store". [KI1]

3.1.9. Theme 3: Barriers to the Implementation

Barriers identified by KIs were categorized into two sub-themes: online training and capacity issues, and technical-related challenges.

3.1.10. Online Training and Capacity Issues

It was revealed that most complained online training sessions which offered during COVID-19 pandemic due to the travel limitation in Nov 2021. While online training offers convenience, it may lack the interaction and quick problem-solving capabilities of in-person sessions.

"When training is conducted online, we encounter certain limitations, including a poor internet connection that disrupts the session flow and hinders our ability actively engage. Moreover, the online format is challenging for us in maintaining full concentration, which could impact our ability to grasp the content with 100% focus". [KI 8]

3.1.11. Technical-Related Challenges

Some emphasized the constraints associated with internet connectivity, technical proficiency, and software feature limitation when managing batches in the mSupply mobile application run on tablets. One informant shared her experience with network issues while using software to manage inventory, as it relies on network connectivity to ensure essential data is available and accessible at every level.

"One challenging aspect has been the synchronization of data between offline and online servers, particularly due to internet connection issues". [KI 14]

One software feature limitation explained by two key informants were as follows:

"There is one thing in the system (mSupply tablet), it displays the same item (e.g. condom) with difference batch numbers as a whole amount so it is difficult for us when issuing products. [KI 1]

"It would be better if I could choose batch number when issuing the products in the system (mSupply tablet)". [KI 2]

3.2. Discussion and Recommendation

The findings that not all facilities utilize the implemented system for ordering health commodities, despite having the ability to analyze available stock, average monthly consumption, and suggested order quantities within the system, raises important questions about the utilization of this valuable tool. One potential explanation for this concern could be administrative-related matters involving project officers who must approve the requested order quantities from facilities to align with project goal. Therefore, further coordination meetings among facilities, central and technical teams are needed to identify the best options to enable the smooth ordering of products through the system and reduce the double workload of facility staff.

Regarding data recording practices, some KIs should have recorded reallocation and local procurement transactions in mSupply, as on ICC, contributing to noteworthy challenges in data entry practices and reporting accuracy. To mitigate this problem, a targeted approach that includes refresher or on-the-job training focusing on this kind of scenario with clear instructions on data entry expectations at both central and service delivery levels is needed to organize for enhancing data use and decision-making processes.

In addition, the observed challenges regarding the reporting burden predominantly arise from the diverse educational backgrounds, varying skill sets, and limited numbers of staff. Many of them come from different academic backgrounds unrelated to health, resulting in limited skills and competency. The study showed that most have been designated to manage LMIS activities and other program responsibilities leading to operational insufficiency. Similarly, a study carried out in Ethiopia demonstrated that insufficient healthcare staff was among the challenges that influenced the LMIS performance[4]. These findings underscore the need for targeted capacity building initiatives to enhance their technical skills and competency, ensuring more efficient reporting processes. In a response to this obstacle, it is recommended to establish a collaboration between central and technical support teams by verifying the monthly stock Excel reports submitted by health facilities with the stock reports generated from the system, beginning with a small sample of two to five health facilities.

The findings revealed that the limited stock at CWH caused stock-out conditions at lower-level health facilities. Therefore, it is vital to comprehensively review the current quantification and forecasting (Q/F) process in the procurement cycle to explore the shortage of health products at CWH. This review should focus on identifying potential bottlenecks and areas for improvement to enhance the efficiency and accuracy of forecasting. Additionally, it is crucial to reinforce data-reviewing practices at every level of the supply chain. By prioritizing rigorous data reviews, the supply chain can ensure the reliability and consistency of information, leading to more informed decision-making and optimized inventory management. This approach of Q/F process review and data reinforcement is a collaborative effort that not only contribute to the overall effectiveness of the supply chain but also highlight the importance of each stakeholder's contribution to this process.

One limitation of this study is its focus solely on project health facilities of non-government organizations, which provide specific healthcare services for key populations within the country.

Consequently, the results may not be fully representative of the entire non-governmental sector. However, the findings offer valuable insights for organizations aiming to enhance their supply chain systems in similar contexts. By evaluating the recording process of LMIS utilizing a tablet module, this study serves as a reference for organizations considering electronic LMIS implementation at their service delivery level.

Lastly, a cost analysis is recommended for further research, as it helps with the estimation of the necessary budget for supply chain investments.

4. CONCLUSION

It is evident that there is an opportunity for improvement in logistics management activities, including ordering and reporting. This is because certain health facilities were unable to operate at their full capacity as intended due to program-related obstacles and the requirement for competent staff. In order to address these challenges, both the central level and stakeholders collaborated with the health facilities.

The factors including in-person training sessions, supportive supervision and, information technology had a significant effect on efficient supply chain operations. Therefore, it is necessary to invest in capacity-building programs, monitoring, and evaluation, as well as software customization in order to improve the supply chain performance of health facilities.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Knowledge, Attitudes, Practices, and Challenges in the Use of Pharmacoeconomic Evaluations among Hospital Pharmacists Involved in the Pharmacy and Therapeutics Committee in Selected Tertiary Private Hospitals in the National Capital Region

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ABSTRACT

As healthcare undergoes rapid evolution globally, the accompanying rise in the cost of medicines and services underscores the importance of using pharmacoeconomic tools, especially in developing countries like the Philippines. This study aims to assess the application of pharmacoeconomic evaluations in selected hospitals within the Philippines, focusing on knowledge, attitudes, practices, and challenges among hospital pharmacists. Using convenience sampling, data was collected from 11 out of 30 tertiary private hospitals situated in the National Capital Region (NCR). A total of 24 hospital pharmacists participated by completing questionnaires focusing on four key parameters of pharmacoeconomic evaluations: knowledge, attitudes, practices, and challenges associated with utilizing these tools. Among the hospital pharmacists involved in this study, a substantial proportion (87.5%) reported utilizing pharmacoeconomic evaluations in their practice. Common practices included identifying medication costs and benefits and making formulary recommendations based on pharmacoeconomic indices, primarily focusing on initial evaluation stages. Respondents identified challenges such as limited understanding hindering effective application, with pharmacists predominantly focusing on traditional evaluations of efficacy, safety, and acquisition. Additionally, the complexity of pharmacoeconomic concepts and a lack of training to overcome them were noted. Improved training and comprehension in pharmacoeconomics are necessary to address these limitations.

KEYWORDS: Challenges; Knowledge; Hospital Formulary; Pharmacoeconomics; Pharmacy and Therapeutics Committee; Practices

1. INTRODUCTION

A recent upsurge in global healthcare expenditures prompted increased attention towards medication costs and evaluation[1]. The necessity for appropriate economic measures is further augmented by the dilemma of selecting from an array of available medications when confronted with budgetary constraints[2]. The process of evaluating pharmaceuticals was expanded to address these concerns, including considerations for economic implications besides treatment efficacy and safety[3].

Pharmaceutical evaluations made within an economic context fall under the concept of pharmacoeconomics[3]. As a branch of health economics[4], it primarily aims to evaluate the costs and consequences of alternative pharmaceutical interventions using different techniques[5]. Four of the most widely used techniques include cost-effectiveness analysis (CEA), cost-benefit analysis (CBA), cost-minimization analysis (CMA), and cost-utility analysis (CUA). This approach bridges economics, medicine, and humanity to achieve optimal allocation of healthcare resources.

Despite global challenges in affording and accessing quality healthcare, pharmacoeconomics empowers healthcare professionals to make informed decisions. By understanding the pharmacoeconomics of drug therapies, professionals can develop strategies to reduce medication costs and optimize patient care, potentially improving patient outcomes and quality of life[6]. Pharmacoeconomics can also guide drug use policies and influence prescribing practices to achieve better patient outcomes at lower costs[7, 8].

Furthermore, pharmacoeconomics allows for comparisons between different treatments, aiding in selecting preventive measures and established treatments[9]. This information is crucial for developing clinical practice guidelines, as demonstrated by managed care organizations implementing cost-effective programs[10]. Pharmacists can also leverage pharmacoeconomics to design patient-centered disease management programs[11].

However, underutilizing pharmacoeconomics is a concern in developing nations due to limited knowledge, infrastructure, and trained analysts[12, 13]. Robust data collection on drug usage, safety, and pricing are essential for effective implementation. Without such data, decisions regarding drug selection lack a strong foundation, leading to increased costs without improved treatment outcomes[14].

The knowledge of pharmacoeconomics is at a stage of infancy, and developing countries need help to afford to invest money in the healthcare sector to get reliable and continuous data regarding usage, safety and pricing of drugs. Pharmacoeconomics is a branch of medicine in which rational decisions are based on evidence. A robust data-gathering machinery should be in place for enough beneficial evidence to accumulate with the use of medicine. The collection of data should be a continuous, reliable, and transparent process. In the absence of strong data-gathering machinery in the country, decisions regarding the selection of drugs are made on poor rationale, and the system ends up paying more with no benefit in the treatment of disease[14].

A 2017 study reported that the pharmacoeconomic capacity within the Philippines remains poor[15]. To our knowledge, no subsequent literature presented a progress report on the extent of the country's pharmacoeconomic competence. For this reason, this study investigates how Pharmacy and Therapeutics Committee (PTC) hospital pharmacists in private tertiary hospitals within the National Capital Region (NCR) of the Philippines utilize pharmacoeconomic evaluations. The research focuses on four key areas: knowledge, attitudes, practices, and challenges related to these evaluations. Specifically, the study explores whether pharmacists' years of experience correlate with their understanding and perspective on using pharmacoeconomic evaluations. It will also assess the prevalence of the use of these evaluations among these pharmacists. Furthermore, the research will identify the most common practices employed by pharmacists from different hospitals when conducting pharmacoeconomic evaluations. Finally, the study will determine the most significant challenges that hinder the use of pharmacoeconomic evaluations in these tertiary private hospitals.

Embracing pharmacoeconomic principles is vital for achieving the long-term goal of affordable, safe, and effective healthcare for all. Selecting, utilizing, and monitoring cost-effective medications ensures high-quality therapy at an acceptable price for the most significant number of people[16].

It is our collective responsibility to embrace the principles of economics in the health industry so that the vision of affordable, safe, and adequate healthcare can be delivered for a long time. Today, the rationalization of pharmacotherapy is needed. Effective, safe, and economical drugs must be selected, used, and monitored to ensure high-quality therapy at an acceptable price for as many people as possible [16].

2. MATERIALS AND METHODS

2.1. Research Design

This paper is a descriptive quantitative study. It entails the need to show the traits of the participants or the relevant phenomenon without changing any of the variables. It is also essential to measure these variables to gain insights regarding the knowledge, attitudes, practices, and challenges

related to the application of pharmacoeconomic evaluations in selected hospitals in the National Capital Region.

This study focuses on hospital pharmacists in the PTC. The collected categorical data from the respondents were described using frequencies and percentages, while the collected continuous data were described using means and standard deviations. Responses to the questionnaires were also presented using frequencies and percentages.

2.2. Respondents

The participants in this research project are hospital pharmacists engaged in the PTC within selected tertiary private hospitals in the NCR. The choice of tertiary private hospitals is deliberate, considering their reputation for leading in advanced research and innovation and their crucial role in medical education and training. The participants include hospital pharmacists serving in the PTC, and their involvement in the drug selection processes is essential for understanding the utilization of pharmacoeconomics in these hospitals. The decision to focus on hospital pharmacists engaged in PTC is purposeful, as they represent a pivotal cohort for evaluating pharmacoeconomic knowledge. These professionals are crucial in shaping and executing drug policies within healthcare institutions.

Inclusion Criteria

The inclusion criteria in this study are the following: (1) private hospitals that are located at the NCR, (2) private hospitals that are under tertiary level located at the NCR, and (3) hospital pharmacists that are members of the PTC in private tertiary hospitals located in NCR at present.

Exclusion Criteria

The following statements are the exclusion criteria needed for this study: (1) private hospitals that are not located at the NCR, (2) private hospitals that are under primary or secondary level, and (3) hospital pharmacists that are not a member of the PTC and other members of the PTC such as: infection disease expert, pediatrician, obstetrician-gynecologist, family medicine, public health specialist, psychiatrist, City Health Officer, Public Health Nurse, Midwife, and Medical technologist.

2.3. Sample Size and Sampling Method

The target population for this study are hospital pharmacists involved in the Pharmacy and Therapeutics (P&T) Committee in tertiary private hospitals within the National Capital Region (NCR). Due to limitations in time and the potential for a low response rate within individual hospitals (i.e., lack of pharmacists willing to participate per hospital), a convenience sampling approach is employed.

Initially, all 30 tertiary private hospitals in NCR were identified as potential participants. However, eight hospitals declined to participate in this study, resulting in a final sampling frame of 22 hospitals. Hospitals A, B, C, D, E, F, and G have 1 respondent. Hospital H has 2 respondents. Hospital I has 4 respondents. Hospital J has 5 respondents. Hospital K has 6 respondents. The respondents of this study totals to 24.

Convenience sampling was chosen due to the aforementioned constraints. This method involved selecting participants who are readily available and accessible to these researchers. While convenience sampling may not ensure a perfectly representative sample, it is still a valuable tool for initial research, particularly when resources are limited.

2.4. Instrument

These researchers employed a formal request letter as a means of engaging participants, aligning with the imperative of verifying confidentiality and facilitating effective communication among both the participants and these researchers. Furthermore, informed consent letters were distributed to the respondents to ensure their comprehension of this study's process and subject matter. The informed consent letter contained an introduction, purpose of this research, study participants,

clarification of the procedural aspects, responsibility of participants, duration, risks, benefits, confidentiality, voluntary participation, access to study results, costs to respondents, compensation, and contact information. Simultaneously, a questionnaire from a study entitled "Assessing the Application of Pharmacoeconomic Evaluations in Medicines Management by Hospital Pharmacists in Nigeria: A Cross-Sectional Survey"[17] authored by Theophilus Ehidiamen Oamen, Kanayo Patrick Osemene, and Romanus Maduabuchi Ihekoronye, was adapted to serve as the principal data collection instrument.

However, to validate the questionnaire, a panel of two experts on pharmacoeconomic evaluations performed content validation following the studies of Kovacic (2017)[18] and Polit (2007)[19]. Experts were asked to rate the relevance of each item on the instrument using a four-point ordinal scale: 1 – not relevant, 2 – somewhat relevant, 3 – quite relevant, and 4 – highly relevant. For each item, an item-level CVI (I-CVI) was computed by dividing the number of experts who gave a rating of 3 or 4 (relevant) by the total number of experts. A comment section was provided after each item to allow for additional feedback. Items with an I-CVI greater than 0.78 were kept while items with an I-CVI near 0.78 were revised. On the other hand, items with a low I-CVI were deleted.

Based on the item-level content validity index per question in this study, four questions—S2.8, S2.9, S2.14, and S2.15—received a score of 0.00 and were recommended for deletion. Consequently, they were removed from the survey questionnaire distributed to the participants.

Meanwhile, eight questions—S2.4, S2.13, S3.5, S3.6, S3.7, S5.10, S6.1, and S6.2—received a score of 0.50 on the item-level content validity and were recommended for revision. As a result, these researchers revised these questions based on the guidance provided by the validator's comments and concerns, to enhance the alignment of the specific questions with the objectives and purpose of this study, as well as to better resonate with the real-life situations encountered by hospital pharmacists who are members of the PTC.

The same panel of experts was asked to evaluate the instrument a second time using the same four-point ordinal scale. I-CVI values were calculated again for all items. Average scale-level (S-CVI/Ave) was also calculated to determine the overall content validity of the instrument. Items scored as relevant (an I-CVI > 0.78) were added together and divided by the total number of items to calculate S-CVI/Ave. The recommended value for S-CVI/Ave was 0.90. This time, the validator confirmed that the questionnaires were better aligned with the context of Philippine hospital pharmacists who are members of the PTC in selected tertiary NCR hospitals.

This questionnaire was given to participants, with their responses subjected to analysis through a statistical treatment of data. These research tools provided significant advantages for directing the methodical gathering of data regarding the knowledge, attitudes, practices, and challenges of hospital pharmacists engaged in PTC activities. This pertains specifically to the utilization of pharmacoeconomic evaluations within selected private tertiary hospitals in the National Capital Region (NCR) at a single point in time.

2.5. Ethical Procedures Applied

This research endeavor prioritized ethical conduct throughout the data collection process. To ensure transparency, researchers declared any potential conflicts of interest before and during the study, as well as during interactions with participants. Data privacy was paramount, with researchers adhering strictly to the Data Privacy Act of 2012. This ensured confidentiality for both participants and the participating hospitals. Anonymity was guaranteed, and access to the collected data was restricted to the research team, which included a statistician and research advisors. The data will be used exclusively for this research project and will be destroyed upon the thesis defense completion.

Prior to survey administration, all participants received an Informed Consent Form. This document comprehensively outlined the study, including the target population, participant responsibilities, the research purpose, procedures, anticipated duration, potential risks and benefits, confidentiality measures, voluntary participation, access to research results, and any associated costs or compensation. Completion of the Informed Consent Form was mandatory before participants could submit the surveys, ensuring informed consent from all participating hospital pharmacists.

The researchers acknowledged potential risks for participants. Completing the surveys might demand valuable time and resources, potentially leading to increased stress and fatigue that could impact their primary duties. Additionally, factors such as participants' mental, physical, and emotional state, along with their comprehension of the questions and topic, could introduce bias and inaccurate responses. To mitigate these risks, researchers ensured appropriate timing for survey administration, guaranteeing contact with the designated person before hospital visits. Tokens of appreciation, such as snacks, were offered during questionnaire collection. Ample time was allotted to minimize participant discomfort, and researchers provided a brief explanation of the study's objectives and benefits to ensure alignment with participants' time constraints and comfort level.

The voluntary nature of participation was emphasized throughout the process. Participants were informed of their right to withdraw at any point without repercussions. No costs were associated with participation, and researchers treated participants with respect and consideration, prioritizing their well-being and preventing any physical or mental harm. Potential risks, such as compromised confidentiality, threats to professional standing, or disruptions to workplace dynamics were also communicated. To address these concerns, researchers took measures to minimize the impact on participants' professional responsibilities, offering ample time for survey completion, and maintaining a strictly professional tone in all communication.

Finally, given the involvement of human participants, the researchers sought ethical approval from the UST Faculty of Pharmacy Research Ethics Committee to guarantee adherence to established ethical standards.

2.6. Data Analysis

The collected categorical data from the respondents were described using frequencies and percentages, while the collected continuous data were described using means and standard deviations. Responses to the questionnaires were also presented using frequencies and percentages.

Following the methodology of Oamen et al. (2021)[17], means and standard deviations were used to summarize the responses of the respondents per question of every section of the questionnaire. A ranking of the attitudes of hospital pharmacists toward the application of pharmacoeconomic tools, practice of pharmacoeconomic evaluations among respondents, and challenges to the use of pharmacoeconomic evaluations among respondents was presented based on the mean scores per question of each section. The student’s t-test, Mann-Whitney U test, analysis of variance, and Kruskal-Wallis H test were then used to compare the responses of the participants for each question of the section pertaining to the knowledge of pharmacoeconomic tools/concepts of hospital pharmacists, as applicable.

For statistical analyses that required testing of hypotheses, a 5% significance level was used as a threshold to determine whether to accept or reject the null hypotheses. All analyses were conducted using STATA version 16.1 and Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Prevalence of the Use of Pharmacoeconomic Evaluations among PTC hospital pharmacists working in tertiary private hospitals in NCR

Table 1 presents the demographic profiles of the respondents. In terms of years of practice, 16 (66.67%) have 5 years or less, 3 (12.50%) have 6 to 10 years, 2 (8.33%) have 11 to 20 years, 2 (8.33%) have 21 to 30 years, and 1 (4.17%) has 31 years and above. In addition, among the 24 respondents, 21 (87.50%) stated that they practice pharmacoeconomic evaluations in their institutions – a positive trend towards pharmacoeconomic use.

Table 1. Demographic profiles of the respondents.

Profiles	Frequency (n = 24)	Percentage
Years of practice		
5 years and below	16	66.67%
6 years to 10 years	3	12.50%
11 years to 20 years	2	8.33%
21 years to 30 years	2	8.33%
31 years and above	1	4.17%
Practice of pharmacoeconomic evaluations in respondent's institution		
Yes	21	87.50%
No	3	12.50%

[1]Frequencies and percentages are presented for categorical variables, while the means and standard deviations are presented for continuous variables

3.2. Knowledge of Pharmacoeconomic Tools or Concepts of Pharmacy and Therapeutics Committee Members

Table 2 presents participants' responses to the statements about their knowledge of pharmacoeconomic tools or concepts. Among the statements, knowledge on the strategic pricing for pharmaceuticals, return on investments, and cost-minimization analysis were highly rated by the participants. On the other hand, the respondents rated naturalistic economic evaluation of pharmaceuticals, cost-utility analysis, economic modeling in medicine selection, and economic evaluation alongside clinical trials for new medicines the least in terms of knowledge.

Strategic pricing for pharmaceuticals, having a mean of 3.79, had garnered the highest rank on the knowledge of pharmacoeconomic tools of the PTC members. This is such an important concept to concentrate on, as pricing policies should clearly prioritize securing high-quality product supply security, as well as providing consumers and health systems with affordable and equitable access to quality-assured pharmaceutical products. Furthermore, it will ensure value for money based on improved population health outcomes[20].

In addition, return on investment has ranked 2nd among all the pharmacoeconomic tools/ concepts being utilized by the hospital pharmacists involved in the PTC. Hospital pharmacists are not used to estimate an ROI, as it can be beneficial to argue his/ her case with his financial director[21]. However, the results show it garnered a mean of 3.00. Besides, ROI is an essential concept, as the financial benefits of healthcare quality improvement (QI) are increasingly being assessed using return on investment (ROI)[22].

Leading to the third rank is the cost- minimization. This entails assessing drug prices to determine the least expensive medicine or therapy method and considering the expense of preparing and giving a dosage. Cost-minimization analysis, ranking third, involves assessing drug prices to determine the most cost-effective therapy. This aligns with the "Generics Act of 1988," which emphasizes supplying drugs at the lowest cost, especially to indigent patients. Despite the use of cost-effectiveness analysis (CEA) in the Philippines' official formulary, the country's pharmacoeconomic capability remains low, warranting guidance from international authorities. Naturalistic pharmacoeconomic studies, which gather data on patient compliance, received the least response, indicating a mean of 2.42. Cost-utility analysis, economic modeling in medicine selection, and economic evaluation alongside clinical trials face challenges due to resource constraints, infrastructure limitations, and expertise shortages.

Table 2. Knowledge of pharmacoeconomic tools or concepts of Pharmacy and Therapeutics Committee Members

Statement	Responses[1]				Mean Score	Rank
	Poor	Fair	Good	Excellent		
1	-	9 (37.50%)	14 (58.33%)	1 (4.17%)	2.67 (0.56)	7th/8th
2	-	6 (25.00%)	15 (62.50%)	3 (12.50%)	2.88 (0.61)	4th/5th
3	-	5 (20.83%)	15 (62.50%)	4 (16.67%)	2.96 (0.62)	3rd
4	1 (4.17%)	10 (41.67%)	11 (45.83%)	2 (8.33%)	2.58 (0.72)	11th
5	-	13 (54.17%)	7 (29.17%)	4 (16.67%)	2.63 (0.77)	9th/10th
6	1 (4.17%)	9 (37.50%)	12 (50.00%)	2 (8.33%)	2.63 (0.71)	9th/10th
7	1 (4.17%)	13 (54.17%)	9 (37.50%)	1 (4.17%)	2.42 (0.65)	12th
8	-	3 (12.50%)	19 (79.17%)	2 (8.33%)	3.79 (3.90)	1st
9	1 (4.17%)	7 (29.17%)	15 (62.50%)	1 (4.17%)	2.67 (0.64)	7th/8th
10	-	8 (33.33%)	15 (62.50%)	1 (4.17%)	2.71 (0.55)	6th
11	-	6 (25.00%)	15 (62.50%)	3 (12.50%)	2.88 (0.61)	4th/5th
12	-	3 (12.50%)	17 (73.91%)	3 (12.50%)	3.00 (0.52)	2nd
Mean of means					2.75 (0.64)	

[1] Frequencies and percentages are presented for categorical variables, while the means and standard deviations are presented for continuous variables.

This is prevalent, especially that "Generics Act of 1988" declared the policy of the State to ensure the adequate supply of drugs with generic names at the lowest possible cost and endeavor to make them available for free to indigent patients, and to promote, encourage and require the use of generic terminology in the importation, manufacture, distribution, marketing, advertising and promotion, prescription and dispensing of drugs.

Despite its role in addressing health finance concerns, a 2017 study reported that the pharmacoeconomic capacity within the Philippines remains poor[15]. International authorities must nevertheless provide guidance when using stronger pharmacoeconomic techniques, even though the country's official formulary already uses CEA. However, it ranked 4th/5th only in the knowledge of pharmacoeconomic concepts tools and has a mean of 2.88.

Naturalistic pharmacoeconomic studies are mostly non-interventional. Unlike interventional clinical trials, observational studies offer the opportunity to gather data on patient compliance[23]. Having said that, it had the least response rate, garnering a mean of 2.42.

Cost-utility analysis, economic modeling in medicine selection, and economic evaluation alongside clinical trials for new medicines have the lowest response rates due to the insufficient resources, infrastructure, and expertise. Furthermore, challenges stated on Table 5 can also add up to these circumstances

3.3. Attitudes of Hospital Pharmacists who are Members of the Pharmacy and Therapeutics Committee Toward Application of Pharmacoeconomic Tools

Table 3 presents participants' responses to the statements regarding their attitudes toward the application of pharmacoeconomic tools. The highest-rated statement was the one pertaining to their belief that these concepts are beneficial to hospital pharmacy practice. This is followed by their belief that identifying and valuing the costs and benefits of alternative drug regimens will improve the quality of decision-making for patients' drug therapy and their willingness to undergo training to enhance their know-how in pharmacoeconomic concepts. On the contrary, the statements "cost of drugs should not be the main factor when deciding which drug to use for a specific person's treatment," "analyzing the costs and benefits of different medications is too complicated for the

setting where I work," and "I doubt if we can accurately measure and put a value on the hidden costs and benefits in what we do" ranked the least among the respondents.

Attitudes comprise cognitive (knowledge), affective (emotions), and behavioral components[24]. Hence, the results from Table 3 regarding the pharmacists' attitudes on pharmacoeconomics can be associated with the results gathered from Table 2 about their knowledge of pharmacoeconomic tools and concepts. The mean score for Table 3 is 3.86, which leans towards a more positive category (agree), while the mean score for Table 3 provides a mean score of 2.75, indicating that the knowledge of the respondents ranges from fair to good. This shows that the respondents may have a positive attitude toward pharmacoeconomics; however, their knowledge is still insufficient, which may have affected their answers in Table 3, as one of the highest-rated statements in this table is their willingness to undergo training, recognizing that there is still room for more learning and improvement.

Conversely, attitude serves as a vital precursor and/or predictor of behavior[24]. Thus, despite the results of their attitudes, it is not strong enough to induce a behavioral change. Therefore, the results in Table 3 regarding their practice on pharmacoeconomic evaluations showed that pharmacists practice more on theoretical than practical application.

Table 3. Attitudes of hospital pharmacists who are members of the Pharmacy and Therapeutics Committee toward application of pharmacoeconomic tools

State-ment	Responses[1]					Mean Score	Rank
	Strongly disagree	Disagree	Cannot say	Agree	Strongly agree		
1	-	-	1 (4.17%)	10 (41.67%)	13 (54.17%)	4.50 (0.59)	1st
2	-	-	3 (12.50%)	10 (41.67%)	11 (45.83%)	4.33 (0.70)	2nd
3	-	-	4 (16.67%)	13 (54.17%)	7 (29.17%)	4.13 (0.68)	4th
4	-	-	3 (12.50%)	13 (54.17%)	8 (33.33%)	4.21 (0.66)	3rd
5	-	3 (12.50%)	14 (58.33%)	3 (12.50%)	4 (16.67%)	3.33 (0.92)	6th
6	-	11 (45.83%)	9 (37.50%)	2 (8.33%)	2 (8.33%)	2.79 (0.93)	7th
7	-	1 (4.17%)	8 (33.33%)	12 (50.00%)	3 (12.50%)	3.71 (0.75)	5th
Mean of means						3.86 (0.94)	

[1]Frequencies and percentages are presented for categorical variables, while the means and standard deviations are presented for continuous variables.

3.4. Association between the Years of Experience of PTC Hospital Pharmacists and with Their Knowledge and Attitudes toward the use of Pharmacoeconomic Evaluation

Table 4 presents the results of the statistical tests which verify whether statistically significant differences exist in the mean scores for knowledge, which has a value of 0.147, attitude, 0.619, 0.542 value for practice, and challenges that have 0.542 among the respondents when grouped according to their years of practice. Based on the results, we find no statistically significant differences in the mean scores among the groups. The range of years of practice might be too narrow to detect a significant range between the values of knowledge, attitudes, challenges, and practices; at the same time, the years of practice in hospital pharmacy do not have any differences because the use of pharmacoeconomic evaluation is still progressing and in the infancy stage despite its impact on pharmaceutical practice[12].

Table 4. Comparison of knowledge, attitude, practice, and challenges among hospitals

Variables	p-values[1,2]
Knowledge of pharmacoeconomic tools and concepts	0.242
Attitude toward the application of pharmacoeconomic tools	0.329
Practice of pharmacoeconomic evaluations	0.339
Challenges to the use of pharmacoeconomic evaluations	0.074

[1] The analysis of variance or Kruskal-Wallis H test were used to test whether there are statistically significant differences in the mean scores between the groups, as appropriate.
[2] A p-value threshold of 0.05 is used to determine whether to accept or reject the null hypothesis. If the generated p-value is less than 0.05, the null hypothesis is rejected. ** signifies that the p-value is statistically significant at the 1% level, while * signifies that the p-value is statistically significant at the 5% level.

3.5. Most Common Pharmacoeconomic Evaluation Performed by PTC Hospital Pharmacists Working in Tertiary Private Hospitals in NCR

Table 5 presents participants' responses to the statements pertaining to their pharmacoeconomic evaluations practice. The members were asked to rate their level of agreement with ten statements on a scale of "strongly disagree" to "strongly agree". The mean score for each statement is shown in the table. Moreover, the table also shows the rankings of the statements based on the mean scores. Among the statements, brainstorming to identify costs and benefits of each medication therapy (statement 1), deciding which costs and benefits are significant (statement 2), making recommendations for Hospital Formulary based on pharmacoeconomic indices (statement 9), and evaluation of how much better someone's health gets by looking at both the quality and length of their life ranked the highest (statement 10). On the other hand, adopting appropriate pharmacoeconomic assumptions (statement 8), assigning monetary value to costs and benefits (statement 3), and specifying a set of options for each medication therapy ranked the least (statement 4).

Table 5. Practice of pharmacoeconomic evaluations among Pharmacy and Therapeutics Committee Members

State-ment	**Responses**[1,2]					**Mean Score**	**Rank**
	Strongly disagree	**Disagree**	**Cannot say**	**Agree**	**Strongly agree**		
1	-	1 (4.76%)	1 (4.76%)	13 (61.90%)	6 (28.57%)	4.14 (0.73)	1st
2	-	-	4 (19.05%)	13 (61.90%)	4 (19.05%)	4.00 (0.63)	2nd
3	1 (4.76%)	-	14 (66.67%)	1 (4.76%)	5 (23.81%)	3.43 (1.03)	9th
4	-	1 (4.76%)	9 (42.86%)	11 (52.38%)	-	3.48 (0.60)	8th
5	-	1 (4.76%)	6 (28.57%)	13 (61.90%)	1 (4.76%)	3.67 (0.66)	5th/6th
6	1 (4.76%)	1 (4.76%)	6 (28.57%)	9 (42.86%)	4 (19.05%)	3.67 (1.02)	5th/6th
7	-	1 (4.76%)	7 (33.33%)	13 (61.90%)	-	3.57 (0.60)	7th
8	-	1 (4.76%)	12 (57.14%)	8 (38.10%)	-	3.33 (0.58)	10th
9	-	-	7 (33.33%)	9 (42.86%)	5 (23.81%)	3.90 (0.77)	3rd/4th
10	-	1 (4.76%)	4 (19.05%)	12 (57.14%)	4 (19.05%)	3.90 (0.77)	3rd/4th
Mean of means						3.46 (1.08)	

[1] Only the responses of respondents who are practicing pharmacoeconomic evaluations in their institutions are included in the table.
[2] Frequencies and percentages are presented for categorical variables, while the means and standard deviations are presented for continuous variables.

Table 5 suggests that P&T committee members who use pharmacoeconomic evaluations are more likely to focus on the initial stages of the evaluation process, such as identifying costs and benefits. They may be less likely to delve into the more complex stages, such as assigning a monetary value to those costs and benefits.

3.6. Most Common Challenges in the use of Pharmacoeconomic Evaluations Perceived by PTC Hospital Pharmacists Working in Tertiary Private Hospitals in NCR

Table 6 shows participants' responses to the statements pertaining to their challenges in using pharmacoeconomic evaluations. Among the statements, limited understanding of pharmacoeconomic concepts (statement 1), fixation on traditional focus on clinical efficacy, safety, and acquisition cost alone (statement 6), complexity of concept (statement 3), and inadequate skilled hands to train pharmacists in pharmacoeconomic concepts (statement 10) ranked the highest. On the other hand, lack of competence to evaluate available evidence (statement 5), not having enough helpful rules and conditions in the working place (statement 2), limited data on local references or competitors (statement 4), and poor administrative support ranked the least (statement 7).

Table 6. Challenges to the use of pharmacoeconomic evaluations among hospital pharmacists who are members of the Pharmacy and Therapeutics Committee Members

State-ment	Responses[1,2]					Mean Score	Rank
	Strongly disagree	Disagree	Cannot say	Agree	Strongly agree		
1	-	1 (4.76%)	2 (9.52%)	13 (61.90%)	5 (23.81%)	4.05 (0.74)	1st
2	-	3 (14.29%)	3 (14.29%)	15 (71.43%)	-	3.57 (0.75)	9th
3	-	-	5 (23.81%)	14 (66.67%)	2 (9.52%)	3.86 (0.57)	3rd/4th
4	-	1 (4.76%)	5 (23.81%)	15 (71.43%)	-	3.67 (0.58)	7th/8th
5	-	3 (14.29%)	4 (19.05%)	14 (66.67%)	-	3.52 (0.75)	10th
6	-	-	2 (9.52%)	17 (80.95%)	2 (9.52%)	4.00 (0.45)	2nd
7	-	3 (14.29%)	2 (9.52%)	15 (71.43%)	1 (4.76%)	3.67 (0.80)	7th/8th
8	-	-	6 (28.57%)	14 (66.67%)	1 (4.76%)	3.76 (0.54)	5th
9	-	-	9 (42.86%)	9 (42.86%)	3 (14.29%)	3.71 (0.72)	6th
10	-	-	6 (28.57%)	12 (57.14%)	3 (14.29%)	3.86 (0.65)	3rd/4th
	Mean of means					3.77 (0.67)	

[1] Only the responses of respondents who are practicing pharmacoeconomic evaluations in their institutions are included in the table.
[2] Frequencies and percentages are presented for categorical variables, while the means and standard deviations are presented for continuous variables.

Despite respondents demonstrating a fair to good knowledge of pharmacoeconomic tools and concepts, as indicated in Table 2 with a mean score of 2.75, Table 5 reveals that participants recognize their limited understanding of pharmacoeconomics as the top prevailing challenge to their practice of pharmacoeconomic evaluation. While this may seem contradictory, it is important to distinguish between having knowledge and understanding. Knowledge implies being aware of information, facts, or concepts, while understanding involves comprehending the relevance of information and being capable of linking it to other concepts as well as applying them in practical situations[25]. The participants' limited understanding of pharmacoeconomics as a challenge is consistent with studies conducted by Alsultan (2011)[26] and Suh et al. (2020)[27], indicating that depletion of knowledge and lack of expertise pose significant risks to the limited application of pharmacoeconomic evaluations[23], [24]. This could be viewed as pharmacists being familiar with the

concepts of pharmacoeconomic evaluations such as CMA, CBA, CEA, and CUA. Nevertheless, when these concepts are put into practice to evaluate specific medications and interventions, their limited understanding is a barrier to their effective performance. Therefore, this indicates a necessity for the participants to enhance their understanding of pharmacoeconomic principles and their existing knowledge. Another challenge that respondents perceive is their tendency to rely on traditional approaches to evaluate clinical efficacy, safety, and cost acquisition of drugs, such as clinical trials. However, these approaches often fail to assess the drug's overall cost-effectiveness[27]. This can be attributed to the poor pharmacoeconomic capacity within the Philippines[15]. A number of other factors challenge respondent's pharmacoeconomic practice, including the complexity of the pharmacoeconomic concept and the lack of skilled hands available to train pharmacists in the field.

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental discussion that can be drawn. The related publication should be referred to and discussed with proper references.

4. CONCLUSION

The use of pharmacoeconomic evaluations across hospitals in the Philippines remains largely undocumented in existing literature. This is the first study covering the knowledge, attitudes, practices, and challenges among PTC hospital pharmacists in the employment of pharmacoeconomic evaluations in the Philippines.

Despite the field’s infancy stage, tertiary private hospital pharmacists exhibit fair knowledge and attitudes toward pharmacoeconomics. The field’s recent emergence may explain the minimal knowledge and attitude gaps among pharmacists with varying lengths of service under the PTC. However, the adoption of pharmacoeconomic evaluations has already been taking place in several institutions in the country. Among the practices commonly performed were identifying and brainstorming potential costs and benefits associated with medications and making hospital formulary recommendations based on pharmacoeconomic indices. Most observed activities related to pharmacoeconomic evaluations concentrate on primary stages, with less emphasis on more intricate tasks like valuing both costs and benefits in monetary terms. These results align with the common challenges encountered in conducting such evaluations, including the inherent complexity of pharmacoeconomic principles and inadequate training to tackle this complexity.

Thus, institutions should provide training opportunities and conduct seminars covering pharmacoeconomics and its associated methods. Reinforcements from international organizations with established pharmacoeconomic systems may also be sought to improve the country’s systems. Higher education curricula for pharmacy courses should also incorporate pharmacoeconomic concepts more comprehensively. Finally, as this study only collected data from a limited number of hospitals and respondents, it is recommended that future researchers cover a broader scope of institutions and an increased number of participants.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Cost-Utility Analysis of Ticagrelor versus Clopidogrel in Patients with Acute Coronary Syndromes Undergoing Percutaneous Coronary Intervention from a Thai Healthcare Perspective

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ABSTRACT

The meta-analysis revealed that the presence of the *CYP2C19*2* allele is associated with decreased efficacy of clopidogrel, resulting in reduced platelet inhibition and a consequent increase in the risk of major cardiovascular events (HR=1.57; 95% CI 1.13-2.16 P=0.006). Meanwhile, ticagrelor, a novel P2Y12 inhibitor, is unaffected by *CYP2C19* polymorphism but has a higher cost. The selection of optimal antiplatelet therapy for patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) poses challenges for clinicians and policymakers. This study aimed to evaluate the cost-utility analysis comparing ticagrelor plus aspirin (ASA) with both generic and original clopidogrel plus ASA in ACS/PCI patients.

A one-year decision tree and a lifetime Markov model were developed to simulate the progression of a typical cohort of ACS/PCI patients. The analysis compared the universal clopidogrel strategy and the universal ticagrelor strategy. Input parameters regarding costs, utilities, and transitional probabilities were obtained from published studies, including randomized controlled trials, systematic reviews, and meta-analyses. Costs were expressed in 2024 dollars (USD) with a Thai willingness-to-pay threshold (WTP) of 160,000 baht per quality-adjusted life year (QALY) gained ($4,320) from a healthcare perspective. One-way and probabilistic sensitivity analyses were conducted to assess parameter uncertainty.

Base–case results showed that the universal ticagrelor strategy was cost-saving compared to both original and generic clopidogrel, yielding higher QALYs and lower costs. One-way sensitivity analysis revealed that the incremental cost-effectiveness ratio (ICER) was most sensitive to the transition probability of remaining in the post-myocardial infarction (MI) state with ASA monotherapy. Probabilistic sensitivity analysis indicated a 100% probability of ticagrelor being cost-effective compared to clopidogrel at the Thai WTP.

From a healthcare perspective, ticagrelor would be a cost-effective treatment option for ACS/PCI patients compared to clopidogrel for reducing major cardiovascular events.

KEYWORDS: Acute Coronary Syndrome; Ticagrelor; Clopidogrel; Cost-Utility Analysis; Economic Evaluation

1. INTRODUCTION

Cardiovascular disease, including acute coronary syndrome (ACS), is a leading cause of global morbidity and mortality[1]. Among the various treatment strategies for ACS, percutaneous coronary intervention (PCI) is pivotal in restoring blood flow to the affected coronary arteries, thereby reducing myocardial damage and improving clinical outcomes. According to the Thai ACS Guidelines 2020, dual antiplatelet therapy (DAPT) is recommended for ACS patients undergoing PCI

to optimize platelet inhibition, reduce the risk of stent thrombosis, and improve overall cardiovascular outcomes. This therapy involves the use of a P2Y12 inhibitor, either ticagrelor or clopidogrel, in combination with aspirin (ASA). All patients received dual antiplatelet therapy, consisting of either ticagrelor or clopidogrel in combination with aspirin, for 12 months following their last percutaneous coronary intervention (PCI) and continued with low-dose aspirin daily thereafter unless contraindicated[2, 37].

Clopidogrel is a P2Y12 platelet receptor antagonist that has been widely used for a long time and is associated with cytochrome *P450 2C19* (*CYP2C19*) metabolism. Genetic variations, especially the *CYP2C19*2* allele - which has a population prevalence ranging from 20% to 65% - can significantly reduce the effectiveness of clopidogrel. This allele induces a splicing defect that results in the complete loss of enzyme activity[3,4]. A meta-analysis suggested that individuals carrying the *CYP2C19*2* allele experience reduced platelet inhibition, consequently increasing the risk of major cardiovascular events (MACE) (HR=1.57; 95% CI 1.13-2.16, P=0.006)[5]. Ticagrelor, an alternative P2Y12 inhibitor, offers advantages in ACS patients regardless of *CYP2C19* gene. The PLATO trial demonstrated the superiority of ticagrelor over clopidogrel in reducing MACE (HR = 0.84; 95% CI 0.77 to 0.92; $p < 0.001$). However, ticagrelor was associated with a slightly increased risk of major bleeding (HR = 1.11; 95% CI 1.03 to 1.30; p = 0.008)[6]. However, the cost of ticagrelor is still expensive, and the lack of cost-effectiveness information has been hindered policymakers in making rationale resource allocation decision. Therefore, this study aimed to evaluate the cost-utility analysis comparing ticagrelor plus aspirin (ASA) with both generic and original clopidogrel plus ASA in ACS/PCI patients.

2. MATERIALS AND METHODS

A hybrid decision tree and Markov model was developed to evaluate the cost-utility of ticagrelor plus aspirin (ASA) compared with both generic and original clopidogrel plus ASA in ACS/ PCI patients. The analysis was conducted from the healthcare payer's perspective.

2.1. Target Population

The models simulated cohorts of 62-year-old ACS patients undergoing PCI, reflecting the age profile typical in the Thai Registry[7].

2.2. Intervention and Comparator

The analysis compared two treatment strategies:

1) Universal clopidogrel strategy: All patients receive clopidogrel 75 mg daily (with a loading dose of 300 mg prior to PCI) in combination with aspirin (81-325 mg) daily for 12 months.
2) Universal ticagrelor strategy: All patients receive ticagrelor 90 mg twice daily (with a loading dose of 180 mg prior to PCI) in combination with aspirin (81-325 mg) daily for 12 months.

All patients received dual antiplatelet therapy (one of the two medications above and aspirin) for 12 months after the last PCI and low-dose aspirin daily thereafter unless contraindicated.

The term "universal" refers to the approach where all patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) are treated with the same medication regimen (either ticagrelor or clopidogrel) regardless of their genetic variations or other individual factors.

2.3. Model Structure and Model Assumptions

A one-year decision tree and a lifetime Markov model were developed, building upon validated models from the PLATO study, which included Thailand as one of the multi-center study[6]. The model was used to determine lifetime costs and health outcomes in ACS/ PCI patients receiving

ticagrelor plus aspirin (ASA) compared with both generic and original clopidogrel plus ASA. From this, the model started with newly diagnosed ACS/ PCI patients. Figure 1.a demonstrated the decision tree model displaying two treatment strategies, which were ticagrelor plus ASA and clopidogrel plus ASA. Patients received one year of treatment in the short-term decision tree model, assuming no rebound effect remaining in Markov cycles. Survivors would transit to a lifelong Markov model, continuing with Aspirin monotherapy. In the universal clopidogrel strategy arm, patients were grouped based on their *CYP2C19* genotypes into those who carry the loss-of-function (LOF) alleles (LOF carriers) and those who do not (non-LOF carriers). The probabilities of major adverse cardiovascular events (MACE), stent thrombosis, and major bleeding in the universal clopidogrel group was estimated according to the risk associated with different *CYP2C19* genotypes (with or without *CYP2C19*2* allele).

In one-year decision tree, patients in all arms could die from cardiovascular cause or fatal bleeding, while survivors might experience various outcomes, including no event, major bleeding, stent thrombosis (ST), non-fatal myocardial infarction (MI), or non-fatal stroke. It was assumed, based on a low incidence rate in the PLATO trial, that patients could not experience major bleeding, ST, MI, and stroke concurrently within the first year[6]. Patients experiencing stent thrombosis would undergo repeated PCI. Bleeding events, excluding those related to coronary artery bypass grafts, were defined as intracranial or retroperitoneal bleeding or bleeding requiring a transfusion of 4 units or more (with a decrease in hemoglobin of 5 g/dL or more)[9]. Bleeding events and stent thrombosis were not considered from the second year onward due to their rarity during this period[6].

After the decision tree, patients entered the long-term Markov model. Starting from the second year, a yearly cycle. Figure 1.b demonstrated three Markov model simulated disease progression over the patient's lifetime. It was assumed that post-one-year event rates under aspirin monotherapy were equal regardless of initial treatment strategies in the first year.

- Patients encountering bleeding or stent thrombosis in the one-year decision tree would progress to the "No event" stage in Markov 1 (M1). Patients who are in “No event” state in Markov model could progress to health state of “non-fatal MI”, “post-MI”.

- Patients experiencing “MI” in the first year entered the “post-MI” stage in Markov 2 (M2).

- Patients experiencing “stroke” in the first year entered to the “post-stroke” stage in Markov 3 (M3). Patients who developed “post-MI” could progress to health state of “non-fatal MI”, “non-fatal stroke” or “death”. Patients who developed “post-stroke” could progress to health state of “non-fatal stroke” or “death” .

Survivors of MI and stroke were assumed to remain in the corresponding stage for one year before transitioning to subsequent stages. Additionally, patients were assumed not to transition from the “stroke” or "post-stroke" state to the "MI" state since the “MI” health state had a lower risk, an improved quality of life, and was less costly than the stroke health state.

2.4. Model Parameter

2.4.1 Transitional probabilities

The model inputs were collected by searching PubMed and Scopus with specific keywords, including "acute coronary syndrome," "CYP2C19", "genotype," "clopidogrel," "ticagrelor," "antiplatelet agent," and "QALY." Selection criteria included 1) publications in English, 2) trials involving ACS patients undergoing PCI, and 3) reporting of clinical event rates or hazard ratios. Meta-analyses and randomized clinical trials were preferred sources.

Model inputs, detailed in Table 1, included one-year event rates for the universal clopidogrel strategy (75 mg daily) in carriers and noncarriers of *CYP2C19*2* alleles (loss-of-function alleles; LOF alleles), mainly obtained from a multi-center clinical trial involving over 13,000 ACS patients undergoing PCI[6]. Ticagrelor event rates were assumed to be consistent across genotypes[9]. The event rates for ticagrelor users regarding a specific cardiovascular event were multiplied by the baseline risk of events with clopidogrel treatment by the hazard ratios representing ticagrelor treatment effects[10, 11].

Based on the assumption that patients are no longer on the study medications after one year, the identical transition probabilities for the second year onwards were applied to both treatment

strategies in the Markov model. Transition probabilities for other health states were drawn from published articles, with specific costs and health utility values assigned to each state based on relevant research.

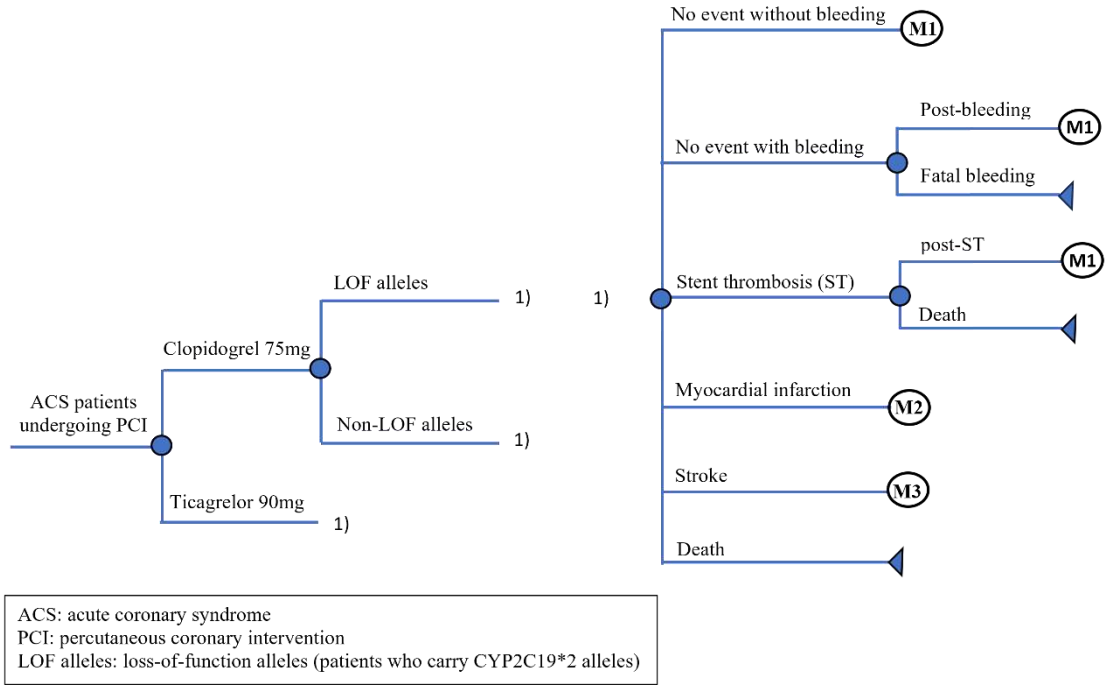

Figure 1 a. One-year decision tree

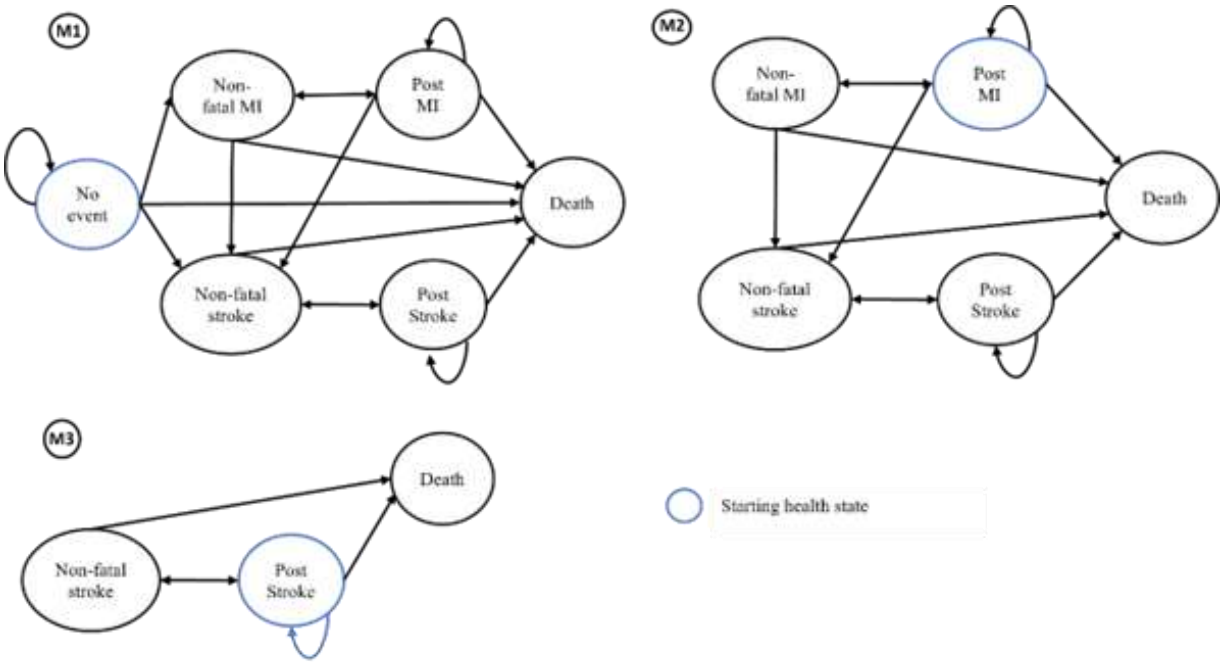

Figure 1 b. Markov model

Table 1 Input parameters

Parameter description	Distribution	Base-case values	Lower	Upper	Source
Discounting rate for costs	Beta	**0.03**	0.0000	0.0600	Chaikledkaew U[19]
Discounting rate for outcomes	Beta	**0.03**	0.0000	0.0600	Chaikledkaew U[19]
Transitional probability at 1-year decision tree (first year)					
(Base case) Clopidogrel in LOF carrier					
Bleeding	Beta	**0.0540**	0.0262	0.1593	Wallentin[6]
Fatal bleeding	Beta	**0.0040**	0.0037	0.0048	Wallentin[6]
Stent thrombosis	Beta	**0.0218**	0.0214	0.0229	Wallentin[6]
Non-fatal MI	Beta	**0.1430**	0.1333	0.1726	Luo Y[20]
Stroke	Beta	**0.0490**	0.0452	0.0589	Luo Y[20]
CV death	Beta	**0.0430**	0.0401	0.0519	Chen M[21]
Death from any cause	Beta	**0.0460**	0.0430	0.0556	Chen M[21]
(Base case) Clopidogrel in non-LOF carrier					
Bleeding	Beta	**0.0200**	0.0185	0.0244	Wallentin[6]
Fatal bleeding	Beta	**0.0040**	0.0037	0.0049	Wallentin[6]
Stent thrombosis	Beta	**0.0130**	0.0117	0.0170	Wallentin[6]
Non-fatal MI	Beta	**0.0720**	0.0670	0.0859	Luo Y[20]
Stroke	Beta	**0.0220**	0.0205	0.0264	Luo Y[20]
CV death	Beta	**0.0230**	0.0214	0.0278	Chen M[21]
Death from any cause	Beta	**0.0290**	0.0267	0.0350	Chen M[21]
Ticagrelor (twice daily) versus clopidogrel (general patients receiving 75mg clopidogrel)					
Bleeding	Beta	**0.0306**	0.0261	0.0431	Kang[22], Wallentin[6]
Fatal bleeding	Beta	**0.0042**	0.0039	0.0050	Canon[23], Wallentin[6]
Stent thrombosis	Beta	**0.0133**	0.0121	0.0168	Canon[23], Wallentin[6]
Non-fatal MI	Beta	**0.0542**	0.0515	0.0617	Canon[23], Wallentin[6]
Stroke	Beta	**0.0082**	0.0056	0.0158	Kang[22], Kazi[24]
CV death	Beta	**0.0140**	0.0134	0.0162	Canon[23], Wallentin[6]
Death from any cause	Beta	**0.0156**	0.0149	0.0175	Canon[23], Wallentin[6]
Transitional probability of aspirin monotherapy in Markov modeling					
From "No event" to "MI"	Beta	**0.0190**	0.0176	0.0231	Peng Y[25]
From "No event" to "Stroke"	Beta	**0.0030**	0.0028	0.0036	Peng Y[25]
From "No event" to "CV death"	Beta	**0.0036**	0.0033	0.0043	Li Y[26]
From "MI" to "Post MI"	Beta	**0.9546**	0.9683	1.0000	
From "MI" to "Stroke"	Beta	**0.0040**	0.0037	0.0048	Karnon[27]
From "MI" to "CV death"	Beta	**0.0414**	0.0387	0.0509	Karnon[27]
From "Post-MI" to "Post MI"	Beta	**0.9071**	0.8695	0.9987	
From "Post-MI" to "MI"	Beta	**0.0529**	0.0491	0.0639	Karnon[27]
From "Post-MI" to "Stroke"	Beta	**0.0043**	0.0040	0.0053	Karnon[27]
From "Post-MI" to "CV death"	Beta	**0.0357**	0.0332	0.0426	Karnon[27]
From "Stroke" to "Post-stroke"	Beta	**0.9641**	0.9846	1.0000	
From "Stroke" to "CV death"	Beta	**0.0359**	0.0334	0.0431	Karnon[27]

From "Post-stroke" to "Post-stroke"	Beta	**0.8400**	0.7921	0.9662	
From "Post-stroke" to "Stroke"	Beta	**0.0640**	0.0596	0.0770	Karnon[27]
From "Post-stroke" to "CV death"	Beta	**0.0960**	0.0893	0.1150	Karnon[27]
Genetic test characteristics					
Prevalence of *CYP2C19*2 allele* (LOF) carriers	Beta	**0.4962**	0.4628	0.5967	Rattanaporn S[29]
Costs					
One-year decision tree - Ticagrelor					
Annual drug cost	Gamma	**981**	918	1,162	DMSIC[18]
No event	Gamma	**3,421**	3,198	4,129	Nikolic[13], Arthorn[17]
Bleeding	Gamma	**7,804**	7,241	9,472	Nikolic[13], Arthorn[17]
Fatal bleeding	Gamma	**11,761**	10,947	14,095	Nikolic[13], Arthorn[17]
Stent thrombosis	Gamma	**7,567**	7,065	9,117	Nikolic[13], Arthorn[17]
MI	Gamma	**10,695**	9,965	12,930	Nikolic[13], Arthorn[17]
Stroke	Gamma	**12,370**	7,065	9,117	Nikolic[13], Arthorn[17]
CV death	Gamma	**7,556**	7,050	8,968	Nikolic[13], Arthorn[17]
Death	Gamma	**3,500**	3,250	4,145	Nikolic[13], Arthorn[17]
One-year decision tree - Clopidogrel					
Annual drug cost (brand)	Gamma	**756**	705	903	DMSIC[18]
Annual drug cost (generic)	Gamma	**9**	8	11	DMSIC[18]
No event	Gamma	**3,505**	3,285	4,178	Nikolic[13], Arthorn[17]
Bleeding	Gamma	**6,043**	5,628	7,146	Nikolic[13], Arthorn[17]
Fatal bleeding	Gamma	**11,761**	10,905	14,096	Nikolic[13], Arthorn[17]
Stent thrombosis	Gamma	**7,110**	6,660	8,416	Nikolic[13], Arthorn[17]
MI	Gamma	**10,238**	9,577	12,169	Nikolic[13], Arthorn[17]
Stroke	Gamma	**10,342**	6,660	8,416	Nikolic[13], Arthorn[17]
CV death	Gamma	**6,444**	6,026	7,658	Nikolic[13], Arthorn[17]
Death	Gamma	**3,585**	3,356	4,310	Nikolic[13], Arthorn[17]
Markov model					
No event	Gamma	**590**	550	698	Nikolic[13], Arthorn[17]
MI	Gamma	**5,852**	5,448	6,972	Nikolic[13], Arthorn[17]
Post MI	Gamma	**4,806**	4,494	5,679	Nikolic[13], Arthorn[17]
Stroke	Gamma	**4,065**	3,772	4,874	Nikolic[13], Arthorn[17]
Post stroke	Gamma	**2,280**	2,115	2,720	Nikolic[13], Arthorn[17]
Utility values in 1-year decision tree					
No event	Beta	**0.8860**	0.8401	0.9928	Nikolic[13]
Stent thrombosis	Beta	**0.7900**	0.7424	0.9230	Garg P[14]
Nonfatal MI	Beta	**0.8320**	0.7811	0.9613	Nikolic[13]
Stroke	Beta	**0.7350**	0.6908	0.8616	Nikolic[13]
Death	Beta	**0.2510**	0.2336	0.3036	Nikolic[13]
Non-fatal bleeding disutility	Beta	**0.0410**	0.0380	0.0487	Sullivan PW[15]
Utility values in the Markov model					

No event	Beta	**0.8750**	0.8234	0.9888	Grima DT[28]
Non-fatal MI	Beta	**0.8120**	0.7601	0.9441	Grima DT[28]
Post MI	Beta	**0.8120**	0.7541	0.9408	Grima DT[28]
Non-fatal stroke	Beta	**0.7370**	0.7111	0.8061	Grima DT[28]

2.4.2. Clinical Effectiveness

Clinical effectiveness data were sourced from published studies and the PLATO trials [6, 22, 23].

2.4.3. Utility

Utilities in the one-year decision tree and Markov model were derived from EQ-5D data collected within the PLATO study[6, 13]. The utility of stent thrombosis and disutility scores of non-fatal major bleeding events were sourced from published articles[13-15].

2.4.4. Costs

In our model, cost analysis was conducted from the perspective of the Thai healthcare payer, covering only direct medication cost. These included expenses for study medication, concurrent procedures, and resources related to bleeding incidents. Resource utilization data from the PLATO trial[6, 13] and unit costs from a Thai database were used to determine healthcare costs for the one-year decision tree and the Markov model1[6, 17]. Costs for ticagrelor and clopidogrel were obtained from the Drug and Medical Supply Information Center[18] (DMSIC) in Thailand, including a 7% VAT. All costs were standardized to US dollars for the year 2024.

2.5. Model Analysis

Lifetime costs and quality-adjusted life years (QALYs) were computed and expressed as the incremental cost-effectiveness ratio (ICER), representing the ratio of incremental costs to incremental QALYs. Based on Thai guidelines, 3% annual discounting rate applied to both costs and outcomes. A treatment strategy was considered cost-effective if its associated ICER was less than 160,000 baht per QALY gained[19].

2.6. Sensitivity Analyses

To ensure the model's reliability, a one-way sensitivity analysis was conducted to assess parameter uncertainties depicted in a tornado diagram. Additionally, probabilistic sensitivity analysis, employing Monte Carlo simulations, evaluated the impact of uncertain parameters across distributions, illustrated in a cost-effectiveness acceptability curve (CEAC).

3. RESULTS AND DISCUSSION

3.1. Base Case Results

The one-year event rates of MACE, stent thrombosis, and major bleeding in each study arm were calculated. In the universal clopidogrel strategy, the rates of non-fatal MI, non-fatal stroke, cardiovascular death, stent thrombosis, and major bleeding are 0.1072, 0.0354, 0.0329, 0.0174, and 0.0369, respectively. Conversely, in the universal ticagrelor strategy, the rates are notably lower at 0.0542, 0.0082, 0.0140, 0.0133, and 0.0306, respectively. This suggests that ticagrelor may offer superior outcomes across all measured parameters compared to clopidogrel.

The life-long cost and QALYs of each antiplatelet strategy are shown in **Table 2**. The universal ticagrelor strategy yields an extra 0.11 QALYs, saves $4,316 versus generic clopidogrel,

and $3,565 versus original clopidogrel. The results suggested that ticagrelor strategy was cost-saving compared to both original and generic clopidogrel, yielding higher QALYs and lower costs.

Table 2. Base-case results

	Ticagrelor	Clopidogrel	Incremental	ICER
Ticagrelor vs generic clopidogrel				
Costs (US dollars)	30,704	34,269	-3,565	Dominate
QALYs	13,16	13,05	0.11	
Ticagrelor vs original clopidogrel				
Costs (US dollars)	30,704	35,020	-4,316	Dominate
QALYs	13,16	13,05	0.11	

3.2. Sensitivity analysis

The one-way sensitivity analysis showed robust base-case results across all inputs, except for the transitional probabilities of remaining in "Post MI" in the Markov model. Tornado graphs depicted the ten most influential variables (Figure 2.a). Furthermore, probabilistic sensitivity analyses, presented as cost-effectiveness acceptability curves, indicated that at a willingness-to-pay threshold of $4,320 per QALY (160,000 baht per QALY), the universal ticagrelor strategy had a 100% probability of being cost-effective compared to the universal clopidogrel strategy (Figure 2.b).

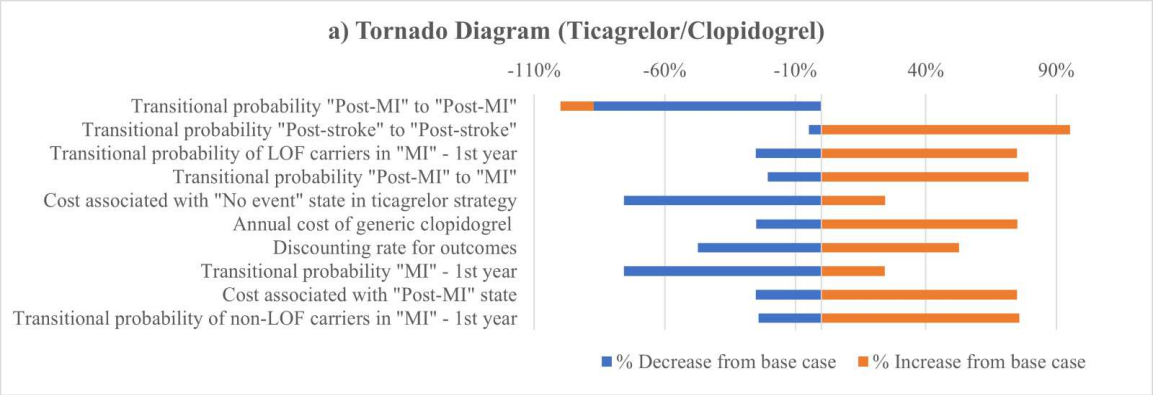

Figure 2. a Tonardo diagram

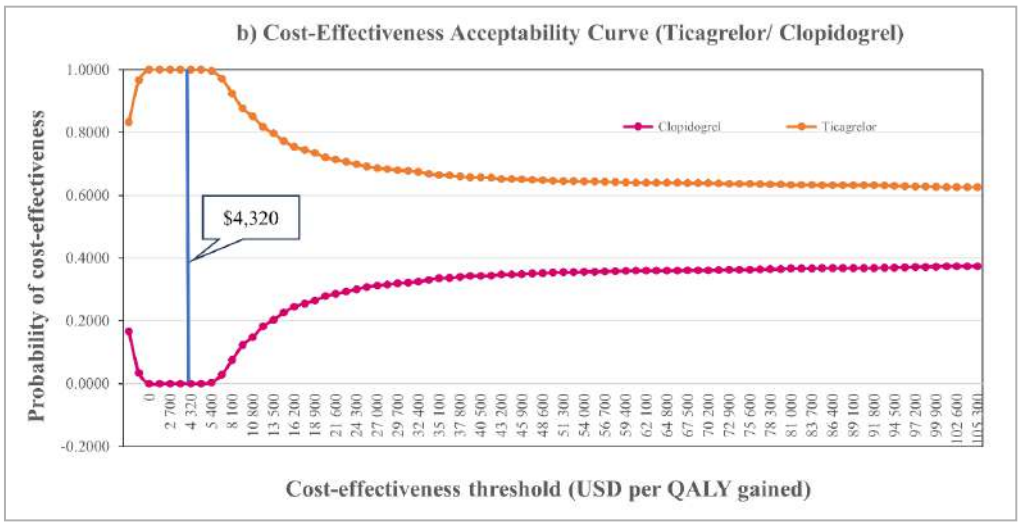

Figure 2. b Cost-effectiveness acceptability curves (CEAC)

3.3 Discussion

This study employed a widely used decision tree and Markov model to assess the cost-utility analysis of antiplatelet therapy for ACS patients. Base case analysis showed that ticagrelor strategy was cost-saving compared to original and generic clopidogrel in ACS/ PCI patients. The results align with similar studies on ticagrelor and clopidogrel in various countries[30-35].

However, it is important to acknowledge several limitations within the study. Firstly, the assumption that generic clopidogrel performs with equal efficacy and safety as its original may be challenged because most generic clopidogrel are not of equivalent quality in real-world practice. Secondly, reliance on previously published data, particularly from PLATO trials, may not fully reflect real-world ACS/ PCI management in Thailand. Moreover, uncertainties may arise from estimating standard errors (10% of mean values) for transitional probabilities and costs in sensitivity analysis. Thirdly, the Markov model's structure does not allow patients to experience multiple cardiovascular events in their lifetime, which may not accurately reflect the studied cohort in real life. Finally, adopting a healthcare perspective, which solely considers direct medical costs, may overlook direct non-medical expenses like transportation and indirect costs such as lost productivity. Previous studies underscored the significant economic burden ACS patients, and their families bear due to productivity losses, with indirect costs outweighing direct healthcare expenses[36]. Hence, excluding these cost components underestimates ACS's true societal impact in Thailand. Therefor further study should consider the societal perspective and perform budget impact analysis in order to completely guide resource allocation decision.

4. CONCLUSION

From a healthcare perspective, ticagrelor would be a cost-effective treatment option for ACS/PCI patients compared to clopidogrel for reducing major cardiovascular events.

5. ACKNOWLEDGMENT

From a healthcare perspective, ticagrelor would be a cost-effective treatment option for ACS/PCI patients compared to clopidogrel for reducing major cardiovascular events.

Conflict of interest

The authors declare that they have no conflict of interest.

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Analysis of Direct Medical Costs in Patients Undergoing Knee Joint Surgery at the Hospital for Traumatology and Orthopaedics in Ho Chi Minh City, Vietnam

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ABSTRACT

Knee joint surgery is a complex procedure that significantly reduces pain and substantially improves the function of the knee joint. However, knee joint surgery is a complex, specialized technique in orthopedic trauma surgery, which entails high costs. This study aimed to analyse costs and factors associated with increased costs among patients receiving knee joint surgery. The cost analysis study from a health sector perspective used retrospective data from the Hospital for Traumatology and Orthopaedics during the period of 2021-2022. The direct medical costs for an inpatient knee joint surgery, included bed costs, medication costs, surgical costs, consumable material costs. The study converted cost to 2022 annual values based on the Vietnam Consumer Price Index (CPI). The study collected patient demographic details and other factors that could affect the direct medical cost. In 2021-2022, the Hospital for Traumatology and Orthopaedics performed knee joint surgery on 964 patients, with an average age of 56.3±17.2 years. Most patients underwent surgery under health insurance coverage. Knee osteoarthritis (73.3%), total knee replacement surgery (74.07%) were the predominant diagnoses and procedures. Consumable material costs accounted for the highest proportion of costs, while bed costs were the lowest. Over the two years, the average cost per case was higher in 2021 compared to 2022 ($2,782 vs. $2,984). Multivariate GLM analysis showed type of surgery, the year of surgery had statistical significance in the patient's total cost. Compared to patients undergoing other surgeries, patients with knee arthroscopic surgery incurred $491 more, while those with knee replacement surgery incurred $2,985 more. On average, compared to 2021, knee surgeries performed in 2022 were $270 cheaper. The cost of knee joint surgery was high, and consumable material costs constituted the largest share of the costs. The cost depended on the type of surgery and slightly changed over the years.

KEYWORDS: Cost Analysis; Direct Medical Cost; Knee Joint Surgery

1. INTRODUCTION

When conventional treatments using medications prove ineffective, people with chronic, severe knee pain must have knee joint surgery. Knee joint surgery is a complex procedure that not only significantly reduces pain but also substantially improves the function of the knee joint[1]. There are two common types of knee joint surgery: knee arthroscopic surgery or knee replacement surgery (also called knee arthroplasty). Knee arthroscopy is a minimally invasive surgical technique that enables physicians to visualize the knee joint without needing a large skin incision or extensive soft tissue disruption. It serves diagnostic and therapeutic purposes, facilitating the identification and treatment of various knee-related issues[2]. Knee replacement surgery is a procedure where damaged components of the knee joint are substituted with metal or plastic components. It is primarily

employed to alleviate pain and stiffness in the knee joint resulting from osteoarthritis. Some research results in the United States have shown that knee replacement surgery is cost-effective at low and high levels of improvement. The rate of knee replacement surgeries has significantly increased over the past decade. A study by Navarro Espigares JL et al. (2008) found the total cost of knee replacement surgery to be lower (6,865.52 €) compared to hip replacement (7,891.21 €)[3]; Nwachukwu BU et al. (2015) found the total cost of knee replacement surgery to range from $36,756 (2005) to $52,175 (2011)[4]. Knee replacement surgery has a symptom improvement rate of over 85% and a long-term failure rate of less than 1% per year[5].

However, the financial burden associated with knee joint surgery is considerable, especially in developing countries like Vietnam. The economic strain on patients and the healthcare system underscores the need for a thorough cost analysis to understand and mitigate these costs. By examining various cost components such as bed costs, medication costs, surgical costs, consumable material costs, the study provide a comprehensive understanding of the economic impact on patients. Additionally, it aims to identify and analyse related factors that may influence these costs, such as patient demographics, comorbidities, and type of the surgery. Understanding these associations is crucial for healthcare providers and Public Health Insurance to optimize resource allocation, improve cost-efficiency, and ensure better access to quality care for patients undergoing knee joint surgery.

This study aimed to analyse the direct medical costs incurred by patients undergoing knee joint surgery at the Hospital for Traumatology and Orthopaedics in Ho Chi Minh City, Vietnam, with the following objectives: (1) Analyse the direct medical costs of knee joint surgery; (2) Analyse the factors associated with direct medical costs in knee joint surgery.

2. MATERIALS AND METHODS

2.1. Study Design

A cost analysis study was conducted on patients diagnosed and treated for knee joint surgery at the Hospital for Traumatology and Orthopaedics in Ho Chi Minh City, Vietnam, from 2021 to 2022. The study collected retrospective data on direct medical costs from the health sector perspective and the patient characteristics from the hospital database and record from January 2021 to December 2022. The study also collected patient characteristics data (gender, age, accommodation, health insurance, levels of health insurance benefits, age, primary diagnosis, comorbidities, type of surgery) and cost (hospital bed costs, medication costs, surgery fees, consumable material costs).

The study retrieved data from patients' electronic medical records and stored data in .csv file format. For research purposes, the study segmented the data into two files: (1) Information on patients and treatment cost data; and (2) Hospital drug lists and medical services provided by the Hospital for Traumatology and Orthopaedics during the period 2021 - 2022.

2.2. Study Participants

All inpatient medical records at the Hospital for Traumatology and Orthopaedics during the period of 2021-2022 were considered for inclusion in the study based on the selection criteria and exclusion criteria. Inclusion criteria included: (1) Patients who performed knee joint surgery in the Department of Lower Limb Orthopedics; (2) Treatment period from January 01, 2021, to December 31, 2022; (3) Records with adequate required information. Exclusion criteria included: patients were transferred to another hospital or died during treatment.

2.3. Statistical Analysis

The study utilized STATA software to analyse data regarding patient characteristics, costs, and construct models analysing factors associated with direct medical costs in knee joint surgery.

Analyse the direct medical costs of knee joint surgery

The direct medical costs of knee joint surgery are detailed in descriptive statistics, calculated for each cost component based on patient characteristics and by year.

Total Direct Medical Costs =Bed costs+Medication costs+Surgical costs+Consumable material costs

The study converted direct medical costs to 2022 annual values based on the Vietnam Consumer Price Index (CPI)[6]. The costs are presented as 2022 US Dollars ($) and Vietnamese Dong (VND)[7]. Although cost variables often exhibit skewed distributions, presenting results as medians may not accurately assess the average treatment cost. Therefore, the study presents the results as mean values (Standard Deviation).

Analyse the factors associated with direct medical costs in knee joint surgery

To examine the association between overall inpatient treatment costs and patient variables, such as demographics, primary diagnoses, comorbidities, and type of surgery, the study utilized generalized linear regression (GLM) models with link log and family gamma.

3. RESULTS AND DISCUSSION

3.1. Patient Characteristics

The demographic characteristics of knee joint surgery patients at the Hospital for Traumatology and Orthopaedics during the 2021-2022 period are presented in Table 1. The number of knee joint surgery cases in 2022 (656 cases) was nearly double that of 2021 (308 cases). The difference in the cases between 2021 and 2022 is due to the COVID-19 epidemic in Vietnam until early 2022. There was a slight difference in the proportion between males and females, with females accounting for a significant majority (73.76%). The average age of the study population was 56.3 (SD: 17.2). The proportion of patients from Ho Chi Minh City (25.52%) was lower than those from other provinces. The result showed that patients prefer to perform surgery at hospitals in big cities (including Ho Chi Minh City). Regarding Health Insurance, most patients underwent surgery under Health Insurance coverage (95.75%). In terms of levels of Health Insurance benefits, the most common levels of health insurance benefits over the two years were 80% (72.73% in 2021 and 75.71% in 2022), 100% (22.73% in 2021 and 17.84% in 2022), and 95% (4.55% in 2021 and 7.01% in 2022).

Futhermore, the analysis results indicate that knee osteoarthritis was the most prevalent primary diagnosis, accounting for the highest proportion (214 patients, 69.48% in 2021 and 493 patients, 75.71% in 2022), followed by sprain (52 patients, 16.88% in 2021 and 79 patients, 12.04% in 2022). The lowest proportion of primary diagnoses was inflammation (1.62% in 2021 and 1.98% in 2022). This finding was similar to others as knee osteoarthritis was the most common type of arthritis diagnosed[8]. Diabetes mellitus (12.34% in 2021 and 12.65% in 2022) and hypertension (38.31% in 2021 and 33.38% in 2022) were the most prevalent comorbidities. Research suggested that just over 50% of people with type 2 diabetes will develop osteoarthritis[9]. An extensive nationally representative survey found a substantial correlation between hypertension and arthritis, including both rheumatoid arthritis and osteoarthritis[10]. In knee joint surgery over the past two years, total knee replacement surgery accounted for the highest number, with 714 cases out of a total of 964 cases (74.07%).

Table 1. Demographic characteristics

Characteristics	2021 N = 308, N (%)	2022 N = 656, N (%)	Total N = 964, N (%)	p-value
Gender				
Male	74 (24.03)	179 (27.29)	253 (26.24)	0.283[a]
Female	234 (75.97)	477 (72.71)	711 (73.76)	
Age				
Mean (SD)	56.4 (17.7)	56.2 (17.0)	56.3 (17.2)	0.833[b]
Accommodation				
Other provinces	219 (71.10)	499 (76.07)	718 (74.48)	0.099[a]
Ho Chi Minh City	89 (28.90)	157 (23.93)	246 (25.52)	
Health Insurance				
Yes	294 (95.45)	629 (95.88)	923 (95.75)	0.758[a]
No	14 (4.55)	27 (4.12)	41 (4.25)	
Levels of Health Insurance benefits				
80%	224 (72.73)	493 (75.15)	717 (74.38)	0.091[a]
95%	14 (4.55)	46 (7.01)	60 (6.22)	
100 %	70 (22.73)	117 (17.84)	187 (19.40)	

Note: [a] Chi-square test, [b] Independent Samples T-Test

3.2. Analyse the Direct Medical Costs of Knee Joint Surgery

Table 2 presents direct medical costs per case of knee joint surgery at the Hospital for Traumatology and Orthopaedics from 2021 to 2022, categorized by year of surgery.

On average, the cost per case in 2021 was higher than in 2022. There were 69,376,140VND ($2,984) per case in 2021 versus 64,690,830VND ($2,782) in 2022, despite the number of patients in 2022 being more than twice that of 2021 (656 versus 308 patients). The increased surgical costs in 2021 may be due to several contributing factors. The COVID-19 pandemic in 2021 increased healthcare expenses due to higher costs for personal protective equipment and sanitation amid supply chain shortages. By 2022, supply chains recovered, and additional safety protocols were eliminated, so the cost of knee joint surgery decreased. However, compared to other countries, the cost of knee joint surgery in Vietnam is relatively low. A study conducted in Canada in 2020 showed that the average surgical costs were $10,476.53 (*equivalent to 2022 US Dollar: $9,005.17), with the majority of surgery-related costs associated with the operation, hospitalization, and post operative care[11]. A study by Delanois, Ronald E et al in the USA showed that patients who underwent total knee replacement surgery had a mean inpatient care costs of $20,728 ($26,370.99*) in 2012 and $16,463 ($20,006.39*) in 2015 ($p < 0.0001$)[12].

Table 2. Direct medical costs per case of knee joint surgery

Variables		Bed costs	Medication costs	Surgical costs	Consumable material costs
Year of surgery					
2021 (N=308)	Mean ± SD	2,307,812 ± 1,248,234VND **($99,25 ± $53.68)**	2,314,959 ± 1,326,319VND **($99.56 ± $57.04)**	8,831,054 ± 3,803,095VND **($379.80 ± $163.56)**	55,922,320 ± 33,682,250VND **($2,405 ± $1,449)**
2022 (N=656)	Mean ± SD	2,061,728 ± 1,095,607VND **($88.67 ± $47.12)**	2,212,212 ± 1,338,344VND **($95.14 ± $57.56)**	8,859,199 ± 3,998,044VND **($381.01 ± $171.94)**	51,557,660 ± 27,281,900VND **($2,217 ± $1,173)**

In the cost structure, there was a general trend of decreased costs in 2022 compared to 2021 across most categories, except of surgical costs, which remained stable. Consumable material costs accounted for the highest proportion, while bed costs accounted for the lowest proportion. The consumable material costs were the highest because of the need for specialized, high-quality surgical implants and equipment, stringent sterilization protocols, importation expenses, and the adoption of advanced medical technologies.

3.3. Analyse the Factors Associated with Direct Medical Costs in Knee Joint Surgery

To analyse the association between the total cost and patient characteristics such as demographic, primary diagnosis, comorbidity, and surgical type-the study employed a generalized linear regression model (GLM). Multivariate regression was used to analyse factors associated with the cost of knee replacement surgery costs by simultaneously incorporating statistically significant factors identified in the univariate regression model into the multivariate regression model. The results are present in Table 3.

After adjusting for other variables, two variables significantly impacted the total costs of patients: type of surgery and year of surgery. On average, compared to 2021, knee surgeries performed in 2022 were 6,269,500VND ($270) cheaper. Compared to patients with other types of surgery, patients undergoing knee arthroscopic surgery had an additional average cost of 11,421,200VND ($491); patients undergoing knee joint replacement surgery had an additional average cost of 69,415,400VND ($2,985). The complexity of knee replacement surgery often requires longer operative times, specialized equipment, and a more extensive post-operative care regimen, contributing to higher costs. Knee replacement surgery is more complex, takes longer, requires special equipment, and leads to long post-operative care that contributes to increased payments. The shorter stays in the hospital for patients undergoing knee arthroscopy reduced hospital costs compared to those who undertake knee replacement surgery. On the other hand, after undergoing knee replacement surgery, patients have to go through a comprehensive rehabilitation program aimed at restoring strength and helping them move and use the operated limb normally again. Such rehabilitation could increase costs associated with knee replacement surgeries at large. Conversely, knee arthroscopic surgery may necessitate less invasive rehabilitative measures, thus cutting expenses even further.

The study analyzing direct medical costs in knee joint surgery at the Hospital for Traumatology and Orthopaedics in Ho Chi Minh City, Vietnam has some limitations. The study only collected data for two years, so the limited sample size and the possibility of selection bias may further limit the generality of the study. The study focused on direct costs that excluded significant indirect costs, such as work loss, worker replacement, and reduced productivity from illness and disease. The retrospective of the study is based on historical data, which may be less accurate. Additionally, its cross-sectional design does not account for long-term results and costs.

4. CONCLUSION

In conclusion, the direct medical cost per knee joint surgery patient was 69,376,140VND ($2,984) in 2021 and 64,690,830VND ($2,782) in 2022. Consumable material costs accounted for the highest proportion (about 80%), followed by surgical costs and medication costs, while bed costs accounted for the lowest proportion. Two variables significantly impacted the total costs of patients: type of surgery and year of surgery. Among the two highest proportions of knee joint surgeries in hospitals, knee replacement surgery had an additional average cost of 69,415,400VND ($2,985), while knee arthroscopic surgery was 11,421,200VND ($491). Knee surgeries performed in 2022 were 6,269,500VND ($270) cheaper due to the increased healthcare expense of COVID-19 impact. Further research is necessary to help refine and validate findings over time.

Table 3. Multivariable regression analysis of direct medical costs in knee joint surgery

Variable		Mean	Multivariable regression analysis			
			Coefficient	p-value	95% CI	
					Lower 95%	Upper 95%
Gender	Male	49,066,300VND ($2,110)	**Ref**			
	Female	72,280,260VND ($3,109)	-769,500VND ($-33.09)	0.285	-2,179,400VND ($-93.73)	640,300VND ($27.54)
Accomnodation	Other provinces	71,093,560VND ($3,058)	**Ref**			
	Ho Chi Minh City	64,506,990VND ($2,774)	-591,700VND ($-25.45)	0.283	-1,671,000VND ($-71.86)	487,600VND ($20.97)
Health Insurance	Yes	67,973,480VND ($2,923)	**Ref**			
	No	25,988,110VND ($1,118)	1,877,100VND ($80.73)	0.384	-2,346,400VND ($-100.91)	6,100,600VND ($262.37)
Knee Osteoarthritis	No	21,519,520VND ($925)	**Ref**			
	Yes	82,425,060VND ($3,545)	4,509,900VND ($193.96)	0.384	-2,346,400VND ($-100.91)	6,100,600VND ($262.37)
Bilateral knee osteoarthritis	No	63,337,670VND ($2,724)	**Ref**			
	Yes	82,962,840VND ($3,568)	284,700VND ($12.24)	0.293	-3,887,400VND ($-167.19)	12,907,300VND ($555.10)
Knee Joint Surgery Performed	No	66,168,570VND ($2,846)	**Ref**			
	Yes	66,341,820VND ($2,853)	1,212,300VND ($52.14)	0.142	-407,700VND ($-17.53)	2,832,400VND ($121.81)
Hypertension	No	58,231,440VND ($2,504)	**Ref**			
	Yes	80,990,860VND ($3,483)	-287,800VND ($-12.38)	0.573	-1,288,800VND ($-55.43)	713,200VND ($30.67)
Diabetes	No	64,480,450VND ($2,773)	**Ref**			
	Yes	78,082,790VND ($3,358)	-868,300VND ($-37.34)	0.195	-2,182,400VND ($-93.86)	445,700VND ($19.17)
Other types of surgery		**Ref**				
Knee arthroscopic surgery		73,074,650VND ($3,143)	11,421,200VND ($491)	<0.001	8,289,900VND ($356.52)	14,552,500VND ($625.86)
Knee replacement surgery		22,779,730VND ($980)	69,415,400VND ($2,985)	<0.001	65,007,400VND ($2,795.78)	73,823,300VND ($3,174.92)
Year of surgery	2021	69,376,140VND ($2,984)	**Ref**			
	2022	64,690,830VND ($2,782)	-6,269,500VND ($-270)	<0.001	-7,316,600VND ($-314.67)	-5,222,300VND ($-224.6)

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Conflict of interest

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Cost-Effectiveness Analysis of Fixed-Dose Combination versus Free-Equivalent Combination in Hypertension Treatment for Outpatients: a Case Study at a Regional Hospital in Southern Vietnam

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ABSTRACT

Combination regimens have become the primary strategy for hypertension treatment, showing higher efficacy in blood pressure control than monotherapy. However, more medications can reduce patient adherence. Fixed-dose combination (FDC), which are combinations of two or more active drugs in a single dosage form, has demonstrated as a promising strategy to mitigate the issue. Although the use of FDC will increase the medications cost, it is hypothesized to be cost-effectiveness when the total treatment costs are evaluated. This study aims to evaluate the cost-effectiveness of FDC versus free-equivalent combination (FEC) in hypertension treatment for outpatients at a regional hospital in Southern Vietnam. The cost-effectiveness analysis used real world data from patient medical records from 2017 to 2023. The direct medical costs were allocated using the GLM model for hypertension and comorbidities treatment. The effectiveness was evaluated with Kaplan-Meier survival analysis to determine proportion surviving free of cardiovascular events. Results were expressed as the incremental cost-effectiveness ratio (ICER), and uncertainty was assessed through probabilistic sensitivity analysis. The outpatient cost of FEC was 1.33 times higher compared to FDC. Kaplan-Meier and Cox analyses indicated that patients receiving FEC treatment, compared to those receiving FDC treatment, had hazard ratios for cerebrovascular disease, ischemic heart diseases, other forms of heart disease of 1.02 (95% CI: 0.7-1.48), 1.05 (95% CI: 0.9-1.22), 0.58 (95% CI: 0.29-1.17) respectively. In terms of the survival probability of cerebrovascular disease, the cost-effectiveness analysis showed that FDC is dominant compared to FEC. On the other hand, the ICERs were 1,837,454 VND for a 1% proportion surviving free of ischemic heart diseases, 332,070 VND for a 1% proportion surviving free of other forms of heart disease. The use of FDC was a cost-effective strategy in prevention of cerebrovascular disease compared to FEC among patients with hypertension.

KEYWORDS: FDC; Fixed-Dose Combination; FEC; Free-Equivalent Combination; Hypertension; Cost-Effectiveness

1. INTRODUCTION

Hypertension has emerged as a predominant global cause of mortality, accounting for an estimated 9.4 million deaths annually[1]. It stands as a significant risk factor for cardiovascular diseases, including myocardial infarction, stroke, chronic kidney disease, and peripheral artery disease[2–5]. The prevalence of hypertension has risen dramatically, affecting approximately 1.13 billion individuals worldwide in 2015, with projections indicating a surge to 1.56 billion by 2025 [6,7]. In Vietnam,

hypertension poses a substantial public health challenge, with the annual number of affected individuals increasing by an estimated average of 1% between 1980 and 2017, potentially reaching 11 million by 2025[8].

