LEADING THE WAY
LEADING THE WORLD
KHALĪFAH • AMĀNAH • IQRA' • RAHMATAN LIL-ĀLAMĪN
INTERNATIONAL MULTI-AWARD WINNING INSTITUTION FOR SUSTAINABILITY

JOURNAL OF PHARMACY

Volume 5 Issue 1, January 2025

E-ISSN: 2773-5664
https://journals.iium.edu.my/ktn/index.php/jp

IIUM JOURNAL OF PHARMACY

About the Journal

The Journal of Pharmacy considers research findings from fundamental research to clinical investigations as original articles, systematic reviews, meta-analyses, general reviews and mini-reviews. The Journal provides a platform for Pharmacists, Researchers, Academicians, and Practitioners who are highly motivated in contributing to the Pharmacy and Pharmaceutical Sciences disciplines.

The journal welcomes submissions from **all over the world** with **no article processing charges**.

The scope of this journal includes all areas related to pharmacy interest and not limited to drug development, pharmaceutical/medicinal chemistry, drug targeting, structure-based drug design, computational chemistry, genomics, proteomics, pharmacogenomics, bioinformatics, pharmacology, toxicology, pharmacokinetics, pharmaceutical analysis, pharmaceutical technology, drug delivery, drug formulation, biopharmaceutics, industrial pharmacy, pharmacognosy, natural product research, cosmeceutical, nutraceutical, pharmacy practice, pharmacoeconomics, pharmacoepidemiology, clinical pharmacy, hospital pharmacy, social and administrative pharmacy.

The Journal of Pharmacy, published biannually (***January and July***), is a ***double-blind peer-reviewed*** open-access journal of the Kulliyyah of Pharmacy, International Islamic University Malaysia (IIUM).

SUBMISSION OF A MANUSCRIPT

A manuscript should be submitted online to the IIUM-Journal of Pharmacy website at https://journals.iium.edu.my/ktn/index.php/jp/about/submissions. Further correspondence on the status of the paper could be done through the journal website.

IIUM JOURNAL OF PHARMACY

Volume 5, Issue 1, January 2025

Table of Content

*Corresponding author

Journal of Pharmacy

The challenges of using *Escherichia coli* as a host in recombinant insulin production

Muhammad Taher

Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.

Editorial

Article history:

Received: 14 January 2025
Accepted: 28 January 2025
Published: 31 January 2025

doi: 10.31436/jop.v5i1.380

Diabetes mellitus is a metabolic disease characterised by elevation of blood glucose level which leads to serious damage to the blood vessels, eyes, heart, kidney, and nerves affecting about 830 million people worldwide. The most common diabetes is type 2 which usually happened in adults when the body becomes resistant to insulin, or the body does not produce enough insulin. Type 1 diabetes mellitus is dependent on insulin which required accessible and affordable insulin (Diabetes, 2025).

In Malaysia, the prevalence of diabetes mellitus (DM), depends on factors such as gender, age, and ethnicity, with women, the elderly, and the Indian community having the highest prevalence of DM. In the 103,063 participants that made up the study's sample, the combined prevalence of diabetes by gender in the population-based studies was 13.80% for men and 14.54% for women, while the combined prevalence of prediabetes was 11.40% for women and 10.98% for men (Akhtar et al., 2022). For age, from this study, it can be observed that the prevalence of diabetes showed a notable upward trend as people aged, rising from 3.16% in the 20–29 age group to 13.71% in the 30-45 age group, 25.66% in the 46–59 age group, and 33.45% in the 60 and older age group (Akhtar et al., 2022). Ethnicity and races can also affect the prevalence of DM. The subpopulation of Indian had the greatest prevalence of diabetes which is 25.10%, among all ethnic groups, followed by Malay with 15.25%, Chinese with 12.87%, Bumiputera with 8.62%, and others with 6.91%. The prevalence demands oral hypoglycaemic agents (OHAs) market size in Malaysia at USD282.22 million in 2025 with a CAGR of greater than 3% during forecast period (2025-2030). The drugs are mainly fall under the following segment: biguanides, alpha-glucosidase inhibitors, dopamine-d2 receptor agonists, sodium-glucose cotransport-2 (SGLT-2) inhibitor, dipeptidyl peptidase-4 (DPP-4) inhibitors, sulfonylureas, and meglitinides (*Malaysia Oral Anti-Diabetic Drug Market Size | Mordor Intelligence*, 2025).

*Corresponding author's email: mtaher@iium.edu.my

Beside of OHAs, insulin is a very important agent in diabetic management. Insulin as a biopharmaceuticals has become a high demand biotherapeutic which is valued USD 310 billion and projected to reach USD 550 billion by 2029, growing at a compound annual growth rate (CAGR) of 10% (Research and Markets 4 min read, 2025). The therapeutic insulin was first derived from animal sources, however, due to a shortage of time to produce enough insulin and immunogenicity, scientists developed a new technique, which is using recombinant deoxyribonucleic acid (rDNA) technology. The steps involved in the synthesis of human insulin using rDNA include the identification and insertion of the insulin gene into suitable vectors followed by transfection into *Eschericia coli (E. coli)* as the expression system.

The discovery of the DNA cloning technique by Stanley Cohen and Herbert Boyer marked the beginning of genetic engineering, which led to the development of recombinant proteins with therapeutic applications from DNA recombinant technology. Several downstream processes are also involved until the final product of active human insulin is produced. Additionally, the advancement of molecular biology allows for the modification of human insulin to become more pharmacologically favourable, thus resulting in insulin analogues such as insulin isophane (intermediate-acting), short-acting insulin, ultra-rapid-acting insulin, long-acting insulin, inhaled insulin, and U-500 insulin.

Throughout the years, insulin production has gone through a massive evolution using *E. coli* as a host. In both research and the biotechnological field, *E. coli* remains a widely used host for the production of recombinant proteins. Despite the advantages offered by using *E. coli*, such as high yield, low cost, ease of management, minimal media requirement, and fast growth rate, researchers faced a lot of obstacles to improve the production of insulin using the *E. coli* expression system (Baeshen et al., 2014) compared to other expression systems, like mammalians cells and yeasts.

According to Kim et al., this system exhibited a number of drawbacks, including mispairing disulfide bonds, which leads to the production of inactive proteins, incorrect folding, and protein expression in inclusion bodies. These, in turn, will result in low production yields. In this case, *E. coli* host strains have been genetically modified to regulate the cellular redox environment in order to prevent the mispairing of disulfide bonds (Kim et al., 2021).

For effective secretion, drug efficacy, reducing immunogenicity and increased stability, the majority of proteins employed in drug therapy need to undergo post-translational modifications such as glycosylation, oxidation of methionine, misfolding and aggregation, proteolysis, and glutamine deamidation of asparagine and glutamine (Jenkins, 2007). However, *E. coli* expression system lacks post-translational modifications (e.g., is incapable of forming disulfide bonds). Arya et al. stated that *E. coli* is unable to perform N- or O-linked glycosylation as it lacks the machinery that involves hundreds of genes and proteins located in the Golgi apparatus and endoplasmic reticulum. Furthermore, it also has no capacity to perform other modifications like phosphorylation, acetylation, and disulfide bridges for proper

protein folding (Arya et al., 2008). Glycosylation is crucial in the pharmaceutical industry because it is frequently required for proper protein glycosylation, and it is desirable to pursue cost-effective bioproduction by using the simplest purification techniques (Ferrer-Miralles & Villaverde, 2013). Moreover, glycosylation will also increases the bioactivity of the foreign protein and its stability, with the closer glycosylation pattern to the native one resulting in the production of a more active and stable protein (Waegeman & Soetaert, 2011). If it is not possible to use *E. coli* as the expression host, other less convenient expression systems need to be applied (Table 1). Simple eukaryotic cells like yeast have the ability to be glycosylated, but they frequently use different glycosidic residues than what is needed for that particular circumstance (Flaschel & Friehs, 1993). Waegeman and Soetaert have also mentioned that yeast strains are particularly ideal for the synthesis of more complex mammalian proteins because, unlike bacteria, they are capable of performing eukaryotic glycosylation patterns (Waegeman & Soetaert, 2011).

Table 1. The advantages and challenges of insulin production in different hosts*

Host	**Advantages**	**Challenges**
Escherichia coli	High yield, rapid growth, well-understood genetics	Inclusion body formation, complex refolding required
Saccharomyces cerevisiae	Post-translational modifications, well-established system	Requires optimization of fermentation conditions
Pichia pastoris	High cell density growth, efficient secretion, fewer impurities	Stability of expression cassettes, optimization needed
CHO cells	Extensive post-translational modifications, high yield	High production costs, longer development times
Transgenic plants	Cost-effective, scalable, long-term stability	Regulatory hurdles, extensive purification required

*The table was generated by Scopus AI (https://www.scopus.com/pages/ai?query=insulin%20production&isExample=false)

Another issue that can be highlighted resulting from the lack of posttranslational modification in *E. coli* is proteolytic maturation. Based on Kamionka, preproinsulin required proteolytic modification to cleave the peptide in the endoplasmic reticulum, leaving the B-chain attached to the A-chain by two disulfide bridges to become fully active (Kamionka, 2011). This issue presented a significant challenge for recombinant human insulin expression, which led to various engineering strategies being implemented. Using a single expression construct, complete proinsulin is produced. For instance, Eli Lilly and Aventis designed an innovative technique that involves generating a single chemically synthesised cDNA encoding for human proinsulin in *E. coli*, purifying, and then removing the resultant C-peptide using proteolytic digestion (Baeshen et al., 2014). It has been supported by Kamionka, who discovered that following the purification of

proinsulin, the development of a disulfide bridge, the elimination of proteolytic C-peptide, and N-terminal methionine cleavage results in the formation of a physiologically active recombinant human insulin. Since this method was thought to be more practical and effective for producing insulin on a wide scale, humulin, the first recombinant insulin was invented to treat diabetes mellitus (Kamionka, 2011).

Furthermore, the strain's failure to secrete the recombinant protein into the culture medium presents another challenge when employing *E. coli* as an expression host (Kleiner-Grote et al., 2018). Inclusion bodies (IBs), which are related to insoluble misfolded protein aggregates, may result from the accumulation of protein in *E. coli* (Baeshen et al., 2014). Furthermore, according to Bathwa et al. (2021), the target protein starts to misfold and finally aggregates into IBs when the rate of recombinant protein expression exceeds the host cell's capacity to control post-translational modifications and protein folding (Bhatwa et al., 2021). To overcome this problem, a few approaches have been tested, as suggested by Baeshen et al. (Baeshen et al., 2014) to use molecular chaperones to help in proper protein folding and increase protein solubility. In this cooperative context, some chaperones inhibit protein aggregation, while others aid in the refolding and solubilisation of misfolded proteins. Not only that, chaperones such as GroEL, GroES, DnaK, DnaJ, and Grp can also reduce aggregation by facilitating the breakdown of proteins that are unable to fold correctly (Carrió & Villaverde, 2003).

In conclusion, the emergence of recombinant DNA technology has become one of the greatest turning points in the development of insulin production. Multiple hosts have been used to express insulin with their significant characteristic, both advantages and drawbacks. However, the drawbacks of using *E.coli* have opened many opportunities to create innovation in molecular biology to improve the product.

References

Akhtar, S., Nasir, J. A., Ali, A., Asghar, M., Majeed, R., & Sarwar, A. (2022). Prevalence of type-2 diabetes and prediabetes in Malaysia: A systematic review and meta-analysis. *PLOS ONE*, *17*(1),e0263139.https://doi.org/10.1371/journal.pone.0263139

Arya, R., Bhattacharya, A., & Saini, K. S. (2008). *Dictyostelium discoideum*—A promising expression system for the production of eukaryotic proteins. *The FASEB Journal*, *22*(12), 4055–4066. https://doi.org/10.1096/fj.08-110544

Baeshen, N. A., Baeshen, M. N., Sheikh, A., Bora, R. S., Ahmed, M. M. M., Ramadan, H. A. I., Saini, K. S., & Redwan, E. M. (2014). Cell factories for insulin production. *Microbial Cell Factories*, *13*(1),141.https://doi.org/10.1186/s12934-014-0141-0

Bhatwa, A., Wang, W., Hassan, Y. I., Abraham, N., Li, X.-Z., & Zhou, T. (2021). Challenges Associated With the Formation of Recombinant Protein Inclusion Bodies in Escherichia coli and Strategies to Address Them for Industrial Applications. *Frontiers in Bioengineering and Biotechnology*, *9*, 630551.https://doi.org/10.3389/fbioe.2021.630551

Carrió, M. M., & Villaverde, A. (2003). Role of molecular chaperones in inclusion body formation. *FEBS Letters*, *537*(1–3), 215–221. https://doi.org/10.1016/S0014-5793(03)00126-1

Diabetes. (2025). https://www.who.int/health-topics/diabetes

Ferrer-Miralles, N., & Villaverde, A. (2013). Bacterial cell factories for recombinant protein production; expanding the catalogue. *Microbial Cell Factories*, *12*(1), 113. https://doi.org/10.1186/1475-2859-12-113

Flaschel, E., & Friehs, K. (1993). Improvement of downstream processing of recombinant proteins by means of genetic engineering methods. *Biotechnology Advances*, *11*(1), 31–77. https://doi.org/10.1016/0734-9750(93)90409-G

Jenkins, N. (2007). Modifications of therapeutic proteins: Challenges and prospects. *Cytotechnology*, *53*(1–3), 121–125. https://doi.org/10.1007/s10616-007-9075-2

Kamionka, M. (2011). Engineering of therapeutic proteins production in Escherichia coli. *Current Pharmaceutical Biotechnology*, *12*(2), 268–274. https://doi.org/10.2174/138920111794295693

Kim, Y. S., Lee, H.-J., Han, M., Yoon, N., Kim, Y., & Ahn, J. (2021). Effective production of human growth factors in Escherichia coli by fusing with small protein 6HFh8. *Microbial Cell Factories*, *20*(1), 9. https://doi.org/10.1186/s12934-020-01502-1

Kleiner-Grote, G. R. M., Risse, J. M., & Friehs, K. (2018). Secretion of recombinant proteins from *E. coli*. *Engineering in Life Sciences*, *18*(8), 532–550. https://doi.org/10.1002/elsc.201700200

Malaysia Oral Anti-Diabetic Drug Market Size | Mordor Intelligence. (2025). https://www.mordorintelligence.com/industry-reports/malaysia-oral-anti-diabetic-drug-market

Research and Markets 4 min read. (2025, January 13). *Biotherapeutics Market Insights 2025, Analysis and Forecast to 2030: Insulin Demand Soars Amid Diabetes Epidemic, Sees $40 Billion Opportunity*. Yahoo Finance. https://finance.yahoo.com/news/biotherapeutics-market-insights-2025-analysis-114000447.html

Waegeman, H., & Soetaert, W. (2011). Increasing recombinant protein production in Escherichia coli through metabolic and genetic engineering. *Journal of Industrial Microbiology & Biotechnology*, *38*(12), 1891–1910. https://doi.org/10.1007/s10295-011-1034-4

Journal of Pharmacy

Students Perceptions, Satisfactions and Challenges on Problem-Based Learning (PBL): A Survey Study Among Final Year Pharmacy Students

Engku Nuraishah Huda E. Zainudin[1*], Fakhira Damia Khaidir[2], May Khin Soe[1], Nurul Asyiqin Yusof[1], and Khairool Azizul Mohammad[3]

[1]*Department of Basic Medical Sciences, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[2]*Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[3]*Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuh Persiaran Tun Khalil, 26300 Kuantan, Pahang Malaysia.*

Abstract

Introduction: Problem-based learning (PBL) is being adopted more frequently in pharmacy education to enhance skills such as problem-solving, and self-directed learning. However, thorough research on pharmacy students' perceptions and satisfaction towards PBL in Malaysia remains inconclusive. This study aims to explore students' views, satisfaction, and obstacles regarding PBL in pharmacy education especially to improve critical thinking, interpersonal skills, and problem-solving skills. **Method**: A cross-sectional study using a validated closed and open-ended questionnaire consisting of 22 statements was distributed among 84 final-year pharmacy students. **Results**: Eighty-four final-year pharmacy students participated in this study. Findings revealed that the majority agreed with statements on benefits that PBL significantly contributed to their knowledge acquisition (100.0%), stimulated the exploration of basic scientific concepts (98.8%), and enhanced their understanding in selecting both pharmacological and non-pharmacological approaches in disease management (97.6%, 95.2% respectively). The consensus extended to the belief that PBL fostered interactions with peers (91.7%), improved problem-solving skills (95.2%) and enhanced interpersonal skills (95.2%). Students expressed confidence in their instructors' clarity (92.9%) and clear answers to questions (90.5%). Most felt comfortable participating in discussions (69%), and nearly all students were satisfied with their group members' cooperation (95.2%). Although, most findings highlight positive aspects of PBL, this study also identified three major challenges in PBL implementation, namely 1) insufficient time for PBL preparation, 2) lack of teamwork, and 3) difficulty in finding adequate reading materials. **Conclusion**: Overall, these findings highlight positive aspects of the PBL experience among respondents. Future research should consider diverse student populations, conduct longitudinal studies, and explore instructors' perspectives.

Article history:

Received: 16 May 2024
Accepted: 30 September 2024
Published: 31 January 2025

Keywords:

problem-based learning
pharmacy
education
students' perception
satisfaction
challenges

doi: 10.31436/jop.v5i1.169

*Corresponding author's email: engkunuraishah@iium.edu.my

Introduction

Over the years, employers and professional organisations have urged higher education institutions to produce pharmacy graduates with appropriate competence and knowledge in response to the continuous emergence of newly developed drugs and the ability to keep up with treatment guidelines that are constantly updated. Hence, there is an increased number of educational institutions implementing or planning to implement problem-based learning, including a great number of health science programmes utilising problem-based and/or case-based learning methodologies (Sistermans, 2020). This pedagogical approach has gained popularity in health science programmes particularly due to its emphasis on active learning and the development of critical thinking skills. It encourages students to apply theoretical knowledge to practical scenarios, fostering a deeper understanding of the subject matter. This learning method has been progressively and widely employed in pharmacy schools after the Accreditation Council for Pharmacy Education (ACPE) announced the curricular requirements for pharmacy schools in the year as early as 2000. According to ACPE Standards 2025 (2024), educational outcomes that will be observed include students' foundational knowledge of medications and pharmacy practice, problem-solving abilities, and communication skills. These standards signify the importance of implementing PBL in pharmacy education. Throughout this article, the term PBL will refer to problem-based learning.

According to Yew & Goh (2016), PBL lessons feature small groups of four to eight students collaborating to solve real-world situations called 'clinical cases, and students will do self-study research on their own with little or no supervision from teachers. This approach fundamentally opposes the conventional way of delivering lectures and learning solely from the slides provided. PBL also promotes teamwork and communication skills as students work together to analyse and solve complex clinical cases. Previous studies analysed several articles on the process of PBL and found that there are promising results when PBL is applied in education (Yew & Goh, 2016; Ibrahim et al., 2018). Therefore, by using this method, students are engaged in the learning process and are able to gain a deeper comprehension of the material as well as critical thinking and problem-solving. Furthermore, positive outcomes were shown in a case study of collaboration between American pharmacy students and three pharmacy schools in Italy which have not yet implement PBL during the research time (Montepara et al., 2021). Although the number of studies approving the PBL method gradually increased, a systematic review of pharmacy education in China found that the PBL approach had not been integrated into the Chinese education system as of 2016. Nevertheless, survey results indicated that most students at these institutions expressed greater enthusiasm for PBL methods when compared to traditional teaching methods (Zhou et al., 2016)

While the existing research supports the benefits of PBL, limited studies have explored students' perceptions, satisfactions, and challenges specifically among pharmacy students, particularly at the advanced stage of their education. This gap in existing studies calls for further investigation to determine the impact of PBL on final-year pharmacy students' learning outcomes and professional development. Thus, this preliminary survey study aims to assess students' perceptions, satisfaction, and challenges related to Problem-Based Learning (PBL) in pharmacy education among final-year pharmacy students.

Methods

Study Design

This cross-sectional survey study employed an online survey to collect data on students' perceptions and experiences with problem-based learning. Self-administered closed and open-ended questionnaires were distributed, targeting final-year students from the Kulliyyah/School of Pharmacy (KOP) at the International Islamic University Malaysia (IIUM).

Study Subjects

Final-year pharmacy students from Kulliyyah of Pharmacy, IIUM, were chosen as they have the most exposure to problem-based learning throughout their pharmacy education. Participation was voluntary and anonymous, with informed consent obtained from all participants before data collection. Yamane's formula is used to calculate minimum sample size as seen in Eq. (1). The total population of final-year pharmacy students is 107, calculated with a 5% margin of error, determined a minimum recommended sample size of 84. Accordingly, 84 final-year pharmacy students were selected as study subjects.

$$n = \frac{N}{1 + Ne^2} \quad (1)$$

n = Sample size

N = Population size

Survey Instrument

The survey questionnaire, developed based on research objectives and relevant literature, included both closed-ended and open-ended questions. Covering demographics, experiences with problem-based learning, and perceptions of its advantages, the questionnaire addressed knowledge acquisition, critical thinking, and clinical competence, along with challenges in implementation. Feedback from the supervisor, co-supervisor, and two expert lecturers in survey research informed the final version.

The questionnaire, designed and published in English, comprised 24 questions divided into four sections:

Section A: Captured socio-demographic background (name, gender and email) of respondents.

Section B: Consisted of 18 items assessing students' perception on of problem-based learning, utilizing a 4-point Likert scale (1 = Strongly Disagree, 2 = Disagree, 3 = Agree, 4 = Strongly Agree).

Section C: Included 4 items assessing students' satisfaction in implementing problem-based learning, covering aspects such as facilitator, time, and teamwork, using a 4-point Likert scale.

Section D: Featured two open-ended questions exploring students' perspectives on challenges in implementing problem-based learning and their suggestions for improvement in the overall experience.

Ethical Considerations

Ethical approval for the survey study is obtained from the IREC committee. Confidentiality of participants' responses will be ensured, and all data will be anonymized to protect their identities.

Statistical Analysis

Statistical Package for Social Sciences programme, version 26.0 (IBM SPSS Statistics for Windows, v.26; IBM Corp, USA) was used to analyse the data. As this is a study of one sample group and the hypothesized population mean is not predetermined, only descriptive statistics were utilised to summarise and describe the main features of the dataset. Responses of strongly disagree and disagree were under disagreement (SD/D), and responses of strongly agree and agree were under agreement (SA/A) as seen in Tables 2 and 3. The mean scores ± standard deviations for each item were also tabulated. All p values ≤ 0.05 were considered statistically significant.

Thematic Analysis

An inductive approach was used for the open-ended questions, meaning that the themes are developed from the responses. In general, this method consists of six steps (1) familiarization with data (2) generation of initial codes, (3) searching for themes (4) reviewing themes, (5) defining themes, and (6) final analysis (Braun & Clarke, 2006). The analysis begins with a search for patterns and data that can be identified with each other and combined to form themes. The purpose of this analysis is to explore issues experienced by the students during PBL and how to overcome the challenges.

Results

Instrument reliability and validity

The content validity of the final questionnaire version was assessed by two lecturers who specialise in survey research. Each lecturer was asked to judge the degree of relevancy using a rating scale objectively and constructively (1 = the item is not relevant to the measured domain, 2 = the item is somewhat relevant to the measured domain, 3 = the item is quite relevant to the measured domain, 4 = the item is highly relevant to the measured domain). The Content Validity Index (CVI) was calculated, and it was found that the S-CVI/Ave for relevancy is 0.88. The results of CVI from two lecturers were greater than 0.80, which is the minimum accepted CVI value for two experts (Yusoff, 2019). Based on the above calculation, it is concluded that I-CVI, S-CVI/Ave meet a satisfactory level, and thus the scale of the questionnaire has achieved a satisfactory level of content validity.

The internal consistency (Cronbach's Alpha) for Section B and Section C is 0.865 and 0.763, respectively. Both values scored above 0.7 and thus, considered reliable (Taber, 2017).

Table 1: Demographic data of participants

Characteristics		n (%)
Gender		
	Male	23 (27.4%)
	Female	61 (72.6%)

A substantial portion of Year 4 students (n=84, 78.5%) actively participated and completed the survey where more than half of the students were female (n=61, 72.6%). Overall, respondents displayed predominantly positive views regarding the integration of PBL into the pharmacy curriculum. The mean scores of students' responses indicated favourable opinions on the effectiveness of PBL (3.36 ± 0.32) and their satisfaction with it (3.24 ± 0.49).

Table 2: Student's Perception on PBL (n= 84)

Survey items	SA/A[1] n (%)	SD/D[2] n (%)	Mean ± SD	p-val
PBL can encourage me to study basic scientific concepts.	83 (98.8)	1(1.2)	3.68 ± 0.495	0.0
PBL can help me gain knowledge	84 (100)	0	3.83 ± 0.375	0.0
PBL can increase my understanding in choosing a pharmacological approach in disease management.	82 (97.6)	2 (2.4)	3.73 ± 0.499	0.0
PBL can increase my understanding in choosing a non-pharmacological approach in disease management.	80 (95.2)	4 (4.8)	3.58 ± 0.585	0.19
I feel satisfied learning by PBL approach.	78 (92.9)	6 (7.1)	3.42 ± 0.625	0.22
I feel more confident expressing opinions.	71 (84.5)	13 (15.5)	3.10 ± 0.670	0.00
I become more interested to learn the topic.	83 (98.8)	1 (1.2)	3.50 ± 0.526	1.00
PBL can help me build interactions with other people.	77 (91.7)	7 (8.3)	3.50± 0.649	1.00
PBL encourage me to be cooperative in groups.	81 (96.4)	3 (3.6)	3.61± 0.602	0.10
PBL develops student-facilitator interaction.	78 (92.9)	6 (7.1)	3.40± 0.661	0.19
I prefer learning by traditional lecture method only.	13 (15.5)	71 (84.5)	1.94 ± 0.812	0.00
I do not like doing assignments in groups.	23 (27.4)	61 (72.6)	2.04 ± 0.898	0.00
PBL increase my knowledge on how to find problems related to drug therapy.	83 (98.8)	1 (1.2)	3.60 ± 0.518	0.09
PBL can increase my performance in clinical reasoning.	82 (97.6)	2 (2.4)	3.54 ± 0.548	0.55
PBL increases my knowledge on how to use clinical practice guidelines for evidence-based recommendations.	84 (100)	0	3.73 ± 0.449	0.00
PBL improve my problem-solving skills.	80 (95.2)	4 (4.8)	3.45 ± 0.629	0.49
PBL can develop my interpersonal skills.	80 (95.2)	4 (4.8)	3.44 ± 0.588	0.35
PBL stimulates individual research related to the topic of the case.	80 (95.2)	4 (4.8)	3.44± 0.665	0.41
Overall mean ± SD =			**3.36 ± 0.32**	**0.0**

[1]*Strongly agree, Agree*
[2]*Strongly disagree, Disagree*

Table 2 presents the mean scores and standard deviations illustrating students' perceptions of PBL for each item. Notably, all participating students (n=84, 100.0%) believed that PBL significantly contributed to their knowledge acquisition ($p<0.001$). They expressed consensus that PBL effectively stimulated the exploration of basic scientific concepts (n=83, 98.8%). Additionally, a substantial majority agreed that PBL enhanced their understanding in selecting both pharmacological and non-pharmacological approaches in disease management (n=82, 97.6% and n=80, 95.2% respectively). Furthermore, a significant percentage of students reported increased interest in learning the subject matter (n=83, 98.8%) and acknowledged that PBL facilitated individual research related to the case topic (n=80, 95.2%). The consensus extended to the belief that PBL improved problem-solving

skills (n=80, 95.2%) which indicates a strong majority of participants recognized the effectiveness of PBL in enhancing their ability to tackle problems efficiently. A noteworthy majority (n=77, 91.7%) agreed that PBL fostered interactions with peers and enhanced interpersonal skills (n=80, 95.2%).

However, a subset of respondents expressed reservations, with 27.4% (n=23) disliking group assignments, while a minority (n=13, 15.5%) indicated a preference for traditional lecture methods over PBL.

Table 3: Demographic data of participants

Survey items	SA/A[1] n (%)	SD/D[2] n (%)	Mean ± SD	p-value
The instructor discusses the task clearly.	78 (92.9)	6 (7.1)	3.29 ± 0.593	0.001
The instructor answers the questions presented clearly.	76 (90.5)	8 (9.5)	3.26 ± 0.623	0.001
I am comfortable in voicing out my opinions during discussion.	58 (69)	26 (31)	3.01 ± 0.736	0.000
My group members are helpful in completing the task.	80 (95.2)	4 (4.8)	3.40 ± 0.583	0.138
Overall mean ± SD =			**3.24± 0.49**	**0.000**

[1] *Strongly agree, Agree*
[2] *Strongly disagree, Disagree*

Table 3 provides the results obtained for students' mean scores and SD regarding satisfaction. It was shown that many of the students (n=78, 92.9%) believed that their instructor discussed the task clearly and answered the presented questions clearly (n=76, 90.5%). About two-thirds of them (n=58, 69%) are comfortable in voicing out their opinions during discussion. Almost all students were satisfied with their group members and agreed that they are helpful in completing the task (n=80, 95.2%).

Students' responses to the question were analysed and a total of three major themes were derived from the analysis as shown in Figure 1.

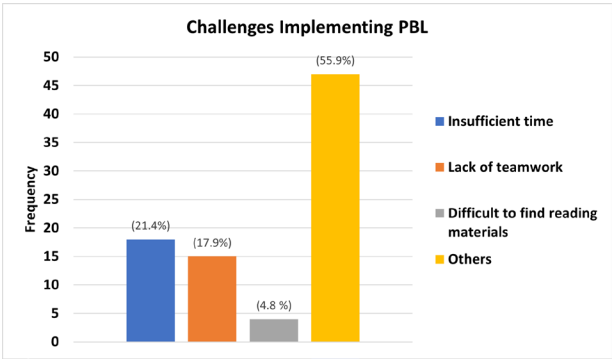


Fig. 1: Thematic analysis for challenges implementing PBL

Challenges in preparing and during PBL

Insufficient time for PBL preparation

The predominant challenge identified by respondents when reflecting on the hurdles associated with learning through the PBL method was insufficient time for preparation. In the open-ended section, participants cited various instances of time constraints such as *'Limited time to prepare'* and *'Sometimes, lack of time to prepare because schedules were packed with other assignments*. Additionally, some students highlighted the difficulty of coordinating schedules with group members, making it challenging to find suitable times for collaborative discussions. This underscores the multifaceted nature of time-related issues, including individual preparation and group coordination.

Lack of teamwork

Despite having a high percentage in satisfaction response, a recurrent theme that came up is the lack of teamwork. The usual practice among students is to initially divide the questions among members and each of them will read through the answers in their own time before the presentation. However, some of them expressed dissatisfaction as the members only focused on their part and did not fully understand the whole case study. A respondent also admitted doing that by responding, *'Actually, most of the time when doing PBL, I only understand my part that I was assigned to instead of other questions/parts. Maybe it is because I did not have enough time to understand other member's part. Besides, the group members are not responsive when I want to ask*

in WhatsApp group.'

This revelation suggests that, despite overall satisfaction, there exists room for improvement in fostering a more collaborative and comprehensive approach to PBL within student groups.

Difficulty in finding reading materials

Some participants encountered challenges in locating relevant references, struggling to find answers in available resources. One respondent expressed their uncertainty, stating, *'We were not sure the information we extracted from different sources is related to our questions or not, as we cannot do the discussion with our lecturer beforehand'*. Other respondents stated *'Sometimes, it is difficult to search for references'* and *'I did not find the answer in any resources'*.

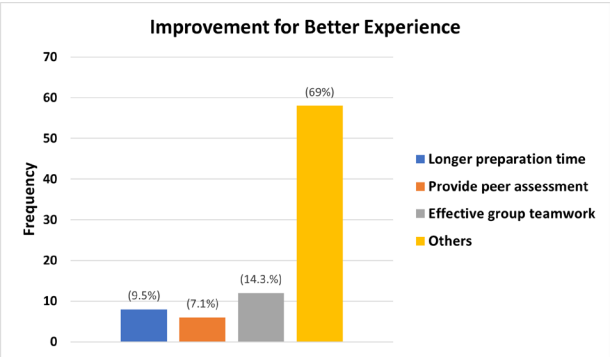


Fig. 2: Thematic analysis on improvement for better experience

Students' responses to the question were analysed and a total of three major themes were derived from the analysis as shown in Figure 2.

Student's opinions on PBL improvisation

Longer preparation time

As anticipated from the challenges highlighted earlier, a recurring theme emerged, emphasizing the need for extended preparation time. Responses uniformly underscored the necessity for more time to comprehend the case, engage in group discussions, and prepare materials for effective presentation.

Provide peer assessment

Among various responses, six students mentioned their desire for peer assessment, reflecting students' interest in offering constructive feedback to their peers regarding their group work performance. This theme suggests a collective eagerness among students to actively contribute to evaluating each other's contributions.

Effective group teamwork

Students shared that the PBL experience could significantly improve with cooperative, proactive, and supportive group members. Responses highlighted the importance of active participation and communication within the group, with suggestions such as *'Maybe more interaction between each other and speak out when having problems or any dissatisfaction'* and *'Everyone has to be prepared on the topic discussed and not covering their part only'*.

Discussion

In this study, the consensus among final-year pharmacy students indicates a belief in the effectiveness of PBL for enhancing knowledge acquisition and critical thinking skills. The statistical analysis results underscore the potential of PBL to enhance teaching and learning, particularly by fostering independent thinking, curiosity, and improving interpersonal skills with both group members and instructors. These findings align with previous studies conducted among medical and nursing students (Ibrahim et al., 2001; Putri & Sumartini, 2021), suggesting that PBL is a preferred method for health science education. Likewise, Trullàs et al. (2022) concluded in their study that PBL is superior compared to traditional methods in enhancing students' skills for social interaction, communication, problem-solving, and self-learning. It also does not negatively impact academic achievement, in fact, in many instances, it improves it.

Examining Table 1 data reveals a notable agreement among students, with 98.8% expressing that PBL encourages the study of basic scientific concepts. This aligns with a related study where 89.6% of students perceived PBL as stimulating the learning of basic sciences content (Ibrahim et al., 2018). Additionally, students reported a heightened

inclination toward self-research when assignments are presented with 'real-life cases' in the coursework (Elkalmi et al., 2020). Existing literature supports this, indicating that students are more motivated and interested in learning when PBL is integrated into the curriculum compared to traditional lectures alone (Ibrahim et al., 2018; Joseph et al., 2015). While PBL demonstrates a high potential to enhance the academic performance of pharmacy students, as noted by Galvao et al. (2014), it is important to acknowledge that this method may not necessarily translate into improved professional skills. The study suggests that educators should consider integrating other teaching methods and practical experiences to ensure the holistic development of well-rounded pharmacy professionals.

As depicted in Table 2, most students expressed favourable opinions about the performance of their instructors. These results align with previous studies, emphasizing the significance of instructors undergoing intensive training before program implementation to ensure the successful achievement of objectives (Ibrahim et al., 2018; Elkalmi et al., 2020; Putri & Sumartini, 2021). However, the requirement for additional training could be unfavourable to some lecturers. This has been seen in a study where satisfaction responses from instructors were not as positive as the students and this may be because of the additional workload and lack of administrative support (Trullàs et al., 2022). Instructors equipped with essential skills play a critical role in ensuring the successful implementation of PBL by effectively guiding and facilitating student learning. Their expertise and ability to create a supportive learning environment significantly enhance the overall effectiveness of PBL. These findings underscore the importance of ongoing professional development and training opportunities for instructors to continually enhance their instructional practices in PBL settings.

PBL sessions, like any other teaching style, may have a few disadvantages. As can be seen in Figure 1, students complained of insufficient time for preparation. An earlier study found that students found PBL to be time-consuming (Joseph et al., 2015). Another study also found that pharmacy students across all academic years consistently brought up the issue of time management during a program that implemented PBL as a learning method (Moseley et al., 2020). It is important to bear in mind that the time allocated to work on the cases in the study is only four days while for this current study, the cases are assigned to students at least a week before the presentation. Nevertheless, Ghani et al (2021) believe that students should know how to prioritise in finishing tasks according to their importance and urgency. In other words, time management is a skill that every student should have to achieve the best result in acquiring knowledge and completing tasks within the time given. This is supported by several studies that found time management behaviours were significantly positively related to the academic achievement of students (Nasrullah & Khan, 2015; Razali et al., 2018). As previously discussed, PBL involves students collaborating with group members to solve cases provided by the instructor. Teamwork is crucial for maximizing the potential of PBL, from task division to presentation day. With advancements in technology, students now have the flexibility to conduct discussions either online or face-to-face. To ensure effective collaboration and prevent "free riders," each group should document brainstorming sessions and record each member's contributions (Sistermans, 2020). Effective teamwork in PBL does not only enhances problem-solving skills but also fosters critical thinking and communication among group members. Teamwork is essential in PBL because it brings together diverse perspectives, promotes collaboration and communication, and utilizes the unique strengths of each member, which ultimately can enhance problem-solving effectiveness and leads to a deeper understanding of the subject matter.

This study also identified that student's ability to access relevant resources to assist in the PBL process was a barrier for some students. Nasr and Wilby (2017) discovered that when PBL is first introduced to a university in Qatar, one of the weaknesses in the PBL process is the availability of resources. Some researchers believed that students should have the skill to obtain and manage learning resources to expand their understanding of the content (Ghani et

al., 2021). Retrieving relevant and high-quality reading materials can be a barrier for some students. Educators should guide students on effective information search strategies and curate suitable materials when students encounter challenges in locating resources that align with their PBL tasks (Joseph et al., 2015).

In terms of recommendations for enhancing PBL, six students expressed interest in peer assessment. They emphasized the importance of constructive feedback among peers regarding group-work performance. This collective eagerness indicates a strong commitment to evaluating each other's contributions. This suggestion aligns with the insights of Ghani et al. (2021), who advocate for providing students with the opportunity to offer constructive feedback on their group members' performance during sessions. Peer assessment not only encourages active engagement with peers' work but also instills a sense of responsibility and accountability within the group. Implementing this approach allows instructors to establish a collaborative learning environment where students can learn from each other's strengths and weaknesses, ultimately enriching their overall educational experience. For future research, it will be beneficial to include instructors' perspectives on students' performance during implementation of PBL as the finding will assist in gaining more insight on the effectiveness of the learning approach. This recommendation had also been applied by previous studies and some interesting points can be found. Antonella (2023) conducted in-depth interviews with two lecturers regarding the benefits of PBL and found that they felt PBL technique promotes teamwork, integrates different domains of knowledge, increases motivation, and improve students' attitudes. Another study in Indonesian private university conducted by Ghufron and Ermawati (2018) revealed that the teachers believed PBL encourage self-directed learning, reducing nervousness, increasing self-confidence and motivation, increasing responsibility, facilitating idea sharing, increasing student engagement, promoting problem-solving, and cultivating a positive learning attitude. Although these studies are not related to pharmacy curriculum, the findings align with other studies that focused on the the impact of PBL on student learning outcomes.

Despite the valuable insights gained from this study, certain limitations should be acknowledged. Notably, the exclusive focus on final-year pharmacy students may limit the venerability of findings to students in other academic years. Additionally, the study centred on a single pharmacy school, restricting the broader applicability of results to all pharmacy students in Malaysia.

Conclusion

The study highlights positive student perspectives and satisfaction levels, highlighting the beneficial impact of problem-based learning (PBL) on their educational experience. Despite these positive findings, several challenges persist, including insufficient time for preparation, lack of teamwork, and difficulty in accessing relevant reading materials. To improve the PBL implementation, both students and instructors can overcome these challenges by refining case development, optimizing the process execution, and adopting effective assessment strategies. This iterative refinement process has the potential to consistently enhance the effectiveness of PBL, fostering continuous growth in students' knowledge acquisition and problem-solving skills within pharmacy education.

Authors contributions

The authors confirm their contribution to the paper as follows: manuscript preparation, study conception and design: E.N.H.E. Z, M. K. S.; data collection: F. D. K.; analysis and interpretation of results: E.N.H.E.Z, K.A.M, F.D.K., draft manuscript preparation: F. D. K. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval statement (if applicable)

The human study protocol was approved by the IIUM Research Ethics Committee (ID No: IREC

2023-179 and date of approval: 24 Oct 2023).

Informed consent statement (If applicable)

Informed consent was obtained from all subjects involved in the study.

Conflict of interest

The author has no conflicts of interest to report.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author have used Grammarly, Paperpal and Copilot to improve readability and language.

References (APA 7th style)

Accreditation Council For Pharmacy Education. (2024). Accreditation Standards And Key Elements For The Professional Program In Pharmacy Leading To The Doctor Of Pharmacy Degree. Standards 2025.

Elkalmi, R. M., Elnaem, M. H., Suhaimi, A. M., Elshami, A. M., Nahas, A. F., & Jamshed, S.Q. (2020). Perceptions and attitudes of pharmacy students towards introducing research project-based learning module in a Malaysian public university. Pharmacy Education, 20, p127-134.

Ghani, A. S. A., Rahim, A. F. A., Yusoff, M. S. B., & Hadie, S. N. H. (2021). Effective Learning Behavior in Problem-Based Learning: a Scoping Review. Medical Science Educator, 31(3), 1199–1211. https://doi.org/10.1007/s40670-021-01292-0

Ghufron, M. A., & Ermawati, S. (2018). The Strengths and Weaknesses of Cooperative Learning and Problem-based Learning in EFL Writing Class: Teachers and Students' Perspectives. In International Journal of Instruction (Vol. 11, Issue 4, pp. 657–672). http://repository.ikippgribojonegoro.ac.id/461/1/EJ1191701.pdf

González-Lillo, E. A. (2023). Tutors' and Students' Views on Learning and Feedback in Problem-Based Learning. European Journal of Educational Research, volume–12– 2023(volume–12–issue–2–april–2023), 705–717. https://doi.org/10.12973/eu-

Hussain, M., Sahudin, S., Abu Samah, N. H., & Anuar, N. K. (2019). Students perception of an industry based approach problem based learning (PBL) and their performance in drug delivery courses. Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society, 27(2), 274–282. https://doi.org/10.1016/j.jsps.2018.11.009

Ibrahim, M. E., Al-Shahrani, A. M., Abdalla, M. E., Abubaker, I. M., & Mohamed, M. E. (2018). The Effectiveness of Problem-based Learning in Acquisition of Knowledge, Soft Skills During Basic and Preclinical Sciences: Medical Students' Points of View. Acta informatica medica: AIM : journal of the Society for Medical Informatics of Bosnia & Herzegovina : casopis Drustva za medicinsku informatiku BiH, 26(2), 119–124. https://doi.org/10.5455/aim.2018.26.119-124

Montepara, C. A., Woods, A. G., & Wolfgang, K. W. (2021). Problem-based learning case studies: Delivery of an educational method and perceptions at two schools of pharmacy https://doi.org/10.1016/j.cptl.2021.01.026

Moseley, L. E., Ford, C. R., & Wilkins, E. B. (2020). Using Focus Groups to Explore Evolving Perceptions of Student Pharmacists' Curricular Experiences. American journal of pharmaceutical education, 84(1), 7122. https://doi.org/10.5688/ajpe7122

Nasr, Z. G., & Wilby, K. J. (2017). Introducing problem-based learning into a Canadian-accredited Middle Eastern educational setting. Currents in Pharmacy Teaching and Learning, 9(4), 719–722. https://doi.org/10.1016/j.cptl.2017.03.027

Putri, S. T., & Sumartini, S. (2021). Integrating Peer Learning Activities and Problem-Based Learning in Clinical Nursing Education. SAGE Open Nursing, 7, 237796082110002. https://doi.org/10.1177/23779608211000262

Razali, S. N. A. M., Rusiman, M. S., Gan, W. S., & Arbin, N. (2018). The Impact of Time Management on Students' Academic Achievement. Journal of Physics: Conference Series, 995, 012042. https://doi.org/10.1088/1742-6596/995/1/012042

Sistermans, I. J. (2020). Integrating competency-based education with a case-based or problem-based learning approach in online health sciences. Asia Pacific Education Review, 21(4), 683–696. https://doi.org/10.1007/s12564-020-09658-6

Trullàs, J.C., Blay, C., Sarri, E. et al. (2022). Effectiveness of problem-based learning methodology in undergraduate medical education: a scoping review. BMC Med Educ 22, 104 https://doi.org/10.1186/s12909-022-03154-8

Yew, E. H. J., & Goh, K. (2016). Problem-Based Learning: An Overview of its Process and Impact on Learning. Health Professions Education, 2(2), 75–79. https://doi.org/10.1016/j.hpe.2016.01.004

Yusoff, M. S. B. (2019). ABC of Content Validation and Content Validity Index Calculation. Education in Medicine Journal, 11(2), 49–54. https://doi.org/10.21315/eimj2019.11.2.6

Zhou, J., Zhou, S., Huang, C., Xu, R., Zhang, Z., Zeng, S., & Qian, G. (2016). Effectiveness of problem-based learning in Chinese pharmacy education: A meta-analysis Approaches to teaching and learning. BMC Medical Education, 16(1). https://doi.org/10.1186/s12909- 016-0546-z

Journal of Pharmacy

Optimising Palm Olein-Based Betamethasone 17-Valerate Emulsions for Scalable Manufacturing and Stability

Thazin Win[1], Mohd Rushdi Abu Bakar[1,2*], Farahidah Mohamed[1], Muhammad Taher Bakhtiar[1], and Md. Zaidul I. Sarker[3]

[1]*Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[2]*IKOP Pharma (IKOP Sdn. Bhd.), Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[3]*Northern Marianas College, Finasisu Lane, Saipan, 96950, Northern Mariana Islands, USA.*

Abstract

Introduction: Palm olein has been used as an excipient in the formulation of topical emulsions due to its rich source of natural antioxidants that can lead to better skin health and higher stability upon storage. Despite its potential as a topical drug delivery vehicle, the practical implementation of manufacturing 20% (w/w) palm olein-in-water emulsions for commercial purposes has not been explored extensively, and obtaining experimental data on scale-up studies would be helpful in facilitating this realisation. **Methods:** This research work established and optimised the manufacturing process parameters for the production of cream and lotion formulations containing betamethasone 17-valerate, utilising palm olein as the vehicle, with scale-up from lab-scale 5 kg batches to pilot-scale 80 kg batches. Design of experiments (DoE) where response surface methodology as well as three-level, two-factors (32) full factorial design were used to develop statistical models for representing the possible relationships between factors: homogenisation time and speed, and responses: particle size and phase separation. **Results:** The findings established that the quadratic model was the most suitable model as it could predict the interactions between factors and responses in an accurate manner as well as suggest the optimum operating conditions. The optimum homogenisation time and speed were found to be 40 minutes and 3400 rpm, respectively. These conditions produced emulsions with the smallest particle size (3.2 μm ± 0.03) and the least phase separation value (29.7% ± 0.35). **Conclusion:** The study successfully demonstrated the potential to scale up the manufacturing of 20% (w/w) palm olein-in-water emulsions for commercial purposes. The optimised parameters, obtained through DoE, facilitate the large-scale production of stable emulsions containing betamethasone 17-valerate.

Article history:

Received: 25 January 2024
Accepted: 25 January 2025
Published: 31 January 2025

Keywords:

Palm olein
Scale-up
Stable emulsions
Full factorial design
Betamethasone 17-valerate

doi: 10.31436/jop.v5i2.281

*Corresponding author's email: rushdi@iium.edu.my

Introduction

Palmisone® products are topical cream and lotion of betamethasone 17-valerate (BV17) which are developed for treating corticosteroid-responsive skin diseases such as eczema and psoriasis. Unlike conventional BV17 products which were prepared using mineral oils as drug delivery vehicle, Palmisone® products were prepared using palm olein, which is the liquid-fractionated part of palm oil extracted from the mesocarp of *Elaeis guineensis*. In order to be used as the drug delivery vehicle, palm olein was homogenised into an oil-in-water emulsion consisting of 20 % (w/w) palm olein-in-water emulsion stabilised with 25 % (w/w) of Span® 20 and Tween® 20 non-ionic surfactant mixture at an effective hydrophile-lipophile balance (HLB) value of 10. The use of palm olein as topical drug delivery emulsion is advantageous since it contains natural antioxidants that can lead to better skin health. It is also stable upon storage due to the presence of natural anti-oxidants (Ramli et al., 2017).

Previous research works have witnessed the superior stabilities and efficacies of 20 % (w/w) palm olein-in-water emulsion when compared to three commercial samples utilising conventional vehicle bases, such as white soft paraffin and liquid paraffin (Ahmad et al., 2018). The present study aims to establish and optimise the manufacturing process parameters for pilot-scale production of topical cream and lotion of BV17 with consistent quality using palm olein as drug delivery vehicle. Since the study comprehensively outlines the practical procedures employed for process optimisation, the research described here serves as a practical reference for optimising procedures in similar processes.

In this research, we used 67.36 kg of 20 % (w/w) palm olein-in-water emulsion to produce 80 kg of Palmisone® products. Response surface methodology (RSM) in conjunction with full factorial design (FFD) was employed to study the influences of two factors (homogenisation speed and time) on two critical responses - particle size and phase separation. These parameters are crucial as they directly impact the stability, bioavailability, and overall performance of the emulsion. The aim was to develop statistical model equations for estimating the optimum conditions in order to generate the smallest particle size and the least percentage of phase separation. RSM demonstrates the relationship between factors and responses by interpreting the effect of factors on responses or processes through mathematical models whereas FFD is used to investigate the importance and the ranges of factors. Despite its low resolution, FFD is a simple and sufficient method for screening a large numbers of experimental parameters (Jankovic et al., 2021, Ćurić et al., 2013, Singh et al., 2011).

Materials and methods

Materials

Refined, bleached and deodorised palm olein, propylene glycol, chlorocresol and BV17 powder were purchased from Plant Succeed Engineering Sdn. Bhd. Tween® 20 and triethanolamine were supplied by Merck Sdn. Bhd. Carbopol® 940 powder was provided by Eurochemo Pharma Sdn. Bhd. Span® 20 was purchased from Hefei TNJ Chemical Industry Co., Ltd. Honey melon fragrance oil was generously provided by IKOP Pharma. All the starting materials used were pharmaceutical grade.

Method

The process optimisation study was performed in the following steps, which were adapted from the methodology outlined by Singh, Ćurić and their co-workers (Singh et al., 2011, Ćurić et al., 2013). The emulsions were prepared using the homogenous mixing equipment (HME) (YC-HM-100, Yenchen, China) available in the Production Department at IKOP Pharma.

Step 1: Define critical formulation stages

Palmisone® products were formulated using four sequential stages: preparation of 1 % (w/w) Carbopol® 940 stock solution (Stage 1), preparation of BV17 slurry (Stage 2), preparation of 20 % (w/w) palm olein-in-water emulsion (Stage 3) and preparation of Palmisone® products (Stage 4).

According to the earlier formulation developmental studies conducted by Win (2015), the

preparation of 20% (w/w) palm olein-in-water emulsion (Stage 3) is the most critical part because it is the base vehicle in which active pharmaceutical ingredient and all other pre-mixed excipients are finally incorporated to obtain the final product. Moreover, the critical quality attributes of finished product are closely derived from the parent base emulsion. For instance, the particle size has a great impact on the diffusion of BV17 from the vehicle into the stratum corneum which in turn affects the therapeutic efficacy of Palmisone® products (Ahmad et al., 2018). Therefore, it is important to determine the best possible manufacturing process parameters in Stage 3 in order to produce 80 kg pilot batch of Palmisone® products with consistent quality and stability.

Step 2: Select critical manufacturing parameters

The purpose of this phase is to determine the appropriate design factors ((Singh et al., 2011, Ćurić et al., 2013). The particle size of the dispersed phase and the phase separation are amongst the most vital parameters in determining the stability of an emulsion (Faria-Silva et al., 2020). These two parameters were chosen as the responses in this study and the factors were further determined based on the manufacturing parameters which had the most influential effect on the chosen responses.

Win (2015) and Mohd Nawi (2018) had reported that homogenisation process parameters such as homogenisation temperature, speed, duration and depth of emulsifier shaft embedded inside the emulsion would greatly influence the properties of palm olein emulsion. Since the emulsifier shaft of the homogenous mixing equipment (HME) cannot be adjusted due to the built-in equipment design, the depth of emulsifier shaft was treated as constant in this research work. Similarly, the homogenisation temperature was maintained at 25 ± 5 ºC as recommended by Win (2015). Therefore, the adjustable parameters, such as homogenisation speed and duration were chosen as the independent variables or factors. The ranges of independent variables were selected based on the HME's homogenising capacities as suggested by the equipment's manufacturer and knowledge attained from trial experiments.