In current hypertension management, monotherapy has proven insufficient in achieving target blood pressure levels, necessitating higher doses that elevate the risk of adverse effects. Conversely, combination pharmacotherapy has emerged as the primary strategy for enhancing blood pressure control, demonstrating superior efficacy compared to monotherapy [9]. Traditionally, the strategy involved combining individual monotherapy tablets, known as Free Equivalent Combination (FEC) therapy. However, a shift towards using fixed-dose combination tablets has become evident and has shown to significantly improve drug compliance compared to FEC. Fixed-dose combination (FDC) drugs hold promise in reducing treatment costs and simplifying patient regimens compared to FEC drugs. This approach is increasingly endorsed in hypertension treatment guidelines, showcasing improved blood pressure-lowering capabilities, and minimized side effects compared to high-dose monotherapy [10]. In 2019, the World Health Organization (WHO) added several FDCs to the Essential Medicines List for hypertension treatment.[11] Furthermore, research findings provide scientific evidence underscoring the potential efficacy and cost-effectiveness of FDCs in hypertension treatment.

Le Van Thinh Hospital is a primary healthcare facility in Southern Vietnam. With the escalating number of hypertensive patients and extensive medication usage at Le Van Thinh Hospital, there was an increasing demand to strike a balance between cost and treatment effectiveness in hypertension management. Based on the actual usage of antihypertensive medications at the hospital, the utilization rate of FEC drugs remained high, particularly with Losartan, Perindopril, and Amlodipine. Among FDC drugs, the combination of Perindopril and Amlodipine had the highest utilization rate. As the treatment landscape shifted towards FDC therapy from monotherapy, various challenges emerge for the Department of Pharmacy in forecasting and supplying medications and for physicians in adopting new treatment paradigms. Therefore, the objective was to evaluate the cost-effectiveness of FDC (Amlodipine + Perindopril) with FEC in outpatient hypertension treatment, providing valuable evidence for drug selection in clinical practice.

2. MATERIALS AND METHODS

2.1. Study Design

Cost-effectiveness analysis study compared two treatment groups: (1) a fixed-dose combination (FDC) of Amlodipine (a calcium channel blocker) and Perindopril (an Angiotensin II receptor blocker - ARB), and (2) a control group receiving free-equivalent combination (FEC) therapy with single-agent Amlodipine and Perindopril. The study was based on real data collected from all patient prescriptions at Le Van Thinh Hospital during the period from 2017 to 2023. Direct medical costs were allocated using the GLM model for hypertension and comorbidities treatment. Effectiveness was evaluated using Kaplan-Meier survival analysis to determine the proportion of patients surviving free of cardiovascular events. Results were expressed as the incremental cost-effectiveness ratio (ICER), and uncertainty was assessed through probabilistic sensitivity analysis.

2.2. Study Participants

A retrospective cross-sectional study was conducted using all electronic medical record data of hypertensive patients at Le Van Thinh hospital giai đoạn 2017 - 2023, meeting the following inclusion and exclusion criteria:

Inclusion criteria:

- Hypertensive patients (diagnosed with one of the following ICD-10 codes: I10, I11, I12, I13, I15) receiving outpatient care covered by Health Insurance at Le Van Thinh hospital.
- Outpatient and inpatient treatment between 01/01/2017 and 30/06/2023
- Patients receiving continuous treatment for a minimum of 12 months.
- Time interval between two treatment sessions not exceeding 90 days

Exclusion criteria:

- To mitigate the impact of Covid-19, patient data from 01/01/2021 to 31/12/2021 was excluded from the study
- Lack of treatment information.

2.3. Data Analysis

2.3.1. Treatment Cost Analysis

The study analysed direct medical costs based on the payer's perspective perspective (Health Insurance) included various cost components: drug costs, hypertension drug costs, hospitalization costs, paraclinical costs, medical examination costs, laboratory costs, other costs (imaging costs, blood costs, medical supplies costs, and surgical procedure costs). The total cost of hypertension treatment includes (1) The allocated cost for hypertension (2) The allocated cost for comorbidities [12]. To estimate these two cost groups, the study employed a generalized linear model with Gamma distribution and loglink function, applied to individual patient-level cost data [13, 14, 15] . The estimated equation for the cost of treating hypertension is established as follows:

$$cost_{id_j} = \left[c_{i|d_j=1.d_{k_{k\neq j}}} = 0, d_{kl_{kl\neq j}} = 0 * 0.d_{jk_{k\neq j}} = 1 * 0\right] - \left[c_{i|d_j=0.d_{k_{k\neq j}}} = 0, d_{kl_{kl\neq j}} = 0 * 0.d_{jk_{k\neq j}} = 0 * 0\right]$$

Concurrently, to assess the correlation between comorbidities and the cost of treating hypertension, the study formulated an equation to compare the combined cost of treating each individual comorbidity and hypertension with the cost of treating each comorbidity independently.

2.3.2. Treatment Effectiveness Analysis

The effectiveness of hypertension treatment has normally been evaluated based on blood pressure values of patients at specific stages. However, when retrieving data from the electronic record, blood pressure values could be missing or the extraction process may encounter difficulties, leading to low reliabiliity and high risk of bias. Therefore, this study evaluated the treatment effectiveness of the treatment and control groups using incurred events including: (1) Cerebrovascular complications (I60-I69); (2) Ischemic heart diseases (I20-I25): (3) Other heart diseases: cardiac arrhythmias (I32-I52).

The study employed survival analysis methods with Kaplan-Meier estimation to assess treatment effectiveness while adjusting for confounding factors that included patient age and gender variables. Hazard ratios (HR) and confidence intervals (CI) were also calculated using Cox regression models. Kaplan-Meier analysis results were tested with the log-rank test at a 95% confidence level.

2.3.3. Cost-Effectiveness Analysis

The results of the cost-effectiveness analysis were the additional costs and effectiveness of the FDC group (treatment group) compared to the FEC group (control group). Subsequently, the Incremental Cost-Effectiveness Ratio (ICER) was calculated. The ICER index, indicating the increased cost per unit of increased effectiveness, was estimated using the formula:

ICER=CostT− CosrCEffectivenessT− EffectivenessCostCostT− CosrCEffectivenessT− EffectivenessC

Where: T is the FDC group (treatment group); C is the FEC group (control group). Costs included outpatient treatment costs (using the allocated cost for hypertension) and inpatient treatment costs. Effectiveness was survival probability of cardiovascular events.

The study employed probability Sensitivity Analysis (PSA) to evaluate the simultaneous effects. In PSA, each input variable of the model had a certain degree of uncertainty and was

described by a probability distribution. The study used 100,000 Monte Carlo iterations to record different cost and effectiveness value pairs. Results were presented through estimated points of ICER values along with 95% confidence intervals, cost-effectiveness acceptability curves, and cost-effectiveness planes with quadrants of estimated ICER values.

3. RESULTS AND DISCUSSION

3.1. Treatment Cost

The study included 177,998 outpatients from January 2017 to June 2023. Of these, 153,544 patients were older than 18 years. Patients using antihypertensive drugs totaled 66,963, and 37,707 met treatment compliance criteria. Data on age and gender were recorded for these 37,707 patients. Patient ages ranged from 18 to 106 years, with an average age of 62.32 (±11.88) years; 87.08% were over 50 years old. The sample comprised 58.31% male (21,986) and 41.69% female (15,712) patients, indicating a 16.62% higher prevalence of hypertension in men. The average number of comorbidities was 1.13 (±1.46). The most common comorbidities were dyslipidemia (E78) with 2,883 cases, diabetes mellitus (E10-E14) with 1,115 cases, and heart failure (I50) with 1,088 cases.

The results of the analysis of total costs and cost components allocated for hypertension treatment and comorbidities are presented in Table 1. For each examination, hypertensive patients paid 741,492 ± 1,241,100 VND ($30.59 ± $51.20). Of this, the allocated cost for hypertension was 341,739 ± 447,900 VND, and the allocated cost for comorbidities was 329,880 ± 327,763 VND ($13.61 ± $13.52).

Table 1. Total costs and cost components allocated for hypertension treatment and comorbidities

	Total cost	**The allocated cost for hypertension**	**The allocated cost for comorbidities**
Average ***(per person visit)***	741,492VND ($30.59)	341,739 VND ($14.10)	329,880 VND ($13.61)
Standard deviation	1,241,100 VND ($51.20)	447,907 VND ($18.48)	327,763 VND ($13.52)
Median ***(per patient visit)***	531,747 VND ($21.94)	287,309 VND ($11.85)	251,664 VND ($10.38)
Q1	378,635 VND ($15.62)	209,787 VND ($8.65)	126,522 VND ($5.22)
Q3	707,721 VND ($29.20)	377,151 VND ($15.56)	446,800 VND ($18.43)

** The costs are calculated in VND (Vietnamese Dong) and $ (US Dollar)*

3.2. Treatment Effectiveness

The Kaplan-Meier and Cox analysis results indicate that The study analyzed the survival probability of cerebrovascular disease, ischemic heart diseases, and cardiac arrhythmias in hypertensive patients using FDC and FEC. The Kaplan-Meier and Cox analysis results indicated that patients treated with FEC had a higher risk of experiencing cerebrovascular disease and ischemic heart diseases compared to those using FDCs, with hazard ratios of 1.02 and 1.05 (95% CI), respectively. However, for cardiac arrhythmias, the risk with FEC was lower, with a hazard ratio of 0.58.

1. Cerebrovascular complications (I60 - I69)

	coef	exp(coef)	se(coef)	exp(coef) lower 95%	exp(coef) upper 95%	p
Control	0.02	1.02	0.19	0.70	1.48	0.92

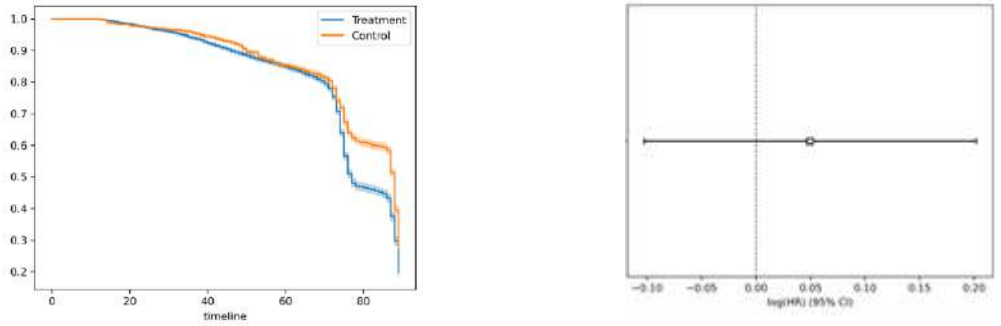

Figure 1. The Kaplan-Meier and Cox analysis results of cerebrovascular diseases

2. Ischemic heart diseases (I20 - I25)

	coef	exp(coef)	se(coef)	exp(coef) lower 95%	exp(coef) upper 95%	p
Control	0.05	1.05	0.08	0.90	1.22	0.52

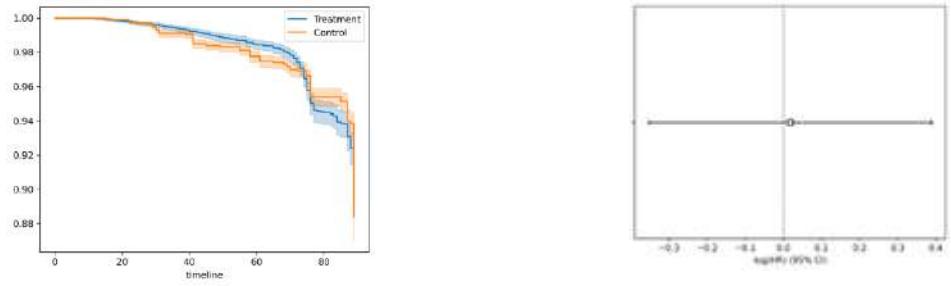

Figure 2. The Kaplan-Meier and Cox analysis results of Ischemic heart diseases diseases

3. Other heart diseases: atrial fibrilliation (I32-I52)

	coef	exp(coef)	se(coef)	exp(coef) lower 95%	exp(coef) upper 95%	p
Control	- 0.55	0.58	0.36	0.29	1.17	0.13

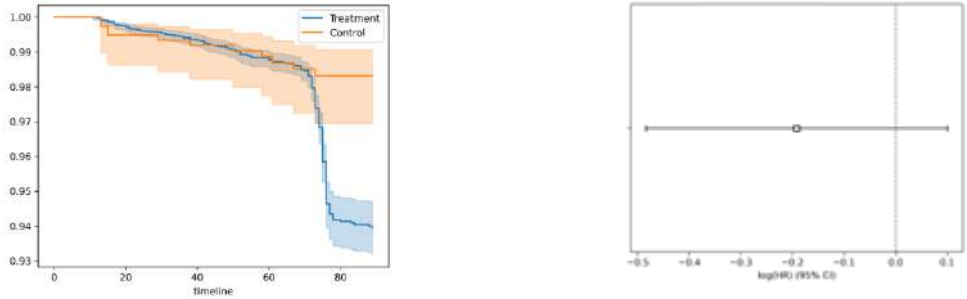

Figure 3. The Kaplan-Meier and Cox analysis results of atrial fibrilation diseases

3.3. Cost-Effectiveness Analysis

The base case analysis results show that when using FDC, it reduces the average total treatment cost in a year by 2,756,181 VND ($113.70) compared to FEC. In terms of effectiveness, FDC increases the survival probability from cardiovascular events by 0.3% compared to FEC and reduces the rates of avoiding non-cerebrovascular events and ischemic heart diseases, and cardiac arrhythmias by 1.5% and 8.3%, respectively. Therefore, considering the effectiveness rate of surviving free of cerebrovascular disease, FDC reduces costs and increases effectivness, or FDC was a dominant regimen compared to FEC. Regarding other outcomes, the ICERs were 1,837,454 VND ($75.80) for a 1% proportion surviving free of ischemic heart diseases, 332,070 VND ($13.70) for a 1% proportion surviving free of other forms of heart disease.

Table 3. Cost-effectiveness analysis results

	Fixed-dose combination (FDC)		**Free equivalent combination (FEC)**		**Δ**
	Mean	**SD**	**Mean**	**SD**	
Cost					
Average annual treatment cost	10,898,006 VND ($449.59)	8,694,430-15,101,677) ($358.68 - $623.01)	13,654,187 VND ($563.29)	(9,583,225-20,445,865) ($395.35 - $843.48)	-2,756,181 VND ($113.70)
Effectiveness					
The proportion surviving free of I60-I69(%)	95,2	(94,2 - 96,6)	94,9	(94,0 - 96,2)	0,3%
The proportion surviving free of I20-I25 (%)	95,9	(95,1 - 97,1)	97,4	(0,969 - 0,980)	- 1,5%
The proportion surviving free of I32-I52 (%)	53,5	(46,0 - 67,1)	61,8	(55,4 - 73,2)	- 8,3%
ICER					
The proportion surviving free of I60-I69(%)	**Dominant**				
The proportion surviving free of I20-I25 (%)	**1.837.454** VND **($75.80)**				
The proportion surviving free of I32-I52 (%)	**332.070** VND **($13.70)**				

4. CONCLUSION

In conclusion, studies have consistently demonstrated the cost-effectiveness of FDC, particularly Amlodipine + Perindopril, compare to FEC in hypertension management. FDC therapy reduced overall treatment cost and enhances survival rates following cerebrovascular disease events compared to FEC therapy. These results underscore the importance of using FDCs in hypertension management to reduce the economic burden and improve patient outcomes.

Conflict of interest

The authors declare that they have no conflict of interest.

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SP-1703106-P

Correlation Analysis of Factors Influencing Customer Loyalty in Retail Pharmacy Chains: A Cross-Sectional Study in Vietnam

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ABSTRACT

In the current era, health has become a growing concern for everyone, leading to a rise in the number of pharmacies in residential areas. This has created a fiercely competitive market, compelling pharmacy chains to implement effective elements and strategies to retain customers. Our research aims to assist pharmacy chains in better understanding the elements that lead to customer retention and higher-quality service. Additionally, evaluate and improve quality assurance guidelines and practices, which will result in patient-centered treatment and client involvement. Analyze factors affecting customer loyalty to pharmacy chains in Can Tho City from 2023 to 2024. The data for this study was collected through interviews with customers who purchased medication from pharmacy chains. A pre-designed set of questions was used to gather information. A total of 747 participants who met the research criteria were included in the study. There were 4/32 variables eliminated after conducting the Cronbach's Alpha test. The Kaiser-Meyer-Olkin (KMO) coefficient, ranging from 0.886 to 0.91, indicated that the data was statistically significant and met the criteria for Exploratory Factor Analysis. The results satisfied the requirements for total variance extracted (>50%) and the Eigenvalue coefficients were all greater than 1 (from 1.014 to 5.385, $p<0.05$). Confirmatory Factor Analysis and Structural Equation Modeling were consistent with market data on factors affecting customer loyalty. The analysis revealed that pharmacy chain brands, price, facilities, convenience, and employee knowledge had a positive influence on loyalty. Importantly, all factors demonstrated a statistically significant impact ($p<0.05$). This analysis successfully identifies and models the factors influencing customer loyalty to the services provided by pharmacies, thereby advancing research on the impact of dedication, trust, and barrier transformation on customer loyalty.

KEYWORDS: Influencing Factors; Loyalty; Pharmacy Chain; Satisfaction

1. INTRODUCTION

Across various industries, studies have demonstrated that significant value is created when companies build loyalty with their customers, employees, and shareholders[1]. Trust, commitment, and satisfaction have a significant and positive influence on customers' loyalty attitudes. Additionally, these attitudes have been found to impact actual customers positively and significantly loyalty behaviors[2]. Over recent years, patients have shown a growing interest in the quality of health services. While ensuring accessibility was once the primary focus in meeting patient demands, the increased

supply has expanded patient options[3]. In the pharmaceutical industry, customer loyalty is essential for pharmacy chains to attract and retain customers. Currently, due to economic challenges and prolonged hospital treatment times, more and more people with non-critical illnesses tend to visit local pharmacies for consultation. Retail pharmacies and pharmacy chains play an important role in the pharmaceutical industry, supplying medications to individuals. Loyalty is defined as "the degree to which a customer exhibits repurchasing behavior from a service provider, possesses a positive attitudinal disposition toward the provider, and considers using only this provider's services when a need for this service arises"[4].

According to BMI, Vietnam was ranked 13th out of 175 countries with the fastest-rising pharmaceutical spending marketplaces in the world in 2013[5]. The demand for pharmaceuticals is increasing rapidly due to high economic growth, rising per capita income, and heightened awareness of health issues among the population. As a result, many retail pharmacies and pharmacy chains such as Long Chau, Pharmacity, and others began to expand dramatically. However, customer loyalty becomes more difficult to obtain as drugstore chains employ increasingly distinctive techniques to adapt to the ever-changing nature of the pharmaceutical market[6]. Retaining loyal customers is challenging when competitors are implementing customer attraction strategies to increase their market share. If pharmacy chains cannot meet customer needs, or provide poor services, they will struggle to succeed in a crowded market.

There have been few studies on customer loyalty to pharmacy chains in the Mekong Delta. Therefore, our study was conducted to help pharmacy chains understand the factors that contribute to client retention and improve service quality. The purpose of our research is to identify the factors affecting customer loyalty to pharmacy chains in Can Tho City.

2. MATERIALS AND METHODS

2.1. Research Subjects

People buy medicine at the chain pharmacy system in Can Tho City during 2023-2024.

2.2. Selection Criteria

Customers who have bought drugs at least once from pharmacies of pharmaceutical retail chains. Respondents must have practical experience with and an interest in the chain's products and services[7].

2.3. Exclusion criteria

Respondents providing incomplete survey responses were excluded.

2.4. Theoretical Foundation

We selected the "Theory of Planned Behavior (TPB)" as our theoretical foundation. This theory, founded and developed by social psychologist Ajzen, holds significance in the field of mentality and behavioral science"[8]. TPB is widely used in research aimed at predicting and explaining human behaviors[9].

Besides subjective norms and behavioral control, another factor that can influence patient loyalty is trust. The relationship marketing literature has revealed that trust is a key factor in building customer loyalty[10,11]. Since then, the research team has built a research model as Figure 1 below:

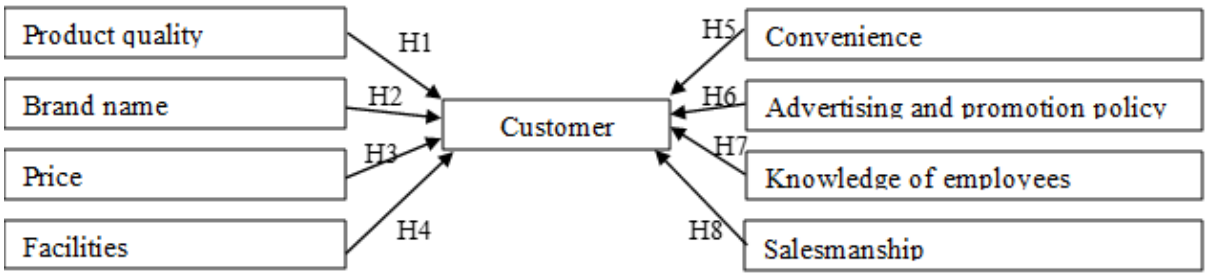

Figure 1. Research Model.

Hypothesis H1: Product quality has a positive impact on customer loyalty to the pharmacy chain system. Hypothesis H2: Brand name has a positive impact on customer loyalty to the pharmacy chain system. Hypothesis H3: Price has a positive impact on customer loyalty to the pharmacy chain. Hypothesis H4: Facilities have a positive impact on customer loyalty to the pharmacy chain system. Hypothesis H5: Convenience has a positive impact on customer loyalty to the pharmacy chain system. Hypothesis H6: Advertising and promotion policy has a positive impact on customer loyalty to the pharmacy chain system. Hypothesis H7: Knowledge of employees has a positive impact on customer loyalty to the pharmacy chain system. Hypothesis H8: Salesmanship has a positive impact on customer loyalty to the pharmacy chain system.

2.5. Design Survey Questionnaires and Measurement Scales

This study utilized a cross-sectional descriptive research design and collected data through direct phone interviews, Google forms or printed questionnaires with clients who purchased drugs at pharmacy chains or hospitals in Can Tho City. The interviews were conducted using a set of questions to evaluate loyalty and factors influencing clients' decisions to return to the pharmacy chain for future purchases. The survey questionnaire system is designed based on outcome-oriented questions. Some previous studies included additional clarifying questions, such as: Does subjective norm affect customer loyalty? Does perceived behavioral control affect customer loyalty? Does trust affect customer loyalty? Or identify some key factors that make the difference between service quality and satisfaction[11-13]. Quality is a model-related evaluation while satisfaction is determined by various external signals including price, reputation, promotions, employee attitudes, and employee skills. Regression analysis was also performed to test the proposed conceptual model and hypotheses.

The first part of the questionnaire combines loyalty with human-related factors (gender, year of birth, living area, occupation, and career income) to identify statistically significant factors that have an impact on the scale/dependent variable. Respondents were required to fill in the available spaces with their information. The second part involves assessing a combination of factors affecting customer loyalty in chain systems. These factors include product quality (3 items), pharmacy chain brand (3 items), price (3 items), facilities (3 items), convenience (3 items), promotional programs (3 items), along with the professional knowledge of the staff (3 items), salesmanship (4 items)[14]. To evaluate these factors, a relatively popular measurement scale in scientific research questionnaires was used to verify individual opinions, behaviors, and perceptions. In this study, responses to the questions were scored on the following scale: 1 = "strongly disagree", 2 = "disagree", 3 = "neutral", 4 = "agree", 5 = "completely agree"[15].

The scale achieved reliability with a Cronbach's alpha coefficient of ≥ 0.6 and a total variable correlation coefficient of > 0.3[16]. In Exploratory Factor Analysis (EFA), the criteria for sample size appropriateness include a KMO (Kaiser-Meyer-Olkin) coefficient ranging from 0.5 to 1, a significant Bartlett test ($p < 0.05$), and a factor loading of at least 0.3[17]. A loading factor of 0.5 indicates good statistical significance, with a total extracted variance $\geq 50\%$. Eigenvalues greater than 1 are retained. Confirmatory Factor Analysis (CFA) assesses the fit of a measurement model to actual data, enhancing reliability and validity, and refining model suitability[18]. Structural Equation Modeling (SEM), a second-generation statistical technique, analyzes multidimensional relationships between

variables, visually representing these relationships and improving theoretical predictions by specifying measurement properties and latent variable relationships[19-20].

2.6. Sample Size

To conduct Exploratory Factor Analysis (EFA), a large sample size is required and is determined based on the minimum sample size and the number of variables included in the analysis. Following Carpenter's recommendation of a minimum observation/variable ratio of 5:1, with 32 questions in the adjusted survey, the calculated minimum sample size was 160[16]. In this study, the actual sample size was 747, meeting the sample size conditions.

2.7. Survey Methods

Convenient random sampling was employed using survey methods through direct phone interviews, Google forms, or printed questionnaires with customers who purchased medicines at pharmacy chains or met the sampling criteria in Can Tho City during the 2023-2024 period. Participants completed the surveys based on real-life experiences while purchasing and using services at these chains[21].

Table 1. General characteristics of research subjects.

Sample Characteristics		**Frequency (n=747)**	**Percentage (%)**
Gender	Male	282	37.8
	Female	465	62.2
Career	Agriculture	40	5.4
	Sales executive	72	9.6
	Public servant	89	11.9
	Office employee	33	4.4
	Businessman	71	9.5
	Others	442	59.2
Earnings	Every month	508	68.0
	Every year	17	2.3
	Others	222	29.7
The pharmacy chain is your first choice when having needs	Yes	603	80.7
	No	144	19.3
Expense	< 1.96$	179	24.0
	1.96$ – 7.85$	424	56.8
	7.85$ – 19.62$	89	11.9
	19.62$ – 39.24$	38	5.1
	> 39.24$	17	2.3
How many times do you buy per month?	< 3	512	68.5
	3 – 5	179	24.0
	5 – 10	31	4.1
	> 10	25	**3.3**

2.8. Data analysis

Data were analyzed using SPSS 26.0, using descriptive statistical data analysis to calculate frequency, mean, and standard deviation. Correlation analysis was also performed to assess the degree of correlation between variables. Finally, AMOS software was used for moderation-mediation analysis, which included regression calculations, Confirmatory Factor Analysis, and Structural Equation Modeling[22-24].

3. RESULTS AND DISCUSSION

3.1. General Characteristics of the Study Sample

Table 1 shows that most study subjects are female (62.2%), while male customers account for a lower proportion (37.8%). Regarding qualifications, the majority of customers hold an Intermediate level (47.9%).

Table 2. Results testing of the Cronbach Alpha scale.

Variable-total correlation			
Survey variables	**Coefficient of correlation of total variables**	**CA coefficient when eliminating variables**	**Cronbach's Alpha coefficient (CA)**
PQ1	0.847	0.845	0.909
PQ2	0.843	0.849	
PQ3	0.772	0.908	
BN1	0.868	0.892	0.931
BN2	0.851	0.909	
BN3	0.870	0.898	
P1	0.980	0.981	0.989
P3	0.983	0.979	
F1	0.967	0.969	0.982
F2	0.950	0.980	
F3	0.964	0.971	
C1	0.978	0.963	0.983
C3	0.983	0.960	
ACP1	0.940	0.932	0.963
ACP2	0.929	0.939	
KE1	0.860	0.913	0.936
KE2	0.884	0.894	
KE3	0.861	0.912	
SS1	0.998	0.996	0.998
SS2	0.998	0.996	
SS3	0.997	0.996	
SS4	0.991	0.997	
L1	0.933	0.969	0.975
L2	0.929	0.970	
L3	0.932	0.969	
L5	0.940	0.969	
L6	0.903	0.972	
L7	0.897	0.972	

3.2. Testing the Reliability of the Cronbach Alpha Scale

As shown in Table 2, several factors were eliminated based on their impact on the Cronbach's Alpha (CA) coefficient: Factors P2, C2, ACP3, and L4 were eliminated because the CA coefficient upon removing this variable is higher than the current CA coefficient. In contrast, the remaining factors were retained as they met the required conditions.

Cronbach's alpha values for CSR, Trust, and Customer Loyalty (including both Loyalty and Common Behavior) are presented in Table 3.

Table 3. Descriptive statistics results and reliability (N = 747).

	Cronbach's Alpha	**Mean**	**SD**
PQ	0.909	4.0843	0.73034
BN	0.931	4.1156	0,75855
P	0.989	3.9670	0.74921
F	0.982	4.0995	0.68956
C	0.983	4.856	0.95695
ACP	0.963	4.2597	0.80238
KE	0.936	4.0437	0.90386
SS	0.998	4.1201	0.85279
L	0.975	4.1329	0.89426
Valid N (List wise)			

3.3. Results of EFA confirmatory factor analysis

3.3.1. Results of EFA exploratory analysis of independent variables

The extracted variance value surpassed 70%, reaching 93.839%. Simultaneously, the coefficient KMO = 0.886 ($0.5 \leq KMO \leq 1$) and the significance level value (Sig.) of the Bartlett test was 0.000, meeting the condition of being less than 0.05). The six groups of factors extracted at Eigenvalue = 1.014 (> 1) all meet the conditions. Consequently, we conducted an EFA analysis for the subsection factors in the independent components as inferred from Table 4.

According to Table 4, the subsections were re-divided into three new factors based on the correlations between the subsections and the factors. Therefore, the variables retained after EFA analysis fully satisfy the condition that the loading factor is greater than 0.5, indicating their significant role and practical meaning.

The values in Table 5 indicate the analysis results show satisfaction with a KMO value of 0.910. Barlett's test with Sig value = 0.000, and Eigenvalue = 5.385 (> 1) mean that one factor was extracted. The total variance extracted was 89.742%, which satisfies the condition of greater than 50%. As a result, EFA analysis was conducted with observed variables of the dependent component "(L): Loyalty".

3.4. Results of Confirmatory Factor Analysis CFA

According to the CFA results, the model appears to be a good fit for the actual data, with TLI= 0.965, CFI= 0.973, GFI= 0.902 (> 0.9), RMSEA= 0.064 (< 0.08) and Chi-square/df= 4.050 (< 5) (Figure 2). This reflects the unidirectionality of the measurement scales, as explained by Hu and Bentler[25] on Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives, Structural Equation Modeling.

Table 4. Results of EFA Exploratory Analysis of the independent variables.

Rotated Component Matrix[a]						
	1	2	3	4	5	6
SS1	0.863					
SS3	0.862					
SS4	0.858					
SS2	0.849					
KE1	0.822					
KE2	0.800					
KE3	0.791					
C3	0.752					
C1	0.750					
F1		0.912				
F2		0.907				
F3		0.903				
ACP2			0.931			
ACP1			0.928			
ACP3			0.915			
PQ3				0.845		
PQ2				0.824		
PQ1				0.806		
BN2					0.796	
BN1					0.724	
BN3					0.714	
P1						0.956
P3						0.946

Table 5. Results of Exploratory EFA Analysis of the dependent variable.

Component Matrix[a]	
L2	0.962
L1	0.960
L3	0.957
L5	0.953
L7	0.937
L6	0.913

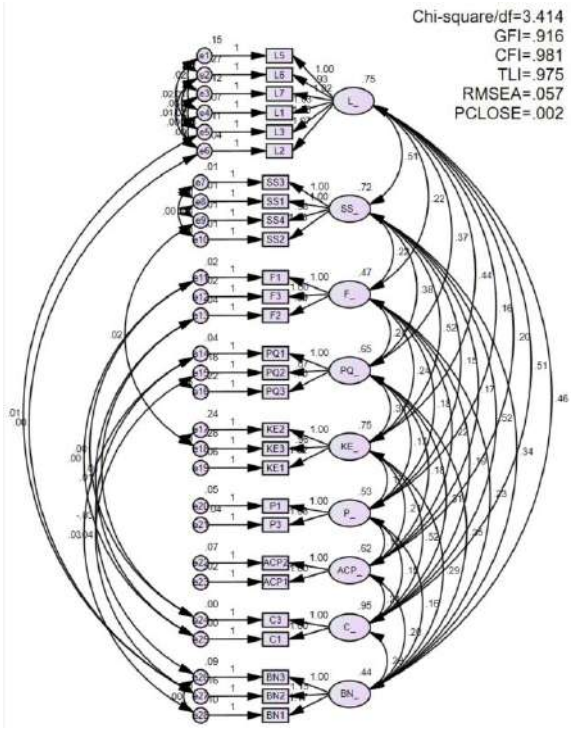

Figure 2. Description of Confirmatory Factor Analysis results.

3.5. SEM Test Results

Figure 3 shows the Good Fit Index (GFI) ranges from 0 to 1. The model result of the GFI value is 0.895, indicating a good fit. The squared error RMSEA reaches a value of 0.067, which is considered satisfactory. Current standards in reports allow RMSEA to be less than 0.08 with 95% confidence. The Comparative Fit Index (CFI) value exceeding 0.9 indicates a good fit, with higher values representing a better fit. The model result, CFI = 0.971, is considered good. A TLI index approaching 1 indicates higher suitability. The model result, TLI = 0.962, is considered good.

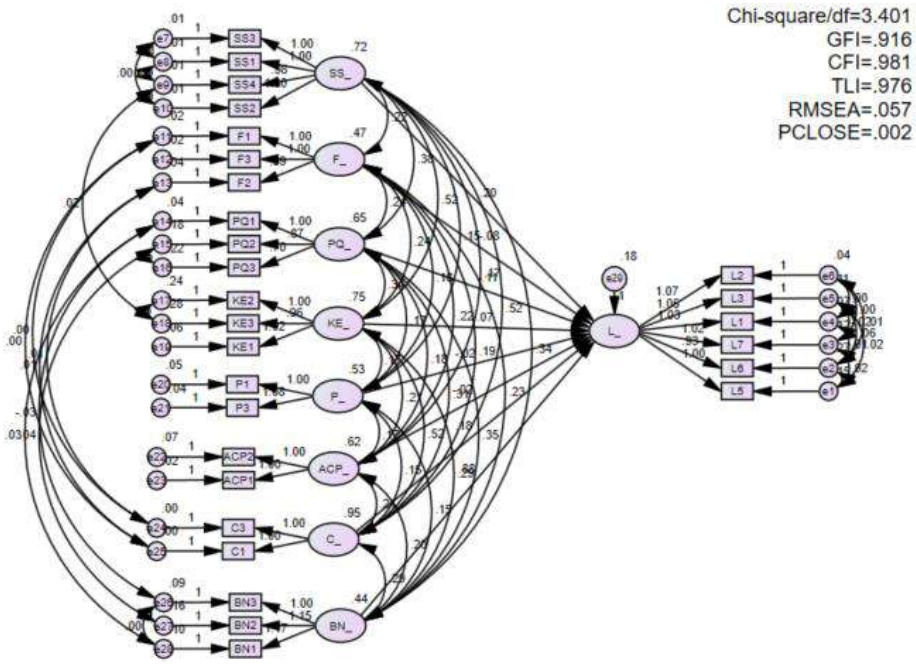

Figure 3. Description of SEM Test results.

3.6. Unstandardized Regression Coefficients (Estimates)

The results depicted in Table 6 illustrate the impact factors as follows: The pharmacy chain's brand, price, facilities, convenience, and employee knowledge all have a positive and statistically significant impact on loyalty, with significance levels <0.05.

3.7. Discussion of Factors Affecting Customers' Repurchase at Pharmacy Chains in Can Tho City

According to the survey results, we have identified several factors that, in line with existing literature[28], influence customers' return to retail pharmacy chains, which are discussed in detail below.

Regarding product quality, most survey participants believe that the products sold at pharmacies have a shelf life of over six months and demonstrate good treatment effectiveness. However, a small portion of participants feel that the products at the pharmacy do not fully meet their needs. Various activities, including professional drug guidance and consulting services, have been implemented to provide buyers with comprehensive information. These efforts aim to enhance consumers' knowledge about the quality of products at pharmacy chains[29].

Table 6. Description of Hypothesis Testing results.

			Estimate	**S.E.**	**C.R.**	**P**	**Hypothesis**
L_	<---	PQ_	0.007	0.019	0.361	0.718	H1: Rejected
L_	<---	BN_	0.524	0.034	15.195	***	H2: Accepted
L_	<---	P_	0.045	0.014	3.182	0.001	H3: Accepted
L_	<---	F_	0.042	0.019	2.246	0.025	H4: Accepted
L_	<---	C_	0.116	0.015	7.904	***	H5: Accepted
L_	<---	ACP_	-0.013	0.015	-0.893	0.372	H6: Rejected
L_	<---	KE_	0.101	0.020	5.153	***	H7: Accepted
L_	<---	SS_	0.185	0.021	8.818	***	H8: Accepted

The brand of the pharmacy chain significantly impacts customer attraction and retention. Almost all our survey participants express trust in the quality of products attributed to the pharmacy chain's brand because they believe that a product sold at a branded pharmacy chain has acceptable quality, which aligns with a study by Mohammadzadeh[27]. Brand image is an asset formed through the relationship between the pharmacy chain and customers, so most customers will make purchasing choices based on product availability and brand image[30]. This is demonstrated by successful examples such as Long Chau, Trung Son, and other well-known retail pharmacy chains in Can Tho City. Therefore, retail pharmacy chains should develop strategies for brand development to attract and retain many customers.

In terms of facilities and convenience, the pharmacy chain with airy space, display shelves with fully arranged price lists, a shopping website, a return policy, and the geographical location of the pharmacy chain are all elements that survey participants find highly appealing. The physical environment and all tangible aspects have a strongly positive influence on satisfaction and experience[31]. Promotional advertising policies are also regarded as a tangible feature since they can offer clients information about the type of service they expect and can play a vital role in enhancing customer satisfaction and generating long-term profits. Other tangible elements can include consultation rooms, equipment, and promotional materials used in a retail pharmacy chain[26].

Price significantly impacts customer return in pharmacy chains, as it directly impacts their subconscious perceptions of product value. Survey respondents agree that lower prices increase return likelihood, and they compare prices between stores to appreciate those with reasonable prices. Therefore, pharmacy chain businesses should develop a pricing strategy that can contribute to

building the brand image of the pharmacy chain, encouraging customers to make repeated purchases[30].

Most participants express satisfaction with pharmacy chain employees' knowledge and sales skills, particularly in providing advice on drug prices, quality, dosage, usage, and potential side effects, and show interest in their attitude and consulting skills. Hung and his associates found that in situations where there was no opposition, there was a high belief in pharmacists' competence to prescribe antibiotics without a prescription and that they had the essential skills for patient consultation and administering antibiotics when needed[32]. For that reason, it shows that customers' trust in pharmacists has a very positive influence on the trust of the pharmacy chain, ultimately affecting customers' repurchasing behavior.

According to Castaldo et al.'s study, trust in community pharmacists is the primary driving force that creates customer satisfaction. This trust not only directly influences satisfaction levels but also serves as a crucial factor, whether directly or indirectly, in building trust for pharmacy chains[33]. Therefore, pharmacy chains should develop their staff's competencies, skills, and attitudes by carefully selecting and training employees. According to Mohammadzadeh et al.'s study, customer satisfaction also increases if community pharmacists spend more time on consultation services[27].

When considering customer loyalty, the process of building loyalty is intricately tied to the quality of pharmacy services. The presence of a knowledgeable employee with a positive attitude plays a crucial role in enhancing customer satisfaction, making them more inclined to return for future purchases[28]. Additionally, meeting customer needs by delivering outstanding service quality is recognized as a key factor in creating customer loyalty. This not only fosters customer satisfaction but also significantly contributes to shaping the image, reputation, and brand of pharmacy chains[33]. Customer satisfaction is a part of their brand experience, which can lead to loyalty and long-term relationships between brands and customers. These factors interact with each other, contributing to customer satisfaction with the pharmacy chain[30].

The aforementioned factors have a positive influence on customer returns to pharmacy chains in Can Tho City. It is imperative for pharmacy chains to develop comprehensive plans and policies. Being able to neutralize these factors will ensure the success and development of pharmacy chains in the future.

4. CONCLUSION

According to this survey, customer loyalty to a particular pharmacy depends on the range of products it supplies, competitive prices, and competent and friendly staff. The results indicate that providing additional services at pharmacies also plays a crucial role in building client loyalty. Faced with increased competition in this sector, the availability of a wide variety of goods, the organization of regular promotions, and the introduction of additional, attractive services are critical for the success of pharmacies.

To gain loyal customers, pharmacies should strategically implement competitive strategies focused on delivering the optimum combination of service elements valued by clients, thereby establishing a unique competitive edge. Additionally, pharmacies should demonstrate adaptability and align with client expectations to achieve success and create a long-term customer network.

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Conflict of interest

The authors declare that they have no conflict of interest.

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The level of influence of factors affecting the Customer's decision-making for purchasing online healthcare products in Ho Chi Minh City, Vietnam.

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ABSTRACT

Introduction: The trend of using online purchasing for healthcare products is increasing. This research is based on the Theory of Planned Behavior (TPB), the Theory of Risk Perception (TPR), and the Technology Acceptance Model (TAM).

Objective: The study was conducted to determine the level of influence of factors affecting Customers' decision-making regarding online healthcare product purchasing in Ho Chi Minh City, Vietnam.

Methods: Data was collected from 323 customers through a direct survey using a 5-level Likert scale questionnaire from June 2022 to December 2022. Seven factors affect customer decision to purchase healthcare products online: (1) Perceived usefulness; (2) Convenience; (3) Personal attitude; (4) Subjective norms; (5) Perceived behavioral control; (6) Perceived risks on product quality and (7) Perceived risks on service quality. Exploratory factor analysis and multivariate linear regression were used to analyze data and their interactions.

Results: 83.3 % of participants were female, and 59.8% were between 31 and 40. The ranking of positive influencing factors is as follows: Perceived usefulness (β = 0.264); Convenience (β = 0.179); Personal attitude (β = 0.167); Subjective norms (β = 0.162); Perceived behavioral control (β = 0.148) with the percentages were 22.56%, 15.30%, 14.27%, 13.85%, and 12.65%, respectively. The ranking of negative influencing factors is as follows: Perceived risks on product quality (β = -0.144) and Perceived risks on service quality (β = -0.106), with 12.31% and 9.06%, respectively.

Conclusions: The most important positive factor affecting customers' online healthcare product purchasing decisions was perceived usefulness. This research can help pharmaceutical companies grasp the trend of buying healthcare products online, build more effective marketing plans and sales strategies, and ensure the quality of products sold to customers.

KEYWORDS: healthcare products online; factors affecting Customer's decisions; Ho Chi Minh City.

1. INTRODUCTION

The 4.0 revolution has had a strong impact on the pharmaceutical industry. Patients can easily search for the nearest reputable pharmacies and quickly buy healthcare products 24/7 without leaving home. E-commerce with online sales tools such as websites and healthcare applications is increasingly popular with users because of its convenience and comprehensiveness. Online shopping is the purchase of products and services through the Internet [1].

Online purchases are consumer behavior through online stores or websites that use online purchases[2]. Research on customers' decision to purchase healthcare products in Ho Chi Minh City is

based on the Theory of Planned Behaviour (TPB)[3]. In online shopping, a personal attitude refers to good or bad consumer reviews about using the Internet to purchase goods or services from retail websites. Personal attitudes positively and strongly impact customers' online purchasing decisions, as agreed upon by numerous empirical studies[3,6]. Subjective norms are formed by two factors: (1) beliefs about influencers assuming that this individual should carry out the behavior, and (2) motivation to conform to these influencers[3]. Subjective standards positively impact consumers' intentions to buy online[6,7]. Cognitive control of behavior is defined as the ease or difficulty of performing the behavior, which depends on the availability of resources and the opportunities to perform the claimed behavior that positively affects the intention to shop online[3,5].

Consumer behavior is influenced by a two-factor risk perception: risk perception related to products/services and online transactions[8]. Perceived risk refers to consumers' perceptions of uncertainty and the consequences of engaging in a particular activity[9]. The risk of buying online may be that the product is of a different quality[1,10]. Product risk in online shopping can be high because customers cannot check and test product quality and cannot change other products immediately[11]. To complete an online transaction, it is necessary to provide personal information, but if left unchecked, this information is lost, and users may experience inconvenience or risk having funds stolen from bank accounts or e-wallets. The risk of disclosing or selling personal information to other businesses, especially account numbers, is becoming a growing concern for online shoppers[12,13]. A negative relationship between perceived risk and online purchase intent has been found in numerous studies[14,15.] Purchasing products and services online and through e-commerce is fast, convenient, and saves time and costs. At the same time, it is possible to experience a variety of products, many different brands, in stark contrast to in-person shopping[16]. The perception that usefulness impacts a customer's online purchase intent has been demonstrated in numerous studies[17,18]. Nowadays, customers can choose their favorite products anywhere with just a smartphone or technological devices; consumers can search, choose products, and easily place orders, so the convenience factor positively impacts customers' online purchase intent proof[13,14].

Based on the Theory of Planned Behaviour (TPB), Theory of Perceived Risk (TPR), Technology Acceptance Model (TAM), current situation, and related research results, the author proposes a model consisting of 7 different factors affecting customers' decision to buy healthcare products online in Ho Chi Minh City includes: (1) Perceived usefulness; (2) Convenience; (3) Personal attitude; (4) Subjective norms; (5) Perceived behavioral control; (6) Perceived risks on product quality and (7) Perceived risks on service quality. This study was conducted to (1) understand the characteristics of customers who purchase online healthcare products and (2) measure the impact of factors influencing their purchasing decisions.

2. MATERIALS AND METHODS

2.1. Materials

The study subjects are customer characteristics and factors influencing the decision to purchase healthcare products online. Respondents are customers living in Ho Chi Minh City. Participants were excluded if they met the following criteria: (1) younger than 18 years, (2) declining consent, (3) unable to understand written or oral expression in Vietnamese.

2.2. Research Methodology

A cross-sectional descriptive study was carried out, using a direct survey using a Likert scale 5-level questionnaire, from (1) Strongly disagree to (5) Strongly agree. The questionnaire consisted of (1) general information from the respondents and (2) information about customers' decisions to buy healthcare products online, with 43 statements. Research concept scales are referenced from related studies, revised through preliminary research, and expressed as statements.

Data was collected from customers in Ho Chi Minh City from June 2022 to December 2022, collecting and excluding incomplete questionnaires, ensuring that the minimum sample size meets the requirements for the study. The study uses non-probability sampling methods with a convenient

sampling technique to save time and cost, in which quota sampling will perform overall grouping according to 2 criteria: (1) having purchased healthcare products online and (2) living in Ho Chi Minh City. The survey sample size is calculated using the following formula:

$$n = k \sum_{j=1}^{m} p = 5 x \sum_{j=1}^{8} p = 215$$

There:

n: Minimum sample size to determine
k: The ratio of observations to an analytical variable is 5:1 or 10:1
m: number of scales
pj: number of observed variables

Since the number of observed variables in the study model is 43 and the coefficient k is 5:1, the minimum sample size required for the study is 215. The sample size required for quantitative research depends on many factors, such as the data processing method or the required reliability. To perform a multiplicity regression analysis, the sample size is usually calculated using the formula $n \geq 50 + 8k$ (k is the number of independent variables of the model). Since the number of independent variables in the study model is 7, the minimum sample size required is 106. Considering the requirements of EFA (Exploratory Factor Analysis), multiple regression analysis, and time constraints, a sample size of 323 would be appropriate.

Using SPSS 22.0 software for entry, encoding, cleaning, descriptive statistical analysis, Cronbach's Alpha scale reliability testing, EFA analysis, and multiple linear regression.

3. RESULTS AND DISCUSSION

3.1. General Information

Of the 323 responses, women (83.3%) were higher than men (16.7%). The survey age group of 31- 40 years had the highest rate (59.8%). The majority of respondents have an income of 5-10 million/month (40.6%) and mainly live in Binh Tan District (15.2%), District 12 (14.6%), and Tan Phu District (13%).

The factors that customers are interested in when buying online are listed as comments of previous buyers, accounting for the highest proportion (21.2%), followed by promotions/discounts (21.0%), reputable and popular sellers (19.1%), sellers' stars rating (17.4%), accompanying services (exchange/return/refund) (15.3%) and some other factors (6.1%). Among the online shopping platforms commonly used by customers, Shopee accounted for the highest proportion (43.8%), followed by Lazada (27.2%), Tiki (14%), Sendo (1.7%), Amazon (1%), other platforms (10.3%).

For online healthcare products buying platforms that customers can know, including chain pharmacy websites (Long Chau, Pharmacity, Phano) (45.6%) and mobile applications that are well known in the drug retail market today, such as Long Chau (15.5%), Pharmacity (15.5%), and An Khang (9.6%).

3.2. The Results of Reliability

Cronbach's Alpha was used to check the reliability of each variable before collecting an accurate sample of 323 respondents. Therefore, the 48 samples are to collect data for prediction tests, identify errors, and assess the scale of questionnaire quality. The reliability test for each variable with a total of 43 questions. The results showed that all observed variables met the requirements for inclusion in the questionnaire to conduct official quantitative research.

The Cronbach's Alpha value of the 8 factors ranges from 0.813 to 0.960 (Table 1), showing that these factors are acceptable and excellent measurements. The questionnaire has high reliability, stability, and consistency during the research process. The observed variables of all factors are closely related and correlated with each other.

Table 1. Result in Cronbach's Alpha of variables in the scale

Influencing factors	Number of observed variables	Cronbach's Alpha
Personal attitude (PA)	6	0.905
Subjective norms (SN)	4	0.896
Perceived behavioral control (PBC)	6	0.937
Perceived usefulness (PU)	7	0.934
Perceived risks on product quality (PRPQ)	5	0.887
Perceived risks on service quality (PRSQ)	6	0.960
Convenience (CV)	6	0.955
Customer's decision-making for online healthcare product purchasing (CD)	3	0.813

The results of the EFA analysis in second times of independent variables according to the Principal Component Analysis (PCA) extraction method, Varimax rotation shows that all 38 observed variables (PA1 and PA6 were eliminated due to the discriminant validity is not guaranteed) meet the value requirements, specifically: KMO coefficient = 0.805 > 0.5; Bartlett test with Sig. = 0.00 < 0.05 (95% confidence); Eigenvalue = 1.618 > 1; total variance extracted 78.298% > 50%, indicating statistically significant factors; the factor loading is > 0.5, proving that these observed variables are reliable, convergent validity discriminant validity. Thus, seven factors extracted 78.298% of the variation in observed variables, and EFA analysis was satisfactory when analyzing the next steps.

The EFA analysis results depend on the method of extracting PCA factors. The Varimax rotation shows that three observed variables meet the requirements: KMO coefficient = 0.701 > 0.5; Bartlett test with Sig. = 0.00 < 0.05 (95 % confidence), total extract variance of 72.816 % > 50 %, Eigenvalue = 2.184 > 1 indicating statistically significant factor; the factor loading is > 0.5, indicating that these observed variables are all reliable.

3.3. Hypotheses Testing

After analyzing EFA, eight factors were formed and included to validate the model. Pearson correlation analysis was used to check the appropriateness of factors in the regression model. The H1-H7 hypotheses were tested by regression analysis.

All independent variables correlate with the dependent variable (Sig < 0.05), and the Pearson r correlation value of the independent variables is advanced to 0. There is a relatively weak correlation among independent variables and no multicollinearity. The dependent variable CD has the highest correlation with the independent variable PU (Pearson coefficient = 0.602) and has the lowest correlation with PRSQ (Pearson coefficient = -0.348). So, the research model is explained by regression analysis from these independent variables.

Regression analysis from the average values of factors tested by Cronbach's Alpha and EFA, using the Enter method, the variables are included simultaneously to select the variable with a sig < 0.05. The coefficient of determination $R^2 = 0.578 > 0.5$ shows that the multivariate linear regression model fits the data set to 57.8%; the variability of the independent variables explains the variation of the dependent variable. The Durbin–Watson coefficient is $1.5 \leq 2.136 \leq 2.5$, so there is no first-order autocorrelation (Table 2).

Table 2. Adjusted R^2 coefficient

Model summary					
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**	**Durbin-Watson**
1	0.760a	0.578	0.568	0.50878	2.136
a. Independent variables (constants), CV, PRPQ, SN, PRSQ, PBC, PA, PU					
b. Dependent variable: CD					

The research model explains that 56.8% of the change in the variable CD is caused by independent variables (R^2 = 0.568), and the remaining 43.2% of the change can be explained by other variables not in the model (Table 2).

Validating the suitability of the regression function: Sig. The value of the F-test is equal to 0.000 < 0.05, so the multiples linear regression model is suitable for the data set, usable, and statistically significant (Table 3).

Table 3: Anova analysis results

ANOVA[a]						
Model		**Sum squares**	**Df**	**Mean Square**	**F**	**Sig.**
	Regression	111.564	7	15.938	61.569	0.000[b]
	Residual	81.541	315	0.259		
	Sum	193.106	322			
a. Dependent variable: QD; b. Independent variables (constants), CV, PRPQ, SN, PRSQ, PBC, PA, PU						

Testing the significance of regression coefficients in the model shows that all the independent variables are statistically significant (the value Sig. = 0.000 < 0.05), and the hypotheses H1, H2, H3, H4, H5, H6, and H7 are accepted (Table 4).

Examining the necessary presumptions for linear regression: The regression model's Durbin-Watson value is 2.136, indicating that the assumption of error independence is upheld (Table 3). The normalized redundancy frequency graph has Mode and median are roughly the same and equal to 0, mean = 2.85E-15 (approximately zero), standard deviation = 0.989 (approximately equal to 1), and the residual values disperse randomly in a range around the 0 axis (mean value of the residual), in a bell chart. Therefore, it can be said that there is no violation of the normative distribution of the residual assumption. The VIF of each independent variable is less than 10 (Table 4), so assuming no correlation between the independent variables is not violated or there is no multicollinearity.

Table 4: Results of multivariate regression analysis using Enter method

	Unstandardized coefficients		**Standardized coefficients**	**T**	**Sig.**	**Collinearity Statistics**	
	B	**Standard error**	**Beta**			**Tolerance**	**VIF**
Constant	0.341	0.272		1.253	0.211		
PA	0.155	0.043	0.167	3.631	0.000	0.636	1.571
SN	0.157	0.045	0.162	3.486	0.001	0.623	1.604
PBC	0.150	0.046	0.148	3.248	0.001	0.643	1.556
PU	0.298	0.052	0.264	5.753	0.000	0.635	1.576
PRPQ	−0.121	0.035	−0.144	−3.427	0.001	0.756	1.323
PRSQ	−0.090	0.036	−0.106	−2.518	0.012	0.751	1.332
CV	0.210	0.046	0.179	4.556	0.000	0.866	1.155

The results demonstrated that the linear multiple regressions between factors and Customer's decision-making for online healthcare product purchasing were appropriate with data and could be used. The Sig. of factors statistically impacted customer decisions (Sig. < 0.05). These factors, including PA, SN, PBC, PU, PRPQ, PRSQ, and CV, are accepted in the regression equation and have a positive effect on the CD variable, except for the PRPQ and PRSQ, which have a negative effect on the CD. However, the Sig. value of the constant 0.211 > 0.05, so the author leaves the constant out of the regression equation. The relationship between the dependent and independent variables is shown through CD = 0.155*PA + 0.157*SN + 0.150*PBC + 0.298*PU - 0.121*PRPQ - 0.090*PRSQ + 0.210*PU. The order of influence of the factors is presented in Table 5.

Table 5. Determining the importance of independent variables as a percentage

No	Factor	Normalization factor	Rate (%)	Order of influence
1	Personal attitude (PA)	0.167	14.27	3
2	Subjective norms (SN)	0.162	13.85	4
3	Perceived behavioral control (PBC)	0.148	12.65	5
4	Perception usefulness (PU)	0.264	22.56	1
5	Perceived risks on product quality (PRPQ)	−0.144	12.31	6
6	Perceived risks on service quality (PRSQ)	−0.106	9.06	7
7	Convenience (CV)	0.179	15.30	2
	Total	1.170	100%	

The accepted research hypotheses are H1, H2, H3, H4, H5, H6, and H7, which can conclude the theoretical model is appropriate to the research data. The research framework is calibrated accordingly (Figure 1).

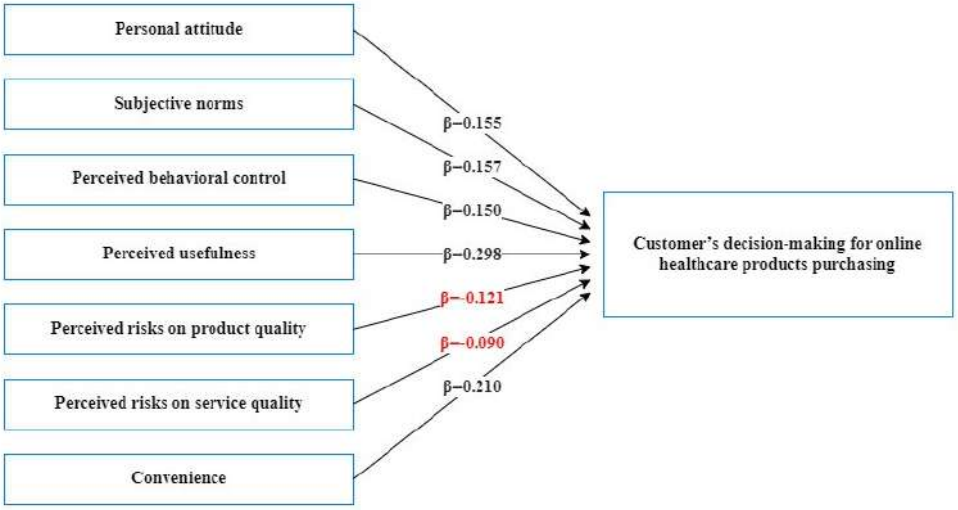

Figure 1. Calibrated research framework

3.3. Discussion

The results show five positive and two negative factors that affect the Customer's decision in Ho Chi Minh City. The Perceived usefulness factor had the most substantial impact (β = 0.264). Customers with the necessary knowledge and ability to make online healthcare product purchases use online shopping services that help them find information about healthcare products quickly, buy healthcare products anytime, anywhere, and receive pharmacists' advice faster; their decision to buy healthcare products online will be decisive. This result is consistent with many other authors' studies[17,18].

With β = 0.179, the Convenience factor is the second most influential factor in the model. Customers find it easier to make decisions about purchasing healthcare products online and the more convenient it is to do so. The results are similar to other studies[14]. Other research mentioned the "risk of convenience" factor that affects online shopping decisions[13]. Customers do not need to leave home to buy drugs or other products at the pharmacy, do not waste time traveling and go directly to the pharmacy, can compare prices of medicinal products, and choose where to sell them. With lower prices and the security of customer information when purchasing medical treatment products, customers will enjoy buying online and easily make quick decisions.

The third level impact factor (β = 0.167) that positively influences CD is Personal attitude. This aligns with both Theory of Planned Behaviour (TPB), Technology Acceptance Model (TAM), and matches the findings of earlier research. Customers will make purchases online when they feel optimistic about shopping online[5,6,19]. Recently, with the development of science and technology, e-commerce platforms have developed in the consumer goods industry, especially after the COVID-19 pandemic, and customers' behavioral attitudes have also changed.

Out of all the factors, the SN factor has the fourth-strongest effect on CD (β = 0.162). This study's factor was considered in terms of the proactive personality, which is crucial when consumers decide whether or not to purchase healthcare products online. People who recommend and encourage online shopping, such as friends, family, and acquaintances, impact how the behavior is carried out. Simultaneously, SN searches actively for information about the intended product on websites, reviews from previous customers, and so on. Making an online purchase is even more straightforward for pharmaceutical consumers if they are provided comprehensive and precise information about related goods and services before purchasing. This outcome is consistent with the earlier research conducted by the author[6,20].

PBC is understood as the level of negative or positive evaluation of an individual when buying drugs online (β = 0.148). Customers with an attitude and mindset that accepts the use of technology and e-commerce have many advantages. They have all the necessary knowledge and abilities to make online drug purchases, CD will increase, and they will have easier-to-perform behavior. These results are consistent with research by many authors[5,6].

PRPQ has a negative effect on CD (β = -0.144). As in the earlier studies, CD is hampered by elevated PRPQ[13]. Furthermore, the author demonstrates that the factor "risk perception" or "risk awareness" is used in other studies[17,18]. This is also a cause for concern because the standard of pharmaceuticals significantly impacts treatment, thereby influencing patients' lives and well-being. As a result, the pharmaceutical industry's prestige and reputation are validated by the highest standards for product quality.

The weakest factor is negatively impacted by PRSQ (β = 0.106). Low service quality carries several risks, including frequent system failure and maintenance stoppage, disruptions to the procurement process, lost products during delivery, reduced payment security due to the potential loss of personal information and money from payment accounts, and a lack of guidance on medicinal products. Customers believe that the higher the PRSQ, the more it impedes their decision to purchase healthcare products online; this finding aligns with the research of other authors[17].

Limitations: The respondents are customers in Ho Chi Minh City. The independent variables in the study model only explained 57.8% of the dependent variable's variability; other factors, such as product variety, price awareness, speed, Internet experience, product features, brand loyalty, etc., were not mentioned in the model. The level of acceptance and electronic word of mouth has not been discussed.

4. CONCLUSION

This study shows that seven factors in the proposed model affect Customers' decision-making regarding online healthcare product purchasing. The ranking of positive influencing factors is as follows: Perceived usefulness (β = 0.264); Convenience (β = 0.179); Personal attitude (β = 0.167); Subjective norms (β = 0.162); Perceived behavioral control (β = 0.148) with the percentages were 22.56%, 15.30%, 14.27%, 13.85%, and 12.65%, respectively. The ranking of negative influencing factors is as follows: Perceived risks on product quality (β = -0.144) and Perceived risks on service quality (β = -0.106), with 12.31% and 9.06%, respectively.

The most important positive factor affecting customers' online healthcare product purchasing decisions was perceived usefulness. The applicability of this research is to help pharmaceutical companies grasp the trend of buying healthcare products online, build more effective marketing plans and sales strategies, and ensure the quality of products to customers.

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Conflict of interest

"I declare that they have no conflict of interest."

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A Systematic Review on Telepharmacy Barriers

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ABSTRACT

Telepharmacy offers remote pharmaceutical services, demonstrating advantages and potential solutions to enhance patients' access to medications and improve coverage in underserved areas. Although telepharmacy is still a relatively new technology with significant advantages and great concepts, implementation can be challenging. To understand more about this evidence gap, this study was conducted to review the factors related to telepharmacy barriers. The systematic review was conducted following the PRISMA guidelines. Studies included were searched using MEDLINE (via PubMed), Scopus, and ScienceDirect databases up to February 2024 and selected based on the inclusion criteria. Search terms were derived from PICo framework, the Population (P) was pharmacist, Interest (I) was telepharmacy, and Context (Co) was barrier. Two independent reviewers selected studies based on inclusion criteria, which were reporting telepharmacy obstacles/ barriers encountered by pharmacists and original research article, and performed data extraction. Descriptive analysis was employed to synthesise the findings. Eleven studies were included in the review. The studies were performed between 2010 and 2024, with more than half conducted in the Asia region. The finding demonstrated that telephone was the most used tool to provide telepharmacy. The most prevalent barrier identified was insufficient technical support or resources. Notably, ten out of eleven telepharmacy studies (91%) were conducted post-COVID-19 (from 2020 onward), with three studies directly addressing telepharmacy's role during the pandemic. Telephone have long been used to deliver pharmacy services, and our review demonstrated that it is still beneficial in telepharmacy. The COVID-19 pandemic influenced the surge in telepharmacy studies and its practice, highlighting its critical role during public health emergencies. Addressing the identified barriers to telepharmacy is essential to fully benefiting from the service and ensuring its successful integration into the healthcare system.

KEYWORDS: Telepharmacy; Pharmacy Service Barrier; Digital Health

1. INTRODUCTION

Digital health has been a widely used approach that utilises standard and novel forms of information and communications technology to address health issues[1]. Telepharmacy, as one type of digital health, offers remote pharmaceutical services, including drug review and monitoring, dispensing, sterile and nonsterile compounding verification, medication therapy management, patient assessment, patient counselling, clinical consultation, outcomes assessment, decision support, and drug information[2, 3].

Telepharmacy has shown its advantages and potential solutions to improve patients' access to medicines and pharmacy service coverage in underserved areas[4-6]. The COVID-19 pandemic in 2020 has led to a surge in global interest and usage for telepharmacy[7]. Currently, the use of telepharmacy

has experienced an accelerated growth, extending beyond underserved and rural populations. Telepharmacy also provides access for patients in both rural and urban area when needed.

Although the goal of telepharmacy is to make healthcare more accessible to patients, there are also possible misuses and concerns in the technology[8]. Transmitting personal and health-related information about patients over the Internet raises data security issues, since patients' privacy must be protected[5]. There is still a lack of information on the safety and quality of the telepharmacy service for patients[9]. Furthermore, the laws, regulations, and policies that govern pharmacy operations are not adequate to deal with the growing industry of telepharmacy[5].

Telepharmacy is relatively still a novel technology with great concepts and potential benefits, however the implementation can be challenging[5]. In order to gain more understanding about this evidence gap, this study was conducted to review telepharmacy barriers encountered by pharmacists. According to the literature review, there is still no systematic review that reports the barriers to telepharmacy implementation.

2. MATERIALS AND METHODS

The protocol for this systematic review was registered with PROSPERO, identification number CRD42023397845. This review was conducted in adherence with the guidelines of The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)[10].

2.1. Search Strategies and Study Selection

The study was searched in MEDLINE (via PubMed), Scopus, and ScienceDirect databases up to February 2024. The search terms were constructed based on PICOS. The search strategy used search terms in the domain of Population (P), which was pharmacist, Interest (I), which was telepharmacy, Outcome (O), which was barrier, and Study Design (S) which was original research. There was no Comparison (C) domain used for the search. All original research articles were included, and there was no restriction on the publication period.

Studies were screened by titles and abstracts followed by full-text review. The studies were included if they met the inclusion criteria: (1) studies reporting telepharmacy-related obstacles or barriers encountered by pharmacists and (2) original research article. The studies were excluded if the full-text were not available and non-English studies. Two reviewers independently selected the studies.

2.2. Data Extraction and Analysis

Data were extracted independently by two reviewers. The data of general information, study characteristics, tools to provide telepharmacy, and the primary outcome which was barriers to telepharmacy were extracted. Descriptive analysis was used to summarise the result of the analysis.

In addition, two reviewers independently performed assessments for risk of bias using Study Quality Assessment Tools (SQAT) of the National Institute of Health11 for cross-sectional and case control studies. Meanwhile, Critical Appraisal Skills Programme Checklist12 was used for qualitative research. Table 1 summarised the general information of the study and Table 2 showed the barriers to telepharmacy.

3. RESULTS AND DISCUSSION

3.1. Characteristics of Included Studies

A total of 604 articles were retrieved from the search of databases: 214 from PubMed, 347 from Scopus, and 43 from ScienceDirect. After screening, eleven articles were eligible to be included in the review, see Figure 1. An overview of the characteristics of the included studies is reported in Table 1. Seven studies were cross-sectional studies[7, 13-18], three were qualitative studies[19-21], and one was case-control[22] study. The studies were performed between 2010 and 2024.

Two studies[13, 18] were conducted in Jordan. The other studies were conducted in Australia[21], Vietnam[14], Serbia[23], Nigeria[15], Qatar[19], United Arab Emirates[7], Malaysia[16], Iran[17], and the United States[22].

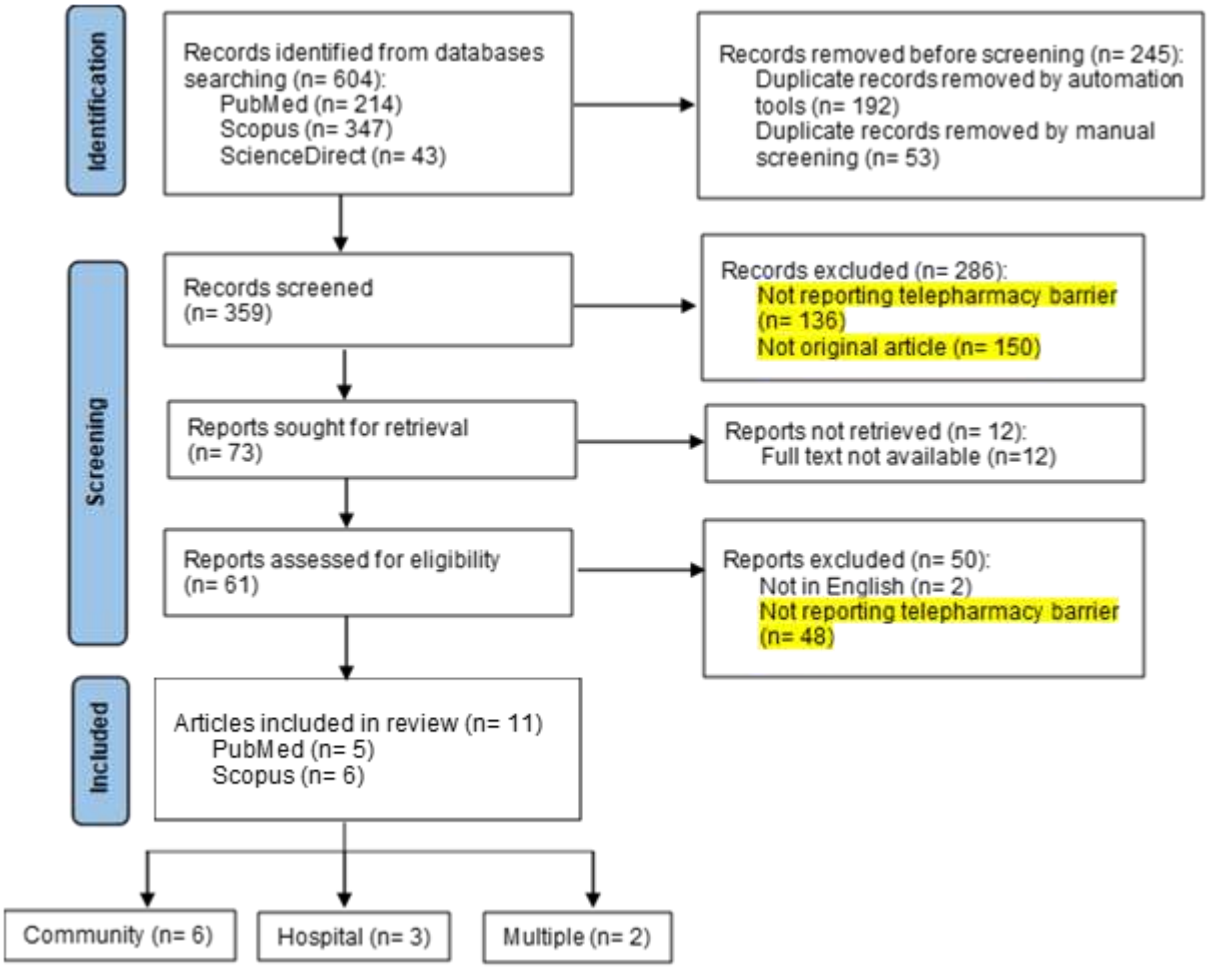

Figure 1. Prism diagram of search process

We categorised the studies based on the pharmacist's working site, with six studies7, 13-16, 23 (55%) conducted in pharmacists from community settings, three studies19, 21, 22 (27%) conducted in pharmacists from hospital settings, and the remaining studies17, 18 conducted in pharmacists from multiple settings, which were community, hospital, industry, and/ or academics, see Table 1.

Table 1. General characteristics of included studies

Author, year published	Study design	Study year (time-period)	Country	Sample size	Tools for telepharmacy
Community setting					
Abu Farha et al., 2024[13]	Cross-sectional study	Apr – May 2023	Jordan	218	Not mentioned
De Tran et al., 2023[14]	Cross-sectional study	Mar – May 2022	Vietnam	747	Not mentioned
Ilkić et al., 2023[23]	Qualitative study	Jan – Mar 2021	Serbia	23	Not mentioned
Nduka et al., 2023[15]	Cross-sectional study	Oct 2021 – Apr 2022	Nigeria	118	Not mentioned
Jirjees et al., 2022 [7]	Cross-sectional study	October 2020	United Arab Emirates	391	Telephone and phone messaging applications (WhatsApp, Messenger, Instagram)
Ng and Sze, 2022[16]	Cross-sectional study	Sep – Nov 2022	Malaysia	217	Not mentioned
Hospital setting					
Chambers et al., 2023[21]	Qualitative study	Apr – Jun 2021	Australia	6	Hospital information system, video conference, and telephone
Alhmoud et al., 2022 [19]	Qualitative study	Nov 2020 – Feb 2021	Qatar	23	Telephone, video conference, phone messaging applications (text message)
Garrelts et al., 2010 [22]	Case control study	Not mentioned	Canada	7	Telephone and hospital information system
Multiple settings					
Muflih et al., 2021 [18]	Cross-sectional study	Mar – May 2020	Jordan	364	Not mentioned
Ameri et al., 2020 [17]	Cross-sectional study	2019	Iran	40	Not mentioned

Table 2. Barriers to telepharmacy

Barrier	Author, year published											Total
	Community						Hospital			Multiple		
	Abu-Farha et al., 2024[13]	De Tran et al., 2023[14]	Ilkić et al., 2023[23]	Nduka et al., 2023[15]	Jirjees et al., 2022[7]	Ng and Sze, 2022[16]	Chambers et al., 2023[21]	Alhmoud et al., 2022[19]	Garrelts et al., 2010[22]	Muflih et al., 2021[18]	Ameri et al., 2020 [17]	
Insufficient technical support/ resources		√		√	√	√	√	√	√		√	8
Lack of imbursement			√	√		√				√	√	5
Lack of time				√	√	√		√		√		5
High workload	√	√		√	√							4
Difficult communication		√	√				√	√				4
Patients' confidentiality and privacy			√	√		√	√					4
Insufficient training		√			√	√						3
Low digital literacy		√					√	√				3
Need to proficient with two different system		√				√			√			3
Lack of regulations and policies for telepharmacy		√		√		√						3
Lack of financial support			√		√	√						3
Lack of expert staff in telepharmacy				√	√	√						3
Limitation in social and emphatic aspects of care	√		√									2
Lack of study about effectiveness and social implication				√						√		2
Reluctance to use technology				√		√						2
Patients' reluctance				√		√						2
No plans for implementing telepharmacy					√						√	2
Low number of staff					√			√				2
Acceptance by other healthcare professional						√		√				2
Higher error rate for dispensing and filling	√											1
Distrust in pharmacists as health professionals			√									1
Complex medication regiments								√				1
Late or missing documentation								√				1

3.2. Tools used to Provide Telepharmacy

The most used tool to provide telepharmacy from the included studies was telephone. Four studies[7, 19, 21, 22] reported the use of telephone to provide telepharmacy. Two studies reported the utilisation of video conference[19, 21], phone messaging applications[7, 19], and hospital information systems[21, 22] to support telepharmacy service, respectively. The remaining seven studies[13-18, 23] did not mention the tool used to provide telepharmay. The studies reported the use of more than one tool to provide telepharmacy service. More detailed information is presented in Table 1.

3.3. Telepharmacy During COVID-19 Pandemic

Ten of eleven studies (91%) were conducted post-COVID-19 pandemic (from 2020 onward). Of them, three studies (30%) were addressing the telepharmacy service during the COVID-19 pandemic. The studies showed that telepharmacy maintained and expanded pharmacy service during the COVID-19 period despite an increase in patients and staff shortage [7, 19].

3.4. Barriers to Telepharmacy

Eight studies[7, 14-17, 19, 21, 22] mentioned insufficient technical support/ resources as the barrier to telepharmacy. The technical support/ resources mentioned including telepharmacy standard and telepharmacy technologies. Other barriers described in the studies were the lack of imbursement[15-18, 23], lack of time[7, 15, 16, 18, 19], high workload[7, 13-15], patients' confidentiality and privacy[15, 16, 21, 23], and difficult communication[14, 19, 21, 23]. More detailed barriers discussed in each study was presented in Table 2.

3.5. Quality Assessment

The quality assessment result showed that all studies had low-risk of bias. All studies clearly defined the purpose and methods while also presented clear results.

3.6. Discussion

The primary purpose of the systematic review was to examine the barriers associated with telepharmacy. The systematic review encompassed eleven articles with diverse methodologies in various regions indicating a global interest in telepharmacy across different scientific perspectives. Telephone have been used to provide pharmacy services for a long time[7], and the systematic review showed the use of telephones in telepharmacy services. This emphasises the accessibility and ease of use of the telephone, making it a favourable tool to provide telepharmacy services. However, the utilisation of newer technologies, such as phone messaging applications and video conferences, is reflecting a growing trend towards digitalisation in healthcare.

The COVID-19 pandemic influenced the surge in telepharmacy studies and its practice, highlighting the critical role of telepharmacy during public health emergency. The adoption of telepharmacy during the pandemic was a response to various challenges, including the need to decrease the risk of infection, address staff shortages, and sustain health system capabilities during COVID-19[4, 7].

The systematic review identified several barriers to telepharmacy's implementation. The most frequent barrier encountered was insufficient technical support and resources[7, 14-17, 19, 21, 22], which suggests the need for more solid infrastructure as well as standardisation of telepharmacy service. As telepharmacy relied heavily on technologies, inadequate technical support would hinder service implementation, which could affect the effectiveness of the service. Meanwhile, the unavailability of telepharmacy standards could cause a variation in practice, which might compromise the effectiveness and reliability of telepharmacy. Additionally, the absence of clear regulations and reimbursement policies complicates telepharmacy integration into healthcare systems, potentially overwhelming pharmacists with increased workloads. Evidence also indicates that high workload[7, 13-15] and limited

time[7, 15, 16, 18, 19] were significant obstacles in telepharmacy practice, underscoring the need for established telepharmacy standards.

Kozlowska et al. found that poor coordination and insufficient skills as barriers to integrating primary and specialist healthcare in the United Kingdom[24]. Further challenges in telepharmacy included a lack of implementation plans and inadequate training, pointing to the necessity for a strategic telepharmacy implementation plan, including training programmes designed for telepharmacy to ensure patient safety and address other barriers like low digital literacy and expert staff shortages in telepharmacy.

Patients' confidentiality and privacy were major concerns in telepharmacy. The Standard for Telehealth Pharmacy Practice stated that telepharmacy practices must implement policies to maintain the security of patient information[25], requiring government and workplace collaboration for effective enforcement.

Transitioning from face-to-face to remote services highlighted communication challenges among pharmacists[14, 19, 21, 23]. Training in both technological use and online communication skills is critical, as studies, like one by Skoy et al., showed that in-person consultations currently surpass telepharmacy in effectiveness, indicating a pressing need for enhanced training in telepharmacy consultations[26].

These barriers collectively indicated the need to address the challenges in telepharmacy. Primary measures to overcome these challenges included making strategic investments in technology, providing comprehensive training for pharmacists, implementing efficient service flow to manage workload, and establishing telepharmacy policies.

This study had several strengths, it provides a comprehensive, up-to-date overview of barriers in telepharmacy implementation, as well as the technology used to provide the service. Additionally, the possibility of single reviewer bias was reduced because two reviewers had high agreement when evaluating the study's eligibility. We also used a broad search strategy that did not restrict studies by publication date, perhaps reducing selection bias.

However, potential publication bias may exist because the field of telepharmacy is currently growing and some new research may not have been published yet. Non-English articles were also excluded from the review, this might lead to language bias as research published in non-English languages was overlooked. The other limitation is that this systematic review focused on the barriers to telepharmacy related only to pharmacists in hospitals or community settings. In reality, telepharmacy could relate to other health professionals, such as physicians and nurses, as well as to patients. To address this limitation, further research should include the perspectives and experiences of these health professionals and patients to provide a more comprehensive understanding of the barriers and facilitators to telepharmacy.

The systematic review provided information regarding the challenges pharmacists encountered in implementing telepharmacy. The findings may be useful to improve telepharmacy implementation plan and ensuring patient safety and effectiveness of the service. Future research regarding how pharmacists overcome the barriers during practice may be useful to plan the strategic measures in broad telepharmacy implementation.

4. CONCLUSION

The COVID-19 pandemic was one of the forces behind the increasing number of telepharmacy studies and telephone was the most used tool to provide telepharmacy. The most prevalent barrier identified was insufficient technical support or resources.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Self-Medication among Myanmar Migrant Workers in Thailand

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ABSTRACT

Self-medication with over-the-counter medicines is becoming increasingly popular including Asian countries. Nevertheless, comprehensive data regarding the prevalence of self-medication among Myanmar migrant workers in Thailand is scarce. This study aimed to investigate self-medication behavior among Myanmar migrant workers in Thailand. A cross-sectional study was conducted among Myanmar migrant workers in Samut Sakhon province, Thailand. A convenience sampling was used to recruit the participants. Data was collected through self-administered questionnaires in January 2024. Findings showed that a total of 384 participants were included in this study. The average age of them was 30± 7.8 (range: 18-55). Nearly two-thirds of them were female. Almost 90% were factory workers. The average household income was 9,211± 4,076 Baht. Two-thirds of them reported taking self-medication within the last three months. More than half of them used self-medication due to their previous experience or personal knowledge (34%) as well as having leftover medicine (29.6%). Headache (54.2%) was the main reason for taking medicine on their own, followed by fever (25.3%) and cough (21.3%) respectively. Thus, nearly 80% of them bought medicine for relieving pain and fever, mostly at drugstores. Healthcare professional consultation was the most common source of information about self-medication (32.4%), followed by friends/family (29.6%). Among demographic variables, gender and household income were found to be statistically significant. Women were more likely to self-medicate compared to men. Additionally, people with a household income of less than or equal to 10,000 Baht were more likely to self-medicate than those with a higher income. This study revealed a high prevalence of self-medication among Myanmar migrant workers when they had minor illnesses. The majority bought their medicine at the drugstore. As a result, the community pharmacist plays a crucial role in giving patients information about drugs and health.

KEYWORDS: Self-Medication; Minor Illness; Drugstore; Myanmar Migrant Workers; Thailand

1. INTRODUCTION

World Health Organization (WHO) defines Self-medication as the selection and use of medicines by individuals to treat self-recognized illnesses or symptoms or the intermittent or continued use of a prescribed drug for chronic or recurrent diseases or symptoms. This broadly includes referring to old prescriptions, referring to prescription of family members, acquiring medication without prescriptions, consulting friends, relatives, neighbors and social groups, sharing medicines[1]. Self-medication involves the use of medicines by the people to treat the self-recognized symptoms. Self-medication becomes an essential part of self-care, social support in illness and first aid in everyday life[2].

The prevalence of self-medication in different countries is varied. Self-medication is practiced globally and in developing, counties reported prevalence rates are much higher with 84% in Pakistan, 78% in Saudi Arabia, 67% in Nigeria, and in India, the prevalence of self-medication was71% and 31% in studies which was conducted in Nagpur and Karnataka respectively[1]. The studies found that the practice of self-medication is different among the migrant population. A systematic review of prevalence and cause of self-medication in Iren stated that the prevalence of self-medication among students was 67%, while in the household and elderly people were 36% and 68% respectively[3]. The prevalence of self-medication among Udupi migrant workers was found to be 68.8% of the total participants[4]. Moreover, the prevalence of self-medication among the migrant workers is found to be the same of higher in male than female[4, 5].

Several studies have focused on different population such as the practice of self-medication among the undergraduate medical and pharmacy students[2, 6], high prevalence of the practice of self-medication among the elderly [7], and migrant worker population[4, 5, 8]. Migrant workers are helpless and exposed to many health issues such as low awareness about local health facilities, climate, and environmental hazards. They have limited protection on health, safety, and security. Non-compliance to treatment, non-utilization of preventive services, poor access to health services was found to be the reasons for poor health-seeking behavior[4].

Many migrants face a lack of health insurance coverage which may lead to a high demand for cheap medicine. For many, the informal health-care option is the most affordable choice. Previous studies have pointed out that migrants' use of health-care services is less than other community members and this may result in self-medication. One major reason for self-medication among migrants is the difficulty in accessing the formal health-care system, and that is mostly related to a lack of health insurance[9]. For the past 3 decades, Thai healthcare system has developed in improving quality of care and increasing the access to primary healthcare services. Despite the Thai government's attempts to offer basic healthcare to all immigrants, an estimated 2.3 million undocumented immigrants still face challenges due to their lack of legal status which means they have difficulty accessing healthcare services[10]. Therefore, this study aimed to investigate the behavior of self-medication among Myanmar migrant workers in Thailand.

2. MATERIALS AND METHODS

2.1. Study Design, Sample and Sampling Method

A cross-sectional survey was conducted in Samut Sakhon province, which has the highest number of migrant workers in Thailand. The target population was adult Myanmar migrants (age ≥ 18 years old) who currently live in Samut Sakhon. A total of 384 participants participated in this study. The eligible participants were collected based on the ability to read, write and speak in Burmese language, those who have lived in Thailand for more than one year, and their willingness to participate in the study. We excluded the participants who have the vision problem to read. Participants were recruited using convenience sampling. Data was collected through self-administered questionnaire in January 2024.

2.2. Instrument and Data collection

The study tool was a self-administered questionnaire developed by the researcher as guided by relevant theories, literature, and previous research[11]. The questionnaire consisted of three parts: self-medication (5 questions), medication literacy (13 questions) and demographic characteristics (11 questions). In this study, only self-medication part and demographic characteristics part were presented.

Participants were recruited using convenience sampling in local community areas of Samut Sakhon province, such as a temple, market, and accommodations near the food factory. The researcher and research assistants (RA) approached Myanmar migrant workers and asked if they were interested in taking part in this study. If so, the researcher and RAs gave them a brief description and

how to complete a questionnaire. Those who were willing to participate in the study completed the self-administered questionnaires. It took about 10-15 minutes.

The questionnaire, participant information sheet, and informed consent form were developed in English and then translated to Myanmar language by the researcher. This study was approved by Faculty of Dentistry and Faculty of Pharmacy Mahidol University Institutional Review Board (COA.No.MU-DT/PY-IRB 2023/062.2109).

2.3. Data Analysis

The data were analyzed using SPSS Statistics for Windows, version 18.0. Descriptive statistics and Chi-square test were used. A p-value of less than 0.05 was considered to be statistically significant.

3. RESULTS

3.1. Demographic Characteristics of Myanmar Migrant Workers

Among the 384 participants, about two-thirds reported practicing self-medication in the past three months. Out of 253, The average age of them was 30.2 ± 7.8 (range: 18-55). The majority were female. Approximately 90% worked in the factory. About one-third completed college or university education or higher. Almost 90% of them had less than or equal 10,000 Baht as a household income. Over half of them had lived in Thailand between 1-5 years. The vast majority were covered by the Social Security Scheme (Table 1).

Table 1. Frequency and percentage of the participants who reported self-medication

Variables	Category	n	Percentage
Age group (n=253)	≤ 25	78	30.8
	26-35	108	42.7
	36-45	56	22.1
	46-55	11	4.3
Gender (n=252)	Male	72	28.5
	Female	180	71.1
Occupation (n=252)	Unemployed	11	4.3
	Factory labor	231	91.3
	Others	10	4.0
Education level (n=253)	Primary school	37	14.6
	Secondary school	121	47.8
	College/ University or higher	88	34.8
	Others	7	2.8
Household income (n=253)	Less than or equal 10000 Baht	220	87.0
	More than 10000 Baht	33	13.0
Duration of living in Thailand (n=253)	1 to 5 years	139	54.9
	More than 5 years	114	45.1
Type of health insurance scheme (n=253)	No insurance	26	10.3
	Social Security Scheme	227	89.7

3.2. Self-Medication among Myanmar Migrant Workers

Nearly one-third of participants practiced self-medication due to previous experience or personal knowledge and leftover medications at home (34.0 % and 29.6% respectively). Drugs which relieves pain and fever were the most common choice of medicine for self-medication which accounted for about 80%. Nearly 76% of them bought medicine at drugstores (Table 2). Headache (54.2%) was the main reason for taking medicine on their own, followed by fever (25.3%) and cough (21.3%) respectively (Figure 1).

Table 2. Self-medication of the Myanmar migrant workers (n=253)

Variables	Category	n	Percentage
Reason of self-medication	From previous experience/personal knowledge	86	34.0
	Recommended by friends/family	26	10.3
	Have leftover medicines at home	75	29.6
	Recommended by staff at a drugstore	45	17.8
	Obtain medicines from friends/family	33	13.0
	Waiting time at health facilities	32	12.6
	Save money	30	11.9
	Save time	24	9.5
	Advertisement	24	9.5
	Convenience	41	16.2
	Others	4	1.6
Drugs for self-medication	Drugs which kill germs	15	5.9
	Drugs which relieve pain and fever	200	79.1
	Drugs which lower body temperature	61	24.1
	Drugs which prevent cough	57	22.5
	Others	19	7.5
Places to obtain drugs for self-medication	Drugstore	192	75.9
	Friends/ family	22	8.7
	Groceries/ stores	72	28.5
	Others	10	4.0

*Participants can choose more than one choice.

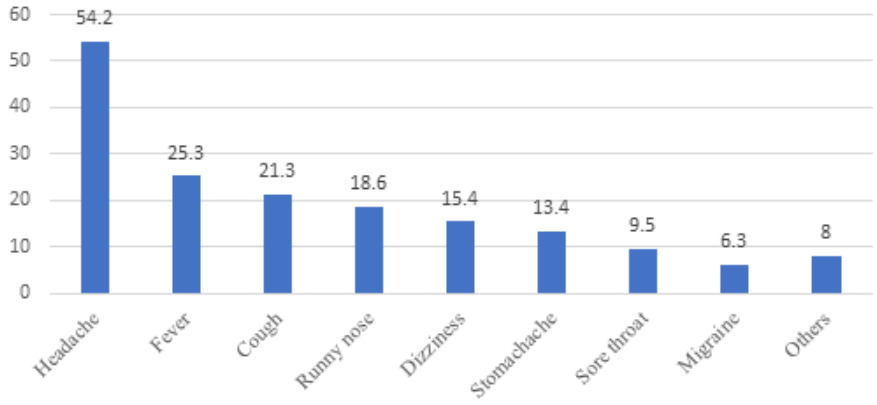

Figure 1. Diseases for self-medication by percentage (n=253)

3.3. Relationship between Demographic Characteristics and Self-Medication of Myanmar Migrant Workers

The results showed that 65.9% (n=253) of the of Myanmar migrant workers practiced self-medication in the last three months. Among demographic variables, gender and household income were found to be statistically significant. Women were more likely to self-medicate compared to men. Additionally, people with a household income of less than or equal to 10,000 Baht were more likely to self-medicate than those with a higher income. However, there was no significant difference between self-medicated person and non-self-medicated person in term of age, occupation, education level, duration of living in Thailand, and insurance scheme. (Table 3).

Table 3. Demographic characteristics on self-medication (n=253)

Variables	Category	% of participants who used self-medication		p value
		Yes (n=253)	No (n=131)	
Age	≤ 25	30.8	35.9	0.718
	26-35	42.7	43.0	
	36-45	22.1	18.8	
	46-55	4.3	3.9	
Gender	Male	28.5	45.8	0.003*
	Female	71.1	54.7	
Occupation	Unemployed	4.3	7.8	0.288
	Factory labor	91.3	86.3	
	Others	4.3	6.1	
Education level	Primary	14.6	9.4	0.144
	Secondary	47.8	61.1	
	College/ University or higher	34.8	28.2	
Household income	Less than or equal 10000	87.0	76.3	0.008*
	More than 10000	13.0	23.7	
Duration of living in Thailand	1 and 5 years	54.9	51.1	0.479
	More than 5 years	45.1	50.0	
Type of insurance scheme	No insurance	10.3	11.7	0.354
	SSS	89.7	87.8	

*Level of significance set was set at $p < 0.05$

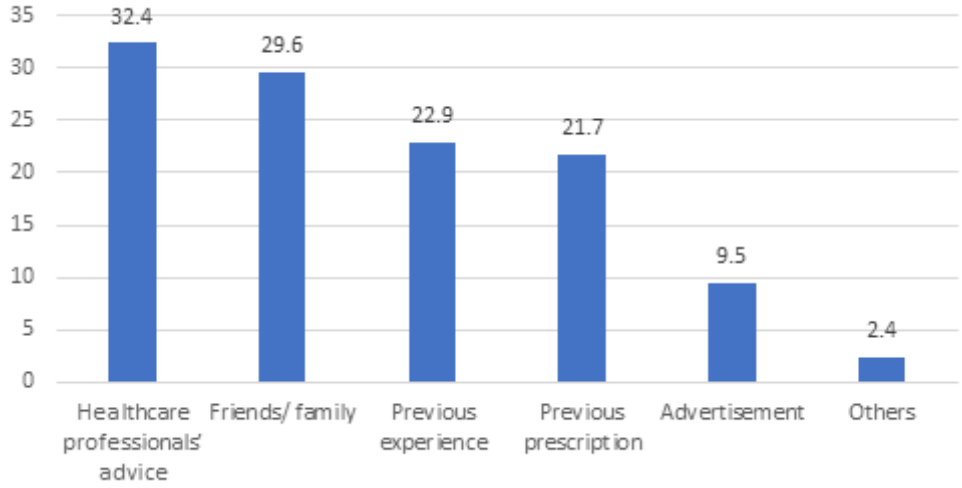

Figure 2. Source of information for self-medication by percentage (n=253)

4. DISCUSSION

Self-medication prevalence differs between nations and respondents, ranging between 38.2% and 67.0%[12-14]. This study showed that Myanmar migrant workers in Samut Sakhon self-medicated at a high level. The finding was consistent with previous studies[4, 15]. Previous experience/personal knowledge and leftover medications at home were the major reasons for self-medication. These reasons were consistent with the previous study[16]. Additionally, lack of time and the inability to take sick leave from work were reasons for self-medication[4]. According to the medication, drugs that relieve pain and fever were the most common choice of medicine, accounting for about 80%. Self-medication with paracetamol for minor illnesses was common among participants, reflecting a cultural belief among Myanmar people in its efficacy for various symptoms. Given the high prevalence of air-conditioned workplaces, increasing susceptibility to colds, and physically demanding jobs potentially leading to back pain, this was particularly concerning. Easy access to painkillers over the counter in grocery stores and drugstores, such as paracetamol, further enables self-medication practices. In contrast to Chautrakarn et al. study[16], NSAIDs and antibiotics were the most often drug used for self-medication.

Approximately 76% of migrant workers purchased medication, mainly from drugstores. This was consistent with a Thai study. It showed that Thai people preferred self-medication by purchasing medicines from drugstores[17]. This may be explained by the cost, accessibility, and location of drugstores in Thailand. Community pharmacists seem to be in a unique position to help and advise the general population. The public had a great deal of trust in community pharmacists and their capacity to provide non-prescription medication advice. Hence, as health professionals, pharmacists were qualified to assist patients with self-care and self-medication[18]. Since this study found pharmacists to be the primary source of information for self-medication, collaboration between the community pharmacist association, health centers, and migrant leaders is recommended.

In terms of demographic factors, household income and gender were found to be statistically significant. Individuals with a household income of less than or equal to 10,000 Baht were more likely to self-medicate compared to those with a higher income. An explanation is that self-medication was low-cost compared to going to the hospital. This is in contrast to a study conducted in Cambodia, which found that the respondents who had higher monthly income were more likely to self-medicate because they had higher purchasing power than the low-income group[12]. Women were more likely to self-medicate compared to men. The results were consistent with a study conducted in Brazil. It showed that women experience higher rates of headaches, muscle pain, and chronic pain conditions, such as migraines and use pain relievers and muscle relaxers from an early age for pain relief during menstruation or dysmenorrhea[19].

There are some limitations that need to be considered. The first limitation could arise from recall bias. We used a three-month recall period, which might lead to bias, and self-reported data on health behaviors or past experiences can be inaccurate. Participants might forget things or be unwilling to share some information. The convenience sampling method could be the second limitation. Difficulty was experienced in recruiting Myanmar migrants for the study. Thus, we chose the specific area, such as around the factory because of the possibility of meeting the sample. Therefore, approximately 90% were factory labor. The sample might not be representative of Myanmar migrant workers, which limits the generalizability of the findings to Myanmar migrants who live in Thailand.

5. CONCLUSION

This finding indicated a high prevalence of self-medication among the Myanmar migrant workers in Samut Sakhon. Mostly, they practiced self-medication according to their previous experience or personal knowledge. Drugs that relieve pain and fever were the most common choice for treating their minor illnesses, such as headaches, fevers, and cough. The majority bought their medicine at the drugstore. Thus, the community pharmacist plays a crucial role in giving patients information about drugs and health. Furthermore, we found a statistically significant relationship between self-medication practice and gender and household income.

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Conflict of interest

The authors declare that they have no conflict of interest.

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The First Impression and Response to the Symptoms, Diagnosis and Treatment of Tuberculosis from the Perspective of Lay People and Healthcare Providers in Indonesia: A Qualitative Approach

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ABSTRACT

Campaigns and programs have been launched and promoted to control and eliminate the spread of tuberculosis in Indonesia for many years. This study aimed to explore understandings and experiences related to the symptoms, diagnosis and treatment of tuberculosis from the perspective of lay people and healthcare providers. Twenty-seven participants were purposively selected. Seventeen sessions of in-depth interview with lay people, tuberculosis cadres, activists and district health office program manager; and two sessions of focus group discussion with healthcare workers were conducted in Depok, West Java, Indonesia. The results were divided into three major themes including the awareness and response to tuberculosis symptoms; the feelings and consequences following tuberculosis diagnosis; and the struggles of undergoing a long-strict tuberculosis treatment. It showed that both lay people and healthcare providers had almost the same understanding of tuberculosis but responded to symptoms, diagnosis and treatment of tuberculosis differently. Lay people had to encounter fear, discomfort and discouragement caused by the stigmatized image of tuberculosis in the community while striving for recovery. Additionally, they had to bear occupational and social relationship risk following tuberculosis infection. For the healthcare providers, lay people's ignorance, dishonesty and non-compliance to the tuberculosis care cascade could lead to drug-resistance and tuberculosis related death. Therefore, it roused their commitment to intensify contact investigation, health education and provision of support for tuberculosis-affected households. The ideas, feelings, emotions and responses of lay people to tuberculosis infection were influenced by their social status and life context. Their knowledge and beliefs grew their perceptions, concerns and stigmatized feelings about tuberculosis. Health education about tuberculosis is essential to raise awareness and control tuberculosis transmission. Furthermore, clinical, financial, social and psychological cross-sector support for tuberculosis-affected households are keys to minimize the misery from the disturbing symptoms of tuberculosis, stigmatization and pressure to finish the treatment.

KEYWORDS: Tuberculosis; Lay People; Healthcare Provider; Understanding; Experience; Stigma

1. INTRODUCTION

Tuberculosis (TB) is a transmissible disease which predominantly damages lungs, allowing its sufferers to experience severe symptoms which greatly disrupt daily life. Referring to its extremely infectious nature, TB was presented in all countries, causing 10.6 million people ill in 2022[1]. Sort of programs have been launched and promoted globally to control and eliminate the spread of TB, such as DOTS strategy (1970-1990's) and Stop TB strategy (2006-2014)[2, 3]. To get aligned with the global target in controlling TB, Indonesia adopted the new National TB Program every five years. It was

intensified with the campaign namely "TOSS TB" (2016) or *Temukan, Obati Sampai Sembuh* aimed to notify, diagnose, treat and cure TB patients and reduce the spread of TB in the community[4]. Since 2000, Indonesia has managed to achieve a slow but constant progress in TB incidence rate, death rates, notification rate and treatment coverage year by year, except during the COVID-19 pandemics where all the progress were disrupted[1, 5].

Despite the urgency to control TB transmission, Indonesia has to manage the widespread stigma and misconception about TB, which was known to give a rise to late diagnosis, treatment withdrawal and even TB related mortality[6-8]. It is important to understand how society thinks and expects regarding TB infection as it can navigate the natural response of someone to the disease. Therefore, this study aims to explore understanding and experiences related to TB and its treatment from the perspective of lay people and healthcare providers so that it can be used as references in developing the practical strategy to control and eliminate TB in the country.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

A qualitative study was conducted from August to October 2023 in Depok, a dense and populated city in West Java province, with 6,549 new TB cases in 2022 in a territorial area of 200.29 km^2, located 27 km from the capital city of Indonesia. Two sub-district community health centers (Puskesmas) were selected considering the high TB prevalence in both adult and children. Participants were purposively selected and classified into lay people group and healthcare provider group. Lay people are defined as a group of people who are assumed to have lay values related to health and tuberculosis' symptoms, diagnosis and treatment. In this study, patients with drug-sensitive pulmonary TB from the selected Puskesmas who already passed the intensive phase of TB treatment and were living with at least two family members/relatives as their household contacts were invited. The adult household contacts were also interviewed to broaden the exploration from lay people perspective. In addition, the researcher purposively invited those who live with children under five to explore how the presence of children influenced their understanding and experience. Healthcare providers referred to people who particularly give their assistance in providing TB services or managing TB control program which consists of healthcare workers, TB program manager from the district health office, TB activists from non-government organizations (NGO) and TB cadres. A minimum of six months experiences in the current position was required to ensure their understandings and real-work experiences related to TB program activities. The selection of study site and the recruitment process were assisted by the representative or head of TB control of each institution who were not involved as participants for data collection process.

2.2. Data Collection and Data Analysis

To get a deeper and better understanding, face-to-face in-depth interview (IDI) and focus group discussions (FGD) were conducted with the duration of 45-60 minutes for each session. Lay people were invited to have IDI where only one participant and the researcher attending the location of IDI to create a relaxing and convenient ambiance for them to speak out their opinion and personal issues about TB. Similarly, the TB cadres, TB program manager and TB activists were also invited for IDI to get detailed information about how they managed their activities with all their perceived perception towards TB. FGD were conducted for healthcare workers from the selected Puskesmas including general physician, pharmacist, nurse, health promotor and medical laboratory analyst, to gain a range of understandings about symptoms, diagnosis and treatment of TB and real-work experiences in providing tuberculosis services. An interview guide was developed based on a review of the literature and discussions within the research team. It addressed six main topics: understanding about TB, transmission of TB, health-seeking behavior, preventive efforts, the impact of TB in daily life, health promotion and education.

The data collection process was fully conducted by the researcher who was a master student with pharmacy background and was belong to tuberculosis team in private hospital previously. The

researcher has ever enrolled in qualitative course before and had experiences joining FGD before. One assistant was hired during the FGD to help taking notes and audio records. Both the researcher and the assistant had no prior relationship with the participants. The entire process of data collection was performed in the national language, *Bahasa Indonesia*. An informed consent was given before the interview/discussion was conducted. Audio records and jotted notes were taken under the participants' permission. The data in this study were analyzed using thematic content analysis. Data were transcribed verbatim into transcript and then translated into English. Transcript were read repeatedly to extract the golden word and generate the sub-themes. The sub-themes were arranged to get the correlation or association with each other in order to form the major theme. All information obtained from the interview and discussion were kept confidentially and will be destroyed once the thesis and original article are completed.

2.3. Ethical Approval

This study was authorized by The Dentistry-Pharmacy Institutional Review Board Mahidol University and The Research and Community Engagement Ethical Committee Faculty of Public Health Universitas Indonesia with approval number Ket-598/UN2.F10.D11/PPM.00.02/2023

3. RESULTS AND DISCUSSION

A total of 30 potential participants were approached but only 27 of them consent to be involved in this study, consisted of seven lay people and 20 healthcare providers. Two TB patients and one household contacts refused the invitation due to personal reasons. This study gained qualitative data from 17 sessions of IDI with 17 participants and two sessions of FGD with five healthcare workers from different professional background in each session. Among the lay people group, three TB patients and four of TB patient's family were interviewed as the household contacts. The healthcare providers group consisted of one TB program manager from the district health office of Depok, two TB activists from two different NGO, seven TB cadres and ten healthcare workers. None of them were found to be positive with TB disease. However, three participants confessed that they had positive result of TB skin test. The detailed characteristics of participants were tabulated, as presented in Table 1.

Lay people and healthcare providers had similar perception related to tuberculosis in almost every dimension but responded to symptoms, diagnosis and treatment of tuberculosis differently. This study divided the result into three parts covering all three retrieved major themes: 1) the awareness and response to TB symptoms; 2) the feelings and consequences following TB diagnosis; 3) the struggles of undergoing a long-strict TB treatment.

3.1. The Awareness and Response to TB Symptoms

Lay people in this study mentioned that they had the experience in caring for family members who suffered from TB or received TB-related information provided by the healthcare workers, social media and also friends who suffered from TB. Thus, they acknowledged TB symptoms and treatment long before TB came into their life. Although TB patients in this study reported that they experienced cough for more than one month and then worsened by the weight loss and decreased stamina in doing their daily routines, still, neither TB patients nor household contacts in this study had ever suspected that those symptoms would lead to TB. Their first attempt to overcome the symptoms was by carrying out self-medication. However, realizing that the symptoms were not getting any better, behind the despair of self-medication that did not work and the disruption of daily life due to severe symptoms, feelings of desperate and worry began to emerge, pushing them to finally decided to see a doctor. From this study, it was found that the urge to get themselves checked actually aroused from the suggestions or request from another parties, in this case their family and co-workers, considering the worsen condition, not because self-awareness.

Table 1. The characteristics of study participants.

No.	Sex	Age	Participation method	Education level	Job	Sputum Test Result	TB Skin Test Result
1	F	36	In-depth Interview	Tertiary	Housewife	Positive	NA
2	F	39	In-depth Interview	Tertiary	Islamic Teacher	Positive	NA
3	M	39	In-depth Interview	Tertiary	Security*	Positive	NA
4	F	24	In-depth Interview	College	Housewife	Negative	Negative
5	M	32	In-depth Interview	Tertiary	Laborer*	NA	NA
6	M	38	In-depth Interview	Tertiary	Printing Staff*	Negative	Negative
7	F	43	In-depth Interview	Tertiary	Housewife	Negative	Negative
8	F	51	In-depth Interview	Primary	Housewife	NA	NA
9	F	46	In-depth Interview	Tertiary	Housewife	NA	Positive
10	F	49	In-depth Interview	Tertiary	Housewife	NA	NA
11	F	51	In-depth Interview	College	Housewife	NA	NA
12	F	43	In-depth Interview	Secondary	Housewife	NA	NA
13	F	40	In-depth Interview	Tertiary	Housewife	NA	NA
14	F	61	In-depth Interview	Tertiary	Self-employed	NA	Negative
15	F	32	In-depth Interview	College	NGO Staff	NA	NA
16	F	42	In-depth Interview	College	NGO Staff/Doctor	NA	NA
17	F	45	In-depth Interview	College	Civil Servant	NA	NA
18	F	57	Focus Group Discussion	College	Doctor	Negative	Negative
19	F	36	Focus Group Discussion	College	Doctor	Negative	Negative
20	F	43	Focus Group Discussion	College	Nurse	Negative	Negative
21	F	47	Focus Group Discussion	College	Nurse	Negative	Negative
22	F	34	Focus Group Discussion	College	Pharmacist	Negative	Negative
23	M	36	Focus Group Discussion	College	Pharmacist	Negative	Negative
24	F	28	Focus Group Discussion	College	Medical Laboratory Analyst	Negative	Positive
25	F	28	Focus Group Discussion	College	Medical Laboratory Analyst	Negative	Negative
26	F	30	Focus Group Discussion	College	Health Promotor	Negative	Positive
27	F	25	Focus Group Discussion	College	Health Promotor	Negative	Negative

*Refer to the breadwinner of the family; F = female; M = male; NA = Not Available

"*Usually, the cough will get better within a week, but at that time the cough didn't get any better even though it had been a month*" – **Male, 38**

"It was upsetting to always listen to the sound of his coughs. We bought medicine again and again but he didn't get any better. What else we should try?" – **Female, 43**

"It was never relieved. And then my family, especially my husband, suggested me to go to the healthcare facilities, to see a doctor and asked about my condition" – **Female, 36**

"*My wife and I got a negative result while my child was positive (for TB). But the cough was still there. Three months later, the medical check-up from the company stated that I was "un-fit" to work due to a lungs disease which later identified as TB*" – **Male, 39**

On the other hand, healthcare providers perceived a prolonged cough as something to be wary of because this symptom was considered as the main symptom related to TB. As a result, all patients with coughs for more than seven days were advised to have themselves checked with sputum test and/or chest x-rays. Apart from that, the healthcare providers expanded their TB screening activities by referring the high-risk person such as people living with HIV, diabetes and malnutrition to get screened and also expanded their activities to various communities such as Islamic study groups and educational institutions so that they could be diagnosed earlier. According to the healthcare providers, most people came to the healthcare facilities when their symptoms were very disturbing and painful. The lack of awareness about the warning symptoms of a disease and the fear of revealing the illness were mentioned to be the possible reasons of why people postponed their visits to the healthcare facilities and resulting in diagnostic delay. Others mentioned that limited access and socio-economic factors took part in resulting the delay.

> *"The more they know about the disease, the more they will overthink about it. How is the treatment? What about the family? They weren't ready. So, they tried to affirm themselves that the disease could heal itself... just an ordinary cough, until finally it gets really bad and they cannot resist, then they just decided to come."* – **Female, 35**
> *"Currently, Mantoux test is also used in the screening of malnutrition and stunting in children. We can see that it leads to a higher completion of target in screening test for children, overpassing 200% of the target"* – **Female, 45**

3.2. The Feelings and Consequences Following TB Diagnosis

In this study, a person's position in a family and their role in society determined feelings and reactions towards TB. Lay people who were also fathers and mothers in the household stated that the presence of TB in their lives caused a concern about their children's health. Among all family members, children were considered as the weakest human being and were susceptible to TB infection, so children must be given extra protection. Female participants from the lay people group stated that as a mother, the presence of TB in their life changed them to become an alert person, protecting children from any harms and threats including themselves of their father. In case where a mother was a TB patient, they chose to stay away from their own children as fear of transmitting the germs, applying unnecessary prevention which led to misconception of preventive effort. Apart from that, due to the weakness and fatigue they suffered, they were unable to take care of household chores or even to babysit their children. Thus, the presence of other people was really needed. Meanwhile, in cases where the TB patient was a father, they more worried about losing opportunity to earn income considering their position as the major earner in the family as TB could cause a productivity loss at work. A mother in this study also concerned about economic hardship caused by TB. Occupational and social relationship risks were haunting following the diagnosis of TB. The stereotype of this disease as a deadly and highly contagious made them afraid of being shunned or laid off from work. As a human being who often socializes, tuberculosis also brings huge changes. In response to TB diagnosis, feelings of low self-esteem or fear of being a burden to others and transmitting the disease to others arose. Moreover, because tuberculosis can be seen from physical changes, it would raise questions from the people around them. Sometimes patients had to hide their illnesses so they can continue doing their jobs or maintain a normal relationship. In this study, it was discovered that women were more likely to withdraw themselves from the community before receiving any discrimination or rejection while men did not bother about social relationship changes as long as they could still earn some money and follow the treatment. On the other hand, health service providers mentioned that disclosing the truth about their disease could have a negative impact on the spread of TB if it was followed by a disregard for the risk of spread in the community by continuing to ignore wearing a mask, not following cough etiquette or not complying with taking medication.

> *"I really became a strict parent. I protected her a lot. Because it was also traumatizing. My husband already had TB, I tried to protect my child from not get infected too. I was really scared... I'm afraid it would infect my child"* – **Female, 24**

"*He thought that he could lose everything. Even people from middle economic level worried about their economy, income, not just about being shunned by friends"* – **Female, 45**
"*My husband was prohibited to work. My biggest fear was in the worst scenario, he could be laid off. Yes, I was stressed out too for some points"* – **Female, 43**
"It's natural. If some people chose to stay away from me, it's okay. Later, if I have recovered, they will be back to normal again just like before" – **Male, 39**
"I slept alone while children were with their father. I also told them that we can no longer eat together at the moment because I was sick" – **Female, 38**
"They never come to my house again and I personally avoided them. My husband still asked me to join him to some wedding invitations, but I always refused. Simply because I am afraid that people will talk about us, about me, behind my husband's back" – **Female, 36**

Healthcare providers played crucial roles in this phase where the patient and his family have just started living side by side with TB. They admitted that they focused on achieving the goals of the tuberculosis control program, particularly increasing treatment success rates, reducing mortality and controlling the transmission. Following the TB diagnosis, healthcare providers had to immediately conduct the contact investigation in order to trace the source of transmission, prevent the further transmission and try to find another potential case. During the contact investigation, sometimes TB cadres experienced dishonesty claiming that the patients did not have TB and then rejected the healthcare providers. Another important thing to do was to keep educating people about TB especially TB patients themselves and their household contacts to increase their knowledge and awareness. Therefore, healthcare providers, following the diagnosis, emphasized that TB was a curable disease, where treatment was provided free of charge and its spread, especially to relatives and friends, could be prevented.

"The best prevention is back to educating the parents, the community, from the very beginning. The worst things can happen if the parents or the community do not aware of TB and about the environment. Back to that, educating is the core" – **Female, 32**
"No matter how hard we try, if patients do not have awareness of the importance of accessing health facilities when they are sick with TB, it will definitely be difficult. Examinations are free, then medicine is also free, but some people are not aware and deliberately being ignorant" – **Female, 47**

3.3. The Struggles of Undergoing a Long-strict TB Treatment

In this study, none of the TB patients were hesitate to start the treatment as soon as they were diagnosed with TB for the sake of their recovery. However, along the way, TB patients and their families had to face some challenges, especially regarding the impact of the treatment to their life, physically, financially and mentally. Lay people confessed that TB treatment worked pretty well in improving their health indicated by the decrease of the symptoms. Along with the positive impacts, there were side effects which were quite disturbing for some patients such as joint pain, nausea and vomiting. Both of health improvements and the side effects of the treatment, indeed, more or less, making people want to withdraw from their treatment. TB services in the PUSKEMAS were offered free of charge to anyone, including the examination and the treatment. However, besides that, lay people complained about the indirect cost which include the transportation costs and the time lost due to the multiple visits for the medication refill. Apart from adherence to the treatment, TB patients and their families must try to control the transmission and protect other family members at home. TB patients were forced to wear masks under any circumstances and started the healthy life style. TB patient's family reported that tuberculosis made the TB patient emotionally unstable, irritable and angry especially when reminded not to do things that could make their condition worse or even just reminded to wear a mask.

"Whether it takes 6 months, 9 months or a year, I will do it. The important thing is to get cured" – **Male, 38**

"They already felt better, feeling recovered (so they stop taking medicine). Other reasons were that he cannot stand the side effect or get bored (of taking medicine)" – **Female, 49**
"(during the treatment) I lose my appetite. I felt pain all over my body. After sitting for a while, I cannot easily stand up because I felt pain in my foot. Now I feel pain and stiffness in my hands" – **Female, 36**
"Why cannot we take the medicine for a month? So, I don't have to go back and forth to the Puskesmas, my mom and my son asked me why I kept returning to the Puskesmas. I mean, even if our relatives took me there but still exist the expense. Moreover, I have to ask someone to look after my children while I go to the Puskesmas" – **Female, 39**
"Sometimes I have to sit and take off my mask for a while. It was hard to breath. I feel tired of wearing mask even when I was sleeping, but I have to in order to protect my child." – **Female, 36**
"She will be angry if I said or did something wrong. So, I should not be wrong at all … This disease made her overemotional, so, I have to hold back my words. We often fight because I remind her to wear a mask, she got offended." – **Household contact, M32**

From the perspective of health service providers, ensuring the TB patients adhere to TB treatment and follow-up visits were very important because non-compliance could lead to treatment withdrawal, treatment failure and even the drug resistance. During the treatment period, lay people were found to have difficulties in fulfilling their needs, such as providing the complementary foods, implementing the healthy lifestyle and creating the healthy environment. Some of them also lack of enthusiasm to finish the treatment as it was never been easy for them. Most of TB patients in the Puskesmas were coming from low-middle economy group, thus, healthcare providers took the initiative to help ease their burden by providing assistance or donations in the form of money or basic necessities, accompanying them on their journey and emphasizing that they had to focus on treatment and healing, not to things that might make them terrify and uncomfortable.

"Please give encouragement, support them to take the medicine, cheer them to get healed. Sometimes, if the sick person doesn't get any supports, they will end up thinking "why should I continue the treatment?" – **Female, 28**
"It is much easier to motivate them to pay attention to nutrition intake and maintain their own enthusiasm to the treatment rather than talking about food/activity restrictions" – **Female, 42**

3.4. Discussion

This study found that all TB patients suffered from the prolonged cough which was pretty much disturbing their daily activities for at least 4 weeks before they got diagnosed. Prolonged cough was occurred in most of TB patients as the major symptoms and apparently become one of the common symptoms or illness which people tend to do self-medication[9-11]. However, it was their own decision to initiate the self-medication in the first place instead of consulting and visiting the physician when the symptom arose. All TB patients were finally accessing the healthcare facilities after sought for a self-treatment by taking over-the-counter medicines for their prolonged cough. A reference to go to the healthcare facilities were often derived from family or friends[12]. Reasons of the preference to self-medication were reported in the previous studies such as assuming that the disease were minor, the experience of similar symptoms and treatment, manifestation of the quick relief and cheaper cost also the easier and shorter access to get the medicine[9, 11]. Besides the benefits of self-medication, it could also lead to the late diagnosis[7].

TB was closely related to stigmatized feeling which forced its sufferers to experiences loses not only in financial sectors but also in social life. The occupational and social risk were following both patients and their family. However, differences in responding the diagnosis of TB were presented depending on the role or position of each person in the family. In this study, we figured out that the fear of losing income and losing friends were the two main issues related to the journey from diagnosis to treatment phase. Female were more concerned about their social life, showing that the stigmatized feelings were more likely to linked with female[13]. Not to mention the risk of infection in

household contacts and their relatives when they started to live with TB patients. In order to protect themselves and their families, TB patients prefer to pull themselves out of the community and focus on the treatment[12].

It was never be an easy journey for them. The side effects of the treatments made them want to stop consuming the TB medicines. Healthcare providers as well as the family held important roles in supporting TB patients. As the closest circle to the patients, household contacts or family were the highest supporters in terms of emotional, practical, financial support and the basic groceries needs[14]. Not only by providing support and assistance, but also in spreading the correct information and increase public knowledge about TB. The lack of knowledge about TB and social support were found to hinder the TB-related adherence[15, 16]. The health promotion was found to be effective in accelerating the progress towards success in TB control program and effective diagnosis and treatment were found to successfully saved millions of lives[17].

Some limitations should be acknowledged in this study. First, the interviewed household contacts were the wives or the husbands of TB patients. In other words, no other perspective inside the family were explored, for instance, the view point of the parents or the siblings of the TB patients, due to the limited access and interaction during the data collection process. Second, this study only focused in two selected sub-district Puskesmas, which could not represent the whole city's situation. There may be vary of impressions and responses to TB in different area, healthcare facility affected by different social status and life context. However, this study successfully achieved a broad and rich data from a thorough exploration about understandings and experiences related to TB symptoms, diagnosis and treatment from various perspectives which can help improving the strategy to raise awareness and boost the TB control program progress towards the national target.

4. CONCLUSION

It was found that the impact of a TB diagnosis on life and employment not only occurs on the TB patients themselves but also on their family. The ideas, feelings, emotions and responses of lay people to tuberculosis infection were influenced by their social status and life context. Their knowledge and beliefs grew their perceptions, concerns and stigmatized feelings about tuberculosis. This study shows that health education about tuberculosis is essential to raise awareness and control tuberculosis transmission. In addition to clinical support, financial, social and psychological cross-sector support for tuberculosis-affected households are keys to minimize the misery from the disturbing symptoms of tuberculosis, stigmatization and pressure to finish the treatment.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this proceeding.

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Assessment of Knowledge, Attitude, and Practices of Pharmacovigilance among Hospital Pharmacists in Metro Manila, Philippines

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ABSTRACT

Despite strict regulations ordained by governing bodies such as the Food and Drug Administration (FDA), drug-related morbidity and mortality as a result of adverse drug reactions (ADRs) are still relevant to this day. In pharmacovigilance, it is the pharmacists' pivotal duty to detect, assess, understand, and prevent adverse effects, prioritizing patient safety. The study aims to assess and determine the correlation between the knowledge, attitude, and practices of hospital pharmacists in pharmacovigilance, and evaluate the pharmacovigilance system in hospitals in Metro Manila based on current practices. A cross-sectional study with a descriptive and correlational design was used to evaluate hospital pharmacists using a questionnaire adapted from Abdulsalim et al. (2023), which was disseminated to 120 hospital pharmacists in selected hospitals in Metro Manila. Responses were analyzed using descriptive statistics and Pearson's R Correlation. Out of 120 respondents, 45% (n=54) and 48% (n=58) showed fair and moderate knowledge, respectively, while 7% (n=8) exhibited poor knowledge. Majority of the respondents displayed a positive attitude (n=120), however, 54% (n=65) showed poor practices. There is no significant correlation between knowledge and attitude and between knowledge and practices, with weak coefficient values of -0.002 and 0.129 and non-significant p-values of 0.987 and 0.161, respectively. Conversely, the correlation between attitude and practice was significant, with a positive value of 0.199 and a p-value of 0.029, indicating a potential relationship between variables. Hospitals in Metro Manila follow most of the minimum requirements set by the FDA, with 79.2% (n=95) reporting that their institution submits all adverse drug events reports to the FDA. The weak correlations suggest that external factors may influence pharmacovigilance. To obtain an operative pharmacovigilance system, interventions should be made to address gaps in the knowledge and practices of hospital pharmacists, as well as in the practices of their respective institutions, ultimately improving patient safety.

KEYWORDS: Adverse Drug Reaction; Hospital Pharmacist; Pharmacovigilance

1. INTRODUCTION

World Health Organization (WHO) defines adverse drug reactions or ADR as unintended and harmful reactions to medicines[1]. The risk of contracting an ADR following the consumption of medicines will never be zero; its possible occurrence is compulsory alongside its desired effects[2]. In many ADR-related cases, costs from inadvertent hospitalization, surgery, and hindered productivity exceeded the medication cost. Pharmacovigilance is done during Phase IV in a typical drug discovery pipeline, whereas the drug's efficacy, safety, and purpose in large populations under real-life conditions are continuously monitored[3]. Moreover, pharmacovigilance further elaborates on the possible expansion or restriction of the drug's therapeutic effects, consideration of its pharmacoeconomic implications, and the identification of unexpected or severe ADRs that have not been determined prior to its regulatory approval of its release for public consumption[4]. In

pharmacists' duties, the responsibility is to detect, assess, understand, and prevent adverse effects, prioritizing patient safety[5].

The practice of pharmacovigilance differs from nation to nation, especially in developing countries. Contextual situations such as the national healthcare budget, types and incidence rates of relevant diseases, and political climate were some of the factors considered[6]. These gaps in the practice of pharmacovigilance led to variation in medical use and the difference in adverse effects experienced by patients, requiring each country to establish its own pharmacovigilance system[7]. In the Philippines, the Food and Drug Administration (FDA) spearheaded pharmacovigilance through the National Pharmacovigilance Center, which is responsible for receiving and processing reports nationwide of suspected adverse drug reactions[8]. However, Philippines, alongside with most Asian countries, had a 'woefully low' culture of ADR reporting[9]. Factors such as the unrecognized reporting process of adverse events, adverse events being misconstrued as 'part of the healing action', and the condescendence of the Filipino population towards unscientific traditional herbal medicines contributed to the often-unutilized pharmacovigilance reporting system initiated by the FDA. Provided that pharmacovigilance was an indispensable dimension of drug discovery and medication safety, it remained questionable whether it was routinely practiced by institutions in the Philippines, let alone individual health professionals such as pharmacists[9].

Hence this study aimed to assess the knowledge, attitude, and practices of hospital pharmacists towards pharmacovigilance. In doing so, the leading causes of hindrance to a functioning pharmacovigilance system can be identified.

2. MATERIALS AND METHODS

2.1. Methods of Research

The study utilized a quantitative and descriptive research design which examined the correlations between the knowledge, attitude, and practices of hospital pharmacists. This is a cross-sectional analysis conducted for hospital pharmacists all throughout Metro Manila, Philippines.

2.2. Subject of Study

Hospital pharmacists working in Manila, Quezon City, Caloocan, Makati, Valenzuela, San Juan, Marikina, Pasay, and Pasig City, Philippines were included in this study. Individuals that do not meet a particular set of characteristics were excluded from the sample based on the study's inclusion and exclusion criteria.

2.3. Sampling Technique

A purposive sampling technique was employed. Based on existing literature, a hospital must have a minimum of 3 pharmacists[10], thus, 3 pharmacists drawn from 40 random hospitals in Metro Manila are likely to respond, leading to a conservative estimate of 120 respondents.

2.4. Statistical Treatment

In performing the statistical analysis, the Statistical Package for Social Science (SPSS) software version 29 was used. Data collected from the survey were encoded in Microsoft Excel and analyzed using descriptive statistics such as mean, standard deviation, frequency and percentages. The correlation between the knowledge, attitude and practices were analyzed using Pearson's R Correlation.

3. RESULTS AND DISCUSSION

3.1. Knowledge of hospital pharmacists about pharmacovigilance

Computed knowledge scores of hospital pharmacists were further classified as poor for scores > 50%, moderate for scores 50-75%, or fair for scores > 75%. Upon analysis, 48% (n=58) and 45% (n=54) of the respondents scored moderate and fair, respectively.

Most respondents (n=72, 60%) correctly defined pharmacovigilance according to the definition by WHO, while 71.1% (n=86) answered correctly when asked about the purpose of pharmacovigilance. The major cause of ADR was drug interactions (n=90, 75%), followed by allergic reactions (n=88, 73.3%). Additionally, out of 120, 94.2% (n=113) of the participants believe that all serious ADRs must be reported. With regards to whom should report ADRs, pharmacists are the leading healthcare professionals (n=114, 95%), followed by doctors (n=107, 89.2%) and nurses (n=89, 74.2%).

Many of the participants are aware of the medication safety processes, with medication history as the most familiar process (n=84, 70%), followed by medication check review (n=78, 65%) and medication reconciliation (n=64, 53.3%). The participants are also familiar with organizations responsible for educating healthcare professionals on safe medication practices, which is led by the World Health Organization (n=108, 90%). Furthermore, a significant number of respondents (n=96, 80%) have knowledge of a center or ADR reporting system in the Philippines. Further details on the results for knowledge are summarized in Table 1.

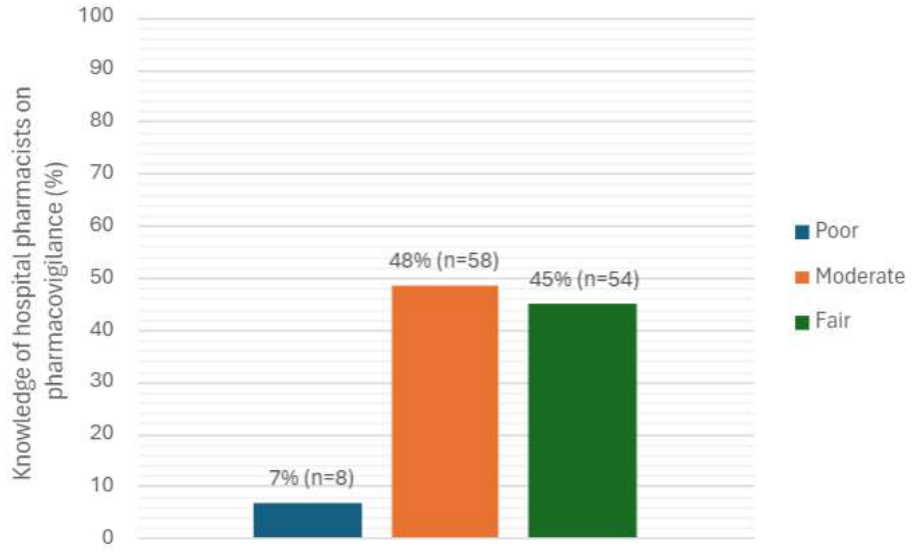

Figure 1. Knowledge scores of hospital pharmacists on pharmacovigilance

Table 1. Knowledge of hospital pharmacists in Metro Manila on pharmacovigilance

Which of the following BEST defines Pharmacovigilance according to the World Health Organization (WHO)?	**n**	**%**
The process of improving drug safety.	5	4.2
The science and activities relating to detecting, assessing, understanding and prevention of adverse effects.	72	60.0
The science of detecting the class and incidence of adverse drug reactions (ADR) after a drug is released to the market.	34	28.3
The science of monitoring adverse drug reactions (ADR) happening in an institution.	9	7.5

Which of the following is the goal of Pharmacovigilance?	**n**	**%**
Calculation of adverse drug reactions (ADR) incidence	2	1.7
Enhancing patient safety in relation to drug use	86	71.7
Identifying predisposing factors to adverse drug reactions (ADR)	13	10.8
Identifying unrecognized adverse drug reactions (ADR)	19	15.8
Which of the following are possible causes of ADRs?	**n**	**%**
Undesirable Effect	62	51.7
Incorrect Administration	57	47.5
Unsafe drug for the patient	39	32.5
Allergic reaction	88	73.3
Drug Interaction	90	75.0
Dosage Modifications (Increase or Decrease)	44	36.7
Which ADRs should be reported?	**n**	**%**
ADRs to herbal products	1	.8
ADRs to new drugs	5	4.2
ADRs to vaccines	1	.8
All serious ADRs	113	94.2
Which of these healthcare professionals are qualified to report ADRs?*	**n**	**%**
Pharmacists	114	95.0
Doctors	107	89.2
Nurses	89	74.2
Dentists	55	45.8
Physiotherapists	29	24.2
Patients	30	25.0
Are you familiar with the following medication safety processes?	**n**	**%**
No	7	5.8
Yes	113	94.2
If YES, which of the following are you familiar with?*	**n**	**%**
Medication Reconciliation	64	53.3
Medication Check Review	78	65.0
Medication History	84	70.0
None of the Above	3	2.5
Are you aware of organizations responsible for educating healthcare professionals on safe medication practices?	**n**	**%**
No	7	5.8
Yes	113	94.2
If YES, which of the following are you familiar with?*	**n**	**%**
Institute for Safe Medication Practices	30	25.0
International Medication Safety Network	14	11.7
World Health Organization	108	90.0
None of the Above	1	0.8
Do you know of any Center or ADR reporting system in the Philippines?	**n**	**%**
No	24	20.0
Yes	96	80.0

**Some results may not total to 100% due to choice given for multiple responses*

3.2. Attitudes of hospital pharmacists towards pharmacovigilance

Attitude scores of surveyed hospital pharmacists in Metro Manila were divided into having a positive attitude for scores ≥ 50% and negative attitude for scores < 50%. The results showed that 100% (n=120) of the respondents scored ≥ 50%, signifying that hospital pharmacists in Metro Manila have a positive attitude towards pharmacovigilance. Among the 120 participants, 100% (n=120) agreed that it is necessary to report ADRs, while 96.7% (n=116) are willing to implement ADR reporting in their practice. On the other hand, 37.5% (n=45) believe that it is not being taught well.

Among the 120 participants, 100% (n=120) agreed that it is necessary to report ADRs. Similarly, all participants believed that ADR reporting will improve and contribute to the healthcare system, and that conducting a medication review can reduce ADR reporting. Most of the respondents (n=118, 98.3%) believe that ADR reporting is a professional obligation of pharmacists, while (n=116, 96.7%) are willing to implement ADR reporting in their practice. On the other hand, 62.5% (n=75) believe that the concepts of ADR reporting and pharmacovigilance are not taught well by healthcare professionals, with 37.5% (n=45) believing that it is not being taught well. The summary of results for attitude is displayed in Table 2.

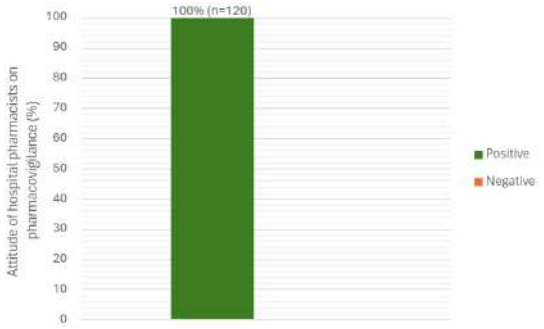

Figure 2. Attitude scores of hospital pharmacists on pharmacovigilance

Table 2. Attitudes of hospital pharmacists in Metro Manila towards pharmacovigilance

In your opinion, do you think it is necessary to report ADRs?	**n**	**%**
No	0	0.0
Yes	120	100.0
In your opinion, is ADR reporting a professional obligation of pharmacists?	**n**	**%**
No	2	1.7
Yes	118	98.3
Do you think ADR reporting will improve and contribute to the healthcare system?	**n**	**%**
No	0	0.0
Yes	120	100.0
Do you think conducting a medication review can reduce ADR reporting?	**n**	**%**
No	0	0.0
Yes	120	100.0
In your own opinion, do you think ADR reporting and pharmacovigilance are taught well by healthcare professionals?	**n**	**%**
No	45	37.5
Yes	75	62.5
Are you willing to implement ADR reporting in your practice?	**n**	**%**
No	4	3.3
Yes	116	96.7

3.3. Practices of hospital pharmacists towards pharmacovigilance

The practice scores of hospital pharmacists in Metro Manila were divided as having good practice for scores ≥ 50% and poor practice for scores < 50%. More than half (n=65, 54%) of the respondents exhibited poor practices on pharmacovigilance, while 46% (n=55) were reported to have good practice. In terms of identifying ADR in any patient, 50.8% (n=61) of the participants answered yes, with 43.3% (n=52) stating that they have only rarely identified ADR in their patients (< 5 times). When asked about the perceived barriers in reporting ADRs, the major factors answered are not knowing how to report ADRs (n=89, 74.2%) and knowing what information to report (n=63, 52%).

In conducting medication reviews with their patients, 55.8% (n=67) answered yes, with 24.2% (n=29) stating that they often conduct them, which is the most frequently practiced by hospital pharmacists. Furthermore, 17.5% (n=21), 12.5% (n=15), and 4.2% (n=5) done it rarely, occasionally, and always, respectively. On the other hand, 44.2% (n=53) never conducted a medication review with their patients. The lack of training on how to conduct a medication review (n=96, 80%) was the most chosen barrier by the respondents. Others saw lack of time (n=69, 57.5%), lack of knowledge by the patients about their medications (n=62, 51.7%), lack of formal process in place (n=51, 42.5%), and language barrier (n=21, 17.5%) in conducting medication reviews. Only two participants (1.7%) did not see a barrier to carrying out the assessment. In terms of identifying ADR in any patient, 50.8% (n=61) of the participants answered yes, with 43.3% (n=52) stating that they have only rarely identified ADR in their patients (< 5 times), 6.7% (n=8) stating that they have reported more than ten times, and 1.7% (n=2) stating that they have reported five to ten times. On the other hand, 49.2% (n=59) stated that they had not identified any ADR in patients. More so, 41.7% (n=50) have reported an ADR, with 33.3% (n=40) having reported less than 5 times, 6.7% (n=8) having reported more than 10 times, and 0.8% (n=1) having reported five to ten times. In contrast, 58.3% (n=70) have no experience reporting ADR, which was experienced by most of the respondents.

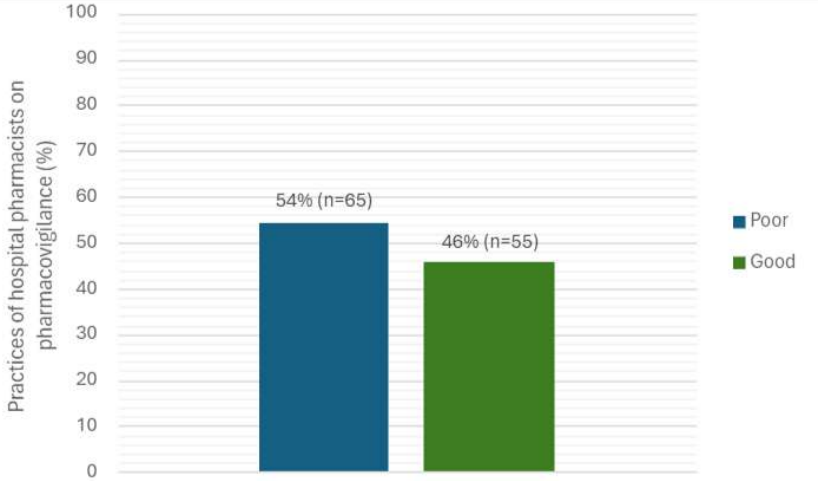

Figure 3. Practice scores of hospital pharmacists on pharmacovigilance

Most of the participants (n=69, 57.4%) prefer to report ADRs via email, while 24.1% (n=29), 12.5% (n=15), 4.2% (n=5), and 1.7% (n=2) prefer direct contact, website, mail/fax, and telephone, respectively. The respondents knew that ADR reports should be reported to the Food and Drug Administration (FDA) (n=104, 86.7%), the Department of Health (DOH) (n=81, 67.5%), their institution (n=66, 55%), and the drug company (n=59, 49.2%). When asked about the perceived barriers in reporting ADRs, the major factors answered are not knowing how to report ADRs (n=89, 74.2%) and knowing what information to report (n=63, 52%). Others were discouraged by patient confidentiality issues (n=54, 45%), the thought that reporting an ADR incident was not important (n=39, 32.5%), and reporting ADR was less important than managing their patients (n=33, 27.5%). Only five respondents (4.2%) thought of reporting ADRs as not part of their job. As part of "incident reports" in the institution where hospital pharmacists belong, 80.8% (n=97) agreed that ADRs were a component of their incident reports, while 19.2% (n=23) disagreed. The results of practices are summarized in Table 3.

Table 3. Practices of hospital pharmacists in Metro Manila on pharmacovigilance

Have you ever conducted a medication review with your patients?	**n**	**%**
No	53	44.2
Yes	67	55.8
If yes, how frequent?	**n**	**%**
Always	5	4.2
Occasionally	15	12.5
Often	29	24.2
Rarely	21	17.5
What are the barriers in conducting a medication review?	**n**	**%**
Lack of Time	69	57.5
Lack of training on how to conduct a medication review	96	80.0
Lack of formal process in place	51	42.5
Language barrier	21	17.5
Lack of knowledge by the patients about their medications	62	51.7
None of the above	2	1.7
Have you ever identified an ADR in any patient?	**n**	**%**
No	59	49.2
Yes	61	50.8
If yes, how frequent?	**n**	**%**
< 5 times	52	43.3
> 10 times	8	6.7
5-10 times	2	1.7
Have you ever reported an ADR?	**n**	**%**
No	70	58.3
Yes	50	41.7
If yes, how frequent?	**n**	**%**
<5 times	40	33.3
>10 times	8	6.7
5-10 times	1	.8
Do you know to whom ADR should be reported?*	**n**	**%**
Department of Health	81	67.5
Food and Drug Administration	104	86.7
Drug Company	59	49.2
The Institution (Hospital)	66	55.0
None of the above	0	0.0
What method would you prefer in reporting ADRs to an ADR Reporting Center?	**n**	**%**
Direct contact	29	24.1
Email	69	57.4
Mail / Fax	5	4.2
Telephone	2	1.7
Website	15	12.5
What factors do you think may be discouraging in reporting ADRs?*	**n**	**%**
Not knowing how to report	89	74.2
Knowing what information to report	63	52.5
Thinking it is not important to report an ADR incident	39	32.5
Managing patients is more important than reporting ADR	33	27.5
It is not part of my job to report ADRs	5	4.2
Patient confidentiality issues	54	45.0
Are ADRs being reported as part of "incident reports" in your institution?	**n**	**%**
No	23	19.2
Yes	97	80.8

**Some results may not total to 100% due to choice given for multiple responses*

3.4. Correlational analysis of the relationships between knowledge, attitude, and practices

The study delved into the intricate relationship between the knowledge, attitudes, and practices (KAP) of pharmacists concerning pharmacovigilance within hospitals situated across Metro Manila. Structured questionnaires were distributed among pharmacists working in diverse hospital settings to ascertain their perspectives. The analysis uncovered weak correlations among the variables under investigation. Specifically, the correlation between knowledge and attitude yielded a negligible coefficient ($r = -0.002$) with a non-significant p-value ($p = 0.987$), indicating an absence of a substantial relationship between these domains. Similarly, the correlation between knowledge and practices exhibited a weak coefficient ($r = 0.129$) with a non-significant p-value ($p = 0.161$), suggesting no statistically significant association. However, a noteworthy finding emerged from the correlation between attitude and practice, where a weak positive correlation ($r = 0.199$) was observed with a significant p-value ($p = 0.029$), implying a tangible link between these aspects. This signifies that while pharmacists' knowledge may not directly impact their attitudes or practices regarding pharmacovigilance, there appears to be a modest association between their attitudes and actual practices in the field.

Table 4. Summary of findings regarding the correlation between knowledge, attitude, and practices

Variable	*r*	Interpretation	*p-value*	Decision	Conclusion
Knowledge and Attitude	-0.002	Weak Negative Correlation	0.987	Failed to Reject Ho	Not significant
Knowledge and Practices	0.129	Weak Positive Correlation	0.161	Failed to Reject Ho	Not significant
Attitude and Practice	0.199	Weak Positive Correlation	0.029	Reject Ho	Significant

Table 5. Practices and training of pharmacovigilance in hospitals

What are the practices of your institution with regards to Pharmacovigilance?	**n**	**%**
Maintains a Pharmacovigilance unit	40	33.3%
Submit all reports of adverse events to the FDA	95	79.2%
Informs the National Pharmacovigilance Center of any amendments in its composition and qualifications	26	21.7%
Encourages healthcare workers to attend pharmacovigilance seminars and trainings	72	60.0%
Has a separate pharmacovigilance unit	12	10.0%
How often does your institution provide seminars and/or training regarding pharmacovigilance?	**n**	**%**
Annually	24	20.0
Biannually	1	0.8
Every ≥ 3 years	13	10.8
Monthly	7	5.8
Never	46	38.3
Quarterly	17	14.2
Semi-annually	12	10.0

3.5. Compliance of the knowledge, attitude, and practice of hospital pharmacists on pharmacovigilance regulations in the Republic of the Philippines

In accordance with the minimum standards issued by the DOH with regards to the practice of pharmacovigilance in hospitals in the Philippines, or Administrative Order 2011-0009, results obtained show that institutions in Metro Manila most of the criteria listed. Submission of all reports of adverse events to the FDA is the most cited criteria, with 79.2% (n=95), followed by encouraging HCWs to attend seminars and training with 60% (n=72).

3.6. DISCUSSION

Most of the respondents (n=72, 60%) properly described pharmacovigilance according to the definition by WHO, in agreement with the study by with 62 percent[11, 12], with 53.4 percent[13] and with 61 percent[14]. Interestingly, results about knowledge of an ADR reporting center were found to be better than other studies, with 80 percent (n=96) of the participants having knowledge of an ADR reporting center in the Philippines as compared to the 35 percent[11], 18 percent[15], 28.6 percent[16], 19.5 percent[17] and 7 percent[12] from other countries.

There were six attitude-related questions; Table 10 and Figure 4 indicate in depth details about the responses to these. It is noteworthy that the respondents showed a favorable attitude towards pharmacovigilance. All respondents (n=120, 100%) think that it is necessary to report ADR cases. This observation is parallel to the similar studies involving pharmacists; 100 percent[18, 19, 20] and 95.2 percent[21]. The case is also similar to the remaining attitude-related questions, whereas respondents have a positive outlook or attitude in terms of ADR reporting. However, in terms of training, a portion (n=45, 37.5%) of respondents think that ADR reporting and pharmacovigilance are not taught well by healthcare professionals, yet the majority (n=75, 62.5%) thinks that ADR reporting and pharmacovigilance are taught well by healthcare professionals. This suggests that there are inconsistencies in the training of hospital pharmacists about ADR reporting, even though they have a favorable attitude and sees the importance of ADR reporting and pharmacovigilance, if they are not taught how to execute it or report it properly, it might be a contributing factor as to why ADR reporting is not exercised frequently in Metro Manila.

There is a low culture of reporting adverse drug reactions (ADRs) in the Philippines[22] that justified the poor practices on pharmacovigilance of hospital pharmacists (n=65, 54%). Despite most respondents identifying an ADR in any patient (n=61, 50.8%), many pharmacists did not report them (n=70, 58.3%) to an ADR Reporting Center. Common barriers to ADR reporting include not knowing how to report ADRs (n=89, 74.2%), not knowing what to report (n=63, 52.5%), patient confidentiality issues (n=54, 45%) and thinking that it is not important (n=39, 32.5%), with not knowing how to report ADRs as the leading factor. Similarly, a study in Saudi Arabia stated that the common factor discouraging ADR reporting was not knowing how to report an ADR, and 33% also thought that not knowing what information to report may discourage pharmacists from reporting ADRs[2]. Factors such as not thinking it was important to report (7.7%) and patient confidentiality issues (4.3%) were also considered to discourage pharmacists from reporting ADRs[23], despite having several options to be averted. Furthermore, some studies also included not knowing where to report as a factor in discouraging pharmacists from reporting ADRs with 34.8 percent[23] and 27.2 percent[24]. Despite this factor, respondents still have an idea of whom ADR should be reported, with 86.7% (n=104) to the FDA, 67.5% (n=81) to DOH, 55% (n=66) to their institutional workplace, and 49.2% (n=59) to the drug company. The majority of the hospital pharmacists were also able to conduct a medication review with their patients (n=67, 55.8%); however, barriers in the process cannot be omitted. These barriers are mainly due to the lack of training on how to conduct a medication review (n=96, 80%), time (n=69, 57.5%), knowledge about patient's medications (n=62, 51.7%), formal process in place (n=51, 42.5%), and language barrier (n=21, 17.5%). Correspondingly, the common challenges perceived by hospital pharmacists in conducting medication review are insufficient training and education (79.8%), and time deficiency (82.7%) due to workforce shortage as 60% of Malaysian pharmacists are working in the public sector[25]. Lack of knowledge about risks and safe use of medicines by patients contributes to the leading causes of drug-related problems that trigger

counseling[26]. The language barrier was the most frequently identified barrier (92%) to access pharmacy services based on a compiled article[27]. No comprehensive literature reviews have found evidence of medication reviews in a hospital setting[28]; however, this may still be a barrier, considering that hospital pharmacists responded to this as an option to be a barrier to conducting a medication review. Moreover, pharmacists offered unsatisfactory counseling, inefficient medication review, and reconciliation. The remaining two participants (1.7%) thought they did not see a barrier to conducting a medication review. In any case that hospital pharmacists would report an ADR to an ADR Reporting Center, most of the respondents prefer to send it through email (n=69, 57.4%).

The results in Table 4 indicate the correlation between the variables knowledge, attitude, and practice of hospital pharmacists on pharmacovigilance. The findings on the knowledge and attitude of hospital pharmacists towards pharmacovigilance have no significant correlation (r = -0.002, p-value = 0.987), which is similar to other studies[4, 29, 30]. A correlation value of 0.129 and p-value of 0.161 suggested a weak positive correlation between knowledge and practice, showing comparable findings from previous studies[31, 13, 16]. A positive weak correlation was also observed between the attitude and practice (r = 0.199, p-value = 0.029) of the hospital pharmacists on pharmacovigilance; this outcome is similar to other studies[32, 33, 34].

The data analysis of pharmacists' knowledge regarding pharmacovigilance regulations in hospitals across Metro Manila reveals a commendable level of understanding and adherence to key principles. A substantial majority of pharmacists accurately identified the definition and goals of pharmacovigilance as outlined by international standards, particularly by the World Health Organization. This recognition underscores a fundamental grasp of the importance of monitoring, detecting, and preventing adverse drug reactions (ADRs) to enhance patient safety and overall healthcare quality. Furthermore, respondents demonstrated a thorough awareness of the diverse causes of ADRs, highlighting their capacity to identify potential risks associated with medication use.

The high percentage of pharmacists acknowledging the necessity to report all serious ADRs indicates a commitment to fulfilling their professional responsibility in ensuring drug safety. Moreover, the recognition of pharmacists as qualified professionals for ADR reporting emphasizes the pivotal role of pharmacists in pharmacovigilance. Collectively, these findings suggest a robust knowledge foundation among pharmacists in Metro Manila hospitals. This strong knowledge base not only enhances patient care and safety but also contributes to the overall integrity and effectiveness of pharmacovigilance practices within hospital settings, ultimately promoting public health and well-being.

4. CONCLUSION

Despite the weak correlations observed in the analysis of pharmacovigilance knowledge, attitudes, and practices, the overall understanding and dedication to pharmacovigilance principles among pharmacists in Metro Manila are evident. The recognition of the importance of reporting adverse drug reactions (ADRs) underscores a shared commitment to patient safety and regulatory compliance within the profession. However, the presence of weak correlations suggests the presence of external factors influencing pharmacovigilance activities, warranting further investigation and targeted interventions.

The correlation analysis between knowledge, attitudes, and practices of pharmacists regarding pharmacovigilance in Metro Manila hospitals revealed weak correlations between these variables, with attitude and practices being the only statistically significant correlation. The weak correlation suggests that factors other than knowledge, attitudes, and practices may influence pharmacovigilance activities among pharmacists in this setting. Despite this, the data analysis indicates a commendable level of understanding and adherence to pharmacovigilance principles among pharmacists, with a strong recognition of the importance of reporting all serious adverse drug reactions (ADRs) and a commitment to fulfilling their professional responsibility in ensuring drug safety.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Meta-Analysis of Observational Studies Using Propensity Score Methods for First-Line Antihypertensive Drug Classes

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ABSTRACT

Antihypertensive medications have been associated with the prevention of cardiovascular events. However, a notable disparity exists in comprehending the relative effectiveness of various medication classes in real-world settings. This study aims to quantify mortality and morbidity effects from different first-line antihypertensive drug classes, including Renin–angiotensin system inhibitor (RASI), beta-blockers (BB), calcium channel blockers (CCB) and diuretics, in observational studies using propensity score (PS) method in hypertensive patients. Systematic searches were conducted in PubMed and Scopus until March 2024, including observational studies utilizing PS methodology comparing first-line drug classes in hypertensive adults. Outcomes included all-cause mortality, cardiovascular mortality, heart failure (HF), hospitalization for heart failure (HHF), stroke, composite cardiovascular events (CCE), and myocardial infarction (MI). Direct meta-analysis was performed with at least three studies sharing similar treatment comparisons and outcomes. Adjusted risk ratios (RR), odd ratios (OR), and hazard ratios (HR) were pooled using a random or fixed-effect model based on the presence of heterogeneity. The risk of bias was assessed using the Non-Randomized Studies of Intervention (ROBIN-I). Publication bias and subgroup analysis were performed. The study included 35 cohort studies involving more than 11 million patients, 19 of which focused on patients diagnosed with Coronavirus Disease 2019 (COVID-19). The mean participant age was 63 years. Compared to non-RASI, RASI reduced mortality (RR 0.51, 95%CI 0.07, 0.96; OR 0.73, 95%CI, 0.62, 0.84; HR 0.9, 95%CI 0.86, 0.94), stroke (HR 0.9, 95%CI 0.86, 0.94), MI (HR 0.84, 95% CI 0.77, 0.91) but not reduce HF. Compared to RASI, diuretics and BB did not reduce mortality, HF, and stroke. CCB increased stroke (HR 1.10, 95%CI 1.00, 1.19) and CCE (HR 1.04, 95%CI 1.02, 1.07), but did not reduce HF, HHF and MI compared with RASI. RASI reduced all morbidity and mortality outcomes except for HF and HHF. RASI, diuretics, and BB show similar effectiveness. CCB were inferior to RASI.

KEYWORDS: Antihypertensive; Hypertension; Meta-analysis

1. INTRODUCTION

Hypertension is a significant modifiable risk factor for global health conditions such as ischemic heart disease, stroke, cardiovascular illnesses, chronic renal disease, and dementia[1]. It accounts for 7.7-10.4 million annual deaths[2].

Ten meta-analyses and an umbrella review of observational studies conducted in patients with COVID-19 have compared Renin–angiotensin system inhibitors (RASI) with non-RASI[3-13]. Among them, only one meta-analysis employed the propensity score (PS) method[6]. Nine meta-analyses of non-randomized controlled trials (non-RCTs) reported that RASI reduced the risk of death compared to non-RASI[3-5, 7, 8, 10-13]. In contrast, in a meta-analysis of non-RCTs that employed the PS method and in an umbrella review, RASI use did not significantly impact death outcomes compared to non-RASI [OR, 1.01, 95% CI: 0.87-1.16 (n=13); OR, 0.90, 95% CI: 0.75-1.08 (n=4), respectively][6, 13]. This suggests that previous evidence have primarily focused on hypertensive patients with COVID-19, without comparing the effectiveness of other drug classes beyond RASI and non-RASI. This limitation restricts the generalizability of their findings to the broader patient population. Additionally, it is critical to gain a more comprehensive understanding of the potential association between the morbidity and mortality effects of different classes of first-line antihypertensive drugs in hypertensive patients with comorbidities.

Recently, the healthcare industry's digitization and increased electronic medical record accessibility have facilitated the analysis of real-world data[14]. This has enabled the extended monitoring of effectiveness and safety outcomes over prolonged treatment durations, gaining significance for healthcare professionals aligning with real-world clinical practice[15]. Therefore, this study aims to (1) quantify the mortality and morbidity effects from various first-line antihypertensive drug classes, including RASI, BB, CCB, and diuretics, in observational studies employing the PS method in hypertensive patients with any comorbidities and (2) perform subgroup analysis to compare the aforementioned outcomes in hypertensive patients with and without COVID-19.

2. MATERIALS AND METHODS

This study was conducted following the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA), and the study protocol was registered on the PROSPERO website (identification number CRD42023454256).

2.1. Data Sources and Search Strategy

A comprehensive search for relevant articles via PubMed and Scopus databases, covering the period from the establishment of these databases up to March 30, 2024. Search terms were derived from PICOS framework, the Population (P) was hypertensive patient, Interest (I) was first-line drug classes, including RASI [i.e., Angiotensin-Converting Enzyme Inhibitors (ACEI) or Angiotensin II Receptor Blockers(ARB)], diuretics (i.e., thiazide diuretics or thiazide-like diuretics), CCBs (i.e., non-dihydropyridine or dihydropyridine CCB), beta-blockers, outcome (O) was all-cause mortality, cardiovascular mortality, heart failure (HF), hospitalization for heart failure (HHF), stroke, composite cardiovascular events (CCE), and myocardial infarction (MI) and study design (S) was any observational study applied PS method. The reference lists of the retrieved studies were also explored to identify further studies. Searching was updated every three months.

2.2. Study Selection

Studies were independently selected through the screening titles and abstracts by two reviewers (AT and PA). If determination of eligibility, the full articles were reviewed. Non-RCTs applying the PS method were included if they met all the following criteria. (1) Adult patients diagnosed with hypertension, with or without any concurrent comorbidities, (2) compared outcomes of interest between any pair of the following first-line drug classes, including RASI (i.e., ACEIs or ARBs), diuretics (i.e., thiazide diuretics or thiazide-like diuretics), CCBs (i.e., non-dihydropyridine or dihydropyridine CCB), beta-blockers; and (3) had at least one of the outcomes of interest including all-cause mortality, cardiovascular mortality, HF, HHF, stroke, CCE, and MI. Any studies published in a foreign language that cannot be translated or do not provide sufficient data for pooling will be eliminated after three unsuccessful efforts to contact the authors. Any disagreements were resolved by consensus.

2.3. Data Extraction

A data extraction form captured the following information: (1) general information, (2) study characteristics, (3) intervention and comparator characteristics, (4) population characteristics, (5) outcome characteristics, (6) characteristics of the method used with PS, (7) data for pooling. Any disagreements were resolved by consensus.

2.4. Risk of Bias Assessment

The risk of bias assessment for each study was performed independently by two reviewers (AT and PA). Given that this review focused on non-RCT studies, the quality assessment using the Risk of Bias in Non-Randomized Studies-of Interventions (ROBINS-I), developed by the Cochrane Bias Methods Group and the Cochrane Non-Randomized Studies Methods Group[16].

2.5. Statistical Analysis

A pairwise meta-analysis was performed if at least three studies or cohorts compared outcomes between the same treatment pair. Treatment effects (i.e., adjusted risk ratio (RR) or adjusted odds ratio (OR) for dichotomous outcomes and adjusted hazard ratio (HR) for time to event outcome] were estimated and pooled across studies using a fixed-effect model (i.e., inverse variance method) if heterogeneity was absent; otherwise, a random-effect model (i.e., DerSimonian and Laird method) was used[17]. Heterogeneity was present if the p-value from Q-test < 0.10 or I^2 > 25%; and An I^2 is classified as low, moderate, or high if it is < 25%, 25-74%, and ≥ 75% respectively[18]. A source of heterogeneity was explored by fitting characteristics of comorbidities. Publication bias was assessed using Egger's tests and funnel plots of at least three studies. If there was asymmetry in the funnel plot, a contour-enhanced funnel plot was generated to identify the cause of asymmetry (i.e., small study effect or heterogeneity)[19, 20].

STATA version 17 (StataCorp, Texas, USA) was used for statistical analyses. A significance threshold p-value < 0.05 was considered for all analyses, except for Cochran's Q test, a p-value less than 0.10 was applied

3. RESULTS AND DISCUSSION

3.1. Identifying of Included Studies

A total of 2073 studies identified, 1787 were screened for title and abstract. Thirty-five studies were eligible see Figure 1. The characteristics of the included studies are summarized in Supplementary Table 1S. All studies had retrospective cohort designs, with samples ranging from 210 to 4,893,591—the mean age of participants ranging from 49 to 80. The included studies focused on hypertensive patients with COVID-19 (n=19), rheumatoid arthritis (n=2), acute myocardial infarction (n=1), HIV (n=1), and breast cancer (n=1).

3.2. Risk of Bias Assessment

The results of bias assessment revealed that most studies were a moderate level of risk. See detail in Supplementary Table 2S.

3.3. Pooling results

3.3.1 The Mortality and Morbidity Effects from Various First-Line Antihypertensive Drug Classes

Thirty-five studies were investigated the mortality and morbidities effects of RASI, diuretics, CCB, and BB. The following details were provided.

All-cause mortality

RASI significantly reduced all-cause mortality compared to non-RASI [(RR 0.51, 95% CI 0.07, 0.96) with homogeneity (I^2=0%, *p=0.75*); (OR 0.73, 95% CI, 0.62, 0.84), with moderate heterogeneity (I^2=73.79%, *p<0.001*); (HR 0.9, 95% CI 0.86, 0.94) with moderate heterogeneity (I^2=68.70%, *p<0.001*). Compared to RASI, diuretics and BB showed similar effectiveness [(HR 1.39, 95% CI 0.58, 2.21) with high heterogeneity (I^2=95.84%, *p<0.001*); (HR 1.48, 95% CI 0.91, 2.05) with high heterogeneity (I^2=79.11%, *p=0.01*), respectively] (**Supplementary figure 1S**). There was no publication bias for all treatment comparisons (**Supplementary figure 8S, Table 3S**).

Heart failure

Compared to RASI, diuretics, CCB, and BB showed similar effectiveness [(HR 1.21, 95% CI 0.21, 2.2) with high heterogeneity (I^2=79.97%, *p<0.001*); (HR 0.98, 95% CI 0.6, 1.37) with moderate heterogeneity (I^2=63.58%, *p=0.04*); (HR 1.77, 95% CI 0.63, 2.9) with high heterogeneity (I^2=86.69%, *p<0.001*), respectively]. There was no difference in the risk of heart failure when comparing between RASI and non-RASI [HR 0.98, 95% CI 0.94, 1.02) with high heterogeneity (I^2=90.24%, *p<0.001*)] (**Supplementary figure 2S**). Publication bias was absent for CCB vs RASI (**Supplementary figure 9S, Table 3S**).

Hospitalization of heart failure

Compared to RASI, CCB showed similar effectiveness [(HR 0.97, 95% CI 0.76, 1.18) with high heterogeneity (I^2=92.09%, *p<0.001*)] (**Supplementary figure 3S**).

Stroke

Compared to RASI, diuretics, BB, and CCB showed similar effectiveness [(HR 1.09, 95% CI 0.71, 1,47) with high heterogeneity (I^2=81.36%, *p<0.001*); (HR 0.78, 95% CI 0.36, 1.19) with moderate heterogeneity (I^2=48.60%, *p=0.12*); (HR 1.10, 95% CI 1.00, 1.19) with moderate heterogeneity (I^2=74.03%, *p<0.001*), respectively]. There was no difference in the risk of stroke when comparing BB versus CCB [(HR 1.19, 95% CI 0.87, 1.52) with homogeneity (I^2=0%, *p=0.46*)] and diuretics versus CCB [(HR 0.91, 95% CI 0.73, 1.08) with moderate heterogeneity (I^2=62.11%, *p=0.03*)]. RASI significantly reduced stroke compared to non-RASI [(HR 0.9, 95%CI 0.86, 0.94) with high heterogeneity (I^2=80.64%, *p<0.001*)] (**Supplementary figure 4S**). There was no publication bias for all treatment comparisons (**Supplementary figure 10S, Table 3S**).

Composite cardiovascular events

CCB significantly increased the risk of CCE compared to RASI ([HR, 1.04, 95% CI 1.02, 1.07) with homogeneity (I^2=0%, *p=1.00*)]. Diuretics significantly increased the risk of CCE compared to CCB [(HR, 1.52, 95% CI 1.20, 1.85) with homogeneity (I^2=0%, *p =0.38*)]. There was no difference in the risk of stroke when comparing RASI and non-RASI [(HR 1.98, 95% CI 0.65, 3.31) with high heterogeneity (I^2=92.41%, *p < 0.001*)] (**Supplementary figure 5S**).

Myocardial infarction

Compared to CCB, RASI and diuretics reduce MI similarly [(HR 0.92, 95% CI 0.84, 1.01) with moderate heterogeneity (I^2=61.83%, *p=0.01*); (HR 0.87, 95% CI 0.63, 1.11) with high heterogeneity (I^2=81.52%, *p<0.001*), respectively]. Diuretics vs RASI had similar effects [(HR 1.11, 95%CI 0.79, 1.42) with high heterogeneity (I^2=80.41%, *p<0.001*). RASI reduced MI compared to non-RASI [(HR 0.84, 95% CI 0.77, 0.91) with high heterogeneity (I^2=91.60%, *p<0.001*)] (**Supplementary figure 5S**). There was no publication bias for all diuretic vs RASI and RASI vs CCB (**Supplementary figure 11S, Table 3S**).

3.3.2. Subgroup Analysis in Hypertensive Patients with and without COVID-19.

Effects of RASI vs non-RASI in hypertensive patients with COVID-19.

One study included 4 cohorts were investigated the all-cause mortality of RASI compared to non-RASI in hypertensive patients with COVID-19. The results suggested that compared to non-RASI, RASI reduced all-cause mortality [(HR 0.89, 95%CI 0.84, 0.94) with high heterogeneity (I^2=93.52%, $p < 0.001$)] (**Supplementary figure 7S**).

Effects of RASI vs non-RASI in hypertensive patients without COVID-19.

Nine studies examined the impact of RASI on all-cause mortality in hypertensive patients with COVID-19. The findings indicated that RASI reduced all-cause mortality compared to non-RASI [HR 0.91, 95% CI 0.83-0.99, with moderate heterogeneity (I^2=40.47%, $p = 0.02$)] (**Supplementary figure 7S**).

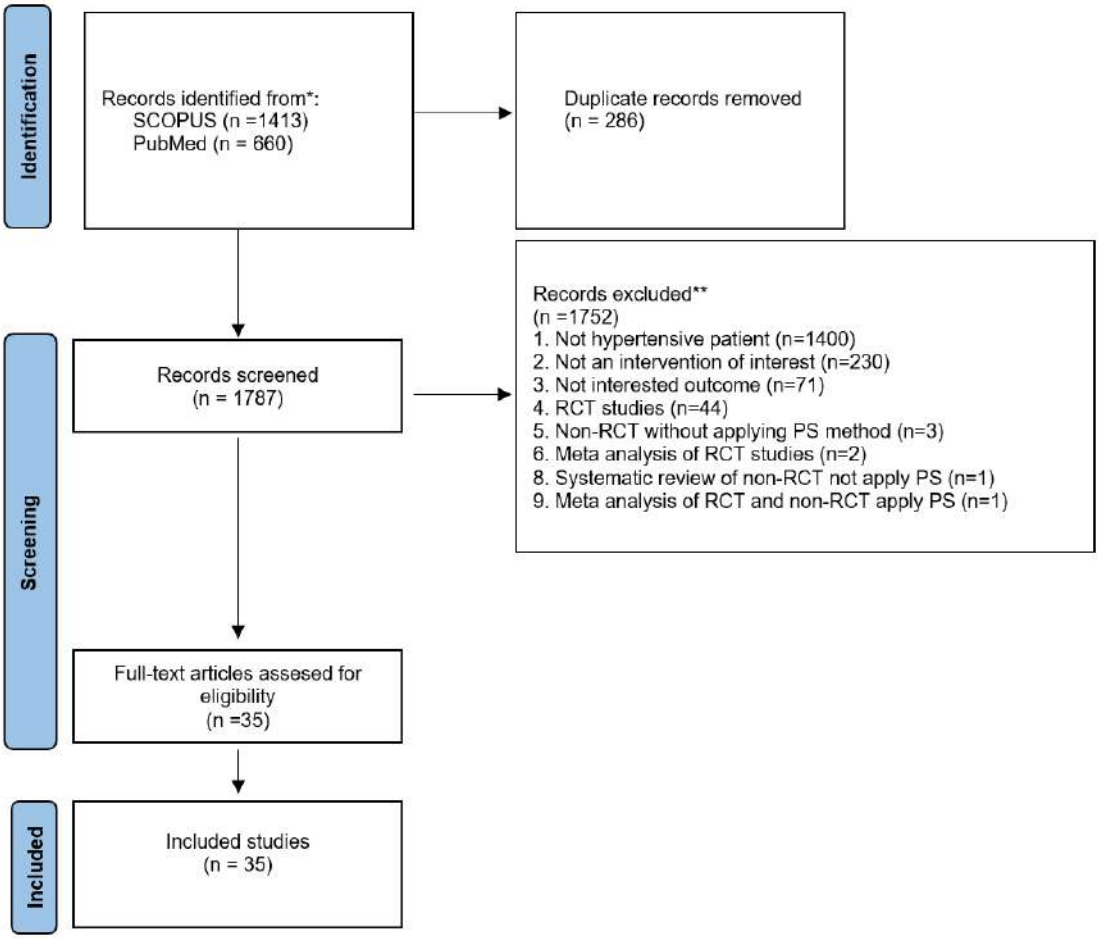

Figure 1. Flow of selection of studies

4. CONCLUSION

In conclusion, our evidence suggests that RASI reduced all morbidity and mortality outcomes except for HF and HHF. RASI, diuretics, and BB show similar effectiveness. CCB were inferior to RASI. The use of RASI in patients with COVID-19 was not associated with an increased death. These findings support the recommendation of major international cardiovascular societies that treatment with RASI should be continued for hypertensive patients with COVID-19.

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Conflict of interest

The authors declare that they have no conflict of interest.