Step 3: Establish experimental model using RSM

The experimental model for the optimisation of homogenisation process was established using the Design-Expert® Software Version 7.0.0 (Stat-Ease Inc., Minneapolis, USA). RSM and three-level, two-factor (32) FFD were used to establish the suitable experimental model (Singh et al., 2011, Ćurić et al., 2013).

Step 4: Finalise experimental design and parameter specifications

The units, notations and targeted values of both independent and dependent variables shown in Table 1(a) were specified in the Design-Expert® software environment and the design matrix is shown in Table 1(b).

Table 1: (a) Variable inputs for the determination of experimental model, and (b) Design matrix for pilot-scale production (67.36 kg) of 20% (w/w) palm olein-in-water emulsion.

(a).

Independent variables	**Unit**	**Notation**	**Coded levels**		
			-1	**0**	**1**
Homogenisation speed	rpm	A	1400	2400	3400
Homogenisation time	min	B	30	40	50
Dependent variables			**Targeted values**		
			Min		**Max**
Particle size	µm	R 1	3.01		5
Phase separation	%	R 2	28.00		40

(b).

Run no.	**A (rpm)**	**B (min)**	**R 1 (µm)**	**R 2 after 24 h (%)**
1	3400	40	3.01	28
2	1400	40	12.54	55
3	3400	30	3.69	40
4	2400	40	3.72	30
5	2400	40	3.73	30
6	2400	40	3.76	30
7	3400	50	3.58	35
8	2400	50	3.91	40
9	1400	30	12.99	60
10	2400	40	3.71	30
11	2400	40	3.83	30
12	1400	50	13.04	59
13	2400	30	3.95	35

Step 5: Perform statistical analysis

The values for coefficient of determination (R^2), predicted and adjusted coefficient of determinations (predicted R^2 and adjusted R^2) were analysed to evaluate the fit quality of the selected model. The residual plots were also visually analysed to examine the goodness of the chosen model. Furthermore, analysis of variance (ANOVA) test was employed to determine the significance of the chosen model based on F-test. In this context, the model was considered significant if the probability associated with the F-test was greater than 0.05 at a 95% confidence interval (i.e. Prob > F). The generated quadratic equations were analysed for suitability in representing the relationship between the chosen factors and each response. For visual representation, the fitted equations for both responses were represented in the forms of contour and response surface plots (Yolmeh et al., 2017).

Step 6: Optimise homogenisation process

To prepare the emulsion, the temperature of HME was maintained at 60 ± 5 °C before commencing the homogenisation. The following starting materials, i.e. 16 kg of palm olein, 3.32 kg of Span® 20 and 0.68 kg of Tween® 20 were mixed with the HME. The mixture was heated to the set temperature and stirred for 15 minutes until the oil and surfactant were homogenous. Upon mixing, the HME was cooled down to 25 ± 5 ºC using a recirculating cooler (HL-35H, Lab Companion, Jeio Tech, Korea) before the addition of 47.36 kg purified water into the mixed oil phase. The stock emulsion was then homogenised.

The experiments were carried out with the parameters as shown in Table 1(b) and the samples were withdrawn at 10-minutes interval throughout the experimental periods (50 minutes) for measuring the particle size and the percentage of phase separation.

In order to measure phase separation, emulsion samples were stored inside 100-mL graduated cylinders to visually monitor the phase separation at room temperature. The mouths of the graduated cylinders were closed with caps to prevent the evaporation of water from the formulations throughout the study period. The percentage of phase volume to total volume of emulsion was determined as the percentage of phase separation of the formulation. In order to investigate the particle size of dispersed phase or palm olein, a calibrated laser particle size analyser (BT-9300H, Dandong Baite Instruments Ltd., China) was used and the result was displayed as D (v,50), i.e. the point at which 50 % of the sample volume possess the same particle size (Win, 2015, Mohd Nawi, 2018).

The measurements were triplicated to determine the average values for each response at specific time and the values were expressed as mean ± standard deviation. The observed average values were then compared against the predicted values generated by the Design-Expert® software.

Step 7: Validate the optimised parameters

When the optimum conditions were determined, three batches of 67.36 kg of 20 % (w/w) palm olein-in-water emulsion were prepared using the optimum homogenisation speed and time obtained from the studies conducted in Step 6. The mean response values were compared to that of the predicted values given by the Design-Expert® software in order to confirm the accuracy, validity and suitability of the optimised parameters (Yolmeh et al., 2017).

Results

The model summary statistics given by the Design-Expert® software shows information such as coefficient of determination (R^2), predicted coefficient of determination (predicted R^2) and adjusted coefficient of determination (adjusted R^2) for linear, interactive (denoted as 2F1) and quadratic models as presented in Table 2. The quadratic model was chosen by the software since this model exhibited the highest R^2.

Additionally, the adequacy of quadratic model was confirmed using ANOVA test. The quadratic equations for particle size (R 1) and phase separation (R 2) given by the ANOVA test were as follow:

$$R\,1 = 3.69 - 4.71*A - 0.017*B - 0.04*A*B + 4.20*A^2 + 0.36*B^2 \quad (1)$$

$$R\,2 = 30.07 - 11.83*A - 0.17*B - 1*A*B + 11.26*A^2 + 7.26*B^2 \quad (2)$$

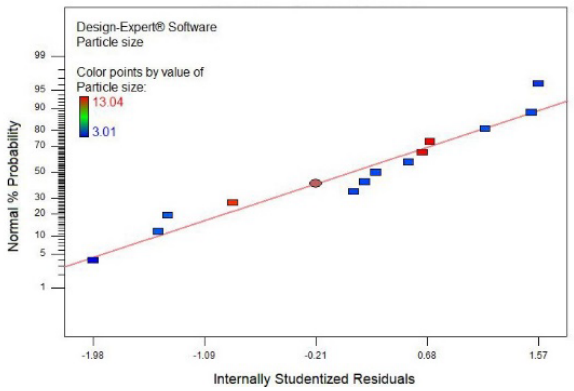
where A is homogenisation speed and B is homogenisation time. The above equations are mathematical descriptions on the true relationship between factors and responses which simulate the possible interactions between factors or combinations of factors on responses (Maran et al., 2013).

Following the ANOVA test, the Fisher's statistical test or F-test was performed to determine the significance of each response using the F-value. The outcomes are summarised in Table 3.

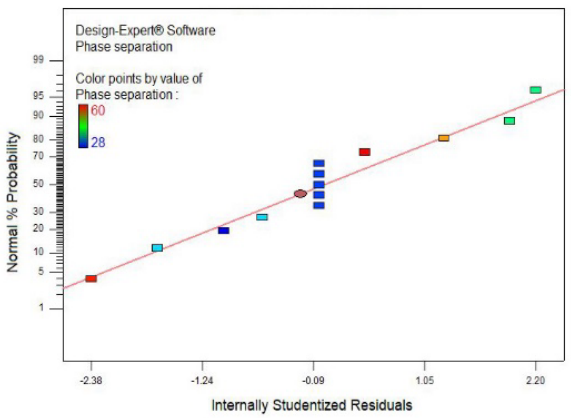
For model validation purpose, statistical parameter such as R^2 is widely used to indicate the suitability of a chosen model. In order to enhance the confidence level of the result, graphical tool was used as well whereby the normal plots of the residuals were constructed to confirm the suitability of quadratic model (Mohr et al., 2022).

Normal probability plots or normal plots are graphical interpretations of how well the data from fitted models are normally distributed. The data can be either raw data or residuals. Residuals from fitted models are the difference between experimental responses and theoretical responses whereas studentised residuals are residuals which are expressed in their standard deviations. Normal plot of residuals is obtained when the normal % probability (y-axis) is plotted against internally studentised residuals (x-axis). The normal plot of residuals for both responses are given in Fig. 1, whereas the predicted against actual plots of both responses are depicted in Fig. 2.

Fig. 3 and Fig. 4 show the visual depiction of the relationship between factors and responses using two-dimensional contour plots as well as three-dimensional response surface plots. Contour plots are simpler interpretations of response surface plots. The contour lines delineate the response which contours both factors at a time. On the other hand, the gridded response surface plots briefly describe the impact of factors on responses from the best-fitted model with a map of contour lines in three-dimensional design. These plots could lead to the identification of optimum operating parameters (factors) for producing the most desirable outcomes (responses) (Lamidi et al., 2023, Singh et al., 2011). In the context of Design-Expert® software, blue zone represents the zone for favourable responses while red zone represents the zone for undesired responses. Factors A and B were plotted at x- and y-axes, respectively for both contour and response surface plots. Unlike contour plots, individual response was plotted at z-axis in each response surface plot. Both contour plots (Fig. 3) and response surface plots (Fig. 4) showed similar trend in which both particle size (R 1) and phase separation (R 2) decreased with increasing homogenisation speed (A) and homogenisation time (B).



(a)



(b)

Fig. 1: Normal plots of residuals for (a) particle size (R 1) and (b) phase separation (R 2) showing normal distribution of studentised residuals and goodness of fit of quadratic model.

Table 2: Results for model summary statistics of both responses (R 1 and R 2) provided by Design-Expert® software.

Model	Particle size (R 1)			Phase separation (R 2)		
	R^2	Predicted R^2	Adjusted R^2	R^2	Predicted R^2	Adjusted R^2
Linear	0.6850	0.6220	0.3646	0.5077	0.4093	-0.0073
2F1	0.6851	0.5801	-0.3907	0.5101	0.3469	-1.4018
Quadratic	0.9994	0.9990	0.9962	0.9820	0.9691	0.8168

Table 3: Analysis of variance results for (a) particle size (R 1), and (b) phase separation (R 2).

(a).

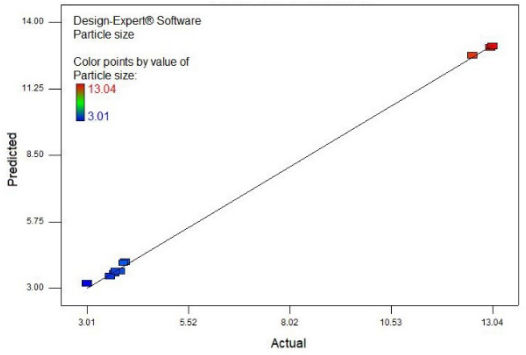
Source	Sum of squares	df	*F*-value	*p*-value (Prob > F)	SE
Model	194.61	5	2446.90	< 0.0001	0.0524
A	133.39	1	8385.59	< 0.0001	-
B	0.00	1	0.10	0.7556	-
A*B	0.01	1	0.40	0.5460	-
A^2	48.80	1	3068.14	< 0.0001	-
B^2	0.36	1	22.33	0.0021	-

A = homogenisation speed, B = homogenisation time, df = degree of freedom, SE = standard error

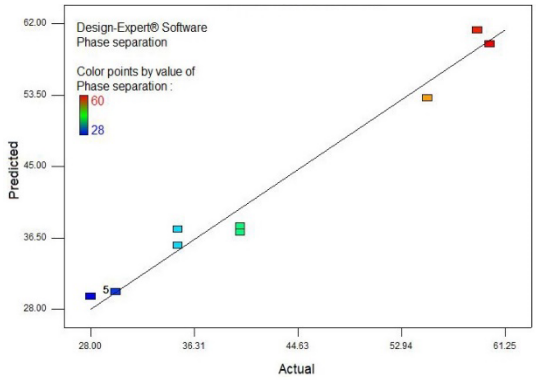
(b).

Source	Sum of squares	df	*F*-value	*p*-value (Prob > F)	SE
Model	1625.24	5	76.25	< 0.0001	0.8573
A	840.17	1	197.10	< 0.0001	-
B	0.17	1	0.04	0.8489	-
A*B	4.00	1	0.94	0.3650	-
A^2	350.09	1	82.13	< 0.0001	-
B^2	145.52	1	34.14	0.0006	-

A = homogenisation speed, B = homogenisation time, df = degree of freedom, SE = standard error

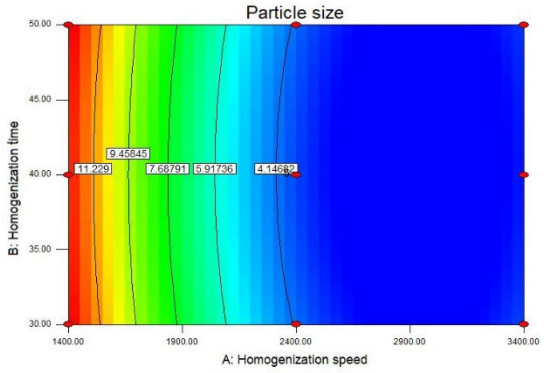


(a)

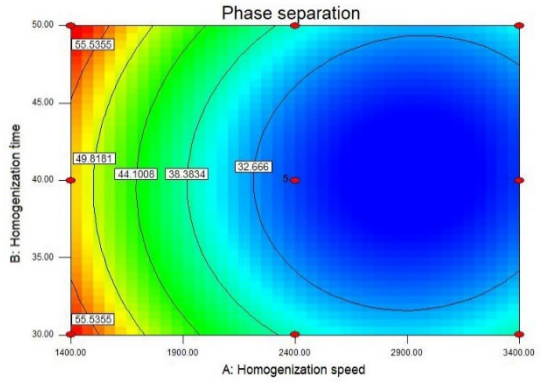


(b)

Fig. 2: Plots of predicted against actual for (a) particle size (R 1) and (b) phase separation (R 2).

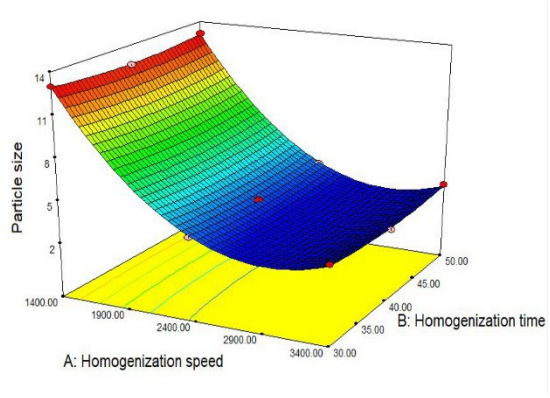


(a)

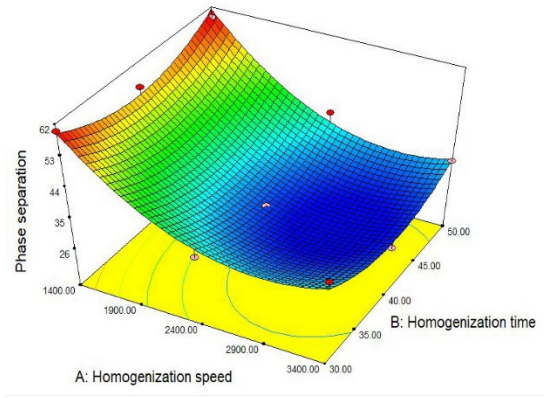


(b)

Fig. 3: Two-dimensional contour plots for (a) particle size (R 1) and (b) phase separation (R 2) against homogenisation speed (A) and homogenisation time (B).



(a)



(b)

Fig. 4: Three-dimensional response surface plots for (a) particle size (R 1) and (b) phase separation (R 2) versus homogenisation speed (A) and homogenisation time (B).

Selection of optimum operating parameters

The Design-Expert® software has provided seven check point batches. The actual responses together with standard deviation values and percentage errors for all check point batches were recorded and summarised in Table 4. Furthermore, the desirability ramp of the optimised process variables for both responses, which focuses on selecting values that maximise the desirability score, is shown in Fig. 5.

Fig. 5: Desirability ramp of optimised process variables for particle size and phase separation.

Table 4: Results for predicted responses, actual responses and percentage errors (values are mean ± SD).

No	Factors		Predicted responses		Actual responses		Error (%)		D
	A (rpm)	B (min)	R 1 (μm)	R 2 (%)	R 1 (μm)	R 2 (%)	R 1	R 2	
1	3400	31	3.54	36.68	3.60 ± 0.10	38.34 ± 0.54	1.65	4.53	1.0000
2	3400	40	3.19	29.45	3.20 ± 0.31	29.66 ± 0.60	0.46	0.71	1.0000
3	3400	44	3.23	30.27	3.25 ± 0.69	30.58 ± 0.19	0.73	1.01	1.0000
4	3400	33	3.41	33.92	3.44 ± 0.43	35.57 ± 1.98	1.02	4.87	1.0000
5	2719	30	3.01	35.18	3.57 ± 0.63	36.13 ± 0.89	18.60	2.69	0.6597
6	2692	50	3.01	34.37	3.59 ± 0.41	38.79 ± 2.52	19.27	12.86	0.6462
7	2664	32	3.01	32.97	3.63 ± 0.55	40.21 ± 2.00	20.60	21.96	0.6322

A= Homogenisation speed, B= Homogenisation time, R 1= Particle size, R 2= Phase separation, D= Desirability

Table 5: Results for the confirmation studies of 67.36 kg of 20 % (w/w) palm olein-in-water emulsions homogenised at 3400 rpm (optimum homogenisation speed) for 40 min (optimum homogenisation time).

Batch No.	Predicted responses		Actual responses	
	Particle size (μm)	Phase separation (%)	Particle size (μm)	Phase separation (%)
1	3.19	29.45	3.20	30.05
2			3.18	29.38
3			3.23	29.56
Mean	-	-	3.20	29.66
Standard deviation	0.13	2.06	0.03	0.35
Percentage error	-	-	0.42	0.72

Validation of optimum operating parameters

The results for three confirmation batches were compared with predicted responses and presented in Table 5.

Discussion

In this study, the quadratic model has been identified as the most suitable model since it gave the highest R^2 for both responses as shown in Table 2. R^2 indicates the total variance in the dependent variables given by the model. Low R^2 implies that there are variations which cannot be explained by the model. Another good indication of significant model is having a difference of 0.2 or less between the predicted and adjusted R^2. It is also a great advantage to have higher adjusted R^2 value than the corresponding predicted R^2 value for a model since it assures high correlation between the theoretical and experimental values (Karch, 2020, Maran et al., 2013). Based on Table 2, it was observed that the quadratic model satisfied the above conditions; hence, it was chosen for the current process optimisation study of 20% (w/w) palm olein-in-water emulsion.

The quadratic equations (1) and (2) were found to effectively capture the relationship between independent and dependent variables since the complete equations including all possible interactions were given by the ANOVA test. It was also observed that homogenisation speed had greater impact on both particle size and phase separation (-4.71 for R 1 and -11.83 for R 2) in comparison to homogenisation time (-0.017 for R 1 and -0.17 for R 2). Owing to the negative signs, larger factors would lead to lower (better) responses (Karch, 2020).

Table 3 shows that the F-value of R 1 (2446.9) was higher than that of R 2 (76.25). The larger the F-values, the better the fitted quadratic equation in describing the variance in the response. The related p-value for each F-value confirms the significance of F-value. The F-value is considered statistically

significant when the value is large and the related p-value is less than 0.05 (Maran et al., 2013). The p-values of approximately 0.0001 were seen for terms such as A, A^2 and B^2, thus showing that they were highly significant statistically. However, low F-value (B = 0.1, A*B = 0.4 for R 1 and B = 0.04, A*B = 0.94 for R 2) and p-values of > 0.05 (B = 0.7556, A*B = 0.546 for R 1 and B = 0.8489, A*B = 0.365 for R 2) were seen in some factors (B and A*B for both responses), thus indicating that these factors were statistically insignificant in the equation although the entire model was proven to be significant. Similar observation was reported by Abdulwahab and Saidat (2013). This happened due to the hierarchical problem which occurred when the developed model contained some factors that were aliased with one another. Nonetheless, the developed model was still applicable since the overall p-value of the model was less than 0.05 (Abdulwahab & Saidat, 2013). Furthermore, the standard error (SE) of R 1 model (0.0524) was found to be lower than that of R 2 (0.8573).

Fig. 1 reveals that all studentised residuals were normally distributed since they were fitted to the diagonal lines for both responses which indicated the goodness of fit of the quadratic model (Maran et al., 2013, Singh et al., 2011). As observed, the internally studentised residuals of R 1 followed the diagonal line more perfectly than R 2. These findings were also verified using predicted against actual plots, as presented in Fig. 2, from which the developed models were proven to be adequate since the data points were distributed along the straight line (Maran et al., 2013). On the other hand, both Fig. 3 and Fig. 4 suggest that higher factors would lead to better responses.

Effect of homogenisation speed and time

The rotor and stator of the homogenising head of HME induced extremely powerful shear forces which can break down the bigger oil droplets into smaller ones and homogenise the emulsion at the same time. The shear force increases with respect to the homogenisation speed. On the other hand, longer homogenisation time would lead to smaller particle size due to longer period of exposure to shearing forces (Win, 2015). This explains the condition where higher factors would lead to lower hence better responses as shown in Fig. 4.

However, it was observed that there was a slight increase in both responses at the end of the experiments (50 minutes). This could be due to the shear-induced flocculation mechanism in which electrical charges appeared on the smaller particles which attracted the adjacent oil droplets and flocculated into bigger particles (Li et al., 2016). In order to avoid this mechanism, homogenisation is routinely carried out at optimum homogenisation speed and time at which emulsions with the smallest droplet size and the least phase separation are produced (Win, 2015). From the visual observation of contour and response surface plots, it was estimated that the optimum homogenization conditions for producing 67.36 kg of 20% (w/w) palm olein-in-water emulsion could be roughly over 3000 rpm for homogenisation speed (A) and approximately 40 minutes for homogenisation period (B). This hypothesis was further confirmed by performing the following studies.

Selection of optimum operating parameters

The percentage error indicates the difference between experimental and predicted values. Lower percentage error dictates higher accuracy of the chosen experimental design. From Table 4, it was found that the least percentage errors recorded were 0.46 % for particle size (R 1) and 0.71 % for phase separation (R 2) in Batch 2. Therefore, the factors from Batch 2 were chosen as the optimum operating parameters because they would lead to the smallest percentage errors in both responses. The batches in which the emulsions were homogenised at speeds of 3400 rpm (i.e. batches 1 - 4) were seen to exhibit higher desirability values in comparison to those with the homogenisation speeds of over 2600 rpm (i.e. batches 5 – 7). Nonetheless, Batch 2 was finally chosen since it had added advantages such as producing emulsions with the smallest particle size (3.20 ± 0.31 μm) and the least percentage of phase separation (29.66 ± 0.60 %) in addition to having the lowest percentage error. Smaller percentage of phase separation was expected from the emulsion having smaller particle size since smaller oil droplets would take longer to undergo aggregation, i.e. the so-called Ostwald's ripening that triggers the phase separation (Zwicker et al., 2015).

On the other hand, higher percentage errors as well as lower desirability values were exhibited by batches where the emulsions were homogenized at speeds less than 600 rpm. High percentage error was generated when the process parameters could not reproduce the predicted response values given by the software. The big difference in predicted and observed responses was attributed to the fact that the homogenisation speed of < 3000 rpm could not produce emulsions of particle size ≤ 3 μm in practical milieu (i.e. 67.36 kg of emulsions). This

phenomenon was caused by the relationship between homogenisation conditions and homogenisation volume. Higher homogenisation volume requires higher homogenisation speed and/or longer homogenisation time to produce emulsion with smaller particle size (Win, 2015). Mohd Nawi (2018) reported that the homogenisation conditions could vary according to the homogenisation volume; therefore, the optimum homogenisation parameters should be customised based on the prepared emulsion amount.

Since the findings of Batch 2 were satisfactory, the optimised homogenisation speed and time were chosen as 3400 rpm and 40 minutes (Batch 2), respectively for preparing 67.36 kg of 20% (w/w) palm olein-in-water emulsion stabilised with 25% (w/w) to oil of Span® 20 and Tween® 20 mixture with effective HLB value of 10. Based on the multiple response optimisation, the chosen optimum operating conditions yielded a desirability value of 1 for both responses. The desirability value ranges from 0 (least desirable) to 1 (most desirable), whereby the value of 1 indicates that the chosen optimum conditions are highly satisfactory to yield the desired outcomes (Shokri et al., 2020).

The results from the validation studies in Table 5 proved that the observed data showed reasonable agreement with the predicted data. This favourable outcome indicated the suitability of quadratic models. These observations also indicated the reproducibility of responses if the optimum homogenisation process parameters were controlled during batch-to-batch operations.

Conclusion

It was evident that the developed quadratic models were able to represent the relationship between process parameters and properties of emulsions. The study revealed that the characteristics of the 20 % (w/w) palm olein-in-water emulsions, such as particle size and phase separation were affected by both speed and duration at which the emulsions were homogenised. Both attributes were found to be inversely proportional to the process variables within certain limits. This study also demonstrated that the favourable outcomes, such as smallest particle size and lowest percentage of phase separation were obtained when the homogenisation speed was set at 3400 rpm for a duration of 40 minutes. These operating parameters were considered as optimum operating conditions which should be controlled for future pilot-batch production of emulsions having similar characteristics.

Authors contributions

Conceptualisation, M.R.A.B. and T.W.; performed the experiments, analysed the data and drafted the paper, T.W.; reviewed and edited the paper, M.R.A.B.; supervision, M.R.A.B., F.M., M.T. and M.Z.I.S. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors thank IKOP Pharma for the permission to carry out studies using its homogenous mixing equipment. Financial support provided by the Malaysian Ministry of Higher Education (PRGS14-005-0015) is also gratefully acknowledged. The first author is indebted to Dr. Kausar Ahmad for her advice and support throughout the course of the work.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that ChatGPT was used to assist in improving the readability and language in certain parts of this work. The authors have reviewed and edited the content as necessary and take full responsibility for the final content of the publication.

References

Abdulwahab, G. and Saidat, O. G. (2013). Isopropyl myristate production process optimization using response surface methodology and MATLAB. International Journal of Engineering Research & Technology. 2, 853-862.

Ahmad, K., Win, T., Jaffri, J. M., Edueng, K., Taher, M. (2018). Palm olein emulsion: a novel vehicle for topical drug delivery of betamethasone 17-valerate. AAPS PharmSciTech. 19, 371-383. https://doi.org/10.1208/s12249-017-0843-9.

Ćurić, A., Reul, R., Möschwitzer, J., Fricker, G. (2013). Formulation optimization of itraconazole loaded PEGylated liposomes for parenteral administration by using design of experiments. International Journal of Pharmaceutics. 448:189-97. https://doi.org/10.1016/j.ijpharm.2013.03.029.