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SUPPLEMENT MATERIAL

Table 1S. Characteristics of included studies

First author	Publication date	Location	Study period	Intervention	Comparator	Population	Number of participants
Marc A. Suchard [1]	2019	Multi-country study	July, 1996 - March, 2018	Diuretic, ACEI, ARB, dCCB, ndCCB	ACEI, ARB, dCCB, ndCCB	General hypertensive patients	4,893,591
Peng Zhang [2]	2020	China	December, 2019 - March, 2020	RASI	Non-RASI	Hypertensive patients with COVID-19	1,128
Jungchan Park [3]	2021	Korea	NR - May, 2020	RASI	Non-RASI	Hypertensive patients with COVID-19	1,905
Catherine G Derington [4]	2021	US	January, 2020 - August, 2020	RASI, ARB	Non-RASI, ACEI	Hypertensive patients with COVID-19	18,550
Sripal Bangalore [5]	2015	US	January, 2004 - December, 2009	ACEI	CCB, TD, BB	General hypertensive patients	53,582
Mauro Gori [6]	2022	Italy	February, 2020 - May, 2020	RASI, ARB	Non-RASI	Hypertensive patients with COVID-19	688
John G Rizk [7]	2022	US	March, 2020 - November, 2020	RASI	Non-RASI	Hypertensive patients with COVID-19	27,556
Álvaro Aparisi [8]	2022	Spain	March, 2020 - NR	ACEI, ARB	Non-ACEI, non-ARB	Hypertensive patients with COVID-19	422
Rohan Khera [9]	2021	US	Outpatient (March 6, 2020 and May 3, 2020) and inpatient (January 5, 2020 and May 10, 2020) – (May 4 and August 2, 2020)	ACEI, ARB	non-RASI, ARB	Hypertensive patients with COVID-19	7,993
Nathalie Gault [10]	2021	France	February, 2020 - June, 2020	ACEI, ARB, RASI	non-RASI	Hypertensive patients with COVID-19	1,160
Steven M Smith [11]	2022	US	February, 2020 - December, 2020	RASI, ACEI	Non-RASI, ARB	Hypertensive patients with COVID-19	13,246
Jae-Geun Lee [12]	2023	Korea	November, 2011-October, 2015	ACEI	ARB	Hypertensive patients with Acute myocardial infarction	4,827
Hack-Lyoung Kim [13]	2022	Korea	January, 2002 - December, 2017	ACEI, ARB, BB, CCB	Diuretics	General hypertensive patients	95,201
Shu-Chen Chien [14]	2015	Taiwan	January, 2000 - December, 2009	ARB	ACEI	General hypertensive patients	79,152
Ju Hwan Kim [15]	2021	Korea	January, 2015 - April, 2020	RASI	Non-RASI	Hypertensive patients with COVID-19	1,290
Ting-Tse Lin [16]	2017	Taiwan	NR	RASI, ACEI, ARB	Non-RASI	Hypertensive patients with Rheumatoid arthritis	27,335
Mingfei Li [17]	2021	Multi-country study	February, 2020 - August, 2020	RASI, ACEI, ARB	Non-RASI	Hypertensive patients with COVID-19	228,722
Jason Roy [18]	2012	US	January, 2001 - December , 2008	ARB	ACEI	General hypertensive patients	22,544
Jean-Claude Tardif [19]	2004	US	January, 1995 - June , 1999	ACEI	CCB	General hypertensive patients	18,199
Jaejin An [20]	2021	US	March, 2020 - September, 2020	RASI	Non-RASI	Hypertensive patients with COVID-19	14,129
Emmanuel Oger [21]	2022	France	2008 - 2014	ARB	ACEI	General hypertensive patients	407,815
Chan Soon Park [22]	2023	Korea	2010 - 2019	ACEI, ARB	Non-RASI, ACEI	General hypertensive patients	2,025,849
María José Soler [23]	2022	Spain	NR - March, 2020	ACEI, ARB, RASI	Non-RASI, non-ACEI, Non-ARB	Hypertensive patients with COVID-19	305,972
Han Saem Jeong [24]	2021	Korea	2013 - December, 2016	ARB	CCB	General hypertensive patients	464,948
Ting Tse Lin [25]	2020	Taiwan	1995-2013	CCB	Non-CCB	Hypertensive patients with Rheumatoid arthritis	27,844
Chi Peng [26]	2021	China	February, 2020 - April, 2020	CCB, RASI, BB	Non-CCB, non-RASI, non-BB	Hypertensive patients with COVID-19	1,449
Shamil Haroon [27]	2021	UK	January, 2019 - January, 2020	ACEI, ARB	CCB	Hypertensive patients with COVID-19	72,071
Zhe Zhang [28]	2023	China	April, 2022-June, 2022	RASI	Non-RASI	Hypertensive patients with COVID-19	763
Kei Sato [29]	2022	Multi-country study	December, 2019-December, 2020	ACEI, ARB	Non-RASI	Hypertensive patients with COVID-19	737
Boshen Yang [30]	2022	Israel	2008-2019	RASI	Non-RASI	General hypertensive patients	15,352
Leah B. Rethy [31]	2021	US	January, 2000-July, 2019	BB, CCB, TD	RASI	Hypertensive patients with HIV	8041
RuiJun Chen [32]	2021	Multi-country study	July, 1996 - March, 2018	ACEI	ARB	General hypertensive patients	2,971,819

Hui-Jeong Hwang [33]	2023	Korea	January, 2008 - December, 2015	CCB, BB, TD	RASI, CCB	Hypertensive patients with Breast Cancer	4,722
Jinwoo Le [34]	2021	Korea	May 15, 2020 - May 27, 2020	RASI, CCB, BB, diuretic	Non-RASI, non-CCB, non-BB, non-diuretic	Hypertensive patients with COVID-19	64,243
Zhongchao Wang [35]	2020	China	February 17, 2020 - March 18, 2020	RASI	Non-RASI	Hypertensive patients with COVID-19	210

NR: not reported, US: United States, UK: United Kingdom

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Table 2S. Risk of bias assessment for non-randomized studies using ROBINS-I

First author	D1	D2	D3	D4	D5	D6	D7	Overall
Marc A. Suchard								
Peng Zhang								
Jungchan Park								
Catherine G Derington								
Sripal Bangalore								
Mauro Gori								
John G Rizk								
Álvaro Aparisi								
Rohan Khera								
Nathalie Gault								
Steven M Smith								
Jae-Geun Lee								
Hack-Lyoung Kim								
Shu-Chen Chien								
Ju Hwan Kim								
Ting-Tse Lin								
Mingfei Li								
Jason Roy								
Jean-Claude Tardif								
Jaejin An								
Emmanuel Oger								
Chan Soon Park [22]								
María José Soler								
Han Saem Jeong								
Ting Tse Lin								
Chi Peng								
Shamil Haroon								
Zhe Zhang								
Kei Sato								
Boshen Yang								
Leah B. Rethy								
RuiJun Chen								
Hui-Jeong Hwang								
Jinwoo Le								
Zhongchao Wang								

D1: Bias due to confounding; D2: Bias in selection of participants into the study; D3: Bias in classification of interventions; D4: Bias due to deviations from intended interventions; D5: Bias due to missing data; D6: Bias in measurement of outcomes; D7: Bias in selection of the reported result

Low risk of bias
Moderate risk of bias
Serious risk of bias
No information

Table 3S. Results of p value of the Egger's test

Statistical type	Intervention	Comparator	Egger test	Publication bias
	All-cause mortality			
RR	RASI	Non-RASI	0.4469	No
OR	RASI	Non-RASI	0.5566	No
HR	RASI	Non-RASI	0.3343	No
HR	Diuretics	RASI	0.5634	No
HR	BB	RASI	NA	
	Heart failure			
HR	Diuretics	RASI	0.0297	Yes
HR	CCB	RASI	0.5541	No
HR	BB	RASI	0.0002	Yes
HR	RASI	Non-RASI	NA	
	Hospitalization of heart failure			
HR	RASI	BB	NA	
	Stroke			
HR	Diuretics	RASI	0.6390	No
HR	BB	RASI	0.2182	No
HR	CCB	RASI	0.4566	No
HR	Diuretics	CCB	0.2708	No
HR	BB	CCB	NA	
HR	RASI	BB	NA	
	Composite cardiovascular events			
HR	Diuretics	RASI	NA	
HR	CCB	RASI	NA	
HR	Diuretics	CCB	NA	
	Myocardial infarction			
HR	Diuretics	RASI	0.0946	Yes
HR	RASI	CCB	0.0286	Yes
HR	Diuretics	CCB	NA	
HR	RASI	Non-RASI	NA	

Noted: NA: not applicable

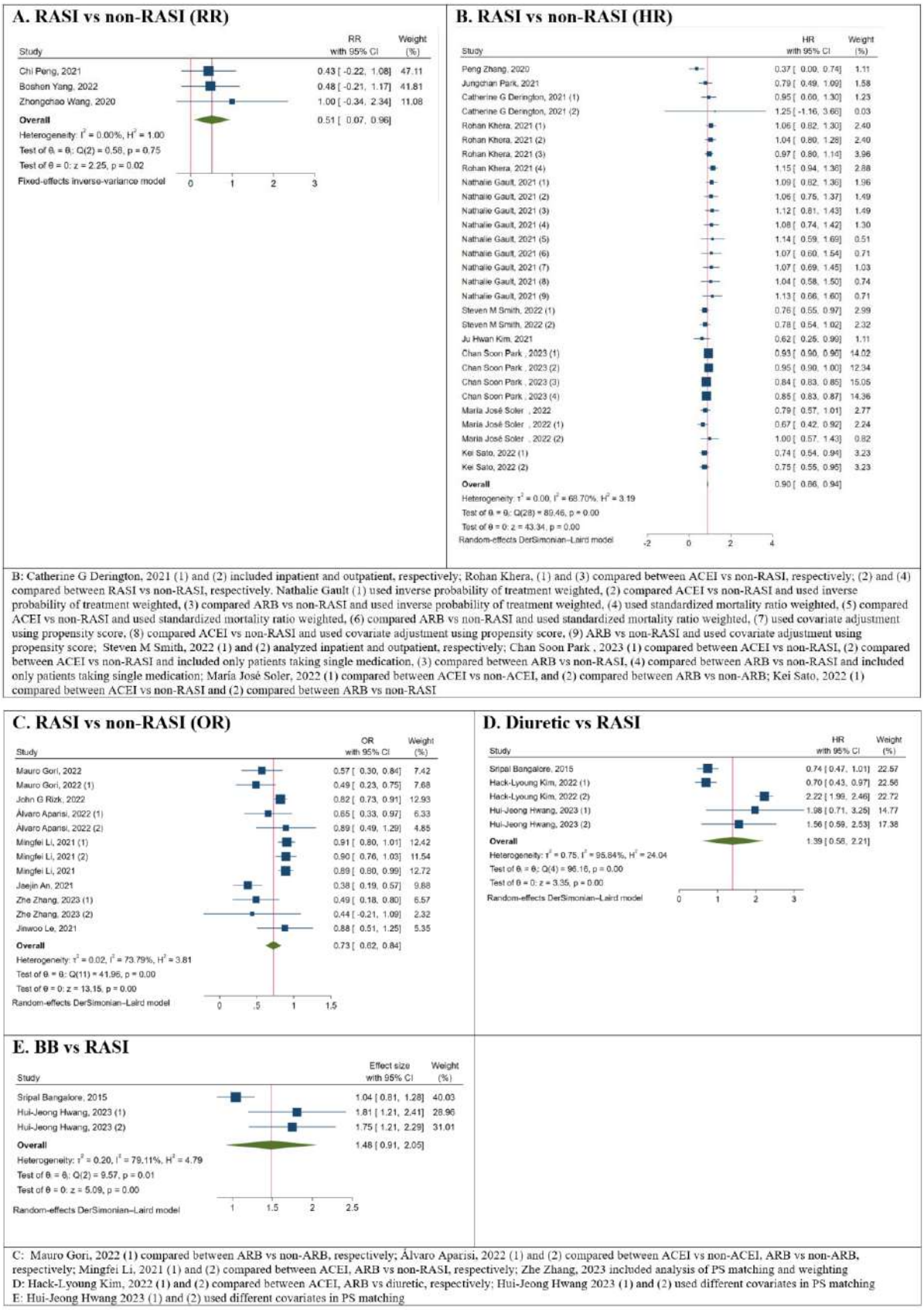


Figure 1S. Pooled RR, OR, HR of all-cause mortality

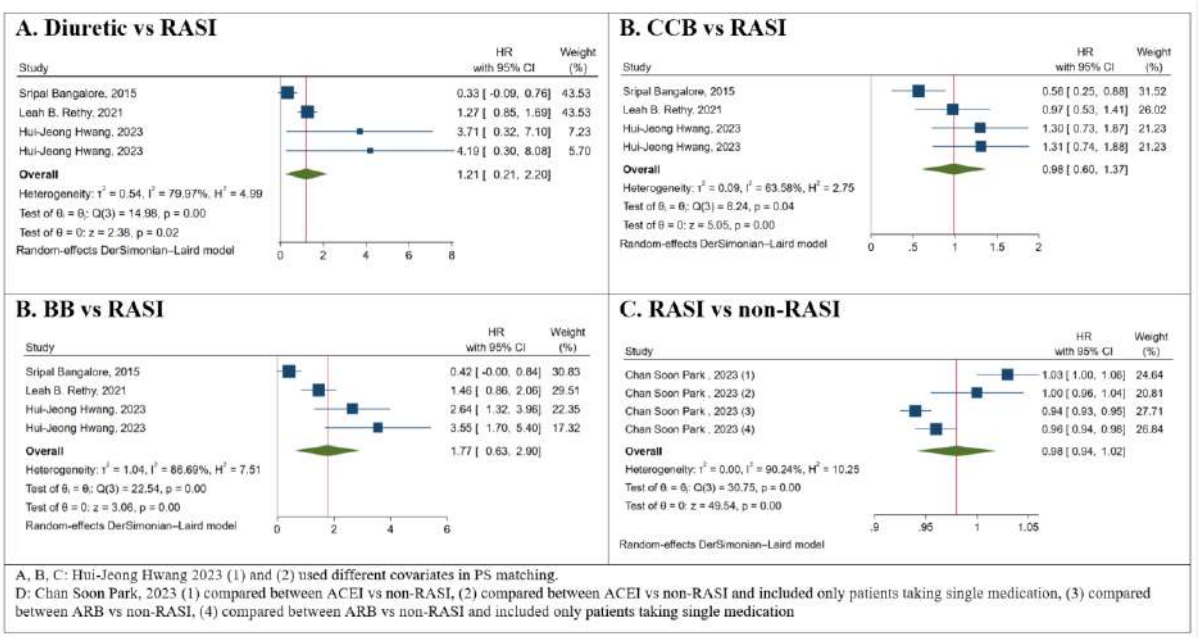


Figure 2S. Pooled HR of heart failure

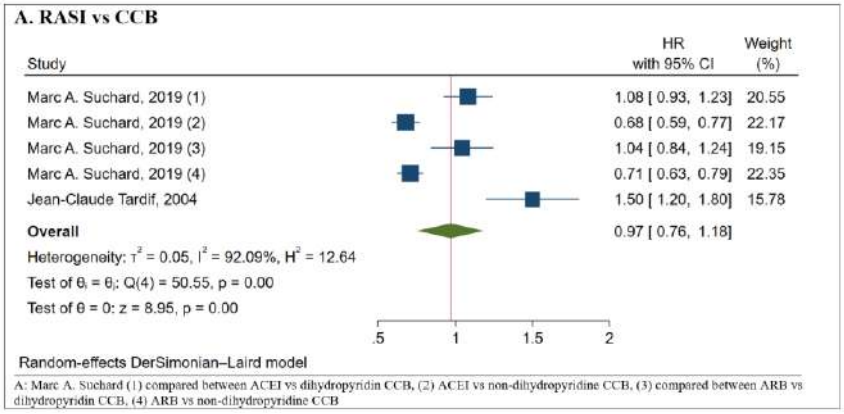


Figure 3S. Pooled HR of hospitalization of heart failure

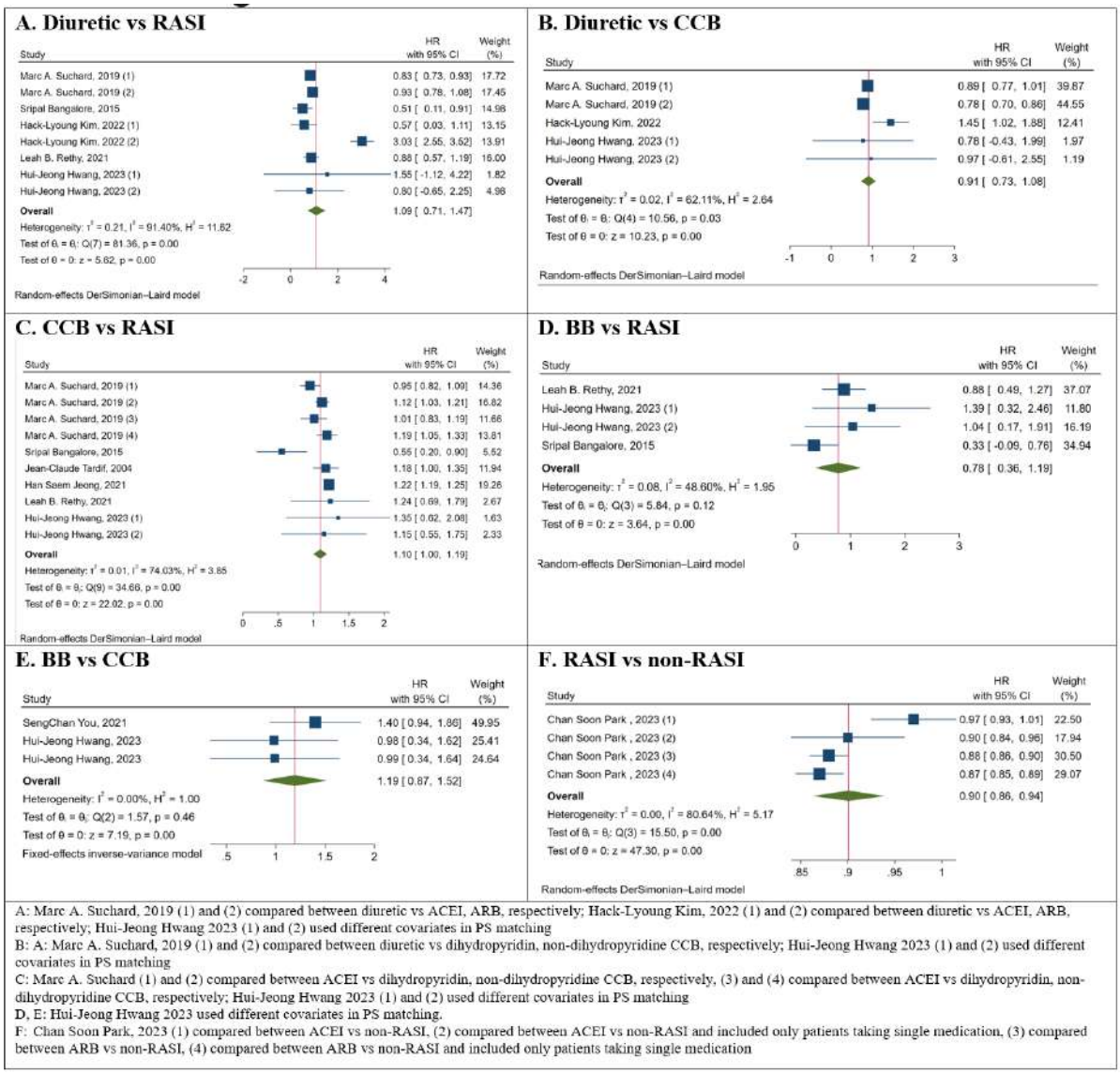


Figure 4S. Pooled HR of stroke

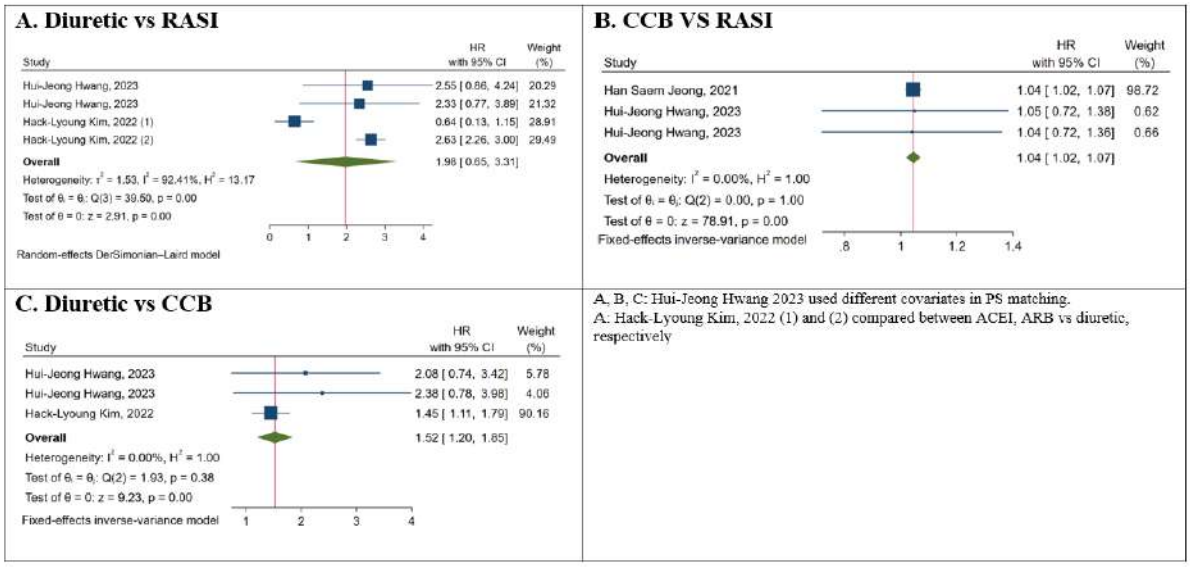
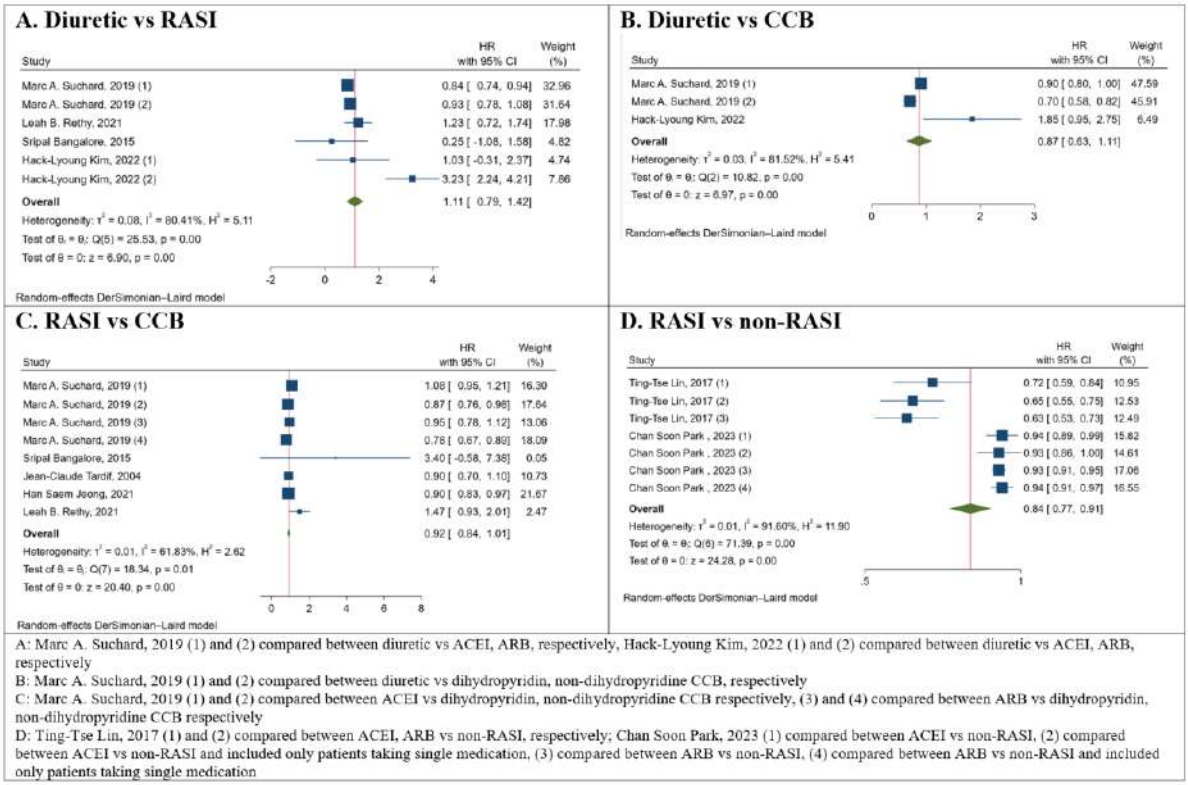


Figure 5S. Pooled HR of composite cardiovascular events



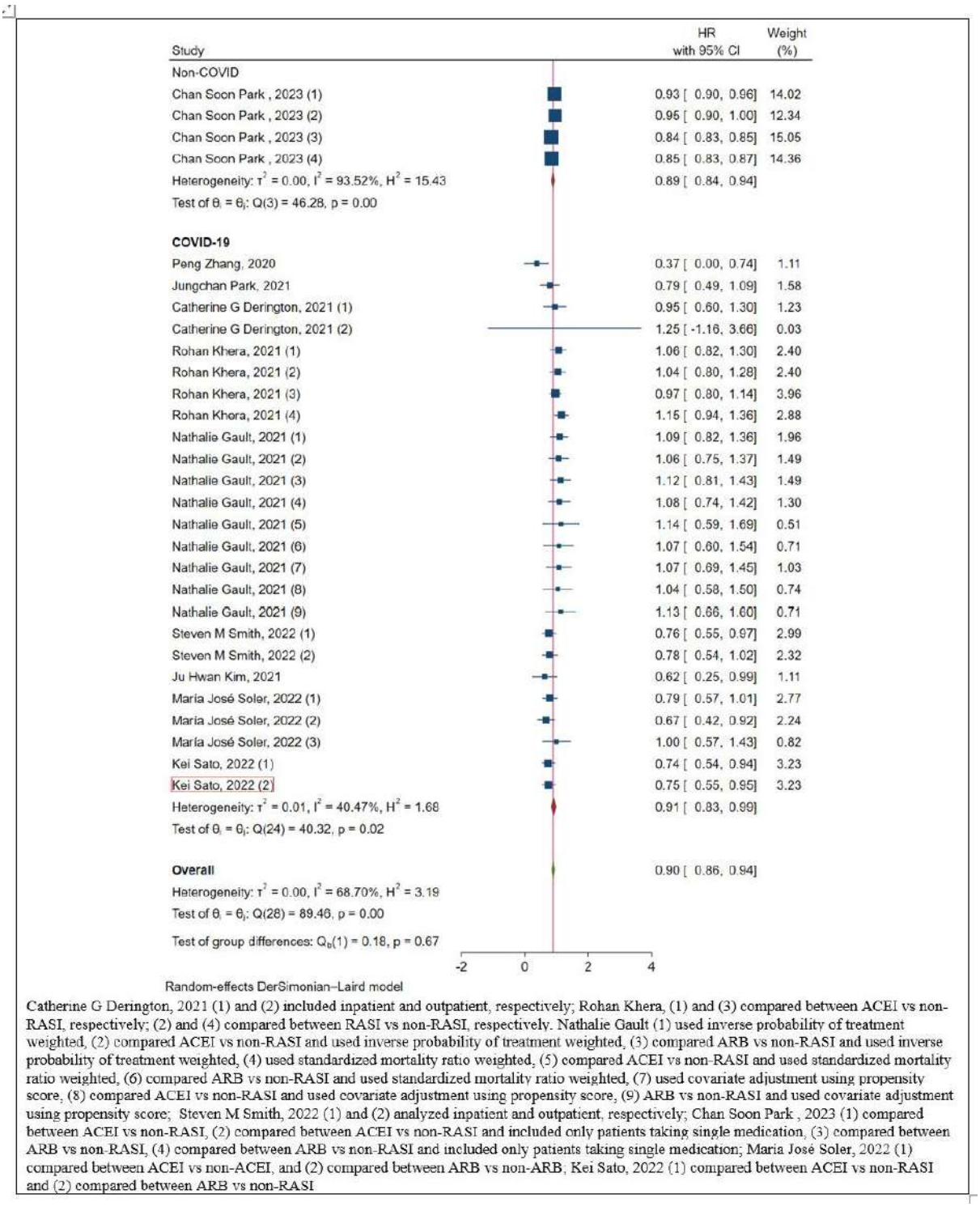
A: Marc A. Suchard, 2019 (1) and (2) compared between diuretic vs ACEI, ARB, respectively, Hack-Lyoung Kim, 2022 (1) and (2) compared between diuretic vs ACEI, ARB, respectively

B: Marc A. Suchard, 2019 (1) and (2) compared between diuretic vs dihydropyridin, non-dihydropyridine CCB, respectively

C: Marc A. Suchard, 2019 (1) and (2) compared between ACEI vs dihydropyridin, non-dihydropyridine CCB respectively, (3) and (4) compared between ARB vs dihydropyridin, non-dihydropyridine CCB respectively

D: Ting-Tse Lin, 2017 (1) and (2) compared between ACEI, ARB vs non-RASI, respectively; Chan Soon Park, 2023 (1) compared between ACEI vs non-RASI, (2) compared between ACEI vs non-RASI and included only patients taking single medication, (3) compared between ARB vs non-RASI, (4) compared between ARB vs non-RASI and included only patients taking single medication

Figure 6S. Pooled HR of myocardial infarction



Catherine G Derington, 2021 (1) and (2) included inpatient and outpatient, respectively; Rohan Khera, (1) and (3) compared between ACEI vs non-RASI, respectively; (2) and (4) compared between RASI vs non-RASI, respectively. Nathalie Gault (1) used inverse probability of treatment weighted, (2) compared ACEI vs non-RASI and used inverse probability of treatment weighted, (3) compared ARB vs non-RASI and used inverse probability of treatment weighted, (4) used standardized mortality ratio weighted, (5) compared ACEI vs non-RASI and used standardized mortality ratio weighted, (6) compared ARB vs non-RASI and used standardized mortality ratio weighted, (7) used covariate adjustment using propensity score, (8) compared ACEI vs non-RASI and used covariate adjustment using propensity score, (9) ARB vs non-RASI and used covariate adjustment using propensity score; Steven M Smith, 2022 (1) and (2) analyzed inpatient and outpatient, respectively; Chan Soon Park , 2023 (1) compared between ACEI vs non-RASI, (2) compared between ACEI vs non-RASI and included only patients taking single medication, (3) compared between ARB vs non-RASI, (4) compared between ARB vs non-RASI and included only patients taking single medication; María José Soler, 2022 (1) compared between ACEI vs non-ACEI, and (2) compared between ARB vs non-ARB; Kei Sato, 2022 (1) compared between ACEI vs non-RASI and (2) compared between ARB vs non-RASI

Figure 7S. Pooled HR of all-cause mortality between hypertensive patients with and without COVID-19

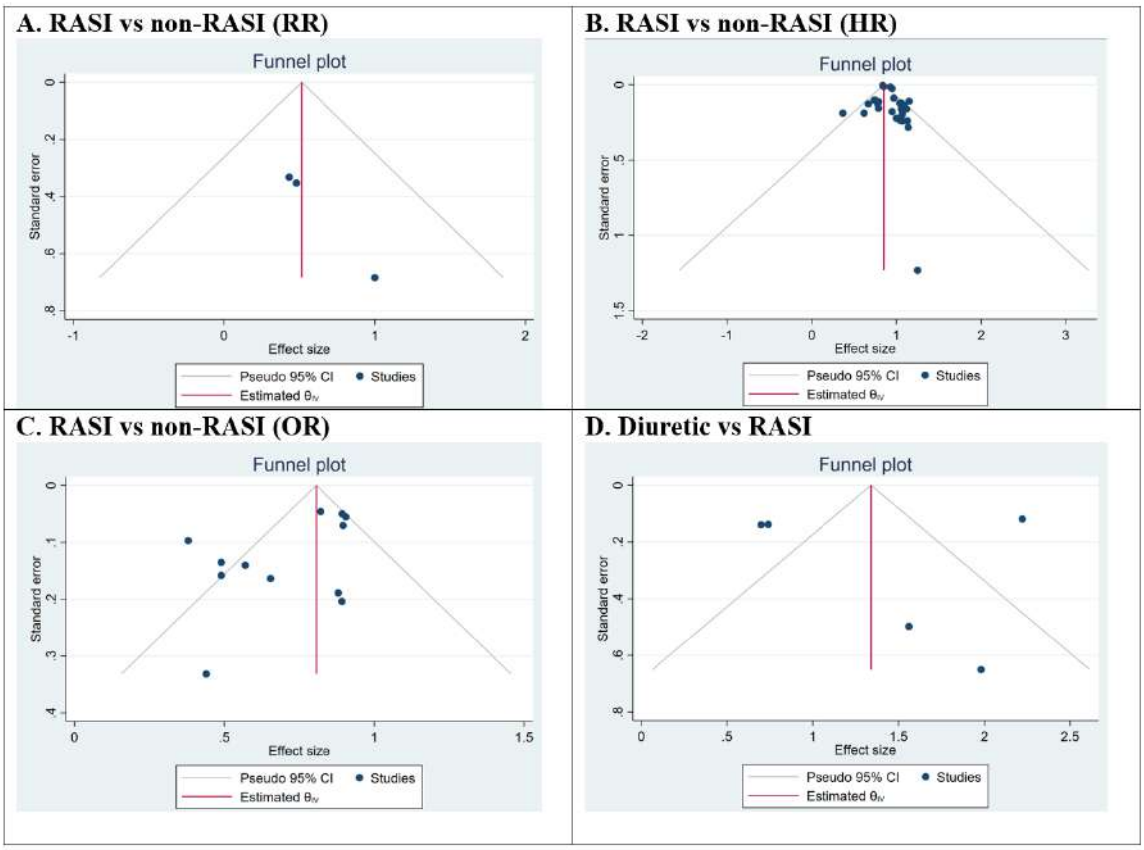


Figure 8S. Funnel plot of all-cause mortality

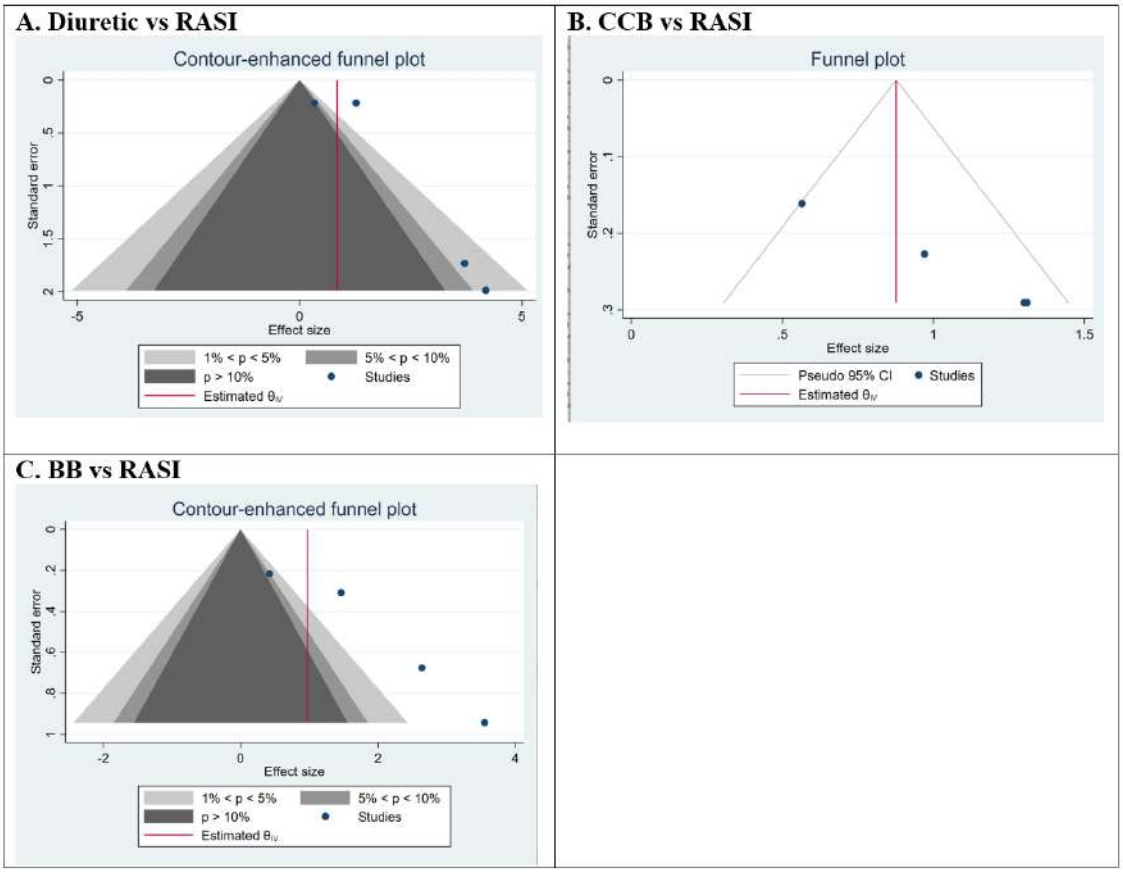


Figure 9S. Funnel plot of heart failure

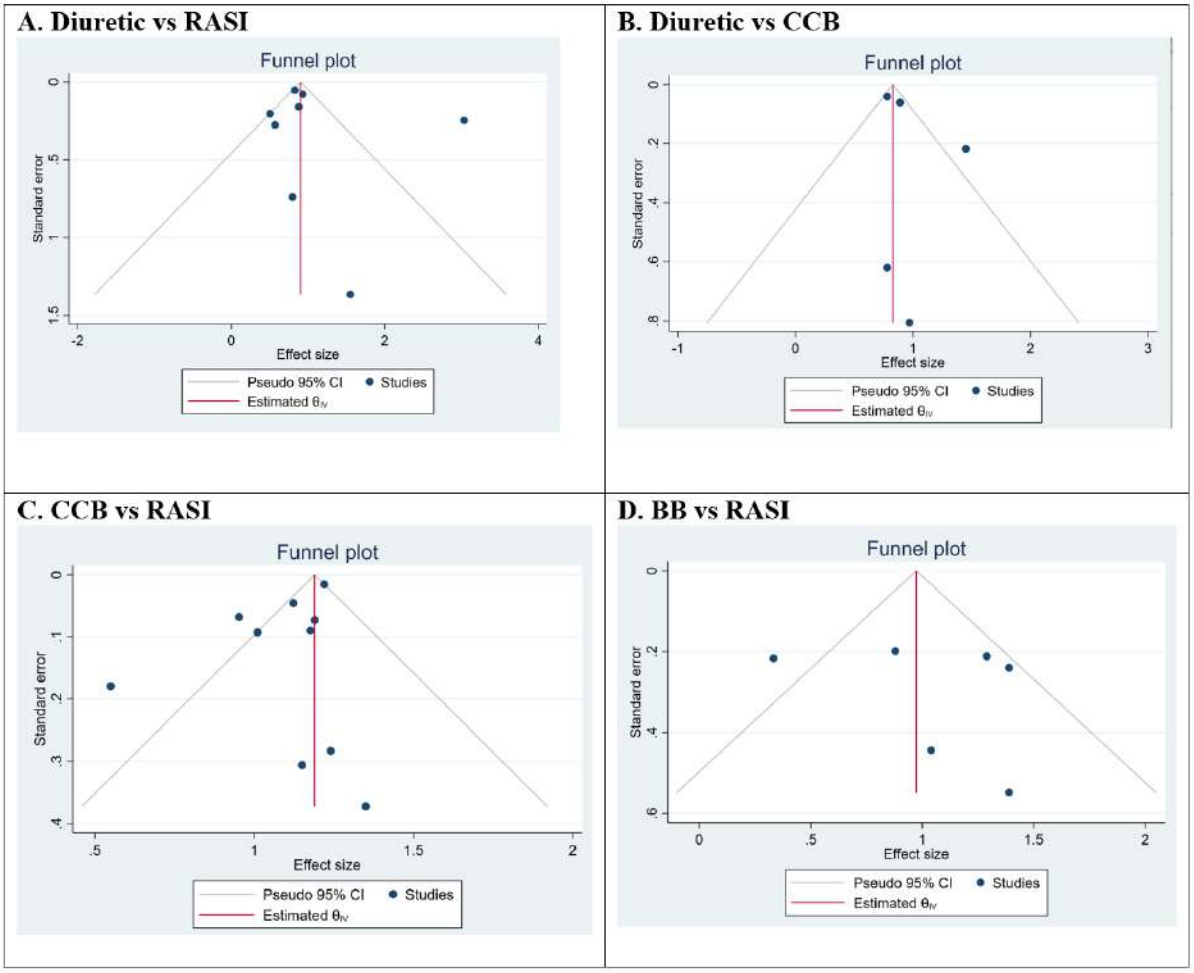


Figure 10S. Funnel plot of stroke

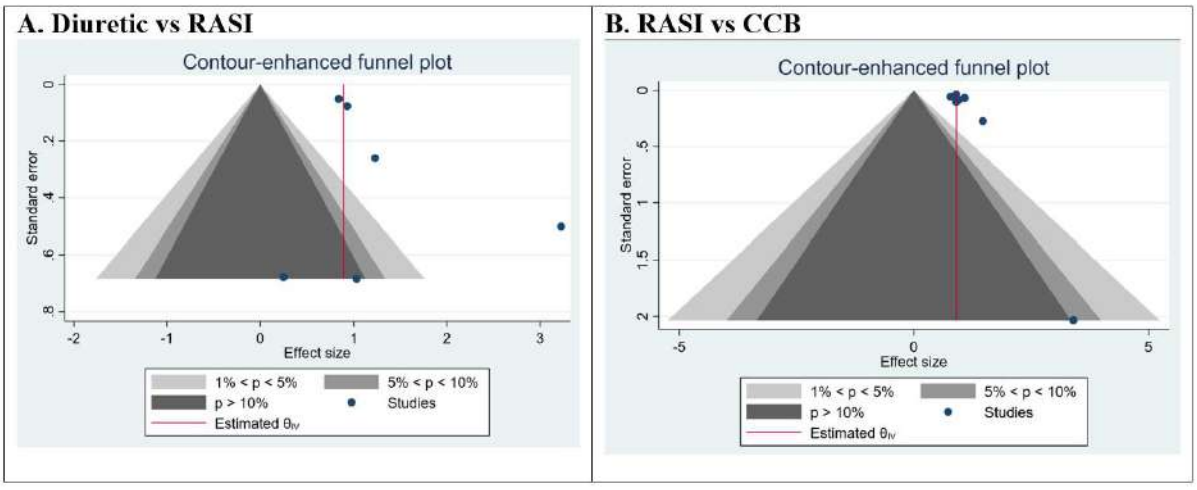


Figure 11S. Funnel plot of myocardial infarction

Pharmaceutical Education & Practice

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Factors Influencing the Score of Core Competency Examination for Pharmacist Licensure among Thai Pharmacy Students at Huachiew Chalermprakiet University

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ABSTRACT

Since 2014, a pharmacist licensure examination in Thailand has been necessary to pass both the core competency exam at the end of the 4th year and the specific competency exam at the end of the 6th year. The Pharmacy Licensure Examination Core Competency 1 (PLE-CC1) is one of the core competency exams. The unpassed PLE-CC1 led to being unable to examine the specific competency exam. This study aims to investigate the factors affecting the PLE-CC1 score and establish a predictive model predicting the PLE-CC1 score of Thai pharmacy students at Huachiew Chalermprakiet University (HCU), which is important for pharmacy students and faculty to plan and prepare for PLE-CC1. This is an analytical study collecting all the data of the students passing PLE-CC1 from the database of the Faculty of Pharmaceutical Sciences at HCU. Factors including gender, age, grade point average (GPAX), grade point average of the pharmacy program (GPA-PP), and frequency of taking PLE-CC1 were analyzed. Statistical analysis was performed by STATA software using descriptive statistics and regression. This study protocol was approved by the Ethic Committee of HCU (No.HCU-EC1380/2566). The PLE-CC1 score of 106 students had the median [interquartile], minimum, and maximum of 67 [9], 60, and 83 scores, respectively. The GPAX, GPA-PP, and frequency of taking PLE-CC1 had significant relationships with PLE-CC1 scores (P-value < 0.05 for all). For the multiple regression analysis, GPA-PP was only a factor significantly influencing the PLE-CC1 scores. The predictive equation was: PLE-CC1 = (8.94 x GPA-PP) + 41.6. Although the minimal GPA-PP is 2.00, which is the graduation requirement, there is a chance of being unable to pass PLE-CC1. The improvement of GPA-PP was the potential key to achieving the pharmacist licensure examination for students to prepare for an examination and for faculty to promote and support their students via the policy.

KEYWORDS: Pharmacist Licensure; Pharmacy Students; Core Competency Examination; Grade Point Average; Thai

1. INTRODUCTION

According to the Pharmacy Professional Act, B.E. 2537, which is the law associating pharmacists in Thailand, no one without pharmacist licensure is prohibited from practicing the pharmaceutical profession in Thailand[1]. In order to obtain pharmacist licensure since 2003, it is necessary to have graduated with a bachelor's degree in the pharmacy program and passed the pharmacist licensure examination[2]. Nevertheless, the Pharmacy Council Announcement No. 17/2556 stated that the pharmacist licensure examination would change to two times, which are the core

competency exam at the end of the 4th year and the specific competency exam at the end of the 6th year. This regulation will begin to apply to those entering the Doctor of Pharmacy (Pharm.D.) program from the academic year 2014 onwards[3].

The Pharmacy Licensure Examination Core Competency 1 (PLE-CC1) is one of the core competency exams. The PLE-CC1 is an examination with multiple-choice questions, and the score of this examination needs to be at least 60% to pass[4]. Therefore, the unpassed PLE-CC1 was disqualified from examining the specific competency exam, which led to being unable to register for pharmacist licensure. Besides, universities both government and private with pharmacy students who graduate but do not pass the pharmacist licensure examination by half may be considered by the pharmacy council to revoke institutional accreditation[5].

The previous study showed that the grade point average was associated with the scores of the pharmacist licensure examination in the era of one-time examinations[6]. The exploration of factors affecting the PLE-CC1 score could help identify ways to improve the PLE-CC1 score. Nonetheless, the pharmacist licensure examination method was changed, and the Thai pharmacy curriculum has also developed accordingly. The Faculty of Pharmaceutical Sciences at Huachiew Chalermprakiet University (HCU) was established along with the founding of the Pharmacy Council of Thailand, and its curriculum has been accredited throughout[7-9]. The Pharm.D. program at HCU, and all universities in Thailand, need to continuously develop and prepare, following the structure of the pharmacy curriculum from the regulations of Pharmacy Council that has been changing. This current situation is different, and the influence of PLE-CC1 has not been investigated. Thus, the objectives of this study were to investigate the factors influencing the PLE-CC1 score and to generate the predictive model of the PLE-CC1 score among Thai pharmacy students at HCU.

2. METHODS

2.1. Study Populations and Data Collection

This analytical study used the data of the students passing PLE-CC1 with their evidence from the database of the Faculty of Pharmaceutical Sciences at HCU. Eligible student information, consisting of the PLE-CC1 score and their characteristics, obtained with consent before they graduated. The correctness of the obtained information is validated by the developed questionnaire re-asking about the PLE-CC1 score and the student number identification for linking their characteristics in the database, reported in the HCU pharmacy project 2024 for graduation[10]. The information in the database identified as the data obtained from the questionnaire was included in the study. The data that missed the PLE-CC1 scores and the student number identification was excluded. The ethical considerations, especially the concern from the Personal Data Protection Act[11, 12], were suggested for revision and determined for approval by the Ethical Committee of HCU and the administrators of the Faculty of Pharmaceutical Sciences at HCU. This research was reviewed and approved by the Ethical Committee of Huachiew Chalermprakiet University, under the approval number HCU-EC1380/2566.

2.2. Data Analysis and Model Evaluation

Statistical analysis was performed by STATA software using descriptive statistics and linear regression. Students' characteristics were determined using descriptive statistics reported in terms of frequencies with percentages for categorical data and means ± standard deviation (SD) or medians [interquartile range: IQR] for continuous data. For regression analysis, the PLE-CC1 scores of individual students were defined as the dependent variable, and factors including gender, age, grade point average (GPAX), grade point average of the pharmacy program (GPA-PP), and frequency of taking PLE-CC1 were tested as covariates of the model by the multiple regression analysis. The statistical power and significance level were set at 0.8 and 0.05, respectively. Consequently, the sample size needs to be at least 100 students for this study[13]. Under the assumption of the least-squares approach with maximum likelihood, the model selection was determined by the P-value, Akaike information criteria (AIC), coefficient of determination (R-square: R^2) or adjusted R^2 (Adj R^2), and error

analysis[13,16]. The model's appropriateness was determined by graphical analysis. The reliability of the final model was assessed by the bootstrapping approach, which performed 1000 replicates under sampling with replacement, providing a 95% confidence interval (95%CI). The external data of fifth-year pharmacy students at HCU who had tested the PLE-CC1 in 2024, obtained from the questionnaire consisting of GPAX and GPA-PP before the test of the PLE-CC1[10], was used to predict the PLE-CC1 score for individual students by the final model. The evaluation of external data was determined by the Pairwise correlation and graphical analysis.

3. RESULTS

3.1. Characteristics

This study included 106 (31%) students from the 338 eligible students in the database, allowing access to their information and answering the questionnaire. All student information had no missing data. One student answered all questions, describing her characteristics such as PLE-CC1 score, gender, date of birth, GPAX, GPA-PP, and frequency of taking PLE-CC1, but she did not define her student identification. This lack of student identification did not affect the data analysis, so this student was also included in this study. All continuous data of characteristics were normally distributed (Shapiro-Wilk W test) and are shown in Table 1 with the categorical data.

For the external data, 33 students consented to provide their PLE-CC1 scores, having the minimum, maximum, mean ± SD, and median [IQR] of 60, 82, 69 ± 5, and 67 [8], respectively. The PLE-CC1 scores were normally distributed (Shapiro-Wilk W test; P-value = 0.25). Their GPAX and GPA-PP had the mean ± SD (minimum, maximum) of 3.18 ± 0.30 (2.62, 3.82) and 2.90 ± 0.39 (2.31, 3.76), respectively.

3.2. The Relationship of PLE-CC1 Scores

The PLE-CC1 scores of the included students had a mean ± SD and median [IQR] of 68 ± 6 and 67 [9], respectively. This study included the students passing PLE-CC1, so the minimal score was 60 while the maximal score was 83. The relationship between PLE-CC1 scores and factors including gender, age, GPAX, GPA-PP, and frequency of taking PLE-CC1 was tested by simple regression. These results are demonstrated in Table 2. The PLE-CC1 scores were significantly associated with GPAX, GPA-PP, and frequency of taking PLE-CC1. The relationship between PLE-CC1 scores and GPA-PP provided the model with the lowest P-value, AIC, and root mean square error (RMSE). According to the maximum likelihood, the model providing the lowest AIC was the most fit. Furthermore, GPA-PP was only a significant factor in the final model after determining the multiple regression analysis. It was transformed into the predictive equation: PLE-CC1 = (8.94 x GPA-PP) + 41.6

Table 1. Characteristics (n=106)

Characteristics		Mean ± SD or Frequencies	[MIN, MAX] or Percentages (%)
Age* (years)		25.44 ± 1.43	[23, 29]
GPAX		3.11 ± 0.30	[2.27, 3.74]
GPA-PP		3.00 ± 0.33	[2.29, 3.71]
Gender			
	Female	87	(82.08)
	Male	19	(17.92)
Track			
	Pharmaceutical care	65	(61.32)
	Industrial pharmacy	41	(38.68)
Type of entrance			
	Admission	56	(52.83)
	Direct Admission	50	(47.17)
Year of entrance			
	2014	6	(5.66)
	2015	19	(17.92)
	2016	26	(24.53)
	2017	30	(28.30)
	2018	25	(23.58)
Reasons of entering			
	By yourself	77	(72.64)
	Not for yourself	29	(27.36)
Habitat			
	Home	6	(5.66)
	On-campus dormitory	10	(9.43)
	Off-campus dormitory	90	(84.91)
Frequency of taking PLE-CC1			
	Once	91	(85.85)
	More than one times	15	(14.15)

GPAX: grade point average; GPA-PP: grade point average of the pharmacy program; PLE-CC1: The Pharmacy Licensure Examination Core Competency 1; SD: standard deviation; MIN: minimal value; MAX: maximal value.
*Age at the time of taking PLE-CC1.

Table 2. Regression analysis for factors affecting PLE-CC1 scores

Factors tested as covariates	Simple regression					Multiple regression
	β	P-value	AIC	R^2	RMSE	P-value
Gender	1.67	0.26	677.07	0.0122	5.84	0.09
Age*	0.36	0.37	677.56	0.0076	5.86	0.99
GPAX	8.94	<0.01	655.82	0.1916	5.29	0.84
GPA-PP	8.53	<0.01	647.57	0.2522	5.08	<0.01
Frequency of taking PLE-CC1	3.43	0.03	673.79	0.0422	5.75	0.23

GPAX: grade point average; GPA-PP: grade point average of the pharmacy program; PLE-CC1: The Pharmacy Licensure Examination Core Competency 1; AIC: Akaike information criteria; R^2: R-square; RMSE: root mean square error; β: Beta coefficient.
*Age at the time of taking PLE-CC1.

3.3. Model Evaluation

The final model was plotted as a fitted plot and a residual plot. The fitted plot demonstrated the linear relationship between PLE-CC1 scores and GPA-PP. The goodness-of-fit plots of the final model are shown in Figure 1. The fitted line of the final model was consistently overlaid with the scattering plot of observed PLE-CC1 scores, and the model fit of the final model was accepted. The residual plot obtained from the residuals and fitted values of the PLE-CC1 scores appeared like a shotgun blast around the zero line of residuals. Thus, the model misspecification was not detected or shown in the systematic trend. Besides, the bootstrapping estimates were comparable to the values of the final model, as demonstrated in Table 3. This result confirmed the reliability of the estimates of the final model.

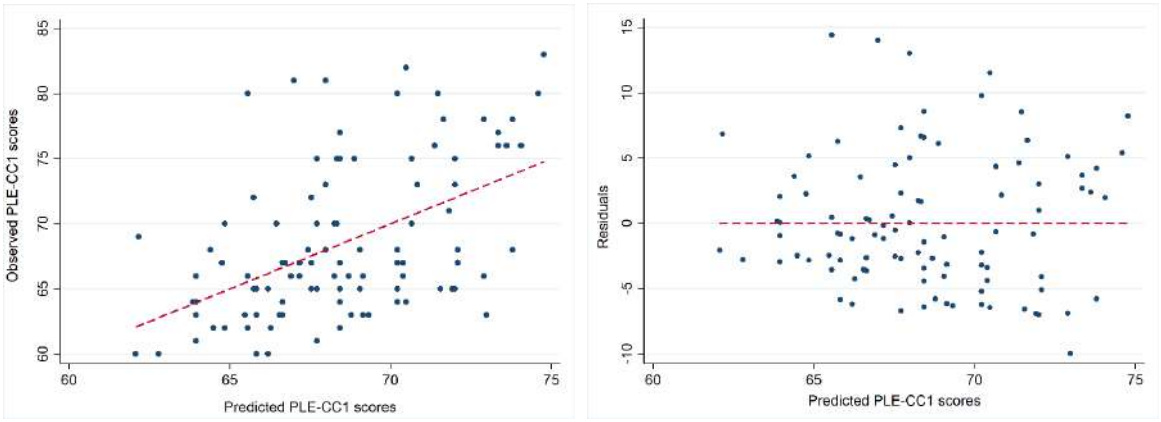

Figure 1. The goodness-of-fit plots of the final model.
[Left] The plot with its fitted dash line shows observed scores versus predicted scores of PLE-CC1.
[Right] The plot with its fitted dash line shows residuals versus predicted PLE-CC1 scores.

Table 3. The estimates of the final model and bootstrapping analyses (1000 replicates)

Parameters	Final model estimates			Bootstrapping estimates	
	B	**Standard error**	**95%CI***	**Standard error**	**95%CI***
GPA-PP	8.94	1.51	5.95, 11.93	1.44	6.12, 11.76
Constant	41.6	4.55	32.57, 50.64	4.23	33.32, 49.89

GPA-PP: grade point average of the pharmacy program; 95%CI: 95% confidence interval; β: Beta coefficient.
*The 95%CI consisted of 2.5th, and 97.5th percentiles.

The students in the external data were predicted to have an individual PLE-CC1 score by using the final model. The minimum, maximum, and mean ± SD of the predicted PLE-CC1 scores were 62, 75, and 67 ± 4, respectively. The correlation coefficient of the predicted PLE-CC1 scores and observed PLE-CC1 scores was 0.5342 (P-value = 0.0014). The external data showed the trend of predictive performance of the final model in the fitted line of the graphical analysis with a positive relationship, as shown in Figure 2.

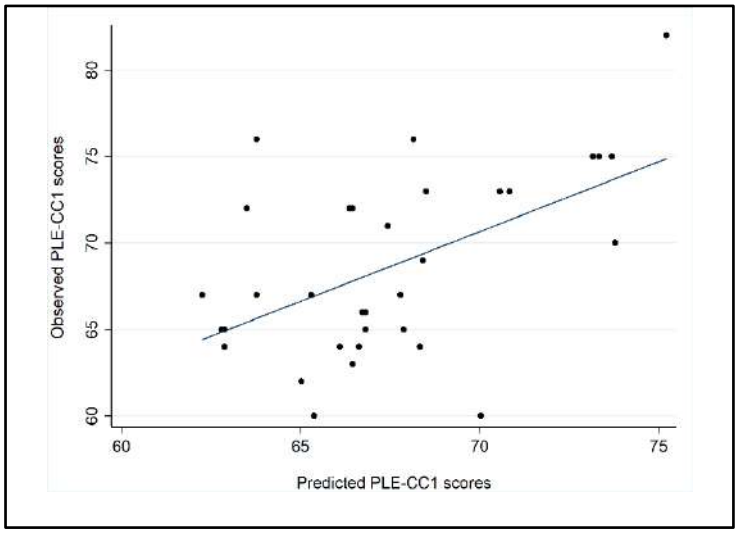

Figure 2. The graphical analysis of the external data

4. DISCUSSION

This is the first study to investigate the factors influencing the PLE-CC1 score with validation and test the predictive performance of the equation at the point of the twice-time examinations for pharmacist licensure. For characteristic results, this study was extremely different from a previous study investigating the once-time examination for pharmacist licensure[6]. The number of students studying the Pharm.D. program from the previous study was lower than ours: 3 (5.2%) versus 106 (100%), respectively. This change was following the paradigm shift in the pharmacy curriculum of Thailand, which changed the Bachelor of Pharmacy (B.Pharm.) to the Pharm.D. program[17, 18]. The Pharm.D. program had an extra year of professional clerkship. This development is expected to improve the pharmacist's competencies, learned through real-world work. The Pharm.D. students were assessed, and their extra-year performances were represented by the clerkship grade point average (CKGPA). The CKGPA was calculated into the GPA-PP[17-20]. Moreover, the results from the previous study, investigating predominantly B.Pharm. students, showed the GPAX affecting the multiple-choice question of the once-time examination for pharmacist licensure. Therefore, the GPA-PP, which was not generally reported in the B.Pharm. program, was masked in the GPAX, influencing the pharmacy licensure examinations[6]. The pharmacy licensure examinations, both once-time and twice-time, were the assessment after passing all program learning outcomes and verification of the pharmacy student, achieving the minimal requirement of the pharmacist's competencies, whereas the PLE-CC1 was the assessment after assessing fourth-year learning outcomes and verification of the pharmacy student, achieving the core competency for pharmacists[3, 17, 18, 21, 22].

The GPAX is one of the quantitative assessments of student academic performance following all program learning outcomes. It consisted of the GPA-PP, grade points for general subjects (required over 30 credits), grade points for basic subjects for the pharmacy profession (required over 30 credits), and grade points for elective subjects (required over 6 credits)[5, 19, 20]. The results in the univariate analysis of our study were similar to several studies among physical therapy, nursing, and medical students, which found that the GPAX correlated with the score of their licensure examination[23-25]. Not only GPAX and GPA-PP, but also the frequency of taking PLE-CC1, related positively to the PLE-CC1 scores in univariate analysis. This result was consistent with other studies, where more frequent examinations contributed to their achievement[26-28]. However, that was the mathematical and statistical determination. In fact, everyone needs to pass their licensure examination for the first time. The analysis of PLE-CC1 scores at only the first attempt was concerned by ethical considerations in this study protocol, and the students who took the PLE-CC1 more than once did not report their PLE-CC1 scores on previous attempts. There might be a reporting bias in our study by selecting only students passing PLE-CC1. For the multiple regression analysis, the frequency of taking PLE-CC1 was not significant for PLE-CC1 scores. The second chance or more for the examination attempts did not improve the PLE-

CC1 scores in the final model. There was only the GPA-PP significantly influencing the PLE-CC1 scores. The R^2 and Adj R^2 of the final model were 0.2522 and 0.2450, respectively. The GPA-PP was the grade calculated directly from the specific subjects for the pharmacy profession[19], such as pharmacology, pharmacotherapy, biopharmaceutics and pharmacokinetics, pharmacy administration, pharmaceutical care, pharmaceutical quality, pharmaceutical technology, pharmaceutical analysis, industrial pharmacy, consumer protection in health products, pharmacy orientation, public health pharmacy, pharmacognosy, research methodology for pharmaceutical sciences, pharmaceutical jurisprudence and ethics, etc. Perception of these subjects' knowledge was the core competency for pharmacists[20, 22, 29-31]. The multiple-choice questions (MCQ) for pharmacy licensure examinations, both the PLE-CC1 and specific competency exam, aimed to predominantly assess the competencies in the cognitive domain of pharmacy students, according to Bloom's taxonomy[30, 32-34], while the objective structured pharmaceutical examinations (OSPE) for pharmacy licensure examinations, which were practical examinations, aimed to assess the competencies in the psychomotor domain[21]. Parallelly, the GPAX included general subjects and elective subjects in the grading calculation. For instance[19], psychology for living, Chinese studies, IT and learning, introduction to statistic, Thai and communication, English for communication, ASEAN studies, leadership and management, and logic reasoning for daily were classified as the general subjects, while music life and society, Chinese philosophy, introduction to business, and academic report writing were classified as the elective subjects. The general and elective subjects endeavored to manifestly improve university learning outcomes in the affective domain, which was not directly assessed by the pharmacy licensure examinations[21]. Thus, the GPA-PP more directly reflected the student's educational performance on competencies for pharmacists than the GPAX.

Females were the major population in the previous study[6]. Our study confirmed that pharmacy students were more likely to be female, with a female-to-male ratio of approximately 80:20. In spite of gender, which was the significant covariate in a previous study among pharmacy students[6], a decade later, our study did not find the significance of this influence, and in-house sub-analysis informed us that the GPA-PP and GPAX were also not affected by gender (T-test, p-value = 0.50 and 0.09, respectively). This finding showed that gender was not a barrier or a condition for the assessment of pharmaceutical sciences education.

The factor of age was not found as a covariate for the score of PLE-CC1 or pharmacy licensure examinations[6]. The pharmacy students in our study had various ages, caused by those who changed the studying program from other universities or faculties and those who had already graduated from other programs. This variation in age might affect educational achievement[35], but this assumption was not affirmed by our study. The route to the licensure examinations was screened and censored by the assessment, followed by the year-level learning outcomes. This result confirmed that age was not a restriction on studying pharmacy education and supported the concept of lifelong learning[18, 36, 37].

Other characteristics were not tested as covariates because the clue of influence was not apparent, leading to an extravagant sample size for testing[13]. Particularly, the study track, which consisted of pharmaceutical care and industrial pharmacy, should not be tested as a covariate because PLE-CC1 was the event that occurred before track selection. However, the analysis with lower statistical power for other characteristics was performed in-house to visualize the trend of the relationship, which indicated that none of the untested characteristics had a relationship with the PLE-CC1 scores.

This study predominantly determined the most fit model by the lowest AIC, which detailed the multi-model determination of both unreported nonlinear and linear functions. The nonlinear function from our data could not be detected during the modeling. Therefore, the linear regression approach was accepted for modeling in this study. After accepting that the model could be the linear function during modeling, the p-value was also determined for the linear functions. Other covariates did not improve the model fit. The final model for prediction of PLE-CC1 scores had GPA-PP in the equation and could explain that every 0.1-point GPA-PP increment or decrement increased or decreased the PLE-CC1 scores by 0.9, respectively. The minimal GPA-PP requirement for graduation at HCU was at least 2.00, used to predict the PLE-CC1 score, and the result from this value of GPA-PP was 59. This predicted value, with its residuals from the predictive equation, might lead to a chance of unpassing PLE-CC1.

It should be noted that this study had some students who were unable to pass some subjects and did not report their grades on previous attempts. Those who have studied until they finish the fourth year of the Pharm.D. program could take the PLE-CC1, according to the announcement from the Pharmacy License Examination Center[4]. The collection of GPA-PP before PLE-CC1 was limited by the unknowns of the unpassed subjects of the students before they took PLE-CC1. For this reason, the data on students' GPA-PP were collected at graduation, but the PLE-CC1 occurred at the end of the fourth year[22]. This might question the logic of the influence of GPA-PP on PLE-CC1. The CKGPA and grades from other specific subjects for the pharmacy profession were assembled into the GPA-PP in this study. The external data were used to check the predictive performance of the final model and the extrapolation of their GPA-PP before taking the PLE-CC1 in the final model[15, 16], which could be substituted by their GPA-PP at graduation for predicting the PLE-CC1 scores. For the bootstrapping analyses, the confidence intervals with standard errors for parameters were checked using the nonparametric technique. The bootstrapping estimates were built on the assumption of an undesignated distribution. They represented the events when the model was applied to several situations to confirm the reliability of the final model.

The results of this study could be applied to several dimensions. For the pharmacy students, the increment in GPA-PP not only indicated academic excellence but also was a predictor of the trend of PLE-CC1 achievement. They could prioritize their study balance and focus on engaging in targeted topics that were specific subjects for the pharmacy profession[22]. The effort for these subjects fortified their fundamental knowledge and honed critical thinking skills essential for navigating the complexities of the PLE-CC1. For the faculty of pharmaceutical sciences, faculty staff were pivotal in fostering an environment and other components conducive to academic achievement and the readiness for PLE-CC1. The strategies might be to provide students with the necessary resources, guidance, or encouragement to transcend in their educational pursuits[38]. The administrators of faculty might use the results of this study to determine curriculum enhancements, announce the policy for the provision of academic support services, and establish specific programs tailored for PLE-CC1 preparation. For example, general subjects could develop and focus on application in pharmaceutical sciences, tailored for pharmacy students, and elective subjects for pharmacy students might be designed to specifically support and complement pharmacy knowledge in core competencies. When trends in student characteristics change, the staff of the faculty of pharmaceutical sciences should communicate and coordinate with pharmacy students to find neutral exits. For the Pharmacy Council and Pharmacy Education Consortium of Thailand, the regulatory institutes might announce the changed regulations of the developed structure of the pharmacy curriculum, suiting the need. To illustrate, they might rule to reduce the proportion of subjects not related to pharmacy[20, 29, 39], resulting in pharmacy students increasing their pharmacy competencies. Additionally, collaboration between universities and regulatory institutes could be strengthened to ensure alignment between educational preparation and licensure examination expectations, ultimately helping to have more robust and competent pharmacists.

This study had several limitations. Previously, the scores of pharmacy licensure examinations were freely published in the Pharmacy License Examination Center[40]. Anyone could access the students' scores by matching the name with their identification number in the announcement of test venues and the testing score results, which simultaneously demonstrated their identification number. After 2022, the individual PLE-CC1 scores, other pharmacy licensure examination scores, GPA-PP, and GPAX could not be freely reached, according to the regulation of the Personal Data Protection Act[11, 12]. In consequence, the analysis and reporting of the unpassed scores of PLE-CC1 or GPA-PP for this study were prohibited. However, all authors were interested in the influences, and focused on the route to pass PLE-CC1 more than the higher or lower scores, because the pharmacists who have passed all pharmacy licensure examinations had equal honor and dignity, although they had different PLE-CC1 scores. Secondly, the participants in external data were sparse. The use of other statistical approaches had limitations for analysis[13], and this study procedure could only investigate correlation and trend viewing. Thirdly, the application of these study results for both internal and external use should be cautioned. For internal use, the final model was modeled from the data from the students studying in the previous pharmacy curriculum, which was different from the current program for pharmacy students[9, 39]. For external use, this study performed only one site, HCU, which could not

directly imply other settings. The characteristics and influences might differ in internal and external use. Finally, this study investigated only PLE-CC1. The Pharmacy Licensure Examination Pharmaceutical Care 1 (PLE-PC1), which was the specific competency exam for pharmacist licensure at graduation, was also assessed by MCQ. The application of our study results to PLE-PC1 might be implied with concern. The CKGPA might greatly affect the PLE-PC1, leading to a different predictive equation. Conversely, the influence of the OSPE on the pharmacy licensure examination for core competency (PLE-CC2) or for specific competency (PLE-PC2) could not be implied by these study results[3, 17, 21], due to their classification into different domains. The factors affecting the PLE-CC2 and PLE-PC2 need further investigation. Moreover, the interaction between domains should be explored in further study. The improvement of the psychomotor domain or affective domain might increase the outcomes of the cognitive domain.

5. CONCLUSION

The GPA-PP was the factor affecting the PLE-CC1 score among the Thai pharmacy students at HCU, which could be shown as the predictive equation of PLE-CC1 = (8.94 x GPA-PP) + 41.6. The improvement of GPA-PP could lead to achieving the Core Competency Examination for Pharmacist Licensure. This finding helps the pharmacy students prepare and plan for the examination and assists the faculty in determining the policy or any intervention to support their students.

6. ACKNOWLEDGMENT

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Conflict of interest

The authors declare that they have no conflict of interest.

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Developing International Pharmaceutical Industrial Training in Thailand

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ABSTRACT

The Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo and College of Pharmacy, Rangsit University have signed the agreement to collaborate research and pharmacy education. International pharmaceutical industrial training is developed and encourages students to participate. This is a report of training journey as a preceptor. The training aimed to develop pharmaceutical industry competency for pharmacy students. Six weeks training was organized and planned. The learning activities were scientific knowledge including pharmaceutics, pharmaceutical technology, quality control, and analytical techniques. Students and the preceptor discussed and planned for learning activities to fulfil students' expectations. From 2016 to 2024, there were eleven US pharmacy students engaged in this program. The activities guided students to develop required competency for pharmaceutical industry, for example, project management, teamwork, problem solving and communication skills. In addition, manufacturing visits were arranged for the students. By the end of training program, students gained laboratory skills and experience in formulation development and evaluation. Assessment was performed for both students and the preceptor through web-based evaluation form. The training program also expand viewpoints in pharmacy areas for students and teaching and coaching skills for the preceptor.

KEYWORDS: International pharmaceutical industrial experience; Pharmacy competency; Project-based learning

1. INTRODUCTION

Professional training rotation is required in pharmacy curriculum for Thai students as well as US students. The Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo have signed a memorandum of understanding (MOU) with College of Pharmacy, Rangsit University (RSU) since 2013. The activities were started with student exchange program (in bound US to Thailand). Since doctor of pharmacy degree (Pharm D.) was patient-oriented and focus on clinical care, the international pharmaceutical industrial rotation has broadened US pharmacy students' experience in non-patient care electives. Pharmacy students had learned formulation development, obtained manufacturing experience, including appreciated for different cultures. In addition, the program also strengthened the preceptor in training management.

Competency for pharmaceutical technology training requires combination of scientific knowledge, technical skills and regulatory compliance[1]. Scientific knowledge included principles of drug formulation, drug delivery systems, pharmacokinetics, and pharmacodynamics. Pharmacy students studied this knowledge through various courses depending on their program in the university. The technical skills focused on hand-on experience with equipment used in pharmaceutical research and development including skills in chromatography and spectroscopy which are crucial for quality control of pharmaceutical products. As a role of an academic preceptor, we used project-based learning

(PBL) approach which focused on drug formulation and development. This required the understanding of drug formulation techniques, various drug dosage forms, pre-formulation studies, formulation optimization, and stability testing under pharmaceutical regulations and guidelines issued by regulatory authorities such as Food and Drug Administration (FDA) in the United States compared with in Thailand. In addition, compliance with Good Manufacturing Practice (GMP) is also essential to ensure the safety, quality and efficacy of pharmaceutical products.

PBL approach was assigned students to engage in problems or questions that were real-world relevance[2]. Students worked on interdisciplinary collaboration, literature search, problem-solving and communication skills. Students worked together in groups to brainstorm ideas, divide tasks, and hand on the experiment. Students were promoted to develop critical thinking skills as they analyzed information, evaluated options, and made decisions to solve problems encountered during the project.

2. METHODS

2.1. Training Plan

The rotation schedule was planned by College of Pharmacy, University of Hawaii at Hilo and arranged the coordinator for handling the document. College of Pharmacy, Rangsit University (RSU) as a host country developed key personnel to work with international students. Six-week training was based on students interests and time available. The learning activities for each week were planned (Table 1).

Table 1. Learning plan for pharmaceutical industrial training.

Week	Learning topics	Competency requirements
1	- Orientation - Laboratory safety - Search for information from library database - Pharmacopeia - Instrument techniques	- Literature search - Laboratory skills
2	- Raw material identification - Qualitative and quantitative analysis of raw materials	- Quality control and pharmaceutical analysis
3	- Pre-formulation study - Design of experiment	- Pharmaceutics and research and development skills
4	- Formulation development - In-process control - Stability study	- Pharmaceutical technology - Problem solving abilities
5	- Formulation development (cont.) - Formulation evaluation - Gather all results and discussion - Statistical analysis	- Analytical skills - Team management
6	- Regulations - Manufacturing visits - Presentation	- Quality management and regulatory compliance - Communication skills

2.2. Learning Activities

The activities were included laboratory safety and skills, assignment for the project, visiting manufacturing and presentation. The topics were instrument techniques, formulation development, formulation evaluation, and regulatory affairs. Students worked together with Thai students. Students were encouraged to set up their goals for the training and the preceptor guided them through the activities to fulfil their expectations.

2.3. Assessment

The preceptor evaluated students through web-based evaluation form both midpoint and final evaluation. Students were evaluated in the professionalisms, attitude, behaviors, ethics, responsibility, communication abilities, literature search, laboratory skill, and scientific knowledge. The evaluation score was ranged from unsatisfactory to excellent in four levels. The presentation of the student was evaluated in four level also and in the category of presentation content and organization, review and application of literature, presentation style and ability to ask and answer questions. On the contrary, students gave feedback and shared their opinions to the preceptor. Student evaluation of the preceptor and site was categorized into 5 Likert scales from strongly disagree to strongly agree.

3. RESULTS AND DISCUSSION

3.1. Learning Activities

There have been eleven US pharmacy students attended the exchange program since 2016. There was a recess during 2020 to 2023 due to pandemic of coronavirus. The students were 5 male and 6 female and in the age 25-28 years old. The ethnicity of the students was American, African American and Asian American. Learning plan was developed and organized with RSU faculty members as the preceptor. The learning activities were planned as showed in Table 1. However, the schedule was flexible depending on students' goals and other circumstances. By the end of the training (week 5 or 6), the preceptor arranged manufacturing visit for students to obtain industry experience. During the past 8 years, we visited pilot plant, local traditional medicine company, globalized cosmetic and dietary supplement manufacturing.

Students planned the experiments, gained laboratory skills, understand the principles of pharmaceutical technology. Students had the opportunity to attend the international conference that RSU was a host in year 2017. Students visited Thailand Herb Expo 2019 and Thailand Inventors' Day 2024. In addition, students were encouraged to participate in social activities and sightseeing. Students had experience to work with different cultures and see a different side of pharmacy.

The rubric evaluation form assessed students' performance for mid- and end-point evaluation to demonstrate personal and professional growth. The preceptor gave formative evaluation to ensure that students had appropriate advice to make progress on their experiment and project. The summative assessment included an oral presentation to the preceptor and to the home institution and wrote one-page abstract.

Table 2. Student evaluation of preceptors.

Evaluation items	Mean	SD
The preceptor clearly stated the objectives, expectations and requirement of the rotation.	4.50	0.84
The preceptor was available for consultation with me in a regular and timely manner.	4.67	0.52
Keeping in mind the work responsibilities of the preceptor, I felt the preceptor spent adequate time with me.	4.67	0.52
The preceptor was courteous and respectful to students, patients and other health care professionals.	4.83	0.41
The preceptor was enthusiastic about teaching.	4.67	0.52
The preceptor was concerned about what I was learning.	4.50	0.84
The preceptor encouraged self-directed learning.	4.67	0.52
The preceptor asked me thought-provoking questions.	4.67	0.52
The preceptor provided opportunities for me to ask questions.	4.67	0.52
The preceptor provided feedback on my progress in a timely manner.	4.50	0.84
The preceptor provided feedback in a supportive and constructive manner.	4.67	0.52
The overall quality of the preceptor was:	4.80	0.45

3.2. Reflection from Students

Eleven students who completed the training, only six of them shared their responses as anonymous using evaluation form designed by University of Hawaii at Hilo through web-based system. Overall quality of the preceptor was very good. During training schedule, all students met toward their expectations. From students' opinion, the preceptor was willing to explain concepts and clarify anything that they did not understand. For site evaluation there were adequate learning resources and equipment although students needed to share some glassware and laboratory equipment. Suggestions from the students were shared in a form of communication between students and the preceptor such as a group chat or Line application to communicate questions and concerns.

Table 3. Student evaluation of site.

Evaluation items	Mean	SD
This site demonstrates a commitment to the education of pharmacy students.	4.83	0.41
At this site, there was adequate access to learning and information resources for the student.	4.67	0.52
At this site, there was enough equipment and technology to enhance/facilitate the education environment for the student.	4.50	0.55

4. CONCLUSION

To develop international pharmaceutical industrial training focused on advanced pharmacy practice experience (APPE) required three main partners; pharmacy students (home institution), preceptor (host), and manufacturing[3]. Since the preceptor site was research laboratory in the academic institution, the training schedule collaborated with pilot plant at RSU. Research at the preceptor site focused on medicinal plants, thus the training project was relevant to formulation development and quality control of traditional medicine and dietary supplements. This international APPE exchange program helped students to gain competency, experience, and perspective of pharmacy. The program also promoted international relations and pharmacy education.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Grit Matters?: Exploring the Correlation Between Grit Levels and Pharmacy License Exam Success

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ABSTRACT

Academic excellence and resilience are crucial in the demanding field of pharmacy. Grit, defined as perseverance and passion for long-term goals, has been shown to influence achievement. However, the relationship between grit levels and licensure exam performance among Thai pharmacy students remains unexplored. This study was to assess grit levels and investigate the association between grit components (perseverance and passion) and licensure exam performance among Thai pharmacy students eligible for the 2024 exam at Burapha University. This observational study utilized an online survey of pharmacy students at Burapha University who were eligible for the 2024 licensure exam. The pre-survey consisted of 21 items across three sections: Grit (10 items), Preparedness (5 items), and Demographics (6 items). The post-survey had only one question regarding their exam result (Pass or Fail). Data were analyzed using SPSS version 26. Institutional review board approval was obtained for this study. Most respondents (n=23) were female (78.26%), in the pharmaceutical care track (56.52%), sixth-year students (95.65%), with a cumulative GPA of 2.50-2.99 (47.83%) and previous semester GPA of 3.50-4.00 (69.57%). The average grit score was 3.29 (SD=0.58), higher than 30% of referenced adults, with passion at 3.10 (SD=0.55) and perseverance at 3.48 (SD=0.76). The logistic regression model predicting exam success included passion and perseverance as predictors, demonstrated good fit (Hosmer-Lemeshow p=0.99), and explained 42% of variance (Nagelkerke R2=0.42). Perseverance was positively associated with passing (B=2.84, SE=1.45, Wald=3.84, p=0.05), suggesting a 17-fold increase in odds for each one-unit increase. This study provides valuable insights for pharmacy educators and students, emphasizing the crucial role of perseverance in achieving academic excellence and success in licensure exams. By fostering perseverance and other components of grit, pharmacy programs can better equip students with the resilience and determination necessary to navigate the demanding field of pharmacy.

KEYWORDS: Grit; Pharmacy Students; Licensure Exam; Thailand

1. INTRODUCTION

The pharmacy profession demands not only academic excellence but also a resilient and determined mindset to overcome its inherent challenges. Grit, defined as perseverance and passion for long-term goals, has emerged as a key psychological factor influencing academic achievement and professional success across various domains[1]. Angela Duckworth's seminal work on grit as a predictor of academic performance has sparked significant interest in understanding the role of perseverance and passion in educational settings. Numerous studies have consistently shown that grit, characterized by a combination of perseverance and passion, plays a crucial role in predicting success beyond traditional measures of intelligence or talent[2,3,4].

Within healthcare education, investigations into the relationship between grit and academic performance have gained traction. Studies in medical education suggest a positive correlation between grit and academic achievement, with grittier medical students demonstrating higher resilience and success in licensure examinations[5,6]. However, limited research has specifically examined this association within the context of pharmacy education and licensure exams. Pharmacy licensure exams, such as the Thai Pharmacy Council License Exam, serve as crucial assessments determining entry into the profession. These exams require not only extensive subject knowledge but also the ability to persist through rigorous preparation and examination challenges.

Previous research has demonstrated the effectiveness of the grit scale in identifying factors influencing student outcomes in pharmacy programs[7,8]. Consistently, these studies show that students with higher grit achieve greater academic success, evidenced by higher GPAs, increased retention rates, and fewer career changes[2]. However, research also suggests that grit is dynamic and can be influenced by various individual factors such as gender, age, marital status, year of study, family income, family history of attending university, family relationships, and motivations for pursuing pharmacy[2,7,9,10].

Despite the acknowledged significance of grit in academic achievement and its demonstrated influence in healthcare education, there remains a gap in understanding its specific impact on pharmacy students' success in licensure exams, particularly within the Thai educational context. Investigating the levels of grit among Thai pharmacy students and its potential correlation with their performance in the pharmacy license exam could provide valuable insights into the factors influencing success in this critical assessment.

This study seeks to bridge this gap by exploring the relationship between grit and success in pharmacy license exams among Thai pharmacy students. It aims to contribute empirical evidence to the existing literature by examining the levels of grit in this specific cohort and investigating the association between grit components (perseverance and passion) and their performance in licensure examinations.

2. MATERIALS AND METHODS

2.1. Population and Samples

The study population consisted of pharmacy students at Burapha University who were eligible to take the licensure examination in 2024. A convenience sampling technique was employed, with voluntary participation from eligible students.

2.2. Study Instrument

This study consisted of two surveys. The first, a pre-survey, was an adapted version of Duckworth's (2009) previously published Short Grit Scale (Grit-S), with the addition of one question for passion ("My interests change from year to year") and another for perseverance ("I have overcome setbacks to conquer an important challenge"). In addition to the grit items, the questionnaire gathered demographic and academic information from participants, including gender, year of study, academic track, cumulative GPA, previous semester GPA, and reasons for choosing the pharmacy program. The second survey, the post-survey, had only one question regarding their exam result (pass or fail). Face validity was established through faculty review, with revisions made to improve appropriateness as needed. Three experts evaluated the content validity, yielding an acceptable content validity index (CVI) of 0.97. Internal consistency reliability was assessed through a try-out with 30 non-sample participants, calculating Cronbach's alpha (0.72).

2.3. Data Collection and Analysis

Research assistants shared the survey link (Google Forms) with eligible participants before their license exam, scheduled from March 22 to 24, 2024 (pre-surveys were distributed between March 1 and 15, 2024), and after the exam result announcement on April 26, 2024 (post-surveys were distributed between April 27 and May 7, 2024). Upon survey completion, the responses underwent a data completeness check. Descriptive and correlation analyses were then performed using SPSS version

26. The study was reviewed under Exemption Determination, project number HS031/2567. Overall, the outcomes of this study included exploring the concept of grit and its relationship with licensure exam success using the adapted version of the Grit Scale.

3. RESULTS AND DISCUSSION

3.1. Participants' Characteristics

Out of 178 eligible pharmacy students, 23 students (response rate = 12.92%) responded to both the pre- and post-surveys. The majority were female (78.26%), enrolled in the pharmaceutical care track (56.52%), and sixth-year students (95.65%), with one student reporting "Other" for their academic year. Most had a cumulative GPA of 2.50-2.99 (47.83%) and a previous semester GPA of 3.50-4.00 (69.57%). The students' reasons for choosing the pharmacy program were presented as shown in Table 1.

Table 1. Reasons Participants Chose the Pharmacy Program

Reasons	N	Percent
Family/friends recommendation	7	30.43%
Only available/reasonable choice	8	34.78%
Interest/passion	7	30.43%
Others	1	4.35%

3.2 Grit Levels and Its Association with the Licensure Exam Success

The average grit score among respondents was 3.29 (SD=0.58), higher than 30% of referenced adults[5]. The passion component had a mean score of 3.10 (SD=0.55), while the perseverance component had a mean of 3.48 (SD=0.76). An average score of each grit assessment item is presented in Table 2.

The results of the analyses are detailed in a report outlining the opportunities identified. For the logistic regression analysis, the model included the two components of grit: passion and perseverance. These variables were selected to examine their association with the outcome variable, which was success in the licensure exam (pass or fail). The odds ratio effectively indicates the likelihood of passing the licensure exam under specific conditions. The logistic regression model, logit(P) = -7.51 - 1.67(Passion) + 2.84(Perseverance), demonstrated a good fit (Hosmer-Lemeshow p=0.99) and explained 42% of the variance in exam outcomes (Nagelkerke R^2=0.42). Perseverance was positively associated with passing the licensure exam (B=2.84, SE=1.45, Wald=3.84, p=0.05), suggesting a 17-fold increase in the odds of passing for each one-unit increase in the perseverance score. Conversely, passion had a negative association with exam success (B=-1.67, SE=1.82, Wald=0.84, p=0.36).

Table 2. Grit Assessment Survey Questions

Item	Mean	SD
1. New ideas and projects sometimes distract me from previous ones.	3.00	0.85
2. Setbacks don't discourage me. I don't give up easily.	3.57	1.20
3. I often set a goal but later choose to pursue a different one.	3.26	0.96
4. I am a hard worker.	3.35	0.98
5. I have difficulty maintaining my focus on projects that take more than a few months to complete.	3.13	1.22
6. I finish whatever I begin.	3.83	0.83
7. My interests change from year to year.	2.87	0.97
8. I am diligent. I never give up.	3.26	1.05
9. I have been obsessed with a certain idea or project for a short time but later lost interest.	3.26	0.92
10. I have overcome setbacks to conquer an important challenge.	3.39	0.89

Note: Items 1, 3, 5, 7, and 9 correspond to passion, while items 2, 4, 6, 8, and 10 correspond to perseverance.

3.3. Discussion

The findings of this study highlight the crucial role of perseverance, a key component of grit, in predicting success in the pharmacy licensure examination. Students who exhibited higher levels of perseverance were significantly more likely to pass the exam, underscoring the importance of fostering this trait in pharmacy education.

The positive association between perseverance and exam performance aligns with previous research in healthcare and other academic domains, which has consistently demonstrated the influence of grit on academic achievement[2,4]. Perseverance, characterized by sustained effort and determination, equips students with the resilience necessary to overcome the challenges and rigors of the licensure exam preparation process.

Interestingly, while passion was included in the regression model, it did not emerge as a significant predictor of exam success in this study. This finding suggests that, within the context of licensure exams, perseverance may play a more prominent role than passion in determining outcomes. Licensure exams often demand intense focus, sustained effort, and the ability to persist through difficult and demanding periods of preparation, which aligns more closely with the perseverance aspect of grit.

The results of this study have significant implications for pharmacy educators and curriculum development. By recognizing the importance of perseverance, pharmacy programs can implement strategies to foster and cultivate this trait among students. This may include incorporating grit assessments, providing targeted interventions for students with lower perseverance levels, and creating learning environments that promote resilience, determination, and a growth mindset.

Furthermore, the findings underscore the potential value of incorporating grit assessments and interventions early in the pharmacy curriculum. By identifying students who may benefit from targeted support in developing perseverance, educators can provide proactive assistance, potentially improving students' chances of success in the licensure exam and their future professional endeavors.

While the study accounted for potential confounders such as gender, track, cumulative GPA, previous semester GPA, and reasons for choosing the pharmacy program through correlation analyses, the possibility of unmeasured confounding variables cannot be ruled out. Additionally, the low response rate and reliance on self-reported data are limitations that should be acknowledged. However, the use of a validated and adapted Grit Scale, along with appropriate statistical analyses, represents a strength of this study.

Conflict of interest

Declare conflicts of interest or state “The authors declare that they have no conflict of interest.”

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Pharmaceutical Technology and Drug Delivery

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Comparison of Physicochemical Characteristics, Nutritional Value, and Antioxidant Activity of Collagen Extracted from Fresh and Dried White Type Jellyfish of Myanmar Marine Source

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Abstract

Nowadays, collagen is one of the most popular active ingredients in cosmeceutical products, and marine collagen is an attractive alternative source because it is metabolically compatible, water-soluble, lacks religious constraints, and is free of harmful pathogens. Collagen is the major protein of jellyfish, which is rich in protein. This study was aimed at extracting collagen from fresh and dried white-type jellyfish, Lobonemoides gracilis Light from Myanmar marine source then comparing their properties for use in cosmeceutical products. The acid hydrolysis method was used for collagen extraction then evaluated in vitro and compared for their yield percentage, solubility, pH, viscosity, moisture content, and ash content of both extracted collagens. Moreover, Ultraviolet absorption and Fourier Transformation Infrared spectrophotometry were also used for the identification of their chemical properties. The Association of Official Analytical Chemists method and 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay method were used for nutritional value and antioxidant activity evaluation. The yield percentages were 1.23±0.01 % from fresh jellyfish and 0.86±0.01 % from dried jellyfish. The viscosity of both extracted collagen behaved in a pseudoplastic nature when tested with different shear rates. Ultraviolet absorption spectra showed the same maximum absorption at 232 nm because of the presence of hydroxyproline, proline, and glycine amino acids. For nutritional value, protein content percentages were 95.69 % and 66.93 %, respectively. Antioxidant activities were expressed in 50 % radical scavenging activity, which was 5.31±0.07 µg/mL for fresh jellyfish and 5.83±0.03 µg/mL for dried jellyfish. In vitro results showed that collagen from both jellyfish has antioxidant activity due to the presence of hydroxyproline, proline, and glycine amino acids. However, the protein content of collagen from fresh jellyfish was higher than that of collagen from dried jellyfish. This study proved that jellyfish collagen can be used in cosmeceutical products as a bioactive ingredient for antioxidant activity and skin nourishment.

Keywords: Collagen; jellyfish; *Lobonemoides gracilis* Light; Myanmar marine source; cosmetic products; antioxidant

1. INTRODUCTION

Nowadays, collagen is widely used in pharmaceuticals, bioengineering, biomedical wound healing, nutraceuticals, and cosmetics [1]. In addition, collagen is a promising biopolymer for the removal of skin defects, deforming scars, pigmentation, and wound healing [2]. However, its application is restricted by the risk of viral and prion infections, and religious and ethical constraints [3]. Collagen derived from marine has identical properties to human collagen. Therefore, marine collagen can be said as an alternative collagen source with fewer human infectious diseases such as bovine spongiform

encephalopathy (BSE) transmission, foot and mouth disease (FMD), and transmissible spongiform encephalopathy (TSE) [4].

The most common marine sources of collagen are Sea anemones, Corals, Sponges, Starfish, Octopus, Cuttlefish, Sea urchins, Sea cucumber, Jellyfish, Squid, Mussels, and Shell [5]. Among marine organisms, jellyfish are rich in proteins and collagen is jellyfish's major protein [6]. The distribution ranges of white-type jellyfish are the Philippines, Myanmar, Vietnam, Thailand, Peninsular and East Malaysia, and Indonesia [7]. The family and genus of white-type edible jellyfish are Lobonemidae and *Lobonemoides* and there are three species: *L. gracilis* Light, *L. robustus* and *L. sewelli* Rao [8]. Kramp, 1961 suggest that *L. gracilis* is a younger stage and juvenile form of *L. robustus* [9].

The most commonly used collagen extraction methods are alkaline hydrolysis, acid hydrolysis, and enzyme hydrolysis. The alkaline hydrolysis method is extraction using sodium chloride or guanidine hydrochloride and low-yield extract is a disadvantage of this method [10]. The acid hydrolysis method of collagen yields better extraction than the alkaline hydrolysis method [11]. Moreover, organic acids can solubilize non-crosslinked collagens, and break inter-strand cross-links in collagen, which leads to a higher solubility of collagen. Therefore, organic acids, especially acetic acid, are commonly used to extract collagen for better yield [12]. The enzymatic hydrolysis method by pepsin, papain, and collagenase removes the non-helical extremities and increases the solubility of collagen. Although this method is preferred, it can cause irreversible denaturation of the collagen [13].

Therefore, this study aimed to extract the collagens from fresh and dried jellyfish by acid hydrolysis and compare their properties for use in cosmeceutical products.

2. MATERIALS AND METHODS

The fresh white-type jellyfish, *Lobonemoides gracilis* Light was collected from the fishing ground of Myeik township (12°26'N98°36'E), Myanmar then, the specimen was identified and confirmed by the authorized zoologist. The dried jellyfish were prepared by preserving the fresh jellyfish with sodium chloride in a well-closed, air-tight container for two weeks.

2.1. Extraction of collagen

The jellyfish (fresh and dried) were plunged in 10 % ethanol solution (1:10) (w/v) ratio for 48 hrs under stirring with medium changes twice a day at 4 °C. Then they were rinsed with distilled water and cut into small pieces. Then they were plunged with 0.1 M NaOH solution (1:10) (w/v) ratio and changed every 2 hrs for 6 hrs at 4 °C. These chemically treated samples were cleaned several times with distilled water until pH 7.0 [14].

After this, the pretreated jellyfish were macerated in 0.5 M acetic solution (1:15 w/v) for 72 hrs under stirring at 4 °C. After 72 hrs, the mixture was filtered through two layers of cheesecloth. The supernatants containing acid-soluble collagen were precipitated by 0.9 M NaCl and left overnight. The resultant precipitates were separated by centrifugation for 1 hr and filtered by two layers of Whatman filter paper (No. 4). The acid-soluble collagens were resuspended in distilled water for 48 hrs. Then, the solutions were freeze-dried [15].

The yield % can be calculated by using the following equation:

$$\text{Yield of collagen} = \frac{weight\ of\ collagen\ (g)}{weight\ of\ wet\ skin\ (g)} \times 100$$

2.2. Determination of the physicochemical characteristics of extracted collagen

2.2.1. Determination of moisture content

The moisture content of extracted collagen was determined by an automatic moisture analyzer. The procedure was carried out according to the standard operating procedure of the moisture analyzer. About 2 g of collagen powder was used then the temperature, 105 ºC, and program no. 1 were selected.

2.2.2. Determination of ash content

The procedure was carried out according to the Method No 930.05, AOAC, 2005 of ash determination. Three grams of collagen powder was heated to 550 ± 25 °C in a muffle furnace until it became white. After the ash had been obtained, the crucible was cooled down in desiccators and weighed [16]. The percentage of total ash was calculated by the following equation.

$$\text{The percentage of total ash} = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

2.2.3. Determination of solubility

A solubility study was conducted by dissolving collagen in 10 mL of deionized water at different weight ranges (0.1- 1 g). Collagen was added into deionized water and stirred until fully dissolved. The maximum weight of fully dissolved collagen was recorded [17].

2.2.4. Determination of pH

The pH of different concentrations of collagen solution (1 to 10 %) was determined by a pH meter (Mettler Toledo, Switzerland). The electrode was first calibrated with standard buffer solutions. The pH of different concentrations of collagen solution was determined in triplicate and results were expressed as Mean ± SD.

2.2.5. Determination of viscosity

The viscosity of different concentrations of collagen solution (1-10 %) was determined by Brookfield viscometer (ATAGO, Japan). The viscosity of each concentration was evaluated by using spindle TL7 at 100 RPM, 60 RPM, 50 RPM, 30 RPM, 20 RPM, and 10 RPM. Each experiment was repeated three times and the mean and standard deviation were recorded.

2.2.6. Identification by Fourier Transform Infrared (FTIR) spectra

The FTIR spectra of the extracted collagen were carried out by FTIR spectrophotometry (PerkinElmer Spectrum Two, USA) in the range of 4000 to 400 cm^{-1} and compared with the spectrum of standard collagen.

2.2.7. Identification by Ultraviolet (UV) absorption measurements

The UV absorption spectra of extracted collagen were analyzed with a UV-Vis spectrophotometer (Shimadzu) according to the procedure described by Liao et al., 2018 with a few slight modifications. Collagen was dissolved with 0.5 M acetic acid and the UV spectrum was measured at wavelengths ranging from 200 to 400 nm. 0.5 M acetic solution was used as a blank and then UV spectra of collagen from fresh jellyfish, collagen from dry jellyfish, and standard collagen were compared [18].

2.3. Determination of nutritional value

The nutritional values of these collagens were evaluated according to the AOAC, 2005. Protein content was determined by the Kjeldahl method, No. 978.04, AOAC, 2005 [16]. Crude fat was determined by extracting the sample with petroleum ether for a minimum of 4 hrs in a Soxhlet extraction apparatus according to Method No. 930.09, AOAC, 2005 [16]. Crude fiber was determined by digesting one gram of sample in refluxing 1.25 % sulphuric acid and 1.25 % sodium hydroxide by Method No. 930.10, AOAC, 2005 [16]. The carbohydrate content was determined by difference. Addition of all the percentages of moisture, ash, protein, crude fat, and crude fiber was subtracted from 100 %.

2.4. Determination of the antioxidant activity of extracted collagen by DPPH assay

The standard solution and test sample solutions were prepared by mixing 1 ml of 60 µM DPPH solution and 1 ml of different concentrations (1 µg/mL, 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, and 10 µg/mL) of standard and test samples. The blank solution was also prepared by mixing 1 mL of DPPH solution and 1 mL of distilled water. These solution mixtures were kept at 40 ˚C for 30 minutes then the measurement of absorbance was done at 517 nm using a UV visible Spectrophotometer, 1601 (Shimadzu) [19]. Absorbance measurements were done in triplicate and calculated to obtain the % inhibition using the following formula and IC_{50} was calculated from the relationship curve of concentration and % inhibition [20].

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A\ control - A\ test}{A\ control} \times 100$$

$A_{control}$ = the absorbance of the control solvent (DPPH)
A_{test} = the absorbance in the presence of the tested sample

3. RESULTS

3.1. Extraction and yield percent

In this study, the yield percent of collagen from fresh and dried jellyfish are 1.23±0.01 % and 0.86±0.01 %, respectively. The yield percent of collagen extracted from fresh jellyfish is significantly ($p < 0.001$) higher than that of collagen from dried jellyfish.

3.2. Physicochemical characteristics of extracted collagen

3.2.1. Moisture content and ash content

After freeze-drying, the moisture contents are 2.01 % for collagen extracted from fresh jellyfish and 4.08 % for collagen extracted from dried jellyfish. The collagen extracted from fresh jellyfish contains only 0.33 % ash content and the ash content of collagen extracted from dried jellyfish was 7.40 %.

3.2.2. Solubility

They are freely soluble in water because there is no precipitation when evaluating the solubility test at different weight ranges (0.1 - 1 g).

3.2.3. pH

When evaluating the pH of extracted collagen, both collagens are slightly acidic and decrease the pH of the solution when increasing the concentration. The results of the pH of both extracted collagens were shown in Table 1.

Table 1. Comparison of pH of extracted collagens from fresh and dried jellyfish

Concentration	pH (Mean ± SD)		*p* value
	Sample 1	Sample 2	
1 %	6.59 ± 0.02	6.37 ± 0.01	< 0.001
2 %	6.43 ± 0.02	6.24 ± 0.01	< 0.001
4 %	6.33 ± 0.02	6.22 ± 0.01	< 0.001
6 %	6.31 ± 0.01	6.20 ± 0.01	< 0.001
8 %	6.29 ± 0.01	6.20 ± 0.01	< 0.001
10 %	6.26 ± 0.01	6.16 ± 0.01	< 0.001

Sample 1 = Collagen extracted from fresh jellyfish
Sample 2 = Collagen extracted from dried jellyfish

3.2.4. Viscosity

The *in vitro* viscosity test showed that both of the extracted collagen were pseudoplastic in nature when increasing the shear rate. Each experiment was repeated three times then, the mean and standard deviation were recorded and the results were shown in Table 2.

Table 2. Comparison of viscosity of extracted collagens from fresh and dried jellyfish at 100 RPM, 60 RPM, 50 RPM, 30 RPM, 20 RPM, and 10 RPM

Concentration	Viscosity (cp)											
	100 RPM		60 RPM		50 RPM		30 RPM		20 RPM		10 RPM	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
1 %	1.77 ± 0.15	4.87 ± 0.25	2.90 ± 0.10	6.10 ± 0.10	4.00 ± 0.10	6.27 ± 0.06	5.53 ± 0.06	6.80 ± 0.10	5.70 ± 0.20	7.53 ± 0.21	22.40 ± 0.10	25.03 ± 0.15
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
2 %	2.50 ± 0.10	5.50 ± 0.10	3.20 ± 0.23	6.57 ± 0.25	4.30 ± 0.10	6.93 ± 0.06	5.50 ± 0.10	9.50 ± 0.10	6.80 ± 0.10	10.73 ± 0.06	23.80 ± 0.10	27.50 ± 0.20
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
4 %	4.10 ± 0.17	5.60 ± 0.10	5.67 ± 0.15	6.90 ± 0.10	6.40 ± 0.20	7.73 ± 0.15	6.93 ± 0.15	12.00 ± 0.10	9.67 ± 0.15	14.47 ± 0.15	24.10 ± 0.44	37.43 ± 0.15
p	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
6 %	4.47 ± 0.12	5.80 ± 0.10	6.50 ± 0.20	7.00 ± 0.10	8.43 ± 0.15	8.57 ± 0.21	8.77 ± 0.23	12.40 ± 0.10	13.13 ± 0.15	14.47 ± 0.15	33.77 ± 0.12	43.30 ± 0.17
p	<0.001		<0.05		>0.05		<0.001		<0.001		<0.001	
8 %	5.10 ± 0.10	5.93 ± 0.15	7.90 ± 0.10	10.40 ± 0.10	10.50 ± 0.10	10.67 ± 0.15	12.70 ± 0.10	13.43 ± 0.21	14.03 ± 0.12	15.87 ± 0.21	43.53 ± 0.15	47.37 ± 0.15
p	<0.001		<0.001		>0.05		<0.05		<0.001		<0.001	
10 %	5.33 ± 0.15	10.67 ± 0.15	11.27 ± 0.15	11.50 ± 0.20	12.57 ± 0.21	13.63 ± 0.31	13.70 ± 0.10	16.00 ± 0.10	16.20 ± 0.10	18.53 ± 0.21	52.60 ± 0.36	54.20 ± 0.20
p	<0.001		>0.05		<0.05		<0.001		<0.001		<0.001	

3.2.5. Fourier Transform Infrared (FTIR) spectra

The FTIR spectra of both of the extracted collagens (Figure 1) indicated the presence of the same five major amide groups which are secondary amide, amide B, amide I, amide II, and secondary aliphatic amine. Table 3 shows the functional group and their wavelength of standard collagen and collagens extracted from fresh and dried jellyfish.

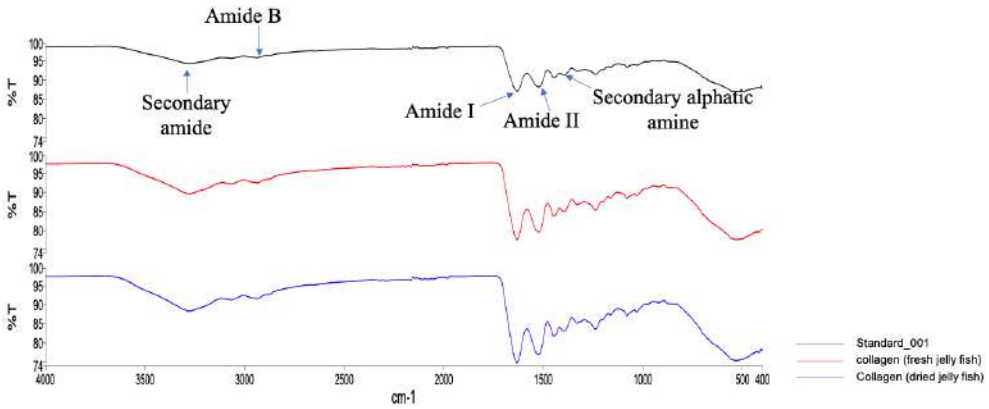

Figure 1. FTIR spectra of standard and extracted collagen

Table 3. Functional groups and their wavenumbers in FTIR spectra of standard collagen and extracted collagens from fresh and dried jellyfish

Functional groups	Wavenumber (cm^{-1})			Vibration mode
	Standard collagen	Sample 1	Sample 2	
Secondary amide	3266.60	3268.7	3301	N-H Stretching
Amide B	2946.40	2929.7	2926.5	C-H Stretching
Amide I	1633.67	1651.87	1651.87	C=O Stretching
Amide II	1531.77	1533.51	1533.51	N-H Bending
Secondary aliphatic amine	1243.35	1244.3	1243.35	C-N Stretching

3.2.6. Ultraviolet (UV) absorption measurements

According to Figure 2, the maximum absorption peak of standard and extracted collagens was observed at 232 nm and the UV spectra of them were identical.

3.3. Nutritional value

The evaluation of nutritional value indicated that the extracted collagens were rich in protein, low content in fat and carbohydrate, and no fiber. The result of nutritional value was shown in Table 4.

Table 4. Determination of nutritional value of extracted collagens from fresh and dried jellyfish

No.	Nutritional value	Percent determined	
		Sample 1	Sample 2
1.	Protein	95.69 %	66.93 %
2.	Crude fat	1.17 %	0.84 %
3.	Crude fiber	0.00 %	0.00 %
4.	Carbohydrate	0.80 %	20.75 %

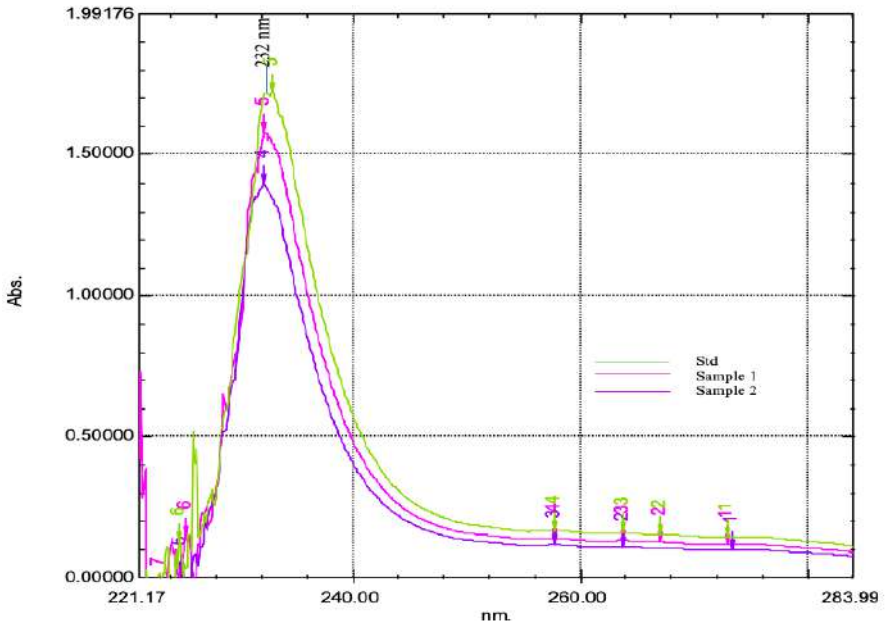

Figure 2. UV spectra of standard collagen and extracted collagens from fresh and dried jellyfish

3.4. The antioxidant activity

The results of % inhibition at different concentrations were shown in Table 5 and the collagens extracted from fresh jellyfish and dried jellyfish showed 78.13 ± 0.17 % and 70.65 ± 0.08 % scavenging activity at the concentration of 10 µg/mL while 98.97 ± 0.58 % of scavenging activity of standard ascorbic acid was observed at this concentration. From the calculation of the relationship curve of concentration and percent inhibition, IC_{50} of extracted collagens was higher than that of ascorbic acid and these values were 5.31 ± 0.07 µg/mL for collagen extracted from fresh jellyfish, 5.83 ± 0.03 µg/mL for collagen extracted from dried jellyfish, and 0.87 ± 0.04 µg/mL for standard ascorbic acid and these results were shown in Figure 3.

Table 5. Percent inhibition of standard ascorbic acid and extracted collagens from fresh and dried jellyfish

Concentration (µg/mL)	%Inhibition (Mean ± SD)		
	Standard ascorbic acid	Sample 1	Sample 2
1	44.13 ± 0.32	28.33 ± 0.29	26.36 ± 1.02
2	61.55 ± 0.19	40.89 ± 0.38	39.14 ± 0.57
4	78.58 ± 0.09	43.44 ± 0.36	42.51 ± 0.33
6	89.42 ± 0.26	52.14 ± 0.29	50.57 ± 0.22
8	97.42 ± 0.42	53.25 ± 0.32	54.25 ± 0.29
10	98.97 ± 0.58	78.13 ± 0.17	70.65 ± 0.08

4. DISCUSSION

Pretreatment with 10% ethanolic solution removes the cuticle and fat therefore, the extracted collagens contain low crude fat. Pretreated with sodium hydroxide removes impurities and non-collagenous matter. Moreover, sodium hydroxide is acceptable for pre-treating skin because it causes swelling, which improves collagen extraction by increasing the mass transfer rate in the tissue matrix [12]. However, the dried jellyfish was difficult to swell therefore the yield percent of this is lower than that of fresh jellyfish.

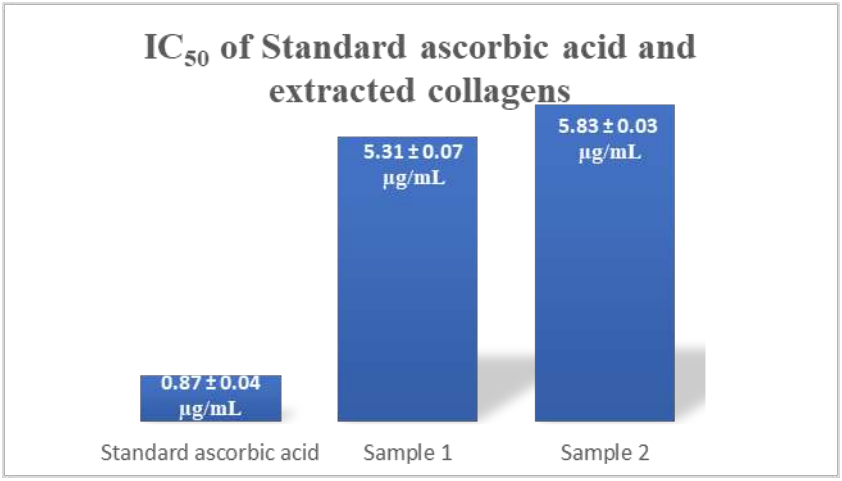

Figure 3. Comparison of IC_{50} of Standard ascorbic acid and extracted collagens from fresh and dried jellyfish

The total ash method can measure the amount of material remaining after ignition [21]. The low ash value means the contamination of extraneous residue in the extract is less [22]. The collagen extracted from fresh jellyfish contains less amount of extraneous residue and has purity. The extracted collagens were finally hydrolyzed and dried by Freeze dryer. Therefore, both of the obtained collagens have good solubility properties.

The slightly acidic nature of collagen is due to the extraction method of acid hydrolysis. The pH of collagen extracted from dried jellyfish is significantly ($p < 0.001$) lower than that of collagen extracted from fresh jellyfish. The pH value of collagen extracted from fresh jellyfish was acceptable according to the 2014, Indonesian National Standard for collagen range, 6.5 to 8 [23].

Pseudoplastic nature is a decrease in viscosity with an increase in shear rates and this nature was observed in both collagens. A higher viscosity value was observed when increasing the collagen concentration and the viscosity of collagen extracted from dried jellyfish was ($p < 0.001$) higher than that of collagen extracted from fresh jellyfish when evaluated with 10 RPM.

In the FTIR study, the spectra of collagen from both fresh and dried jellyfish are similar to the spectrum of standard collagen. The secondary amide is created by N-H stretching and this vibration was detected at about 3300 cm^{-1} and this functional group was observed in both of the extracted collagens and standard collagen. At 2922-2931 cm^{-1}, the amide B peak was discovered due to the C-H stretching and these bands were identified in the FTIR spectra of both of the extracted collagens at 2929.7 and 2926.5 cm^{-1} respectively [24]. The amide I peak is located between 1680 and 1630 cm^{-1} and is created by stretching vibration of the carbonyl group (C=O) [25]. In this study, this functional group was detected in both of the extracted collagens. Peaks at about 1530 cm^{-1} are amide II bands in all of the samples which is N-H bend coupled with C-N stretching vibration [24]. At 1250-1020 cm^{-1}, the secondary aliphatic amine was observed due to C-N stretching and these bands were detected in the FTIR spectrum of extracted collagen at 1244.3 and 1243.35 cm^{-1} respectively [25].

The primary structure of the peptide is the order of the specific amino acid residues and part of a collagen sequence is Gly-Ala-Hyp-Gly-Pro-Hyp-Gly-Ala-Hyp-Gly-Ala-Hyp-Gly-Pro-Val-Gly-Pro-Ala-Gly-Lys-Ser-Gly-Asp-Arg-Gly-Glu-Thr-Gly-Pro-Ala-Gly. A collagen may be recognized by its the relative frequencies of the different amino acid residues [26]. When acetic acid, lactic acid, citric acid, and pepsin were used in the extraction of collagen, collagen and proteins generally have strong UV absorption due to their peptide connections and side chains. The maximum absorbance of collagen at 230 nm was due to glycine, proline and hydroxyproline [27].

From the nutritional value, collagen extracted from both fresh and dried jellyfish are rich in protein but the protein content of collagen extracted from fresh jellyfish is higher than that of collagen extracted from dried jellyfish.

Percent inhibition of extracted collagens was increased with an increase in concentration. The smaller the IC_{50} value, the higher the antioxidant activity, therefore the antioxidant property of extracted

collagens was lower than the standard ascorbic acid. Between the extracted collagens, IC_{50} of collagen extracted from fresh jellyfish was significantly ($p < 0.001$) lower than that of collagen extracted from dried jellyfish. Amino acids such as glycine, proline, and hydroxyproline have a high level of solubility in fat and provide antioxidant activity [28]. N- or C- peptide endings in these amino acids can interact with lipid molecules and donate protons to free radicals [29].

5. CONCLUSIONS

In this study, both of the extracted collagens have the same functional group and UV absorption pattern according to the FTIR spectrum and UV spectrum. However, the nature of the sample influences the yield percent, physicochemical characteristics, nutritional value, and antioxidant activity. From the nutritional value study, the protein content of collagen from fresh jellyfish was higher than that of collagen from dried jellyfish. *In vitro* antioxidant activity evaluation results showed that collagen from both jellyfish has antioxidant activity due to the presence of hydroxyproline, proline, and glycine amino acids. However, the IC_{50} of collagen extracted from fresh jellyfish was lower than that of collagen from dried jellyfish. This study proved that jellyfish collagen can be used in cosmeceutical products as a bioactive ingredient for antioxidant activity and skin nourishment. But, the properties of collagen extracted from fresh jellyfish were superior to the collagen extracted from dried jellyfish.

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Conflict of interest

The authors declare no conflict of interest.

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Formulation and Characterization of Cream Containing a Combination of Apigenin and Tomato Powder For Overcoming Xerosis on the Heels of the Feet

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ABSTRACT

Background: Xerosis is a condition of dry and cracked skin that usually occurs on the heels of the feet due to reduced water content in the stratum corneum due to loss of skin lipids and natural moisturizing factors. Tomatoes and apigenin are efficacious for moisturizing the skin and treating heel xerosis with a very strong antioxidant value (IC50<50 ppm). This study aims to formulate O/W (Oil in Water) type cream preparations and determine their effectiveness in overcoming heel xerosis.

Methods: Mix the oil phase (cera alba, lanolin, and stearic acid) with the water phase (TEA, propylene glycol, sodium metabisulfite) which has been melted at a temperature of 70ºC while stirring. Add apigenin dissolved first with propylene glycol and dissolve the tomato powder with distilled oil phase into a hot mortar, then stir. Comparison of apigenin and tomato concentration control formulas F1 (10%:5%); F2 (7.5%:7.5%); and F3 (5%:10%). Examination of cream includes a homogeneity test, pH measurement, emulsion type test, and preparation stability test with stability parameters such as odor, color, and texture during accelerated storage. The effectiveness of the cream was tested on 30 volunteers who had xerosis of the heel for four weeks by applying the cream twice a day to the heel of the foot. The safety of the cream was determined through an irritation test using a usage test conducted on 30 volunteers.

Results: The results showed that a combination of apigenin and tomato cream could be formulated into a cream that was homogeneous and stable during 6 cycles of storage. The irritation test showed that no one had irritation on their skin. The best heel recovery was seen in F1 which was able to reduce heel xerosis.

Conclusions: This study concludes that all preparation formulas combined with apigenin powder, and tomatoes can reduce the level of skin dryness and F1 (Apigenin and tomato concentrations 10%:5%) has the best ability to overcome xerosis of the heel and meets all the requirements for the physical properties of the cream.

KEYWORDS: *Cream; Apigenin; Solanum lycopersicum* L.; *Xerosis*

1. INTRODUCTION

Dry skin or xerosis is a dry and cracked skin condition that usually occurs on the heels of the feet due to reduced water content in the stratum corneum due to loss of skin lipids and natural moisturizing factors. Statistical figures for cracked heels in Indonesia are not found but in the United States, It is reported that as many as 20% or 44 million people aged 21 years and over experience cracked heels. Dry skin or xerosis is a dry, cracked skin condition that usually occurs on the heels of the feet due to reduced water content in the stratum corneum due to loss of skin lipids and natural moisturizing factors. This condition not only makes the heel of the foot look less attractive but is very risky because fissures in the heel can cause complications such as infection and ulceration so that the

heel looks black, hardens, bleeds easily, makes it uncomfortable because it causes pain when walking and interferes with activities[1]

Efforts to overcome xerosis on the heels of the feet can be made by using moisturizing cosmetic products. The term back to nature or returning to using natural active ingredients is one strategy that can be used to avoid the negative impact of using chemicals in cosmetics and has been proven to be more effective and safer. Skin moisturizing formulations consisting of plants rich in antioxidants are good for treating dry skin caused by free radicals where high UV radiation causes skin cells to absorb radiation and produce reactive oxygen species (ROS) which can damage DNA and cell walls[2] . Tomato (*Solanum lycopersicum* L.) is a plant that has strong antioxidant properties with an IC50 value of 60 ppm. The antioxidants contained in tomatoes are lycopene, flavonoids, vitamin C, and vitamin E[3]. Lycopene has strong antioxidant activity, as an antiaging, and skin protector, can inhibit enzymes that degrade the main component of elastin fiber, and contains amino acids which are natural moisturizing components. In research Yusuf, 2018[4] cream containing tomato lyophilizate can provide a moisturizing effect.

Apigenin is a natural plant flavone contained in several fruits, herbal plants, and vegetables[5]. Apigenin is a very strong antioxidant with an IC50 value of 5.18 ppm, apigenin can repair skin damage caused by UV rays and has anti-inflammatory and anti-carcinogenic properties. Choi's research (2016)[6] concluded that creams containing apigenin can increase skin density, elasticity, and moisture[7].

The benefits of tomatoes and apigenin are widely available for skin health, but there has been no research that combines and shows the effectiveness of tomatoes and apigenin in moisturizer formulations to treat xerosis. Based on this, the author formulated a combination of apigenin powder and tomato (*Solanum lycopersicum* L.) as a natural moisturizing cream ingredient used to treat heel xerosis

2. MATERIALS AND METHODS

2.1. Materials

This research used several tools including a knife, Ohaus analytical balance, porcelain cup, test tube, tube clamp, mortar, stamper, glass object, pH meter, cream pot, water bath, microscope, Brookfield viscometer (NDJ-8S), oven (DHG -9053A), skin moisture checker (SK-8), magnetic stirrer.

The ingredients used in this research were apigenin powder (Hefei Dielegance Biotechnology Co., Ltd) purity 98,21%, tomato fruit, stearic acid, cera alba, lanolin, TEA, propylene glycol, sodium metabisulfate, methylene blue, distilled water, HCl, Mg powder and ethanol 96%.

2.2 Methods

This type of research is experimental research with research stages, namely sample preparation, powder making, and making heel cream preparations using a combination of tomato powder and apigenin. Check the quality of cream preparations and test the effectiveness of cream preparations on xerosis on the heels of the feet.

3. RESULTS AND DISCUSSION

3.1. Physical Properties

Results of homogeneity tests carried out on cream preparations F0, F1, F2, and F3. The cream preparation does not contain coarse grains on the glass object, so the homogeneity test results are declared homogeneous, that is, the active substance has been mixed evenly with the additional substances in the cream formulation and has not changed during storage. This is also influenced by the mixing and stirring process of the cream preparation. When mixing the oil and water phases of the cream preparation, it must be at a high temperature, namely 70°C. This mixing process is carried out in a heated mortar and stamper to facilitate the mixing process of the two phases. After the cream base is formed, continue with the addition of the active ingredients in it with consistent stirring.

The viscosity test results of cream preparations at F0, F1, F2, and F3 meet the required viscosity value range. A good cream viscosity value according to SNI 16-4399-1996 ranges from 2,000-50,000 cps. The viscosity value is inversely proportional to the spreadability value, if the viscosity increases the spreadability will decrease.

The pH test results of cream preparations F0, F1, F2, and F3 meet the required pH value range. According to the National Standardization Agency, the quality requirements for cream comply with Indonesian national standards, namely having a pH of 3.5 – 8.0. If the preparation is too acidic compared to the skin's pH, it will cause irritation and if the preparation is too alkaline it is feared that it will make the skin dry.

The highest spreadability test results for cream before storage were F3, namely 4.8 cm, and after storage, namely 4.4 cm. Based on Table 1 the spreadability test results of F0, F1, F2, and F3 meet the requirements for a good spreadability test value, namely 4-7 cm. gravity values obtained for F0, F1, F2, and F3 in three replications or repetitions. From the research results, the specific gravity values have an average of around 0.95-1.005 g/ml so that the specific gravity values can be said to be appropriate because meets SNI 16-4399-1996 which ranges from 0.95 to 1.05[12].

Table 1. Evaluate the physical properties of the preparation

Parameter	Result			
	F0	**F1**	**F2**	**F3**
Organoleptic	White color, typical cream aroma, semi-solid texture	Light cream color, weak tomato aroma, semi-solid texture	Cream color, typical tomato aroma, semi-solid texture	Orange color, strong tomato aroma, semi-solid texture
Homogeneity	homogeneous	homogeneous	homogeneous	homogeneous
Viscosity test	3962 cps	4006,67 cps	4130 cps	4403 cps
pH Test	6.74	6.82	7.01	6.92
Stability (cycle)	Unstable (6th)	Stable	Stable	Stable
Spreadability test	4.7 cm	4.6 cm	4.2 cm	4.2 cm
Specific gravity test	0.95 g/ml	1.005 g/ml	0.99 g/ml	0.97 g/ml
Test the cream type	O/W	O/W	O/W	O/W

F0 F1 F2 F3

Figure 1. Results of a Cream Preparation Combining Apigenin Powder and Tomatoes (***Solanum lycopersicum*** L.,). F0 negative control, F1 (10% apigenin and 5% tomato), F2 (7.5% apigenin and 7.5% tomato), F3 (5% apigenin and 10% tomato)

3.2 Results of tests on the effectiveness of cream preparations in treating heel xerosis

The measurement results are in Table 2. From the measurement results it can be seen that the initial condition of moisture on the skin of all groups of volunteers experienced cracked heels and dehydration (loss of water content) seen from the percentage of moisture content before use, showing that the moisture level of the respondents' skin was (<30%) so it falls into the dry category. The formula with a higher percentage of apigenin, namely F1 (10% apigenin and 5% tomato) has the best ability to treat heel xerosis.

Table 2. Humidity Value

Formula	Volunteer	Humidity Value (%)				
		Before	0 week	2 weeks	4 weeks	After
F0	1		13,4	18,2	20,4	
	2		10.8	15,00	22,5	
	3		15,6	21,4	27,9	
	4		14,1	16,0	19,8	
	5		20,8	26,8	31,2	
	6		17,2	21,2	24,9	
	Average± SD		13,59±6,97	19,77±4,36	24,45±4,46	
F1	1		11,8	49,8	59,6	
	2		14,2	39,4	39,6	
	3		12,1	29,9	30,6	
	4		12,2	41,6	57,7	
	5		15,3	50,0	61,8	
	6		15,0	55,3	58,1	
	Average±SD		13,43±5,51	44,33±9,19	51,23±12,90	
F2	1		13,7	33,7	49,0	
	2		17,1	50,8	53,3	
	3		11,4	39,4	54,6	
	4		10,8	33,4	43,9	
	5		12,9	43,8	53,0	
	6		12,3	33,8	49,1	
	Average±SD		13,03±2,24	39,15±7,05	50,49±3,97	
F3	1		14,2	28,5	34,6	
	2		16,6	38,5	42,2	
	3		13,6	29,4	39,7	
	4		14,4	25,3	30,4	
	5		13,7	37,9	38,4	
	6		17,1	25,5	37,2	
	Average±SD		14,93±1,52	30,85±5,92	37,08±4,14	
F+	1		14,6	53,3	56,8	
	2		10,9	53,5	56,1	
	3		12,2	45,7	56,5	
	4		11,3	46,0	49,2	
	5		12,0	46,9	53,3	
	6		11,8	37,1	57,6	
	Average±SD		12,13±1,299	47,08±6,04	54,92±3,16	

4. CONCLUSION

A combination of apigenin and tomato powder can be formulated in a cream preparation with oil in water (O/W) type with good cream characteristics according to SNI (16-4954-1998), does not irritate the skin and has good stability in storage cycling test 6 cycle, efficacious in treating heel xerosis in volunteers for 1 month of use with the formulation that has the best ability in treating heel xerosis is FIII (% apigenin and 10% tomato).

5. ACKNOWLEDGMENT

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Conflict of interest

We declare that we have no conflicts of interest.

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PD-0102105-P

Influence of Particle Size Distribution, Thickening Agents and Tonicity Modifiers on Rheological Properties and Dissolution Profiles of Mangiferin Suspensions

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ABSTRACT

Physico-chemical properties of suspensions, including particle size distribution and rheological properties, may have a significant influence on drug release with downstream effects on biological performance. This study aimed to evaluate the impacts of formula on physico-chemical properties and dissolution of mangiferin suspensions. Mangiferin suspension formulations with three levels of particle size distribution were prepared by grinding wet-milling. Thickening agents and tonicity modifiers were then introduced at different concentrations for suspension stabilisation. Particle size distribution (D[4,3], SPAN) was determined by laser diffraction, and rheological properties (storage modulus, yield stress, viscosity) were characterised using a DHR2 rheometer. The *in vitro* dissolution was evaluated by stirring technique and *in vivo* study was performed on experimental rabbits. Thickening modifiers possessed significant impacts on the rheological properties of mangiferin suspensions, the effects likely stemmed from the size-dependent binding affinity of mangiferin particles to polymer chains. The smaller the mangiferin particle size the higher viscosity, storage modulus, and yield stress were observed when Carbopol 974P was used as a thickening agent. Such effects were minimal when cellulose derivatives (HPMC E6, HPC EF) were employed. Furthermore, reducing Carbopol concentration or increasing NaCl (tonicity modifier) level inversely influenced on thixotropic behaviour of suspensions. Rheological experiments also demonstrated that Carbopol-based suspensions exhibited thermal stability in stress conditions. Correlations between rheological properties and drug dissolution were elaborated. The yield stress of suspensions exhibited an inverse effect on *in vitro* dissolution. In the *in vivo* study, mangiferin levels in tear fluid were found to be highest in the eyes treated with the largest particle formulation or with the lowest viscosity formulation, the greatest difference was two folds observed at 5 mins after instillation. Mangiferin particle size, thickening agents, and tonicity adjusting agents significantly impact on rheological properties, dissolution, and in vivo performance of the suspensions.

KEYWORDS: Mangiferin, Suspension, Particle size, Dissolution, Rheological properties

1. INTRODUCTION

Mangiferin is a natural antioxidant that exhibits significant antiviral activity against Herpes simplex virus, especially on acyclovir-resistant strains. However, the compound is poorly soluble and has low permeability across biological barriers that necessitates strategies to enhance drug absorption. Particle size reduction is a widely used technique to improve drug apparent solubility and dissolution (1, 2), however, this approach potentially alters physicochemical characteristics with downstream effects on clinical outcomes (3, 4). For instance, in a high-energy milling process, size-reduced particles can exist in non-stable polymorphism, thereby facilitating rearrangement in crystalline structure towards a stable form that is often accompanied by fluctuations in dissolution processes. Particle size reduction in suspension formulation can also possess impacts on drug-excipient interactions with further influence on physical states, dissolution profiles and product stability (4). Such characteristics are also

governed by thickening agents that exhibit different stabilising mechanisms, such as by forming gel and enhancing suspension viscosity (polyacrylic derivatives) or by adsorbing onto particle surfaces to create hydrophilic steric coats (cellulose derivatives). Drug-excipient interactions are also prone to solution ionic strength that is influenced by pH or tonicity adjustment. As such, any impacts of formula compositions on critical quality attributes should be carefully evaluated during the drug development stage.

In this research, we aimed to study the roles of particle size distribution, thickening agents and tonicity adjusting agents in modifying physicochemical properties, *in vitro* and *in vivo* dissolution of MGF suspensions. The results herein can be used to develop ophthalmic suspensions containing mangiferin.

2. MATERIALS AND METHODS

2.1. Materials

Mangiferin (97,58%) was purchased from Nature Vietnam Pharmaceutical Joint Stock Company. Carbopol 934, and Carbopol 974 P were acquired from Corel Pharma Chem (India); hydroxy propyl cellulose E6 (Methocel E6), and hydroxypropyl cellulose EF (Klucel EF) were purchased from Ashland, USA; Acetic acid, ethylenediaminetetraacetic acid disodium, hydrochloric acid, mannitol, sodium chloride, and sodium hydroxide (analytical grade) were purchased from Xilong Scientific Co., Ltd, China. Acetonitrile and methanol (HPLC grade) were provided by Fisher Chemical, USA). Adult white New Zealand rabbits (3.0 - 4.0 kg in weight) were raised in the Animal Centre, Pharmacology laboratory, Hanoi University of Pharmacy and were employed for *in vivo* dissolution test. Animal use and care were approved by the Scientific and Ethics Committee, Hanoi University of Pharmacy (No. 2023.07/PCT-HDDD).

2.2. Methods

- MGF suspension preparation. MGF at three levels of particle size distribution (PSD), namely submicron (D[4,3] = 0.44 µm; SPAN = 0.94); fine (D[4,3] = 1.81 µm; SPAN = 2.64); and coarse (D[4,3] = 6.12 µm; SPAN = 2.28) were prepared by wet grinding technique. Milling and formula parameters were optimised to control MGF particle size distribution. Subsequently, suspensions containing MGF particles were mixed with stabiliser (Carbopol 974P; Carbopol 934; HPMC E6, HPC EF), tonicity adjusting agent (NaCl; mannitol) and other components where relevant.

- Particle size determination. Particle size distribution - PSD (D[4,3], SPAN) was determined by laser diffraction using a background subtracting method (5). Samples were also analysed by a light microscope for morphology and size determination.

- Physicochemical characterisation. The crystallinity was examined by DSC and XRD. Molecular interactions were examined by FT-IR. Rheological properties of suspension were characterized using a DHR 2 rheometer. Amplitude oscillation experiments were performed at strain variation (0.01% - 200%) to calculate yield stress; in flow sweep, experiments were performed at shear rate variation (0.001 - 500 s^{-1}) to calculate viscosity; in oscillation frequency sweep, experiments were alternatively performed at 1 Hz and 30 Hz frequency for thixotropic illustration; in temperature ramp, experiments were performed at multiple 5 - 45 ^{o}C cycles to evaluate thermal stability.

- *In vitro* dissolution assay was tested by a stirring method (6), using 10 mL of isotonic phosphate buffer pH 7.4 as testing media. At each time point, media was withdrawn for HPLC analysis of MGF concentration.

- *In vivo* dissolution study was performed on a rabbit model. After instilling drugs into the rabbit's eyes, tear fluid containing MGF was adsorbed onto paper strips. To minimize any chance of collecting undissolved particles, one side of each paper strip was placed onto the conjunctival epithelium around the eye punctum (upper conjunctival de sac). The API was further extracted by a solvent system prior to HPLC analysis. An equation correlating MGF peak area versus the amount of MGF loaded onto paper strips was elaborated: $y = 124.2x + 1.2145$; ($R^2 = 0.9999$). The method to quantify MGF in tear fluid was fully validated according to US-FDA and ICH-M10 guidelines.

3. RESULTS AND DISCUSSION

3.1. MGF crystallinity evaluation in samples with different PSD

High-energy input of ball milling potentially alters polymorphism of materials. In this research, MGF at three ranges of particle size was prepared by wet grinding method (namely submicron particles; fine particles; coarse particles). The samples were examined for any change in solid state polymorphism of MGF compared to the starting material. Figure 1A illustrates the DSC and XRD spectra of these samples. DSC thermograms of raw material and coarse particles (Figure 1A.a and Figure 1A.d, respectively) exhibited an exothermal peak at around 268 ^{o}C showing that MGF existed in crystalline form and that the crystalline polymorphism of coarse sample was similar to that from the starting material. However, in spectra of fine and submicron samples (Figure 1A. c and 1A. b, respectively), there was a shift of the melting point of particles towards the lower temperature. The exothermal peaks also exhibited different shapes compared to those from bigger particles showing that there was a change in the polymorphism structure of particles with average size (D[4,3]) < 2 µm. The most significance in Figure 1A. b was the presence of a new melting peak at approximately 150 ^{o}C implied the formation of a new MGF polymorphism in fine particles.

XRD spectra (Figure 1B) also confirmed the difference in solid-state polymorphism of MGF samples. The diffractogram of coarse particles was congruent with that from raw material showing that MGF existed in the same polymorphism structure. Major peaks of MGF (Figure 1B. a) were also exhibited in the diffractogram of fine and submicron particles but at a lower absolute intensity as well as smaller relative intensity (for instance, the ratio between 10.54^{o}/ 12.04^{o} peak height) implying the shift in solid-state structure. The evolution in polymorphism structure could be attributed to the difference in the milling process: the smaller the particles are, the more energy input is required to break down MGF material that resulted in a greater change in the solid-state structure.

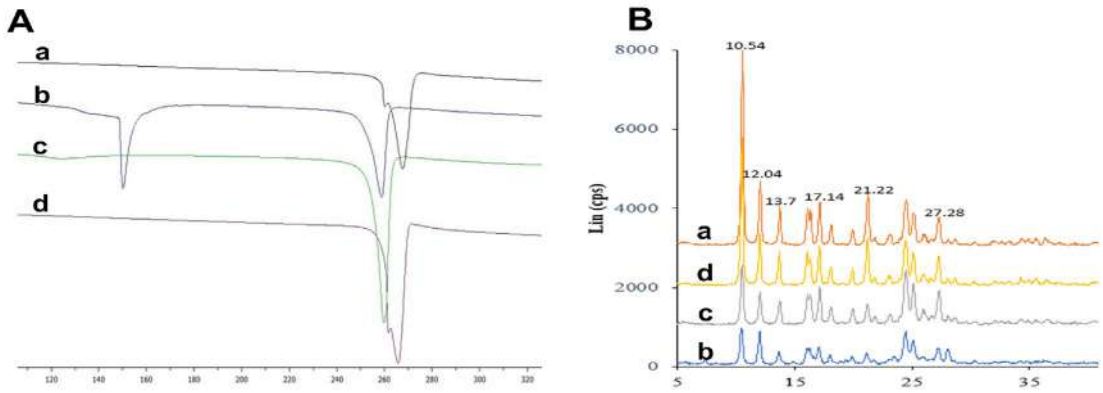


Figure 1. DSC thermograms (A) and XRD spectra (B) of a. MGF raw material; b. Submicron particles; c. Fine particles; d. Coarse particles.

3.2. MGF particles exhibited selective affinity to Carbomer tangle in a reverse size-dependent manner

Table 1 summarises different formulations containing submicron, fine and coarse MGF particles prepared in this study. Two types of thickening agents were employed to stabilise suspensions, i.e. polyacrylic derivatives (Carbopols) and cellulose derivatives (HPMC, HPC). Both polyacrylic polymers exhibited a high degree of crosslinks, however, Carbopol 974 P is polymerised in ethylacetate and Carbopol 934 is polymerised in benzene, potentially leading to different hydration capacities. Similarly, HPMC E6 and HPC EF belong to low viscosity grades but possess different functional groups that might result in different binding affinity onto particle surfaces. The two polymers were intentionally used at different concentrations to obtain suspensions with similar viscosity.

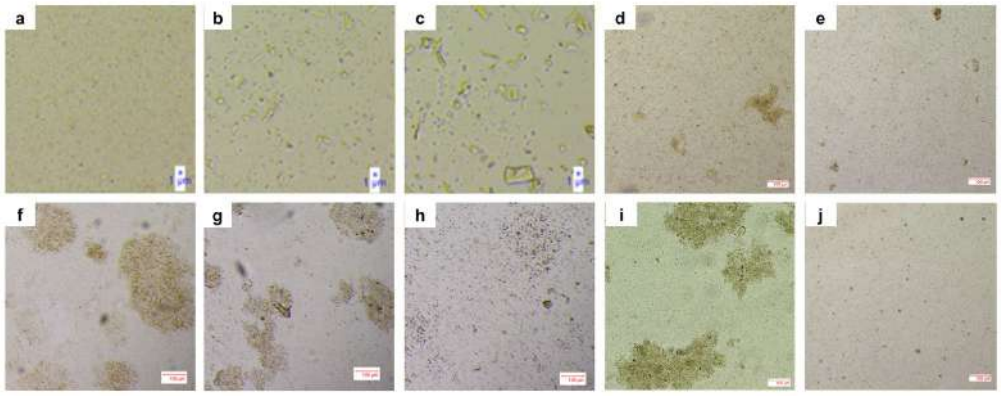
Sodium chloride and mannitol were used to modify suspension tonicity; pH values were adjusted to pH 5.9-6.1. After prepared, MGF suspensions showed typically different physical appearances: Carbomer-based suspensions exhibited a more gel-like state whereas HPMC and HPC-

based suspensions showed more liquid-like properties, implying a possible physical interaction between MGF particles and Carbopols. To elucidate any interaction, first, particle size analyses were performed. Microscopic images (Figure 2A) showed that there were clumps sizing approximately hundreds of micrometres in HD 1-5 formulations (prepared from Carbopols) but these big particles were not observed in either particle before polymer introduction (Figure 2A.a-c) or in suspensions containing HPMC E6/ HPC EF (Figure 2A.e, j). Representative particle size distribution of HD 1-3 determined by laser diffraction (Figure 2B) confirmed the formation of ~ 30-300 µm particles when Carbopol 974P was employed.

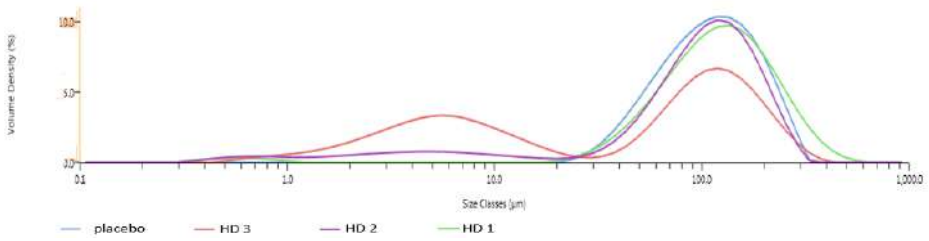
Table 1. Formulations prepared in this study

MGF PSD* (D [4,3]; SPAN)	**Sample**	**Polymer**	**NaCl**	**Mannitol**	**Other ingredients**
0.44 µm; 0.94	HD 1	Carbopol 974P (0.4 %)	0.2 %	2.35%	NaH_2PO_4 (0.06 %) Na_2EDTA (0.01 %) NaOH q.s. pH 5.9 - 6.1 H_2O q.s.
1.81 µm; 2.64	HD 2				
6.12 µm; 2.28	HD 3				
0.44 µm; 0.94	HD 4	Carbopol 974P (0.2 %)	0.1 %	3.00 %	
	HD 5	Carbopol 934 (0.4 %)	0.2 %	2.35%	
	HD 6	HPMC E6 (0.75 %)	0.1 %	3.00 %	
	HD 7	HPC EF (1.0 %)	0.1 %	3.00 %	

**determined before polymer and other ingredients were introduced*



A



B

Figure 2. **A**. Representative microscope images of MGF particles in suspension before (**a**, **b**, **c**) and after introduced to thickening agents (**d-j**). MGF PSD (D[4,3], SPAN) are 0.44 µm, 0.94 (**a**, **d**, **e**, **f**, **i**, **j**); 1.81 µm, 2.64 (**b**, **g**); 6.12 µm, 2.28 (**c**, **h**), respectively. Please refer to Table 1 for formula details: **f**. HD 1; **g**. HD 2; **h**. HD 3; i: HD 5; **e**. HD 6; **j**. HD 7. Notably, scale bars in **a, b,** and **c** were 1 µm and in other panels were 100 µm. **B**. Particle size distribution of HD 1, HD 2 and HD 3 suspension in comparison with placebo.

To further evaluate Carbopol - MGF interactions, FT-IR experiments were performed on HD 1-3 (Figure 3). Due to the presence of a number of -OH functional groups belonging to the aromatic

ring and glucose segment, -OH stretching of MGF material was found at 3500-3100 cm^{-1} with a sharp peak at c.a. 3400 cm^{-1} and a shoulder at c.a. 3200 cm^{-1}. Spectra of MGF raw material also demonstrated a "fingerprint" region for -C=O stretching (~1650 cm^{-1}); -C=C_{aryl} stretching (~1485 cm^{-1}); -C-O-$_{aryl}$ stretching (~1250 cm^{-1}) and -C-O-$_{glucose}$ stretching (~1100 - 1020 cm^{-1}). When reducing PSD of MGF, the intensity of aforementioned -OH stretching decreased correspondingly and a schetching at c.a. 3300 cm^{-1} was pronounced. The shape of -C-O- stretching in the glucose segment also changed significantly with two sharp peaks determined in three MGF suspensions prepared from reduced PSD. Besides, the intensity of stretchings of -C=O, -C=C_{aryl}, and -C-O-$_{aryl}$ also decreased upon reducing PSD implying a possible reduction in aromatic electron density that unfoured intermolecular vibrations. Altogether, the IR spectra confirmed the interaction between MGF molecules on the particle surface and Carbopol molecules. When particle size was reduced, surface area increased proportionally resulting in more pronounced interactions on FT-IR analyses (4).

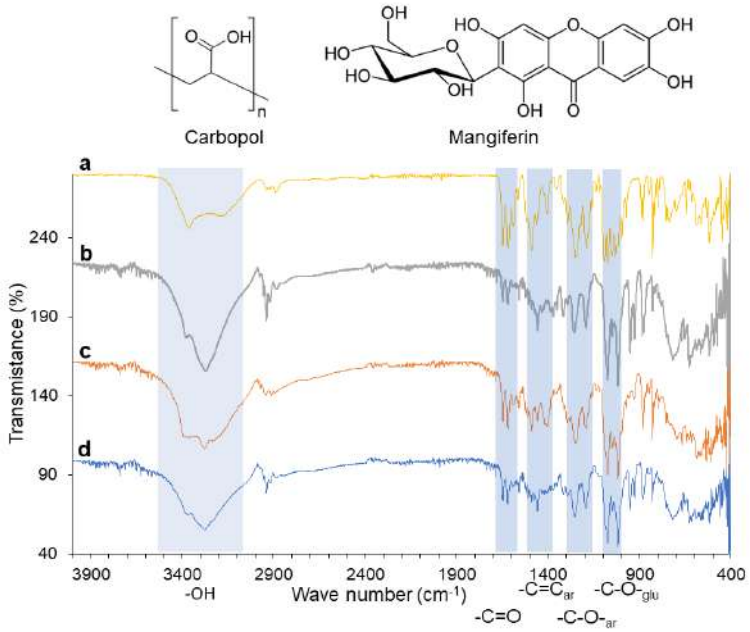


Figure 3. ATR-FT-IR spectra of a. MGF raw material; b. HD 1; c. HD 2; d. HD 3.

3.3. Rheology

Rheological experiments showed that Carbopol-based suspensions exhibited non-Newtonian, shear-thinning properties (viscosity was inversely varied with shear rate). Even though it was less pronounced, shear-thinning properties were exhibited on suspensions containing cellulose derivatives (HPC, HPMC) at shear rates < 10 s^{-1} (Figure 4A). The results were in line with published data on brinzolamide-based suspensions (4). Compared to the placebo, MGF suspensions prepared from Carbomers showed better stress resistance (viscoelastic region shifted towards higher stress, Figure 4B) which was accompanied by higher storage modulus. The difference was more pronounced when reducing particle size and felt in the order: HD 3 < HD 2 < HD 1. At tested concentrations, HPC and HPMC-based suspensions did not show clear trends in storage modulus as a function of oscillation stress. The data imply that Carbopol-based suspensions exhibited more solid-like characteristics than suspensions stabilised by cellulose derivatives did. Suspensions containing Carbopol 974 also exhibited thixotropic properties evidenced by a restoration of dynamic viscosity as shown in viscosity vs. oscillation frequency plots (Figure 4C). The viscosity decreased drastically when the oscillation frequency jumped suddenly from 1 Hz to 30 Hz, and it restored the initial viscosity once the oscillation frequency dropped back to 1 Hz. Such results demonstrated a gel-like property of the suspensions, which ultimately inhibited sedimentation in the suspensions. The storage modulus was greater than the loss modulus at low oscillation frequencies implying the suspensions were a more solid-like system.

The time-independent structure profiles of suspensions confirmed that the suspensions exhibited a network consisting of secondary bonds rather than a chemically cross-linked system or a physically entangled polymer solution (7).

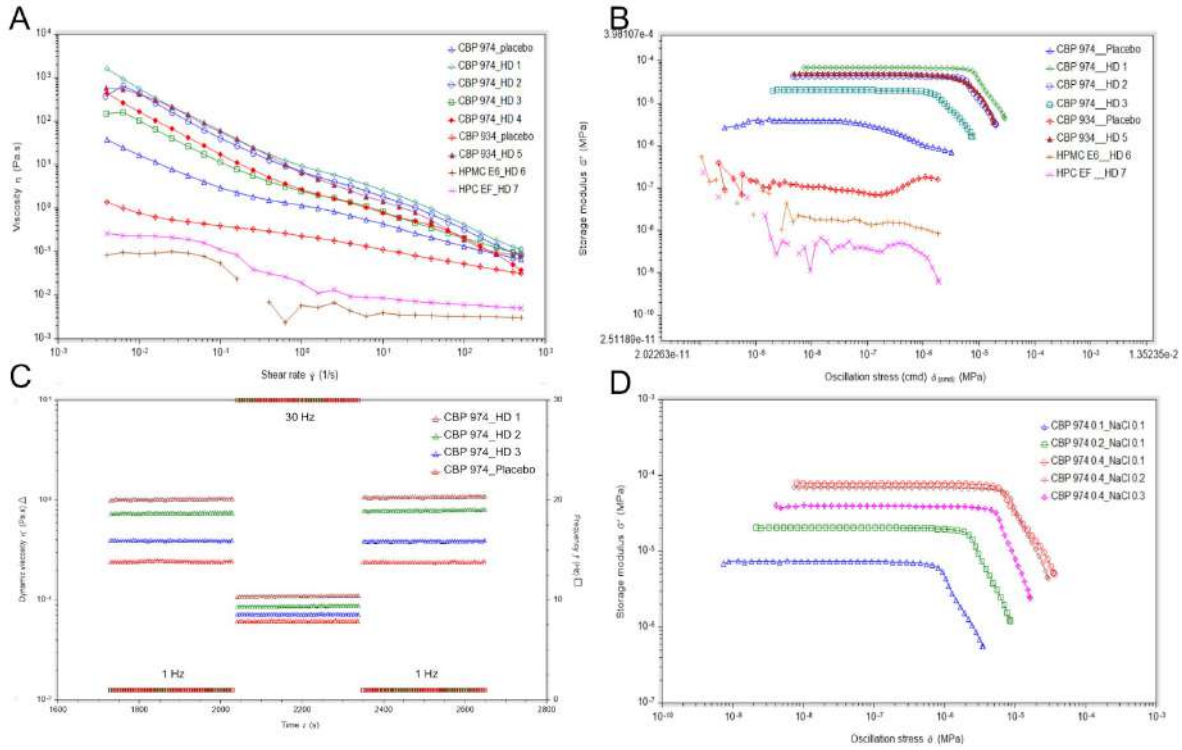


Figure 4. Rheological profiles of mangiferin suspension: A. Viscosity as a function of shear rate; C. Suspension viscosity response to oscillation frequency; B and D: Storage modulus profiles of suspensions as a function of oscillation stress at varying polymers and PSD of MGF (C) or varying Carbopol 974 P and NaCl concentrations (D). Notably, suspensions in (D) were prepared from submicron MGF particles and contained similar ingredients as per other formulations (Table 1).

Table 2 summaried the rheological properties of HD 1-7. Notably, although suspensions HD 1-3 were prepared from the same concentration of Carbopol 974P, the viscosity of HD 1 was the highest, followed by the viscosity of HD 2 and HD 3. This was potentially attributed to the difference in PSD of MGF that resulted in distinguished interaction with Carbopol as discussed in section 3.2: at a significant level of interaction, MGF particles act as gel stabilisers. It can also be inferred from data in Table 2 that Carbopol 974 exhibited higher interactions with MGF particulates than Carbopol 934 did (HD 1 vs HD 5).

Table 2. Summary of rheological properties of MGF suspensions

Sample	Viscosity (Pa.s)		Storage modulus (Mpa)	Tanδ	Yield stress (Mpa)
	Shear rate 0,063 (1/s)	Shear rate 10,000 (1/s)			
HD 1	84,923	2,585	$6,88x10^{-5}$	0,122	$7,72x10^{-6}$
HD 2	63,708	1,873	$4,44x10^{-5}$	0,137	$5,39x10^{-6}$
HD 3	17,180	0,837	$2,03x10^{-5}$	0,155	$1,54x10^{-6}$
HD 4	26,998	0,786	$2,04x10^{-5}$	0,138	$2,11x10^{-6}$
HD 5	94,595	1,416	$5,02x10^{-5}$	0,101	$7,24x10^{-6}$
HD 6	0,079	0,004	Unmeasurable		
HD 7	0,162	0,009			

The impact of concentrations of polymer and tonicity adjusting agent (NaCl) on the rheology of Carbopol-based suspensions prepared from submicron particles was illustrated in Figure 5A. At the same level of NaCl (0.1%), decreasing Carbopol 974 concentrations (from 0.4 to 0.1%) led to suspensions that possessed lower storage modulus (more liquid-like behaviour). Similarly, increasing NaCl concentrations from 0.1% to 0.3% while maintaining levels of Carbopol 974P resulted in less rigid suspensions. These changes can be attributed to the mechanism of Carbomer gel formation that is favoured by high polymer concentrations. On the other hand, Na^+ ion may affix onto polymer tangles that unfavours polymer-polymer interactions, thus reducing gel strength and viscosity (8). Such alterations will further possess influence on the homogeneity of drop weight upon drug dosing (4) (weight of dispensed suspensions from an ophthalmic bottle), as well as *in vivo* dissolution profiles. As NaCl is frequently introduced to adjust system tonicity and Carbopol-based suspensions prepared from different PSD of MGF exhibit distinguished viscosity, a tight governance of salt levels is required. That's said, non-ionic agents, such as mannitol or glucose, can be combined with NaCl for better monitoring osmotic pressure and suspension rheology.

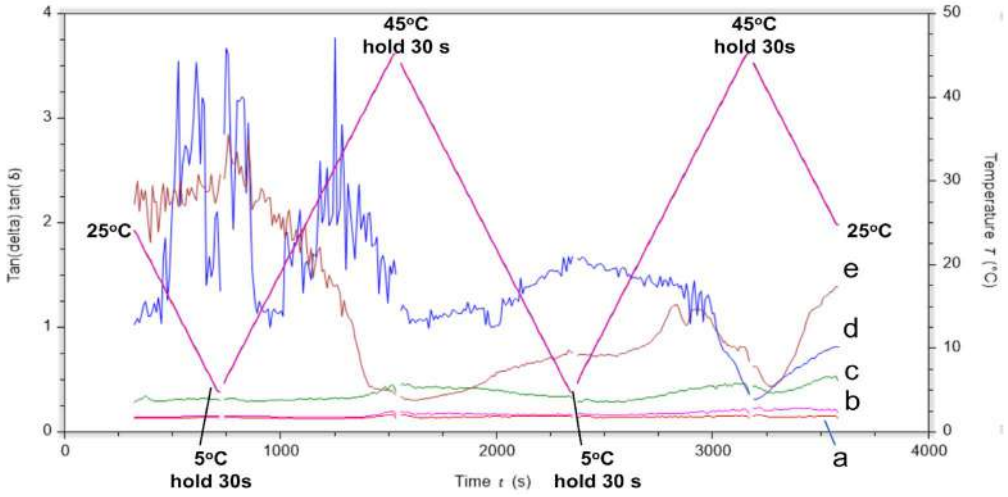


Figure 5. Impacts of temperature on rheological properties (tanδ) of suspensions stabilised by different agents: **a**. Carbopol 974 P 0.4%, NaCl 0.2%; **b**. Carbopol 974 P 0.2%, NaCl 0.1%; **c**. Carbopol 934 0.4%, NaCl 0.2%; Carbopol 974 P 0.4%, NaCl 0.2%; **d**. HMPC E6 1%, NaCl 0.1%; **e**. HPC EF 1%, NaCl 0.1%.

The roles of polymers as suspension stabilisers were under scrutiny by examining rheology in temperature stress. As presented in Figure 5B, two temperature ramps were applied to suspensions containing either Carbopol 974P, Carbopol 934, HPMC E6 or HPC EF. Data showed that Carbopol 974P exhibited the greatest stabilising ability, and tan δ (ratio of storage modulus/ loss modulus) of corresponding suspensions remained almost unchanged after multiple 5 ºC - 45 ºC cycles. At the same polymer concentration (0.4%), Carbopol 934-based suspensions showed less stabilising capacity compared to Carbopol 974 based-counterparts evidenced by a more obvious change in tan δ observed at the end of the second ramp, possibly due to the difference in hydration and microstructure (9). It can be inferred that, the effect of temperature on the physical states of Carbopol-based suspensions, if any, is reversible, and that suspensions can be handled at any temperature in the range 5 ºC - 45 ºC with minor or negligible changes in rheological properties. With minimal gel-like behaviour at test conditions, HPMC E6 and HPC EF based- suspensions exhibited drastic fluctuations in tan δ values, implying that the physical properties of these systems were heavily dependent on storage temperature.

3.4. *In vitro* dissolution

Three suspensions HD 1, HD 2, and HD 3 prepared from disctintive PSD were further examined for dissolution profiles. As can be seen in Figure 6 B, all formulations dissolved completely after 180

minutes. Three dissolution profiles can be distinguished from each other, however the trend was unexpectedly proportional to PSD. In particular, the dissolution rate in HD 1 suspension (prepared from submicron particles) was slower than that from HD 2 formulation (prepared from fine particles); the rate was fastest in HD 3 sample. The result herein could be explained by taking into account the rheological properties of tested samples that stemmed from the different binding affinity of MGF particles onto Carbopol tangles. Because MGF is fairly soluble at pH 7.4 (10), the rate of liberating MGF from polymer chains (that was impacted by particle-polymer interaction) is the rate-limiting step in suspension dissolution. HD 1 formulation exhibited the highest viscosity and yield stress (strongest interaction) thus requiring the most time to be dispersed completely in media. HD 3 suspension, on the other hand, presented as the lowest viscosity sample with the lowest yield stress (weakest interaction) and was quickly distributed into media at several minutes after stirring. The data illustrates that the proposed method was good enough to distinguish formulations with different PSD and that MGF dissolved from suspensions can be retained in the eyes after instilling.

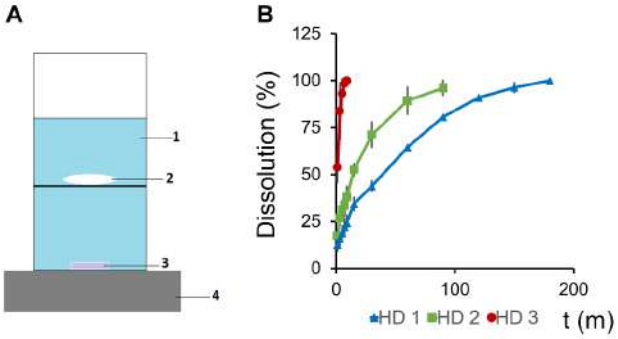


Figure 6. A. Illustration of dissolution apparatus: 1. Dissolution media; 2. Samples; 3. Magnetic stirring bar; 4. Magnetic stirrer. B. *In vitro* dissolution profiles of HD 1, HD 2 and HD 3 (*n = 3, mean ±SD*).

3.5. *In vivo* dissolution

As dissolution is closely associated with drug absorption, three formulations, HD 1, HD 2, HD 3 were further tested on experimental rabbits to probe *in vivo* dissolution. Data showed that tear fluid collected from rabbits treated with HD 3 suspension possessed the highest MGF concentrations compared to those administered with HD 1 or HD 2 suspension, the most significant difference was observed at 5 minutes. However, there was no difference in MGF levels in tear fluid after 30-minute administration albeit MGF retained in the eyes at least two hours post administration. Similar to the *in vitro* dissolution test, the discrepancy in *in vivo* dissolution profiles could be attributed to the distinctive rheological behavior of tested suspensions. HD 3 formulation exhibited the lowest viscosity and MGF can be released/ dissolved more effectively from polymer cluster upon eye blinking, leading to higher drug concentration in the tear. HD 1 suspension existed in a more solid-like material that limited MGF dissolving.

Collectively, the experimental results demonstrated that rheological properties, physical states, and dissolution of MGF suspension heavily depend on formula components, including but not limited to drug PSD, thickening agent, and tonicity modifier. Ophthalmic suspensions may need to be viscous for physical stability and eye retaining. They are also required to be less viscous for easy use and accurate dosing. As such, suspensions containing Carbopol 974 were more desired for further development of mangiferin ophthalmic suspensions.

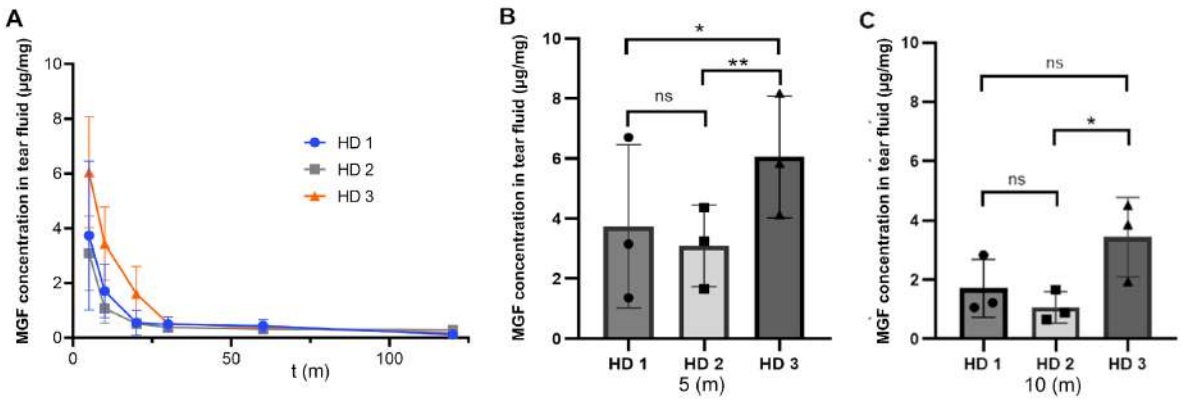


Figure 7. A. *In vivo* dissolution profiles performed on rabbits of HD 1, HD 2 and HD 3 suspensions. B. MGF concentration in tear fluid at 5 min post instillation. C. MGF concentration in tear fluid at 10 min post instillation. A matched two-way ANOVA analyse with Tukey's multiple comparisons - post hoc test was used to evaluate the difference among tested formulations and was processed on GraphPad Prism software, version 9.2.0; (*), $p < 0,05$; (**), $p < 0,01$; ns: not significant; n = 3; error bar: SD.

4. CONCLUSION

Mangiferin particle size, thickening agents and tonicity adjusting agents significantly impact on rheological properties, dissolution, and *in vivo* performance of the suspension.

5. ACKNOWLEDGMENT

Conflict of interest

The authors declare that they have no conflict of interest.

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Dissolution Improvement of Celecoxib by Wet Granulation

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ABSTRACT

Celecoxib is a nonsteroidal anti-inflammatory drug with high selective cyclooxygenase-2 inhibition. It is a Biopharmaceutical Classification System (BCS) class II drug with poor aqueous solubility, resulting in low oral bioavailability. This study aimed to improve the dissolution of celecoxib by wet granulation process. The dissolution of the drug in granules was improved using surfactant, β-cyclodextrin, and hydrophilic polymer at various ratios. The granules were studied in a dissolution test, Granules containing 34 mg of celecoxib were dissolved in 900 ml of phosphate buffer pH 7.4 and X-ray diffractometry was employed to evaluate the physical state of the granules. Results showed that the combination of β-cyclodextrin and PVP could significantly improve the dissolution of celecoxib in pH 7.4 phosphate buffer. The drug in the granules was in an amorphous state, as shown by the X-ray diffractogram. It also formed an inclusion complex with β-cyclodextrin and subsequently established ternary complexes through PVP, providing a marked increase in the dissolution of celecoxib, compared with the pure drug and the inclusion complex without PVP. With an appropriate ratio of beta-cyclodextrin and PVP in granulating liquid, the dissolution of celecoxib in granules could be improved. However, the insights into this complex system still need further investigation.

KEYWORDS: Celecoxib, β-cyclodextrin, hydrophillic polymer, dissolution enhancement, inclusion complex, ternary complex

1. INTRODUCTION

Celecoxib is a nonsteroidal anti-inflammatory drug with high selective cyclooxygenase 2 inhibition. Generally, it is used as an anti-inflammatory, analgesic, and antipyretic drug. Celecoxib is in a Biopharmaceutical Classification System (BCS) class II drug (Seedher et al., 2003) due to its poor aqueous solubility (3-7 µg/ml at 40◦C) (Paulson et al., 2001) resulting in low oral bioavailability (Fong et al., 2016). Celecoxib was found to be highly soluble in methanol and polyethylene glycol (PEG) 400, having a solubility of 113.94 and 414.804 mg/ml, respectively (Seedher et al., 2003). Many attempts have been made to improve celecoxib dissolution through formulation by various techniques. They include complexation with β-cyclodextrin (Rawat et al., 2004; Sinha et al., 2005), nanosuspension using D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) as a stabilizer (He, 2017); solid dispersion using polyvinylpyrrolidone (PVP) K 30 and K60 (Knopp et al., 2016), phosphatidylcholine (Jo et al., 2018) and Soluplus® (Mukesh et al., 2021) as carriers. In this study, the dissolution of celecoxib was improved in the formulation of granules prepared by wet granulation using single or combination of dissolution enhancers as granulating liquid.

2. MATERIALS AND METHODS

2.1 Materials

Celecoxib (Kekule Pharma Limited, Telangana, India), polyoxyl 40 hydrogenated castor oil (Kolliphor® RH40, BASF, Ludwigshafen, Germany), polyvinylpyrrolidone K30 (Nanhang Industrial Co., Ltd., Hangzhou, China), β-cyclodextrin (Cavamax W7, Wacker Chemical Corporation, Iowa, United States), absolute ethanol (GR grade, Duksan Pure Chemicals, Gyunggido, Korea), microcrystalline cellulose PH101 (Avicel® PH-101, Dupont Nutrition, Delaware, United States) and lactose monohydrate (DMV-Fonterra Excipients GmbH & Co. KG, Auckland, New Zeland), were used as received.

2.2 Granules preparation

Celecoxib (CEL) granules were prepared by using the solution of drug and dissolution enhancer(s) as granulating liquids. The compositions are tabulated in Table 1. Briefly, the drug was dissolved into polyoxyl 40 hydrogenated castor oil (HCO) or in the ethanolic solution of polyvinyl pyrrolidone K30 (PVP) and/or β-cyclodextrin to obtain clear solutions. Then, the solution was mixed with 1:1 microcrystalline cellulose-lactose monohydrate (KitchenAid, 5K5SS, Michigan, United States) for 10 min. The final ratio of the drug to the diluent in the mixture was kept at 1:70. After that, the resulting wet mass was passed through a standard sieve with mesh no.16 and dried in a hot air oven at 60°C for 30 min.

Table 1 Celecoxib granules formulations.

Compositions	Ratios
CEL : HCO : PVP	1:15:0, 1:0:15, 1:9:6, 1:6:9, 1:9:0, 1:6:0, 1:0:9, 1:0:6
CEL : PVP : β-cyclodextrin	1:15:0, 1:0:15 1:9:6, 1:6:9, 1:9:0, 1:6:0, 1:0:9, 1:0:6

In addition, the solid dispersion of the drug and selected dissolution enhancers, which provided promising dissolution results of granules, were prepared by dissolving the drug in the ethanolic solution of the dissolution enhancer (s) before evaporation of the solvent in the hot air oven at 60°C for 2 h.

2.3 Dissolution

The dissolution of celecoxib granules was tested in 900 ml of pH 7.4 phosphate buffer at 37 ± 0.5 °C, using USP dissolution apparatus II (Vankel® VK 7000, North Carolina, United States), with a paddle rotating speed at 50 rpm for 120 min. Then, the sample was taken at 5, 10, 15, 30, 60, 90, and 120 min and analyzed by UV-spectroscopy (Shimadzu Corporation, UV-1800, Kyoto, Japan).

2.4 X-ray diffraction

The crystallinity of the samples was characterized using an X-ray diffractometer (Rigaku Corporation, MiniFlex II, Tokyo, Japan). The granules or solid dispersion was gently ground using a mortar and pestle prior to packing in the sample holder and scanned over the 2θ range of 5° to 45° at 30 kV and 15 mA, with 0.05°/s increment.

3. RESULTS AND DISCUSSION

3.1 Dissolution

The dissolution results of celecoxib granules granulated with a single dissolution enhancer at the ratio of 1:15 can be ranked as HCO (94.4%) > PVP (58.0%) > β-cyclodextrin (29.7%). With toxicity concerns of too high amounts of each dissolution enhancer in the formulation, the combination of these

dissolution enhancers was proposed in this study. It was demonstrated that the granules containing the combination of celecoxib: HCO: PVP at the ratio of 1: 9: 6 and 1: 6: 9 gave 49.6% and 37.9% of drug release, respectively. The result, in fact, was lower than that of granules containing the same quantity of HCO or PVP (in the combination of celecoxib: HCO: PVP at the ratios of 1: 9: 0, 1: 6: 0, 1: 0: 9, 1: 0: 6) which gave 81.4%, 39.1%, 60.8%, and 59.3% of drug release, respectively (Figure 1-2). Accordingly, the combination of these two compounds did not help improve the drug release.

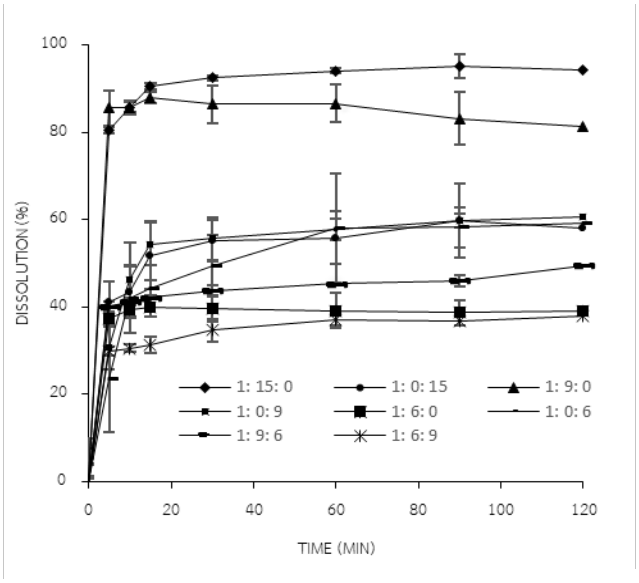

Figure 1. Dissolution results of celecoxib granules containing celecoxib : HCO : PVP at various ratios, 1:15:0, 1:0:15, 1:9:6, 1:6:9, 1:9:0, 1:6:0, 1:0:9, 1:0:6 ratios.

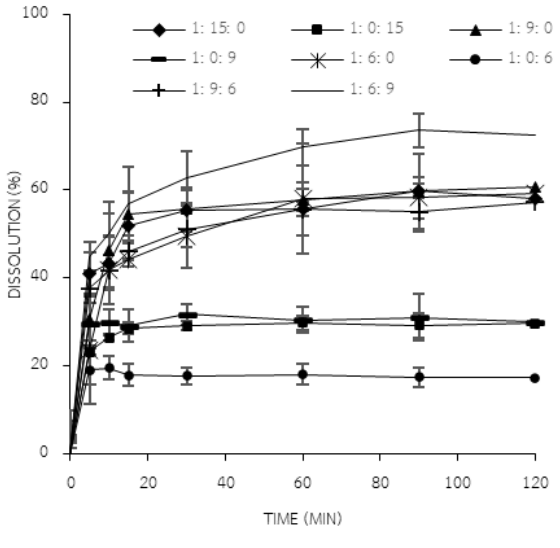

Figure 2. Dissolution results of celecoxib granule containing celecoxib : PVP : β-cyclodextrin at various ratios, 1:15:0, 1:0:15, 1:9:6, 1:6:9, 1:9:0, 1:6:0, 1:0:9, 1:0:6 ratios.

In contrast, the granules containing the combination of celecoxib: PVP: β-cyclodextrin at the ratio 1: 6: 9 provided a higher drug release (72.6%), compared with the granules having the same quantity of PVP or β-cyclodextrin. The granules of celecoxib: PVP: β-cyclodextrin at the ratios of 1: 6: 0, 1: 0: 9 showed 59.3% and 30.1% of drug release, respectively. The improved dissolution results indicated that there was a synergistic effect of PVP and β-cyclodextrin. However, the ratio of PVP and β-cyclodextrin is also a key factor in improving the dissolution of celecoxib. It was observed that the granules of the 1: 9: 6 ratio of celecoxib: PVP: β-cyclodextrin gave only 57.2% of drug release. The result was similar to that was determined for the granules having celecoxib: PVP: β-cyclodextrin at the ratio of 1: 9: 0, from which the drug release was 60.8%.

3.2 X-ray diffraction

The X-ray diffractograms of granules show mainly characteristics peaks of lactose monohydrate, while the diffractograms of other components could not be clearly observed (Figure 3). By analogy with solvent evaporation technique, the drying of granules allowed the drug and the dissolution enhancer to precipitate together and form solid dispersion. Therefore, in this study, solid dispersions of celecoxib: PVP: β-cyclodextrin at both ratios of 1:6:9 and 1:9:6 was employed to identify the solid state of the drug in granules. It was found that the characteristic peaks of celecoxib at $2\theta = 7.2$, 9.5, 10.3, 10.6, 12.6, 14.4 and 15.7° in solid dispersions and physical mixtures were not observed, relative to the diffraction pattern of β-cyclodextrin. However, the peak at $2\theta = 5.0°$ which was detected in the physical mixture, indicated crystalline celecoxib disappeared in the diffractogram of solid dispersions. This indicated that celecoxib was in an amorphous state in these samples.

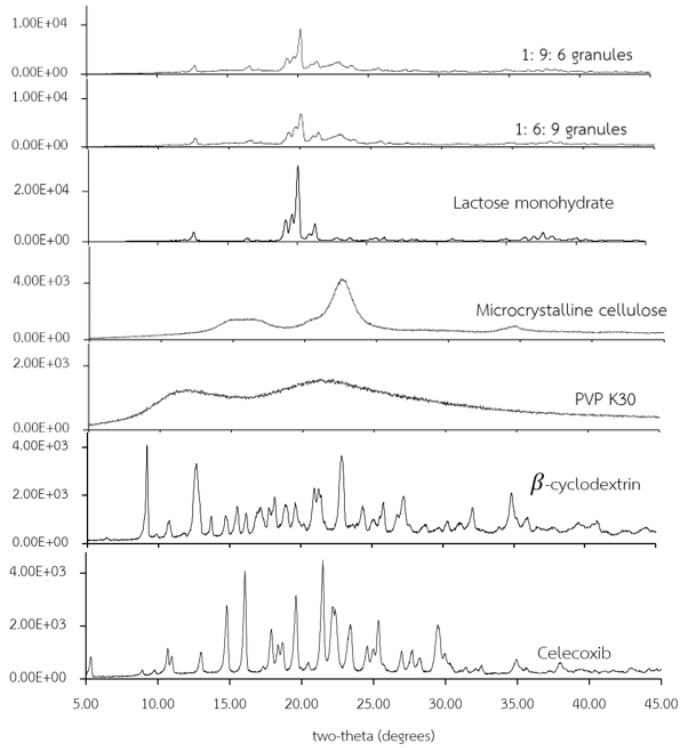

Figure 3. X-ray diffractograms of celecoxib, β-cyclodextrin, PVP K30, microcrystalline cellulose, lactose monohydrate, and granules of celecoxib: PVP K30: β-cyclodextrin.

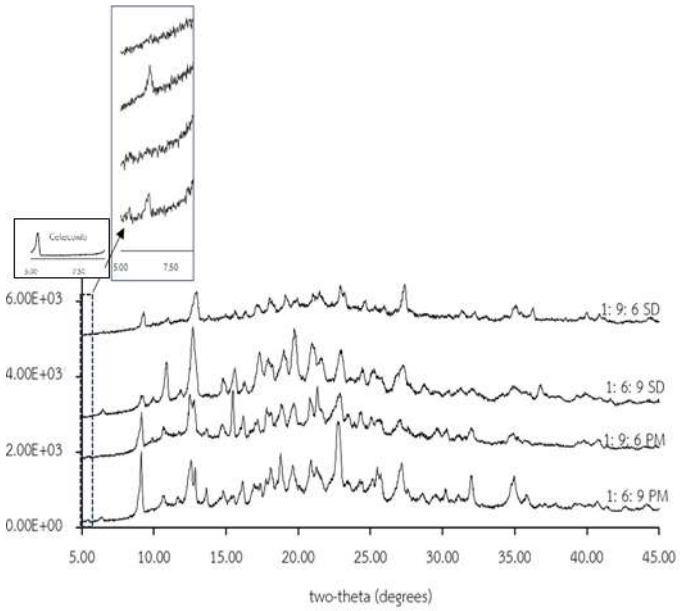

Figure 4. X-ray diffractograms of physical mix (PM) and solid dispersion (SD) of celecoxib: PVP K30: β-cyclodextrin.

The characteristic peak of β-cyclodextrin at 2θ = 12.51, 17.80, 19.60, 22.78, 24.33 and 35.88° showed relatively high peak intensity, and also the peak at 2θ = 6.3° could be detected only in the formulation of celecoxib: PVP: β-cyclodextrin at the ratio of 1:6:9 but not found in 1:9:6 ratio, which corresponds to the quantity of β-cyclodextrin. It is postulated that in this combination which higher

amount of β-cyclodextrin, there was an occurrence of inclusion complexes formed by binding of hydrophobic drug with cyclodextrin as well as ternary complexes of the drug-cyclodextrin-PVP, as previously reported by Bera et al.,(Bera et al., 2016). With a higher proportion of cyclodextrin in combination of celecoxib: PVP: β-cyclodextrin at the ratio of 1:6:9, these complexes could provide the marked increased dissolution of celecoxib (Rawat et al., 2004). . The mechanism was suspected that the inclusion complexes were formed by the hydrophobic drug binding in β-cyclodextrin which is the host molecule, (Manzoori et al., 2005) then the inclusion complexes bound with hydrophilic polymers (PVP). that can form the ternary complexes (Bera et al., 2016) which results in a marked increase in dissolution (72.6%) when compared to the inclusion complex without PVP K30 added, which the dissolution result is only 30.1%. Which is the stabilized effect of PVP that PVP can established greater complexation of drug and β-cyclodextrin by established superior electrostatic interactions and hydrogen bonding to form ternary complexes. (Bera et al., 2016)

4. CONCLUSION

In this study, dissolution of celecoxib could be improved by using the combination of celecoxib: PVP: β-cyclodextrin. The synergistic effect of PVP K30 and β-cyclodextrin was shown at an appropriate ratio due to the formation of inclusion complexes, together with the formation of drug-cyclodextrin-PVP ternary complexes. However, this system requires further investigation.

5. ACKNOWLEDGMENT

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Conflict of interest

The authors declare that have no conflict of interest.

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Evaluating impacts of formula and processing parameters on mechanical properties of microneedles for dermal/ transdermal drug delivery

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ABSTRACT

Microneedle is a viable approach to overcoming the skin barrier for dermal/ transdermal drug delivery with minimal invasion and painlessness. However, controlling microneedle quality, such as puncturing capability, geometric configuration, and drug release characteristics, remains challenging. This study aimed to evaluate the impacts of polymers, plasticizers, drug load, and pressure on the physical strength and skin-puncturing capability of in-house microneedles. Dexamethasone sodium phosphate and mangiferin were used as model drugs to prepare solution-based and suspension-based microneedles, respectively. Microneedles (height:base:pitch 600:200:500 µm) were prepared by solvent casting method using 15x15 array silicone-based pyramidical microneedle molds. The morphology and dimension of the prepared microneedles were examined by using a digital microscope. Microneedle hardness was tested using a CT-3 Texture Analyzer and skin piercing capability was evaluated on both parafilm-based simulated skin and porcine skin. Various polymers were subjected to screening and polyvinylpyrrolidone (PVP K30) was selected for fabrication of microneedle owing to its low viscosity at relatively high concentrations. The hardness and geometric configuration of microneedles were heavily dependent on formula parameters and vacuum pressure. The hardness of microneedles was significantly increased while their sharpness was compromised with the increase in polymer concentration. Plasticizer level posed reverse effects on the mechanical strength of microneedles. At 2% drug loading, suspension-based microneedles exhibited approximately a 50% increase in mechanical strength compared to that of the placebo, while solution-based microneedles did not show any significance. Lower vacuum pressure resulted in sharper and stronger microneedles. Experimental data exhibited that the microneedles containing 30% plasticizer, being processed with 40% PVP K30 solution, possessed sufficient strength to puncture both simulated skin and porcine skin with minimal change in their shape. Polymer, plasticizer ratio, drug loading, and vacuum pressure impacted significantly on the configuration, physical strength, and skin-puncturing capability of the microneedles.

KEYWORDS: Microneedle; Skin puncturing; Dermal drug delivery; Transdermal drug delivery; Physical strength.

1. INTRODUCTION

Transdermal delivery is a minimally invasive method of drug disposition bypassing the first-pass effect and reducing the risks of infection. However, permeation of drugs through the skin is limited by the resistance of the stratum corneum, the outermost layer of the epidermis. Enhancing drug transport across this barrier has been the central topic of topical pharmaceutics and delivery drugs via microneedles (MNs) is considered as one of the most efficient pathways. The vehicle has been established to efficiently pierce stratum corneum, thereby creating microchannels for drugs to be distributed locally or absorbed into the systemic circulation (11). The emergence of therapeutic MNs is illustrated by numerous clinical trials of utilising microneedles for delivering hormones, vaccines, anaesthetics delivery, and for dermabrasion (12).

Mechanical property and piercing capability are considered as two of the critical quality attributes that possess significant impacts on skin puncturing capability and *in vivo* drug distribution. Therefore, controlling these properties are crucial in delivering drugs accurately and effectively. The current study aimed to evaluate impacts of formula and process parameters on the quality of microneedles. Different microneedle arrays were prepared from PVP K30 and PEG 400 by a solvent casting technique, including drug-loaded MNs and their placebo (solution or suspension-based). The prepared MNs were characterised in terms of geometric configuration, mechanical strength, and skin-piercing capability. The experimental results provide fundamental understanding on development of microneedles for dermal/ transdermal drug delivery.

2. MATERIALS AND METHODS

2.1. Materials

Mangiferin, dexamethasone sodium phosphate (Vietnamese pharmacopeia V grade, Vietnam). Hydroxy propyl methyl cellulose E6 (HPMC E6, Methocel E6) was purchased from Ashland, USA. Polyvinyl alcohol 205 (PVA 205) (USP 41 grade) was purchased from Kuraray Inc., Singapore. Polyvinyl pyrrolidone K30 (PVP K30) (USP 41) was purchased from BASF GmbH, Germany. Microneedle moulds (height:base:pitch 600:200:500 µm, 15x15) were purchased from Smicna Pte. Ltd., Singapore. Parafilm PM992 was received from the USA.

2.2. Methods

- Dexamethasone sodium phosphate and mangiferin were used as model drugs to prepare solution-based and suspension-based microneedles, respectively.
- MNs were prepared by solvent casting technique using 15x15 array silicone-based pyramidical microneedle molds. The process was illustrated in Figure 1. Briefly, a polymer solution was loaded on MN mould. A vacuum was then applied to pull the formulation dispersion going deep into the channels. Any exceeding liquid was removed before a base formulation was added up on top. Samples were dried at room temperature for two days before drying at 60°C for another 12 hours.
- Morphology and dimension of the prepared microneedles were examined by using a digital microscope equipped with a digital camera (Eclipse Ci-L, Nikon, Japan).
- The hardness of the microneedles was tested with 3 x 3 needle array using a CT-3 Texture Analyzer (Brookfield, USA) in a “Normal test” mode.
- Skin piercing capability was evaluated on both parafilm-based simulated skin and porcine skin. Briefly, MNs were pressed firmly by a volunteer (approximately force ~30 N) onto an 8 layer-fold parafilm, or to porcine skin, and was held for 5; 10; 30 seconds. The morphology of MNs, parafilm layers and porcine skins were further evaluated by using a microscope. Porcine skin was stained with methylene blue to visualise before imaging.

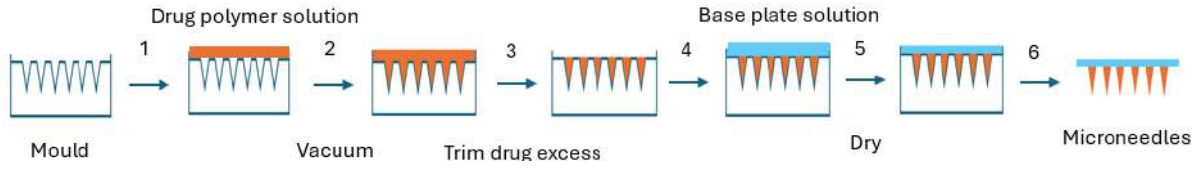

Figure 1. Microneedle fabrication by solvent casting method.

3. RESULTS AND DISCUSSION

3.1. Polymer screening

Experiment data showed that PVP K30 is the most soluble polymer in water and can be used to prepare high-concentration solutions (up to 70%). Although other hydrophilic polymers such as PVA

(H), PVA 205 and HPMC E6 possess good water-solubility (soluble at concentrations up to 20 - 30%), the obtained solutions were much more viscous than the PVP K30 solutions at the same concentrations. To fabricate MNs by solvent casting, low viscosity polymer solution is preferred, therefore PVP K30 was selected. Others are potential candidates to modify/enhance MN characteristics, such as physical strength, drug load, and drug release profiles… where required.

Table 1. Relative solubility of different polymers in water.

Polymer	20%	30%	40%	50%	60%	70%
PVA(H)	+	+				
PVA (205)	+					
HPMC E6	+					
PVP K30	+	+	+	+	+	+

+: formed clear solution after 12 hours of stirring at RT.

3.2. Impacts of processing parameters on MNs

Figure 1 illustrates typical stages of fabricating MNs by the solvent casting method. The impact of vacuum pressure in the 2nd stage on the MNs was evaluated. A solution containing 40% PVP K30 and 12% PEG 400 was chosen as a model formulation (for both needles and base plate). PEG 400 was employed to increase the physical stability of MNs. MN moulds containing polymer solutions and base plate were dried at room conditions (25°C, RH 40% - the 5th stage).

Observation the MNs prepared at different vacuum pressures (i.e. -0.04 bar; -0.07 bar and -0.1 bar), exhibited that needle shape, strength, as well as bubble formation were all influenced by vacuum levels. Images of MNs in Figure 2 showed that applying a high vacuum level (-0.1 bar pressure) resulted in MNs free of visible bubbles and the needles presented a highly degree of sharpness. At lower vacuum levels (-0.04 and -0.07 bar), MNs exhibited large air bubbles mostly located in the base plate, indicating formulation had not filled up the channels on the moulds. The presence of porous structure created by air bubbles did not allow us to perform the mechanical strength test of MNs as they were broken down when applying external force. Therefore, the highest vacuum condition was selected to fabricate MNs.

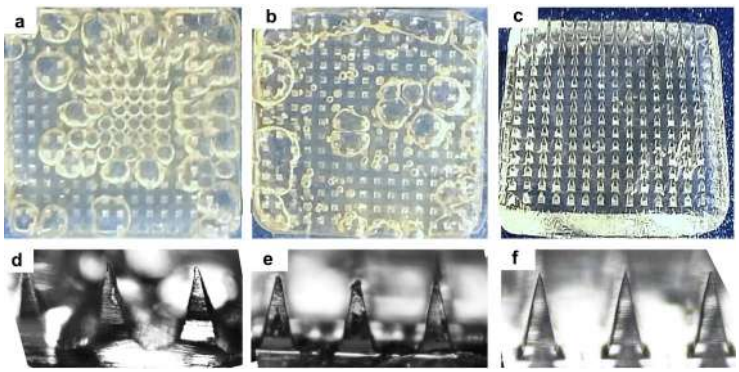

Figure 2. Impacts of vacuum pressure on the physical appearance of MNs: a, d: -0.04 bar; b, e: -0.07 bar; c, f: -0.1 bar. Notably, in d, the presence of bubbles caused light diffraction when taking images.

3.3. Impacts of formula composition on MNs

3.3.1. Influence of polymer and plasticiser concentrations

Once preparation, MNs containing only PVP K30 were stored in a silica gel contained box to prevent moisture adsorption. The needles were found fractured after several day storage. The collapse of needles caused by the lack of plasticiser which led to polymer rearrangement in the microstructure after water was removed. To solve the problem, PEG 400 was introduced in the formulation to stabilise

the physical state structure of MNs. The impact of PEG 400 ratio and PVP K30 concentrations in formulations on MN physical strength was further investigated by testing force resistance capability. MNs were prepared in 3x3 arrays and were tested on a texture analyser. Data were reported as work per needle (Figure 3b); an illustration of the force-travel curve was also presented in Figure 3a.

When the sensor of the instrument moved vertically at a slow speed, the compression force was increased as a function of travel distance and reached the highest point at the end of the travel. Interestingly, all the regression equations correlating force vs distance of tested formulations were polynomial (second order) with $R^2 > 0.99$, showing that the functions were continuous and that implied no sudden change in MN microstructure observed during force application.

Experiments showed that polymer and plasticiser concentrations had impacts on the mechanical strength of prepared MNs illustrated by the endpoint of the force curve. This was also pronounced by comparing the average work required per needle during force compression (Figure 3b). At tested PVP levels, increasing PEG 300 concentration from 10 to 20% (relative to PVP mass) led to a reduction in strength (defined by work required), but the change was less significant when the PEG level was 30% of polymer mass. There was also a trend in increasing force resistance when the polymer level was increased, however, this change was not significant. The result could be attributed to the hygroscopic property of PVP K30 (and PEG 400). The relative humidity at tested conditions was maintained at ~70%, reflecting common environmental conditions in Vietnam, however, this was high enough to compromise needle strength (13). Nonetheless, the impact of polymer and plasticiser levels can be illustrated by the shape of the needlepoint after force application in Figure 4.

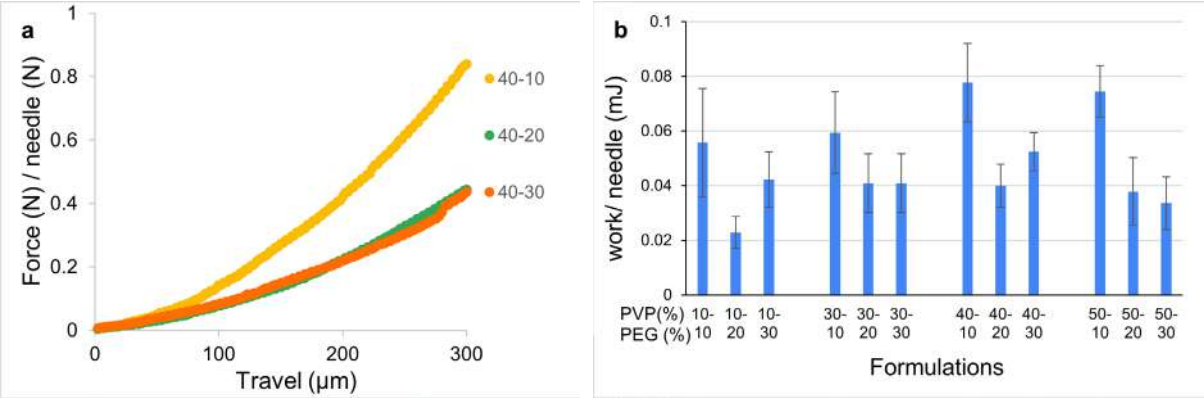

Figure 3. a. Illustration force applied per needle as a function of travel; b. Average work per needle, from at least 2 arrays of 9 x 9 needles. Notably, the ratio of PEG was presented as percentage of PVP mass.

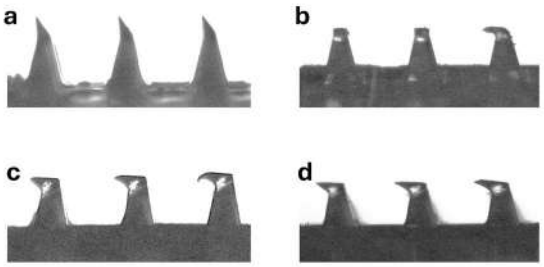

Figure 4. Illustration of MN morphology after physical strength test upon formula variation: a: 30% PVP, 3% PEG; b. 30% PVP, 9% PEG; c. 40% PVP, 4% PEG; d. 40% PVP, 12% PEG. Notably, the ratio of PEG was presented as percentage of PVP mass.

3.3.2. Impacts of drug load

The impact of drug load on MN properties was investigated by evaluating alterations in mechanical strength of solution-based MNs (containing dexamethasone phosphate, 2%) and suspension-based MNs (containing mangiferin 2%, D_{10} = 0.825 µm; D_{50} = 2.54 µm; D_{90} = 5.52 µm). A formulation of 10% PVP K30 and 1.5% PEG 400 was used to minimise the impact of moisture absorption.

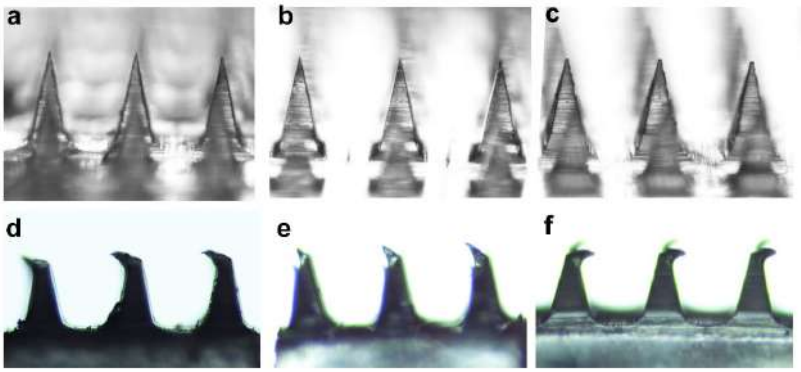

Figure 5. Microscopic images of placebo MNs (a, d); dexamethasone-loaded MNs (b, e) and mangiferin-based MNs (c, f) before and after force applied.