Faria-Silva, A. C., Costa, A. M., Ascenso, A., Ribeiro, H. M., Marto, J., Gonçalves, L. M., Carvalheiro, M., Simões, S. (2020). Nanoemulsions for cosmetic products. Nanocosmetics, 59–77. https://doi.org/10.1016/b978-0-12-822286-7.00004-8.

Jankovic, A., Chaudhary, G., and Goia, F. (2021). Designing the design of experiments (DOE) – An investigation on the influence of different factorial designs on the characterization of complex systems. Energy and Buildings, 250, 111298. https://doi.org/10.1016/j.enbuild.2021.111298

Karch, J. (2020). Improving on adjusted R-squared. Collabra: Psychology, 6(1): 45. https://doi.org/10.1525/collabra.343

Lamidi, S., Olaleye, N., Bankole, Y., Obalola, A., Aribike, E., & Adigun, I. (2023). Applications of Response Surface Methodology (RSM) in Product Design, Development, and Process Optimization. In Kayarogannam, P. (Ed.), Response Surface Methodology - Research Advances and Applications. IntechOpen. https://doi.org/10.5772/intechopen.106763

Li, Z., Lu, P., Zhang, D., Chen, G., Zeng, S., He, Q. (2016). Population balance modelling of activated sludge flocculation: Investigating the influence of extracellular polymeric substances (EPS) content and zeta potential on flocculation dynamics. Separation and Purification Technology. 162, 91-100. https://doi.org/10.1016/j.seppur.2016.02.011.

Maran, J. P., Manikandan, S., Priya, B., Gurumoorthi, P. (2013). Box-Behnken design based multi-response analysis and optimization of supercritical carbon dioxide extraction of bioactive flavonoid compounds from tea (Camellia sinensis L.) leaves. Journal of Food Science and Technology. 52(1), 92–104. https://doi.org/10.1007/s13197-013-0985-z.

Mohd Nawi, M. S. (2018). Palm olein-in-water emulsions stabilized by Span® 20/Tween® 20 surfactants as potential vehicles for drug delivery. [Master's thesis. International Islamic University Malaysia].

Mohr, D. L., Wilson, W. J. and Freund, R. J. (2022). Inferences on a Single Population. Statistical Methods. In Mohr, D. L., Wilson, W. J. and Freund, R. J. (Eds.), Statistical Methods (4th ed., pp. 169–199). Academic Press. https://doi.org/10.1016/b978-0-12-823043-5.00004-7.

Ramli, S., Norhman, N., Zainuddin, N., Mohamad Ja'afar, S. and Abdul Rahman, I. (2017). Nanoemulsion-based palm olein as vitamin E carrier. Malaysian Journal of Analytical Sciences. 21(6), 1399-1408. https://doi.org/10.17576/mjas-2017-2106-22

Shokri, A., Daraei, P., Zereshki, S. (2020). Water decolorization using waste cooking oil: An optimized green emulsion liquid membrane by RSM. Journal of Water Process Engineering. 33, 101021. https://doi.org/10.1016/j.jwpe.2019.101021

Singh, B., Bhatowa, R., Tripathi, C. B., Kapil, R. (2011). Developing micro-/nanoparticulate drug delivery systems using "design of experiments". International Journal of Pharmaceutical Investigation. 1, 75-87. https://doi.org/10.4103/2230-973X.82395.

Win, T. (2015). Characterization of palm olein-in-water emulsion as a vehicle for tropical drug delivery of betamethasone 17-valerate. [Master's thesis, International Islamic University Malaysia].

Yolmeh, M. and Jafari, S. M. (2017). Applications of response surface methodology in the food industry processes. Food and Bioprocess Technology. 10(3), 413-433. https://doi.org/10.1007/s11947-016-1855-2.

Zwicker, D., Hyman, A. A., Juelicher, F. (2015). Suppression of Ostwald ripening in active emulsions. Physical Review E: Statistical, Nonlinear, and Soft Matter Physics. 92, 012317. https://doi.org/10.1103/PhysRevE.92.012317.

Journal of Pharmacy

Perspectives of Pharmacists Towards Patient-Centred Mental Health Pharmacy Services: A Focus Group Study

Nurul Aziera A Rahim[1*], Norny Syafinaz Ab Rahman[1], Khairul Azrul Abdul Rahman[2], Cheong Jia Yi[3], Nur Fazlin Abd Ghafar[4], Yasmin Ibrahim[5], Aida Roziana Ramlan[6] and Syahrir Zaini[1,7*]

[1]*Department of Pharmacy Practice, Kulliyyah of Pharmacy, International Islamic University Malaysia, Kuantan, Pahang.*
[2]*Department of Pharmacy, Sultan Ahmad Shah Medical Centre @IIUM, Kuantan, Pahang.*
[3]*Indera Mahkota Health Clinic, Bandar Indera Mahkota, Kuantan, Pahang, Malaysia.*
[4]*Pekan District Health Office, Kampung Pancur, Pekan, Pahang, Malaysia.*
[5]*Tengku Ampuan Afzan Hospital, Jalan Tanah Putih, Kuantan, Pahang, Malaysia.*
[6]*Pekan Hospital, Kampung Mengkasar, Pekan, Pahang, Malaysia.*
[7]*Qualitative Research Group, Kulliyyah of Pharmacy, International Islamic University Malaysia, Kuantan, Pahang, Malaysia*

Abstract

Introduction: The rising prevalence of mental illness is a growing concern. Integrating pharmacists into mental health care teams could help address this challenge by improving service quality and accessibility for patients. This study investigates pharmacists' opinions regarding treatment care in the Kuantan and Pekan areas and their attitudes toward patient-centred mental health pharmacy services. **Methods:** A qualitative study was conducted with 63 pharmacists around Kuantan and Pekan, Pahang, utilising a focus group discussion (FGD) style. The pharmacists were selected through purposive sampling from 2 general hospitals, 2 district health offices, and 1 teaching hospital. All locations are primary healthcare in Malaysia. The focus group topic guide was created using the relevant literature research. The entire interviews were audio-recorded and then transcribed verbatim. Thematic analysis was performed inductively to analyse the data. **Results:** This study involved 14 focus group sessions with all 63 pharmacists participating. Every interview was in person at the designated sites. From the interviews, 6 key themes were identified. 1. Role of pharmacists in mental health care. 2. Pharmacists-patients' interactions. 3. Interprofessional collaborations with other healthcare workers. 4. Challenges and barriers. 5. Training needs for pharmacists and lastly 6. Improvement on the role of pharmacists and future practice. **Conclusion:** This research explores Malaysian pharmacists' perspectives on mental health treatments. It highlights pharmacists' potential contributions to mental health care, the challenges they face when assisting patients with mental health conditions, and the current limitations of mental health services in Malaysia's primary healthcare settings, hospitals, and Klinik Kesihatan.

Article history:

Received: 14 March 2024
Accepted: 26 March 2024
Published: 31 January 2025

Keywords:

Qualitative study
Perspectives
Hospital pharmacists
Mental health services

doi: 10.31436/jop.v5i1.301

*Corresponding authors' emails: azierahim8892@gmail.com, syahrirz@iium.edu.my

Introduction

World Health Organization (WHO) stated more than 350 million people have already been diagnosed with depression, 60 million people with bipolar disorder, and 21 million with schizophrenia. (Wakida et al., 2017). Mental illness is a state in which a person is unable to function in a community due to abnormal cognition, emotion, or behavioral symptoms (Yeap & Low, 2009). Mental health service is one of the most important departments of primary health care, but due to community stigma, there is a treatment gap and low capabilities to handle mental patients(Henderson et al., 2013), mental health services sometimes being forgotten and the health systems are unable to adequately respond to mental health burden (Wakida et al., 2017). Malaysia adopted a model of care consisting of in-patient and community care. The primary care model is community-based, with 22 established community-based specialized mental health services (MENTARI) and 958 mental health day centers. Additionally, Malaysia has four mental health hospitals and 47 psychiatric in-patient units attached to general hospitals (Raaj et al., 2021). These are all the primary healthcare in Malaysia. During the coronavirus disease 2019 (COVID-19) pandemic, some healthcare workers have already shown effects on their mental health state such as post-traumatic stress disorder (PTSD) and depression (Daryanti et al., 2021). The global prevalence of mental health problems among healthcare workers showed the highest was from nurses (43.7%), then doctors (27.9%) and allied health workers (7%). Post-traumatic stress disorder was the highest mental health problem among them indicated 49%, followed by anxiety, at 40% (Daryanti et al., 2021). The past data from 2020 until early 2021 also suggested that there is psychosocial suffering in the general population and increased distress among healthcare workers, exposed to the early stages of mental disorders. This indicates the importance of mental health services in primary health care and involving pharmacists can be one of the crucial interventions to improve mental health outcomes among mental health patients.

The pharmacist is regarded as the easiest accessed primary healthcare provider (Gan et al., 2018). Mental health patients, who were prescribed psychotropic medications should be monitored and get proper management and advice from a pharmacist. This situation makes pharmacists a crucial addition to the mental health collaborative teams. One study proved that having pharmacists in mental health collaborative teams resulted in good feedback from mental health patients and other health care providers (Bell et al., 2007). Furthermore, taking psychotropic medication might present difficulties such as side effects, worries about reliance, and associated stigma; finding appropriate treatment can be challenging (Maroun et al., 2018). As much as the prevalence of mental illness is high among all ages and generations, taking psychotropic medications also becomes a burden in public health as general. The pharmaceutical Experience Model, developed by Wegremeyer et al. (2014), incorporates these pharmaceutical experiences together with other important themes related to drug use, like adhering to a prescribed regimen and medication load. Although it was developed based on the personal experiences of adult consumers using psychotropic medicine, this model illustrates the relationships between medication experience, medication acceptance, and sickness experience. It is likely to be relevant for a younger demographic (Wheeler et. Al.,2019)

According to the most recent epidemiological statistics, which the Malaysian Ministry of Health provided in 2015, 29% (95% CI 27.9–30.5) of adults had a mental illness. This represents a threefold increase above the 10% prevalence rate in 1996(Ning et., al, 2020).

Pharmacists frequently provide psychotropic medicine and have successfully assisted patients in addressing issues and advancing mental health. Consequently, for this to occur, numerous opportunities for pharmacists to play a more prominent role in mental health care must be made available (Silva et Al., 2018). As you can see in Malaysia, pharmacists play a very small or non-existent role in providing mental health care across all health sectors, including general hospitals, public health clinics, and even private hospitals.

There was previous survey study made by Malaysian researchers found that pharmacists did offer a wide range of services in different types of facilities, but the scope and depth of these services may be improved (Abousheishaa et al.,2022). Therefore, from this study, as a stepping stone to the next level for pharmacists' involvement in mental health care to improve, we will investigate the views of pharmacists in the Kuantan and Pekan areas,

regarding treatment care and attitudes about patient-centred mental health pharmacy services.

Materials and methods

Study settings and recruitment

A qualitative, focus group discussion among registered pharmacists was conducted for this study. A total of 14 Focus group discussions (FGD) methods were held from August 2023 until December 2023 at Kuantan, Pahang. The qualitative study was chosen to explore in-depth more about their perspectives on current mental health services in our primary healthcare in Malaysia and their experiences when dispensing and encounter with mental health patients (McMillan et.,al. 2020). The pharmacists included were practiced pharmacists from primary healthcare in Malaysia which were government hospitals, Klinik Kesihatan, and Sultan Ahmad Shah Medical Centre (SASMEC). The study was conducted at Klinik Kesihatan Indera Mahkota, Kuantan, Hospital Tuanku Ampuan Afzan (HTAA), Klinik Kesihatan Pekan, Hospital Pekan, Pahang and SASMEC. Ethical approval for this study was obtained from the Medical Research and Ethics Committee (MREC) of Ministry of Health (MOH) Malaysia, with the identification number of: NMRR ID-23-01687-IE3. The sample size of this study was 63 pharmacists overall. This study used a purposive sampling. For their participation in the study, all pharmacists had signed written consent forms. Participants were recruited based on the inclusion criteria below All participants agreed to participate out of their own free will no one refused to participate. Pharmacists' inclusion criteria Must be a registered pharmacist, practicing in different facilities such as a hospital setting, several government health clinics, and a multi-centre hospital, Sultan Ahmad Shah Medical Centre @ IIUM, and working in any pharmacy department at least 2 years.

Data Collection

14 groups, consisting of 4-6 pharmacists in each group, were interviewed using a focus group study topic guide. The duration for each session was approximately 45 to 55 minutes. The topic guide is mostly open-ended. Before creating the interview topic guide, a comprehensive evaluation of the literature was done to address the research questions.

The phenomenology approach served as the foundation for the interview. The open-ended questions for the pharmacists primarily centred on their experiences with mental health patients, the services they have received for both physical and mental illnesses, any recommendations they may have for expected services for mental health patients, their thoughts on patient-centred mental health pharmacy services, and any expectations they may have for education when working with mental health patients. Before the interview, all participants were required to sign the consent form. All the interviews were audio-recorded by using the application "Voice Recorder" on the smartphone. Interviews were conducted by two pharmacist researchers (NAR and SZ).

Data Analysis

All the recorded audios were transcribed verbatim. The main author analysed the transcriptions independently first by using inductive thematic analysis through ChatGPT. After getting the main themes from the saturated data with the thematic analysis, the main author and her co-investigator re-read the transcription returning to pull out the pertinent remarks, convert them into a code for the corresponding primary ideas that had previously emerged, and condense them by using macro views in Microsoft Word (Ose et., al, 2016). The important comments/codes were highlighted and coded into their respective themes. The quotes were trimmed and edited to give clarity and conciseness. All data analyses were independently verified by a co-author and the co-investigator to ensure accuracy and reliability. These recurring themes served as a basis for collecting data and making judgments.

Results and discussion

14 groups with a total of 63 pharmacists were interviewed in this focus group discussion (FGD). 4 groups consisting of 16 pharmacists were interviewed on 19 September 2023 at the Meeting room, Klinik Kesihatan Indera Mahkota, Kuantan. All of them were pharmacists from Pejabat Kesihatan Daerah Kuantan. 3 groups consisting of 17 pharmacists were interviewed on 20 September 2023 at the Meeting Room, Hospital Tuanku Ampuan Afzan, Kuantan. Another 3 groups consisting of 16 pharmacists were interviewed on 3 October 2023 at Meeting room Pejabat Kesihatan Daerah Pekan and another 3 groups of 12 pharmacists from Hospital Pekan were interviewed

on 4 October 2023. Another last group consisted of 4 pharmacists from SASMEC and their interview was conducted on 12 December 2023. All of them were conducted face-to-face. The mean interview duration was 47 minutes, with a range of 38 to 1 hour 11 minutes. Table 2 below shows the participants' demographic data.

Table 1: Participants' Demographic Data

Age	21 -30 years old	16
	31 – 40 years old	35
	41-50 years old	12
Gender	Male	13
	Female	50
Race	Malay	57
	Chinese	4
	Indian	2
Current work pharmacy unit	Outpatient pharmacy	41
	Drug information services	1
	Inpatient pharmacy	11
	Logistic pharmacy	5
	Satellite pharmacy	1
	Others (Ketua Jabatan, Clinical & MTAC)	4
Number of years working (current unit)	1 – 5 years	57
	6 – 10 years	6
Total no. of years working	1 – 5 years	24
	6 – 10 years	21
	>10 years	18

All the participants revealed to have experience in dispensing medications to mental health patients. Data analysis showed 6 main themes that emerged from the focus group discussion (FGD) such as: 1. Role of pharmacist in mental health care. 2. Pharmacists – patients interactions. 3. Interprofessional collaborations with other healthcare workers (Doctors, Medical Assistant (MA) & Nurses). 4. Challenges and barriers. 5. Training needs for pharmacists and lastly 6. Improvement in the role of pharmacy and future practice. Each theme consisted of several subthemes. All these themes and subthemes were provided along with the verbatim quotes extracted from the interview transcriptions. All the related quotes extracted and being coded below to reflect the themes and subthemes that emerged from them. Some of the quotes are being edited for clarity and conciseness. The quotes from the participants will be provided below and the participants will be identified as P1 until P63.

1. Role of pharmacist in mental health

The interviewees shared their opinions regarding pharmacists' roles in mental health care. Each gave their opinions on the roles that pharmacists should play going forward in providing all rounded services in mental health treatments. The subthemes that appeared were; 1.1 to ensure compliance of medications among mental health patients. 1.2 dispensing correct dosage. 1.3 counselling on side effects of medications. One participant mentioned there was a role of pharmacists in ward and clinical department. Pharmacists were seen tagging around with psychiatrist doctors and also checking their medication in ward directly. other pharmacists in inpatient department or other departments only involved during the ward check which occur monthly or once in a 3 months.

"….We check carefully during ward check sessions if staff nurse or doctor use the medication, whether they record every time they use the medications or not, are they tally or not, DD especially because it is the most important. So if there are MTAC pharmacists or clinical pharmacists who can attach there, maybe they can do this kind of checking regularly. Don't have to wait until 3 months, or 6 months for a ward check. Make it as another routine for clinical pharmacists in a psychiatric ward." (P22)(M)

1.1 To ensure the compliance of mental health patients

The participants gave several opinions on the role of the pharmacist which usually only stressing about the compliance of medications especially towards mental health patients.

"We know that patients are mentally unstable, so whether they comply with their medications or not. So to ensure their compliance, for me, we have to carefully observe and make sure patients are complying with their medication……." (P1)(M)

Another participant also emphasized that compliance is important to make sure that there is no relapse of symptoms in patients and to ensure that the treatment given is effective in controlling patients' symptoms.

"*….for pharmacists directly involved. Because it is true haaa..how the patients taking their medications and we can counsel on that. Then the efficacy of the treatment, the factors that contributed to it, patients must comply with their medications, their adherence towards the medications, so the role of pharmacy here is pretty much important." (P8)(F)*

"For me, role of pharmacist in mental healthcare is more towards how to ensure patient more compliance with their medication because.. ha.. ha.. mental health in Malaysia I think emphasized more about compliance right , so ha…counselling about medication is also important." (P38)(F)

1.2 Dispensing on the correct dosage

Even though the keyword is the dose of medications, it is still related to the medication regimen as a whole.

"….involve indirectly, for example about the dose, compliance, and medication" (P14)(F)

There is also one of the participants who feels worried when some doctors in the psychiatric ward just simply increase the dose without checking with the pharmacist first.

"If following guideline, the dose must take about 2 weeks to increase, but the doctor in ward, less than 1 week, already changed the dose." (P22)(F)

It is not only about the dose or regimen of the medications, but the dosage form of the medications also one of the role of pharmacists when dispensing the psychotropic medications.

"The role of pharmacists itself is already important, especially when dispensing the medications at the counter, usually simple things like the dosage form of the tablet, color, if the shape of tablet changed also, the patients might be more particular compared to other patients" (P49)(F)

"Based on my experience, when we dispensed the medications, we are more concerns on telling the patients how to consume the medications…." (P62)(M)

1.3 Counselling on the side effects of the medications

Psychotropic medications are very well known for their side effects. The most common are drowsiness, weight gain, headache and dizziness. . Some of the participants expressed concerns about the side effects that patients might get after taking the psychotropic medications so counselling them on how to handle the side effects is also one of the most important roles of pharmacists and they are only limited to counsel the side effects' part.

"Our role other than to supply medication at the clinic aa, and the counselling part only limited on the medications that might have side effects on patients." (P33)(F)

"Like us pharmacists, can only take part the role on talking about medication and side effects as well as pharmacotherapy part to reconfirm certain diagnosis." (P48)(F)

"…..So when we have the side effect of the medications, it is our role to explain the side effects, especially if the patients taking the atypical psychotropic medications, the old generation one." (P49)(F)

"When Dispensing, we must know everything about the medications, the characteristics of the medications, and also the possible side effects that might occur and the precaution if the side effects occur." (P62)(M)

There is also one of the participants who stated that sometimes, their role is only about dispensing medication, not that much on the side effects counselling as patients in Klinik Kesihatan are usually already stable.

"So the role of the pharmacist only dispense the medication, not so much intervention on the side effects, because patients are stable and have been on with the medication for so long." (P40)(F)

2. Pharmacists-patient interactions

The participants discussed several types of interactions with mental health patients. The interactions with mental health patients are very limited only when dispensing medications.

"…If me, I just treat mental health patients almost the same as other types of patients. If they got mad or quite rude when talking to us, I just kept quiet and not reciprocate their rudeness, just talk as usual" (P2)(F)

"If we encounter with mental health patients, we just act

as outpatient pharmacist only….." (P14)(F)

The participants also discussed the interactions between pharmacists and patients usually about the side effects and their medications in general.

"….I remembered there is one patient, he always said that his medications is not enough 1 or 2 tablets. Their medication is DD, so it is weird if it is not enough because DD is usually always being recorded when we want to give to patients." (P8)(F)

"With the right skills and better understanding, we can manage, we can have better understanding of the patient, so that we don't have to trigger that much. I feel because we do not understand their conditions, so that is why…..." (P31)(F)

"They mostly talked about medications, about side effects, or they are asking the effectiveness of the medications, is it the medication okay or not, they are not really informing doctor, so one of the role of pharmacists also always informing the doctor." (P58)(F)

Pharmacists sometimes try to do the counselling sessions or have more time to listen to them but difficult due to time constraints and mainly just giving the medications.

"Just like my colleagues said, if stable patients, we can interact with them like normal persons, because we know their history, we know how to tackle them. Usually, we will ask them a lot of questions, and listen to them while they tell us their story. Sometimes they just need someone to listen to……" (P9)(F)

"…We are bound to our working time and we only have 2 hours only for dispensing the medications, so we cannot ask a lot of questions to them. For example if u said that you can dispensed only for 5 patients in a day, then you can ask a lot of question and counselling during dispensing. So our time frame not allowing this." (P46)(F)

The interactions with mental health patients are also either positive emotions from the interactions such as empathy, sympathy, or trying to understand them better or very bad experiences such as quite dangerous misbehaviour, rude and yelling behaviour, very difficult to entertain during dispensing.

"Maybe because of the stress, I do feel empathy actually. So pity, because we didn't even know their background story, their history of why they get the mental illness. If they are born with the mental illness, then maybe we can understand them, but those who already old but still stressed and have the mental illness" (P1)(M)

"……because for patients, if they cannot sleep, it is already a big issue for them. Their life feels so much disturbing because of that. So the way we respond to them…." (P23)(F)

"Sometimes patients are like..with us..maybe they are stressed waiting for their medications for too long, so when we dispensed to them, they dind't even bother to listen to us. Sometimes they cried, like that."(P15)(F)

"……Yeah, got some patients who threatened us, not like serious like threatening to kill us or what, just threatened to file a complaint. But patients who are taking DD is not this rude usually." (P18)(F)

"There is one case also, at outpatient pharmacy, that patient was got so mad that she just pull out one of our pharmacist's scarves. That time I just calling for 'code grey' to the whole hospital, because patient is so uncontrolled and aggressive. Every month will have the same issue with this same patient, she will act so dramatic in front of the counter, she is actually a patient from other hospitals, she just take the medications here, and when the date is near for her to come, to collect her medications, we will always get ready, because she always have issue with us. She will act aggressive, she do not want to wait, her medications has DD, but she cannot wait." (P18)(F)

"I have a lot of experience with mental health patient when I was in HTAA. That time I have bad experience with psychiatric patient and they have some stigma while at HTAA. Patient there are very difficult to entertain, a lot of demand….." (P36)(F)

There is also a situations of their interactions when pharmacists do not even know how to interact back to mental health patients.

"There is one patient, who currently taking medications from us, she was like, looks stable, actually stable,but she suddenly said to me,'life is not meaningful anymore, I just want to die.', so at that time, I do feel shocked. To counsel back err….want to interview her more but I do not know how. Quite negative." (P13)(F)

Sometimes mental health patients are always accompanied by their caregivers. Communication with their caregiver is also very important. Just like how they communicate with mental health patients, they also emphasize the importance of communicating well with patients' caregivers for treatments..

"…..We will always see when we encounter aggressive and rude mental health patients. They sometimes just

throw the prescription slips at us, and got angry without any reason even though they are being accompanied by their caregiver. Their caregivers sometimes look ashamed…..." (P1)(M)

"….We give example, we give 15 tablets for 30 days, and we ask caregiver if is it not enough? The caregivers said, if patients look unstable, then I give another 1 tablet. Haaa..so I think our part as a pharmacist is over there. We encounter the situations by telling back to the doctor about the situations." (P31,F)

There is only 1 participant that mentioned his experience with mental health patients at their ward.

"….They talked a lot. Based on my experienced while visiting their ward at previous workplace, They are talking to me about their own life experience, not many of them asking me about their medications. They just talked to me how and why they end up being a mental health patients." (P53)(M)

3. Interprofessional collaborations with other health care workers (doctors, nurses and medical assistants)

The collaboration between pharmacists and other healthcare workers is one of the most important components that need to be done first to build the MTAC of psychiatry.

3.1 Interprofessional collaborations in general

One participant showed agreement on having the pharmacists collaborate with other healthcare workers dealing with the increasing cases of mental illness.

"Nowadays many people are being exposed with what is depression and so on, so actually a lot of people experience some of the depressions symptoms. People who look like have a great career but actually has a depression. We actually do not know this. So I think if MTAC being established, actually it is a good idea, because we can consider that many people have the mental illness nowadays." (P9)(F)

One of the participants also stated that if need to establish MTAC, doctor's cooperation is always the most important factor.

"Must have the cooperations from the doctor, and doctor I think always welcome this opportunity. To collaborate with us. So, we have to do this together to established the MTAC Psychiatry." (P24)(F)

Not only with doctors but also medical assistants and nurses because a lot of things such as space to do the counselling session during MTAC also need everyone's cooperation.

"Something simple like a place/space to set up for MTAC sessions, comfortable enough to do the counselling, for me needs the cooperation and collaboration from other staff such as nurses and MA." (P22)(F)

When doing the inter-collaboration between doctors and pharmacists, the need to distinguish roles between two of them is so much necessary to prevent any overlapping.

"Until now, if there is any interventions, all of them are coming from the doctor, not from the pharmacists and doctor also not query us anything about the regimen or dose of medication to us. Means there is a lot of gap there and need a clarification on the role for both of us." (P25)(F)

"We do not even know what doctor has counsel the patients, so if we also counsel the same patients, afraid its not the same thing with what the doctor already said to the patients"(P62)(M)

3.2 The communication between pharmacists and other healthcare workers

The participants shared what kind of communication happened between them and other healthcare workers, especially doctors and psychiatry. Some of them talking about the drug-drug interactions because some of mental health patients also have other comorbidities.