Figure 5 presents the morphologies of the prepared MNs under microscopes. Examination on individual microneedle shape showed that the suspension-based MNs were less sharp at the tips, while the solution-based MNs were relatively sharper and were similar to that of the placebo. The physical strength test showed no significant change in force - travel curves of these MNs meaning needles were only bent at the top without fracture. The average work required per needle during 0.3 mm travel was 0.035 ± 0.013 mJ (blank MNs); 0.035 ± 0.011 mJ (solution-based MNs); 0.048 ± 0.012 mJ (suspension-based MNs). This result indicated that the presence of mangiferin in polymer solution (suspension form) could potentially increase the physical strength of MNs.

3.4. Evaluation of skin piercing ability of fabricated MNs

MNs should have sufficient physical strength to overcome skin barriers, most importantly stratum corneum, before drug can be liberated. Herein, two models were employed, namely parafilm-based simulated skin and porcine skin, to test mechanical strength of our in-house MNs. Microscopic observation of skin and needle was used to characterise the insertion capability of MNs.

3.4.1. Using a parafilm base-simulated skin model

Figure 6a, b illustrates typical morphologies of MNs (40% PVP; 12% PEG) before and after being pressed onto 8 layers of parafilm, in a 30% humidity room. Needles could pierced three parafilm layers (about 300 µm in depth) under an approximate 30N press (14). Significantly, the needles were intact after the test. These data showed that the MNs were strong enough to pierce parafilm layers with minimal change in morphology, and a low humidity condition was necessary to perform insertion studies of PVP-based MNs.

Figure 6d-f presents three top parafilm layers with hole structures created by MNs. The hole size in each layer can be employed to evaluate the homogeneity of needles. This also showed that the parafilm model can be applied to distinguish MNs possessing different mechanical strengths before performing skin insertion tests .

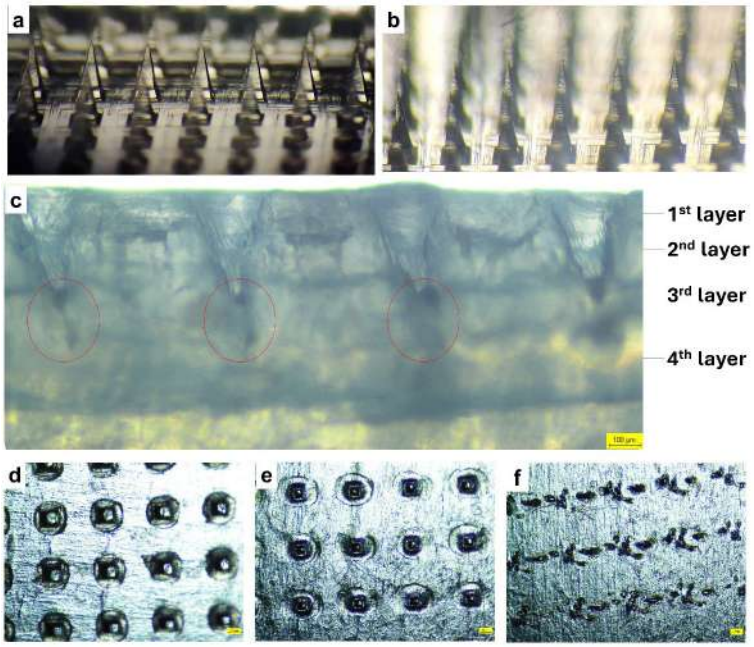

Figure 6. Microarray before (a) and after (b) parafilm piercing assay; c-f: parafilm-simulated skin after microneedle application: c. from the side view (red ovals denote area were affected by needles when applying force); d-f. from the top view of 1st, 2nd and 3rd layer.

3.4.2. Using a porcine skin model

The MNs used for the parafilm-simulated skin test (Section 3.4.1) were then employed for the insertion study on porcine skin in a 30% RH condition (Figure 7). MNs could pierce the skin creating holes that were visible even after 5-second insertion. As shown in Figure 7e, pierced holes appeared as white area that were not stained by methylene blue. This could be attributed to the high surface tension of the methylene blue solution that delayed itself from diffusing into microchannels created by applying MNs. Nonetheless, by gently pulling the solution into such holes, we managed to capture microchannels created by MNs in Figure 7f, insertion depth was found to be approximately 300 µm.

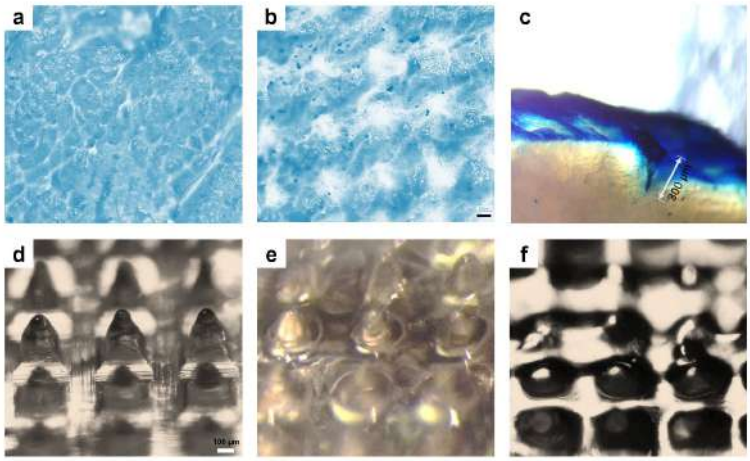

Figure 7. Representative images of the surface of control, untreated skin (a) and microneedle inserted skin (b) after staining with methylene blue (30-second insertion, white area referred to pierce points by needles); c. illustration of a microchannel created by one needle after insertion. Microneedle morphology after different insertion times: d. 5 seconds; e. 10 seconds; f. 30 seconds.

No fractured or bent needle was found after applying MNs on the skin. Such results sugested that the MNs possessed sufficient mechanical strength. Further, needles were quickly dissolved upon skin piercing stemming from the highly soluble property of PVP K30: all the needles were blunt after 5-second insertion, and most of them disappeared after 10 or 30-second insertions. Compared to the parafilm-simulated skin model, the porcine model could better simulate the *in vivo* conditions where the water in the skin (~65%) accounted for dissolving MNs. The water solubility of polymers should be carefully considered when formulating MNs. The experimental results demonstrated that the PVP-based MNs herein could be further developed for dermal and transdermal drug delivery. Both piercing tests could be utilised for MN characterisation.

4. CONCLUSION

This study proposed a methodology for efficiently testing the mechanical strength of microneedles, which could be utilised during the development of therapeutic microneedles. Vacuum pressure and composition of MNs (i.e. polymer, plasticiser ratio, and drug loading) possessed significant impacts on the configuration, physical strength, and skin-puncturing capability of the microneedles.

5. ACKNOWLEDGMENT

Conflict of interest

The authors declare that they have no conflict of interest.

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PD-0102113-P

Halal by Design Verification of Raw Materials and Evaluation of Emulgel from Clove Oil (*Syzygium aromaticum L*)

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ABSTRACT

Eugenol is the main component of clove oil which has the potential as an anti-inflammatory agent. (1) Background: To improve comfort and effectiveness of use, clove oil is developed into an emulgel preparation. This study aims to develop a clove oil emulgel formulation as an anti-inflammatory with a Halal by Design approach; (2) Methods: The clove oil emulgel was prepared using the fusion method. Raw materials used in the emulgel formulation were ensured to have documents to ensure no contamination from prohibited substances. (3) Results: The research results show that the preparation is milky white, has a thick liquid texture, with a pH of 4.44 which is suitable for facial skin pH. The average minimum content test is 8.85, with no container weighing less than 90%. It exhibits pseudoplastic flow properties, which are not yet ideal, consistent spreadability at the eighth minute, and adhesive properties of 78.7 seconds, indicating that the longer the emulgel adheres to the skin, the more substances are absorbed. (4) Conclusions: Based on the research results, it can be concluded that the emulgel formulation with clove flower essential oil shows good results and meets the quality requirements of the preparation according to the compendia.

KEYWORDS: Anti-inflammatory; Halal; Formulation; Clove oil; Emulgel; *Syzygium aromaticum*

1. INTRODUCTION

Halal products are all types of objects made from elements that are permissible according to the Shari'a, so they can be used, whether for consumption, usage, or daily use.1 Consumer protection for the availability of halal products in Indonesia is guaranteed by Law (UU) Number 33 of 2014 concerning Halal Product Guarantees (JPH). Business actors are required to carry out certification no later than October 17 2024 as stipulated. However, the implementation of the JPH Law is still not running perfectly due to bureaucratic problems. The formulation of a drug is very complex, and the Ministry (of Health) is not yet ready to see whether there are halal or haram elements in a drug[1,2].

Halal medicine is one of the standards that need to be met, considering that Indonesia is predominantly Muslim. Technological developments in the drug manufacturing process are now increasingly advanced and make consumers unaware of the content of medicinal ingredients on the market, therefore it is necessary to pay attention to the critical points of halal medicine, such as: ensuring the halalness of the active ingredients, excipients and auxiliary ingredients used; production facilities used specifically for halal products only; there is no chance of being mixed and contaminated with haram materials; halal packaging materials used; carry out the process of cleaning and laundering equipment according to sharia, as well as allowing halal auditors to carry out the process to be audited directly and determine its halalness. The critical point of product halal can be a reference in producing halal products before submitting the halal product certification process to BPJPH. If the pharmaceutical industry says that as long as a substance is a haram it is a good medicine and its use can still be tolerated, it cannot be corrected, because "as long as there is a substance that has the same properties as the haram

substance, then the substance is still declared haram", perhaps this is the principle that must be applied in enforcing the JPH Law on medicinal products.

Inflammation is a normal protective response to tissue injury caused by physical trauma, damaging chemicals, or microbiological substances. Signs of inflammation are redness, swelling, heat, pain, and loss of function[3]. Anti-inflammatories are drugs that can eliminate inflammation caused by non-microorganisms. The principle of anti-inflammatory action is to inhibit the action of enzymes that cause the inflammatory process to occur[4]. Synthetic drugs commonly used as anti-inflammatories are NSAIDs (Non-Steroidal Anti-Inflammatories) which generally have the side effect of gastric ulcers[5]. Therefore, it is necessary to look for innovations for anti-inflammatory treatments with relatively fewer side effects, for example, drugs made from plants.

Clove (*Syzygium aromaticum* L) grows widely in Asian countries such as China, Sri Lanka, and Indonesia. Parts of the clove plant such as leaves, flowers, and stalks contain essential oil with the main component of the phenol group, namely eugenol[6]. Clove essential oil with the active ingredient eugenol acts as an anti-inflammatory, with the mechanism of inhibiting prostaglandin synthesis and neutrophil chemotaxis[7]. Eugenol in clove oil has the potential to suppress the action of NF-kB, which is one of the receptors in the inflammatory pathway[8]. Eugenol exhibits the same anti-inflammatory effects as COX antagonists (indomethacin)and a selective COX-2 antagonist (celecoxib)[9].

Oil-based preparations are felt to be less effective and less comfortable for patients; therefore, to adjust the comfort and aesthetics of oil-based formulas, emulgel dosage forms are made. Emulgel is a semisolid preparation that has quite good stability because the stability of the emulsion is increased by the addition of a gelling agent[10]. These considerations refer to the physical stability of the compound and preparation when it becomes the final product, and emulgel for skin for dermatological use have several favorable properties such as being thixotropic, non-greasy, easy to spread, removable, emollient, non-staining, long shelf life, environmentally friendly, transparent and pleasant appearance[11].

2. MATERIALS AND METHODS

2.1. Tools and materials

The tools used in this research are a water bath, analytical balance (Ohaus CP 214), pH meter (Ohaus ST 300G), refractometer (Abbe DR-M4), viscometer, magnetic stirrer, TLC plate, chamber glass, spreadability test equipment, minimum content test equipment.

The materials used in this research were: clove flower essential oil, eugenol (Ghimas), cera alba (Green Pharmacy Indonesia), liquid paraffin (Merck), span 80 (Merck), tween 80 (Merck), glycerin (Smart Lab Indonesia), benzoic acid (Smart Lab Indonesia), distilled water (Brataco), and CMC Na (Merck)

2.2. Raw Material Verification

Verification of raw materials is carried out using 4 parameters, namely documents on the halalness of the raw materials used to prepare emulgel. The next parameter is verification of the clove oil used, including organoleptic, refractive index, specific gravity, and solubility in 70% ethanol, the results of which will be compared with the Indonesian National Standard (SNI).

2.3. Evaluation of Finished Products

Evaluation includes 4 parameters for emulgel preparations, namely organoleptic test, pH test[12], viscosity, and minimum content tests. Organoleptic tests include shape, smell, and color[13]. The pH test is carried out to see the acidity level of the cream preparation to ensure the cream preparation does not irritate the skin and ensures the stability of the active substance and the effectiveness of the preservative. The pH of a topical product is said to be safe, namely if it is still within the neutral pH range or following the skin's pH[14]. This viscosity test aims to determine the viscosity level of the cream preparation[15].

3. RESULTS AND DISCUSSION

3.1. Raw Material halal documents

The results of the halal verification are shown in Table I

From Table I, it can be concluded that the clove oil emulgel raw material that we make has HALAL results

Table I. Halal Verification Results

Raw material	Halal Documents	Results	Source
Clove oil	Halal Certificate	Halal	KMA No. 1360 of 2021
Cera alba	Halal Certificate	Halal	KMA No. 1360 of 2021
Liquid paraffin	Halal Certificate	Halal	KMA No. 1360 of 2021
Span 80	Manufacturer COA	*Subhat*	List of Food Additives and Their Halal Status
Tween 80	Manufacturer COA	*Subhat*	List of Food Additives and Their Halal Status
Glycerin	Halal Certificate	Halal	Halal Watch Word
Benzoic acid	Halal Certificate	Halal	KMA No. 1360 of 2021
Aquadest	Halal Certificate	Halal	LPPOM MUI
CMC Na.	Halal Certificate	Halal	KMA No. 1360 of 2021

3.2. Verification of Clove Flower Oil Ingredients

Verification of raw materials for clove flower oil is carried out to ensure the quality and purity of the clove flower oil used[16]. Verification of clove flower oil ingredients refers to the SNI for clove flower oil including organoleptic tests, refractive index, specific gravity, and eugenol content. The results of the clove flower oil verification test can be seen in Table 2.

Table 2. Verification results of clove flower oil

No	Test Name	Results	SNI
1.	Organoleptic	Form: solution; Color: clear yellow; Smell: typical of cloves	Form: solution; Color: clear yellow; Smell: typical of cloves
2.	Refractive Index	1.5298/0.1896 g/mL/RSD	1.529-1.537 g/mL
3.	Solubility	Soluble in 70% ethanol by comparison oil: 70% ethanol = 1: 2	1:2
4.	Specific Gravity	1.0347	1.04-1.07
5.	Analysis of levels using TLC densitometry	83% Eugenol in clove flower essential oil	80-95%

Organoleptic oil quality testing is carried out by observing color, shape, and odor. Refractive index testing was carried out to see the quality of clove flower essential oil. In this study, the refractive index value of clove flower oil was obtained which met the requirements for the refractive index of clove flower oil according to the SNI 06-4267-1996. The clove flower oil solubility test was carried out to see the solubility of the oil in 70% ethanol. The solubility test results showed the solubility of clove flower oil in 70% ethanol in a ratio of 1:2, following SNI. Specific gravity is often used as a standard for essential oils on the market, one of which is clove flower essential oil. Clove flower oil itself is widely circulated in Indonesia, so the quality of clove flower oil has been determined in SNI. Therefore, products that use clove flower oil as their basic ingredient need to carry out oil quality testing, one of which is the specific gravity to ensure the quality and purity of the clove flower oil used. The specific gravity test is a test carried out by comparing the weight of oil at the same volume and temperature,

namely 15°C. According to SNI, clove flower oil has a specific gravity of 1.04-1.07. The specific weight value of clove flower oil in the test carried out was 1.0347. This result shows that the clove flower oil sample used did not meet the clove flower oil standards set by SNI

The qualitative test to analyze the presence or absence of eugenol compounds in essential oils is checked using the thin-layer chromatography method. Based on the provisions in the Indonesian Herbal Pharmacopoeia Edition II[17], to identify eugenol from clove flower essential oil, use a stationary phase in the form of a silica gel GF254 plate and a mobile phase in the form of Toluene: Acetone with a ratio of 10:2. Qualitative testing was carried out using the KLT method using the appropriate mobile phase and stationary phase, by spotting the replication sample 3 times and also the eugenol standard, the Rf value of the standard was 0.75. It is known that the Rf value for replication samples 1, 2, and 3 is 0.75. This indicates that the clove flower essential oil sample contains eugenol compounds because it has the same Rf value as standard eugenol. Testing for eugenol content in essential oil samples can be determined using the densitometry method, by drying the KLT plate that has been eluted with the mobile phase, and then reading the AUC value using a tool in the form of a densitometer. It was found that the average eugenol content of the 3 replication samples was 83%, with a CV value of 31.08%. It can be interpreted that this value is not good because a good CV value is less than 5%. A high CV value indicates that the measurement data obtained is very varied and not uniform.

3.3. Product Testing

Testing of product quality is carried out to see the success of the emulgel product being made. Table 3 shows the results of the quality tests of the preparation, including pH, viscosity, and minimum content tests.

Table 3. Test results on the quality of emulgel products.

No	Test Name	Condition	Results
1.	pH	4.5-6.5 (FI VI, 2020)	4,54
2.	Viscosity	6000-50000 (SNI Emulgel)	21199.75/30.0175 (cPs/RSD)
3.	Minimum Content	Average contents of 10 containers in stage 1 testing, Not less than equal to 90% of the dosage label claim	99.34 % of label claim

The pH test is carried out with the help of a special pH meter for semisolid preparations. The pH test is carried out by calibrating with pH 7 and 10 first. Then a pH test was carried out on the semisolid preparation (emulgel) and the result was 4.01, so the pH of this preparation was not in the range. It is known that the pH of skin preparations is between 4.5-6.5[18] because if it is too acidic, it will irritate the skin and if it is too alkaline it will result in dry skin. It is possible that the pH of the emulgel preparation is not within the range or is too acidic due to the ingredients in the formulation. Viscosity is a preparation test to see the resistance to flow of a system so that if a fluid has a greater viscosity, the greater force is required by the fluid to flow[18]. According to SNI 16-4399-1996, the viscosity value of a good emulgel preparation is 6000-50000 cPs. The viscosity test on the clove flower oil emulgel preparation had an average value of 21199.75 cPs/30.0175 RSD, these results indicate that the viscosity of the emulgel preparation meets the requirements set by SNI. However, the RSD results show that the emulgel viscosity has a high value, meaning that the emulgel preparation is not homogeneous.

The minimum content test is carried out after the emulgel preparation has been put into the container. In the minimum test with a net weight of 5 grams, 10 containers were taken at the initial stage[19], if the average result is not less than 90% of the label then it meets the requirements. The average yield we obtained from 10 containers was 99.34% of 5 grams or 4.967 grams.

4. CONCLUSION

The results of the research carried out can be concluded that clove oil emulgel uses halal ingredients so that it obtains halal results using the method halal by design.

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Conflict of interest

"The authors declare that they have no conflict of interest.

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Effects of Internal Structure on Buoyancy and Drug Release of Customized Control Release 3D Printed Floating Tablet Containing Levodopa and Carbidopa

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ABSTRACT

Levodopa, a narrow absorption window drug, is the first-line treatment for Parkinson's disease. It is usually taken with carbidopa to minimize its peripheral metabolism. Additionally, individual-based dose adjustment is required throughout the treatment. This study aimed to fabricate 3D printed floating tailorable tablets containing levodopa and carbidopa, as well as to investigate the effects of hollow volume (HV), infill percentage (IP), and wall thickness (WT) on buoyancy and drug release. Dual drug-loaded filaments were prepared using a single-screw hot melt extruder. Hollow 3D tablets with various HVs, IPs, and WTs were designed and then fabricated by using a standard 3D fused deposition modeling printer. The mechanical strength of filaments was characterized by using a CT3 texture analyzer. The thermal stability and polymorphism of formulations were characterized by thermogravimetric analysis, differential scanning calorimetry, and powder X-ray diffraction. The buoyant force was measured using the resultant weight method. Drug content and dissolution in the simulated gastric fluid of the two drugs were analyzed simultaneously by HPLC. Both levodopa and carbidopa were thermally stable and partially amorphized during the process. The filaments were uniform regarding drug load (57.67 ± 4.42%) and diameter (1.716 ± 0.008 mm). The bending resistance force (4.097 ± 0.082 N) was sufficient to facilitate a smooth 3D printing process. All prepared printlets possessed a great buoyant force (238.55 – 823.23 µN) and the drug release was controlled for up to 10 hours. The buoyant capacity and drug release were co-variant with HV, while they were inversely variant with IP and WT. This study demonstrated the capability of developing tailorable floating tablets containing levodopa and carbidopa. The dose, drug release, and buoyancy of the dosage forms could be customized based on personalized therapeutic needs of patient for treatment of Parkinson's disease.

KEYWORDS: Melt extrusion; three-dimensional printing; levodopa; carbidopa, floating drug delivery system.

1. INTRODUCTION

Levodopa is the gold-standard treatment for Parkinson's disease. It is prescribed to treat all stages of the disease[1]. Oral levodopa posed pharmacokinetic limitations, such as poor bioavailability and short half-life, stemming from its peripheral metabolism and narrow absorption window[2]. Levodopa is mainly absorbed in duodenum and jejunum, facilitating by active transportation for large neutral amino acids[3]. There are two popular strategies to enhance the bioavailability of the drug, namely, dosing in combination with a catechol-O-methyltransferase and formulation in gastroretentive drug delivery systems (GRDDS)[4–8]. Residing dosage form in the stomach would allow drugs release gradually into the upper part of the small intestine which is the optimal absorption site of levodopa[9].

Among the various explored GRDDS, floating drug delivery systems (FDDS) are the most practical approach since they do not hinder the physiological activities of the gastrointestinal system. The dosage form could float based on either low density (zero lag time) or bubble generation (with lag time). Zero lag time formulations are more advanced than the others because they could minimize the risk of being unable to refloat after submerging. Foam and hollow structures are usually utilized to formulate immediate FDDS. To formulate immediate FDDS, an air compartment can be incorporated within the system, which is hardly achieved by using conventional pharmaceutical technologies. Such complex structure, however, may be generated by using 3D printing technology [10–14].

3D printing, or additive manufacturing, enables the fabrication of three-dimensional objects by depositing material layer by layer in a defined path according to the designed models. Among the 3D printing techniques, fused deposition modeling (FDM) is the most popular due to its relatively affordable cost, simplicity, and accessibility[15]. This technique uses polymeric filaments containing active ingredients, which are often prepared by melt extrusion, as feedstocks. During the 3D FDM process, the filament tip is typically heated to a molten state and then pushed through the nozzle of a 3D printer. A prominent advantage of the technology is its capability to fabricate customizable dosage forms for personalized treatment [10,12].

This study aims to fabricate customizable floating printlets containing levodopa and carbidopa by conjugating hot melt extrusion and 3D printing technology. Additionally, the effects of the internal structure, HV, IP, and WT on buoyancy and drug release were explored.

2. MATERIALS AND METHODS

2.1. Materials

Levodopa (LD) was purchased from Zhejiang Wild Wind Pharma, China. Carbidopa (CD) was purchased from Divis' Laboratories, India. Klucel LF (hydroxypropylcellulose, HPC) was gifted from Ashland, USA. Methanol HPLC grade was purchased from ThermoFisher, USA. Other chemicals were the salts, used for the preparation of the dissolution medium and HPLC mobile phases, were analytical grade.

2.2. Thermal analysis

2.2.1. Thermogravimetric analysis

The raw materials, physical mixtures, filaments, and printlets were subjected to thermogravimetric analysis (TGA) by using a TGA/DSC 1 system (Mettler Toledo, Switzerland). The system was purged with nitrogen gas at a flow rate of 30 mL/min. Samples weighing 5–10 mg were loaded into zirconia cups and placed onto the sample position of the system. The samples were then stabilized at 25 ºC for 2 minutes before heating to 300 ºC at a rate of 10 ºC/min.

2.2.2. Differential Scanning Calorimetry

DSC experiments were performed using DSC 1 StarSystem (Metler Toledo, Switzerland). Samples weighing 4–5 mg were transferred into aluminum pans and hermetically sealed. Subsequently, they were placed in the heating and an inert environment was maintained by purging nitrogen at a flow rate of 20 mL/min. Samples were stablized at 25 ºC for 2 minutes, before heating to 350 ºC at a rate of 10 ºC/min.

2.3. Preparation of filaments using hot melt extrusion (HME) technology.

Dual drug-loaded filaments were prepared by using a single-screw extruder equipped with a 1.7 mm circular die opening, a filament winding motor, a volumetric feeder, and a conveyor. Materials were first sieved separately through a #35 mesh sieve. Physical mixture of HPC, levodopa, and carbidopa with a ratio of 50:40:10 was weighed and mixed using a mortar and pestle to form a

homogenous mixture. The extruder was heated to 120 ºC and further equilibrated for 15 minutes. Screw speed and conveyor belt speed were set at 36 rpm and 42 cm/min, respectively. Subsequently, the homogenized physical mixture was fed to the extruder at a constant rate of 96 mg/min. The formulations were softened inside the barrel under the effect of heat. The molten mass was conveyed forward and pumped through the die. Once going out of the die, the extruded strands were cooled and solidified at room temperature. They were collected and stored in a desiccator at room temperature of 25°C to avoid any water sorption.

2.4. Mechanical characterization of filaments

The mechanical strength of the filament was evaluated using a texture analyzer (Brookfield CT3, USA) equipped with an adjustable three-point bend test or compression test module. The instrument allows to measure both horizontal and vertical deformation force of the filaments.

2.4.1. Three-point bend (3PB) test

The mechanical strength and brittleness of the extruded filaments were evaluated using Repka – Zhang method [16]. The circular filaments were cut into 5.0 cm-long segments and placed on the holder with a 2.5 cm gap. The probe was moved to the top of the filaments and pushed down at a speed of 1 mm/s. The instrument started recording when reaching the trigger force of 0.05 N and stopped at a distance of 10 mm. The test was repeated 10 times.

2.4.2. Compression test

Compression tests that simulate the feeding process of the filament through the printing head were. The filaments were cut into 5.0 cm-long segments and placed vertically on the sample support. The probe was moved down and compressed filament at a speed of 0.1 mm/s. The instrument started recording once the trigger force of 0.05 N reached and stopped at a distance of 2.0 mm. The test was repeated 10 times.

2.5. Model design

The objects were designed using Fusion 360, version 2.0.18961 (Autodesk, USA). To prepare floating dosage forms, objects were designed as hollow models. The 3D models will be saved as a .stl file format, then sliced and converted to g-code file format using Cura version 4.11.0 (Ultimaker, Geldermalsen, The Netherlands). The g-code files contained the instructions for the 3D printer, such as the directions of the print head, nozzle temperature, layer height, infill percentage, wall thickness. Finally, the g-code files will be transferred to the 3D printing software installed on a control computer.

2.6. Fused deposition modeling three-dimensional (FDM 3D) printing

The designed models were printed using a standard 3D printer equipped with a 0.4 mm hot end extruder printhead. The settings for printer were configured as follows: print bed temperature: 40 °C; nozzle printing temperature: 180 °C; nozzle traveling speed: 20 mm/s; layer height: 0.10 mm. First, filament was loaded into the 3D printer, which was preheated to setting temperate. When reaching the hot nozzle, the filament tip was melted and then deposited on a build plate, creating one layer of the object. The nozzle then moves up, and another layer is deposited. By repeating these steps in a layer-by-layer manner, final object was obtained.

2.7. Drug release studies

In vitro release of printlets was performed using a dissolution system equipped with USP apparatus II following the guidance for dissolution test of current USP [17]. Test conditions include: media volume of 900 ml of 0.1N HCl, a temperature of 37 ± 0.5 ºC, and a stirring speed of 50 ± 2 rpm. At 0.5,

1, 2, 3, 4, 6, 8, 10 hours, 4 mL dissolution media was withdrawn and 4 mL fresh media was added. Next, the samples were filtered through a 0.45 µm nylon membrane. The concentrations of levodopa, and carbidopa in the samples will be quantified by HPLC. The experiments were conducted in triplicate.

2.8 Drug content analysis

Samples were cut into small pieces (1-2 mm per side). A sample amount containing approximately 100 mg of levodopa and 25 mg of carbidopa was accurately weighed. It was then transferred into a 50 mL volumetric flask and dissolved completely with 0.1N HCL. Levodopa and carbidopa in the samples were analyzed using an HPLC system (Infinity 1200 series, Agilent) equipped with a diode-array detection detector. The HPLC program was developed referencing the USP 43 [17]. Levodopa and carbidopa were separated by a GL Sciences InertSustain C18 (15 mm × 4.6 mm, 5 µm) reverse phase column. The mobile phase was a mixture of methanol and phosphate buffer pH 3.0 (20 mM NaH_2PO_4 and 10 mM Na_2HPO_4) at a volume ratio of 5:95. The flow rate was 1.0 ml/min, and injection volume was 10 µL. The signal was detected at a wavelength of 280 nm.

2.9. Buoyancy kinetics

Buoyant force of each unit dose was determined by the "resultant weight" method referenced from Vo's study [18]. A five-point calibration curve and the regression equation were established from the counterpoise weight and the measured "resultant weight". A printlet was placed in a dissolution vessel containing 900 mL of 0.1N HCl solution. The solution temperature was maintained at 37 ± 0.5 °C and stirred at a speed of 50 rpm. At 0.08, 1, 2, 3, 4 hours, the vessel was removed from the dissolution system to measure the floating force of the unit dose. The vessel was then placed back into the dissolution system, waiting for the next measurement. The experiments were conducted in triplicate.

2.10. Powder X-Ray Diffraction (PXRD)

Polymorphism of levodopa and carbidopa in samples will be investigated using an X-ray diffractor (D8 Advance, Bruker, Germany). The diffractograms were collected with the following parameters: step width of 0.02°/s, scanning range from 5° to 60° on a 2θ scale; generator tension (voltage) of 40 kV; generator current of 40 mA.

2.11. Uniformity

The sample uniformity was evaluated in terms of size, weight, and drug content [19]. The drug content of samples was determined by HPLC (Infinity 1200 series, Agilent). Dimensions of the filaments and printlets were measured 5-10 times using a digital caliper (Insize 1114-200A, China). The weight variation was determined by weighing five printlets using an analytical balance (Mettler Toledo ab204, Switzerland).

3. RESULTS AND DISCUSSION

3.1. Thermal analysis

High temperatures used in the HME and FDM 3D printing processes may cause drugs and polymers to degrade. To prevent these degradations, it was necessary to carry out these processes at temperatures below the degradation temperatures, which can be determined using thermal analysis.

The TGA thermograms (Fig. 1A) demonstrated that the degradation temperatures of pure LD and CD were 290 °C and 204 °C, respectively. HPC has excellent thermal stability, even when exposed to temperature as high as 300 °C. In addition, there was no significant mass loss at HME temperature of 120 °C and 3D printing temparature of 180 °C, except for a small weight loss of carbidopa (4%) at a range of 100-130 °C caused by water evaporation. These findings confirmed that HME and 3D printing

could be processed without any significant degradation. Further proof of the materials' and filaments' thermal stability was supplied by the DSC data.

The DSC diagram of CD (Fig. 1B) showed a broad endothermic peak between 100 - 140 °C for water evaporation, which was the same as the TGA result. The endothermic peaks at 295 °C for LD and 204 °C for CD suggested that LD and CD crystals melted at these specific temperatures. These results align with values reported in the literature for LD (295°C [20,21]) and CD (203–208°C[22]). The thermograms did not show any abnormal thermal events throughout the temperature range of 25 – 180 °C, showing that the materials were stable during the HME (120 °C) and 3D printing (180 °C) processes.

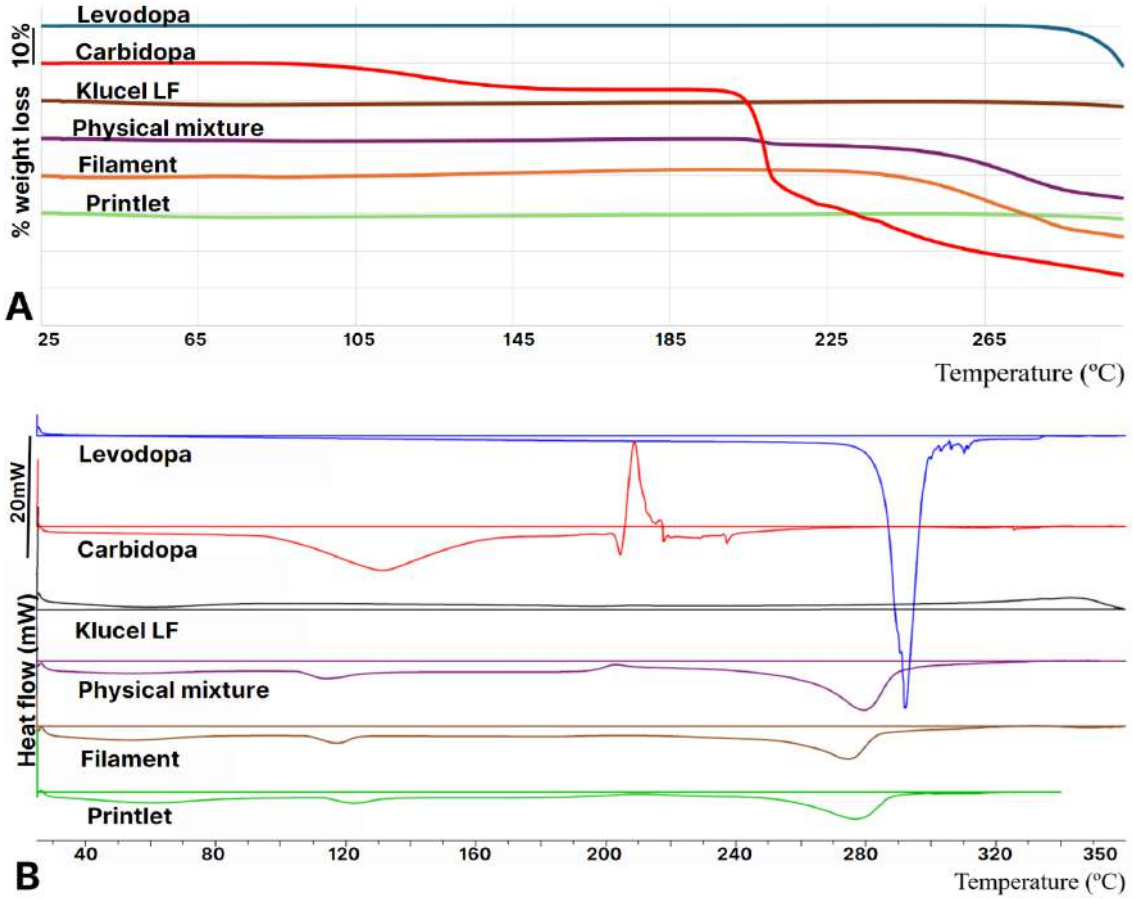

Figure 1. The TGA thermogram (A) and DSC diagram (B)

3.2. XRD analysis

On the PXRD diffractogram, characteristic peaks were recorded at 2θ = 21.22 º, 24.92 º, 25.86º, 18.38 º for LD, and peaks at 2θ =9.74 º; 18.62 º ;19.58 º for CD. These sharp peaks indicated that LD and CD were in crystalline form. These peaks were still detected in the filament and printlet, but their intensity decreased, indicating a partial transformation of LD and CD into the amorphous form. Additionally, the PXRD spectra of CD showed the re-crystallization of this drug during printing process. The CD signal was almost absent in the filament's PXRD spectra, but it was detected in the printlet one. This recrystallization may be a result of high temperatures used in the 3D printing process.

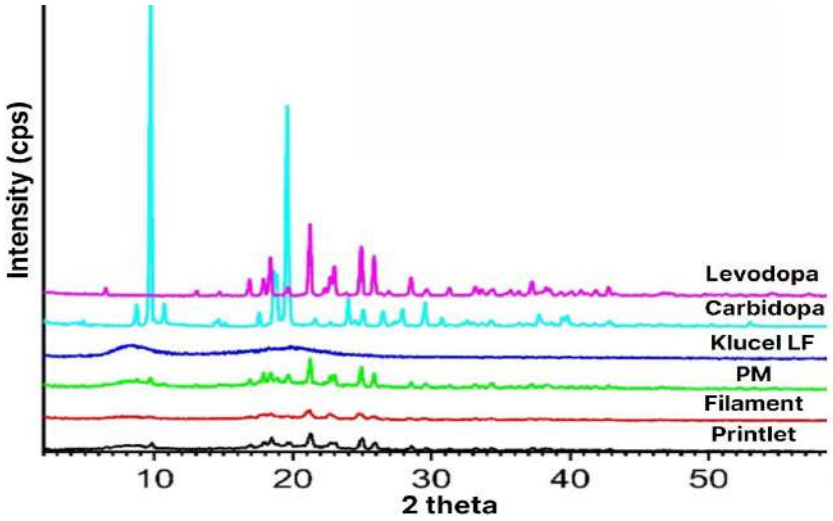

Figure 2. PXRD diffractograms

3.3. Preparation and characterization of filaments

The extruded filament exhibited a smooth surface, perfect circularity, great homogeneity, and appropriate mechanical characteristics. The filament diameter was 1,716 ± 0,008 mm, allowing for easy integration into standard commercial printers. The filament homogeneity, in terms of diameter (RSD = 0.49%), and drug contents (RSD =8.23% for LD and 6.18% for CD), directly enables for producing the consistent objects. The filaments also possessed good mechanical strength, with its bending resistance force of 4.097 ± 0.082 N and vertical compression strength of 8.676 ± 0.394 N. This allowed them to deal with compression and pushing force, resulting in a continuous delivery of filament during the printing process.

3.4. Three-dimensional printing and characterization of printlets

3.4.1. Effect of internal structure on buoyancy of the printlets.

To evaluate the impact of the internal structure on the performance of dosage form, total of nine formulations (Table 1) were designed. All of them were caplet-shaped with the same size of 12x6x4 mm. To achieve buoyancy and desired weight, the hollow volume, infill density, shell thickness, and top/bottom thickness were varied in the range of 38 to 58 mm^3; 50 to 100%; 0 to 0.8 mm, 0 to 0.4 mm, respectively.

Table 1. Effect of internal structure on printlet's weight and floating force

Fomulation	Hollow volume (mm^3)	Infill percentage (%)	Shell thickness (mm)	Top/bottom thickness (mm)	Weight (g)	Floating force at 5 minutes (µN)
F1	38	100	0.4	0.2	0.273 ± 0.005	0
F2	48	100	0.4	0.2	0.260 ± 0.007	115.35 ± 22.98
F3	58	100	0.4	0.2	0.248 ± 0.005	149.38 ± 37.81
F4	48	70	0.4	0.2	0.243 ± 0.002	141.81 ± 24.45
F5	48	50	0.4	0.2	0.225 ± 0.018	212.02 ± 96.64
F6	48	50	0	0.2	0.196 ± 0.007	616.39 ± 37.42
F7	48	50	0.8	0.2	0.233 ± 0.010	182.64 ± 20.92
F8	48	50	0.4	0	0.202 ± 0.015	313.47 ± 6.19
F9	48	50	0.4	0.4	0.229 ± 0.010	242.85 ± 27.11

All nine formulations had a smooth surface (Figure 3) and showed good uniformity in terms of size (RSD in the range of 0.7-2.7% for all three dimentions), weight (RSD = 1.7%), and drug content (RSD = 3.22% for LD, and 8.17% for CD). The RSD of the size, weight, and drug content of 3D printed object indicated the accuracy and consistency of the printing process. These formulations were then tested *in vitro* for their ability to float.

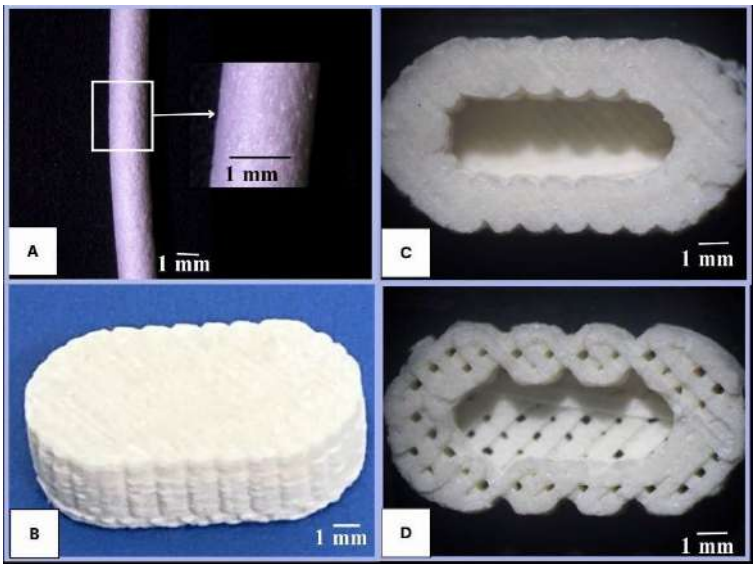

Figure 3. Imagine of filament and printlets. A: Surface of filament; B: printlet (size of 12 mm x 6 mm x 4 mm); C: cross-section of printlet with 48 mm^3 hollow volume; 100% infill, 0 mm shell; 0.2 mm top/bottom thickness; D: cross-section of printlet with 50% infill, 0 mm shell; 0 mm top/bottom thickness.

The results revealed that 8 out of 9 formulations floated immediately stemming from the low density of the hollow structure. The effect of hollow volume on buoyancy was shown in Fig. 4A. Printlets with air chambers of 48 mm^3 (F2) and 58 mm^3 (F3) floated immediately, with their buoyancy ranged from 115.35 µN to 149.38 µN, and gradually increased over time. However, printlets with air chambers of 38 mm^3 (F1) did not show any evidence of floating due to their high density. This finding was similar to previous publications [10,12].

The buoyancy of the printlets depended not only on hollow volume, but also on the infill percentage, which was the ratio of solid material was deposited inside the 3D printed model. This parameter could be set from 0% (the empty object) to 100% (the solid object). Additionally, the shell, the top, and the bottom layers acted as barriers, and their thickness controlled the buoyancy and drug release. The data revealed that lowering these parameters may enhance the floating capabilities of printlets. The greatest floating force was achieved by F6, a printlet with 50% infill and a shell thickness of 0 mm; however, it was unstable and quickly decreased. This was due to the lack of shell, allowing dissolution media to enter and replace the air inside the infill. As the result, polymer swollen which led to increase the density of the system and then reduce buoyancy. In contrast, the buoyancy of printlets with 100% infill (F1 -F3) was slightly increased over time. This was due to the reduction in prinlets' size and density, which caused by the dissolution.

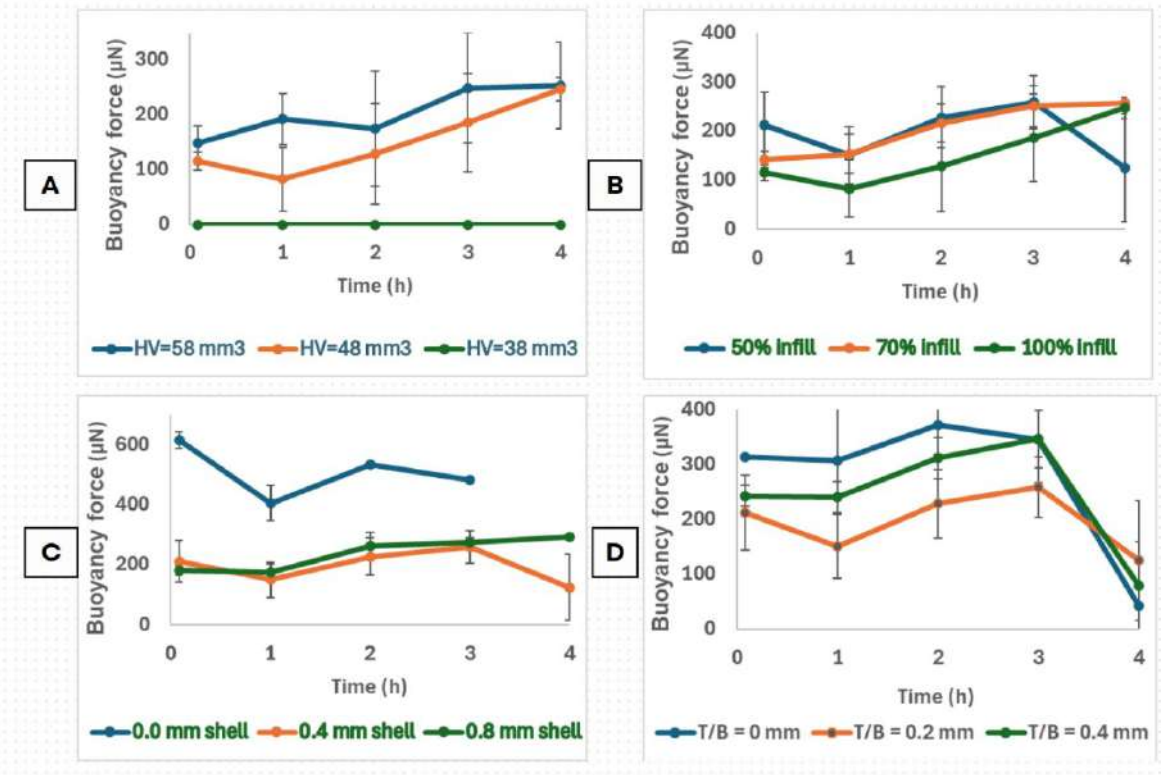

Figure 4. The effect of internal structure on buoyancy of the printlets
A: hollow volume; B: infill density; C: **shell** thickness; D: top/bottom thickness

3.4.2. Effect of internal structure on dissolution profile of the floating printlets

Fig. 5 showed that shell thickness and top/bottom thickness had little effect on the percentage of LD and CD released. However, the hollow volume and infill density had a significant impact. Printlets with 50% infill released 80% in 4h, while others required about 6h to achieve the same level. LD and CD released faster in low infill formulations due to their porosity structures, allowing for quicker penetration of the dissolution media into the printlet matrix, boosting the dissolution of polymer and drugs. The finding was consistent with the earlier studies [10,12].

The hollow volume of printlets not only controlled its buoyancy, but also influenced on dissolution rate. Fig. 5 showed that once the wall was dissolved (about 1 hour), air chamber was exposed, allowing media to enter the hollow part. An increased hollow volume provided a larger surface area, hence leading to a faster release rate. Despite the exception of F1, which had the smallest air chamber but the fastest release rate. This was because F1 sank and the whole caplet was submerged in water. Therefore, the printlet dissolved at a faster rate compared to the floating F2 and F3 printlets.

4. CONCLUSION

Customizable FDDSs containing levodopa and carbidopa were successfully formulated using 3D printing conjugated with melt extrusion technology. The printlets could float immediately, and the buoyancy was maintained until the hollow structure was disrupted. The unit dose, floating force, and drug release profile of the printlets could be tailored by customizing the internal structure. This study suggested that FDM 3D printing is a promising technique for the fabrication of tailorable FDDS that meet the individual needs of Parkinson's patients.

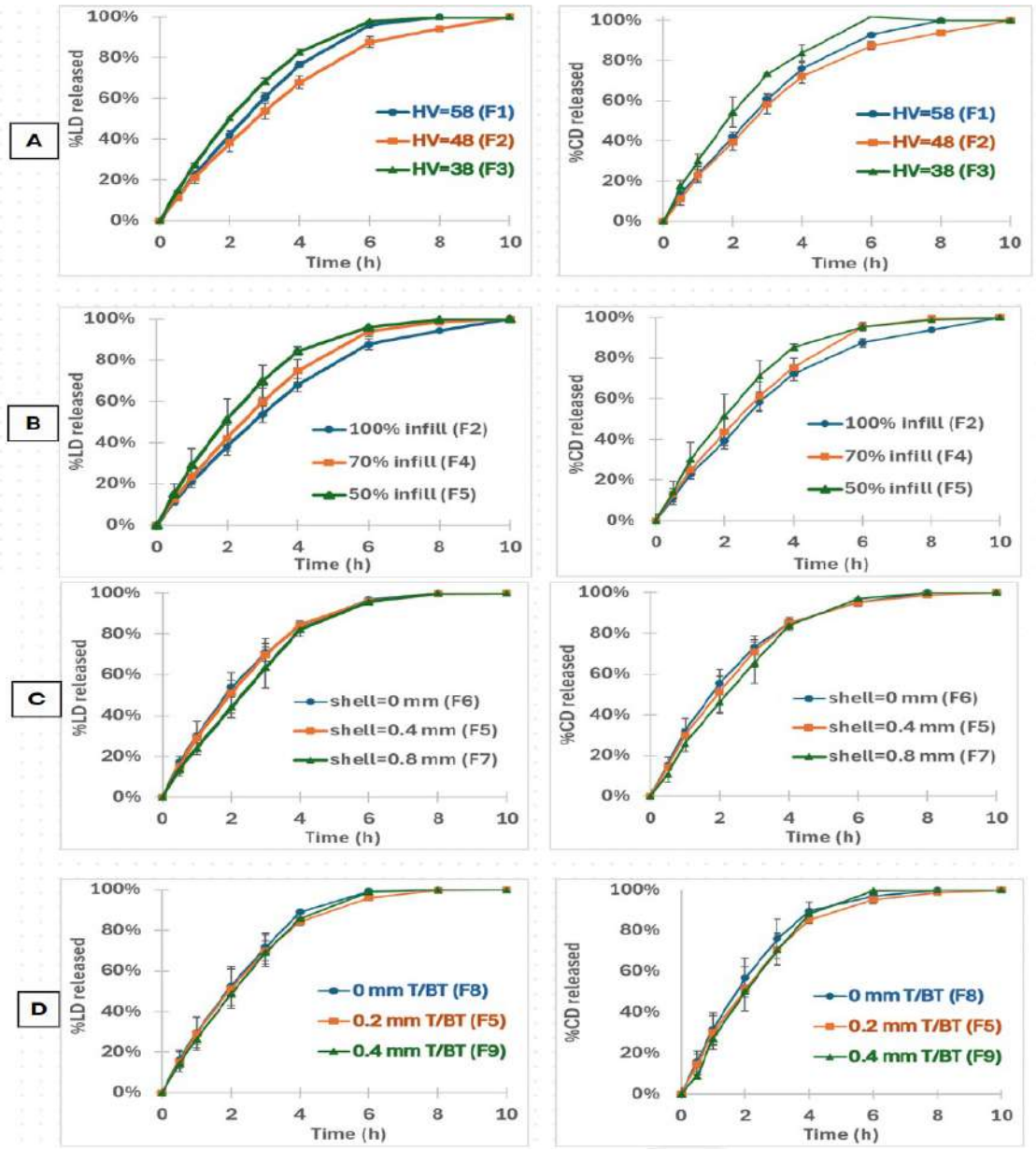

Figure 5. The effect of internal structure on LD and CD release
A: hollow volume; B: infill density; C: shell thickness; D: top/bottom thickness

5. ACKNOWLEDGMENT

Conflict of interest

The authors declare that they have no conflict of interest.

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Effect of Polymer Types and Concentrations on the Characteristics of Alginate-Based Microspheres Containing Vitexin and Isovitexin

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ABSTRACT

Vitexin and isovitexin are bioactive flavones known for diverse pharmacological effects. Both compounds have low oral bioavailability in gastrointestinal tract. Microspheres prepared using biodegradable polymers like alginate offer promising delivery systems to enhance flavones' bioavailability. Microsphere properties, influenced by various factors in preparation process, including polymer types and concentrations, can be controlled to achieve desired microsphere. This study aimed to identify impacts of alginate types and concentrations on mean size, encapsulation efficiency, and *in vitro* release of microspheres loading compound of purified vitexin-isovitexin (1:1) extract. Alginate-based microspheres were prepared using water-in-oil emulsion technique, followed by external gelation. Nine formulations employing one of three alginate types (low/medium/high viscosity) at determined concentration (1%, 2% and 3%) were carried out. The obtained microspheres were evaluated in terms of particle mean size, encapsulation efficiency, and *in vitro* release at pH 7.4. Vitexin-isovitexin was quantified by UV-Vis-spectroscopy at 269 nm. The morphology was examined by SEM, and FT-IR was used to analyze functional groups of chemical ingredients on microspheres. All microspheres obtained had mean sizes of 1.28±0.38 to 4.55±1.18 µm and narrow size distributions. Although concentration of alginate in range of 1-3% had no significant impact on encapsulation efficiency, varying alginate types led to considerable differences in vitexin-isovitexin encapsulation efficiency. Using low-viscosity alginate at 1% resulted in maximum encapsulation efficiency of 5.94%. The *in vitro* release study showed that all formulated microspheres released vitexin-isovitexin almost completely in controlled manner for 4 h. Microspheres were spherical with smooth surfaces, and FT-IR spectra of microsphere highlighted functional groups of alginate and vitexin-isovitexin. These results pointed out influences of polymer on size and encapsulation efficiency of alginate-based microspheres. Furthermore, *in vitro* release of vitexin-isovitexin was thoroughly examined. These findings can be references for studies aiming to optimize the use of alginate to prepare microspheres through emulsification process.

KEYWORDS: alginate; vitexin; isovitexin; microspheres.

1. INTRODUCTION

Vitexin and isovitexin are flavones, a major subclass of natural flavonoids, and have been commonly isolated together in a mixture. These two compounds are isomers of each other, as both are mono-*C*-glycosylated derivatives of apigenin. While vitexin is characterized as an apigenin-8-*C*-glucoside, the glucosyl moiety binds to the aglycone through a C-C linkage at the C6 atom in the case of isovitexin. Hence, the two compounds share almost all chemical properties, i.e., chemical formula ($C_{21}H_{20}O_{10}$), molecular weight (432.3775 g/mol), one sugar moiety (*β*-D-glucopyranose), and seven aromatic hydroxyl groups in a molecule[1,2]. Compared to the aglycone apigenin and its other *O*-glycosylated derivatives, vitexin and isovitexin exhibit higher stability and antioxidant and anti-diabetic

effects in most cases[3]. Unfortunately, vitexin and isovitexin are poorly absorbed in the digestive tract[4], which means they possess low oral bioavailability[5] and are hardly degradable under the influence of normal digestive enzymes.

Decades of research have suggested that vitexin and isovitexin are strong candidates for further drug discovery studies and subsequent clinical trials; however, it is still a major challenge to enhance the efficacy of vitexin and isovitexin, which is necessary for designing a suitable drug delivery system to protect against degradation and controlled release of substances. It has been demonstrated through studies that several drug delivery systems, including liposomes[6], microemulsions[7], microspheres from β-CD[8], nanoparticles[9,10], and microparticles[11], may efficiently increase solubility and regulate release *in vitro*. Studies that concentrate on either pure vitexin or a combination of vitexin and other compounds found in medicinal herbs are becoming more common, however there are not many that look at both vitexin and isovitexin at the same time.

Given the aim of producing an optimal carrier system for hydrophobic compounds such as vitexin and isovitexin, biodegradable polymers such as sodium alginate are commonly used to prepare microspheres due to their advantages of stability, reduced volatility, release characteristics, and environmental conditions[12]. Furthermore, no published paper utilizes an alginate-based microsphere where the carriers of these two compounds have been located. Meanwhile, various parameters in the preparation influence microsphere properties, specifically polymer types, and concentrations.

Thus, this study aimed to identify the impacts of sodium alginate types and concentrations through the microspheres loading a purified extract containing simultaneously vitexin and isovitexin preparation. The microsphere characteristics, including the mean size, encapsulation efficiency, and *in vitro* release, were evaluated to analyze the impact of polymer alginate types and concentrations. Then the formulation could be controlled to achieve the desired microsphere.

2. MATERIALS AND METHODS

2.1. Materials

Three types of alginates divided by their viscosity namely: low viscosity (100-200 cps of 1% solution), medium viscosity (300-400 cps), high viscosity (500-600 cps) were purchased from TCI (Japan); A purified (>95%) compound of vitexin and isovitexin extract from *Mung bean* seed coat, was bought from Napro (Vietnam). The extract with the ratio of vitexin and isovitexin as 1:1 determined by HPLC method following the in-house standard, and the ratio of vitexin and isovitexin did not varied during the preparation process. Other chemical agents such as isooctane, Tween 80, Span 80, calcium chloride, and acetone were provided by Fisher (USA).

2.2. Preparation of microspheres

The method of preparing alginate-based microsphere was ion gelation through the water-in-oil emulsification stage. Nine formulations at determined concentrations 1%, 2% and 3% w/w of each sodium alginate type (low/medium/high viscosity) were dissolved in distilled water and magnetically stirred overnight. Exactly 100 mg of vitexin-isovitexin extract was dispersed into a sodium alginate solution and then mixed completely to form a homogenizer suspension. Sodium alginate suspension containing vitexin-isovitexin extract added dropwise gently into 40 g isooctane oil phase containing 4.4 g span 80 using a high speed homogenizer (IKA T25 – Digital Ultra-Turrax, Germany) at 7200 rpm for 5 min to create a W/O emulsion. Then, 10 mL Tween 80 solution and 20 g $CaCl_2$ 10% (w/w) solution were added to the W/O emulsion and continued homogeneous for 3 min and 15 min, respectively. The alginate-based microspheres were formed and solidified using acetone for 10 min in the magnetically stirred. Then, centrifugation was performed at 10000 rpm for 5 min, followed by vacuum filtration using a 0.45 µm filter to obtain alginate-based microspheres that were washed triple with acetone and dried at room temperature. The alginate-based microspheres were stored in proper condition before evaluation in the subsequent stages.

2.3. Particle size and size distribution

The microsphere size distribution and mean particle size were determined experimentally using Zetasizer Nano Size Malvern (UK) with a scattering angle of 12.8º. The dry microspheres were well dispersed in distilled water by ultrasonic for 5 min. The measurement operation was repeated for 3 samples and the average value was calculated.

2.4. Microspheres encapsulation efficiency

An accurate mass (10.0 mg) of the alginate-based microspheres was suspended in 100 mL phosphate buffer (PBS pH 7.4) and stirred at 1000 rpm at room temperature until the microspheres were dissolved completely to release the active ingredient. The content of vitexin-isovitexin was determined by UV-Vis-spectroscopy Shimadzu UV - 1601PC (Japan) at 269 nm. The measurement was conducted in triple, and each sample was quantified three times. The encapsulation efficiency was calculated as the content of vitexin-isovitexin in alginate-based microspheres and shown as the average value.

2.5. *In vitro* release study

A method for evaluating the *in vitro* drug release using a dissolution apparatus Labinda DS 1400 (India) was developed. Approximately 100 mg of alginate microspheres were suspended in 500 mL PBS pH 7.4 solution and maintained at 37 ºC under stirring at 100 rpm. At predetermined time intervals including 0.5 h, 1 h, 2 h, 3 h, and 4 h, the sample (10 mL) were collected from a release medium, and the same volumes of fresh medium were replaced. Aliquots were analyzed for absorbance using UV-Vis at 269 nm. All experiments were measured in triplicates, and the percentage of the released amount of vitexin-isovitexin was calculated against the time.

2.6. Particle morphology and FT-IR spectroscopy

The morphology of alginate-based microspheres was examined using scanning electron microscopy (SEM), JEOL-JSM-IT200 (Japan). The SEM photographs were taken at different magnifications at room temperature and analyzed using an acceleration voltage of 5kV. FT-IR spectra of pure vitexin-isovitexin extract, sodium alginate, and alginate-based microspheres were obtained to analyze the changes in functional groups and intermolecular interactions of ingredients in the microspheres.

2.7. Statistical analysis

All analyzed data were shown as mean ± SD. One-way analysis of variance (ANOVA) and t-test were used to test the statistical significance with the significance determined at a level of p=0.05.

3. RESULTS AND DISCUSSION

3.1. Effect on particle size

The microspheres obtained from nine formulations (F1 to F9) had mean sizes of 1.28 ± 0.38 to 4.55 ± 1.18 µm, as shown in Table 1, and narrow size distributions. The ANOVA analysis demonstrated that the sizes of the microspheres from all formulations were not significantly different ($p > 0.05$), irrespective of the alginate types or concentrations. Previous studies[13-15] reported that the microsphere size was significantly influenced by stirring speed in the process, which is explained by the higher shear force resulting in smaller emulsion droplets in the continuous phase (isooctane phase). A report by Caetano LA *et al*[16] also revealed that the microsphere size may be affected by stirring speed and polymer characteristics. In this study, when the stirring speed was fixed at 7200 rpm, and the

alginate viscosity to was varied from 100 cps to 600 cps, there was no statistically significant differences in the size of the alginate-based microspheres.

Table 1. Particle size and encapsulation efficiency of vitexin-isovitexin extract microspheres

Formulation	Alginate type	Alginate concentration	Particle mean size (µm)	D_{50} (µm)	Encapsulation efficiency (%)
F1	Low viscosity	1%	1.46 ± 0.03	0.69 ± 0.01	5.94 ± 0.11
F2	Low viscosity	2%	1.33 ± 0.21	0.78 ± 0.02	4.64 ± 0.48
F3	Low viscosity	3%	1.68 ± 0.73	0.96 ± 0.22	4.33 ± 0.55
F4	Medium viscosity	1%	4.55 ± 1.18	1.18 ± 0.19	3.53 ± 0.10
F5	Medium viscosity	2%	2.99 ± 0.13	1.37 ± 0.30	2.66 ± 0.03
F6	Medium viscosity	3%	1.28 ± 0.38	0.79 ± 0.05	4.48 ± 0.05
F7	High viscosity	1%	1.77 ± 0.41	0.79 ± 0.03	1.99 ± 0.16
F8	High viscosity	2%	1.87 ± 0.14	0.90 ± 0.09	2.73 ± 0.14
F9	High viscosity	3%	1.85 ± 0.69	0.81 ± 0.16	2.72 ± 0.07

3.2. Effect on encapsulation efficiency

It is observed that the alginate types had no significant impact on encapsulation efficiency ($p = 0.89$). Meanwhile, varying alginate concentrations from 1-3% w/w led to considerable differences in vitexin-isovitexin encapsulation efficiency ($p = 0.02$). Employing low-viscosity alginate at 1% resulted in a highest encapsulation efficiency of 5.94%. The encapsulation efficiency of vitexin-isovitexin tended to decrease as the alginate intrinsic viscosity increased. The mechanism of this phenomenon could be referred to the previous study by Elsa AK *et al*[11]. Using a higher alginate concentration, the denser polymeric structure would be formed, leading to a faster solidification of the microspheres, causing the microspheres with lower encapsulation efficiency.

3.3. Effect on *in vitro* release profile

Figure 1 pointed out that 18.32-25.31% of vitexin-isovitexin was released from all nine formulations in the first thirty minutes, which is considered as a burst release of vitexin-isovitexin encapsulated on the surface of microspheres. Then, microspheres experienced a lag phase until 1 h, whereas the alginate gel structure was swollen. In the next three hours, microspheres released vitexin-isovitexin almost completely (94.39-127.93%) in a controlled manner. The formulation F4 using medium-viscosity alginate at 1% w/w demonstrated the highest *in vitro* release rate.

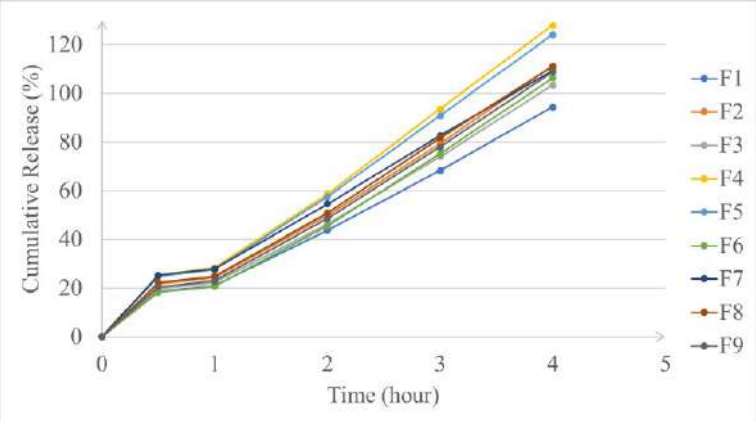

Figure 1. The *in vitro* release profiles of vitexin-isovitexin extract microspheres

The release kinetics of vitexin-isovitexin from the microspheres were examined using release data from 1 h to 4 h. The results demonstrated that the alginate-based microspheres followed the first-order kinetic with relatively high coefficients of determination (Table 2).

Table 2. The *in vitro* release results of vitexin-isovitexin extract microspheres

Formulation	First-order kinetic equation (*)	R^2	Formulation	First-order kinetic equation (*)	R^2
F1	y = 24.471x – 4.3183	0.9990	**F6**	y = 28.651x – 9.6539	0.9974
F2	y = 29.062x – 6.3674	0.9977	**F7**	y = 27.229x + 0.5245	0.9999
F3	y = 27.106x – 6.2922	0.9980	**F8**	y = 28.959x – 5.2528	0.9988
F4	y = 33.385x – 6.3371	0.9991	**F9**	y = 28.686x – 7.0889	0.9983
F5	y = 32.248x – 5.5728	0.9993			

*Calculated for the cumulative release data from 1 h to 4 h

3.3. Particle morphology and FT-IR spectroscopy

It could be demonstrated from the SEM images that the microspheres were spherical in shape. The surface of the microsphere was smooth without having visible pores.

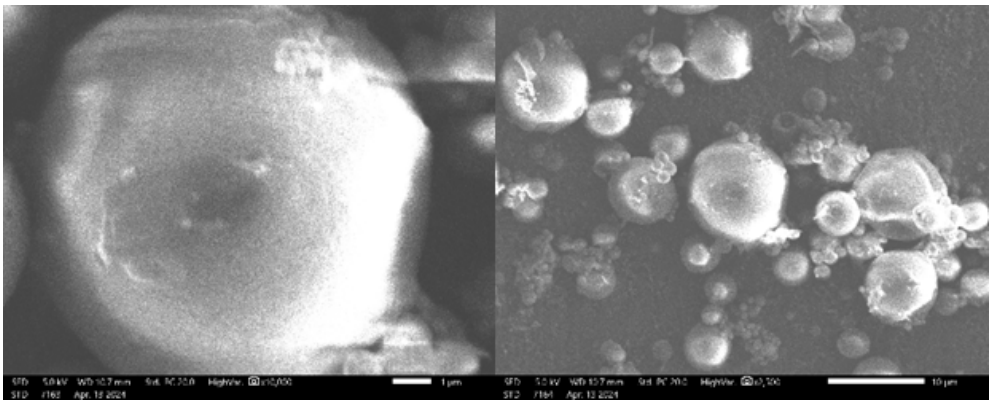

Figure 2. The SEM images of microspheres

The FT-IR spectra of sodium alginate, purified vitexin-isovitexin extract, and obtained microspheres were examined. The sodium alginate spectra (the orange line) showed characteristic bands at 3433 cm^{-1} (OH), 2924 cm^{-1} (CH), 1630 cm^{-1} (COO-asymmetric), 1416 cm^{-1} (COO-symmetric), and 1031 cm^{-1} (C-O-C). The purified vitexin-isovitexin extract (the green line) showed characteristic bands corresponding to 3383 cm^{-1} (OH), 1654 cm^{-1} (CO), as well as the characteristic bands of aromatic double bonds at 1614, 1508 and 1429 cm^{-1} (Figure 3). The FT-IR spectra of vitexin-isovitexin extract microspheres (the blue line) highlighted the functional groups of alginate and vitexin-isovitexin, indicating that a physical barrier was formed between alginate and two flavones to control the release of encapsulated compounds.

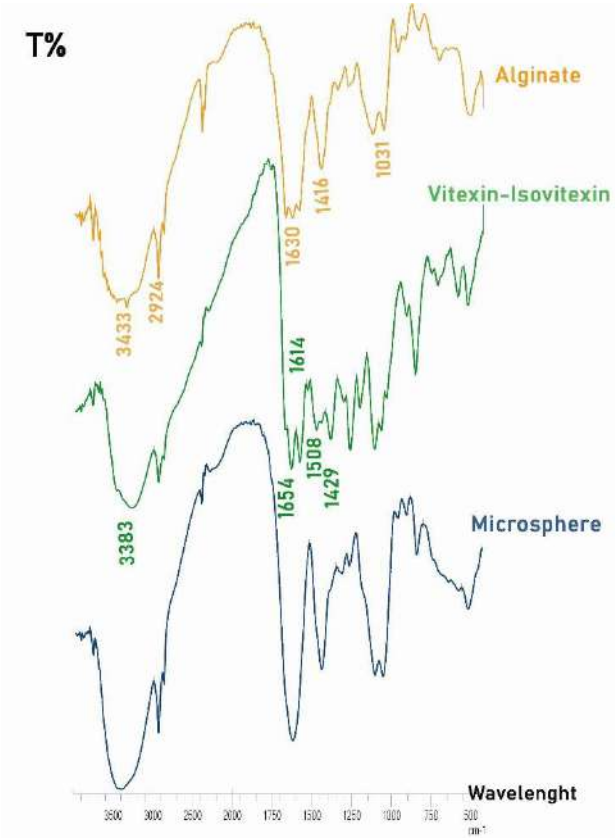

Figure 3. The FT-IR spectra of sodium alginate, purified vitexin-isovitexin extract, and vitexin-isovitexin extract microspheres

4. CONCLUSION

The results from this study demonstrated the impact of polymer properties on the size and encapsulation efficiency of the obtained alginate-based microsphere. Furthermore, the *in vitro* release of vitexin-isovitexin from microspheres was completely and in a controlled manner. These findings can be references for studies aiming to optimize the use of alginate to prepare microspheres through the emulsification process.

5. ACKNOWLEDGMENT

Conflict of interest

The authors declare that they have no conflict of interest.

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Fabrication of Tailorable Controlled Release Printlets of Methylprednisolone using a 3D Fused Deposition Modeling

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ABSTRACT

Treatment with methylprednisolone for children requires dose adjustment according to body weight, symptom severity, and clinical response. 3D printing has been emerging as a novel platform for manufacturing personalized medicines. This study focused on developing tailorable methylprednisolone tablets for pediatric treatments using a 3D Fused Deposition Modeling (FDM) platform. Filaments were prepared using a single-screw melt extruder, where polyvinyl alcohol and glycerol were the main polymer and plasticizer. 3D models were designed using 3D Builder and sided using Cura 5.4.0 software. The internal structure and unit dose of printed tablets (printlets) were customized using model design and printing parameters. Tailorable printlets were fabricated using a standard 3D FDM printer. Mechanical strength and uniformity of the filaments were characterized using a CT3 Texture Analyzer and digital caliper, respectively. The morphology and surface characteristics of the filaments and printlets were examined using a stereo microscope. The thermal stability of formulations was characterized by differential scanning calorimetry (DSC), thermalgravimetric analysis (TGA), and HPLC. Drug content and dissolution were analyzed by HPLC. The filaments exhibited good uniformity, smooth surface, and robust mechanical properties (i.e. bending and vertical compression resistance). Tailorable printlets were successfully fabricated with a minimal deviation from the designed models (RSD < 4%). Correlations between unit dose and design parameters were successfully derived by geometric calculation and regression approaches. The drug release was governed by the internal design that could be controlled for up to 12 hours. The current study proposed a viable paradigm for preparing personalized medications for pediatric patients.

KEYWORDS: Methylprednisolone; 3D FDM; pediatric; extrusion process; tailorable dosage form.

1. INTRODUCTION

1.1. Digital medical care paradigm.

Patient's clinical responses to a drug may be greatly different from patient to patient. Such variations stem from inter-subject differences in race, weight, age, diet, medical record, etc. Therefore, customizing medication to satisfy individual needs is critical to ensure safety and efficacy of a treatment regimen. The importance of digital technology to health care is widely recognized and confirmed by its benefits where information of patient's disease history, records of medication and medical intervention, and records of clinical test index are fully managed[1]. Digital medical care provides fundamental information for individualized treatment which helps to optimize efficacy and minimize side effects of medications. Based on such a comprehensive database along with clinical index and diagnosis, doctors can quickly prescribe medication with personalized dose, drug combination, and requirement of drug release kinetics [2].

Conventional pharmaceutical technologies were designed for mass production where manufacturing processes are developed to ensure the highest uniformity of all unit doses. Such a

manufacturing model makes tailoring medication unpractical, and all patients are treated with an identical tablet. The individualized treatment paradigm requires a new manufacturing platform by which dose and drug release kinetics can be flexibly designed to satisfy individual needs. 3D printing technology has been emerging as a novel technology satisfying such requirements of individualized medicine fabrication[3-5]. It can be integrated into a digital healthcare paradigm, sending prescriptions directly to certified pharmacies to print individualized medications. This technology allows people to fabricate dosage forms with precise doses to meet each patient's therapeutic requirements with minimum human involvement in the process [6]. Owing to its inherent flexibility this technology offers means to tailor dose, composition, and drug release kinetics for each treatment stage of a particular patient [7,8]

Treatment for the pediatric population often requires a high level of medicinal customization to ensure the efficacy and safety of the patients. Methylprednisolone (base form), a potent anti-inflammatory corticosteroid, has been widely used in treating various fundamental and severe conditions in children, such as asthma, arthritis, and other inflammatory disorders. However, adjusting the dosage and formulation suitable for children encounters challenges due to the limited available pharmaceutical products on the market[9,10]. Tailoring the drug for individuals could enhance treatment efficacy, reduce adverse drug reactions, and improve patient compliance.

This study aimed to develop a tailorable dosage form containing methylprednisolone for the pediatric population utilizing the 3D FDM platform. Printing filaments were prepared by using hot-melt extrusion technology to utilize its high drug-loading capability. Correlations between design parameters and dug content per unit dose were established utilizing both design of experiment (DOE) and geometric calculation approaches. Impacts of the internal structure of the dosage form on drug release characteristics were also investigated. The proposed paradigm is a viable approach to fabricate tailorable dosage forms for individualized medications.

2. MATERIALS AND METHODS

2.1. Materials

USP grade methylprednisolone (Xianju Pharma, China) was sponsored by Merap Group (Hanoi, Vietnam); pharmaceutical grade polyvinyl alcohol (PVA) was purchased from Anmol Chemical (India); glycerin, acid phosphoric, tetrahydrofuran, absolute ethanol, methanol either analytical grade or HPLC grade were procured from chemical trading companies in Vietnam.

2.2. Methods

2.2.A. Reformulations and Characterization of filaments and printlets

Thermogravimetric analysis (TGA): Thermal stability of raw materials and formulations was evaluated using a TGA/DSC 1 system (Mettler Toledo, Switzerland). Inert environment in analysis chamber was maintained by purging the system with nitrogen gas at a flow rate of 30 mL/min. Samples, 5–10 mg, were weighted and loaded into a zirconia cup which was then positioned into the analysis chamber of the system. The temperature was held at 25 ºC for 2 minutes to stabilize the sample before heating to 300 ºC at a ramp rate of 10 ºC/min. Sample weight vs. temperature was recorded by integrated software.

Differential Scanning Calorimetry: DSC experiments were performed using DSC 1 StarSystem (Metler Toledo, Switzerland). An amount of 4-5 mg of sample was loaded into aluminum pans which were then hermetically sealed. The sample pans were subsequently placed into the heating chamber where the inert environment was maintained by a 20 mL/min nitrogen purging flow. Samples were stabilized by holding the system temperature at 25 ºC for 5 minutes. The temperature of the samples was then increased to 300 ºC at a ramp rate of 10 ºC/min. Sample-blank differential heat flow vs. sample temperature was acquired by integrated software.

Surface properties: The morphology of the printing filament samples was assessed through observation under 4x magnification lens stereo microscope (Euromex Steroblue, Netherlands). Images were captured using the built-in digital camera.

Uniformity: Filament diameter was measured using a 0.01 mm resolution digital caliper (1114-200A, Insize, China). The measurements were repeated 15 times, and the average and relative standard deviation were calculated.

Drug load: Drug load of the filament was determined using HPLC, as the method described assay of printlets. Briefly, filaments were cut/ground, dissolved, and diluted by using a mixture of ethanol : water (4:1). They were filtered through a 0.45 µm membrane before injecting 10 µl into an HPLC system equipped with a C18 column (150 mm x 4.6 mm, 5 µm packing L1) and a diode-array detection (DAD). Samples were eluded using a mobile phase composed of water : methanol : tetrahydrofuran : phosphoric acid (50 : 50 : 1.5 : 0.1) at flow rate 1.2 ml/min, and detection wavelength 247 nm.

Bending strength: The bending resistance force of the filament was measured by using a CT3 texture analyzer (Brookfield, USA). Filaments were cut into 5 cm-long segments and placed perpendicularly onto the two sample supporting ridges and the blade shape probe. The measurements were performed with the following parameters: distance between the two ridges: 4 cm; probe velocity before, during, and after contacting the sample: 0.1 mm/s; probe displacement distance: 10 mm; trigger force of a measurement 0.05 N. The bending strength of the filament was the force causing the filament's perpendicular deformation[11].

2.2.2. Printlets fabrication.

Cylindrical models of methylprednisolone (MP) tablets were designed using Autodesk Fusion 360 software (Autodesk, USA) and saved in ".stl" format. The models were then sliced using Ultimaker Cura 4.11.0 software (Ultimaker B.V., Geldermalsen, Netherlands). The dosage forms were subsequently printed using a customized 3D FDM printer (Kingroon KP3S Pro), equipped with a 0.4 mm print head nozzle. The printing parameters were set as follows: printing temperature at 180°C; build plate temperature at 45°C; printing speed at 20 mm/s; layer thickness of 0.1 mm; filament density of 100%. The printing capability was assessed based on observation of issues during the printing process such as filament squeezed, breaking, or snapping. A filament was considered printable if no such issues occurred after 6 consecutive dose units were printed.

2.2.3. Design of experiment

A 3-level full factorial design was utilized to plan the experiments. The DOE formulations were generated using the Modde 8.0 software (Umetrics, Sweeden). The DOE formulations were then prepared and characterized. Subsequently, the independent factors were correlated with the drug content and weight of the printlets. The Power 2 polynomial model was used for elaborating regression correlation equations using the Modde 8.0 software. The significance of models and each independent factor were statistically assessed based on corresponding p-values. The prediction power of the models was judged based on R2 and Q2 values calculated in the data fitting process.

2.2.4. Characterization of printlets.

Dimension uniformity: The height and diameter of 5 printlets were measured. Uniformity was assessed based on the relative standard deviation of the measurements.

Uniformity of Mass: 5 consecutive printlets were weighed and the average and relative standard deviation.

Deviation between printed samples and design model: Calculate the relative standard deviation of the dimensional differences between the printed products and the design model.

Dissolution: the dissolution experiment of the printlets was performed using Pharmatest PTWS-610 dissolution system (Pharmatest, USA) according to the method referencing from USP 43, under Methylprednisolone tablet monograph. The dissolution parameters were as follows: USP

apparatus II, dissolution medium was 900 ml deionized water, rotation speed 75 ± 2 rpm; temperature 37 ± 0,5 ºC. The concentrations of dissolution samples at predetermined time points were determined by HPLC, and then the corresponding percentage of drug dissolved was calculated.

Assay: The drug content of the samples and the concentration of methylprednisolone in dissolution media were analyzed by HPLC method referencing a published study [11]. Samples were dissolved using a mixture of ethanol : water (4:1) and diluted, if needed, to a suitable concentration using the same solution. Samples were then filtered through a 0.45 µm membrane before injecting into an Agilent 1200 HPLC system (Agilent Technology Inc., Germany). The chromatography analysis was performed with the following setting: Inertsil column C18 (150 mm x 4.6 mm), 5 µm particles (packing L1); a diode-array detection (DAD) wavelength 247 nm; flow rate: 1.2 ml/min; injection volume: 10 µl. The samples were eluded by the mobile phase composed of water : methanol : tetrahydrofuran : phosphoric acid (50 : 50 : 1.5 : 0.1). The chromatographic signals were acquired and analyzed by using the ChemStation software suite (Agilent Technology Inc.)

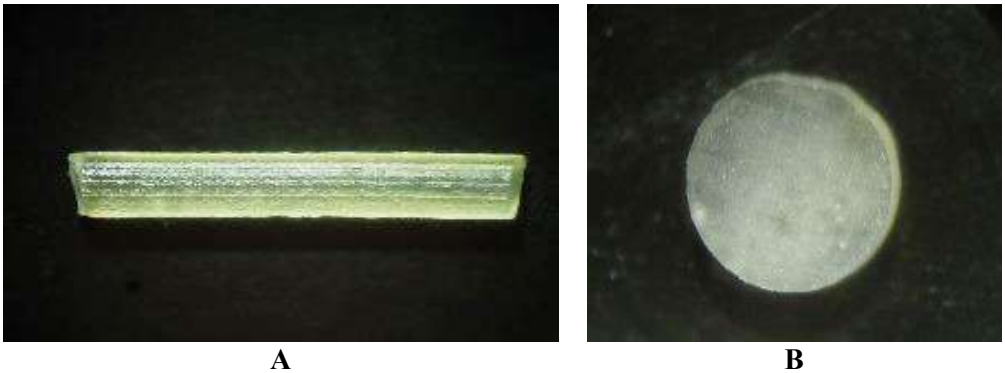
Data collection and report: measurement and analysis were performed at least 3 replicates. Statistical calculations were performed using Microsoft Excel 365. Results were reported as averages and standard deviations from the replicates.

3. RESULTS AND DISCUSSION

3.1. Preparation of printing filaments and printlets.

Thermalgravimetric analysis examination exhibited that the weights of MP, PVA, physical mixture, filaments and printlets samples were lost less than 2% when they were heated to 250ºC, suggesting the samples were thermally stable. There was a small endothermic peak at 230ºC and a larger one at 250ºC on the DSC thermogram of MP raw material, indicating that it existed as polymorphic crystals. No other thermal event was detected in the thermogram of all examined samples, concurring with the TGA results that the samples were thermally stable.

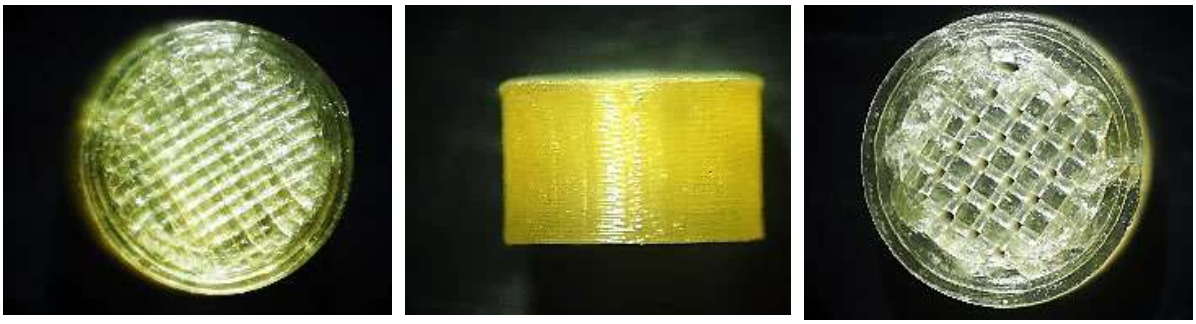
Through a series of preliminary and screening studies, PVA (a biocompatible polymer [5,12]) was chosen as a matrix formation polymer; glycerin was used as a plasticizer, and drug load of the filament was fixed at 5%. The extrusion process of the PVA-Glycerin-MP blend (weight ratio 90: 5: 5) yielded extruded strands with a uniform diameter of 1.82 ± 0.03 mm. The strands or filaments were opaque and possessed a smooth surface (Figure 1A). The cross-sectional of the filaments was dense and perfectly round as shown in Figure 1B. Such properties of the filaments facilitated the 3D printing process.



A **B**

Figure 1: Digital images of the prepared filament: A) surface, B) cross-section.

The prepared printlets were identical to their designs. They appeared in short cylindrical shape, free from defects, with a smooth surface and uniform glossy appearance (Figure 2 A, B). The edges of printlets were well-defined and the cross-section was uniform (Figure 2C). Such that indicated a high precision and consistency of the printing process.



A B C

Figure 2: Digital pictures of fabricated printlet taken at different angles: A) from the top, B) from side view, C) cross-section of a 50% infill printlet.

The dimensions, shape, and internal structure of the dosage form could be customized by 3D design. Meanwhile, the shell thickness (defined as a dense outer layer of objects), infill density (defines the percentage of internal volume of objects that will be filled with printing materials) and print head itinerary (define patterns the print head follows on each sliced layer of the object) could be set by operation parameters. With numerous inputs, the printlets could be highly customized in terms of drug content, shape, and drug release properties satisfying the therapeutic requirements of individuals.

3.2. Tailoring strength of printlets.

A 3-level full factorial design model was applied to plan the experiment that generated 12 DOE formulations of the printlets. Each formulation was printed with at least 6 replicates. No issue or error was observed during the printing process of all formulations, the printlets were identical to their design. The printing process was consistent and reproducible. As shown in Table 1 the prepared printlets possessed good uniformity in terms of dimension, weight, and drug content with RSD < 4%. Within the variation ranges, the dosage strength of each printlet can be varied from 6 mg to nearly 21 mg.

Table 1: The impact of tablet size on drug content

Sample ID	Design		Actual dimension (n=5)		Weight (mg) (n=5)	Drug content (mg), (n=3)
	Height (mm)	Diameter (mm)	Height (mm)	Diameter (mm)		
N1	4	5.6	3.86 ± 0.07	5.64 ± 0.04	122.1 ± 4.1	6.1 ± 0.16
N2	4	7.2	3.89 ± 0.03	7.27 ± 0.06	201.9 ± 8.8	10.1 ± 0.27
N3	4	8.8	3.87 ± 0.01	8.90 ± 0.01	295.2 ± 3.9	14.8 ± 0.20
N4	5	5.6	4.86 ± 0.06	5.71 ± 0.03	157.9 ± 2.5	7.9 ± 0.13
N5	5	7.2	4.92 ± 0.04	7.26 ± 0.07	256.9 ± 2.1	12.5 ± 0.11
N6	5	8.8	4.92 ± 0.02	8.97 ± 0.10	387.4 ± 1.7	19.1 ± 0.19
N7	6	5.6	5.86 ± 0.04	5.67 ± 0.03	187.1 ± 0.7	9.4 ± 0.14
N8	6	7.2	5.86 ± 0.08	7.25 ± 0.05	300.7 ± 8.2	15.0 ± 0.26
N9	6	8.8	5.86 ± 0.06	8.72 ± 0.01	422.2 ± 7.0	21.1 ± 0.36
N10	5	7.2	4.96 ± 0.04	7.16 ± 0.07	247.2± 3.3	12.18 ± 0.17
N11	5	7.2	4.94 ± 0.04	7.12 ± 0.07	254.5 ±4.2	13.13 ± 0.21
N12	5	7.2	4.95 ± 0.04	7.15 ± 0.07	252.4 ±3.8	12.89 ± 0.19

To successfully develop individualized medicines, the dose strength of each printlet needs to be accurately controlled. In this experiment, the dose strength was designed to be controlled by the height and thickness of the printlets, which several mouse clicks on computers could assign. The correlation between dose strength and dimension of the printlets was evaluated by two approaches, namely DOE and geometric calculation. Both of them came up with good prediction equations.

Table 2: DOE regression results (confident level: 95%)

Term	Weight		Dose strength	
	Coefficient	P	Coefficient	P
Constant	252.27	0.000	12.6667	0.000
d	79.48	0.000	4.02386	0.000
h	37.03	0.000	1.9313	0.000
d^2	5.30	0.029	0.317274	0.010
d*h	9.82	0.001	0.579545	0.000
R2	0.997		0.997	
$R2_{adj}$	0.995		0.996	
Q2	0.733		0.719	
P	0.000		0.000	
$P_{lack\ of\ fit}$	0.188		0.529	

The results of DOE regression were shown in Table 2. Statistical analysis exhibited that the established regression equations were significant (p=0.000) and dependable for correlating the independent factors with the output variables. The correlation could be visually elaborated by the established response surfaces as shown in Figure 3. The good of fit (R2 > 0.99) and good of prediction (Q2 > 0.71) suggested that the regression models could accurately calculate the input factors from the outcomes and vice versa. Such models allow healthcare workers to fabricate printlets with predetermined dose strength accurately, without mistakes.

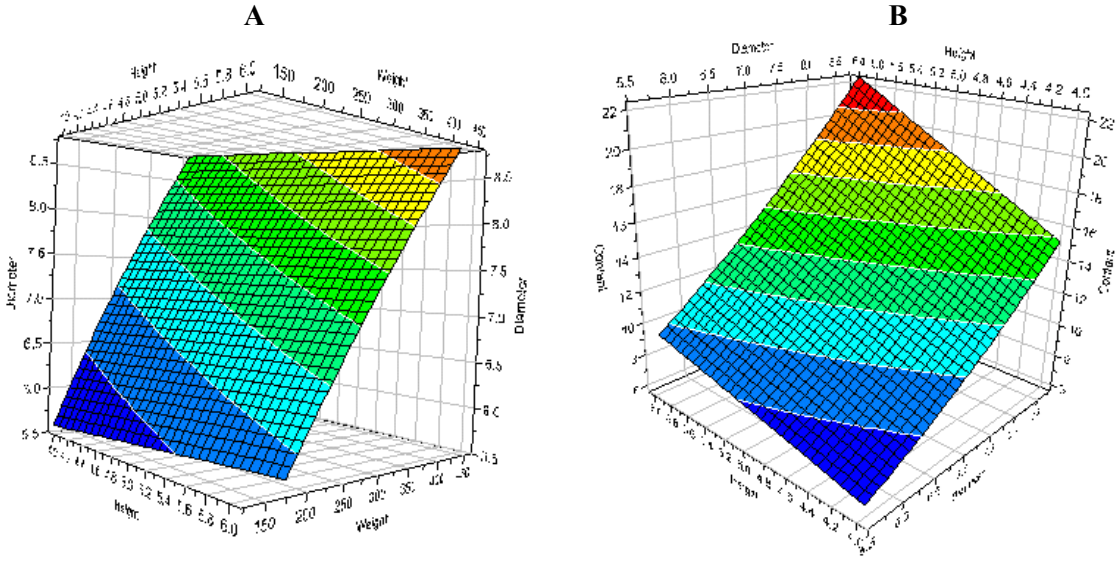


Figure 3: Response surfaces visually describing the correlation between dimension and printlet weight (A); dimension and dose strength of printlets (B).