"Or sometimes they also asked us about patients, if the patient got seizures is it still under psychiatry part.. so basically they just asking about drug-drug interaction." (P1)(M)

And some of them talking to pharmacist about the dose of medications whether it is suitable or not with their patients.

"We just interact with doctor only by call through telephone. Only when there is any changes of medications or not." (P61)(M)

"Secondly if the doctor is the one ask us if those medications suitable or not with their patients. No..not really suitable or not the medications, but more to the strength of the medications. For example, we don't really keep lamotrigine 50mg, we only keep 100mg, so if they want to give low dose like 25mg, can cut the tablet or not." (P61)(M)

"If inpatient, they always query when patients are suicidal, they ask us the lethal dose. hmm lethal dose or, toxic dose, no exact answer, we just keep on treating based

on patients' respond and symptom of toxicity." (P62)(M)

The interviewee also shared that nurses sometimes are the ones that have the least communication with pharmacists in terms of how to use the medications/injections of the psychotropic medications.

"They are all professional already, they do not need us to show them how to use..nurses are the one that did not ask us anything..most of the time..(laughing)" (P21)(M)

Another interviewee shared one of the communications that she encountered was the doctor asking her to check the patient's previous medications because that patient being admitted to ward at that time. Other interviewees just encounter doctor asking them about the medications related questions in inpatient pharmacy department rather than outpatient pharmacy department

"There have been one doctor asking me that time, at ward, yeah, psychiatric doctor, he ask me to check patient's previous medications." (P29)(F)

"Our services, doctor did not ask us so many questions. Just asking about the medications' quota and availability……"(P31)(F)

"Let's say if non-psychotropic medications are controlled items at HTAA, but the medications are an old medication of the patients from other hospitals, so we will query back to the doctor to double confirm, so in that sense for medication history and others, we only involve in this kind of situations only." (P31)(F)

"…..yeah.. if talking about intervention,after that they ask us is there any alternatives medications for this patients….." (P32)(F)

3.3 The need for a multidisciplinary approach

The multidisciplinary approach means that one patient got a treatments from a multiple health professionals from several disciplines. One interviewee shared there is a multidiscipline service in her Klinik Kesihatan (KK).

"A counsellor from KBKK will come to KK UTC and the counsellor will see mental health patients at KK UTC. But the counsellor will just give counselling, that mental health patients still need to go to HTAA to get the medications. ..." (P5)(F)

Interviewee P5 also added that she believes that a multidisciplinary approach could give a lot of benefit to the patients and also to us.

"Not sure if we can do this services or not. But from my own knowledge, this mental health services can be like 2 in 1 services. For example, methadone clinic, sometime methadone patients also a psychiatry patients. So we can do the counselling during methadone clinic. Another example like if that patient attend 'Klinik Berhenti Merokok'(KBM), and he is also a mental health patient, so we can dispensed and counsel at the same time. If the patients are also diabetes patients, haa then we can do another 2 in 1 counselling." (P5)(F)

One participant suggested the collaboration between pharmacists, other healthcare workers, and mental health patients should not be limited only to the treatment part, but also other activities that can heal our minds or doing charity work together. Agree on the establishment of MTAC Psychiatry but still need to look at the rapport from a doctor.

"All the patients actually have their own talents, some of them knew how to sew, and she sell the things that she sew. Knitting, and dancing to Zumba… and everyone is joining their activity. They welcome anybody to join them. Some of the patients still looks not okay, but some of them looks okay. I also not sure. Maybe the one that stable did the activity. For MTAC, if you want to do it, better take the stable one, depends on the one that doctor will refer, if we really want to do the MTAC, must look at the rapport from the doctor." (P10)(F)

According to one interviewee, a specialised doctor had previously asked for inter-collaborations such as MTAC in his hospital; therefore, it is hoped that a psychiatrist expert would also request MTAC Psychiatry.

"Previously, MTAC-MTAC that being developed here are being requested by their own respective specialist doctor, for example, diabetes, respiratory disease, INR clinic and warfarin clinic. So, they are all being requested. They are necessary to have the interventions from pharmacist for their patients. But for psychiatry patients, we still do not have the request yet. Hopefully soon." (P61)(M)

4. Challenges and barriers

There are also some subthemes of challenges and barriers that emerged. 5 subthemes can be derived from these themes which are staff shortage, lack of awareness and recognition for pharmacists, limited resources, time and budget, unclear role of pharmacy, and stigma.

4.1 Staff shortage

The interviewees consistently highlighted the shortage of pharmacists, indicating the seriousness of the staffing issue. Almost every group mentioned these challenges.

"We do not have enough staffs actually (chuckle)"(P3)(F)

"If looking at the workload now, we should add on more staff (laughing)"(P3)(F)

"There are a lot of things need to be taken care of. Because you see, if 1 pharmacist can handle maximum 2 type of MTAC. Maximum. Actually normally one MTAC per 1 pharmacist. That's why it is already more than capacity. Like I said, all the reports and times that needed to talk with patient. The time is not really enough. A lot of workload. With your actual work to dispense some more.." (P1)(M)

"The problem with KKM is when we added new staff, we have to add also more services. Haaaa..the reason we add more staff is to reduce workload, so if they as me to add more services also, it is still the same thing."(P7)(M)

4.2 Lack of awareness and recognitions for pharmacists

Some of the interviewees mentioned that pharmacists still lack of awareness about mental health patients. All the terms used by psychiatry doctor looks unfamiliar.

"Maybe there are some terms that being used is not really familiar and not enough exposure, knowledge about mental health…."P3)(F)

"….maybe lack of knowledge, because we are not that exposed with mental health patient" (P11)(F)

One participant mentioned that one of the reason why a lot of pharmacists did not want to explore new services and become specialist in that new field because they felt not motivated to do their MTAC happily and enthusiastically.

"Our MTAC service should have something like an incentive when we have to be the charge of it. Truthfully (everybody laughing) so, if there is an incentive, everybody wants to do it. Right? Like example must have some certificate to show that this pharmacist specifically well trained..like 'Post-Basic'. Like if we have Post-Basic we will get money, like special allowance? Haa allowance.. so even if our workload increases, but then the salary still the same, why should we increase the workload? So if we have something like post basic, then we have to do more work, but at least got additional allowance. Haa" (P7)(M)

"..there is no post-basic for pharmacists at all. Even Assistant pharmacists have the post-basic….."(p7)(M)

4.3 Limited Resources, Time & budget

Stable human resources, enough time, and a budget are the most important components in every working organization to ensure the efficiency and productivity of the job.

Hospital policies or SOPs must be clear to be the resources for pharmacists to do their job efficiently while handling mental health patients.

"If outpatient here, there is no special SOP when handling mental health patients. Everything is just based on our kindness and judgment. If we see some aggressive patients disturb other patients also, so we have to give their medications first." (P61)(M)

"I think we must have one guideline because if we want to dispense, actually we worried a lot. First, we feel like we are invading their privacy, secondly, being judgemental just like I said just now. So, when we dispense and want to tell them the indications for each of their medications, we agree there must have a guideline for this matter." (P61)(M)

Another SOP that is always being mentioned by interviewees is all dangerous drugs classification (DD) must be recorded in and out. That is the reason why the waiting for them to get the medications is sometimes very long.

"….The waiting time for mental health patients, always longer than other patients because most of their medications involved dangerous drug(DD), so the process to bring out DD must always be recorded, so it takes time." (P61)(M)

The participants also showed concerns about if there is an integrated module for future MTAC programs, there will be limited access to patients' file

"Another thing is if we do want to use the current integrated module for MTAC implementations, between prescriber and pharmacy, before this we can see all the patients' notes, but now we cannot anymore. So it is privacy. they deny all access toward mental health patients." (P61)(M)

When this access is denied, pharmacists cannot see their progressions while taking the medications. Other than that, They are talking about limited time spent with patients either while dispensing the medications or even in a counselling room doing the existing MTAC.

"I was in MTAC DM right, I can only just see on several patient in one day. One patients already take up half an hour. So one day up to 4 patients only. Clinic of course got a lot of patients." (P62)(M)

It was based on some of the participants' experiences while dispensing medications to mental health patients was not getting enough interactions with them. .

"Patient psychiatric who did a suicide attempt… but when it times for us to counsel or getting the history taking and everything, we are not directly interact with patient. So always with their caretaker. Patient doesn't really talk with us…." (P4)(F)

Different environments, such as hospitals and Klinik Kesihatan, also differ in certain ways, such as workload, patient volume, and the absence of specialised psychiatry in the Klinik Kesihatan setting.

"If want to compare between KK setting and hospital setting, of course there is difference. Hospital has more patients and everything than Klinik Kesihatan. Same with our experience also……"(P15)(F)

4.4 Unclear role of pharmacists

One person brought up the pharmacist's lack of participation in the patient's care.

"…we don't really have much involvement in patients' treatment. Unless there is a drug-drug interaction or patient complaint, ' I just start this medication, but why it happen like this'. Then we will check….".(P1)(M)

"Haaa..is it only about adherence? If adherence maybe at the counter also we can see. I don't know, its not clear the role." (P58)(F)

4.5 Stigma

One of the interviewees mentioned that they try not to be as stigmatized as possible by being not judgemental toward mental health patients.

"We do not want to be judgemental towards them. If that patient want to tell us and share their story to us or even that patient does not want to tell me anything, that's okay. We also do not want to ask more than that about their conditions" (P63)(M)

"For me, stigma sometimes always there, because when talking with them, I don't really believe them 100%. They look okay but I don't know."

The participants also mentioned being afraid of getting into patients' privacy too much or exposing them as mental illness patients as it also disturbs their privacy.

"If we want to dispense theur medications,what is the things that we can tell to them? Because we afraid as whatever we said during dispensing will invading their privacy and confidentiality" (P61)(M)

Patients also sometimes have their own level of stigma towards themselves

"….Then we know certain patients have their own stigma. Sometimes when we told them, they looks okay, but sometimes they are not okay….." (P61)(M)

5. Training needs for pharmacists

The participants showed us that training among pharmacists is very well much needed to everyone. One of them tell us how MTAC training usually takes place.

"When we get trained, we must also have a preceptor. So basically preceptor is like a master for everyone. Other states also same this, for example hepatitis B, we will be trained at Hospital Selayang, for 2 weeks. So if psychiatry, if you want to have the clinical part, have to train at the hospital that specializes first, here in Malaysia, we have to find somehow. We have to train first, then that person will go to clinical and train everybody else…."(P18)(F)

"….of course need some kind of training like full courses of learning and also attachement/tagging and not only with doctors, but tagging with other healthcare workers too, nurses, MA…."(P49)(F)

Another participants elaborated again the time needed to have the MTAC training is quite long actually to be well certified

"MTAC now got 2 phase. Phase 1, 2 weeks training plus with 6 months in total. Not sure if eco training is just 2 weeks also.." (P23)(F)

One participant said that training to interact with mental health patients is also important.

"Our way to... Interact. Our way of responding to patients' complaints towards us or anyone, it is important. We cannot simply ignored." (P23)(F)

Many participants expressed what kind of training they want to get to learn more about mental illness disease. They were asking a well-rounded kind of training to handle mental health patients.

"…prefer something like courses with hands on training. 1 day courses but still very interactive." (P31)(F)

"The other day, I get the resources on how deal if patients attack us…." (P29)(F)

"..Need to learn on how to communicate efficiently. We learn On how to be assertive in a way. Learn what is the coping mechanism and its example….."(P31)(F)

"….tagging, tagging at the place that already establish like psychiatry clinic, because when we want to set up MTAC that have no guideline at all, we have to bring the established one and follow them."(P56)(M)

"I think we better have a courses than theory, but better if we do the technical and practical part..."(P53)(M)

Another participant showed concerns if we get the training, we must make sure that the service is established first.

"If in terms of training… of course we can go to training, but we have to see first where is the place that it should be practice. Sometime when we already become specialist in certain field, then the facilities that we work suddenly not give that services." (P41)(M)

6. Improvement on the role of pharmacists and future role

One of many things to be improved in the future is the guidelines and SOP suitable for pharmacists to give the best services to mental health patients. A participant expressed that the absence of standard operating procedures and guidelines for dispensing medications at the counter causes pharmacists to feel uncertain and uneasy about doing so.

"haa..there is no SOP, ok if patient rude, at least we can tell them, okay puan, if puan still act like this, we have to call our guard. For example, have this kind of sop, if there is patient who act aggressive, we can tell them, puan, puan is now disturbing my work as government worker, so if puan still insists and being stubborn like this, we will call the guard. Atleast if like this happen again, at least at that time we know what to do, but right now? Right now if anything happens, haaa we are the one become stressed, sometime leave the dispensing counter for a while, went inside and screaming because become too mad already."(P18)(F)

In the future, pharmacists will also need to play a bigger role in psychiatry medications.

"..Have to be more involved. I feel if want to refer patients to MTAC, need to be more involved and training, with the medications, interactions among doctor with patients, we have to be tagging with them. If only at outpatient, just accept the prescriptions, I think we just dispensed the medication just like we dispensed other medications, we do not even know about the mental illness diseases and the indications for every patients..."(P28)(F)

"We should join the first aid programe on Psychology first aid courses. haa..because sometime they ask us to join, they do a program, but at that time was during flood. We listen to the victims of flood, but that time I just join with the counsellor here, not with other pharmacists" (P33)(F)

"I mean, all of us need to be involved, integrated, I do have an experience before this when I did a group counselling with the counsellor, at that time me, counsellor and patients. So we will discuss, either everything is okay or not. So we know the progression of the patients, so we as a pharmacist also can take part, medication okay or not, so at least there will be the positive outcomes."(P53)(M)

The interviewee also highlighted the benefits of establishing a dedicated MTAC Psychiatry at local health care, particularly at the hospital and Klinik Kesihatan:

"If the dispensing part, counselling part, is done at the clinic done by Pharmacist MTAC, this can reduce the congestions that always happen at the pharmacy counter. Usually, if there is delay, the waiting time also increases. We do have our own KPI on waiting time part."(P31)(F)

In the future, one participant also showed some interest in getting better skills communication to deal with mental health patients

"maybe we will have different approach, counselling technique and also communication skills are very important element in terms of pharmacist, another things mental health patients usually a long term one, so every patient has their own approach, not everybody is one for all or all for one, the approach must be individualized." (P53)(M)

Discussion

This qualitative study investigated the perspectives and experiences of pharmacists from different settings regarding mental health services in pharmacy services. During the interview, 6 main themes were found out. Most participants talked about pharmacists' limited role in mental health care, the lack of pharmacists-patient interactions, the importance of collaborations with other healthcare workers, and the training needs for pharmacists in mental health care.

The first theme is about the role of pharmacy in mental health care. A lot of interviewees mentioned

that the role of the pharmacy is only to dispense the medications at the outpatient clinic counter and make sure mental health patients comply with their medications. These specialised chemists' roles at community mental health facilities have been described in the past as involving medication dispensing and education, as well as collaborating with doctors and nurses to optimise drug therapy.

Other than that, they also mentioned that just a few mental health patients did ask them about the side of medications they are taking. Consistently, from another study, also demonstrated expertise in classic pharmacists' tasks, such as information provision, in line with earlier findings, preparing, distributing, and teaching patients about the proper and safe use of the medication (Mohiuddin et al., 2019). The participants said that ensuring compliance is always pharmacists' main duty in any setting. Ensuring compliance is important for mental health patients to prevent them from getting relapsed state and to make patients have a better quality of life. In addition, studies have shown that pharmacist-led initiatives, which have mostly centred on teaching and supervision, are successful in enhancing antidepressant drug adherence (Al-Jumah et al., 2012).

Another subthemes for the first theme is to ensure the correct medication and dose when dispensing. This is to prevent any medication error occur. The last subtheme is pharmacists are seen only to counsel patients about the side effects of medication even though most of them claim that it is also very rare for patients to talk with them first about the side effects of medication, but it still happens from time to time. Through the provision of pharmacological care interventions, pharmacists can also actively contribute to the field of mental health treatment (Mohammed et al.,2016). Medication review services, like the Home Medicines Review program in Australia, involve pharmacist-led medication reviews in conjunction with general practitioners and offer another way for pharmacists to contribute to multidisciplinary healthcare, including mental healthcare. Pharmaceutical care interventions can include these types of services (Chen et al.,2016).

This led to theme number 2, pharmacist-patient interactions. Most of the participants claimed that the only place they try to communicate with mental health patients is at the dispensing counter at the outpatient clinic, the topic conversations are also very short and pharmacists only say what kind of medication they will get at that time. However, it is still a problem with the terms used to them while dispensing the medication to them because pharmacists try not be judgemental and take care of their privacy. The pharmacists always encounter most of mental health patients are rude and quite demanding. These mental health patients become more irritated because the waiting time at the counter is taking so long. However, most of the pharmacists also emphasized that they need training for them to communicate better with mental health patients Effective counselling sessions can result from improved communication, and pharmacists are required to employ effective counselling techniques, such as providing patients and carers with verbal and written information (Chen et al.,2012). Finding from Abousheishaa et al., 2022, showed however, that the majority of pharmacists did not give the patients' or carers' educational or adherence-supporting resources. Maybe as a result of the chemists' lack of access to trustworthy information sources or the absence of patient demand, which have both been widely mentioned as obstacles to the delivery of pharmaceutical treatment in this study.

The third theme is about the interprofessional collaborations between pharmacists and other healthcare workers. From the interviews, the participants showed interests and asking the cooperations from the doctors and other healthcare workers to treat mental health worker. If MTAC is being established, pharmacists in the interviews give an opinion about the clear role of them and between doctors. This to prevent any redundant job scope and make sure all the pieces of information we give to patients is align with one another. The second subtheme from this theme is the communication between pharmacists and other healthcare workers. The participants shared that doctors only communicate with pharmacists only asking about the availability of the medications, the alternatives of the medications, drug-drug interactions, history of patients' previous medications, and some of them asking about side effects of the medications. Based on other studies, they might think that pharmacists' job scope is only limited to preparing and dispensing medications (Aldhafeeri et al.,2022). There is study that tell us from a point of view other healthcare workers, that a lack of cooperation between doctors and pharmacists, pointing out the dearth of pharmacists

at hospital wards thus leads to a significant gap in their interactions (Kempen et al.,2020). Most of the participants agree on the multidisciplinary approach for mental health patients that involves pharmacists. Other study also shows the same result as pharmacists were highly encouraged to provide pharmaceutical services to patients directly instead of just focusing on medications administrations records to ensure maximum patient care and prevent any drug-related problems (Mohiuddin et al.,2019)

The fourth themes from these interviews is the challenges and barriers among pharmacist in mental health services. The first subthemes, that is very common across all the focus group discussions is shortage of staff. This issue has been a challenge for a long time and to a lot of many departments in hospitals or clinics. Increasing workload also leads to a shortage of staff. The policy in KKM somehow stated that if want to add more staff to certain departments, they must provide new services. The participants suggest to add staff so that MTAC of psychiatry could be established and this is quite frustrating situations among pharmacists as they need more staffs to reduce the workload not the other way around. The second subthemes are the lack of awareness among pharmacists about mental health patients. Most pharmacists are not really exposed much to mental health patients and mostly they only encounter between themselves only at the outpatient clinic. All the awareness program about mental health in a hospital or clinic, they rarely invite pharmacist to join them together. Mostly the activities or the charity activities are only among the patients, doctor, nurse and MA. This was supported by other study showed that pharmacists lack the requisite training to practise mental health, maybe as a result of pharmacy universities' inability to offer such instruction. Therapeutics is the foundation of psychiatry education, and internships in the field are scarce, particularly for pharmacists (Aldhafeeri et al.,2022).

The third subthemes are the lack of resources, time and space to do the mental health pharmacy services. Lack of SOPs and guideline about handling mental health patients one of the most significant barriers seen among pharmacists. Even though the participants show some positive attitude to provide pharmacy services towards mental health patients, but they still feel reluctant as they felt they are still lack of knowledge to manage them. They did not know how to respond if patient shows unpredictable behaviour in front of the pharmacy counter. This is the same result on study by Soliman et al., (2020), pharmacists showed more negative views towards treating patients with depression than physical disorders, despite their confidence in many areas of working with patients who had depression. The participants also explained that pharmacists only can access up to their medication's history and current medications. Patient history and other cases notes are being denied. This will lead to pharmacists cannot even see their progressions. Pharmacists only can communicate mostly at the outpatient clinic counter during dispensing the medication, because there is KPI of waiting time, the participants claimed that they do not have enough time to ask patients more questions. Another subtheme is the unclear role of pharmacists if MTAC psychiatry being developed. The participants hope there will be no redundant in terms of our role as a pharmacist to doctors' role. The last subthemes are stigma. One of the participants showed that he tries hard not to be judgemental as mental health patients also deserved some privacy and he felt no stigma at all. Most of the participants showed not really have a stigma or not, but they have to control themselves especially when the patients might be aggressive or difficult to handle.

The fifth themes from these interviews are training needs. Most of the participants agreed that all pharmacists need training on how to deal mental health patients. In order for pharmacists to help the management of mental health issues and crises within the parameters of their area of practice, they must receive adequate training. In addition, well-trained pharmacists can help lessen the stigma associated with mental illness and increase access to and knowledge of mental health treatments, all of which can be obstacles to receiving mental healthcare (El-Den et al., 2021). They give several suggestions on what kind of training that they should have especially on how to communicate with mental health patients. The training should be hands on, or one day courses of continuous pharmacy educations (CPE) and interactive sessions. Some of the participants suggests to directly tagging with psychiatry doctor in ward or clinic because they believe face-to-face training is more effective. The training also should include the general guideline about the mental illness treatment and the standardised SOPs on how to handle them. This was supported by other study in Saudi Arabia, efforts are underway to provide specific training

programmes for each sector of the mental health services industry as well as to standardise and grow mental health services throughout the country (Koenig et al.,2014). One of the participants shared on the latest guideline of standard MTAC training from KKM. The training should be 2 weeks at other facilities with the preceptor there, then another 6 weeks to apply what have been trained and after that have to train another pharmacist also. This should be applied to if want to establish the MTAC in future. For example, one training program called Mental Health First Aid (MHFA) has been used worldwide and gained recognition as a pertinent and essential for all healthcare workers when dealing with mental health patient. Evidence of MHFA training in pharmacy extends back to 2011, when self-reported knowledge, confidence, attitudes, and behaviours among MHFA-trained pharmacy students increased in a controlled trial on Australian pharmacy students (O'Reilly et al., 2011).

The last theme found from this study is the improvement role of pharmacists in the future. Most of the participants hope that in the future there will be a standardised guideline and SOPs for them to handle mental health patients. Other than that, they hope that they can be more involved in mental health patients' treatment together with other healthcare workers. This is the same finding with another study that recommends that to strengthen the bonds between HCPs and pharmacists and address issues stemming from inadequate communication between the two, pharmacists should engage in more direct and team-based patient care (Aldhafeeri et al.,2022). Based on the current situation in the pharmacy at the outpatient clinic, the number of patients always increasing and always affects the KPI of waiting time for patients to receive their medications. One of the participants said that if there is MTAC Psychiatry, it will reduce the congestion of patients at the pharmacy counter because their prescriptions will be dispensed during MTAC sessions. There will be many future roles of pharmacists in Malaysia that can be adapted from other countries' practices. For example, the results of a recent systematic review of community pharmacist-led depression screening for adults indicated that more thorough, high-calibre research was required to show the cost-effectiveness and clinical implications of this approach, even though pharmacists could identify people living with undiagnosed depression using screening tools (Miller et al., 2020). Another example in Nova Scotia, Canada, The Bloom Program was created to improve the way chemists treat patients who have a personal history of addiction and mental illness with the improvement of 78% medication issues. A 27-month demonstration project was used to assess the Bloom Program, demonstrating the significant responsibilities that pharmacists may play in pharmaceutical efficiency, instruction, and assistance in navigating the healthcare system (Murphy et al., 2019).

Conclusion

This qualitative study offers the opinions of the pharmacists in the Kuantan and Pekan, Pahang area, about their perspectives on current mental health services in their facilities, their own experience and toward mental health patients, psychiatry doctors, and other healthcare workers. This study shows that pharmacists can always show various potentials and be the most important components in our healthcare systems, either at the hospital level or primary care. It has been proposed that specialty counters, private counselling rooms, and MTAC Psychiatry will enhance mental health services in every hospital and primary care setting. In the end, it was determined that ongoing assistance from other healthcare professionals was required for a successful procedure. To enable pharmacists to be integrated into mental healthcare, further research is needed to show the therapeutic results and financial viability of these roles teams frequently in a lot of practice environments. Pharmacists are dedicated to developing the best plans to give patients the best care possible despite the obstacles. The early descriptions of pharmacists were always about optimizing the use of drugs and providing education about medicines. As a result, mental health pharmacists are now essential members of the clinical management teams in inpatient and outpatient settings in the 21st century.

This study only limited at Kuantan & Pekan, Pahang area. There might be differences or additional perspectives and opinions form pharmacists at other regions, urban area or rural area. Despite that this study consisted quite large samples and the saturations data is quite saturated from this study.

Authors contributions

The corresponding authors NA and SZ provided the main objectives of this study and the methodology framework for the research project. She and her other co-authors (KAAR, CJY, NFAG, YI and ARR) performed the focus study group interview with the participants at respective allocated locations. The author NA transcribed and performed the data analysis, and her co-author SZ validated them. the author NA was writing the original draft preparation, SZ; supervised and reviewed the draft. All other co-authors listed proofread the draft. SZ and NSAR; was doing the project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript."

Acknowledgements

The authors extend sincere gratitude to Sultan Ahmad Shah Medical Centre @ IIUM (SASMEC @ IIUM) as the funder for the SASMEC Research Grant (SRG), with project ID SRG21-032-0032. Additionally, we want to thank to all the pharmacy staff at SASMEC @ IIUM, PKD Kuantan, Pahang, Hospital Tengku Ampuan Afzan (HTAA) Kuantan, Pahang, Hospital Pekan, Pekan, Pahang and PKD Pekan, Pahang for all their cooperations and commitments to this study.

Ethical approval statement

This study was approved under the Medical Research and Ethics Committee (MREC) of Ministry of Health (MOH) Malaysia, with the identification number of: NMRR ID-23-01687-IE3.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Conflict of interest

The main author NA and other investigators declares no conflict of interest throughout the study

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author declared the usage of AI which is ChatGPT. It is used to improve readability and language. After using the ChatGPT, NA reviewed and edited the content as needed and took full responsibility for the content of the publication.

References

A Aldhafeeri, B.D. (2022). Qualitative analysis of healthcare providersand administrators' perceptions, expectations, barriers, and facilitators towards pharmacists in mental healthcare in Saudi Arabia. *Pharmacy education*,22(1), 142-154. https://doi.org/10.46542/pe.2022.221.142154

Abousheishaa et al. Psychiatric pharmaceutical care service across Malaysian hospitals: results from a cross-sectional study. BMC Health Services Research (2022) 22:321 https://doi.org/10.1186/s12913-022-07681-4

Al-Jumah KA, Qureshi NA. Impact of pharmacist interventions on patients' adherence to antidepressants and patientreported outcomes: a systematic review. Patient Prefer Adherence. 2012;6:87-100. https://doi.org/10.2147/ppa.s27436

Almanasef, M. (2021). Mental health literacy and help-seeking behaviours among undergraduate pharmacy students in abha, saudi arabia. Risk Management and Healthcare Policy, 14, 1281–1286. https://doi.org/10.2147/RMHP.S289211

Bell, J. S., Rosen, A., Aslani, P., Whitehead, P., & Chen, T. F. (2007b). Developing the role of pharmacists as members of community mental health teams: Perspectives of pharmacists and mental health professionals. *Research in Social and Administrative Pharmacy*, *3*(4), 392–409. https://doi.org/10.1016/j.sapharm.2006.10.005

Braun V, Clarke V. Using thematic analysis in psychology. Qual Res Psychol. 2006;3(2):77–101

Chang, C. M., Li, Y., Or, L. L., Pikkarainen, M., Pölkki, T., Wang, W., Lau, S. T., & He, H. G. (2020). A Qualitative Study Exploring Singaporean Parents' Perceptions on

PreParing their Child for Surgery. Journal of Pediatric Nursing, 54(2020), e69–e77. https://doi.org/10.1016/j.pedn.2020.05.004

Chung, A., Vieira, D., Donley, T., Tan, N., Jean-Louis, G., Kiely Gouley, K., & Seixas, A. (2021). Adolescent Peer Influence on Eating Behaviors via Social Media: Scoping Review. Journal of Medical Internet Research, 23(6), e19697. https://doi.org/10.2196/19697

Daryanti, I., Imanuel, S., Septriani, I., Advani, S., Oktaria, S., Suarilah, I., & Lin, C. (2021). International Journal of Nursing Studies Global prevalence of mental health problems among healthcare workers during the Covid-19 pandemic : A systematic review and. *International Journal of Nursing Studies*, *121*, 104002. https://doi.org/10.1016/j.ijnurstu.2021.104002

Devraj, R., Gupchup, G. V, & Henson, D. (2019). Mental Health Literacy of Pharmacy Students Compared to Nursing and Medical Students. INNOVATIONS in Pharmacy, 10(4), 2. https://doi.org/10.24926/iip.v10i4.2091

El-den S, Collins JC, Chen TF, O'Reilly CL. Pharmacists' roles in mental healthcare: Past, present and future. Pharmacy Practice 2021 Jul-Sep;19(3):2545.

Gan, Y. L., & O'Reilly, C. L. (2018). Community pharmacists' attitudes and opinions towards supplying clozapine. *International Journal of Clinical Pharmacy*, *40*(5), 1116–1130. https://doi.org/10.1007/s11096-018-0676-y

Henderson, C., Evans-Lacko, S., & Thornicroft, G. (2013). Mental illness stigma, help seeking, and public health programs. *American Journal of Public Health*, *103*(5), 777–780. https://doi.org/10.2105/AJPH.2012.301056

Institute for Public Health. National Health & Morbidity Survey 2015. Volume II: Non-Communicable Diseases, Risk Factors & Other Health Problems. Ministry of Health Malaysia, 2015

Kempen, T., Kälvemark, A., Sawires, M., Stewart, D., & Gillespie, U. (2020). Facilitators and barriers for performing comprehensive medication reviews and follow-up by multiprofessional teams in older hospitalised patients. European Journal of Clinical Pharmacology, 76(6), 775-784. https://doi.org/10.1007/s00228-020-02846-8

Koenig, H., Al Zaben, F., Sehlo, M., Khalifa, D., Al Ahwal, M., Qureshi, N., & AlHabeeb, A. (2014). Mental Health Care in Saudi Arabia: Past, Present, and Future. Open Journal of Psychiatry, 04(02), 113-130. https://doi.org/10.4236/ojpsych.2014.42016

Liu, K. S. N., Chen, J. Y., Sun, K.-S., Tsang, J. P. Y., Ip, P., & Lam, C. L. K. (2022). Adolescent Knowledge, Attitudes and Practices of Healthy Eating: Findings of Qualitative Interviews among Hong Kong Families. In Nutrients (Vol. 14, Issue 14). https://doi.org/10.3390/nu14142857

Maroun RA, Thackeray LA, Midgley N. Meaning and medication: a thematic analysis of depressed adolescents' views and experiences of SSRI antidepressants alongside psychological therapies. BMC Psychiatry. 2018;18(1):374.

Martí-García, C., Fernández-Férez, A., Fernández-Sola, C., Pérez-Rodríguez, R., Esteban-Burgos, A. A., Hernández-Padilla, J. M., & Granero-Molina, J. (2023). Patients' experiences and perceptions of dignity in end-of-life care in emergency departments: A qualitative study. Journal of Advanced Nursing, 79(1), 269–280. https://doi.org/10.1111/jan.15432

McMillan SS, Stapleton H, Stewart V, Wheeler AJ, Kelly F. A qualitative study exploring opportunities for pharmacists to connect with young mental health consumers. J Am Pharm Assoc (2003). 2020;60(5S):S23–33

Miller P, Newby D, Walkom E, Schneider J, Li SC. Depression screening in adults by pharmacists in the community: a systematic review. Int J Pharm Pract. 2020;28(5):428-440. https://doi.org/10.1111/ijpp.12661

Mohammed MA, Moles RJ, Chen TF. Impact of Pharmaceutical Care Interventions on Health-Related Quality-of-Life Outcomes: A Systematic Review and Meta-analysis. Ann Pharmacother. 2016;50(10):862-881. https://doi.org/10.1177/1060028016656016

Mohiuddin, A. (2019). Psychiatric Pharmacy: New Role of Pharmacists in Mental Health. Scholarly Journal of Psychology and Behavioral Sciences, 2(4). https://doi.org/10.32474/SJPBS.2019.02.000144

Murphy AL, Gardner DM, Jacobs LM. The patient experience in a community pharmacy mental illness and addictions program. Can Pharm J (Ott). 2019;152(3):186-192. https://doi.org/10.1177/1715163519839424

Ning C. The economic cost of mental disorders in Malaysia. Lancet Psychiatry 2020; 7(4): e23

O'Reilly CL, Bell JS, Kelly PJ, Chen TF. Impact of mental health first aid training on pharmacy students' knowledge, attitudes and self-reported behaviour: a controlled trial. Aust N Z J Psychiatry. 2011;45(7):549-557. https://doi.org/10.3109/00048674.2011.585454

Ose, S.O. (2016). Using excel and word to structure qualitative data. *Journal of Applied Science*, 1-16. DOI: 10.1177/1936724416664948

Pharmacy SCoSPiMH. Standards of Practice for Mental Health Pharmacy. J Pharm Pract Res. 2012;42(2):142–5.

Shaeraine R, Sujesha N, Myelone T, John L. Mental disorders in Malaysia: an increase in lifetime prevalence BJPSYCH INTERNATIONAL (2021) 18:4. doi:10.1192/bji.2021.4

Silva SN, Lima MG, Ruas CM. Pharmaceutical interventions in mental health: a review of the literature to support evidence-informed policymaking. Res Social Adm Pharm. 2018;14(10):891e900.

Soliman, M. (2020). Pharmaceutical Care in Depression: A Survey of Stigma, Confidence, Attitudes, and Barriers. Risk and management and healthcare policy, :13 2611–2620 . w.dovepress.com/ by 77.220.193.115

Wakida, E. K., Talib, Z. M., Akena, D., Okello, E. S., Kinengyere, A., Mindra, A., & Obua, C. (2018). Barriers and facilitators to the integration of mental health services into primary health care: A systematic review 11 Medical and Health Sciences 1117 Public Health and Health Services. In *Systematic Reviews* (Vol. 7, Issue 1). https://doi.org/10.1186/s13643-018-0882-7

Werremeyer A, Skoy E, Kelly GA. Use of photovoice to understand the experience of taking psychotropic medications. Qual Health Res. 2017;27(13):1959e1969.

Wheeler A, Crump K, Lee M, Li L, Patel A, Yang R, et al. Collaborative prescribing: A qualitative exploration of a role for pharmacists in mental health. Research in Social and Administrative Pharmacy. 2012;8(3):179–92

Wheeler A, Fowler J, Hattingh L, Kelly F. Exploring the role of community pharmacy in supporting mental health consumers and carers. Available at: http://6cpa.com.au/resources/fifth-agreement-rd/mental-healthproject/. 2019

Yeap, R., & Low, W. Y. (2009). Mental health knowledge, attitude and help-seeking tendency: A Malaysian context. *Singapore Medical Journal*, *50*(12), 1169–1176.

Zaini, S., Bharathy, H. A. M., Sulaiman, A. H., Gill, J. S., Hui, K. O., Huri, H. Z., Shamsudin, S. H., & Guan, N. C. (2018). Development of a strategic tool for shared decision-making in the use of antidepressants among patients with

major depressive disorder: A focus group study. International Journal of Environmental Research and Public Health, 15(7). https://doi.org/10.3390/ijerph15071402

Appendix A

Table A1: FGD topic guide (overview)

Welcoming speech from the research interviewer with her supervisor and co-supervisor (if there is any).
"Assalamualaikum and hello to everyone. My name is ……. as your interviewer today. Here is my supervisor for this research Dr……. thank you again for participating in this interview. Every respond and answers, we are really appreciating that."
Overview for the overall topic of the interview
Our topic is about implementing pharmacy services in mental health services.
The result from this interview will be one of the sources of information regarding perspectives of pharmacists in mental health pharmacy services.
Thank you for willing to participate in this interview and sharing some opinions about this topic.
The ground rules of this focus group and assurance of confidentiality
There are no right or wrong answers.
Only differing points of views.
We are tape recording and one person speaking at a time. All the recordings will not to be share with any organizations or anyone as this is solely for research purpose only.
We are on a first name basis.
You do not need to agree with others, but you must listen respectfully as others share their views.
My role as an interviewer will be to guide the discussion.
So, here are the questions (Table A2):

Table A2: FGD topic guide (main questions)

Current Roles in mental health services in your place:
What do you consider the current role of pharmacist in mental health care?
Tell me about your experiences with mental health patients. Tell me more about your interactions and relationship with the patients.
Collaborations within HCP:
In your opinion, what say you on the statement that pharmacists also should be included in the mental health care besides psychiatrists?
What do you believe to be the key benefits of having a pharmacist in a multidisciplinary mental health team?
Barriers & Facilitators to the provision of care:
What do you see as the facilitators of mental health pharmacy services?
What do you see as barriers of mental health pharmacy services? In your opinion, what are the reason/s for pharmacist not having a prominent role in mental health primary care?
Perceptions of what mental health pharmacy services can be expected to provide for the patient:
What mental health pharmacy services do you expect to be provided to the patients in your facilities?
What ideas do you have for improvement of (mental health pharmacy) services provided in your facilities?

References: (McMillan *et al.*, 2020; Morris, Wong and McKinlay, 2021; Adam and Keers, 2022; Crespo-Gonzalez *et al.*, 2022)

Journal of Pharmacy

In vitro antiplasmodial activity of six plants against chloroquine-sensitive and resistant strains of *Plasmodium falciparum*

Samuel Korsah[1]*, Nathaniel Nene Djangmah Nortey[2], John Antwi Apenteng[1], Felix Kwame Zoiku[3], Jessica Korsah[1], Miriam Tagoe[1], David Ntinagyei Mintah[1], Nana Adwoa Boamah-Danso[1], Kanati Perry[1] and Prince Antwi[1]

[1] *Department of Pharmaceutical Sciences, School of Pharmacy, Central University, Accra-Ghana.*
[2]*Department of Natural Product Sourcing and Herbarium, Institute of Traditional and Alternative Medicine, University of Health and Allied Sciences, Ho, Ghana.*
[3]*Noguchi Memorial Institute for Medical Research, University of Ghana.*

Abstract

Introduction: The effectiveness of the first-line malaria treatment has been affected by drug resistance and adverse side effects leading to a limited number of treatment options. This calls for the search for alternative antimalarial agents. The study evaluated the *in vitro* antimalarial activity of six plants frequently used in herbal antimalarial products in Ghana against chloroquine-sensitive strain (3D7) and chloroquine-resistant strain (DD2) of *Plasmodium falciparum*. **Method:** Aqueous extracts were prepared from the plants by decoction and freeze-dried. A fluorescence-based SYBR Green assay was used to evaluate the antimalarial activity of the extracts against *Plasmodium falciparum* strains 3D7 and DD2. Also, the cytotoxic effects (CC_{50}) of the plant extracts against red blood cells were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) rapid calorimetric assay technique. **Results**: *Alstonia boonei*, *Cryptolepis sanguinolenta,* and *Azadirachta indica* were the most effective mono-herbal extracts with IC_{50} of 8.64 µg/mL, 6.12 µg/mL, and 5.22 µg/mL respectively against 3D7 lab strain and 8.47 µg/mL, 5.12 µg/mL and 5.22µg/mL respectively against DD2 lab strain. The aqueous extracts of *Paullinia pinnata, Citrus aurantiifolia,* and *Tetrapleura tetraptera* exhibited moderate activity against both lab strains with IC_{50} values of 24.72 µg/mL, 34.89 µg/mL and 14.94 µg/mL respectively against 3D7 strain and 14.84 µg/mL, 31.01 µg/mL and14.74 µg/mL respectively against DD2 strain. All plant extracts exhibited no cytotoxicity against RBC (>100 µg/mL, except *Cryptolepis sanguinolenta* with CC_{50} 92.7 µg/mL). Moreover, except *Paullinia pinnata, Citrus aurantiifolia* and *Tetrapleura tetraptera* (with low selectivity index: SI < 10), all the plants displayed a good selectivity index (SI>10). **Conclusion**: All six frequently used antimalarial plants in monotherapy possess significant antimalarial activity against *Plasmodium falciparum* (3D7) and (DD2) strains. The data obtained from this study support the folkloric and frequent use of these plants in several herbal antimalarial products on the Ghanaian market.

Article history:

Received: 10 June 2024
Accepted: 23 October 2024
Published: 31 January 2025

Keywords:

In vitro antimalarial
Antiplasmodial
Resistant strains
Cytotoxicity
Plant extracts

doi: 10.31436/jop.v5i1.319

*Corresponding author's email: korsahjnr@gmail.com

Introduction

Malaria is a major tropical disease with high morbidity and mortality, affecting about 3 billion of the world's population, particularly those residing in Asia, Latin America, and Sub-Saharan Africa. The sub-Saharan Africa region carries a disproportionate share of 90% of the global malarial load and accounts for 92% of the total malaria deaths(Wang, 2022).

A major setback in the treatment of malaria has been the development of resistance by the parasite to most single-molecule allopathic drugs used as well as the cost of its management. Also, pharmaceuticals of herbal origin are acquiring more relevance on the global market due to increased awareness of the side effects of synthetic drugs and Africa, especially which has compelled reputable pharmaceutical corporations to introduce herbal based formulations to the market (Chaitanya, 2021).

Herbal medicines have a long history, almost as far as human history. The majority of these plant-derived pharmaceuticals were discovered through traditional treatments and Indigenous people's folk knowledge, and some of them could not be replaced despite enormous advances in synthetic chemistry (Rizvi et al., 2022).

People around the world have developed their techniques for prevention and treatment of diseases (Aslam et al., 2016). In Asian and African nations, about 80% of people depend on native therapeutics for their main healthcare (Karole et al., 2019). Malaria is a disease caused by a plasmodium parasite, transmitted by the bite of infected mosquitoes (White, 2022) . There are 229 million cases of malaria worldwide and more than 400,000 people die from the disease each year, according to the World Health Organization (2020, 1997). In the WHO African Region, *Plasmodium falciparum* is the most chronic and lethal species infecting people, accounting for 96% of malaria cases and fatalities in 2020 in the 29 endemic African nations (Essoh et al., 2022). The rise of drug-resistant parasites in some regions of the world threatens the effectiveness of artemisinin-based combinations, which have been a successful weapon in efforts to eradicate malaria (Ouji et al., 2018).

In vitro, antimalarial assay necessitates continuous cultivation of *P. falciparum* and any other lab strains. It enables a quantitative evaluation of intrinsic drug sensitivity that is based on microscopic parasitemia evaluation and inhibitory concentration(IC) determination (Chetia, 2019). By comparing the inhibition of parasite growth in drug-exposed cells to *Plasmodium* drug-free control cultures, antimalarial medication activity is assessed, and Sigmoid dose-response curves are produced to evaluate the efficacy of compounds in terms of IC after testing the active substances in the primary tests in serial drug dilution. Microscopic tests have been replaced by other techniques such as the hypoxanthine 3 incorporation method (microculture radioisotope technique), the flow cytometry assay (SYBR Green I-based), colorimetric ELISA tests (pfLDH assay, pfHRP2 assay), and fluorescence assay (pfGFP-based, SYBR Green I-based). These approaches are generally straightforward, necessitate fewer time-consuming test steps, and offer high throughput, but they call for pricey equipment.

The research was carried out based on most frequently used plants in herbal antimalarial products on the Ghanaian market as reported in our previous work (Nortey et al., 2023), which are *Alstonia boonei* (leaves), *Azadirachta indica (leaves)*, *Citrus aurantiifolia* (leaves), *Cryptolepis sanguinolenta* (roots), *Paullinia pinnata* (leaves) and *Tetrapleura tetrapetra* (fruits). These plants were collected and screened against chloroquine-sensitive (3D7) and resistant (DD2) strains. All the plants have previously been evaluated for their antiplasmodial activity and antimalarial bioactives identified, however, a few of the plants have yet to be assayed against the 3D7 and DD2 strains (Table 1). *Plasmodium falciparum* has developed resistant strains to chloroquine due to a mutation in the *Pfc*rt gene present in the parasite's food vacuole (Turschner & Efferth, 2009). They possess a neutral threonine instead of the positively charged Lysine at position 76 of the *Pfc*t gene. This mutation causes reduced accumulation of chloroquine within the food vacuole and allows chloroquine to diffuse away from the food vacuole by a steep downward concentration gradient (Sanchez et al., 2004) (Cooper et al., 2002) (Sanchez et al., 2004).

This study sought to provide the scientific basis for their selection in the formulation of polyherbal preparations and compare their antiplasmodial effects against 3D7 and DD2 strains of *Plasmodium falciparum*.

Table 1: Previous antimalarial reports on the plants against chloroquine sensitive(3D7) and chloroquine resistant strains (DD2) of *Plasmodium falciparum* strains

Plants	Known antimalarial phytochemicals present	Extraction method	*In vitro* assay (IC_{50}, µg/mL)		References
			3D7	DD2	
Paullina pinnata	Alkaloids, terpenoids flavonoids, saponin, and coumarins	N/A	N/A	N/A	(Fred-Jaiyesimi & Anthony, 2011)
Citrus aurantiifolia	Flavonoids, coumarins, terpenoids and saponins	Maceration (aqueous)	71.31	N/A	(Bapna et al., 2017) (Laksemi et al., 2023) (Ettebong et al., 2019)
Azadirachta indica	Flavonoids, saponin, coumarins, terpenoids (Nimbin, azadirachtin) and alkaloids	Maceration (Ethanol)	7.52	N/A	(Deshpande et al., 2014)
Alstonia boonei	Alkaloids, triterpenes, flavonoids, saponins, terpenoids and coumarins	N/A	N/A	N/A	(Omoya & Oyebola, 2019)
Tetrapleura tetraptera	Flavonoids, saponins, alkaloids, saponins, coumarins and terpenoids	Maceration (Ethanol)	13.0	10.1	(Nsofor et al., 2023) (Lekana-Douki et al., 2011)
Cryptolepis sanguinolenta	Alkaloids (cryptolepine and cryptoquindoline), saponins, flavonoids, terpenoids and coumarins.	N/A	N/A	N/A	(Opoku-Agyemang et al., 2022)

N/A, No report available

Materials and methods

Collection and processing of plant materials.

The leaves of Azadirachta indica and Citrus aurantifolia were collected and harvested locally in the Ablekuma area, Accra, Ghana. The leaves of Paullinia pinnata, Alstonia boonei, and fruit of Tetrapleura tetraptera were harvested locally in the Nkwatia area, Kwahu, Ghana. The dried roots of the Cryptolepis sanguinolenta plant were purchased from the Centre for Scientific Research, Mampong in March 2023. The collected specimen was authenticated by Miss Miriam Tagoe, the head of the School of Pharmacy Herbarium, Central University, and voucher number CUC/F/NK/003, CUC/L/NK/007, CUC/R/AM/002, CUC/L/A/009, CUC/L/NK/008 and CUC/L/A/006 were assigned to the Tetrapleura tetraptera, Alstonia boonei, Cryptolepis sanguinolenta, Azadirachta indica, Paullinia pinnata, and Citrus aurantiifolia respectively and the samples deposited at School of Pharmacy Herbarium, Central University, for future reference.

Sample extraction

The leaves of *A. indica, P. pinnata, C. aurantifolia*, and *A. boonei* were washed gently under running water and air-dried for about 7 days. The roots of *C. sanuginolenta* were sliced into pieces, washed under running water, and air-dried for about 7 days. The dried pods of *T. tetraptera* were washed under running water, chopped into sizeable amounts, and washed again, then air dried for about 5- 10 days. The aqueous extract of the leaves, roots, and fruits of *A. indica*, *P. pinnata, C. aurantifolia, A. boonei*, *C. sanguinolenta*, and *T. tetraptera* respectively were obtained by decoction according to the Ghana Herbal Pharmacopeia. The leaves were boiled with water for about twenty minutes and bark for about thirty (Busia.ed, 2007). The cooled extracts were filtered and freeze-dried at the Noguchi Memorial Research Institute. The freeze-dried powdered *T. tetraptera*, *A. boonei*, *C. sanguinolenta*, *A. indica, P. pinnata,* and *C. aurantifolia* were assigned TTE, ABE, CSE, AZE, PPE and CAE code names respectively. Plant extracts were stored in a refrigerator until needed for use.

Phytochemical test

Phytochemical components of the respective plants were identified using conventional techniques outlined (Evans, 2009).

In vitro antiplasmodial assay

Preparation of extract and Parasite

The extracts and parasites were prepared as described in previous work by (Amengor et al., 2024). A working solution was serially diluted using Roswell Park Memorial Institute Medium 1640 (RPMI 1640) to obtain the following concentrations: 100 µg/ml, 50 µg/mL, 25ug/ml, 12.5 µg/mL, 6.25 µg/mL, 3.13 µg/mL, 1.56, 0.78 µg/mL, and 0.39 µg/ml. The parasites were obtained from the Immunology department of Noguchi Memorial Institute for Medical Research, University of Ghana. P. falciparum asexual cultures were maintained as described in (Trager & Jensen, 1976). The parasites were plated based on the description in (Gathirwa et al., 2008). This procedure was repeated for all extracts.

Extract plating and SYBR green assay

With artesunate (ATS) as the standard drug, 100mL of each of the already prepared concentrations in 2.4.1 were plated in duplicates in an already labeled (1 to 11) 96 well coastal plate. 15ng/mL of this standard drug was serially diluted and plated alongside the extracts. 100 µL of parasite mix with 2% hematocrit and 1% parasitemia were added to each treated labeled well from the 2 to 10 (Test wells). A parasite +2% hematocrit +1% parasitemia mix only was added to well no. 11 as a negative control. The procedure was repeated for the rest of the extracts. The plates were carefully arranged in a modular Chamber and gassed for 5min with a gas mixture as described in (Amengor et al., 2024). The plates were subsequently maintained at 37 °C for 72 hrs. After 72hrs, the plates were removed and assayed by the addition of 100 µL lysing buffer containing SYBR Green to each of the wells. The mixture in the plates was carefully mixed and incubated in the dark for 30-60 minutes. The plates were then read using a FLUOstar OPTIMA Fluorometer plate reader (software version 2.20) at 470nm and 520nm wavelengths.

Cytotoxicity studies

The extracts were tested for toxicity to RBCs using a modified version of the tetrazolium-based colourimetric technique. 100 µL of each extract was prepared to obtain concentrations ranging from 6.25 µg/mL to 100 µg/mL. These concentrations were instilled in triplicate into the wells of a 96-well microtiter plate. 100 μL of uninfected RBCs were subsequently put into each well. The plates were incubated and maintained and the optical densities of wells were measured based on the description in described in (Amengor et al., 2024) (Ikem et al., 2020). Extracts, culture medium, and uninfected RBCs were subtracted from the optical densities by running control experiments for each parameter independently alongside the main experiment. Artesunate which is a known antimalarial drug was used as a positive control in the cytotoxicity assay

Statistical Analysis

Each product was tested in a duplicate and the median inhibitory dose (IC_{50}) and median cytotoxic concentration (CC_{50}) values of the extracts against asexual Plasmodium falciparum was estimated from dose-response curves by non-linear regression analysis using Graph pad Prism version 7.0 Software (Graph Pad Software, San Diego, CA, USA).

Results and discussion

Phytochemical screening of aqueous extracts of plant samples.

Phytochemicals are active biologically compounds found in plants (Agidew, 2022). Some of the significant phytochemicals are coumarins, tannins, saponins, alkaloids, and glycosides. Table 2 contain the phytochemical analysis of the aqueous extracts of the plant samples.

Antiplasmodial activity

The sequel of *in vitro* antiplasmodial assay of aqueous extracts of the six selected medicinal plants against *Plasmodium falciparum* 3D7 and DD2 lab strains are graphically represented on Fig. 1. IC_{50} values of the 3D7 lab strain were generally higher than those of DD2 lab strain.

Table 2: Phytochemical screening results of aqueous extracts of plant samples.