The mass and dose strength of printlets could also be calculated from geometric parameters of the printlets. Initially, parts with different densities were determined, then their volume was calculated individually based on dimension parameters. The mass of each part was then calculated by multiplying the volume with its density before summing up the mass of all parts of a printlet. Applying

this method to 12 DOE formulations resulted in an excellent match between the calculated mass and actual weight of the printlets (Figure 4), as well as between the calculation and actual dose strength. The correlation coefficients of both mass and dose strength of the printlets were great (R^2 >0.995) and all calculated values deviated less than 5% from their actual values.

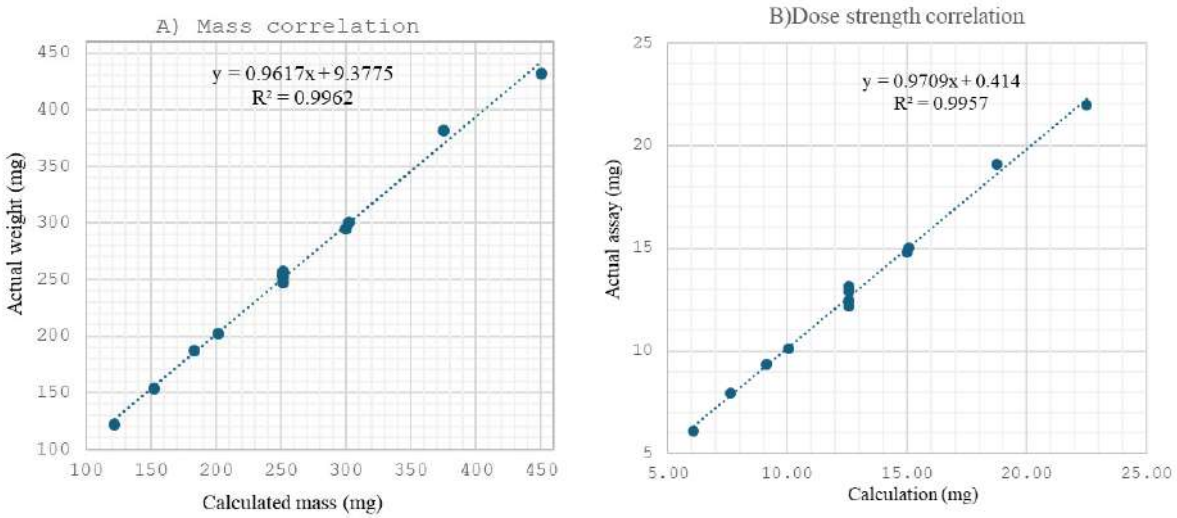


Figure 4: Correlation plots of: A) actual weight (by weighing the printlets) vs calculated mass; B) actual dose strength (determined by HPLC) vs. calculated values.

3.3. Potential for tailoring dissolution kinetics.

To demonstrate the capability of tailoring dissolution profiles, printlets with 5.0 mm height and 7.2 mm diameter were fabricated with 0.8 mm wall thickness and four infill levels from 30% to 100%. The experimental results demonstrated that the dissolution profiles of the printlets were greatly influenced by the infill density. Dissolution of the printlets could be extended from 3 hours to 8 hours. Increasing the infill density would result in more prolonged drug release kinetics. The dissolution of the printlets could be further accelerated by reducing the wall thickness, and vice versa. Additionally, substituting hydrophilic plasticizer glycerin with a more hydrophobic plasticizer, such as castor wax or stearic acid, could drastically prolong the drug release profile of the printlets. The experimental results suggested that the dissolution profile of the dosage form could be flexibly customized by design, printing parameters, and additives.

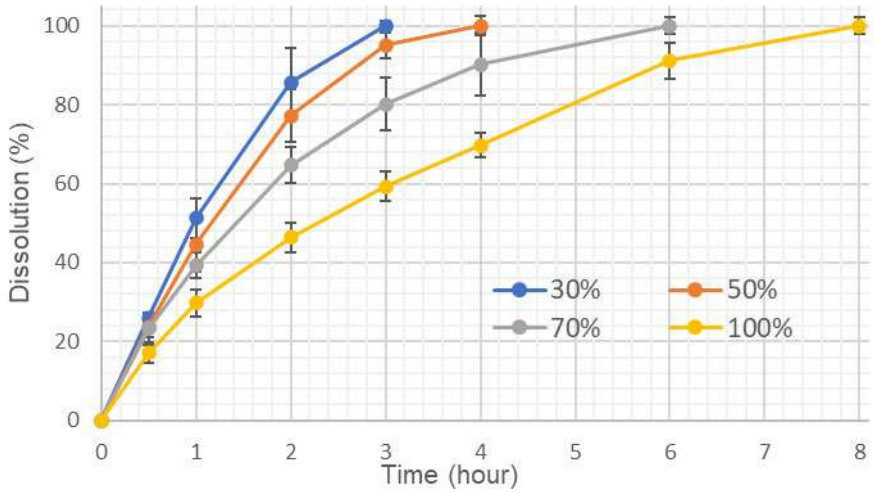


Figure 5: Dissolution profiles of 0.8 mm wall printlets with various infill densities

4. CONCLUSION

Tailorable dosage forms containing methylprednisolone were successfully developed using the 3D FDM platform. The dose strength of the printlets could be accurately tailored by controlling design utilizing either regression equations or geometric calculations. Drug release kinetics of the printlets were customizable based on defining infill density of the design. Such flexible adjustments for the developed printlets may not be feasible for the current pharmaceutical manufacturing technologies. The proposed paradigm is a viable approach to the preparation of individualized medications, especially for treatment in the pediatric population.

5. ACKNOWLEDGMENT

Conflict of interest

The authors declare that they have no conflict of interest.

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Biopharmaceutical Sciences and Pharmaceutical Biotechnology

BB-0602001-O

Association Between Shorter Telomeres and Risk of Non-Communicable Diseases

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ABSTRACT

Non-communicable diseases (NCDs) pose a major global health concern, accounting for 71% of total deaths worldwide. These conditions are characterized by an accelerated aging phenotype, often associated with telomere attrition. Leukocyte telomere length (LTL) is recognized as a reliable indicator for predicting age-related pathological conditions, including cardio-metabolic diseases. This study aimed to examine LTL in Thai patients with NCDs compared to age-matched healthy volunteers and determine its association with NCD risks. A total of 252 patients with NCDs, including hypertension (n=147), diabetes mellitus (DM, n=81), cardiovascular diseases (CVDs, n=18), and various cancers (n=6), and 20 age-matched healthy controls were recruited. Relative telomere length in blood leukocytes was measured using quantitative real-time polymerase chain reaction. Study participants were categorized based on the median distribution of LTL in healthy volunteers into those with shorter LTL (<1.894, n=187) and those with longer LTL (≥1.894, n=65). Compared to age-matched healthy controls, patients with hypertension, DM, and CVDs exhibited significantly shorter LTL (P=0.005, P=0.033, P=0.030, respectively). In patients with cancers, LTL was longer than in controls, but this difference was not statistically significant. After adjusting for age and gender, individuals with shorter LTL had a significantly higher risk of DM, with a 2.08-fold increase compared to those with longer LTL (OR=2.083, 95% CI: 1.038, 4.178, P=0.039). Conversely, no significant associations were found between shorter LTL and risks of hypertension, CVDs, or cancers. Collectively, our findings reveal a direct link between shorter telomeres in blood leukocytes and DM risk, suggesting that LTL could serve as an aging biomarker for predicting the development of NCDs.

KEYWORDS: Telomere length; Aging biomarker; Non-communicable diseases (NCDs); Diabetes mellitus (DM)

1. INTRODUCTION

Non-communicable diseases (NCDs) pose a significant challenge to global health, accounting for approximately 71% of all deaths worldwide annually[1]. The diseases are caused by a combination of genetic, physiological, environmental, and lifestyle factors. The main NCDs consist of cardiovascular diseases, cancers, chronic respiratory diseases, and diabetes. Among NCDs, diabetes mellitus (DM) is rapidly increasing, significantly impacting health, society, and the economy. There are multiple factors contributing to the rise in diabetes cases worldwide. These factors contributing to health issues encompass aging populations, urbanisation, reduced physical activity, poor diets, genetic predisposition, and socioeconomic challenges like limited healthcare access[2-3]. Moreover, DM is a significant cause of cardiovascular diseases (CVDs), kidney failure, blindness, and amputations[4], placing substantial burdens on healthcare systems. The onset and progression of DM are influenced by the effects of aging, which include the accumulation of oxidative damage and a decrease in antioxidant capacity[5-6].

Aging is characterized by various physiological changes, such as a decrease in insulin sensitivity and an increase in oxidative stress. Previous studies have shown that telomere attrition, a marker of biological aging, was associated with chronic hyperglycemia and oxidative stress, leading to genomic instability and cellular dysfunction[7-10]. Thailand is experiencing an increasing challenge with NCDs, and DM is a major issue that needs attention. The prevalence of DM has been on the rise due to factors such as rapid urbanization, dietary changes, and an aging population. In 2014, approximately 8.9% of Thai adults suffered from DM, and it is expected that this number will continue to increase[11]. Accordingly, the objective of this study was to examine leukocyte telomere length (LTL) in Thai patients with NCDs compared to age-matched healthy volunteers and to determine the possible association between LTL and NCD risks.

2. MATERIALS AND METHODS

2.1. Study subjects

This study included 252 patients with NCDs, comprising 147 patients with hypertension, 81 patients with DM, 18 with cardiovascular diseases (CVDs), and 6 patients with various cancers, alongside 20 age-matched healthy control subjects. Informed consent was obtained from each participant. The study protocol adhered to the ethical standards outlined in the Declaration of Helsinki and received approval from the Ethical Committee on Human Research of the Faculty of Dentistry and Faculty of Pharmacy, Mahidol University, as well as the Faculty of Medicine, Mahidol University. DNA samples were collected by the Center for Medical Genomics, Ramathibodi Hospital, and subsequently transferred to the Faculty of Pharmacy, Mahidol University. Clinical data will be retrieved from the automated digital database of Ramathibodi Hospital.

2.2. Measurement of telomere length

Telomere length in DNA extracted from peripheral blood leukocytes was assessed using a quantitative real-time PCR technique, as outlined by Cawthon et al. [12] This method involves calculating the ratio of telomere repeats (T) to single-copy genes (S), employing the 36B4 gene, which encodes the acidic ribosomal phosphoprotein (PO). The resulting T/S ratio serves as an indicator of the average telomere length. PCR amplification was conducted in 10 µl reactions using the QuantStudio™ 3 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The primers for telomere repeat amplification were: telomere forward 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGGTTTGGGTT-3' and telomere reverse 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'. For the single-copy gene, the primers were: single-copy gene forward 5'-CAGCAAGTGGGAAGGTGTAATCC-3' and single-copy gene reverse 5'-CCCATTCTATCATCAACGGGTACAA-3'. The quantities of telomere repeats and single-copy genes in each sample were normalized to a reference DNA sample obtained from a single individual. This reference was incorporated into each measurement to ensure consistency and control for inter-assay variability.

2.3. Statistical analysis

Statistical analyses were performed with the SPSS statistical package, version 26.0 (SPSS Inc., Chicago, IL, USA). The differences in continuous variables represented as median with interquartile range (Q1, Q3) among groups were executed using Kruskal–Wallis H test, whereas the differences in categorical variables represented as number with percentage were assessed using Chi-square test. Multivariate logistic regression analysis with adjustments for confounding factors including age and gender was undertaken to determine the possible associations between shorter telomeres and risks of NCDs. Statistical significance was determined by considering a P-value <0.05, based on a two-tailed test, for all analyses.

3. RESULTS AND DISCUSSION

3.1. Baseline characteristics of study participants

A total of 252 patients with NCDs, including hypertension (n=147), DM (n=81), CVDs (n=18), and various cancers (n=6), and 20 age-matched healthy controls were included in this study. There were no statistically significant differences in mean age and gender ratio between patients with NCDs, including those with hypertension (mean age=60.05±3.60 years, 79.19% males), those with DM (mean age=61.03±4.29 years, 80.25% males), those with CVDs (mean age=62.78±5.01 years, 83.33% males), and those with cancers (mean age=64.67±6.41 years, 83.33% males), and healthy controls (mean age=60.05±3.60 years, 80.00% males) (*P*=0.073, *P*=0.174, respectively).

3.2. LTL in NCDs patients and healthy controls

Compared to age-matched healthy controls [1.894 (1.553, 2.428)], median LTL was found to be significantly shortened in patients with hypertension [1.240 (0.780, 2.000)], DM [1.465 (0.803, 1.988], and CVDs [1.335 (0.365, 2.053] (*P*=0.005, *P*=0.033, *P*=0.030, respectively) (Figure 1). In patients with cancers [2.115 (1.555, 2.538)], LTL was longer than in healthy controls, but this difference was not statistically significant (Figure 1).

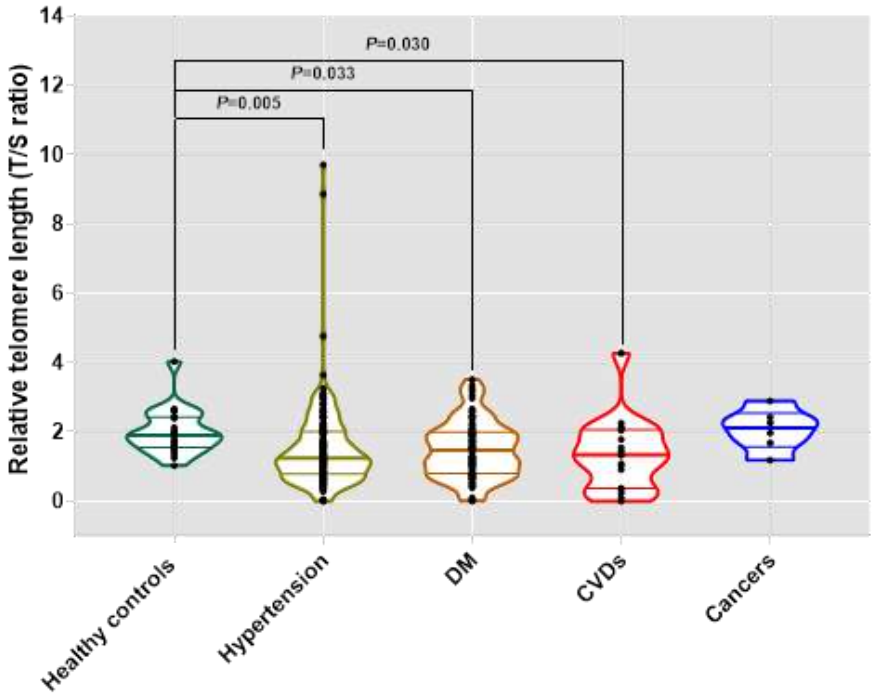

Figure 1. LTL of NCDs patients and aged-matched healthy controls. Abbreviations: CVDs, cardiovascular diseases; DM, diabetes mellitus.

3.3. Association between shortened LTL and risks of NCDs

Based on the median distribution of LTL in healthy volunteers, study participants were categorized into those with shorter LTL (<1.894, n=186) and those with longer LTL (≥1.894, n=86). The association between shortened LTL and risks of NCDs is detailed in Table 1. Individuals with shorter LTL had a significantly higher risk of DM, with a 2.08-fold increase compared to those with longer LTL (OR=2.083, 95% CI: 1.038, 4.178, *P*=0.039), after adjusting for age and gender. Conversely, there were no notable relationships discovered between shorter LTL and the risks of hypertension, CVDs, or cancers.

Table 1 Association between shortened LTL and risks of NCDs.

LTL	NCDs		Adjusted model		
	Yes (%)	**No (%)**	**OR**	**95% CI**	***P*-values**
Hypertension (n=147)					
Shorter LTL	106 (72.11%)	80 (64.00%)	1.554	0.913, 2.645	0.104
Longer LTL	41 (27.89%)	45 (36.00%)	Reference		
DM (n=81)					
Shorter LTL	66 (81.48%)	131 (68.59%)	**2.083**	**1.038, 4.178**	**0.039**
Longer LTL	15 (18.52%)	60 (31.41%)	Reference		
CVDs (n=18)					
Shorter LTL	13 (72.22%)	173 (68.11%)	1.367	0.459, 4.072	0.574
Longer LTL	5 (27.78%)	81 (37.89%)	Reference		
Cancers (n=6)					
Shorter LTL	2 (33.33%)	184 (69.17%)	Reference		
Longer LTL	4 (66.67%)	82 (30.83%)	4.304	0.702, 26.402	0.115

P-values marked with bold indicate statistically significant associations between shorter LTL and susceptibility to NCDs. OR was adjusted for age and gender. Abbreviations: CI, confidence interval; CVDs, cardiovascular diseases; DM, diabetes mellitus; LTL, leukocytes telomere length; OR, odd ratio.

Chronic hyperglycemia in diabetes induces oxidative stress and inflammatory responses, which accelerate telomere erosion. This notable shortening of telomeres in diabetic patients highlights their increased susceptibility to complications such as hypertension and CVDs. The study uncovered that after adjusting for age and gender, individuals with shorter LTL had a significantly higher risk of DM compared to those with longer LTL. In line with our findings, previous studies showed telomere shortening in patients with DM[13]. However, no significant associations were found between shorter LTL and the risks of hypertension and CVDs after adjusting for age and gender. This indicates that shorter LTL may be a more sensitive and specific biomarker for DM compared to other NCDs. This finding establishes a direct link between shorter LTL and the risk of DM, suggesting that LTL could be a robust marker for DM. In the context of hypertension, patients exhibited a marked reduction in LTL compared to healthy controls, consistent with existing literature that associates hypertension with telomere shortening due to increased oxidative stress and inflammation[14]. Similarly, patients with cardiovascular diseases showed significantly shorter LTL compared to controls. The relationship between CVDs and telomere length is well-documented[15], with the pro-inflammatory state and oxidative stress associated with CVDs contributing to telomere shortening, emphasizing the importance of telomere maintenance for cardiovascular health. Interestingly, the study included a smaller cohort of patients with various cancers, who had longer LTL than controls, although this difference was not statistically significant. This complexity suggests that the relationship between telomere length and cancer might be influenced by various factors, necessitating further research to fully understand the specific impact on telomere length.

The study's findings have several clinical implications. LTL measurement could serve as a non-invasive biomarker for identifying individuals at higher risk for DM. The significant association between shorter telomeres and DM emphasizes the potential of LTL as a predictive marker for the onset and progression of diabetes-related complications. Interventions aimed at reducing oxidative stress and inflammation might help in preserving telomere length and potentially mitigating disease risk. Lifestyle modifications, such as improved diet, regular physical activity, and smoking cessation, may also contribute to telomere maintenance and overall health. Furthermore, the study highlights the need for longitudinal research to establish causal relationships and explore the effectiveness of telomere-targeted therapies. Understanding the mechanisms of telomere shortening in NCDs, particularly how DM exacerbates other conditions, could lead to novel therapeutic strategies aimed at preventing or delaying the onset of these diseases.

4. CONCLUSION

In conclusion, our findings revealed a direct link between shorter telomeres in blood leukocytes and DM risk, suggesting that LTL could serve as an aging biomarker for predicting the development of NCDs.

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Identification of Biomarkers for Treatment Resistance in Breast and Ovarian Cancer Patients Using Transcriptome Datasets

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ABSTRACT

Breast cancer and ovarian cancer are highly prevalent and life-threatening diseases affecting women globally, characterized by complexity and heterogeneity. Furthermore, women who have experienced breast cancer are also at risk of developing ovarian cancer. To identify transcriptomic biomarkers for the prediction of disease-free survival (DFS) and overall survival (OS) in breast and ovarian cancer patients. We examine transcriptomic data specific to breast and ovarian carcinoma, exploring gene expression patterns associated with the treatment resistance in these cancers. The biological and genetic data generated via high-throughput screening of two patient datasets were retrieved. Patients were divided into two groups, i.e., recurrence and disease-free, based on their DFS status. Differential gene expression (DGE) analysis was performed to compare the differences between the recurrence and disease-free groups, categorized by a fold change (FC) and p-value. Enrichment pathway analysis unveiled the biological pathways linked to drug responsiveness. Additionally, we analyzed the prognostic ability of the candidate gene level to the patient survival rate using the Kaplan-Meier analysis and Cox regression model. The enriched pathways of up-regulated genes in both breast and ovarian cancers were related to the hormone biosynthetic process. On the other hand, the immune response pathway was associated with down-regulated genes. The survival analyses demonstrated that the expression levels of the candidate biomarkers notably impacted patients' DFS. The final biomarkers served as independent prognostic markers. RASGRP1 was associated with a good prognosis in breast cancer. In contrast, SEMA5B was associated with a poor prognosis in ovarian cancer. We proposed novel transcriptomic biomarkers for predicting responses to the treatment and enhancing treatment strategies for both breast and ovarian cancers.

KEYWORDS: Breast cancer; Ovarian cancer; Biomarker; Recurrence; Disease-free survival; Overall survival

1. INTRODUCTION

Breast carcinoma is the most prevalent malignant tumor found globally. It originates from epithelial cells lining the terminal duct lobular unit. Cells that stay within the basement membrane of these units and the draining duct are categorized as in situ or noninvasive. Invasive breast cancer refers to cancer cells that have spread beyond the basement membrane into surrounding normal tissue. Traditionally, invasive breast cancers were classified as ductal or lobular based on their origins from ducts or lobules. However, it is now known that both invasive ductal and lobular breast cancers stem from the terminal duct lobular unit, rendering this classification outdated. Breast cancer treatment is determined based on the molecular subtype, regardless of whether it is in the early or advanced stages. Typically, treatment begins with surgical removal of cancer. Following surgery, additional therapies

such as radiation, chemotherapy, and hormone therapy are often administered. In some cases, chemotherapy or hormone therapy may be given before surgery to reduce the size of the cancer and facilitate its removal[1,2]. Ovarian cancer is a significant cause of cancer-related deaths among women globally, with more than 310,000 new cases and approximately 200,000 deaths reported in 2020. Epithelial ovarian cancer, which encompasses a diverse range of molecular and histological characteristics, accounts for about 90% of ovarian cancer cases. The genetic connection between the ovaries and breasts is significant, particularly *BRCA1* and *BRCA2* gene mutations, which have been linked to elevated risks of both breast and ovarian cancer. Consequently, women diagnosed with ovarian cancer may be advised to undergo genetic testing and counseling to address their breast cancer risk. Similarly, women diagnosed with breast cancer, especially those with a family history of breast or ovarian cancer, may also be recommended genetic testing and counseling to manage their ovarian cancer risk[3]. Transcriptomics technologies encompass the methods employed to investigate an organism's transcriptome, which comprises all RNA transcripts. The genetic information of an organism resides in its DNA genome and is expressed through transcription[4].

2. MATERIALS AND METHODS

2.1. Data and patient characteristics

We used the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases to retrieve the data and comply with ethical guidelines for data sharing and reporting. All participants in these studies provided informed consent for their data to be used in research. The gene expression profiling data for breast and ovarian cancer patients, their corresponding clinical details, and validated RNA-seq results were downloaded from cBioPortal and GEO databases, respectively as public databases. Breast cancer patients were divided into two datasets based on their treatments: RCH (Radiotherapy + Chemotherapy + Hormone therapy) and RCTH (Radiotherapy + Chemotherapy + Targeted therapy + Hormone therapy). Ovarian cancer patients were also divided into two datasets: GSE165808 and GSE143897. The clinical and genetic data of the patient were retrieved, including RNA-seq gene expression, disease-free status, overall survival, and other available clinical data.

2.2. Differentially Expressed Genes (DEGs)

To assess gene expression differences between different recurrent status, we analyzed patient genomic data to identify Differentially Expressed Genes (DEGs). Patients in each dataset were classified into two groups by DFS status (comparing recurrence and disease-free). Gene expressions of each gene were compared between the two groups of patients based on the DFS using R program. Genes were declared as DEGs when $|FC|>2$ and the adjusted p-value<0.05. Therefore, the common genes in both breast and ovarian cancer were obtained for subsequent analysis.

2.3. Gene set enrichment analysis

DEGs based on common genes in both breast and ovarian cancer were subjected to enrichment analysis using gene ontology (GO). For this study, a significance threshold of p-value < 0.05 was selected and applied for subsequent analysis.

2.4. Survival and Cox regression analysis

To identify genes associated with patients' disease-free status (DFS) by comparing recurrence and disease-free states, as well as overall survival (OS) by comparing deceased and living patients, the patient samples will be divided into two groups based on the median expression of the gene (high vs low expression). Gene expression data was underwent survival analysis using the Kaplan-Meier plotter using mRNA breast and ovarian cancer database to analyze DFS and OS, incorporating hazard ratios (HRs), 95% confidence intervals (95% CIs), and log-rank p-values. Genes significantly associated with DFS and OS were initially identified using univariate Cox regression analysis.

Subsequently, significant genes underwent multivariate Cox regression survival analysis to adjust for clinical factors in both breast cancer (age, stage, menopausal status, lymph node involvement, ER, PR, HER2 status) and ovarian cancer (age, stage, platinum status), identifying genes associated with either better or worse prognosis.

2.5. Statistical analysis

The nonparametric Mann–Whitney U test and Pearson's Chi-square were utilized for the statistical analysis of associations between gene expression and patient characteristics. Survival rate curves for two groups DFS (comparing recurrence and disease-free states) and OS (comparing deceased and living patients) were generated using the Kaplan–Meier method and compared using the log-rank test. Cox's proportional hazards model was employed for univariate and multivariate analyses of prognostic biomarkers for treatment recurrence and patient survival. The EZ package for R version 4.4-0 was used for statistical analysis (Figure 1).

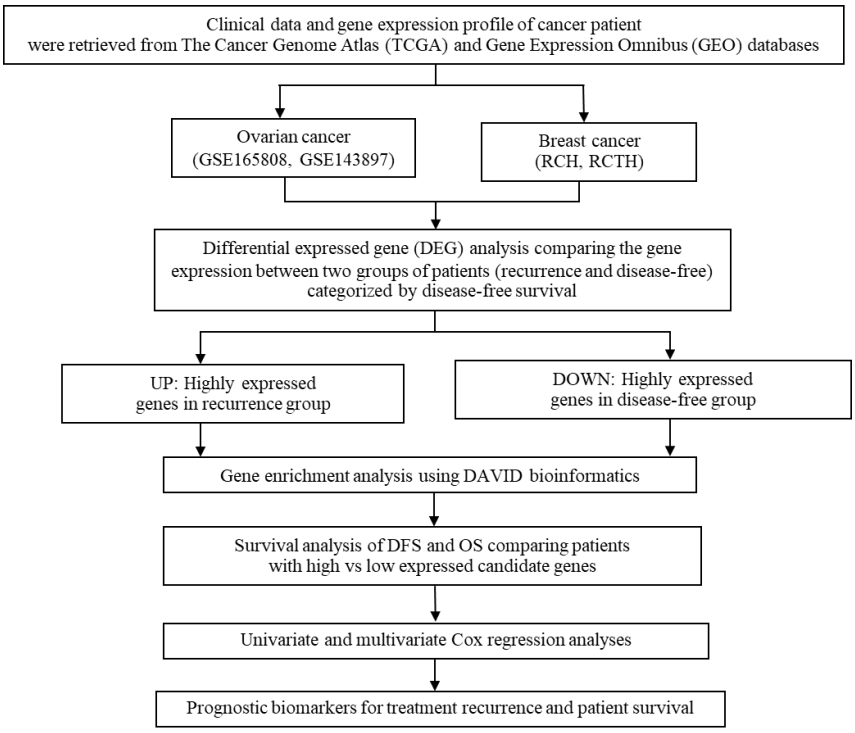

Figure 1. Research design of the study.

3. RESULTS AND DISCUSSION

3.1. Numbers and characteristics of patients

The cases for breast cancer included in this study were patients having RNA-seq gene expression and DFS data, female gender, and available data of received treatments including chemotherapy, hormone therapy, radiotherapy, and targeted therapy (Table 1).

Table 1. The characteristics of breast cancer datasets (TCGA, 187 patients).

Clinical characteristics of patients (N=187)	Recurrence N (%) 20 (10.7)	Disease-free N (%) 167 (89.3)	P-value
Age at diagnosis (year)			
Median (IQR)	60.5 (44.5-62.5)	53 (46.0-63.0)	0.72[b]
Tumor stage			
Stage I	2 (10.0)	18 (10.8)	0.16[c]
Stage II	7 (10.9)	91 (54.8)	
Stage III	11 (55.0)	57 (34.3)	
Menopause status			
Premenopause	6 (30.0)	58 (36.5)	0.56[c]
Perimenopause	1 (5.0)	4 (2.5)	
Postmenopause	13 (65.0)	97 (61.0)	
Number of positive lymph nodes			
<6	10 (55.6)	127 (85.8)	<0.01[c]
6 to <10	4 (22.2)	7 (4.7)	
≥10	4 (22.2)	14 (9.5)	
ER status[a]			
Negative	4 (22.2)	6 (3.8)	0.01[c]
Positive	14 (77.8)	153 (96.2)	
PR status[a]			
Negative	6 (33.3)	29 (18.4)	<0.01[c]
Positive	12 (66.7)	129 (81.6)	
HER2 status[a]			
Negative	7 (50.0)	87 (59.6)	0.68[c]
Positive	3 (21.4)	27 (18.5)	
Equivocal	4 (28.6)	32 (21.9)	
Treatment regimen			
Radiotherapy + Chemotherapy + Hormone therapy	15 (75.0)	141 (84.4)	0.34[c]
Radiotherapy + Chemotherapy + Hormone therapy + Targeted therapy	5 (25.0)	26 (15.6)	

[a]Status was assessed by Immunohistochemistry, [b]Mann-Whitney U-test, [c]Pearson Chi-square

3.2. Differentially Expressed Genes (DEGs) in both breast and ovarian cancer patients

Analysis of DEGs was performed between recurrence and disease-free patients. To conduct the comparisons, the disease-free group was designated as the reference, highlighting the DEGs in the recurrence group. Fold-change (FC) indicates the ratio of mRNA expression of recurrence over that of disease-free groups. A total of 20,531 gene symbols were identified from the collected samples. The numbers of up-regulated DEGs for RCH, RCTH, GSE165808 and GSE143897 were 332, 593, 392, and 231, respectively, while the numbers of down-regulated DEGs were 272, 324, 339 and 163, respectively. These were identified with a p-value < 0.05 and |FC| > 2.

3.3. Pathway and Enrichment Analysis

Gene Set Enrichment Analysis (GSEA) was utilized to detect pathways significantly enriched (p-value < 0.05) in common gene expression datasets for both breast and ovarian cancer. Subsequently, the enrichment scores were normalized considering the variation in the gene dataset. 82 genes showed

common up-regulated gene expression (Figure 2A), while 43 genes showed common down-regulated gene expression (Figure 2B). The main functions of the up-regulated DEGs in cancers were mainly enriched in hormone biosynthetic and cell motility pathways, with 24 related up-regulated genes (Figure 3A). Conversely, the main functions of the down-regulated DEGs in cancers were mainly enriched in immune response and chemokine-mediated signaling pathways, with 17 related down-regulated genes (Figure 3B).

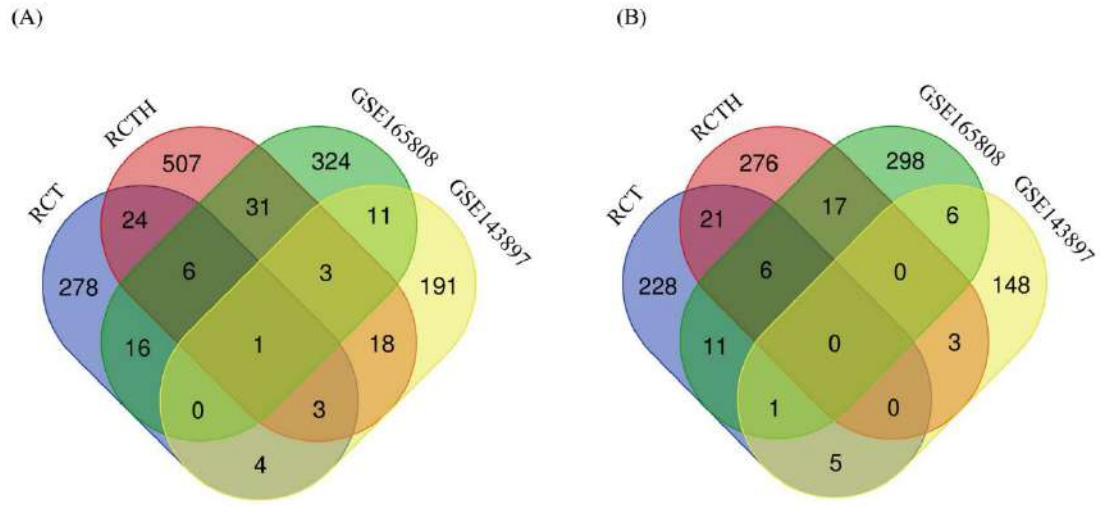

Figure 2. Venn diagrams indicate the numbers of up-regulated (A) and down-regulated (B) genes from breast and ovarian cancers.

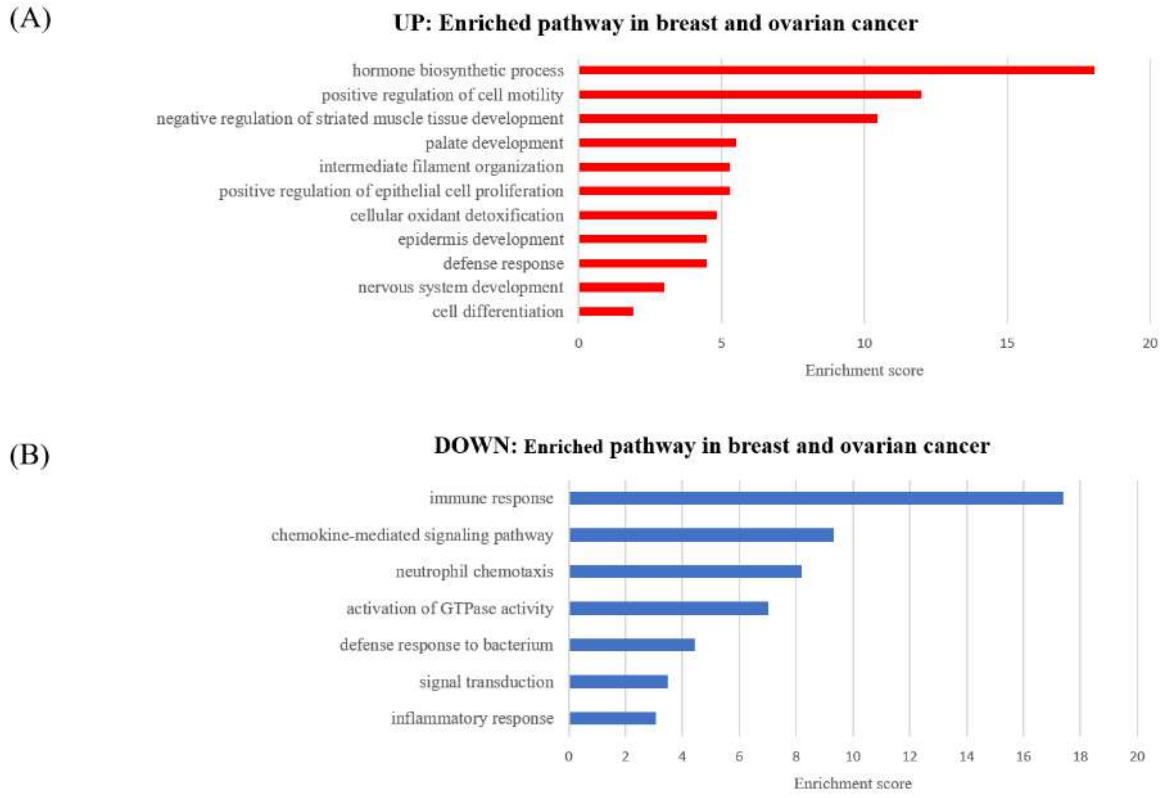

Figure 3. Enrichment pathways of common biomarkers in breast and ovarian cancers.

3.4. Genes associated with survival of patients with breast and ovarian cancers

Survival analysis was conducted to determine the survival rates of patients based on disease-free status data. In breast cancer, the expression of RASGRP1 has been linked to the enrichment of GTPase activity activation and signal transduction, both of which play crucial roles in cancer development and response to therapies (Figure 3B). The expression of RASGRP1 was down-regulated in breast and ovarian patients with recurrence. Interestingly, breast cancer patients with high expression of RASGRP1 showed improved disease-free survival outcomes. In ovarian cancer, the expressions of ZNF750 and SEMA5B were up-regulated in recurrence and they were in a similar enriched pathway, i.e., cell differentiation (Figure 3B). On the other hand, the HLA-DRB5 gene is down-regulated and was in immune response enrichment pathways. In addition, ovarian cancer patients with high expressions of ZNF750 and SEMA5B showed poor disease-free survival outcomes, whereas high expression of HLA-DRB5 showed good disease-free survival outcomes. However, none of candidate biomarkers for DFS was associated with OS of breast and ovarian cancer patients.

3.5. Prognostic significance of genes in with breast and ovarian cancers

Univariate and multivariate Cox regression analyses were conducted to assess the prognostic significance of genes in both breast and ovarian cancer (Table 1 and Table 2). In the multivariate analysis of disease-free survival (DFS), which included factors such as age, menopause status, tumor stage, lymph node status, ER, PR, and HER2 status for breast cancer, as well as age, tumor stage, and platinum status for ovarian cancer. RASGRP1 expression and ER status were identified as independent prognostic predictors for longer DFS of breast cancer patients (Figure 4A). SEMA5B expression and platinum status emerged as independent prognostic predictors for shorted DFS in ovarian cancer (Figure 4B). Univariate and multivariate analyses were conducted on a cohort of 131 breast cancer patients and 46 ovarian cancer patients, for whom complete data were available.

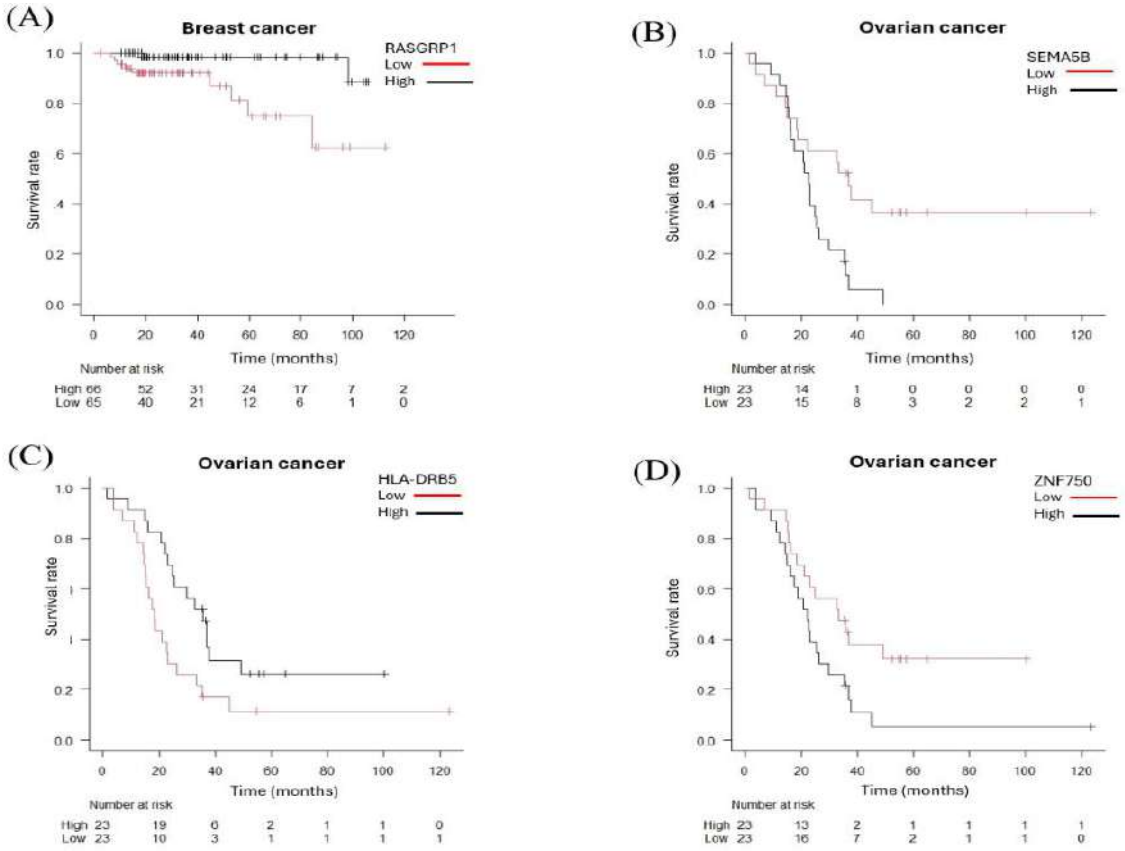

Figure 4. Kaplan-Meier analyses of DFS in breast (A) and ovarian (B, C, D) cancers based on expression level (low, black; red, high) of candidate biomarkers.

Table 2. Univariate and multivariate analyses of breast cancer with DFS.

Variable	Univariate analysis (N=131)			Multivariate analysis (N=131)		
	Hazard ratio	95%CI	P-value	Hazard ratio	95%CI	P-value
RASGRP1						
Low (≤ Median)	1	Reference		1	Reference	
High	0.13	0.02-0.64	0.01*	0.16	0.03-0.82	0.03*
Age						
≤ 55	1	Reference				
> 55	1.68	0.51-5.66	0.39			
Stage						
I	1	Reference				
II	0.91	0.10-8.24	0.93			
III	2.07	0.25-17.29	0.50			
ER status						
Negative	1	Reference		1	Reference	
Positive	0.09	0.02-0.38	<0.01*	0.12	0.03-0.54	<0.01*
PR status						
Negative	1	Reference				
Positive	0.53	0.15-1.82	0.31			
HER2 status						
Negative	1	Reference				
Positive	1.33	0.26-6.68	0.73			
Equivocal	0.88	0.22-3.57	0.86			
Menopause						
Premenopause	1	Reference				
Perimenopause	3.55	0.39-32.28	0.26			
Postmenopause	1.44	0.40-5.16	0.58			
LN						
<6	1	Reference		1	Reference	
6 to <10	4.14	1.03-16.57	0.04*	1.37	0.29-6.41	0.69
≥10	1.93	0.23-15.90	0.54	1.52	0.18-13.22	0.7

Table 3. Univariate and Multivariate analyses of ovarian cancer with DFS.

Variable	Univariate analysis (N=46)			Multivariate analysis (N=46)		
	Hazard ratio	95%CI	P-value	Hazard ratio	95%CI	P-value
HLA-DRB5						
Low (≤ Median)	1	Reference		1	Reference	
High	0.47	0.24-0.91	0.02*	0.76	0.29-1.97	0.57
SEMA5B						
Low (≤ Median)	1	Reference		1	Reference	
High	2.63	1.29-5.35	<0.01*	2.37	1.05-5.351	0.04*
ZNF750						
Low (≤ Median)	1	Reference		1	Reference	
High	2.07	1.06-4.07	0.03*	1.56	0.64-3.82	0.33
Age						
≤ 54	1	Reference				
> 54	1.94	0.98-3.83	0.06			
Stage						
I	1	Reference		1	Reference	
II	<0.01	0.00-Inf	0.10	<0.01	0.00-Inf	0.10
III	5.20	0.70-38.52	0.11	4.7	0.57-38.84	0.15
IV	28.12	3.07-257.80	<0.01*	3.5	0.23-53.58	0.37
Platinum status						
Sensitive	1	Reference		1	Reference	
Resistant	7.31	3.16-16.92	<0.01*	5.53	1.75-17.47	<0.01*

Women with aging have a higher incidence of breast and ovarian cancers, with well-known risk factors including nulliparity or delayed childbirth, hormone replacement therapy usage, and specific gene mutations. Moreover, a history of breast cancer increases the risk of developing ovarian cancer. This study screened candidate genes from databases using whole transcriptome analysis, identifying common prognostic genes for model construction[5] based on multiple distinct steps. In this study, transcriptomics was employed to investigate gene expression in cancer. Based on enrichment analysis, genes related to the hormone biosynthetic process and positive regulation of cell motility were predominantly found among the up-regulated DEGs. Simultaneously, genes associated with the immune response and chemokine-mediated signaling pathway were mainly found among the down-regulated DEGs. Additionally, numerous studies report that the hormone biosynthetic and cell motility pathways are associated with metastasis, a key factor in cancer progression and spreading[6]. On the other hand, immune response pathways and chemokine-mediated signaling play crucial roles in immune cell recruitment and activation, influencing cancer immune surveillance encompassing both innate and adaptive immune responses. These pathways are critical in cancer suppression, drug sensitivity, and preventing cancer recurrence[7]. Survival analysis was conducted across all survival-associated genes in breast and ovarian cancer patients. We also performed univariate and multivariate Cox analysis of clinical factors to assess their prognostic value. Specifically, *RASGRP1* was found to be overexpressed in long-survival patients, suggesting its potential as a significant tumor suppressor in cancer (HR = 0.13, p-value = 0.01). Previous studies have associated *RASGRP1* with tumor suppression in the colon, where it inhibits EGF-driven proliferation of colonic organoid cultures. Reduced *RASGRP1* levels were predictive of poor clinical outcomes in colorectal cancer patients[8]. In contrast, *SEMA5B* was observed to be overexpressed in short-survival patients (HR = 2.37, p-value = 0.04). This finding could be particularly significant as a poor prognostic biomarker for cancer patients and should be further investigated. Due to the limitation in bioinformatics analyses in this study, experimental validation is necessary to ensure the clinical implementation of these biomarkers.

4. CONCLUSION

This study introduces novel transcriptomic biomarkers for predicting treatment responses. By conducting survival analysis across all genes, we identified the top-performing genes in both breast and ovarian cancer. Specifically, *RASGRP1* was overexpressed in patients with better outcomes. Conversely, *SEMA5B* overexpression correlated with poorer prognosis. This study aims to determine their potential as biomarkers to guide treatment decisions and enhance patient outcomes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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The Constituents Potential from Melinjo Peel (*Gnetum gnemon* L.) as Anti-inflammatory: *In Silico* Molecular Docking and ADME-Tox Prediction

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ABSTRACT

Melinjo peel is reported to contain ascorbic acid, tocopherol, and polyphenols with high anti-inflammatory potential. However, scientific research has not yet been carried out, so in silico is the initial stage in predicting the potential of contained compounds targeting the enzymes cyclooxygenase-2 (COX-2) and inducible Nitric Oxide Synthase (iNOS). This research aims to in silico investigate the specific target for the anti-inflammatory potential of compounds found in melinjo peel and to predict their ADME-Tox properties. Potential interactions of compounds from melinjo peel (ascorbic acid, *trans*-resveratrol, gnetin C, gnemonoside A, and gnemonoside D) to COX-2 and iNOS enzymes using AutoDock Tools 1.5.6. Then, in silico molecular docking results were predicted for pharmacokinetic properties using the pkCSM ADMET descriptors algorithm protocol for used as a drug product. In silico molecular docking results are based on the binding affinity values (ΔG and Ki). Gnetin C has the potential to act as an anti-inflammatory for the COX-2 with values of -9.91 kcal/mol and 54.69 nM. However, rofecoxib is still better as a comparison (drug), namely -10.66 kcal/mol and 15.23 nM. Meanwhile, the iNOS target shows that gnemonoside D and gnetin C have excellent potential of -8.61; 486.01 (kcal/mol) and -7.53; 3030 (nM), respectively, compared to dexamethasone (-6.81; 10210) as a drug. The values are obtained from the clustering histogram. Meanwhile, only gnetin C meets ADME-Tox predictions based on the parameters intestinal absorption (human), CNS permeability, cytochrome P450 (CYP2C9 inhibitor and CYP3A4 substrate), total clearance, AMES toxicity, heRG II channel, as well as hepatotoxicity. The iNOS enzyme is a specific target, and the potential anti-inflammatory compound from melinjo peel is gnetin C (stilbenoid).

KEYWORDS: COX-2; iNOS; melinjo peel; molecular docking; ADME-Tox

1. INTRODUCTION

Acute and chronic inflammation occurs due to the body's defense response to external stimuli, such as pathogens, irritants, and infections[1]. A persistent inflammatory response can lead to chronic diseases, namely cardiovascular, neurodegenerative, and inflammatory bowel diseases[2]. Inflammatory mediation is triggered by reactions of neutrophils, mast cells, eosinophils, macrophages, dendritic cells, and epithelial cells[3], which are induced through the enzymes cyclooxygenase (COX) and nitric oxide synthase (NOS)[4]. COX plays a role in converting arachidonic acid (AA) into prostaglandins, which are divided into two isoforms, namely COX-1 and COX-2[4]. COX-1 is expressed in almost all organs and cells but mostly in platelets and the stomach. Meanwhile, COX-2 becomes a specific isoform when inflammation is induced and plays a role in regulating prostaglandin synthesis[5].

The NOS enzyme can also induce inflammation, which consists of three isoforms (neural NOS, nNOS; endothelial NOS, eNOS; and inducible NOS, iNOS). Overexpression of iNOS is found in the pathogenesis of various inflammatory diseases[6,7]. The COX-2 and iNOS enzyme activity is inhibited with steroid (SAID) and non-steroid (NSAID) anti-inflammatory drugs. NSAIDs that are widely

prescribed, namely the coxib class of drugs (rofecoxib, celecoxib, valdecoxib, and lumiracoxib) to treat pain, fever, and inflammation caused by COX-2[3,8]. Meanwhile, dexamethasone inhibits the expression of the iNOS enzyme[9,10].

However, these drugs have some side effects in the long term, such as gastrointestinal damage (gastric ulcers, bleeding, etc.), liver and kidney dysfunction, and skin diseases[1]. The use of natural products has more pharmacological activity and low toxicity, so it can be a potential source for drug development, one of which is melinjo peel (*Gnetum gnenom*). Melinjo peel has been reported to contain ascorbic acid, tocopherol, and polyphenol compounds with antioxidant activity[11,12] and has excellent potential as an anti-inflammatory and anti-aging[12]. Stilbenoids are the main secondary metabolites found in all parts of *G. gnemon*[13,14]. Melinjo peel has not been studied as an anti-inflammatory, so it is necessary to carry out virtual screening with computer-aided drug design (structure-based method) for initial and specific studies of treatment targets and pharmacokinetic properties with pkCSM ADMET. It is to streamline research time in in vitro and in vivo drug development.

2. MATERIALS AND METHODS

2.1 Data Preparation

Compounds from melinjo peel (ascorbic acid, *trans*-resveratrol, gnetin C, gnemonoside A, and gnemonoside D) as test ligands were obtained from research that has been carried out[12–14]. Meanwhile, the reference ligands using rofecoxib[3] **for COX-2 and iNOS are dexamethasone**[10]. Test and reference ligands were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) with 2D structures in SDF format. Enzymes were downloaded from the Protein Data Bank (https://www.rcsb.org/) with ID codes 5KIR (COX-2) and 2NSI (iNOS).

2.2. Structure-based Drug Design

2.2.1. Preparation of enzymes and ligands

Enzymes are separated from water molecules and native ligands using Discovery Studio 3.5 Visualizer® (DS) to obtain pure enzymes and saved in PDB format (.pdb)[15]. Then, add Kollman charge and hydrogen polar only and save in pdbqt format using AutoDock Tools® 1.5.6 (ADT)[16,17]. Meanwhile, native ligands are separated from enzymes using DS by selecting the script menu, selection, and selecting protein chains A[18], and saving in .pdb format[15]. For test and reference ligands downloaded from PubChem[19,20]. All ligands are added Gasteiger charge, all hydrogen, and merge non-polar using ADT and saved in pdbqt format[21].

2.2.2. Identification of active sites of COX-2 and iNOS

Setting the grid box, namely the grid box size and center coordinate values (x, y, and z), aims to determine the position of active sites of enzymes using ADT. It is also used as a reference for the docking test process and references ligands to enzymes[19,20]. Meanwhile, the docking parameters are based on the Lamarckian Genetic algorithm (100 times) with a value of 27,000; 2,500,000; and 150 for algorithm generation, energy evaluation, and population, respectively[22,23].

2.2.3. Validation of the method based on RMSD

Method validation was carried out by evaluating the root-mean standard deviation (RMSD) value. Additionally, visualization is carried out by overlapping native ligands resulting from crystallography and *re*-docking[19].

2.2.4. Molecular docking of compounds from melinjo peel to enzymes and visualization

The docking process uses ADT, which opens the results with Notepad++® to obtain binding affinity (ΔG and Ki) based on a clustering histogram. For visualization of amino acid residues (bonds of hydrogen, HB and Van der Waals, VdW) using DS[18].

2.3. Prediction of Pharmacokinetic Properties and Toxicity

Prediction absorption, distribution, metabolism, excretion, and toxicity (ADMET) of potential compounds melinjo peel docking results using the pkCSM ADMET descriptors algorithm protocol (https://biosig.lab.uq.edu.au/pkcsm/prediction)[24,25].

3. RESULTS AND DISCUSSION

3.1. Method Validation

In this study, RMSD values were obtained, namely 1.264 and 1.927 Å for the enzymes of COX-2 and iNOS, respectively, which shows that enzymes are valid because values ≤ 2.00 Å[19,26]. It means that the position of the native ligands resulting from re-docking is close to that of the co-crystal results[19,27,28]. In addition, determining the parameters of the grid box and Lamarckian Genetic Algorithm is important because it becomes a reference when docking ligand tests and references to enzymes[19]. This study obtained the COX-2 and iNOS docking coordinate values and the grid box size, as shown in Table 1. The grid box setting aims to determine the active site area of the receptor[29]. Meanwhile, the algorithm method uses Lamarckian Genetic with a value of GA run 100 times[19]. It is to find the best position or conformation during ligand-receptor interactions[19].

Table 1. Parameter values of grid box and Lamarckian Genetic Algorithm.

Enzymes	Docking Coordinate Value			Grid Box Size	Grid Spacing (Å)	Lamarckian Genetic Algorithm
	X	Y	Z			
COX-2	23.287	0.439	34.435	40 × 40 × 40	0.375	100
iNOS	17.652	65.496	24.815	40 × 40 × 40	0.375	100

3.2. *In Silico* Molecular Docking and Visualization

The molecular docking results are binding affinity and bond interactions, as shown in Tables 2 and 3. If the bond value between ligands and enzymes is low, the stronger the bond formed. It is influenced by the stability and strength of non-covalent interactions[19,20].

Table 2. Molecular docking compounds from melinjo peel to COX-2 enzyme.

Compounds	Amino Acid Residue Interactions		ΔG (kcal/mol)	Ki (nM)
	Hydrogen Bonds	Van der Waals Bonds (Hydrophobic)		
Test ligands				
Ascorbic acid	Ile517, Phe518, Arg513, His90, Tyr355, and Ser353	Leu352, Gln192, Ala516, and Val523	-5.48	96550
Trans-resveratrol	Gln192, Ile517, Phe518, Ser353, and His90	Arg513, Ala516, Ser530, Phe381, Tyr385, Trp387, Leu384, Gly526, Met522, Al527, and Tyr355	-8.38	716.91
Gnetin C	Pro514, Tyr385, and Ser530	Asp515, Thr94, Gly354, Gln192, Ser353, Tyr355, Arg120, Val116, Leu359, Leu534, Gly526, Met522, Leu384, Phe381, Phe518, His90, and Arg513	-9.91	54.69
Gnemonoside A	Ser530, Leu352, Phe518, Gln192, Tyr355, Arg120, Gly354, Arg513, Pro86 and Ser353	Ile517, Phe381, Phe209, Thr206, Phe201, Val344, Ala527, Leu93, and Tyr115	+32.60	-
Gnemonoside D	Tyr348, Gly354, Ile517, Tyr355, and Pro86	Tyr206, Phe209, Leu384, Phe381, Gly526, Ala527, Glu524, Val89, Leu93, Gln192, His90, and Thr94	+58.29	-
Rofecoxib	Ser530, Ala527, Arg513, Ile517, and Phe518	Tyr355, Ser353, His90, Gln192, Ala516, Trp387, and Leu531	-10.66	15.23

Table 3. Molecular docking compounds from melinjo peel to iNOS enzyme.

Compounds	Amino Acid Residue Interactions		ΔG (kcal/mol)	Ki (nM)
	Hydrogen Bonds	Van der Waals Bonds (Hydrophobic)		
Test ligands				
Ascorbic acid	Arg381, Trp463, Ile462, and Met120	Ser118 and Ile119	-3.97	1220000
Trans-resveratrol	Pro466, Ser118, and Ile119	Met374, Val465, Trp463, Arg199, and Met120	-5.90	47220
Gnetin C	Pro467 and Trp463	Pro466 and Met120	-7.53	3030
Gnemonoside A	Arg199, Trp463, Arg388, and Ser118	Gln387, Pro467, Pro466, Leu464, Met374, Ile201, Cus200, and Glu377	-3.94	1290000
Gnemonoside D	Tyr491	Trp461, Gly470, Pro466, Met468, Val352, Met355, Arg199, Ala197, and Cys200	-8.61	486.01
Reference ligand				
Dexamethasone	Arg381, Trp463, and Pro466	Val465, Met120, and Ser118	-6.81	10210

Binding affinity consists of the value of free energy of binding, ΔG and inhibition constant, Ki[19]. Table 2 shows that all melinjo peel compounds are ineffective in working on the enzyme of COX-2 because they have ΔG and Ki values greater than rofecoxib as a reference ligand (drug). The gnemonoside A and D compounds do not interact with COX-2 because the ΔG value is > 0. It shows that not all secondary metabolites from melinjo peel have binding affinity to the enzyme's active sites[30]. Meanwhile, in Table 3, all secondary metabolites of melinjo peel can interact with the active sites of the iNOS enzyme so that they have binding affinity values. The gnemonoside D and gnetin C compounds have ΔG and Ki values, namely -8.61 and -7.53 kcal/mol; 486.01 and 3030 nM, respectively, which is very good compared to dexamethasone (-6.81 kcal/mol; 10210 nM) as a comparison (drug). The smaller or minus ΔG value indicates increased activity, so very little energy is needed to form stronger bonds[19,20]. Ki shows the compound's ability to inhibit target macromolecules with a smaller value, meaning the more substantial the inhibitory power[31]. The interaction of ligands with amino acid residues of enzymes shows binding to the active sites of COX-2 and iNOS, where each compound (ligand) has different interactions[20]. Tables 2 and 3 show the HB and VdW between compounds from melinjo peel to COX-2 and iNOS. These compounds form at least three types of HB, namely conventional, carbon, and pi-donor, with various amino acid residues. HB and VdW are the most important types of bonds in biological systems[20].

3.3. ADME-Tox

The predicted results of the pharmacokinetic properties and toxicity of potential compounds of melinjo peel as an anti-inflammatory to the enzyme of iNOS based on in silico molecular docking are shown in Table 4.

Table 4. Prediction of ADME-Tox properties from melinjo peel compounds.

Properties	Gnemonoside D	Gnetin C
Absorption		
Water solubility (log mol/L)	-2.923	-2.946
Caco2 permeability (log Papp in 10^{-6} cm/s)	-0.2	-0.524
Intestinal absorption (human) (% absorbed)	51.047	95.619
P-glycoprotein substrate	Yes	Yes
P-glycoprotein I inhibitor	Yes	Yes
P-glycoprotein II inhibitor	Yes	Yes
Distribution		
VDss (human, log L/kg)	-1.483	-2.046
Fraction unbound (human) (Fu)	0.12	0.103
BBB permeability (logBB)	-1.519	-0.873
CNS permeability (log PS)	-3.819	-2.78
Metabolism		
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	Yes
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	Yes
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No
Excretion		
Total clearance (log mL/min/kg)	-0.147	-0.129
Renal OCT2 substrate	No	No
Toxicity		
AMES toxicity	No	No
hERG I inhibitor	No	No
hERG II inhibitor	Yes	Yes
Hepatotoxicity	No	No

4. CONCLUSION

In silico molecular docking, which investigated the anti-inflammatory potential of melinjo peel (*G. gnemon*), concluded that the specific target was the enzyme of iNOS with the constituents potential being gnemonoside D and gnetin C. Meanwhile, predictions of pharmacokinetic properties showed that only gnetin C (stilbenoid compound) was fulfilled.

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Conflict of interest

None to declare.

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Evaluation of the Wound Healing Activity of Elephant Foot Yam (*Amorphophallus paeoniifolius* Dennst. Nicolson) Aqueous Flower Extract in Zebrafish (*Danio rerio*) Model

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ABSTRACT

Wound healing is crucial for restoring tissue integrity and function following injury. Recent research has explored phytochemicals in *Amorphophallus paeoniifolius* (AP) flower for their potential therapeutic effects, including anti-inflammatory, antioxidant, and antimicrobial activities, highlighting the compound's potential in wound healing. The study aimed to evaluate the wound healing activity of AP through systematic review, in-silico docking, and in vivo wound healing assay in the laser-ablated cutaneous wound in zebrafish. AP flower extract (APAFE) was obtained via hot water extraction and lyophilization. A systematic review identified the phytochemical constituents, which were then confirmed through phytochemical tests. Reverse docking techniques assessed molecular interactions of reported constituents with receptors. Safety evaluation was conducted using FISH OECD 203. Laser-ablated zebrafish (n=10/group) with 1mm^2 cutaneous wound were immersed in APAFE (12.5 to 300 mg/L), allantoin (positive control), and methylene blue (negative control) and observed at 0, 1, 7, 14 and 21 days. APAFE afforded tannins, terpenoids, and anthocyanins. APAFE anthocyanins showed strong binding affinities with transforming growth factor-beta (-7.6 to -9.1 kcal/mol), collagenase-3 (-8.2 to -8.9 kcal/mol), and catalase (-8.1 to -8.7 kcal/mol). LC_{50} exceeded 300 mg/L and did not cause any mortality to zebrafish. APAFE (200 mg/L) enhanced wound healing and closure rate (23.33%±6.11) compared to untreated wounds (-21.61%±10.42) at day 7 ($p<0.05$), comparable to the standard drug allantoin (-10.37%±7.84). Normal wound healing and closure were observed at day 14 and 21 with APAFE treatment. APAFE significantly improved wound recovery and closure rate in zebrafish on 7th day. Docking results, phytochemical analysis, and systematic review support the observed wound healing activity. Further research involving other parameters for wound healing is recommended to substantiate the present results.

KEYWORDS: *A. paeoniifolius*; Dermatology Laser; In silico Molecular Docking; Wound Healing, Zebrafish

1. INTRODUCTION

There is an anticipated high rate of wound infection in postoperative patients in the rural areas of the Philippines caused by inadequate wound cleaning, care knowledge, and resources[1]. Any disturbances such as continuous inflammation, infection, and necrosis in the process of wound healing, which consists of hemostasis, inflammation, proliferative phase, and remodeling, may eventually lead

to the emergence of chronic state or aggravation[2]. Hence, some people resort to using traditional medicines to treat wounds, such as *guava*, *sambong*, and *lagundi* [3].

Herbal remedies are frequently employed as a primary approach to treating various illnesses. A medicinal herb with significant therapeutic potential and pharmacological attributes is *Amorphophallus paeoniifolius* (AP), more commonly known as Elephant Foot Yam. This plant, native to the Philippines, has a well-documented phytopharmacological profile[4,5]. One of the notable aspects of this plant is the aqueous flower extract for its potential therapeutic properties, including anti-inflammatory and antioxidant effects, which play a role in the process of wound healing[6].

In research, the utilization of zebrafish (*Danio rerio*) as an animal model has emerged due to its characteristics and regenerative properties. The adult zebrafish has a skin structure similar to that of mammals, comprising the epidermis, dermis, and hypodermis[7]. This species is popular due to its natural attributes: external fertilization, high offspring numbers, rapid growth, transparent bodies, common genetic profile, ease of experimentation, and straightforward genetic modification processes, making them an exceptional model for investigating the mechanisms behind cutaneous wound healing[8] that are comparable to adult mammals[9].

The objective of the study is to investigate the wound healing activity of AP aqueous flower extract (APAFE) using a zebrafish model through identifying its chemical constituents through systematic review, phytochemical tests, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), binding affinities through *in silico* docking, acute toxicity following Organisation for Economic Co-operation and Development (OECD), effectiveness in enhancing wound healing, and comparison with the standard drug (allantoin).

There is a continuous and growing demand for effective wound-healing treatments as wounds can cause infections, complications, and decreased quality of life. Investigating the wound healing potential of AP responds to the demand of the increasing interest in plant-based and natural remedies and will also contribute to a sustainable healthcare practice, thus enabling a cost-effective and ethical method of studying the effects before proceeding to human clinical trials.

This study was only limited to the wound-healing capability of the APAFE on zebrafish skin. Other parts of zebrafish that may be related to skin anatomy were not included. Moreover, the wound was induced by a laser-ablation technique based on the standard protocol.

2. MATERIALS AND METHODS

2.1. Review of the Compounds with Wound Healing Activity in *Amorphophallus* Genus Aqueous Extract

2.1.1. Search Strategy

The review examined electronic literature databases, including Google Scholar, PubMed, JSTOR, and ScienceDirect, starting from January 2004. Keywords used were "*Amorphophallus paeoniifolius*," "*Amorphophallus*," "Elephant Foot Yam," "flower," "phytochemical," "constituents," and "aqueous extract," combined with Boolean operators. Reference lists from relevant studies were also reviewed. Studies were screened based on inclusion and exclusion criteria. Titles were first screened, followed by abstracts for relevance. Finally, the entire studies were evaluated for data extraction.

The inclusion criteria for the study were:

- Research related to the *Amorphophallus* genus
- Studies showing its phytochemical constituents
- Studies analyzing its aqueous extract
- *Amorphophallus* genus cultivated in Asia and
- Studies published in English

The exclusion criteria were:

- Research not related to *Amorphophallus*
- studies not including its phytochemical constituents
- Studies analyzing other extracts (e.g., methanolic)
- Studies published before January 2004 and
- Non-English publications

2.2. Collection and Extraction of APAFE

The collection and preparation of APAFE were conducted by the botanist and thesis adviser, Prof. Ross D. Vasquez, PhD, in Pilar, Bataan (14.6682° N, 120.5528° E), in August 2023.

Before extraction, the inflorescence parts were separated, rinsed with distilled water, and dried in a food dehydrator at 40-45°C. The dried floral part was ground into fine powder with a domestic grinder and stored in airtight bags. To obtain the aqueous extract, 500 g of the powder was boiled in one (1) L of water and filtered through a vacuum funnel. This procedure was performed by research assistants at the Research Center for the Natural and Applied Science (RCNAS) Laboratory Room 307.

2.3. Phytochemical Properties of *A. paeoniifolius* Aqueous Flower Extract

2.3.1. Test for Anthocyanin

Two (2) mL of 2N hydrochloric acid (HCl) was added to 2 mL of plant extract in a test tube and subjected to a hot water bath. The presence of anthocyanin can be confirmed by the appearance of a pink-red solution[10].

2.3.2. Test for Flavonoids

In a test tube, 1 mL of APAFE was mixed with a few drops of sodium hydroxide (NaOH); an intense yellow color indicates the presence of flavonoids[11].

2.3.3. Test for Phenols and Tannins

Few drops of 1% lead acetate ($Pb(OAc)_2$) was added to 3 mL of APAFE. A yellow precipitate indicates tannins[11].

2.3.4 Test for Terpenoids

One (1) mL of APAFE was added to 2 mL of chloroform ($CHCl_3$) in a test tube. Three (3) mL of concentrated sulfuric acid (H_2SO_4) was dropped along the sides of the test tube. A reddish-brown color confirms terpenoids[12].

2.3.5 Total Phenolic Content

The Folin-Ciocalteu method was used for TPC with minor adjustments. Instruments included a microcentrifuge, beaker, graduated cylinder, micropipette, and a 96-well plate. The sample, containing 1 mcg of APAFE in 1 mL of water, was diluted by adding 50 µL to 950 µL of water. The Folin reagent (50 µL mixed with 800 µL water) is light-sensitive, thus kept in the dark. Sodium bicarbonate ($NaCO_3$) was prepared by mixing 100 mL of water with 20 g of $NaCO_3$ powder. After mixing the sample, Folin reagent, and $NaCO_3$, the reaction was left in the dark for 120 minutes. Absorbance was measured at 750 nm using a Ultraviolet–visible (UV-Vis) spectrophotometer. The gallic acid equivalents (GAE) per gram of phenolic content were calculated using a standard curve of gallic acid in 10 – 60 mg L^{-1} range.

A standard calibration curve was created by plotting absorbance (y) against the concentrations of the gallic acid standard (x). The TPC for each sample concentration was then calculated using Equation 1, which quantifies the amount in milligrams of Gallic Acid Equivalent (GAE) per gram of the sample. This calculation involves multiplying the gallic acid concentration of the sample in parts per million (C), obtained from the standard calibration curve, by the volume of the solution in milliliters (V), and then dividing this result by the sample's mass in grams (W). The total phenolic content was expressed as gallic acid equivalents using the linear equation based on the calibration curve.

$$TPC = \frac{C\ x\ V}{W\ x\ 1000}$$

2.3.5 Total Flavonoid Content

Materials included microcentrifuge tubes, beakers, micropipettes, and a 96-well plate. Quercetin, for the standard calibration curve, was prepared by dissolving 5 mg in 2 mL of methanol (CH_3OH), followed by serial dilutions to five concentrations: 62.5, 125, 250, 500, and 1000 mcg/mL. The extract was prepared by dissolving 2 mg of the sample in 2 mL CH_3OH (1 mg/mL), then diluting 500 µg with CH_3OH. 200 µL of each quercetin and plant extract were diluted to 800 µL with water. CH_3OH was used as a blank. Next, 100 µL of 5% sodium nitrite ($NaNO_2$) was added, followed by 100 µL of 10% aluminum chloride ($AlCl_3$) after 5 minutes, and 200 µL of 1M NaOH after 6 minutes. The volume was adjusted to 2 mL with water. Two hundred µL was transferred to a 96-well plate and triplicated. The absorbance was measured at 510 nm, and the Total Flavonoid Content (TFC) was calculated using the equation below. The results were expressed as quercetin equivalents based on the calibration curve.

$$TFC = \frac{C \times V}{W \times 1000}$$

C is the concentration (ppm)
V is the volume of extract taken (mL)
W is the weight of sample (g)

2.3. Reverse Molecular Docking Analysis of Compounds Related to Wound Healing Activity

2.3.1. Computational Tools

The Python Prescription (PyRx) v0.9.9 software[13]with AutoDock Vina v1.1.2 was used for protein-ligand docking. Compound libraries were screened against drug targets, and the molecular structures were visualized and analyzed using Biovia Discovery Studio and UCSF Chimera 1.17.3.

2.3.2. Preparation of 3D Structure of Ligand and Target Protein

Candidate bioprotein targets' structures were obtained from PubChem, while x-ray crystallographic structures of *Danio rerio* were acquired from the AlphaFold protein structure database. PyRx's AutoDock Tool processed these structures. Ligand preparation utilized PyRx's Open Babel.

2.3.4 Reverse Molecular Docking

A. paeoniifolius compounds were investigated for their potential targets in wound healing using a reverse docking approach carried out through the assistance of Honeymae C. Alos, RPh, MSc, from the Institute of Pharmaceutical Sciences, National Institutes of Health, University of the Philippines Manila. Top 3 ranking molecular targets were identified based on binding models, affinities, and residue interactions on active protein sites, indicating the compounds' potential in wound healing[14,15]. Grid docking, aimed at reducing docking time, was employed by creating a three-dimensional grid around a protein target, where each point represented a potential binding site for a ligand. This process utilized PyRx's AutoDock Vina wizard with the Assisted Model Building with Energy Refinement (AMBER) force field, where a more negative score indicates a higher affinity between the compounds. Energy reduction upon ligand binding to the protein receptor was observed, with the magnitude of the negative Gibbs free energy (ΔG) measuring the interaction strength, complex stability, or affinity to a specific receptor[16]. Discovery Studio (DS) 2021 Client was then used for visualizing and analyzing docking poses and ligand interactions (https://www.3dsbiovia.com/) (Accelrys Inc., San Diego, CA, USA).

2.4. Acquisition of Zebrafish

Zebrafish of mixed gender, aged 4-6 months, weighing approximately 250 mg, were purchased from PET Corner Cartimar in Pasay City, Philippines. They were transported in oxygen-filled plastic bags with adequate water.

2.5. Determination of Acute Toxicity of Zebrafish

Ten fish were randomly assigned to each treatment and control group, totaling 50 for test solution concentrations and 20 for negative and positive controls. The experiment was conducted in appropriate glass aquariums. Test solutions ranging from 200 mg/L to 6.25 mg/L were prepared from a stock *A. paeoniifolius* extract. Controls included a negative control and a positive control with allantoin at 6.25 mg/L, following OECD guidelines. Mortality in controls was kept below 10% for test validity. Fish were measured before and after exposure, with mortality and abnormalities recorded. Data analysis, according to OECD Test Guideline No. 203, involved measuring cumulative mortality, computing LC_{50} values at different time points, assessing concentration-mortality curves, and using statistical methods for data treatment.

2.6. *In Vivo* Wound Healing Assay

2.6.1. Fish Preparation and Distribution

Each of the eight zebrafish groups, comprising 15 randomly distributed fish per group, underwent a 5-minute acclimatization in experimental tanks filled with system water. These groups included a positive-control group treated with 6.25 mg/L of Allantoin, a negative-control group, and six treatment groups exposed to concentrations ranging from 300 mg/L to 12.5 mg/L, with a sample size (n) of 15 for each group. Fish were anesthetized with Tricaine methanesulfonate (1 mg/mL), following University of Michigan guidelines[17].

To prevent gastrointestinal responses, zebrafish fasted for 12 hours before undergoing laser ablation described in the study of Balitaan et al.[18]. Using a DK4.0 Laser Engraver, full-thickness wounds (approximately 1 mm^2) were induced on the left flank, anterior to the anal and dorsal fins. For wound uniformity, the parameters used were 100% for printing power, 260 mm/s for speed, and four scan times. Afterward, 0.1% methylene blue solution was applied, and images were captured using a compound microscope. Fish recovered for 5 minutes in system water before being placed in their respective experimental tanks and returned to their original tanks.

2.6.2. Determination of Treatment Dose and Time Frame

Treatment doses for wound healing were determined based on the study of Zain et al. and results from the acute toxicity test[19]. Within the non-toxic concentration range, treated groups were exposed to concentrations ranging from 300 mg/L to 12.5 mg/L, with allantoin at 6.25 mg/L as the positive control and untreated as the negative control. Time points for wound healing were determined with wound closure progress monitored every 7 days over 21 days, at 0, 7, 14, and 21 days post-wound[19,20].

2.6.3. Wound Healing Assay

After wounding, zebrafish groups were treated with APAFE at six concentrations (300 mg/L, 250 mg/L, 200 mg/L, 150 mg/L, 50 mg/L, and 12.5 mg/L) and Allantoin (6.25 mg/L), added to their tanks with water changes every two days, while the negative control received no treatment.

2.6.4. Euthanasia and Disposal of Zebrafish

Surviving zebrafish after treatment exposure are euthanized using the Rapid Cooling method, involving submersion in 2-4°C tricaine solution for at least 20 minutes. Control fish that survive do not require euthanasia. This method complies with OECD guidelines and the European Directive 2010/63/EU. Fish carcasses are placed in yellow plastic bags and disposed accordingly to minimize environmental impact.

2.6.5. Data Analysis and Comparison of Wound Healing Effects of A. paeoniifolius extract and Allantoin

Wound healing effects were assessed via wound closure percentage (WCP), calculated using ImageJ software for wound area measurements. Data were presented as mean ± standard deviation (SD) and analyzed using one-way Analysis of Variance (ANOVA) to detect differences between treatment, positive control, and negative control groups. Post hoc tests identified specific group differences, with a significance level set at $p < 0.05$. Excel Analysis Toolpak facilitated statistical computations. Additionally, wound healing effects were observed, considering factors like wound size, rubor signs, and skin color[19].

3. RESULTS AND DISCUSSION

3.1. Systematic Review

An initial search across four electronic databases yielded 113,052 studies for the systematic review. However, after applying inclusion and exclusion criteria and screening the full articles, only 14 studies were selected. Figure 1 illustrates the search strategy and the results for each database. Most of the studies focused on the tuber of *A. paeoniifolius*, with none utilizing the flower. Various analytical techniques, including spectrophotometry, chromatography, mass spectrometry, and infrared spectroscopy, were employed in the methodologies.

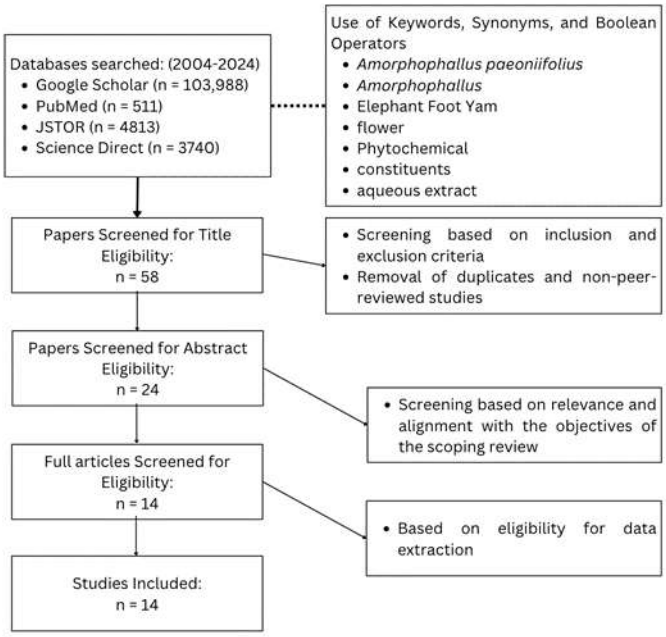


Figure 1. Flow diagram illustrating search strategy

Table 1 summarizes the selected studies, which identified various phytochemicals such as flavonoids, anthocyanins, phenols, tannins, glucomannan, and terpenoids. According to other literature, these phytochemicals exhibit wound healing activities. Flavonoids, in particular, were noted for their anti-inflammatory, angiogenic, re-epithelialization, and antioxidant properties relevant to wound healing[21-22]. They positively regulate Matrix metalloproteinases (MMPs), Ras/Raf/MEK/ERK, PI3K/Akt, and nitric oxide (NO) pathways and mediate the release of inflammatory cytokines and other signaling molecules. Phenolic compounds also show potential as wound healing agents by effectively suppressing the Wnt/β-catenin signaling pathway. Tannins improve wound healing through their antioxidant effects, promotion of wound contraction, fibroblast proliferation, and capillary vessel formation[23]. Terpenoids contribute to the wound healing process due to their anti-inflammatory

properties and capacity to promote re-vascularization and re-epithelialization[24]. The systematic review indicates that these constituents, with their wound healing activities, demonstrate the potential of APAFE in the wound healing process

Table 1. Summary of the methods and results of the selected studies

STUDY SETTING	AUTHOR AND YEAR	ARTICLE TYPE	PART OF THE PLANT	CONSTITUENTS	METHODOLOGY
India	Apurva and Monali (2024)	Review	Tuber	Glucomannan, Phenols, and Flavonoids	Spectrophotometry
India	Bhuvaneswari and Sivasubramanian (2023)	Experimental Study	Tuber	Alkaloids, Carbohydrates, Glycosides, Saponins, Phenolics, Flavonoids, Tannins, Terpenoids	HPTLC, HPLC, Spectrophotometry
India	Dey et al. (2016b)	Experimental Study	Tuber	Phenols Tannic Acid Glucomannan	TPC TFC TGC
India	Dey et al. (2020)	Experimental Study	Tuber	Glucomannan and Betulinic acid	HPTLC
India	Guruprasad and Sachin (2020)	Experimental Study	Leaf	Flavonoids and Tannins	FT-IR
Bangladesh, Saudi Arabia, India, Indonesia, Egypt, USA, Thailand	Islam et al. (2023)	Systematic/ Literature Review	Corm, Tuber, Inflorescence, Leaf, Spathe	Steroids, Flavonoids, Vitamins, Phenolic, Fatty acids and derivatives, Sugar	Not Applicable
Japan	Iwashina et al. (2023)	Experimental study	Leaf	Flavonoids	HPLC, LC-MS, acid hydrolysis, NMR
India	Jain et al. (2009)	Experimental study	Tuber	Alkaloids, Carbohydrates, Proteins, Amino Acids, Phenolic Compounds, Glycosides, Flavonoids	Not indicated
India	Das et al. (2009)	Experimental study	Corm	D-galactose, D-glucose, 4-O-acyl-D-methyl galacturonate, and l-arabinose	Methylation analysis, Periodate oxidation experiment, NMR studies
India	De et al. (2010)	Experimental study	Tuber	Alkaloids, Steroids, Fats and Fixed Oils, Flavonoids, Tannins, Proteins, Carbohydrates	TLC
India	Salunke & Satpute (2018)	Review	Corm	Alkaloids, Tannins, Phenols, Carbohydrates, Glycosides, Flavonoids, Saponin	TLC
India	Shete et al. (2015)	Experimental study	Tuber	Phenol	TPC, FRAP, HPLC
India	Ansil (2022)	Research	Tuber, Corm	Diterpenoids, Triterpenoids Triacontane, Saponin, Rutin, Lupeol, Betulinic acid, Gallic acid, Resveratrol, Quercetin, Stigmasterol, β-sitosterol, Palmitate	Not Applicable

3.2. Extraction and Phytochemical Analysis

The aqueous extract from *A. paeoniifolius* flower appears as a viscous, water-soluble liquid with a rich, dark green color and a pungent odor, indicating the presence of volatile organic compounds. The constituents identified in *A. paeoniifolius*, through the systematic review, were confirmed in the APAFE using various confirmatory tests. Anthocyanins were verified with the HCl anthocyanin test, which yielded a positive result when the solution turned bright red upon the addition of an acidic substance to the APAFE. Flavonoids were confirmed by adding NaOH to the APAFE, which changed the solution's color to an intense yellow, indicating a positive result. Phenols and tannins were confirmed through the lead acetate test, with a positive result marked by the formation of a yellow precipitate. Lastly, terpenoids were confirmed using the Salkowski test, which produced a reddish-brown solution as a positive result. Table 2 shows images of the results of the confirmatory tests. Following the confirmatory tests, quantitative TPC and TFC were performed, calculated from the regression equation of the standard calibration curve, gallic acid ($y = 0.0032x + 0.0718$; $R^2 = 0.9921$) and quercetin ($y = 0.0018x + 0.2408$; $R^2 = 0.9982$) standards, respectively. The TPC yielded a value of

0.081665596 mg GAE/g of sample, while the highest TFC value obtained was 0.72578 mg QE/g of sample. Compared to other studies evaluating the TFC and TPC of aqueous tuber extracts, these values are considerably lower, likely due to the different plant part used, in this case, the flower

Table 2. Results of the Confirmatory Test

Anthocyanin Test	Flavonoids Test	Phenols Test	Tannins Test	Terpenoids Test
				
+	+	+	+	+

*(+) Present ; (-) Absent

3.3. Reverse Molecular Docking

As shown in Table 3, the APAFE flavonoids showed the highest or strongest binding affinities with TGF-β (-7.6 to -9.1 kcal/mol), collagenase-3 (-8.2 to -8.9 kcal/mol), and catalase (-8.1 to -8.7 kcal/mol). TGF-β, a pluripotent cytokine, plays a crucial role in wound healing by regulating granulation tissue production, inflammation, angiogenesis, and re-epithelialization. Therapeutic drugs targeting the TGF-β pathway can enhance wound healing and scarring. Catalase (CAT) eliminates reactive oxygen species (ROS), reducing oxidative damage and promoting wound healing. Collagenase-3 influences wound contraction, initiating re-epithelialization and keratinocyte migration.

3.4. Determination of Acute Toxicity on Zebrafish

The acute toxicity of the APAFE was determined by exposing adult zebrafish to four concentrations of the extract (12.5 mg/L, 50 mg/L, 100 mg/L, 200 mg/L), positive control (6.25 mg/L allantoin), and negative control (methylene blue). This was done by observing the abnormal behaviors of the fish and recording mortalities to determine the LC_{50} of the extract. However, no mortalities were recorded during the observation period. Instead, abnormal behaviors were recorded. Aggressive behavior involving attacking or chasing other fish was observed in all groups, including the controls. Triggers that may have caused this behavior include securing food, territories, and mates[38]. Abnormal bottom distribution was also observed in all groups, where most fish swim close to the tank's base to conserve energy and adjust to the new environment[39]. Aside from these behaviors, hypoactivity, hypoventilation, and hyperactivity were observed in some groups exposed to APAFE concentrations, which may be attributed to adjustment to a new environment, disturbances, and stress. In summary, the LC_{50} of the APAFE cannot be determined due to the lack of a record of mortalities, considering that the APAFE is safe for wound healing experiments. However, abnormal behaviors are recorded due to environmental stressors during the experiment.

Table 3. Predicted binding energy on the target proteins

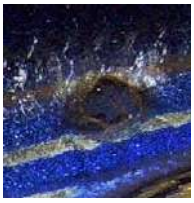
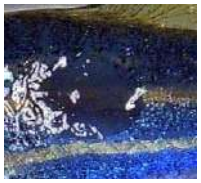
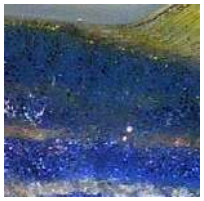
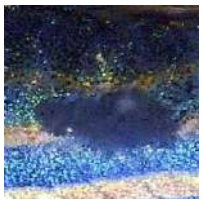
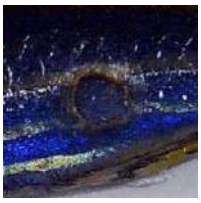
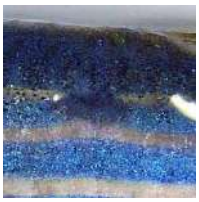
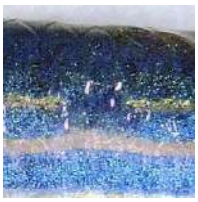
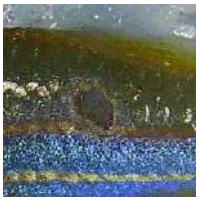
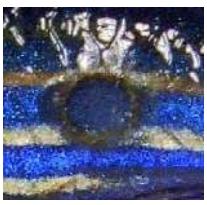
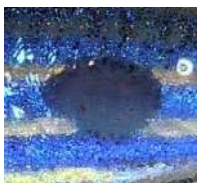
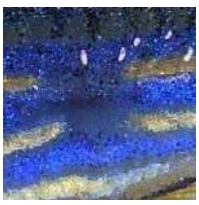
Protein (Source organism: *Danio rerio*)	**Binding Energy** (kcal/mol)
Transforming growth factor-beta	Cyanidin 3-O-rutinoside: -9.1
	Peonidin 3-O-rutinoside: -9.0
	Cyanidin 3-O-glucoside: -8.0
	Peonidin 3-O-glucoside: -8.0
	Vitexin: -7.6
Matrix metalloproteinase 13	Cyanidin 3-O-rutinoside: -7.1
	Peonidin 3-O-rutinoside: -7.0
	Vitexin: -6.9
	Cyanidin 3-O-glucoside: -6.6
	Peonidin 3-O-glucoside: -6.5
Superoxide dismutase	Cyanidin 3-O-rutinoside: -7.8
	Peonidin 3-O-rutinoside: -6.6
	Peonidin 3-O-glucoside: -6.4
	Vitexin: -6.4
	Cyanidin 3-O-glucoside: -6.3
Catalase	Peonidin 3-O-rutinoside: -8.7
	Cyanidin 3-O-rutinoside: -8.6
	Cyanidin 3-O-glucoside: -8.5
	Peonidin 3-O-glucoside: -8.1
	Vitexin: -8.1
Lipopolysaccharide-induced tumor necrosis factor-alpha factor homolog	Cyanidin 3-O-rutinoside: -6.0
	Peonidin 3-O-rutinoside: -6.0
	Vitexin: -6.0
	Cyanidin 3-O-glucoside: -5.4
	Peonidin 3-O-glucoside: -5.4
Interleukin 1 beta	Cyanidin 3-O-rutinoside: -8.8
	Peonidin 3-O-rutinoside: -8.4
	Peonidin 3-O-glucoside: -8.3
	Vitexin: -8.0
	Cyanidin 3-O-glucoside: -7.9
Collagenase 3	Cyanidin 3-O-rutinoside: -8.9
	Peonidin 3-O-rutinoside: -8.8
	Vitexin: -8.5
	Cyanidin 3-O-glucoside: -8.4
	Peonidin 3-O-glucoside: -8.2

3.5. Evaluation of Wound Healing of *A. paeoniifolius* Aqueous Flower Extract

Due to the present relevant phytochemical constituents in the extract and its high binding affinity in selected bioprotein targets, the potential of APAFE was evaluated by conducting an *in vivo* wound-healing assay on zebrafish and observing the wound closure for 21 days.