Phytochemical	CSE	AZE	PPE	TTE	CAE	ABE
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Hydrolysable Tannins	-	+	+	+	+	+
Condensable tannins	+	-	-	-	-	-
Terpenoids	+	+	+	+	+	+
Alkaloids	+	+	+	-	-	+
Flavonoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Anthracene glycosides	+	-	-	-	-	+
Coumarins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Hydrolysable Tannins	-	+	+	+	+	+
Condensable tannins	+	-	-	-	-	-
Terpenoids	+	+	+	+	+	+

KEY: + – phytochemical detected

- – phytochemical not detected.

Cryptolepis sanguinolenta extract (CSE), *Azadirachta indica* extract (AZE), *Paullinia pinnata* extract (PPE), *Tetrapleura tetreptera* extract (TTE), *Citrus aurantifolia* extract (CAE), *Alstonia boonei* extract (ABE)

Cytotoxicity

In these studies, the in vitro cytotoxicity was expounded as follows; low selective index <10 and high (or good) selective index >10 (Obbo et al., 2019). The outcome showed that the aqueous extracts of PPE, TTE, and CSE indicated a low selective index. AZE, CSE and ABE exhibited high selective index as seen in Table 3 below.

Discussion

In this study, the antimalarial potencies of six most frequently use plants to treat malaria were evaluated against chloroquine sensitive and resistant *Plasmodium falciparum.* These plants are mostly used in antimalarial FDA approved herbal products on the Ghanaian market. Plant extracts are particularly essential in medicine, and their biologically active components are thought to be the reason for this(Dar et al., 2023). When taken orally, herbal remedies may have the desired healing effect, but they may also contain other components that could have harmful effects. Phytoconstituents are said to have a wide range of therapeutic advantages, hence it is always important to evaluate the phytoconstituents in plants with ethnomedical qualities (Shaikh & Patil, 2020). A vast source of novel and promising medicines can be found in

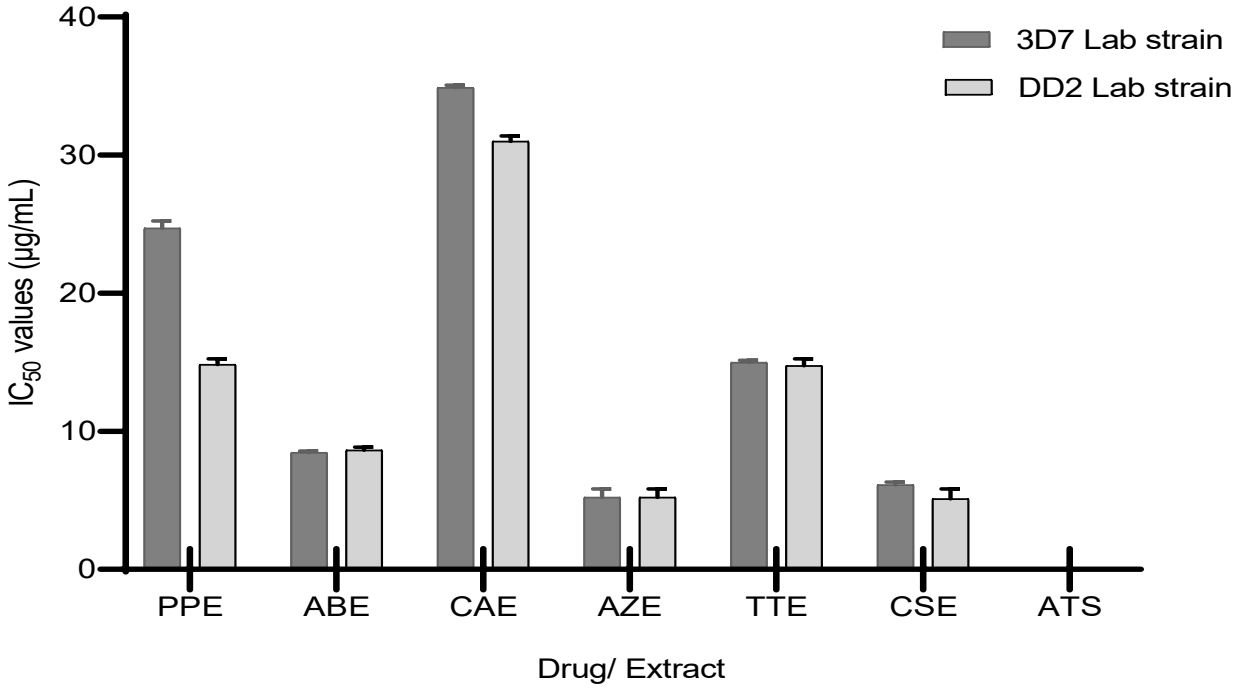


Fig. 1: IC_{50} of the various plant extracts against 3D7 and DD2 lab strains.
Key: *Cryptolepis sanguinolenta* extract (CSE), *Azadirachta indica* extract (AZE), *Paullinia pinnata* extract (PPE), *Tetrapleura tetreptera* extract (TTE), *Citrus aurantifolia* extract (CAE), *Alstonia boonei* extract (ABE) and Artesunate (ATS)

plants, which contain phytochemicals like steroids, glycosides, flavonoids, alkaloids, amino acids, saponins, tannins, and more (Majrashi et al., 2023). In addition to the health benefits associated with macronutrients and micronutrients, phytochemicals are biologically active, naturally occurring chemical compounds found in plants (Agidew, 2022). Alkaloids are responsible for most antimalarial properties of plants. The first successful antimalarial drug was the alkaloid quinine, extracted from the Cinchona tree (Hariyanti et al., 2022). There are studies on the mechanism by which some alkaloids effect antiplasmodial activity, typically amongst them is cryptolepine, found in Cryptolepis sanguinolenta. Flavonoids and saponins possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative agents and antiviral properties (Korsah et al., 2021).

From the studies, significant levels of saponins, tannins, condensed tannins, glycosides, anthracene glycosides, alkaloids, terpenoids, coumarins, and flavonoids were present in *Cryptolepis sanguinolenta* aqueous extract. Similar phytochemical composition was observed in *Azadirachta indica*. Anthracene glycosides were undetected in *Paullinia pinnata* and *Tetrapleura tetraptera* aqueous extract. *Alstonia boonei* aqueous extract showed the presence of saponins, hydrolyzable tannins, tannins, terpenoids, coumarins, flavonols, alkaloids, and anthracene glycosides (Table 2). These results are comparable to previous studies reported on Table 2.

All the plants have previously evaluated for their antiplasmodial activity and their antimalarial bioactives identified, however few of the plants have been assayed against the 3D7 and DD2 strains (Table 1). Again, none of the previous reports compared the antiplasmodial potency of these plants to assist in their selection for formulations in polyherbal preparations. Although, herb-herb polytherapy may not always favor the most performing herbal monotherapy, this kind of comparative studies will serve as a guide for the plant's selection in combination therapy in further studies. The following parameters were used to evaluate the *in vitro* antiplasmodial of the plants: high antiplasmodial activity (IC50 ≤ 10 µg/mL), moderate antiplasmodial activity (IC_{50} of 11-50 µg/mL), low antiplasmodial activity (IC_{50} of 50-100 µg/mL), and inactive (IC_{50} of ≥100 µg/mL) as reported in (Cudjoe et al., 2020).The increasing order of the antimalarial potency of plants as seen on Fig. 1 were as follows; *Azadirachta indica> Cryptolepis sanguinolenta >Alstonia boonei> Tetrapleura tetraptera> Paullinia pinnata> Citrus aurantiifolia* against the two lab strains. *Alstonia boonei, Cryptolepis sanguinolenta, and Azadirachta indica*

exhibited good antimalarial activity in 3D7 and DD2 parasites. Alkaloids isolated from *Cryptolepis sanguinolenta* exhibited significant antiplasmodial activity against K1 chloroquine strain of *Plasmodium falciparum* by (Paulo et al., 2000). Similarly, antiplasmodial studies in *Azadirachta indica* and *Alstonia boonei* by (Deshpande et al., 2014) and (Bello et al., 2009) showed similar activity. Flavonoids and phenolic compounds in medicinal plants have been linked with antimalarial activity and could be the justification for the greater activity exhibited by these plant samples. *Citrus aurantiifolia*, *Paullina pinnata and Tetrapleura tetraptera* showed moderate antimalarial activity against the 3D7 parasite. Results from the studies against these aforementioned plants with moderate antiplasmodial activity were comparable to similar reports in (Lekana-Douki et al., 2011)(Maje et al., 2007) (Bapna et al., 2014). The 3D7 lab strain were observed to be less susceptible in the plants compared to the DD2 labs train. This observation was significant in *Paullinia pinnata* with IC_{50} values of 24.72±0.50 μg/mL and 14.84±0.14 μg/mL against 3D7 and DD2 respectively. *Azadirachta indica* showed similar potency (5.22±0.26 μg/mL) against both of the chloroquine sensitive and resistant strains. In a study by (Oseni & Akwetey, 2012) reported in their *in vivo* studies that the *Azadirachta indica* showed significant *in vivo* chemosuppresion (>70%). *Tetrapleura tetraptera* also showed almost the same activity against the two strains.

RBCs are human cell line or cellular model used to study potential interactions of the blood with other substance. Morphological changes, subsequent disruption of RBC membrane integrity and hemolysis could be used to determine the cytotoxicity of various compounds (Podsiedlik et al., 2020). Cytotoxicity at the preliminary stage is important in finding the possible toxicity of plant extracts or biologically active compounds segregated from plants. In preclinical studies of a compound, ability of a compound to discriminate between the pathogen and host is very important (Elhassanny et al., 2020) (Alven & Aderibigbe, 2019).This discrimination is crucial for the safety and efficacy of the compound. *In vitro* determination of hemolytic properties is a usual and significant technique in assessing the cytotoxicity of chemicals and drugs and its advantages are being affordable, easily obtainable and simple to use (Sæbø et al., 2023). All the plants showed high selectivity for the pathogen hence low or no toxicity against RBC as seen in Table 3. Extracts with high selectivity index offer the potential of been a safer therapy (Obbo et al., 2019).

When the selective index (SI) > 10, it is usually accepted that the compound or extract is non- toxic. *Azadirachta indica, Cryptolepis sanguinolenta, Alstonia boonei* showed the highest selectivity index (SI > 10). Showing the basis for their frequent use in several herbal antimalarial products in Ghana as reported in (Nortey et al., 2023). However, *Paullinia pinnata,*

Table 3: Cytotoxicity (CC_{50}) result against red blood cells

Extracts	CC_{50} Values (μg/mL)	Selective Index
PPE	129.63	5.24
ABE	109.50	12.93
CAE	100.23	2.87
AZE	118.90	22.78
TTE	102.72	6.85
CSE	92.68	15.14
ATS	72.62	> 100

Key: *Cryptolepis sanguinolenta* extract (CSE), *Azadirachta indica* extract (AZE), *Paullinia pinnata* extract (PPE), *Tetrapleura tetreptera* extract (TTE), *Citrus aurantifolia* extract (CAE), *Alstonia boonei* extract (ABE), Artesunate (ATS)

Citrus aurantiifolia and *Tetrapleura tetraptera* exhibited low selectivity index (SI < 10). There is a need for further study on the safety profiles of these plants with low selectivity indexes.

Conclusion

It can be inferred from the studies that all the six frequently used antimalarial plants possess a significant antimalarial activity against the lab strains; chloroquine sensitive (3D7) and chloroquine resistant (DD2) strains. These data provide rationale for their frequent use in several herbal antimalarial products on the Ghanaian market. A study on the combination effects of their polyherbal usage is underway. The data reported will guide how these combinations can be carried out or if it is more economical for practitioners to use the plants in isolation. The safety of the various plants being reported is crucial in guiding their use.

Authors contributions

Conceptualization, Samuel Korsah and Nathaniel Nene Djangmah Nortey.; methodology, John Antwi Apenteng and Felix Kwame Zoiku.; formal analysis, Samuel Korsah.; investigation, Nana Adwoa Boamah-Danso, Kanati Perry and Prince Antwi.; resources, Miriam Tagoe, Jessica Korsah and David Ntinagyei Mintah.; writing—original draft preparation, Samuel Korsah and Nathaniel Nene Djangmah Nortey.; writing—review and editing, Samuel Korsah.; supervision, Samuel Korsah.; project administration, John Antwi Apenteng. Funding acquisition; this work was jointly funded by authors with no external funding. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors are grateful to the Ghana Health Service for their support in the study

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no AI or AI-assisted technologies were used in the writing process

References

Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre*, *46*(1), 87. https://doi.org/10.1186/s42269-022-00770-8

Alven, S., & Aderibigbe, B. (2019). Combination therapy strategies for the treatment of malaria. *Molecules*, *24*(19), 3601. https://doi.org/10.3390/molecules24193601

Amengor, C. D. K., Biniyam, P. D., Brobbey, A. A., Kekessie, F. K., Zoiku, F. K., Hamidu, S., Gyan, P., & Abudey, B. M. (2024). N-Substituted Phenylhydrazones Kill the Ring Stage of Plasmodium falciparum. *BioMed Research International*, *2024*(1), 6697728. https://doi.org/10.1155/2024/6697728

Aslam, M. S., Ahmad, M. S., Mamat, A. S., Ahmad, M. Z., & Salam, F. (2016). An update review on polyherbal formulation: A global perspective. *Systematic Reviews in Pharmacy*, *7*(1), 35-41. https://doi.org/10.5530/srp.2016.7.5

Bapna, S., Ramaiya, M., & Chowdhary, A. (2014). Brine shrimp toxicity and invitro antimalarial activity of Citrus aurantifolia (Christm.) Swingle against Plasmodium falciparum 3D7. *IOSR Journal of Pharmaceutical and Biological Science*, *9*(5), 24-27.

Bapna, S., Satvekar, T., Jadhav, P., & Sawant, M. G. (2017). Evaluation of in Vitro Antimalarial Activity of Calotropis gigantia and Citrus aurantifolia Flower extract against Plasmodium falciparum 3D7. *World Journal of Pharmaceutical Research*, *6*(16), 1619-1626.

Bello, I., Oduola, T., Adeosun, O., Omisore, N., Raheem, G., & Ademosun, A. (2009). Evaluation of antimalarial activity of various fractions of Morinda lucida leaf extract and Alstonia boonei stem bark. *Global Journal of*

Pharmacology, *3*(3), 163-165. https://doi.org/10.13140/RG.2.2.13010.91849

Chaitanya, D. M. (2021). Current Aspects in Pharmaceutical Research and Development. https://doi.org/10.9734/bpi/caprd/v8

Chetia, M. R. D. (2019). In vitro in vivo and models used for antimalarial activity: A brief review. *Asian Journal of Pharmacy and Pharmacology*, *5*(6), 1251-1255.

Cooper, R. A., Ferdig, M. T., Su, X.-Z., Ursos, L. M., Mu, J., Nomura, T., Fujioka, H., Fidock, D. A., Roepe, P. D., & Wellems, T. E. (2002). Alternative mutations at position 76 of the vacuolar transmembrane protein PfCRT are associated with chloroquine resistance and unique stereospecific quinine and quinidine responses inPlasmodium falciparum. *Molecular pharmacology*, *61*(1), 35-42. https://doi.org/10.1124/mol.61.1.35

Cudjoe, E., Donu, D., Okonu, R. E., Amponsah, J. A., & Amoah, L. E. (2020). The in vitro antiplasmodial activities of aqueous extracts of selected Ghanaian herbal plants. *Journal of Parasitology Research*, *2020*(1), 5041919. https://doi.org/10.1155/2020/5041919

Dar, R. A., Shahnawaz, M., Ahanger, M. A., & Majid, I. (2023). Exploring the diverse bioactive compounds from medicinal plants: a review. *J. Phytopharm*, *12*, 189-195. https://doi.org/10.31254/phyto.2023.12307

Deshpande, P. K., Gothalwal, R., & Pathak, A. K. (2014). Phytochemical analysis and evaluation of antimalarial activity of Azadirachta indica. *The Pharma Innovation*, *3*(9, Part A), 12.

Elhassanny, A. E., Soliman, E., Marie, M., McGuire, P., Gul, W., ElSohly, M., & Van Dross, R. (2020). Heme-dependent ER stress apoptosis: a mechanism for the selective toxicity of the dihydroartemisinin, NSC735847, in colorectal cancer cells. *Frontiers in Oncology*, *10*, 965. https://doi.org/10.3389/fonc.2020.00965

Essoh, A. P., Cassiano, G. C., Mandim, F., Gomes, I., Moura, M., Medeiros, M. M., Cravo, P., & Romeiras, M. M. (2022). Studies on the in vitro antiplasmodial activity of endemic and native plants from Cabo Verde. https://doi.org/10.21203/rs.3.rs-1881844/v1

Ettebong, E., Ubulom, P., & Etuk, A. (2019). Antiplasmodial activity of methanol leaf extract of Citrus aurantifolia (Christm) Swingle. *Journal of Herbmed Pharmacology*, *8*(4), 274-280. https://doi.org/10.15171/jhp.2019.40

Evans, W. C. (2009). *Trease and Evans' pharmacognosy*. Elsevier Health Sciences.

Fred-Jaiyesimi, A., & Anthony, O. (2011). Larvicidal activities of the extract and fractions of Paullinia pinnata Linn leaf. *Pharmacognosy Communications*, *1*(2), 37-40. https://doi.org/10.5530/pc.2011.2.7

Gathirwa, J., Rukunga, G., Njagi, E., Omar, S., Mwitari, P., Guantai, A., Tolo, F., Kimani, C., Muthaura, C., & Kirira, P. (2008). The in vitro anti-plasmodial and in vivo anti-malarial efficacy of combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya. *Journal of ethnopharmacology*, *115*(2), 223-231. https://doi.org/10.1016/j.jep.2007.09.021

Hariyanti, H., Mauludin, R., Sumirtapura, Y. C., & Kurniati, N. F. (2022). A review: Pharmacological activities of quinoline alkaloid of Cinchona sp. *Biointerface Research in Applied Chemistry*, *13*(4), 3. https://doi.org/10.33263/BRIAC134.319

Ikem, C. J., Appiah-opong, R., Oli, A. N., Ugwu, M. C., Amoateng, P., Agyemang, K., Nwobodo, D. C., & Esimone, C. O. (2020). In vitro and in vivo antiplasmodial assays of selected Nigerian commercial herbal formulations. *Journal of Herbmed Pharmacology*, *9*(4), 374-381. https://doi.org/10.34172/jhp.2020.47

Karole, S., Shrivastava, S., Thomas, S., Soni, B., Khan, S., Dubey, J., Dubey, S. P., Khan, N., & Jain, D. K. (2019). Polyherbal formulation concept for synergic action: a review. *Journal of Drug Delivery and Therapeutics*, *9*(1-s), 453-466.

Korsah, S., Gbedema, S. Y., Bayor, M. T., Boakye-Gyasi, M. E., Owusu, F. W. A., & Forkuo, A. D. (2021). In Vivo antimalarial activity of Polyalthia longifolia (Annonaceae) leaf extract and assessment of its formulated oral dosage forms. *Evidence-Based Complementary and Alternative Medicine*, *2021*(1), 6519346. https://doi.org/10.1155/2021/6519346

Laksemi, D. A., Tunas, I. K., Damayanti, P. A., Sudarmaja, I., Widyadharma, I. P. E., Wiryanthini, I. A., & Linawati, N. M. (2023). Evaluation of Antimalarial Activity of Combination Extract of Citrus aurantifolia and Honey against Plasmodium berghei-İnfected Mice. *Tropical Journal of Natural Product Research*, *7*(1). https://doi.org/10.26538/tjnpr/v7i1.13.

Lekana-Douki, J. B., Oyegue Liabagui, S. L., Bongui, J. B., Zatra, R., Lebibi, J., & Toure-Ndouo, F. S. (2011). In vitro antiplasmodial activity of crude extracts of Tetrapleura tetraptera and Copaifera religiosa. *BMC Research Notes*, *4*, 1-5. https://doi.org/10.1186/1756-0500-4-506

Maje, I., Anuka, J., Hussaini, I., Katsayal, U., Yaro, A., Magaji, M., Jamilu, Y., Sani, M., & Musa, Y. (2007). Evaluation of the anti-malarial activity of the ethanolic leaves extract of Paullinia pinnata (Linn.)(sapindaceae). *Nigerian Journal of Pharmaceutical Sciences*, *6*(2), 67-72.

Majrashi, T. A., Alshehri, S. A., Alsayari, A., Muhsinah, A. B., Alrouji, M., Alshahrani, A. M., Shamsi, A., & Atiya, A. (2023). Insight into the biological roles and mechanisms of phytochemicals in different types of cancer: targeting cancer therapeutics. *Nutrients*, *15*(7), 1704. https://doi.org/10.3390/nu15071704

Nortey, N. N. D., Korsah, S., Tagoe, M., Apenteng, J. A., Owusu, F. A., Oppong, J., Attah, A. E., & Allotey, S. (2023). Herbs Used in Antimalarial Medicines: A Study in the Greater Accra Region of Ghana. *Evidence-Based Complementary and Alternative Medicine*, *2023*(1), 6697078. https://doi.org/10.1155/2023/6697078

Nsofor, W. N., Nwaoguikpe, R. N., Ujowundu, F. N., Keke, C. O., Uba, M. T. u., & Edom, C. V. (2023). Phytochemical, GC-MS, FTIR and Amino acid profile of methanol extract of Tetrapleura tetraptera fruit. *Journal of Drug Delivery and Therapeutics*, *13*(2), 61-69. https://doi.org/10.22270/jddt.v13i2.5739

Obbo, C., Kariuki, S., Gathirwa, J., Olaho-Mukani, W., Cheplogoi, P., & Mwangi, E. (2019). In vitro antiplasmodial, antitrypanosomal and antileishmanial activities of selected medicinal plants from Ugandan flora: refocusing into multi-component potentials. *Journal of ethnopharmacology*, *229*, 127-136. https://doi.org/10.1016/j.jep.2018.09.029

Omoya, F., & Oyebola, T. F. (2019). Antiplasmodial activity of stem bark and leaves of Alstonia boonei (De Wild). *Journal of Microbiology & Experimentation*, *7*(5), 241-245.

Opoku-Agyemang, F., Dodoo, J. N. O., Hlomador, T. E., Gilday, K., & Amissah, J. N. (2022). Conservation and Sustainable Use of Cryptolepis sanguinolenta. In *Herbs and Spices-New Advances*. IntechOpen. https://doi.org/10.5772/intechopen.108249

Oseni, L., & Akwetey, G. (2012). An in-vivo evaluation of antiplasmodial activity of aqueous and ethanolic leaf extracts of Azadirachta indica in Plasmodium berghei infected balb/c mice. *International Journal of Pharmaceutical Sciences and Research*, *3*(5), 1406-1410.

Ouji, M., Augereau, J.-M., Paloque, L., & Benoit-Vical, F. (2018). Plasmodium falciparum resistance to artemisinin-based combination therapies: A sword of Damocles in the path toward malaria elimination. *Parasite*, *25*. https://doi.org/10.1051/parasite/2018021

Paulo, A., Gomes, E. T., Steele, J., Warhurst, D. C., & Houghton, P. J. (2000). Antiplasmodial activity of Cryptolepis sanguinolenta alkaloids from leaves and roots. *Planta medica*, *66*(01), 30-34. https://doi.org/10.1055/s-2000-11106

Podsiedlik, M., Markowicz-Piasecka, M., & Sikora, J. (2020). Erythrocytes as model cells for biocompatibility assessment, cytotoxicity screening of xenobiotics and drug delivery. *Chemico-Biological Interactions*, *332*, 109305. https://doi.org/10.1016/j.cbi.2020.109305

Rizvi, S. A., Einstein, G. P., Tulp, O. L., Sainvil, F., & Branly, R. (2022). Introduction to traditional medicine and their role in prevention and treatment of emerging and re-emerging diseases. *Biomolecules*, *12*(10), 1442. https://doi.org/10.3390/biom12101442

Sæbø, I. P., Bjørås, M., Franzyk, H., Helgesen, E., & Booth, J. A. (2023). Optimization of the hemolysis assay for the assessment of cytotoxicity. *International journal of molecular sciences*, *24*(3), 2914. https://doi.org/10.3390/ijms24032914

Sanchez, C. P., McLean, J. E., Stein, W., & Lanzer, M. (2004). Evidence for a substrate specific and inhibitable drug efflux system in chloroquine resistant Plasmodium falciparum strains. *Biochemistry*, *43*(51), 16365-16373. https://doi.org/10.1021/bi048241x

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603-608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Trager, W., & Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science*, *193*(4254), 673-675. https://doi.org/10.1126/science.781840

Turschner, S., & Efferth, T. (2009). Drug resistance in Plasmodium: natural products in the fight against malaria. *Mini Reviews in Medicinal Chemistry*, *9*(2), 206-214. https://doi.org/10.2174/138955709787316074

Wang, Y. (2022). Malaria in Sub-Saharan Africa: Current situation and future strategies. *Highlights in Science, Engineering and Technology*, *8*, 215-221. https://doi.org/10.54097/hset.v8i.1130

White, N. J. (2022). Severe malaria. *Malaria journal*, *21*(1), 284. https://doi.org/10.1186/s12936-022-04301-8

Journal of Pharmacy

JOP
Journal of Pharmacy

Sweet Potato Peel Pectin's Potential as a Suspending Agent in Pharmaceutical Formulations: The Effect of Extraction Technique

Frederick William Akuffo Owusu[1], Prince George Jnr Acquah[1], Mariam El Boakye-Gyasi[1], Raphael Johnson[1], Marcel Tunkumgnen Bayor[1], Desmond Asamoah Bruce Otu[1], Samuel Nartey[1], Pearl Osei Akoto[1]

[1]*Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.*

Abstract

Introduction: Sweet potato (*Ipomoea batatas* L. Lam) represents an essential underutilised crop globally. The ubiquitous hydrocolloid, pectin, with versatile pharmaceutical and cosmetic applications has been reported to be abundant in the peels. This study thus examined the impact of acid (citric acid) and alkaline (sodium hydroxide) extraction procedures on the suitability of pectin extracted from sweet potato peels as a biocompatible and economical alternative pharmaceutical suspending agent. **Methods**: Conventional acid and alkaline extraction procedures were utilised in sweet potato pectin extraction followed by characterisation and phytochemical evaluation. The proximate composition, FTIR spectra, secondary metabolites and degree of esterification were determined. Additionally, different concentrations (1% and 2%) of the pectins were utilised in formulating paracetamol suspensions using acacia gum as the reference and assessed for pharmaceutical quality. **Results**: Secondary metabolites were present in all pectins while the yield was 9.04±0.07 and 7.24±0.25 respectively for the acid and alkaline extraction methods. Quality and high methoxyl pectins with significant differences ($p<0.05$) in all characterisation parameters (Equivalent weight=1666.67 and 1250mg/mol; methoxyl content=16.43 and 9.57% respectively) except for ash content (3.12 and 2.95 % respectively) were obtained. FTIR highlighted characteristic functional groups in pectin. Both pectin suspensions demonstrated good however, variable significant differences ($p<0.05$) in flow rates, re-dispersibility, sedimentation rates, and volumes compared to acacia gum. The pH remained mildly acidic (<7) with no physical instabilities. **Conclusion**: The alkaline pectin exhibited a better-suspending property than the acid pectin extract. Nevertheless, they both can be utilised as an alternative to acacia gum as a suspending agent.