Table 5 reveals the stages of wound closure across different treatments for 21 days. Initially (0 days post-wound or dpw), the wound margins darkened without bleeding compared to the surrounding skin. By 7 dpw, the wounds visually appeared larger due to shedding of darkened scales. By 14 dpw, wounds were barely visible in some treatment groups. By 21 dpw, wounds were healed entirely in the positive control and most treatment groups (50 mg/L, 150 mg/l, 200 mg/L, 250 mg/L, 300 mg/L).

Table 5. Progress of Wound Closure in 21 days

	0 dpw	7 dpw	14 dpw	21 dpw
Negative Control				
Positive Control				
300 mg/L				
250 mg/L				
200 mg/L				
150 mg/L				
50 mg/L				
12.5 mg/L				

While visual inspection suggested optimal healing in most treatment groups, similar to the positive control, wound closure assessment should rely on software-based measurements. The wound closure percentage (WCP) was determined by comparing the difference in wound size between the initial day (0 dpw) and subsequent days (7, 14, and 21 dpw) relative to the wound size at day 0.

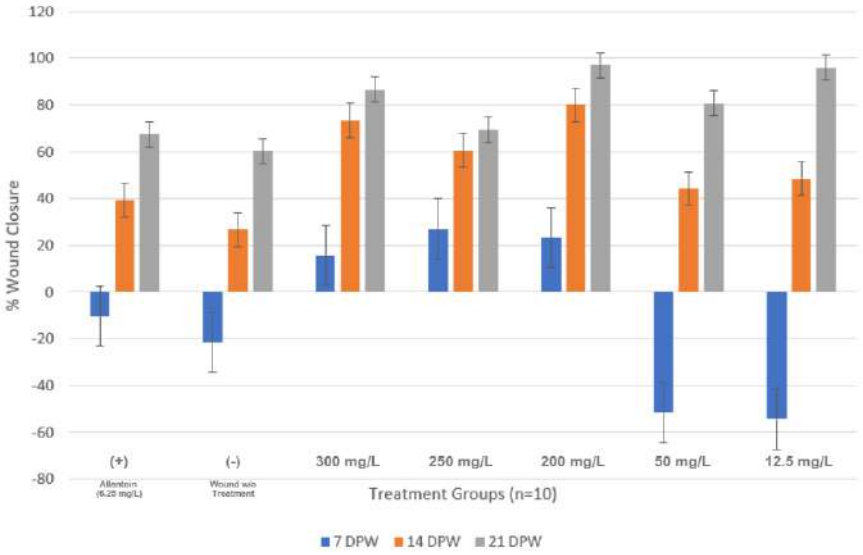


Figure 2. Wound Healing Progress Based on Wound Closure Percentage

Figure 2 shows the progression of wound healing across various concentrations and exposure times based on mean WCP, where a normal wound healing process was observed. Negative values of WCP indicate an increase in wound size area due to certain factors, such as the removal of burnt scales. While the progression of wound closure in all groups improved over time, an ANOVA and post hoc analysis must be conducted to statistically compare the data and determine any significant differences across treated and untreated groups.

There appeared to be no significant difference in wound closure of treated and untreated groups in 14 dpw and 21 dpw. However, a significant difference was observed in wound closure in seven (7) dpw across the concentrations, specifically 300 mg/L, 250 mg/L, 200 mg/L, and 50 mg/L. In addition, significant differences were observed between 200 mg/L and negative control, indicating that the wound-healing activity can be found in the stated concentration. Figure 3 shows the mean WCP of each concentration in seven (7) dpw, where bars marked with an asterisk (*) indicate a statistically significant difference in wound closure ($p < 0.05$).

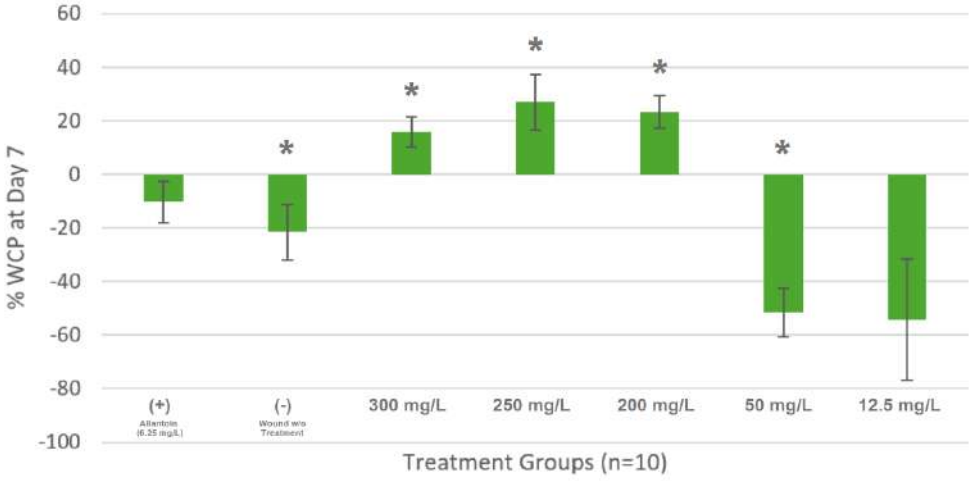


Figure 3. Mean % Wound Closure of Treatment Groups in Day 7

Moreover, no statistically significant difference was recorded between the groups treated with APAFE and the positive group (allantoin 6.25 mg/L) in 7 dpw, 14 dpw, and 21 dpw, indicating that its wound-healing effect is potentially comparable to allantoin. However, no statistically significant difference was also recorded between the negative and positive controls. Theoretically, positive control was expected to differ significantly from negative control. Possible factors that might have contributed to these results are the concentrations and formulation used for allantoin.

In summary, no statistically significant difference was found between groups treated with *A. paeoniifolius* aqueous flower extract and positive control, making the wound healing activity of the extract comparable to allantoin. However, there is a significant difference ($p < 0.05$) between the treated group (200 mg/L) and the untreated group, showing improvement in wound healing progression and potential of wound healing activity

4. CONCLUSION

Considering the general phytochemical tests, the theoretical binding affinities determined from the *in silico* molecular docking, and the systematic review, the APAFE possesses potential healing activities in cutaneous wounds. In addition, the *in vivo* experiment conducted by this study found that there is a significant difference between the negative control (methylene blue) and one of the treatment concentrations (200 mg/L); hence, the APAFE manifested significant wound recovery and closure rate in the laser-induced wounded zebrafish model, potentially due to its flavonoid phytochemical content. To further maximize the investigation of the wound healing effects of APAFE, *in vivo* studies using higher concentrations of the APAFE, exploration of the safety, acute toxicity, and potential superior effectivity of methanolic extract, extended research on the plant's inflorescence, increase in sample size, and utilization of higher quality laser are recommended.

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Conflict of interest

The authors declare that they have no conflict of interest.

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In Vitro Probiotic Potential of Lactic Acid Bacteria (Lab) Isolated from Fermented Foods with Anti – *Helicobacter Pylori* Activity

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ABSTRACT

Helicobacter pylori is known for causing inflammation and gastric ulcers. With their antibacterial activity and probiotic performance, lactic acid bacteria (LAB) have been attracting considerable attention in development as an adjuvant therapy for *H. pylori*. This study aimed to investigate the probiotic potential of lactic acid bacteria (LAB) isolated from fermented foods and their antibacterial activity against *H. pylori* ATCC 43504 *in vitro*. Twenty LAB strains were isolated from fermented foods and identified based on morphological characteristics, Gram staining, and catalase reaction. Acid tolerance (pH2 and pH3), bile tolerance (0.3% w/v bile salts), auto-aggregation and co-aggregation assays were used as restrictive criteria to evaluate the probiotic potential of LAB. The antagonistic activity of LAB against *H. pylori* ATCC 43504 was assessed by using well diffusion assay and the 96-well plate coculture assay urease activity of LAB with *H. pylori* by red phenol method. The findings revealed that all LAB strains exhibited good tolerance at pH2 and pH3 after 3 hours (survival rates > 85%). Fifteen out of twenty LAB strains showed survival rates > 50% after 3 hours in the presence of 0.3% bile salts. Ten out of fifteen and thirteen out of fifteen strains showed auto-aggregation and co-aggregation percentages over 20%, respectively. Six LAB strains (DC1, DC3, DC8, DC9, DC11, and DC16) were identified as potential probiotics for *in vitro* anti-*H. pylori* experiments. All selected strains inhibited *H. pylori*, with DC3 showing the largest zone inhibition diameter (13.67±0.58 mm). In the coculture assay, two LAB strains (DC8 and DC16) showed over 50% inhibition of urease activity, as indicated by the absorbance at 550 nm (OD550). The study reveals that two LAB strains, DC8 and DC16, have significant probiotic potential, effectively inhibiting *H. pylori* ATCC 43504, suggesting LAB as a cost-effective and effective therapy.

KEYWORDS: Probiotics; fermented foods; antimicrobial activity; Lactic Acid Bacteria; *Helicobacter pylori.*

1. INTRODUCTION

Helicobacter pylori (H. pylori) is one of the most common pathogens in the gastric epithelium of humans and results in several diseases such as chronic gastritis, peptic ulcer(16)**.** Infection with *H. pylori* has been related to an increased risk of gastric cancer(17). In 1994, the International Agency for Research on Cancer (IARC) - a subordinate organization of the World Health Organization (WHO), reported that *H. pylori* was classified as a carcinogen (group 1) in humans(18). *H. pylori* infection affects more than half of the world' s population, with the higher prevalence in developing countries(19, 20). Vietnam is in the top 3 countries in Southeast Asia, with the prevalence of H. pylori's infection estimated at approximately 70% in adults**(21, 22)**. Recently, The Maastricht IV/Florence Consensus recommends triple therapy for treating bacterial infections, followed by alternative regimens like bismuth quadruple therapy or sequential therapy. If first-line therapy fails or persists, second-line or rescue therapies may be necessary. Alternative regimens may include bismuth quadruple therapy or sequential therapy(23). However, the effectiveness of these treatments may be undermined by the rise

of antimicrobial-resistant (AMR) strains and treatment failure(24). Adequate treatment compliance was the most strongly linked characteristic with effective eradication. Thus, solving the solution requires investigating some new therapeutic substances with less or no negative effect. Probiotics, which are live microorganisms, are important for maintaining health, especially in the digestive tract(25, 26). Researchers have discovered numerous benefits of probiotics, such as immunomodulation, pathogen prevention, and enhanced barrier function(27). Active microbes present in fermented foods contribute to reducing inflammation and improving the histological conditions of ulcers caused by *H. pylori* infection(28). Many investigations have indicated that lactic acid bacteria (LAB) exhibit anti-*H.pylori* activity relies on their probiotic attributes(29). Overall, LAB probiotics have been shown to suppress *H. pylori* colonization in both *in vivo* and *in vitro* experiments(30, 31). This study was conducted to screen LAB strains possessing antimicrobial activity against *H. pylori* along with probiotic attributes using a multi-step approach. Initially, 20 LAB strains were sourced from the laboratory of Microbiology and Parasitology Department (Faculty of Pharmacy, UMP). These strains were evaluated for their antagonistic potential against *H. pylori* as well as various probiotic characteristics, including tolerance to acidic and bile salt conditions, auto-aggregation, and co-aggregation. Subsequently, the LAB strains demonstrating promising antagonistic activity against *H. pylori* were further analyzed for their anti-*H. pylori* efficacy, and the inhibition of *H. pylori* urease activity. The results in this study indicate the two LAB strains have potential probiotic with anti-*H. pylori* activity.

2. MATERIALS AND METHODS

2.1. Bacteria strains and growth conditions

Twenty (20) strains of LAB were obtained from various vegetable sources, including Vietnamese pickles, garden egg, and cacao. To propagate the lactic acid bacteria, both the liquid and solid portions were blended. A 10 mL aliquot of the blended mixture was added to 90 mL of De Man-Rogosa-Sharpe (MRS) broth and then incubated at 37°C for 24 h in a shaking incubator. After incubation, the suspension was diluted with sterilized distilled water, then the level of absorbance at 600 nm (optical density, OD600) was adjusted to 0.1 to standardize the bacteria count ($1 – 5\times10^8$ CFU/mL). Subsequently, 0.1 mL of the diluted suspension was plated onto MRS agar. Prepare an anaerobic condition by incubating for 72 h at 37°C in AnaeroPack System 2.5-L rectangular jar (Mitsubishi Gas Chemical Co., Inc.) and AnaeroPack-Anaero (Mitsubishi Gas Chemical Co., Inc.). Colonies were subjected to repeated purification on MRS agar plates until uniform colony characteristics were observed for each isolate. Finally, purified strains were selected based on their distinct characteristics. These isolates were either stored at 4°C after streaking inoculation or at -80°C in a medium containing 20% glycerol broth to supply a stable inoculum for the study. *H. pylori* ATCC 43504 was used as a reference strain. *H. pylori* strains were cultured on Brain Heart Infusion (BHI) agar plates containing 10% sheep blood under 5% CO_2 and 10% O_2 conditions at 37°C for 72 h. To activate LAB strains, LAB isolates were cultured in MRS broth at 37°C for 24 h and then subjected to each experimental study.

2.2. Probiotic properties of LAB isolates

2.2.1. Acid tolerance

This assay evaluated the ability of LAB strains to survive in an acidic environment (pH 2 and 3), which reflects their potential to survive the harsh conditions of the stomach. The acid tolerance assay of LAB strains was carried out by the method of Chen et al. (2010), with modifications(29). Briefly, MRS broth was adjusted to different pH (pH 2.0 and 3.0 as tests, pH 7.0 as a control) using 1N HCl. Initially, twenty LAB strains were screened by incubating them in MRS broth at 37°C for 24 h. OD600 was adjusted to 0.1 to standardize the bacteria count ($1 – 5\times10^8$ CFU/mL). The decrease in pH following incubation indicated higher acid production during the stationary phase, suggesting relatively better acid resistance. Following this initial screening, the strains were subjected to incubation at different pH

levels (2.0, 3.0, and 7.0 as a control) for 3 h. OD600 was measured every hour intervals for 3 h. The survival rates of LAB under pH 2.0 and 3.0 conditions were calculated with equation:

$$\text{Survival (\%)} = \frac{A_{LAB}}{A_{control}} \times 100\ \%$$

Where A_{LAB} represents the OD600 values after 1, 2 and 3 h inoculation at pH 2.0 and pH 3.0;
$A_{control}$ represents the OD600 values after 1, 2 and 3 h inoculation at pH 7.0 (control).

2.2.2. Bile tolerance

This assay assessed the ability of LAB strains to tolerate bile salts, which is important for their survival in the small intestine where bile is present. To determine tolerance against bile salts, the results from the pH tolerance test were used as a basis. The LAB strains were adjusted to 10^7–10^8 CFU/mL as described above. The strains were sub-cultured overnight at 37°C in MRS broth containing concentrations of bile salts (0.3%, and 0% as a control, w/v) for 4 h. OD600 values were measured, and the survival rate percentage was calculated with equation:

$$\text{Survival (\%)} = \frac{A_{bile}}{A_{non-bile}} \times 100\ \%$$

Where A_{bile} represents the OD600 values after 3 h inoculation with 0.3% w/v bile.
$A_{non\text{-}bile}$ represents the OD600 values after 3 h inoculation without bile.

2.2.3. Auto-aggregation and co-aggregation assays

Auto-aggregation and co-aggregation assays were performed in accordance with Liu et al. (2022), with modifications(32). LAB strains were grown for 18 h at 37 °C under anaerobic conditions. The bacteria were centrifuged at 8000×*g* for 15 min, rinsed twice with PBS, and re-suspended in PBS. OD600 was adjusted to 0.1 to standardize the bacteria count (1 – 5×10^8 CFU/mL). 4 mL LAB suspension was incubated for 5 h at 37 °C. Auto-aggregation was expressed with equation:

$$\text{Auto-aggregation (\%)} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100\ \%$$

Where A_0 and A_t represent the OD600 values after 0 and 5 h incubation, respectively. This assay measured the ability of LAB strains to aggregate with themselves, which is an indicator of their ability to adhere to intestinal mucosa and colonize the gut.

For co-aggregation assay, *H. pylori* ATCC 43504 were used as pathogenic strains. The LAB strains and the pathogenic strains liquid concentration were adjusted to 10^7–10^8 CFU/mL as described above. Equal volumes (2 mL) of LAB strains and *H. pylori* strains were mixed and vortexed for 10 s followed by an incubation at 37°C for 5 h without shaking. Cell suspensions of each single strain were used as controls. Co-aggregation was expressed with equation:

$$\text{Co-aggregation (\%)} = \left(\frac{\frac{(A_{LAB} + A_{pat})}{2} - A_{LAB+pat}}{\frac{A_{LAB} + A_{pat}}{2}}\right) \times 100\ \%$$

Where A_{LAB}, A_{HP}, and A_{mix} represent the OD600 values of control tubes and mixture after 5 h incubation, respectively. This assay evaluated the ability of LAB strains to aggregate with *H. pylori*, which is important for their potential to displace *H. pylori* from the gastric mucosa.

2.3. Antimicrobial activity of selected LABs against *H. pylori*

2.3.1. Agar well diffusion method

This assay measured the ability of LAB strains to inhibit the growth of *H. pylori* on agar plates. The size of the inhibition zone around each well-containing LAB culture indicates the strength of the antibacterial activity. This assay provides insight into the potential of LAB strains to act as natural antimicrobial agents against *H. pylori*. The inhibitory effects of LAB strains on the growth of indicator strains were assessed using the well diffusion method(33). *H. pylori* ATCC 43503 strain was used as an indicator bacterium. A suspension of *H. pylori* (10^7 -10^8 CFU/mL) was prepared and 50 µL of this suspension was incorporated into 200 mL of MRS agar. This mixture was thoroughly combined and subsequently poured onto a plate. Wells of 7 mm diameter were created on the plates using a sterile borer (7mm)**,** then filled with 80 µL of suspension and the plates were incubated overnight at a temperature of 37°C. After incubation at 37°C for 72 h under anaerobic conditions, the diameters of the inhibition zones were measured.

2.3.2. Inhibition of urease activity

This assay evaluated the ability of LAB strains to inhibit the urease activity of *H. pylori*. Urease is an enzyme produced by *H. pylori* that plays a key role in its survival in the stomach. By inhibiting urease activity, LAB strains could potentially disrupt *H. pylori's* ability to survive in the stomach. This assay provides insight into the potential of LAB strains to interfere with a key virulence factor of *H. pylori*. Urease activity was assessed using the phenol red method, as described by Chen et al. (2010)(29). To analyze inhibitory effects on the urease activity of *H. pylori* by cell-free supernatant (CFS) of LAB, *H. pylori* was resuspended in BHI broth (OD600=0.1) and mixed with 10% CFS incubated at 37 °C for 48 h. MRS broth (pH 7.0) was used as a control of the reaction. Subsequently, 150 µL of urease reaction buffer (20% w/v urea and 0.012% phenol red in phosphate buffer adjusted to a final pH of 6.8) was added to the microtiter plate. By virtue of the enzyme, urease catalyzes the hydrolysis of urea, leading to the production of ammonia, which elevates the pH solutions and induces a color change in the phenol red indicator from yellow to red. The absorbance of ammonia was quantified at 550 nm (OD550)(34). The value at OD 550 was measured after 20 min of incubation at 37°C. The highest urease activities as revealed by OD550.

$$\text{Relative urease activity (\%)} = \frac{A_{550\,(\text{LAB})}}{A_{550\,(\text{control})}} \times 100\%$$

Where $A_{550(LAB)}$ and $A_{550(control)}$ represent the OD550 values of LAB cocultured with *H. pylori* and control (MRS broth with *H. pylori*), respectively.

2.4. Statistical analysis

All results were expressed as the mean and standard deviation of three independent experiments. The one-way analysis of variance (one-way ANOVA), followed by post-hoc Tukey`s test for multiple comparisons. P values < 0.05 were considered significant. Statistical analysis was conducted using Minitab 18.0 software and GraphPad Prism 8.0 software.

3. RESULTS AND DISCUSSION

3.1. Evaluation of potential probiotic properties of LAB strains

3.1.1. Acid tolerance

At medium at pH 2, strain DC8 exhibited the highest survival rate of 98.3%, while strain DC7 showed the lowest survival rate of 87.1%. In the test at pH 3, strain DC9 had the highest survival rate at 101.0%, and strain DC20 had the lowest survival rate at 88.7% (Figure 1 and Figure 2).

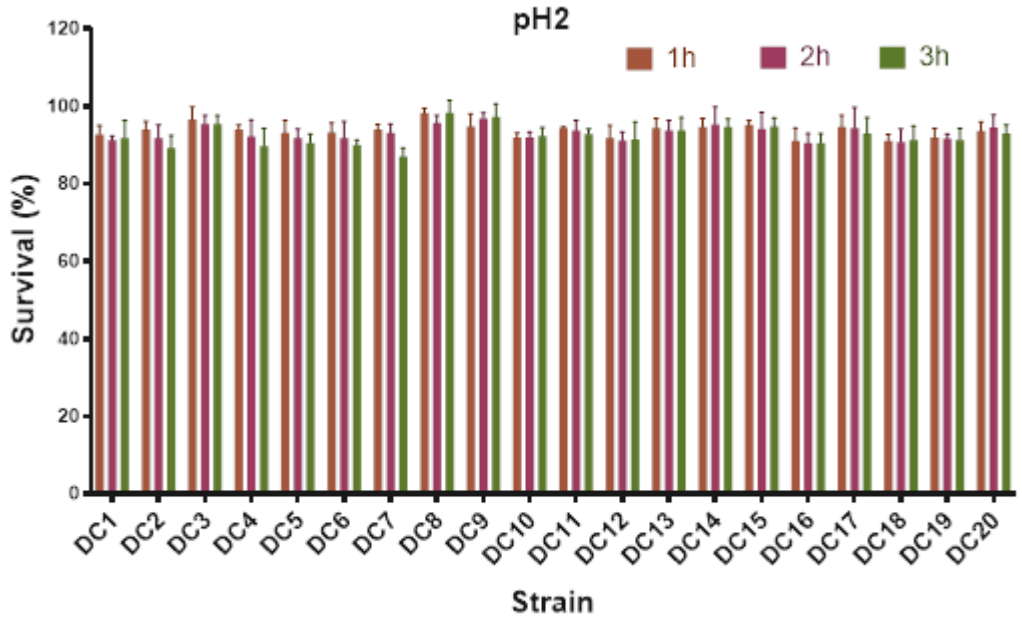

Figure 1. The survival rate of 20 LAB strains under the condition at pH 2.

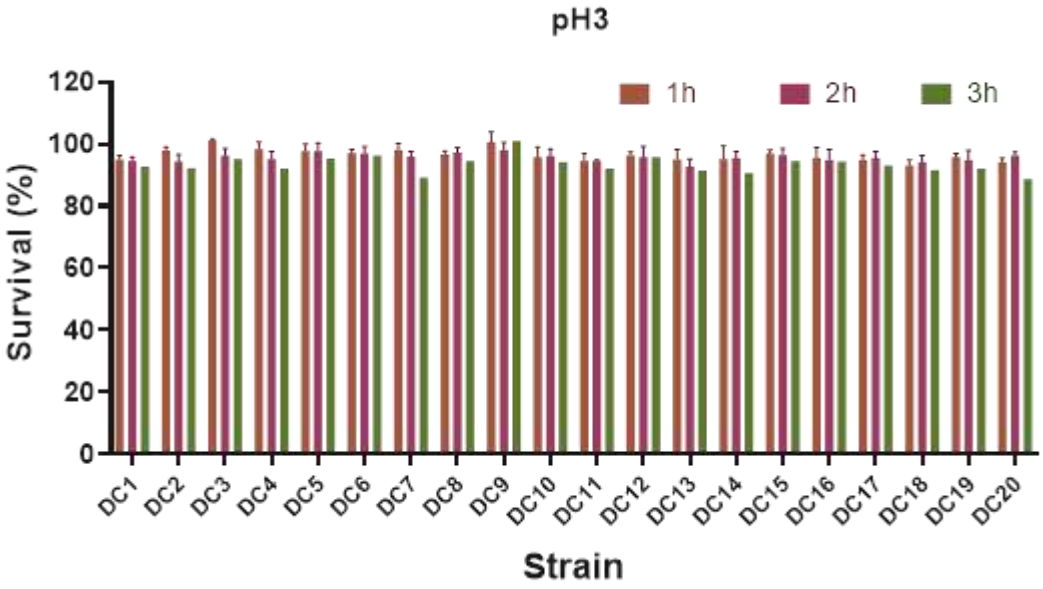

Figure 2. The survival rate of 20 LAB strains under the condition at pH 3.

3.1.2. Bile tolerance

This result indicated that following the 4 h incubation, 15 out of the 20 strains exhibited survival rates exceeding 50% at 0.3% bile concentration (DC1, DC2, DC3, DC4, DC6, DC7, DC8, DC9, DC10, DC11, DC12, DC13, DC16, DC17, and DC19). Among these, strain DC8 demonstrated the highest survival rate, reaching 82.6% (Figure 3).

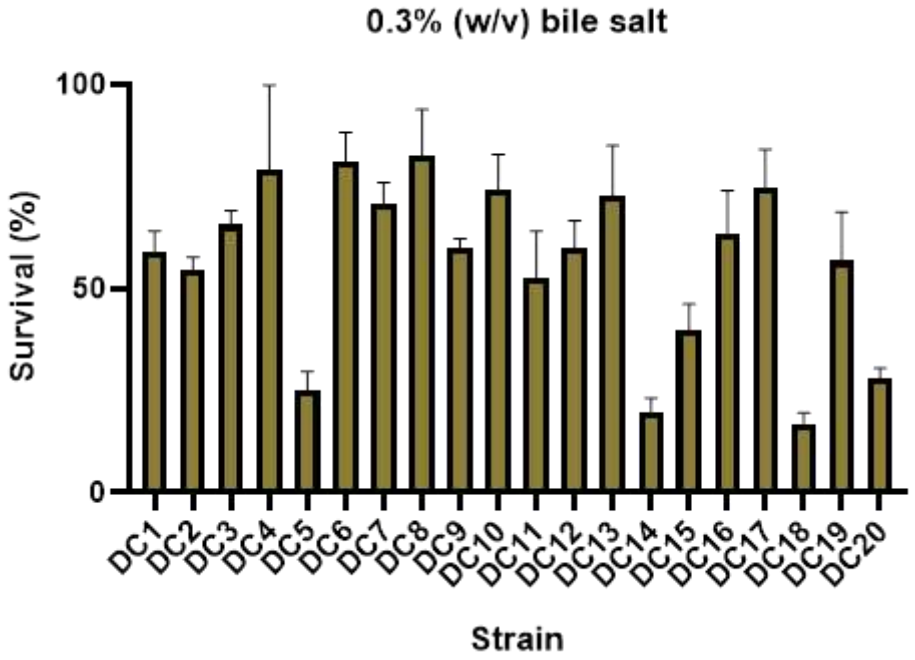

Figure 3. Resistance of lactic acid bacteria in the presence of 0.3% (w/v) bile salts.

3.1.3. Auto-aggregation and co-aggregation

The auto-aggregation assay results (Figure 4A) indicated that among all the strains, DC9 and DC10 exhibited the highest self-adhesion rates of 43.26% and 44.54% respectively, while DC2 showed the lowest rate at 7.20%. Out of all the strains, 10 of them (DC1, DC3, DC6, DC8, DC9, DC10, DC11, DC16, DC17, and DC19) demonstrated a medium auto-aggregation capability, with rates exceeding 20%. Only one strain, DC7, had a low auto-aggregation ability with a rate of less than 20%. The co-aggregation test results (Figure 4B) revealed that strain DC12 exhibited the highest rate at 48.61%, with no significant statistical difference compared to strains DC1, DC3, and DC9. On the other hand, strain DC13 had the lowest co-aggregation rate with *H. pylori* at 5.08%. 13 of the 15 strains exhibited co-aggregation rates above 20%.

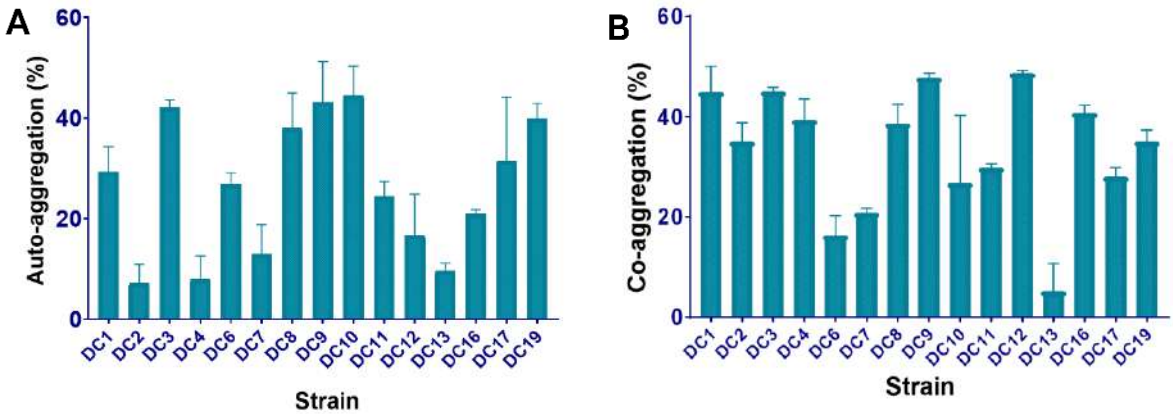

Figure 4. (A) Autoaggregation (%) and (B) Co-aggregation (%) of LAB strains after incubation of 5 h. From the two auto-aggregation and co-aggregation assays, six strains (DC1, DC3, DC8, DC9, DC11, and DC16) demonstrated both a medium auto-aggregation ability and a potential co-aggregation ability with *H. pylori* bacteria. These six strains were chosen for further experiments.

3.2. *In vitro* screening for antagonist activity with *H. pylori*

3.2.1. Well diffusion assay

Among the tested strains, strain DC3 exhibited the largest antibacterial ring diameter (13.67±0.58 mm), while strain DC1 had the smallest antibacterial ring diameter (12.00±0.00 mm).

Table 1. Inhibitory effect of six potential LAB strains on *H. pylori* ATCC 43504

Strain	Zone inhibition diameter (mm)
DC1	12.00±0.00[c]
DC3	13.67±0.58[a]
DC8	13.33±1.15[a]
DC9	12.67±1.15[b]
DC11	13.33±0.58[a]
DC16	12.67±1.15[b]
MRS broth	7.00

3.2.2. Effect of LAB strains on urease activity of *H. pylori*

The urease activity of 6 strains differed significantly (P value < 0.05) between the cocultures of LAB supernatants with *H. pylori* and individual *H. pylori* culture (*H. pylori* with MRS broth pH 7.0). The DC8 and DC16 demonstrated a reduction in relative urease activity by more than 50%. This outcome could be attributed to incongruent growth and culturing conditions or the presence of diverse resistance mechanisms to *H. pylori* of LAB.

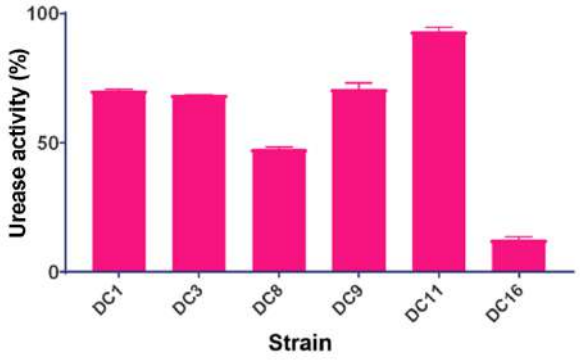

Figure 5. Inhibition of urease activity of LAB supernatant. MRS broth pH 7.0 was used as controls of the reaction.

4. CONCLUSION

In this study, the survival rate of LAB under various conditions was assessed based on optical density measurements at 600 nm (OD600). It is important to note that this method estimates bacterial biomass and does not directly differentiate between live and dead cells. Consequently, the reported survival rates may overestimate the actual number of viable cells, particularly under stress conditions. Future studies should consider incorporating additional methods, such as flow cytometry with viability stains or colony-forming unit (CFU) counts, to provide a more accurate assessment of bacterial viability. This limitation underscores the need for caution in interpreting the survival data and highlights areas for methodological refinement in future investigations into the probiotic potential of LAB. Despite this limitation, our findings provide valuable insights into the viability and potential probiotic properties of the LAB strains isolated from fermented foods, highlighting their potential as adjuvant therapies for *H. pylori* infection. This study was screening the selection of twenty strains of LAB which were isolated

from the traditional Vietnamese food. The screening for probiotic properties revealed that all twenty experimental strains demonstrated good survival ability, even in an acidic pH condition; only fifteen out of the twenty strains exhibited the ability to tolerate bile salts, with strain DC8 demonstrating the highest tolerance. The result indicated that two LAB strains, DC8 and DC16, possess probiotic potential and exhibit in vitro antagonistic activity against *H. pylori*. This demonstrates the potential of using probiotics derived from traditional fermented food to aid in eradicating *H. pylori*.

5. ACKNOWLEDGMENT

Conflict of interest

The authors declare that they have no conflict of interest.

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Prevalence of Microbiological Contamination in Herbal Products: A Public Health Concern

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ABSTRACT

Currently, the popularity of herbal products is surging due to their natural ingredients. To ensure consumer confidence and product safety, regulations are in place. These regulations are established by the Herbal Products Committee, Ministry of Public Health, Thailand. These regulations define the criteria, methods, and conditions for production, import, and selling herbal products. This ensures that the products meet high quality and efficacy standards, ultimately protecting consumer well-being. The aim of this study was to access the adherence of herbal products to microbiological quality control standards established by the Ministry of Public Health, Thailand. The method used in this study were "Microbial Enumeration tests" and "Test for Specified Micro-organisms" according to the Thai Herbal Pharmacopoeia 2020 and British Pharmacopoeia 2020. The criteria were stated by the Ministry of Public Health, Thailand (2021). The sampling period of the product from Microbiology Department, Center for Analysis of Product Quality started from November 2022 – February 2024. During this period, the test performed are total aerobic microbial count (TAMC) and total yeasts and molds count (TYMC). For test for specified micro-organisms, the tests are bile-tolerant gram-negative bacteria, *Salmonell*a spp., *Escherichia coli* and *Clostridium* spp. A total of 35 samples were analyzed. Of these, 18 samples (51.43%) were passed all criteria for every test. Among the remaining 17 samples that failed at least one criterion, the most common failures were TYMC, followed by bile-tolerant gram-negative bacteria and TAMC, respectively. Notably, none of the 35 samples tested positive for *Salmonella* spp., *Escherichia coli* and *Clostridium* spp. Although more than half (18/35) of the samples passed all criteria, nearly half failed at least one test, most commonly exceeding TYMC. Importantly, no *Salmonella* spp., *Escherichia coli* and *Clostridium* spp. were detected. These results indicated a need for the manufacturers to improve quality control practices to ensure consistent adherence to microbiological standards.

KEYWORDS: Herbal products; The Herbal Products; Committee Ministry of Public Health; Thailand; Microbial Enumeration tests; Test for Specified Micro-organisms; Microbiological Testing; Microbiological quality control

1. INTRODUCTION

Herbal products are experiencing a surge in popularity, but many consumers may not be fully aware of their categories. Herbal products encompass various forms of medicine derived from plants, including traditional Thai medicine and modern herbal formulations. These products are used for a range of purposes, from treating and preventing illness to promoting overall health and well-being, such as herbal teas, supplements, and topical applications. While being natural, herbal products are not without potential risks. Incorrect usage, including choosing the wrong herb, dosage, or method for a particular condition, can lead to adverse effects. Additionally, herbal products may cause allergic

reactions, contain harmful toxins, or harbor excessive microbial contamination. These contaminants can cause both acute illness and long-term health problems.

To address these concerns and build consumer confidence, the Ministry of Public Health, Thailand, has established regulations for herbal products. These regulations classify herbal products based on their intended use (oral or topical) and form (e.g., teas, capsules, ointments). This regulation also defined specific criteria, testing methods, and conditions for production, import, and sale. These measures aim to ensure the products are high-quality, efficacious, and meet safety standards. Orally used herbal products (Type B) as defined by the Ministry of Public Health Announcement in BE 2564 (2021) Volume 138, Special Part 294, Page 6 was one of the popular herbal products. The analysis methods and criteria are based on this announcement and utilize established testing methodologies from The British Pharmacopoeia 2020 (Volume I – V for *Salmonella* spp.) and the Thai Herbal Pharmacopoeia 2020 (APPENDIX 10: MICROBIOLOGICAL TEST 10.2 MICROBIAL LIMIT TEST). Specifically, the tests evaluate total aerobic microbial count (TAMC), total yeasts and molds count (TYMC), bile-tolerant Gram-negative bacteria, *Escherichia coli* and *Clostridium* spp. This study aims to assess the adherence of these herbal products to the microbiological quality control standards established by the Ministry of Public Health, Thailand.

2. MATERIALS AND METHODS

The methods used in this study was "Microbial Enumeration tests" and "Test for Specified Micro-organisms" according to the Thai Herbal Pharmacopoeia 2020 and British Pharmacopoeia 2020. The criteria were stated by the Ministry of Public Health, Thailand (2021). This study was focused on the herbal products (Type B) as defined by the Ministry of Public Health. Samples were collected from the Microbiology Department, Center for Analysis of Product Quality, Faculty of Pharmacy, Mahidol University, Thailand from November 2022 to February 2024. During this period, the tests performed were total aerobic microbial count (TAMC) and total yeasts and molds count (TYMC). The tests for specified microorganisms included: bile-tolerant gram-negative bacteria, *Salmonella* spp., *Escherichia coli* and *Clostridium* spp.

2.1. Microbial Enumeration tests (Thai Herbal Pharmacopoeia, 2020)

To conduct the microbial enumeration tests, the sample was serially diluted in a suitable buffer. From each dilution, take 1 mL of suspension and perform the pour plate method using soybean casein digest agar (TSA) and Sabouraud-dextrose agar (SDA); prepare at least duplicates for each dilution. Once the agar has solidified, incubate the TSA plates at 30-35°C for 3-5 days and the SDA plates at 20-25°C for 5-7 days. After incubation, the plate with the highest number of colonies with counts less than 250 colony-forming units (CFU) for TAMC and less than 50 CFU for TYMC was selected. The amount of microbial was calculated back to the original microbial load.

2.2. Test for Specified Microorganisms

2.2.1 Bile-tolerant gram-negative bacteria (Thai Herbal Pharmacopoeia, 2020)

The sample was prepared using 10 g or 10 mL of the test material mixed into 100 mL of Soybean-casein digest broth and incubated at 20-25°C for 2-5 hours. After the incubation, the mixture was inoculated into Enterobacteriaceae Enrichment Broth-Mossel (EE) at the concentrations of 0.1 g/mL, 0.01 g/mL, and 0.001 g/mL, using three tubes for each concentration. The sample was incubated at 30-35°C for 24-48 hours. Then, the sample was subcultured onto Violet red bile dextrose (VRBD) agar and incubated at 30-35°C for 18-24 hours. The colonies on the VRBD agar were observed, and the probable number of bacteria was determined using the reference table provided (Table 1).

Table 1. Probable number of bacteria (1)

Results for each Quantity of Product			Probable Number of Bacteria per g or per mL of product
0.1 g or 0.1 mL	0.01 g or0.01 mL	0.001 g or 0 001 mL	
+	+	+	More than 10^3
+	+	-	Less than 10^3 and more than 10^2
+	-	-	Less than 10^2 and more than 10
-	-	-	Less than 10

2.2.2 Salmonella spp. (British Pharmacopoeia, 2020)

To prepare the sample for *Salmonella* spp. detection, 25 g or mL of the sample was mixed with 225 mL of buffer peptone medium. Then, the mixture was incubated at 30-35°C for 18-24 h. After that, the enriched mixture was transfered for 0.1 mL into 10 mL of Rappaport-Vassiliadis broth and incubated at 30-35°C for 24-48 h. Subsequently, the enriched broth was subcultured onto Xylose-lysine-deoxycholate (XLD) agar and incubated at 30-35°C for 24-48 h. The presence of red colonies with or without black centers appearing on the XLD agar was recorded and subsequently tested to confirm the presence of *Salmonella* spp.

2.2.3. Escherichia coli (Thai Herbal Pharmacopoeia, 2020)

To detect the presence of *Escherichia coli* , 10 g or 10 mL of the samples was mixed with a suitable amount of neutralizing agent. Then, this mixture was transferred for 10 mL into 100 mL of Soybean-casein digest broth and incubated at 30-35°C for 18-24 h. After incubation, the enriched broth was transfered for 1 mL into MacConkey broth and incubated at 42-44°C for 24-48 h. Subsequently, the enriched broth was subcultured onto MacConkey agar and incubated at 30-35°C for 18-24 h. Pink colonies appearing on the MacConkey agar were recorded and subsequently confirmed by subculturing the colonies onto Levine Eosin Methylene Blue (EMB) agar. After incubating for 18-24 h at 30-35°C, the characteristic metallic green sheen on EMB agar typically occurs by *Escherichia coli*

2.2.4. Clostridium spp. (Thai Herbal Pharmacopoeia, 2020)

To test for the presence of *Clostridium* spp., 10 g or 10 mL of the samples was mixed with a suitable amount of neutralizing agent. Then, the mixture was divided into two 10 mL portions and inoculated into Reinforced Medium for Clostridia. One portion of the mixture was heated at 80°C for 10 min. and rapidly cooled it on ice. Both portions (heated and unheated) were incubated under anaerobic conditions at 30-35°C for 48 h. After incubation, both enriched broths were subculture onto Columbia agar supplemented with gentamicin and incubated the plates under anaerobic conditions at 30-35°C for 48-72 h. The presence of colonies on either the heated or unheated enriched broths after subculture suggested the possible presence of *Clostridium* spp. The presence of colonies was further examined using additional methods, such as microscopic examination for spores and biochemical tests for confirmation.

2.3 Determination of the tested results with the standard criteria

The results from The Microbial Enumeration tests and Test for Specified Microorganisms were combined and compared with the microbiological standard criteria mentioned in the regulation of the Ministry of Public Health, Thailand, as showed in Table 2.

Table 2. The microbial contamination criteria of Herbal products containing extracts or medicinal plants (The Herbal Products Committee Ministry of Public Health (2021).

	Criteria		
	TAMC (cfu/g or cfu/ml)	TYMC (cfu/g or cfu/ml)	Specified microorganisms
Herbal products (Oral use)			
B. Herbal products containing extracts or medicinal plants (With or without additives) In addition to type A	Not more than 5×10^4	Not more than 5×10^2	- Bile-tolerant gram-negative bacteria: Not more than 10^2 cfu (1 g or 1mL) - Absence of *Salmonella* spp. (25 g or 25 mL) - Absence of *Escherichia coli* (1 g or 1mL) - Absence of *Clostridium* spp. (1 g or 1mL)

3. RESULTS AND DISCUSSION

Herbal products are becoming increasingly popular due to their natural ingredients. However, concerns remain about the safety of these products, particularly regarding microbiological contamination. While there may not be a lot of reported cases of contamination, it's still important to ensure the safety of these products. This study evaluated the microbiological quality of 35 commercially available oral herbal products. The criteria of each test were following the regulations of the Ministry of Public Health, Thailand. The results showed that more than half (51.43%, or 18 out of 35) of the samples met all established criteria for microbial contamination and absence of specific pathogens (Table 3). For the remaining 17 samples (48.57%) that failed at least one criterion, the most common failures were TYMC, followed by bile-tolerant gram-negative bacteria and TAMC, respectively. Notably, none of the 35 tested samples showed contamination with *Salmonella* spp., *Escherichia coli* and *Clostridium* spp. (Table 3). This indicates a low prevalence of these high-risk pathogens in the commercially available herbal products.

Table 3. The results for sample (35 samples).

Method	Passed the criteria (No. of Sample)	Not passed the criteria (No. of Sample)
Total Aerobic Microbial Count	26	9
Total Yeasts and Molds Count	20	15
Bile-tolerant Gram-negative bacteria	21	14
Salmonella spp.	35	0
Escherichia coli	35	0
Clostridium spp.	35	0

4. CONCLUSION

This study evaluated the microbiological quality control of herbal products according to the regulation of the Herbal Products Committee, Ministry of Public Health, Thailand. Although more than half (18/35) of the samples passed all criteria, nearly half failed at least one test, most commonly exceeding TYMC. Importantly, no *Salmonella* spp., *E. coli* and *Clostridium* spp. were detected. These results indicate a need for the manufacturers to improve quality control practices to ensure consistent adherence to microbiological standards.

5. ACKNOWLEDGMENT

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Conflict of interest

The authors declare that they have no conflict of interest.

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Investigate the Effects of Culture Conditions on Survival, Viability and Activity of *Bacillus Clausii* M31 Spores

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ABSTRACT

Bacillus clausii spores are widely used which renowned for their probiotic benefits, including resilience in the gastrointestinal environment and compatibility with antibiotic therapies. Research into high-density cultivation through medium optimization has employed various statistical experimental designs but which do not emphasize increasing spore yield in culture media, frequently neglecting the functional properties of spores. The fermentation conditions of *B. clausii* strain have not been studied for producing large number of survivals. High activity of spores was underreported. Our study classed the environmental and growth parameters of *B. clausii* M31 (isolated in laboratory in Vietnam) to bolster spore functionality, and stability, and attain optimal spore density in the culture medium. Strains *B. clausii* M31 has growth under conditions: 37°C, 200 rpm, for 18-24 hrs. *B. clausii* M31 were cultured on media with varying ingredients. A Design of Experiments (DOE) which was a factorial design with three central points and 24 runs in JMP Pro software was applied. Impacts of nine medium components on total survival, viability efficiency, and activity of *B. clausii* M31 spores (isolated in laboratory in Vietnam) in cultures were examined across varying concentrations. Results demonstrated significant influence of Glucose and $MnSO_4$ concentrations on *B. clausii* M31 spore formation, stability and production efficiency. Specifically, at Glucose 2.0%, $MnSO_4$ 0.0235%, $CaSO_4$ 0.05% along with controlled agitation and aeration rates, maximal viability and stability were observed. Bioreactor-scale experiments achieved high spore densities of approximately 2.06 log10 CFU/mL and significant sporulation efficiency, antibacterial ring 2.83cm and survival about 75% in pH 3 in 4 h. Optimization of media constituents and culture parameters led to potential spore efficiency for *B. clausii* M31 in fermentative systems, reduced nutrient requirements, increased number spores, and strengthened spore activity. This study had significance in dosing and administration frequency for diverse biotechnological applications involving *B. clausii* M31.

KEYWORDS: *Bacillus clausii* M31; spore, survival; activity; viability; JMP pro

1. INTRODUCTION

Among the species studied are *Bacillus subtilis*, *Bacillus clausii*, *Bacillus cereus*, *Bacillus coagulans*, and *Bacillus licheniformis*. Heat-stable *Bacillus* spores offer an advantage over non-spore-forming species like *Lactobacillus* spp., allowing products to be stored at room temperature in a dry state without compromising viability. This advantage is supported by studies demonstrating that over 10% of implanted *B. clausii* spores can germinate in the small intestine, propagate, and regenerate spores (Abbrescia A. et al., 2015; Elazzazy et al., 2024). These spores are commonly found in environments such as soil, straw, and mud, as well as in the intestines of insects, animals, and humans (Omer AM et al., 2010; Sanders et al., 2018). Numerous studies have indicated positive effects of *Bacilli* on digestive disorders, constipation, and irritable bowel syndrome (Elshaghabee et al., 2017). The activity of spores is a crucial factor influencing the therapeutic effectiveness of the *B. clausii* strain. However, there is currently a scarcity of published research on fermentation conditions and the collection of *B. clausii* spores in Vietnam. Existing studies often focus solely on increasing spore count

without addressing spore activity. Hence, this study seeks to identify optimal conditions for cultivating the *B. clausii* M31 (isolated in laboratory) strain to achieve high survival rates, dense spore populations, and robust spore activity. The ultimate goal is to utilize these findings to establish a reliable source of raw materials and to produce probiotics for both food and pharmaceutical applications.

2. MATERIALS AND METHODS

2.1. Strains and Culture Conditions

Strains *B. clausii* M31 were isolated from the laboratory in Vietnam. Inoculum cultures were cultured in Luria-Bertani (LB) broth (Himedia, India). Subsequently, the culture broth was transferred to the production medium and incubated in a shaking incubator under specific conditions: 37°C, 200 rpm, for 18-24 hrs. Screening of culture medium components.

2.2. Assay for Viable Cell Number Counting

Cell growth was determined by counting the viable number of cells due to the presence of insoluble components in the medium. Samples were taken aseptically for the analysis of viable cell numbers and spore numbers. Samples were appropriately diluted, plated, and then incubated for 18 hrs at 37°C.

2.3. Effect of Working Volume on spores

B. clausii M31 were cultured on media with varying ingredients. Adjustments to pH were made using 6 N NaOH and 3 N HCl. Samples were withdrawn at regular intervals over a 24 hrs period, and the optical densities at 600-610 nm were measured using a spectrophotometer (UV-160A, Shimadzu Co., Japan) to assess the effects of culture conditions on survival, viability and activity of *B. clausii* M31 spores.

2.4. Statistical Media Optimization

Significant components of the original production medium influencing cell growth were identified using the DOE design. Furthermore, the optimal concentrations of these media components were determined utilizing its three-center points. Statistical analyses were conducted using JMP pro software (Version 14, USA).

2.5. Cultivation and optimization of the culture medium

Bioreactor experiments were conducted in a 2.5 L jar fermenter with a working volume of 1.0 L. The medium was steam sterilized, and inoculation was performed with 0.7% (v/v) inoculum. Fermentation was carried out at 30°C with an aeration rate of 1.0 vvm and an agitation rate of 200 rpm for 18-24 hrs.

3. RESULTS AND DISCUSSION

3.1. Assessment of culture medium constituents to facilitate the growth of *B. clausii* M31

Bacillus clausii, a well-recognized bacterial species, has been harnessed in various industrial probiotic applications. Optimization of Culture Medium The core composite matrix experiment sought to investigate the effect of primary culture medium components on *B. clausii* biomass. This study made use of the JMP Pro software design. The results of individual and interactive of these Runs design approach, a series of 24 experiments were conducted to systematically investigate the influence of various factors (X1-X9) which affected to the growth of *B. clausii* M31 was carried out using the statistical JMP design approach. Total 24 experiments were carried out following the design.

*Screening basic production medium components: X1: 0-3 % (w/v) of Glucose, X2: 0-0.2 % of $MgSO_4.7H_2O$, X3: 0-0.05 % of $MnSO_4.4H_20$, X4: 0-0.1 % $CaSO_4$, X5: 0-0.1 % FeSO4; X6: 0-10 % Yeast extract, X7: 0-0.1 % Meat Extract; X8: 0-10 % Peptone; X9: 0-5 % $(NH_4)_2.SO_4$

**Code for component concentrations:

Y1: Total cell density of *B. clausii* M31 in 1 mL suspension (log10 CFU/ mL);

Y2: Number of spores *B. clausii* M31 in 1 mL suspension (log10 CFU/ mL);

Y3: Activity of spores is calculated by inhibition zone (cm) of *B. clausii* spores suspension was accessed for their antibacterial using agar well diffusion method. The assessment of antibacterial efficacy for the isolated *B. clausii* M31 strains involved the measurement of the growth inhibition zone diameter against various pathogenic bacteria (***S. aureus* ATCC 6538)** within an agar plate environment supplemented with probiotics.

Table 1. The results measure of model.

Response	Measures of model	Training	Validation
Total cell Y1	R Square	0.9987	0.9996
	Log likelihood	-39.958	-13.541
Sporulation	R Square	0.9941	0.9868
	Log likelihood	-27.758	-4.951
Activity	R Square	0.9925	0.9462
	Log likelihood	-18.443	0.187

a) b) c)

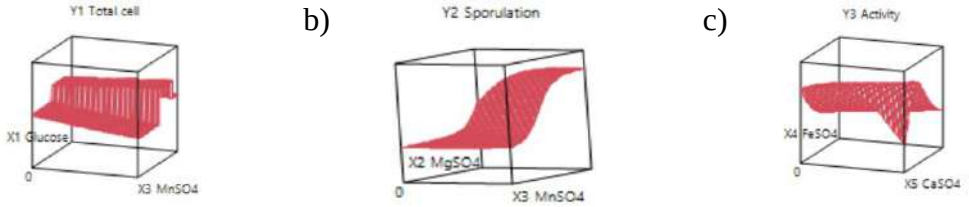

Figure 1. The graph evaluates the influence of independent variables on output variables.

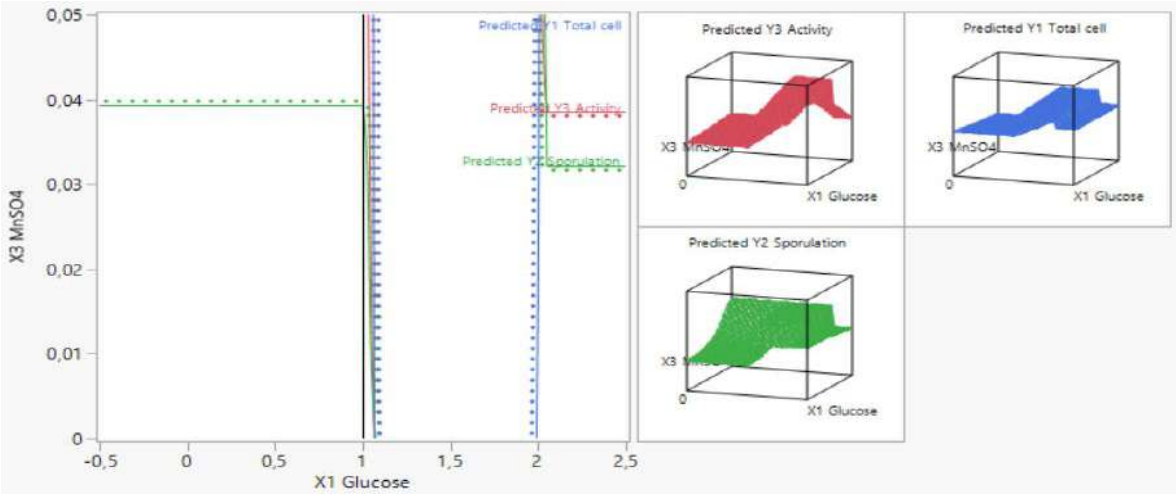

Figure 2. The contour profile of design optimization of *B. clausii* M31 growth.

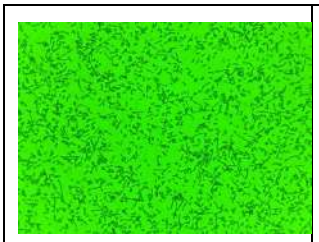	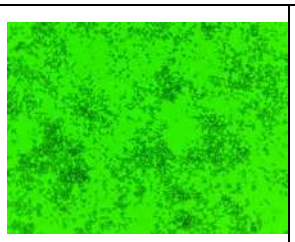	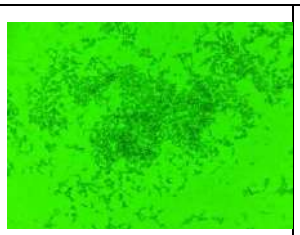	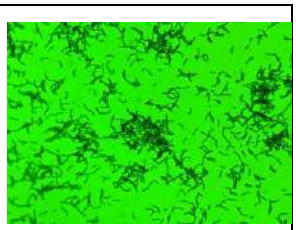
Run 2: The fermented biomass solution comprises primarily vegetative cells and many spores.	Run 14: The fermented biomass solution totally transforms to spores.	Run 10: The fermented biomass solution has few vegetative cells but many spores.	Run 22: Fermented biomass solution includes only vegetative cells, not spores.

Figure 3. Fermented biomass for each formulation through a microscope after 24h culture.

With the target function of maximal biomass, the program forecasts the following environmental parameters: 2.0% glucose (X1), 0.192% MgSO4 (X2), 0.0235% MnSO4 (X3), 0.011g% FeSO4 (X4), and 0.05% CaSO4 (X5), with a total cell density of 2.45 log10 CFU/ml (*Fig. 1*,*2*,*3*). The results of analyzing the agreement between model parameters and experiment demonstrate that the compatibility is 99.87%. The optimization of culture medium for *B. clausii* M31 growth was performed using the JMP software. It predicted that the highest *B. clausii* M31 cell density could be attained (up to 2.45 log 10 CFU/ mL) in the medium containing Glucose 2.0%. The experimental data at the optimal culture medium gave the maximum of 0.0235% $MnSO_4$ (X3), and 0.05% $CaSO_4$ (X5), compatible with the predicted value by the software. The growth of *B. clausii* M31 in optimal medium was increased comparing to that in the initial culture medium.

4. CONCLUSION

The best culture medium for *B. clausii* M31 development was identified by screening and optimizing the key components with the neural JMP design approach. Optimization of media ingredients and culture parameters resulted in exceptional spore efficiency for *B. clausii* M31 in fermentative systems, lowering nutritional requirements while maintaining robust spore activity. When compared to the initial media, the optimal culture medium increased the viability and stability of complete sporulation, as well as the strength of spore activity. This result is very encouraging and merits further scaling up, since it considerably contributes to optimizing dose and delivery frequency for a variety of biotechnological applications utilizing *B. clausii* M31.

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Conflict of interest

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Evaluation of Microbiological Quality in Cosmetics: A Study on Herbal and Non-Herbal Products

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ABSTRACT

Nowadays, cosmetics are widely used by all people, not just women. Cosmetics can be divided into 2 categories: those containing herbal ingredients and those without. Because of various herbs can offer nourishing and beautifying properties, the components in cosmetics should be safe and meet quality standards to ensure consumer safety. This study was aimed to assess the microbial contamination in commercially available cosmetics. All samples were collected from the Microbiology Department, Center of Analysis for Product Quality, Faculty of Pharmacy, Mahidol University, during February 2022 to December 2023. A total of 71 cosmetic samples were submitted for testing. Of these, 58 contained herbal ingredients, while the remaining 13 did not. All samples underwent testing for microbial contamination using standard methods outlined in the United States Pharmacopeia (2023), specifically General Chapters <61>Microbiological examination of nonsterile products: Microbial enumeration tests and <62> Microbiological examination of nonsterile products: Tests for specified microorganisms. Additionally, all samples containing herbal ingredients were specifically tested for Clostridium spp. contamination. The results were required to meet the standards set by the Ministry of Public Health, as published in Royal Gazette, Volume 133, Special Section 72 D. The results showed that, from a total of 71 samples, 58 were herbal cosmetics and 13 were non-herbal cosmetics. The accepted criteria were: The total number of aerobic plate count (TAMC and TYMC) must not exceed 1,000 cfu/g. In addition, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, and Clostridium spp., for sample containing herbal ingredients, should not be found. All samples met the specified criteria. From these results we can concluded that the cosmetics available in the market is meet the quality standard of microbiological contamination. Detection of microbial contamination in cosmetic products is an important criterion for quality control. Passing these criteria can indicate that the cosmetic product has a standardized production process and is safe for consumers.

KEYWORDS: herbal cosmetics; non-herbal cosmetics; Microbial enumeration tests; Test for specified microorganism.

1. INTRODUCTION

Thailand's cosmetics industry is experiencing phenomenal growth, with a multitude of manufacturers catering to both domestic consumers and international export markets. This surge reflects a global trend towards self-care and a growing emphasis on personal appearance. The desire to look and feel one's best transcends traditional boundaries of gender, age, and nationality. In today's image-conscious world, cosmetics empower individuals to enhance their confidence and project a desired image.

The Thai cosmetics landscape is diverse, offering a vast array of products formulated with both natural, herbal ingredients and innovative, synthetic compounds. To ensure consumer safety and promote industry trust, establishing standardized criteria for cosmetic production is crucial. These

standards should not only guarantee the absence of harmful contaminants and allergens but also ensure the product's efficacy and performance as intended. By implementing robust quality control measures, Thailand's cosmetics industry can solidify its position as a global leader in innovation and safety.[1]

2. MATERIALS AND METHODS

2.1. Sample collection

For this study, cosmetic samples were obtained from the Microbiology Division, Center for Analysis of Product Quality, Faculty of Pharmacy, Mahidol University, Thailand. The collection period spanned from February 2022 to December 2023

2.2. Microbial enumeration tests

The method of microbial enumeration test was performed according to USP 2023 General Chapters <61>Microbiological examination of nonsterile products: Microbial enumeration tests.[2] Briefly, prepared 10-fold serial dilution of 10 g of 10 ml sample in sterile Tryptic soy agar (TSA). Take 1 mL of suitable dilution into a sterile petri dish. Pour approximately 15-20 mL of warmed, molten TSA for total aerobic microbial count (TAMC) or Sabouraud dextrose agar (SDA) for total yeast and mold count (TYMC). Gently swirl the media to ensure the distribution of the sample throughout the agar. Allow the agar to solidify completely at room temperature. Incubate the TSA plate at 30-35°C for 3-5 days and SDA at 20-25°C for 5-7 days. All samples were performed in triplicate for each dilution. After incubation, the number of colonies formed on each plate was counted. Culture plates with a colony count between 25 and 250 colony forming unit (CFU) for TAMC and between 15 and 50 CFU for TYMC were used in calculating the TAMC and TYMC. The number of microbial found in the products was calculated and reported in CFU per gram or milliliter of the original sample using the following formula:

$$\text{TAMC or TYMC (CFU/g or mL)} = \frac{\text{(Number of colonies x Dilution factor)}}{\text{Sample weight (g) or Sample volume (mL)}}$$

2.3. Tests for specified microorganisms

2.3.1 Staphylococcus aureus[3]

Tested sample was prepared in a tryptic soy broth (TSB) in a 1:10 dilution. Then, the diluted sample was transfered an appropriate volume to TSB and incubated at 30-35°C for 18-24 h. After incubation, the mixture was subcultured onto Mannitol Salt Agar (MSA) and incubated at 30-35°C for an additional 18-72 h. The MSA plates was examined for the presence of yellow colonies, indicative of mannitol fermentation by *S. aureus*.

2.3.2 Pseudomonas aeruginosa[3]

A 10-fold dilution of the sample was prepared in accordance with the microbial enumeration test protocol. Subsequently, 10 ml of the diluted mixture was transferred into tryptic soy broth (TSB) and incubated at a temperature range of 30-35°C for 18-24 h. Following this incubation, the mixture was subcultured onto cetrimide agar and again incubated at 30-35°C for another 18-24 h. After incubation, the presence of green colonies on the agar was observed.

2.3.3 Candida albicans[3]

The sample was prepared in a 10-fold dilution of TSB. Transfer 10 ml of this dilution into 90 ml of TSB and incubate at 20-25°C for 3-5 days. After that, a portion of the broth was subcultured onto Sabouraud Dextrose Agar (SDA) plates and incubated at 20-25°C for 24-48 h.

2.3.4 Clostridium spp. [3]

The sample preparation in a 10-fold dilution was prepared the same as other microorganisms. The diluted sample was transferred for 10 ml into Reinforced Clostridial Medium (RCM) for 2 tubes. The first tube was heated at 80 °C for 10 min and cooled on ice rapidly. While another tube was left at room temperature. Both tubes were incubated at 35-37°C for 24-48 h in anaerobic conditions by adding liquid paraffin on top of the media. After incubation, the enriched cultures were subcultured onto Columbia agar and further incubated at 30-35 °C for 48-72 h under anaerobic condition.

3. RESULTS AND DISCUSSION

The acceptance criteria for cosmetics was shown in table 1. A total of 71 cosmetic samples were included in the analysis, including herbal and non-herbal containing products. Specifically, the sample set comprised 58 herbal cosmetics and 13 non-herbal cosmetics (Fig.1). The result showed that all samples met the standards criteria of the Ministry of Public Health, Thailand.[4]

Table 1. Acceptance criteria according to the announcement of the Ministry of Public Health Royal Gazette, Volume 133, Special Section 72 D.[4]

TEST	LIMIT
Standard plate count	Total aerobic plate count (TAMC and TYMC) not more than 1,000 cfu/g or cfu/mL
Tests for specified microorganisms	
Pseudomonas aeruginosa	Corresponding in 1 g or 1 mL
Staphylococcus aureus	Corresponding in 1 g or 1 mL
Candida albicans	Corresponding in 1 g or 1 mL
Clostridium spp.	Corresponding in 1 g or 1 mL

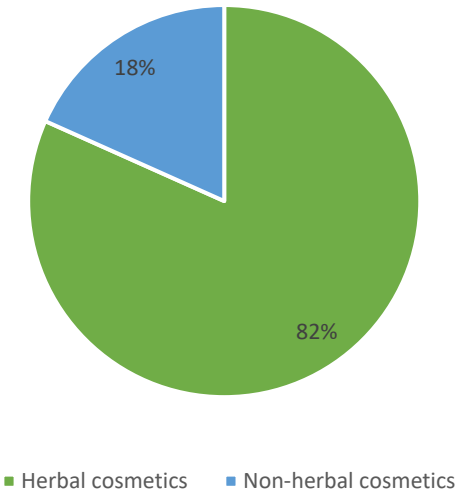

Figure 1. The percentage of herbal and non-herbal cosmetics in this study.

4. CONCLUSION

In Thailand's food and drug regulations, cosmetics need to follow the regulations and meet the standards criteria of the Ministry of Public Health, Thailand. Detection of microbial contamination in cosmetic products is one of the important criteria for product quality control. Passing these criteria can indicate that the cosmetic product has a standardized production process and is safe for consumers. However, to register the cosmetics with the Food and Drug Administration, not only do the microbiological quality criteria need to be met, but also the quantity of each ingredient needs to be passed. The results indicated that cosmetic products in Thailand have good quality in terms of microbiological control. This result should help ensure the confident of consumers in cosmetic products both with and without herbal ingredients in Thailand.[5]

5. ACKNOWLEDGMENT

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Pharmacology, Toxicology, and Physiology

PP-1002101-P

Growth Characteristics and Morphology of Frozen-Thawed Porcine Oviductal Epithelial Cells and Their Application in Cytotoxicity Tests of Kratom Leaf Juice by Using MTT Assay

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ABSTRACT

Porcines are some of the most frequently consumed animals globally. While various aspects of porcines have been extensively examined, scientific research is lacking on the reproductive organs, such as the oviduct. This study aimed to scrutinize the capabilities of porcine oviductal epithelial cells (POECs) for cytotoxicity testing, employing kratom leaf extract as a case study. Kratom has recently been decriminalized in Thailand and credited with pain alleviation, leading to increased consumption of kratom leaf juice. The information will provide data for future research on the porcine reproductive system to support the safe development of kratom-based products. Frozen-thawed POECs were cultured at 2×10^4 cells/well in a 24-well plate and counted every 24 hours for 7 days to determine the growth curve. Cells at the log phase were then treated with kratom leaf juice at concentrations of 5%, 10%, 50%, 75%, and 100% (v/v). Cell viability was determined using MTT assay with absorbance at 540 nm. All experiments were performed in triplicate. The growth rate and doubling time of POECs were 0.0142 cells per hour and 21.30 hours, respectively. From the MTT assay, 5% and 100% concentrations of kratom leaf juice from the red vein increased cell viability, whereas the green vein showed only 5%. Other concentrations decreased cell viability. The IC_{50} value of POECs for the red and green veins is 199.44 and 261.45, respectively. Frozen-thawed POECs maintained epithelial-like morphology. The optimal time for cell experimentation is 24–48 hours post-thawing during the log phase of maximal growth. The subculture can sustain growth indefinitely, showing potential for cell line development. Kratom juice at 5% boosted cell viability, with varying effects on different strains, whereas at higher concentrations, it inhibited cell growth. The results from this study indicated that POECs are a viable option for cytotoxicity testing.

KEYWORDS: Cytotoxicity; Green vein kratom; Porcine oviductal epithelial cells; Red vein kratom

1. INTRODUCTION

Porcines are widely farmed in Nakhon Pathom Province. However, the oviduct of porcine has not been popularly eaten. This research aimed to investigate potentials of porcine oviductal epithelial cells (POECs) in terms of biotechnological application. Previously, the morphology of porcine oviducts was studied using a scanning electron microscope[1] and cells were cryopreservation. In this study, frozen POECs were studied for growth characteristics and toxicity testing capabilities using Thai kratom strains. The information acquired from this research will provide valuable data for future research on the porcine reproductive system and support for safe development of kratom-based products.

2. MATERIALS AND METHODS

2.1 Analyze Growth Characteristics and Morphology of Frozen-Thawed POECs

POECs were cultured in DMEM with 5% fetal bovine serum at 2×10^4 cells/well in 24-well plate and maintained at 37°C, 5% CO_2, 95% air atmosphere, and high humidity. Estimation of viable cells was performed using trypan blue staining and an inverted microscope for every 24 hours, 7 days. The experiments were carried out in triplicate.

2.2 Kratom Leaf Juice Preparation

Mitragyna speciosa Korth. (Kratom) leaves, green vein, and red vein cultivars, at three-year-old age harvesting during February and December from the Kaeng Krachan district, Phetchaburi province, Thailand were used in this study. The leaves were blended in distilled water (1:1; w/v) prior to centrifugation at 3,000 rpm for 5 min. The supernatant was sterilized using a 0.22 µm syringe filter and stored at -20°C.

2.3 Cytotoxicity Test with Kratom Leaf Juice

POECs were incubated with Kratom juice at concentrations 5%, 10%, 25%, 50%, 75%, and 100% (v/v) for 24 hours. Cell viability was investigated using MTT assay[2]. A relationship between concentration and percentage of cell viability was analyzed in relative with control (without Kratom leaf juice addition). The experiments were carried out in triplicate.

3. RESULTS AND DISCUSSION

3.1 Growth Characteristics of Frozen-Thawed POECs

Cells entered log phase in the first 23 hours before entering lag phase between 24-48 hours (Figure 1). Cells were re-entering to log phase (48-72 hours post-thawing), and then decreased after 120 hours. Sub-culturing cells in new containers allowed them to continue multiplying, a continuous cell characteristic. Estimation of growth rate and doubling time resulted in 0.0142 cells/hour and 21.30 hours. When cultured for 24 hours, POECs exhibited an epithelial-like cell morphology, characterized by polygonal shapes and tight cell-to-cell junctions (Figure 2). Some fibroblast-like cells were observed interspersing between epithelial-like cells.

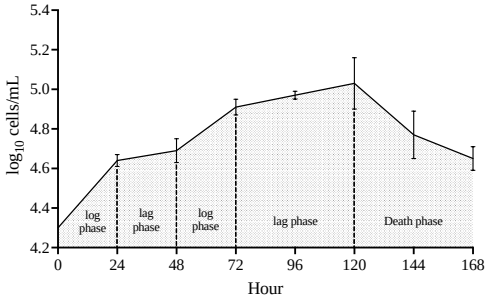

Figure 1. Growth curve of POECs between 0 and 168 hr

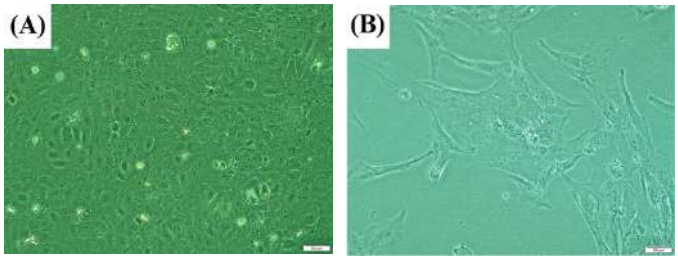

Figure 2. Morphology of POECs cultured in DMEM (A) 24 hr, (B) sub-passaged 2 times

3.2 Cell Viability from Kratom Leaves Juice Treatment

Only 5% and 100% Red vein juice and 5% Green vein increased cell viability (Figure 3), suggesting differential effects on cells from different kratom concentrations. This is consistent with

previous research demonstrating that high concentrations of kratom organoid inhibited nasopharyngeal carcinoma cell growth[3]. It is still unclear that the increased cell number could lead to tumorigenesis or simply increase of normal cells. At 5% and 10% juices, Green vein cell viability was higher than those of Red vein. But, at 50%, 75%, and 100%, Red vein gave higher cell viability, the different effects may result from differences in their components. IC_{50} value of POECs treated with Red and Green veins were 199.44 and 261.45, respectively. IC_{50} values of kratom leaf juice to POECs demonstrated markedly higher values in comparison to other cell lines (Table 1), implying tolerate response mechanism in POECs to kratom leaves. Cell morphology with 5% Red vein addition was elongated but contained numerous vacuoles in the cytoplasm (Figure 4A), where numerous dead cells were seen at with 50% juice (Figure 4B). Interestingly, cells treated with 75% (Figure 4C) and 100% juice were similar to the control group, where cells were in fibroblast shape.

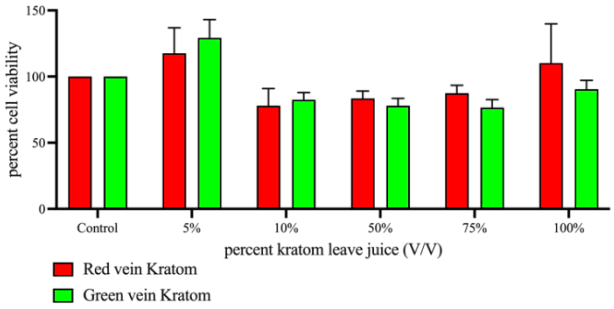

Figure 3. Cell viability of POECs treated with juice from Red and Green vein juices at five concentrations

Table 1. IC_{50} values of Red and Green vein juices against various cell lines

CELL LINE TYPE	IC_{50} (mg/mL)	
	Red Vein kratom	Green Vein kratom
POECs	199.44	261.45
LLC-MK$_2$*	30.63	51.38
Vero*	27.52	28.13
HaCat*	14.04	26.82
Hela*	34.27	41.19

*Results from project research by Kleangklom and Budaumkha (2024)[4] in triplicate.

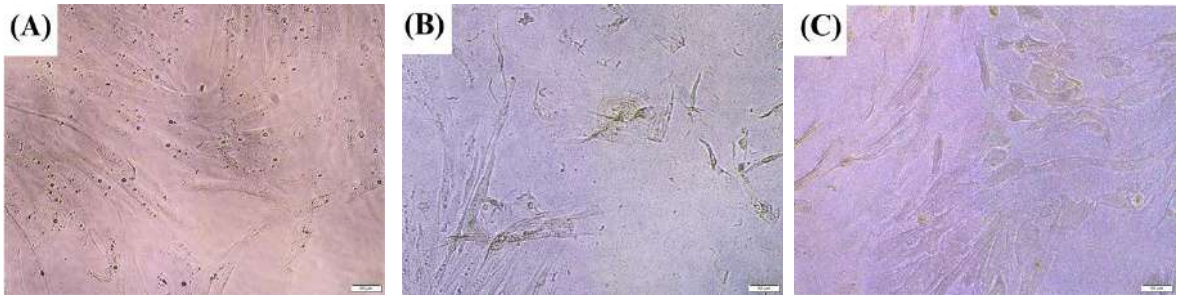

Figure 4. Morphology of POECs when treated with kratom leaf juice at various concentrations for a period of 24 hours. (A) kratom 5%, (B) kratom 50% ,(C) kratom 75%

4. CONCLUSION

Frozen-thawed POECs can grow in culture condition very well showing a characteristic of continuous cells. The growth rate and doubling time were calculated to be 0.0142 per hour and 21.20 hours, respectively. When cultured with kratom leaf juice addition, cells display tolerate specific chemicals present in kratom leaves with IC_{50} values for the Green and Red vein kratom strains at 199.44 and 261.45, respectively. This allowed us to conclude that POECs possess potential properties that are useful in the fields of cell biotechnology and cytotoxicity tests.

5. ACKNOWLEDGMENT

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Pharmaceutical Chemistry

PC-0502005-O

Effect of freeze-drying and spray-drying on the physicochemical composition of *Thunbergia laurifolia* leaf extract

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ABSTRACT

Thunbergia laurifolia leaves are used in Thai traditional medicine for the treatments of fever and mouth ulcers. Among various processing methods, drying techniques play a crucial role in determining the physicochemical properties and chemical composition of plant extract. This study aims to compare the physicochemical properties and chemical composition of *T. laurifolia* leaf extracts obtained from two drying methods: freeze-drying and spray-drying. The dried *T. laurifolia* leaves were boiled in water at 95 °C for 2 h, then filtered and dried on water bath to obtain a concentrated extract. The concentrated extract was separately dried using two drying methods: freeze-drying and spray-drying. For spray-drying, maltodextrin was used as an absorbent while freeze-drying was conducted without any additive. The physical characteristics including color, odor, and physical state of both extracts were manually observed. The water solubilities were evaluated by shake flask method. The chemical characteristics and compositions of both extracts were examined using ultraviolet (UV) spectroscopy, attenuated total reflectance infrared (ATR-IR) spectroscopy, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Extracts obtained from freeze-drying and spray-drying showed no difference in water solubility at 37 °C. Their UV spectra at 200-400 nm, TLC and HPLC fingerprints, and chemical marker contents showed no significant difference. Rosmarinic acid was found to be a major marker in both extracts. IR spectra of both extracts showed similar general fingerprints region, however, the extract obtained from spray-drying showed additional peaks that corresponded to maltodextrin. Extracts from freeze-drying and spray-drying methods had similar physical and chemical properties. Further investigation is required for biological activity testing to support these findings.

KEYWORDS: *Thunbergia laurifolia*; Freeze-drying; Spray-drying; Physicochemical properties; Rosmarinic acid

1. INTRODUCTION

Thunbergia laurifolia Lindl.is commonly known as Rang Jued in Thai. The Herbal teas and capsules of *T. laurifolia* leaf have been used in Thai traditional medicine for treatments of fever, mouth ulcers, and drug addiction[1,2]. The phytochemical composition of *T. laurifolia* leaf have been reported to contain phenolic compound such as apigenin and apigenin glycosides, caffeic acid, gallic acid, vicenin-II, and rosmarinic acid[1,3-6]. Recently, *T. laurifolia* aqueous extract have attracted increasing interest in the pharmaceutical due to its biological activities, such as anti-inflammatory, antioxidant, anti-diabetic, anti-depressant, and anti-dementia activities[7-13].

However, phenolic compounds in liquid forms are often subject to chemical and physical deterioration. Dry forms, achieved through freeze-drying or spray-drying, are frequently used as alternatives. To achieve a dried product, freeze-drying uses freezing and low pressure with the addition of heat (in the amount required to provide the sublimation of frozen water)[14]. Lyophilized particles

containing phenolic compounds are known to be stable over long periods of time because they prevent hydrolytic and oxidative degradation of the active compounds during storage[15]. However, freeze-drying is an expensive and time-consuming method that costs approximately six times more per kilogram of water removed than spray drying. Spray drying is an industrial method that uses hot air to obtain powder from a solution. The aforementioned technique improves the microbiological and biochemical stability of products while lowering storage and transportation costs[14].

The physical features of *T. laurifolia* leaf extracts obtained through freeze-drying and spray-drying were examined in this work. The solubility was assessed using the shake flask method. The chemical properties and contents of both extracts were investigated using ultraviolet (UV) spectroscopy and attenuated total reflectance infrared (ATR-IR) spectroscopy, thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC). This study aimed to help develop standardized protocols for drying *of T. laurifolia* aqueous extract in the pharmaceutical and nutraceutical industries.

2. MATERIALS AND METHODS

2.1. Plant materials

The leaves of *T. laurifolia* was collected from Sa Kaeo province, Thailand. The botanical and taxonomical characteristics of the leaves were identified according to Thai Herbal Pharmacopoeia 2021. The leaves were cleaned and dried in a hot air oven at 60 °C for 2 h, grinded into powder (with No. 20 mesh), and preserved in tight container until use.

2.2. Preparation of freeze and spray-dried extracts

The powdered leaves of *T. laurifolia* (1 kg) were boiled in 10 L of distilled water for 2 hours, then filtered and evaporated to make 1 L of the concentrated *T. laurifolia* leaf extract (TL).

Before the lyophilization, the 200 mL of TL were frozen in freezer at -80 °C for 1 h. In freeze dryer Alpha 1-4 LD plus (Christ, Osterode am Harz, Germany), main drying was carried out at -50 °C and pressure of 0.1 mbar for 48 h. At the end of this period, freeze-dried samples were transferred to dark-capped glass bottles and stored at +4 °C. Production was done in three replicates.

Spray drying of extracts with 10% w/w of maltodextrin was executed in Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) equipped with nozzle of 0.7 mm. The inlet temperature was 185 °C, while outlet temperature was 120 °C. The air flow rate was 45 mm and the rate of liquid feed was 30%. Powdered samples were taken from the machine's flask and stored at 4 °C in dark-capped bottles. Production was carried out in 3 replications.

2.3. Analysis of the spray and freeze-dried extracts

2.3.1. Determination of physical characteristics and water solubility

The physical characteristics of both extracts, such as color, and odor were manually observed. The samples solubility was determined using the shake flask method. Two hundred milligrams of dried extract were dissolved in 100 milliliters of water and shaken continuously at 37 °C for 24 h.

2.3.2. Ultraviolet spectroscopy

Samples for freeze-drying and spray-drying were prepared with concentrations of 0.25 and 0.5 mg/mL in water, respectively. The absorbance was measured spectrophotometrically at a wavelength of 200-400 nm. All measurements were performed with a UV spectrophotometer, UV-1900i (Shimadzu, Kyoto, Japan).

2.3.3. Attenuated total reflectance infrared (ATR-IR) spectroscopy

The IR analysis of freeze-drying and spray-drying samples was performed using Nicolet iS5 spectrometer paired with iD7 ATR accessory (Thermo Scientific, USA). The samples were analyzed in the spectral range 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}.

2.3.4. TLC fingerprint analysis

The sample solutions for freeze-drying and spray-drying were prepared at concentrations of 1 and 2 mg/mL in 50% methanol, respectively. A standard solution of caffeic acid, vicenin-2, and rosmarinic acid was prepared at a concentration of 0.2 mg/mL in 50% methanol. Then, eight microliters of the sample and 3 µL of standard solution were separately spotted on a precoated silica gel 60 GF_{254} TLC plate. The plates were developed in two solvent systems. Solvent system I was chloroform: methanol: formic acid (70:30:5 v/v/v), and solvent system II will be ethyl toluene: ethyl acetate: formic acid (10:9:2 v/v/v). The developing distance was 15 cm. After being removed from the developing chamber, TLC plates were air dried in a fume hood for 30 minutes and examined under white light, UV light (254 and 366 nm), and UV light at 366 nm after spraying with a natural product/polyethylene glycol (NP/PEG) reagent. The phytochemical characteristics of the extracts were compared.

2.3.5. HPLC fingerprint and quantitative analysis of chemical markers

A Shimadzu i-Series LC-2050C (Kyoto, Japan) was used for HPLC analysis. The system is composed of a photodiode array (PDA) detector, quaternary solvent, cooling autosampler, and column oven. The HPLC conditions were as described in the previous study[3]. A Zorbax® SB-C18 (3.0 x 150 mm, 5µm, Agilent, USA) was used with a mobile phase consisting of 0.02% phosphoric acid at a pH of 2.57 and 12% methanol (Merck, Germany) in acetonitrile (Merck, Germany). The flow rate was 0.8 mL/min. with a detection wavelength of 330 nm, and the injection volume was 20 µL.

The sample solutions of freeze-drying and spray-drying were prepared at concentrations of 0.75 and 1.5 mg/mL in water, respectively. The standard mixture of *T. laurifolia* chemical markers, including caffeic acid, vicenin-2, and rosmarinic acid, was prepared at concentrations of 8, 12, and 12 µL/mL, respectively. All solutions were filtrated through a 0.45 µm PTFE syringe filter prior to the HPLC analysis. The HPLC fingerprints of samples ($n = 3$) were compared using visual chromatographic analysis. The content of chemical markers was determined and expressed as mg/100 g of dried leaf.

2.4. Statistical analysis

The similarity analysis of the UV spectrum, IR spectrum, and HPLC fingerprints was performed by calculating the correlation coefficient (r) using Microsoft Excel. All analyses were done in triplicate, and the results were given as the mean ± standard deviation (SD). To compare the significant differences in the markers content at $p < 0.05$, a t-test was used.

3. RESULTS AND DISCUSSION

3.1. Analysis of physical characteristics and water solubility

Extracts from freeze-drying and spray-drying were dark brown and had a distinct odor. The spray-drying sample had a lighter coloration due to the presence of maltodextrin. Furthermore, after 24 hours of shaking at 37 °C, all of the samples were dissolved completely in water.

3.2. Analysis of chemical characteristics using UV, ATR-IR, TLC and HPLC analysis

Figure 1A shows that the UV spectrum of freeze-drying and spray-drying extracts followed a similar pattern, with λ_{max} at 280 and 330 nm, which correspond to the absorption of phenolic and

flavonoid structures, respectively. The IR spectra of both extracts showed a similar general fingerprint region (1000-400 cm^{-1}). However, the spray-dried extract showed additional peaks that belonged to maltodextrin at 1200-1000 cm^{-1} (Figure 1B). TLC chromatograms of both extracts revealed a similar pattern, revealing caffeic acid, vicenin-2, and rosmarinic acid (Figure 1C). Table 1 displays the hR_f values for all markers across the two solvent systems.

Furthermore, all samples had a comparable HPLC fingerprint, with rosmarinic acid being a prominent signal in both extracts (Figure 1D). The HPLC determination of caffeic acid, vicenin-2, and rosmarinic acid contents in both extracts (Table 1) revealed no significant differences (p-value > 0.05). The chemical properties of freeze-drying and spray-drying extracts were statistically evaluated by similarity analysis. The correlation between the UV spectrum, IR spectrum, and HPLC fingerprint of all samples was calculated and reported as a correlation coefficient (r). The results show that those characteristics of freeze-drying and spray-drying extracts are highly comparable, with r-values ranging from 0.975 to 1.00.

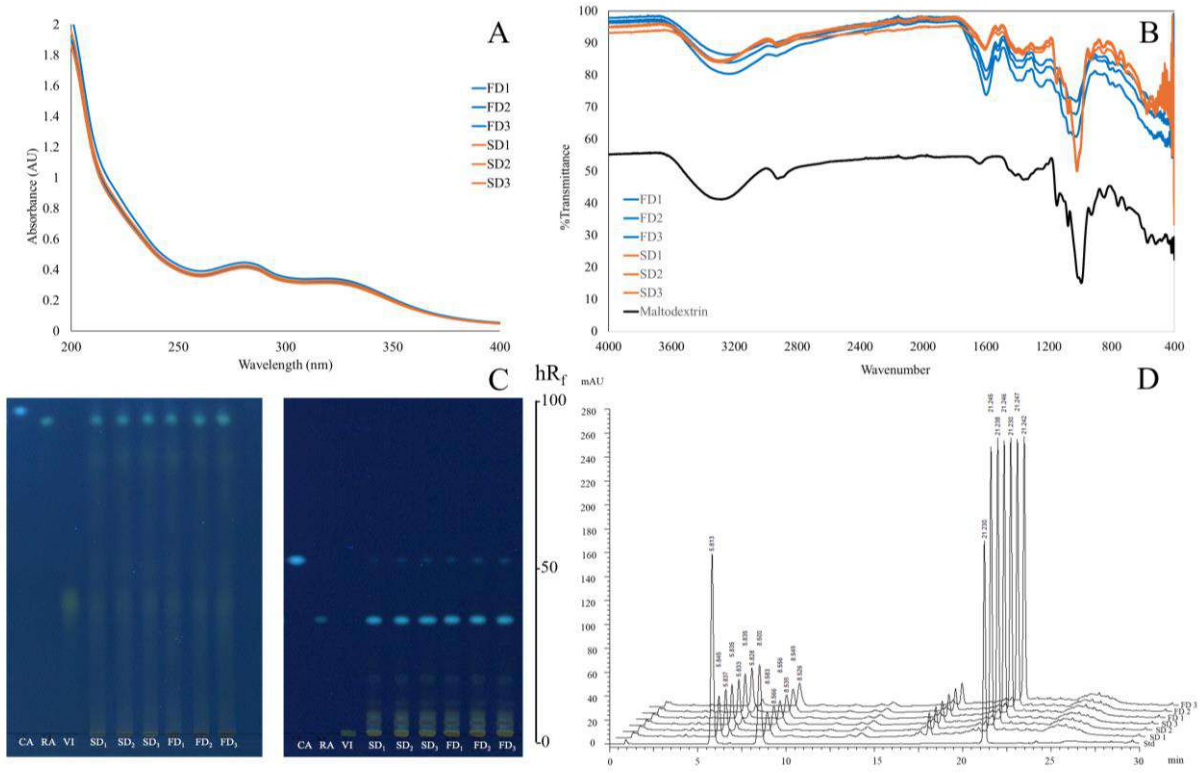

Figure 1. UV spectrum (A), IR spectrum (B), TLC fingerprints (C) and HPLC fingerprints of freeze drying (FD) and spray-drying (SD) extracts of *T. laurifolia* leaf.

Table 1. hR_f value, retention time, and marker content of freeze-drying and spray-drying of *T. laurifolia* leaf extract.

Freeze-drying	TLC System I (hR_f)		TLC System II (hR_f)		HPLC retention time (min)		Marker content (mg/ 100 g dried leaf)	
	FD	SD	FD	SD	FD	SD	FD	SD
Caffeic acid	90 – 93	90 – 93	60 – 64	60 – 64	5.83	5.84	50.17 ± 0.44	51.56 ± 0.33
Vicenin-II	10 – 15	10 – 15	0 – 6	0 – 5	8.54	8.57	118.75 ± 1.26	121.31 ± 0.65
Rosmarinic acid	85 – 89	84 – 89	42 – 46	43 – 46	21.24	21.24	513.47 ± 5.64	523.29 ± 3.49

* FD = freeze-drying, SD = spray-drying

4. CONCLUSION

This study compares the physical and chemical properties of *T. laurifolia* leaf extracts produced by freeze-drying and spray-drying. The findings indicate that freeze-drying and spray-drying methods exhibit similar physical and chemical features. These findings can be beneficial for researchers and manufacturers attempting to improve the drying methods for *T. laurifolia* products employed for pharmaceutical and nutraceutical applications. To verify these findings, further investigation is required regarding biological activity testing.

5. ACKNOWLEDGMENT

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Conflict of interest

The authors declare that they have no conflict of interest.

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