Article history:

Received: 22 May 2024
Accepted: 16 October 2024
Published: 31 January 2025

Keywords:

sweet potato
suspending agent
pharmaceutical excipient
extraction
waste valorisation

doi: 10.31436/jop.v5i1.325

*Corresponding author's email: frederickakuffo.owusu@knust.edu.gh

Introduction

An essential albeit underused versatile crop is sweet potato (Ipomoea batatas L. Lam) (Alam, 2021). It currently ranks seventh on the global essential crops list and behind yam (Dioscorea spp.), Taro (Colocasia spp.), and cassava (Manihot esculenta Crantz) in Ghana. Its capacity to thrive in varied agro-ecologies and water-stress soils coupled with its short growth cycle and functional properties makes it increasingly attractive for commercial and industrial purposes (Ocloo et al., 2011; Sugri et al., 2017). Significant phytochemical and physicochemical attributes of sweet potato tubers such as high mineral contents (iron and calcium) and antioxidant properties have been established in literature highlighting their ubiquitous nutritional benefits and potential for industrial application (Shaari et al., 2021; Shamsudin et al., 2022). Several cultivars of sweet potato (CRI-Apomuden, CRI-Bohye, Resisto, Ukerewe) are reported in Ghana which is generally orange to pale yellow with a maximum maturity period of 4 months (Darko et al., 2020; Ofori et al., 2009; Teye & Abano, 2012). CRI-Apomuden in particular has a short maturity period (3 to 3.5 months) coupled with high beta-carotene contents and serves as an excellent input for industries making it an ideal alternative to current pectin sources (Darko et al., 2020). Commercial and industrial applications of sweet potatoes such as starch and flour production and biofuel generation produce sizable amounts of residues either employed as animal feed or disposed of as waste (Alam, 2021). Specifically, the peels of sweet potatoes account for 15-40% of the initial product mass which ends up in landfills and degrades to generate greenhouse gases. This contributes immensely to environmental pollution and the wastage of substantial resources (Oluyori et al., 2016; Torres & Domínguez, 2020).

Pectin, an abundant plant polymer in the middle lamella of the cell wall of plants, is composed of α-galacturonic acid (GalA) with varying amounts of methyl ester groups, α-L-rhamnopyranosyl backbone and neutral sugars (e.g. Arabinose, galactose) (Garna et al., 2004). Pectin has high-value applications in the pharmaceutical as well as food industries nevertheless, commercial sources are currently limited to apple pomace and citrus peels which exhibit good gelling properties but are disadvantaged by long maturity periods (Boakye-Gyasi et al., 2021). Therefore, there is a need to investigate a new pectin source with ideal molecular weight, degree of esterification, and methoxyl content (Zhang & Mu, 2011). According to Mei et al, sweet potato residues have a dry matter pectin content of 15% (Mei et al., 2010). Furthermore, rhamnogalacturonan I (the hairy section of pectin) is present in significant amounts in industrial sweet potato waste suggesting the potential of sweet potato as a viable source of pectin (Hamidon & Zaidel, 2017).

The physicochemical and functional properties such as the degree of esterification, molecular weight, and GalA content depend on the pectin product structure and are significantly dependent on the method of extraction (Wandee et al., 2019). High methoxyl pectin has been extracted using water, mineral acids, and bases however, due to the salt-bridge linkages with polyvalent metal ions, the extraction of low methoxyl pectin is difficult using similar solvents (Zhang & Mu, 2011). Citric acid is reported to be the least pectin-degrading agent and yields pectin with the best gelling properties (Hamidon et al., 2020; Kliemann et al., 2009). Furthermore, alkaline extraction processes have yielded low methoxyl, and low-molecular-weight pectin via saponification reactions and β-elimination (Hamidon et al., 2020; Wandee et al., 2019).

There are high healthcare expenditures coupled with increased out-of-pocket medicine payments in Ghana largely due to increased importation (70-90%) of drugs (Adebisi et al., 2022; Conway et al., 2019). This impedes the attainment of the World Health Organization's (WHO) Universal Health Coverage and highlights the need to increase local production while utilising indigenous raw materials such as sweet potato peels to address this problem.

Despite the extensive characterisation of sweet potato pectin, there is limited evaluation of the effect of different extraction techniques on the quality of extracted sweet potato pectin and its subsequent utilisation as a pharmaceutical suspending agent (Hamidon et al., 2020; Hamidon & Zaidel, 2017; Zaidel et al., 2015; Zhang & Mu, 2011). Furthermore, current sources of suspending agents such as acacia are plagued with significant disadvantages such as bio-incompatibility, variable viscosity and decreased stability in acidic medium (Nussinovitch, 2009). The objective of this study is therefore to investigate the effect of acid and alkaline extraction procedures on the suitability of pectin extracted from sweet potato peels as a pharmaceutical suspending agent. Suspending agents ensure that dispersed drugs are dispersed long enough for an accurate dose to be dispersed by ensuring slow sedimentation and easy redispersibility (Bamigbola et al., 2017). Previous studies have highlighted the viscosity-enhancing effects of pectin polymers in suspensions and other liquid dosage forms (Chandel et al., 2022; H. Chen et al., 2016; Owusu et al., 2022, 2024; Pacheco et al., 2019). The study has the potential to transform significant waste of sweet potato (*Ipomoea batatas* L. Lam) peels generated into considerable wealth all the while ensuring the diversification of local sweet potato use and its commodification by the pharma industry.

Materials and methods

Materials

Analytical grades of citric acid, 95% Isopropyl alcohol, 95% ethanol, benzoic acid, 0.25N HCl, 0.1N NaOH, phenol red, and NaCl were acquired from UK Chemicals in Kumasi. Paracetamol powder (99%) (Xi'an Henrikang Biotech Co., China), Acacia gum powder (Sigma-Aldrich, Darmstadt Germany). 0.1% Ferric Chloride, Fehling's Solution A and B, and 1% Lead Acetate obtained from the Department of Pharmacognosy laboratory, Kwame Nkrumah University of Science and Technology (KNUST). The Department of Pharmaceutics Laboratory, KNUST provided distilled water.

Method

Sample Collection and Preparation

Simple random sampling was utilised in acquiring ~ 40 kg of fully ripe sweet potato tubers (CRI-Apomuden) from the Ayigya market in the university community which was authenticated at the Department of Horticulture. The average linear dimensions ± standard deviations were 11.6±2.2, 5.7±1.3 and 4.9±1.1 cm respectively for the major, intermediate, and minor diameters. The tubers were thoroughly washed to remove dirt and peeled about 1 mm from the tubers. The peels were sun-dried till crisp and of constant weight for 7 days. For easier extraction, the dried peels were then ground into fine powder using a compact blender (ARMG Aardee, India) and divided into two for the alkaline and acid extraction, and stored in an airtight container.

Extraction of pectin

Pulverised sweet potato peels (150 g) were dispersed in 1000 ml of distilled water (adjusted pH to 1.5 with citric acid) and heated at 90 °C for 60 minutes. The mixture was cooled and subsequently filtered through a cheesecloth. The precipitation of pectin was carried out by adding 1000 mL of 95 % ethanol (about three times the volume of the filtrate that passed through the cheesecloth). Thorough and vigorous stirring of the filtrate was undertaken and then allowed to rest for 30 minutes followed by skimming off the pectin flocculate with a spatula. The excess flocculate was filtered again using a cheesecloth to ensure purity. The extracted acid pectin gel (ACP) was then left to dry at 40 °C in an oven and stored in an airtight zip-lock bag until further analysis. A similar extraction protocol was followed for the alkaline procedure with the initial pH of distilled water being adjusted to 7.4 with NaOH (Owusu et al., 2023).

The pectin yields of the acid (ACP) and alkaline (ALP) pectins were subsequently determined using Equation 1:

$$\%\,Yield = \frac{Weight\ of\ dried\ pectin\ (g)}{Weight\ of\ powdered\ sweet\ potato\ peels\ (g)} \times 100 \quad Eq.1$$

Determination of moisture content, crude protein and ash content

The method described by Ismail and colleagues was used to determine the ash and moisture content. Briefly, an amount of 1 g pectin was ignited at 550 ° C for 4 hours to assess the ash content. For the moisture content, 1 g pectin was oven-dried (105 ° C) (Ismail et al., 2012). The Kjeldahl method was used for the crude protein analysis (Sáez-Plaza et al., 2013). Measurements were obtained on a constant dry weight basis and in triplicates.

Test for Secondary Metabolites

The presence of phenols, tannins, saponins, and glycosides was established using the standard protocols described by (Evans, 2009).

Phenols

An amount of 2 mL 2% pectin solution was treated with five drops of 0.1% ferric chloride solution and observed for a colour change.

Tannins

An amount of 0.2 g pectin was boiled in 20 mL of distilled water and allowed to cool for 2 minutes. Five drops of 1% lead acetate were added and the precipitate was observed.

Saponins

Distilled water (10 mL) was added to 0.2 g pectin and the mixture was filtered. The filtrate was vigorously shaken, and the persistence of the foam for more than 5 minutes indicated the presence of saponins.

Glycoside

Five (5) mL dilute HCl was added to pectin (0.2 g), boiled in a water bath for 5 min, and allowed to cool. 20 % NaOH (20 drops) was added to alkalise the mixture. Additionally, 1 mL of Fehling A and B solutions were added and boiled for 5 minutes. The formation of brick-red precipitates was observed.

Test for Pectin

Before being heated and cooled, a quantity of pectin (1 g) was quickly combined with 9 ml of water to form a gel. NaOH was subsequently added to 5 ml of the pectin solution and allowed to rest for 15 minutes before 1 ml HCl was added and boiled (Owusu et al., 2023).

Characterization of Ipomoea batatas Peels Pectin

Determination of Equivalent Weight

The method described by Rangana was used with minor modifications. To 500 mg pectin in a 250 mL conical flask, 5 mL of ethanol (to moisten pectin) before it was dissolved in 100 mL distilled water. 1g NaCl and 6 drops of phenol red (indicator) were added to the mixture which was then thoroughly mixed to ensure homogeneity and then titrated against 0.1 N NaOH (Ranganna, 1986). Equivalent weight was determined using Equation 2:

$$Equivalent\ weight = \frac{weight\ of\ pectin\ sample\ (g)\ \times 1000mg}{volume\ of\ alkali\ (ml) \times Normality\ of\ Alkali} \quad Eq.2$$

Determination of Methoxyl Content (MeO)

In a stoppered flask, 25 mL of 0.25 N NaOH was added to the neutralized solution from the equivalent weight setup, thoroughly mixed and allowed to rest on the bench for 30 minutes. Subsequently, 25 mL of 0.25 N HCl was introduced into the solution which was titrated to the same endpoint as before after the addition of phenol red and NaCl (Ranganna, 1986).

Equation 3 was used in calculating MeO.

$$methoxyl\ content\ (\%) = \frac{volume\ of\ alkali\ (ml)\ \times\ Normality\ of\ Alkali\ \times\ 31\ \times\ 100}{weight\ of\ pectin\ sample\ (mg)} \quad Eq.3$$

The molecular weight (MW) of the methoxyl group is 31.

The Determination of Anhydrouronic Acid Analysis (AUA)

The method proposed by (Twinomuhwezi et al., 2023) was used:

$$\%\ AUA = \frac{176 \times 0.1y \times 100}{w \times 1000} + \frac{176\ \times 0.1z \times 100}{w\ \times 1000} \quad Eq.4$$

Where 176 indicated the 1 unit of AUA, z and y are the volumes of NaOH used in the determination of equivalent weight and MeO respectively and w is the sample weight.

Determination of Degree of Esterification (DE)

The values of AUA and MeO obtained were substituted into Equation 5 to determine the DE (Twinomuhwezi et al., 2020):

$$\%\ DE = \frac{\%MeO\ \times 176\ \times 100}{\%\ AUA\ \times\ 31} \quad Eq.5$$

Fourier Transformed Infrared (FTIR) Spectroscopy

The spectroscopic analysis of ALP and ACP was determined using an FTIR spectrometer (UATR Spectrum 2, 941333, PerkinElmer, UK). Transmission mode scanning was done on the spectrum between 400 and 4000 cm^{-1}.

Formulation of Paracetamol suspensions using Ipomoea batatas peels pectin as a suspending agent

The method used by (Owusu et al., 2023) was replicated in the formulation of 100 mL paracetamol (5 g) suspensions. The test-suspending agents were ACP and ALP while the reference was acacia gum. Evaluations were done on two concentration levels 1% $^{w}/_{v}$ and 2% $^{w}/_{v}$ with distilled water as the vehicle and benzoic acid (0.1% $^{w}/v$) as the preservative (Table 1).

Table 1: Master Formula for Preparation of Paracetamol Suspension

Ingredient	Quantities
Paracetamol powder	5 g
Benzoic acid (0.1% w/v)	0.1 g
Purified water to	100 mL

Quality Control Tests on Suspensions

Sedimentation volume (F)

Separate portions (50 mL) of formulations were quantitatively transferred into 100 ml measuring cylinders. The sedimentation volume was measured on the first day and then every week for the following three weeks in triplicates (Owusu et al., 2023). Equation 6 was used to determine the sedimentation volume:

$$F = \frac{Ultimate\ volume}{Initial\ volume} \times 100\% \qquad Eq.6$$

Flow rate (f)

Equation 7 was used to determine the flow rate. The flow time was established by measuring the time required for 10 mL suspension to move through the entire length of a 10 ml pipette (Owusu et al., 2023).

$$f = \frac{volume\ of\ pipette\ (ml)}{flow\ time\ (s)} \qquad Eq.7$$

pH of Suspension

An amount of 20 ml of the suspensions was quantitatively transferred into beakers (100 mL) and stirred thoroughly. The pH readings were obtained in triplicates by submerging the electrodes of the pH meter in the suspension (Mettler Toledo, USA) (Owusu et al., 2023).

Ease of Re-dispersibility

A qualitative assessment of the suspension's re-dispersibility was made as described by (Owusu et al., 2023).

Sedimentation rate and volume

The method described by Owusu colleagues was followed to calculate the sedimentation rates of the formulations as described in Equation 8 (Owusu et al., 2023).

$$Sedimentation\ rate = \frac{Change\ in\ volume\ of\ sediment\ (V_1 - V_2)}{T_1 - T_2}$$

Where V1 and V2 represent the initial volume of sediment and the final volume of sediment respectively. T1 and T2 also represent the time it took for V1 to settle to V2.

Statistical Analysis

All the analyses were carried out using GraphPad Prism version 8.0.1. Standard deviations and mean were used to present data while the unpaired student t-test and two-way analysis of variance (ANOVA) were used to determine the p-values where appropriate. Significance was denoted as $p \leq 0.05$.

Results and discussion

The pectin yield and phytochemical constituents of sweet potato peels pectin

Percentage yield of pectin

The pectin yields obtained were 9.04±0.07 and 7.24±0.25 for the acid and alkaline extraction methods respectively as shown in Figure 1. The ACP yield has a significantly higher yield ($p<0.05$) than the ALP which could be accounted for by β-elimination reactions as well as saponification reactions resulting in the galacturonic acid backbone degradation (Wandee et al., 2019). Acid extraction conversely loosens the cell wall matrix resulting in increased

pectin extraction and high levels of galacturonic acid (Zaidel et al., 2015). The yield of sweet potato pectin from cell wall materials under varied conditions has been reported to range from 7.2 and 29.3% indicating the results obtained were comparable (Zaidel et al., 2015). Compared to other pectin sources extracted using similar procedures such as watermelon (yield = 14.1%) and plantain (10.01%-46.55%), the yields were however lower which could be accounted for the variation in plant sources (Otu et al., 2024; Owusu et al., 2022).

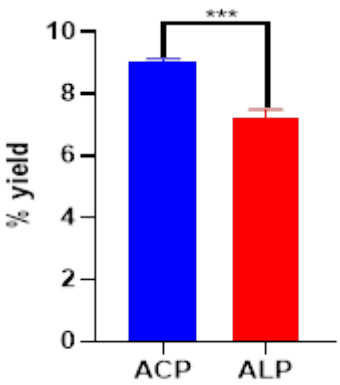


Fig. 1: Statistical analysis on % Yield of pectin extracted by Acid (ACP) and Alkaline (ALP) extraction methods ***P < 0.001 significant difference between ACP and ALP using unpaired student's two-tailed t-test.

Phytochemical constituents of pectin

The phytochemical analysis indicated that the secondary metabolites; tannins, glycosides, saponins, and phenols were present in both ACP and ALP (Table 2). These metabolites particularly phenols and tannins have been implicated in the antioxidant, antineoplastic, and antimicrobial activity of plants. Saponins also exhibit emulsifying properties (Edeoga et al., 2006; Schieber et al., 2003). The pectin identification test concluded the presence of pectin as per the requirements prescribed in the USP (The United States Pharmacopeial Convention, 2021).

Table 2: Phytochemical components in extracted Ipomea batatas peel pectin

Phytochemical Parameters	Observation	Inference
Glycosides	The appearance of a brick-red precipitate was observed for both ACP and ALP	Presence of glycosides
Saponins	The persistence of a froth for 5 minutes for both ACP and ALP	Presence of saponins
Tannins	A greenish-coloured precipitate and a light brown precipitate were formed for both ACP and ALP respectively which remained after the introduction of 5 drops of a solution of 1% ferric chloride	Presence of tannins
Phenols	The appearance of bluish-black colouration for both ACP and ALP	Presence of Phenols

Proximate content and characterisation of Ipomoea batatas peels pectin

Moisture content

In the pharmaceutical sector, moisture content is crucial since it affects powders' flow characteristics and susceptibility to microbial degradation (Ismail et al., 2012; Joel et al., 2018). For ACP and ALP, respectively, the moisture contents were significantly different ($p<0.05$); 16.05%±0.05 and 14.23±0.25 (Table 3). Nevertheless, when stored for an extended duration, ACP shows a higher propensity for microbial growth and poor flow characteristics since it is above the recommended British Pharmacopoeia acceptable moisture content (<15%$^{w}/_{w}$) (British Pharmacopoeia, 2018). Storage in air-tight containers is consequently recommended to prevent early-onset degradation.

Ash content

The ash content is a measure of pectin purity. Decreased ash values translate into higher pectin purity (Ismail et al., 2012). The ash contents of ACP (3.12%±0.1) and ALP (2.95±0.05%) (Table 3), were below recommended standards in literature (<10%), pointing to high-quality pectin in the extracted materials. The values were also non-significant further highlighting that both the alkaline and acid extraction techniques yield high-quality pectin

(British Pharmacopoeia, 2018).

Crude protein content

The crude protein content in Table 3 was significantly different ($p < 0.05$) for ACP and ALP; 5.78%±0.03 and 8.26%±0.05 respectively. Pectin's activating and stabilising qualities, notably in dispersed systems, are directly influenced by the proteinaceous components connected to the pectin polymer chains (Funami et al., 2007; Mada et al., 2022). The high protein content in ALP may be secondary to the increased covalent bonds between the proteins and pectin that caused co-precipitation by the ethanol (Li et al., 2015; Yapo et al., 2007). This supports the AUA content results indicating ACP was significantly purer. The lower crude protein concentrations in ACP can be attributed to protein hydrolysis by the acidic medium (Yapo et al., 2007).

Equivalent weight

The equivalent weight determines the quantity of free galacturonic acid in the pectin. In addition to affecting the gelling properties, the equivalent weight is also a measure of the purity of the pectin. It is reported to be dependent on the plant source, the quality of raw material, and the extraction technique (Ismail et al., 2012; Twinomuhwezi et al., 2020). The equivalent weights were 16667.67±0.00 and 1250.00±0.00 mg/mol for ACP and ALP respectively and as shown in Table 3, were significantly different ($p<0.05$). This confirms the impact of alkaline conditions on the amount of galacturonic acid as discussed earlier. Moreover, because the gel-forming properties of pectin correlate positively with the equivalent weight, the greater values obtained by ACP may translate to enhanced gelling capabilities (Ismail et al., 2012; Twinomuhwezi et al., 2020).

Methoxyl content

The methoxyl content of pectin affects their ability to gel and their set times (Azad et al., 2014). According to Aina *et al.*, values ≥7% are high methoxyl pectins, and this classification depends on the source of the pectin and the extraction method used (Aina et al., 2012). The methoxyl contents of ACP and ALP were 16.43%±0.31 and 9.57%±0.25, respectively, suggesting that both pectins can easily disperse in aqueous conditions and generate high-sugar gels despite the significant difference between them ($p < 0.05$) as shown in Table 3. They may accordingly be utilised as binders, suspending agents, or gelling agents in pharmaceutical formulations (Ismail et al., 2012; Twinomuhwezi et al., 2020).

Anhydrouronic acid content

Anhydrouronic acid (AUA) content affects the pectin structure and the texture of the gel produced. The quality of pectin is also influenced by the AUA (Chan and Choo, 2013). Levels of < 65% are indicative of high concentrations of proteins, starch, and sugars (Ismail et al., 2012; Twinomuhwezi et al., 2020). Despite the AUA values of ACP and ALP being significantly different ($p<0.05$); 103.80%±1.76 and 68.41%±1.42 respectively (as shown in Table 3), they were all > 65%. This suggests that both extracted pectin may have low contents of proteins and other cell wall constituents, however, ACP may be purer than ALP in comparison corroborating the findings in the ash and moisture contents.

Degree of esterification

The extracted pectins had a high degree of esterification (DE) 89.83%±0.17 and 79.41%±0.43 for ACP and ALP respectively as shown in Table 3. The results were comparable to the results of the work by Hamidon and colleagues (58.5%) (Hamidon et al., 2020). The high DE demonstrates that pectin from sweet potato peels can convert into gel at a pH range between 2.9 and 3.5 when sugar is present in concentrations of more than 55%. Additionally, as the ester content rises to 75%, the gel strength of high methoxyl pectin increases (Chen et al., 2014). The pectins' strong gelation and emulsification characteristics are further established by the high degree of esterification. This supports their prospective use as emulsifying agents, gelling agents, and suspending agents in pharmaceutical formulations (Willats et al., 2006).

FTIR Spectra

FTIR was used to characterise ACP and ALP. The peaks corresponding to various functional groups were assigned. Compared to those reported, similar pectin pecks were obtained (Arachchige et al., 2020;

Ogutu & Mu, 2017; Sato et al., 2011).

The FTIR profiles for ACP and ALP are presented in Figures 2a and 2b respectively. The principal bands observed for ACP were 3295.72, 2929.69, 1717.82, and 1626.36 cm^{-1}. These corresponded to O–H stretching vibrations, C–H stretching of the CH_2 group, esters (COOR), and free carboxylic acid moieties (COO^-) respectively. The bands at 1145.33 and 1022.11 cm^{-1} represented rhamnogalacturonan, while the bands at 935.11 cm^{-1} corresponded to the monopyranose component. The CH deformation vibration associated with the primary amide groups ($CO–NH_2$), the carboxylic acid (COO^-), and the methyl ester ($COOCH_3$) were highlighted by the higher peak between 1210.66 and 1398.12 cm^{-1}. The C–C, C–O–C, and C–OH vibration modes of the carbohydrate ring coupled to the glycosidic linkage vibrations of the fingerprint region were identified below the 1500 cm^{-1} range. In similitude, ALP showed corresponding principal bands at 3287.60, 2902.65, 1789.87, and 1618.76, 1307.37 with similar fingerprint regions. The intense peaks in 1000-115 cm^{-1} can be assigned to the rich homogalacturonan content in pectin. The C–O–C vibrations of glycosidic bonds were assigned to the less intense peak around 1146 cm^{-1}. The bands below 1000 cm^{-1} are associated with the neutral sugars, xylose, galactose, and arabinose. The number of ester groups in ALP was relatively higher compared to ACP which could be attributed to the hydrolysis of the ester groups by the acid employed in its extraction (Arachchige et al., 2020; Ogutu & Mu, 2017).

For both ALP and ACP, higher absorbance at 1717.82 cm^{-1} (COOR) was observed compared to 1626.36 cm^{-1} (COO^-). This suggests that the pectins were high methoxyl pectins (DE > 50%) as indicated by the titration method.

Table 3: Proximate content and characterization of *Ipomoea batatas* peels pectin

Parameter	ACP	ALP
Equivalent Weight (mg/mol)	1666.67 ± 0.00^a	1250.00 ± 0.00^b
Methoxyl Content (%)	16.43 ± 0.31^c	9.57 ± 0.25^d
Anhydrouronic Acid Content, AUA (%)	103.80 ± 1.76^e	68.41 ± 1.42^f
Degree of Esterification, DE (%)	89.83 ± 0.17^g	79.41 ± 0.43^h
Moisture Content (%)	16.05 ± 0.05^i	14.23 ± 0.25^j
Ash Content (%)	3.12±0.1	2.95±0.05
Crude Protein (%)	5.78 ± 0.03^k	8.26 ± 0.05^l

The results are presented as a mean ± SD (n=3). Different superscript letters denote statistical significance ($p < 0.05$) using students' two-tailed t-test.

Evaluation of quality control parameters of extracted pectin

pH of suspensions

To assess the stability of suspension dosage forms, pH analysis is crucial (Owusu et al., 2022). Throughout the evaluation period, a reasonably constant weakly acidic pH was observed for all formulations (Table 4). This suggests that pH stability issues may not be a problem during extended storage periods. The lack of any undesirable physical modifications, such as crystal growth development and caking, was further evidence. Moreover, the growth of microbes which can culminate in degradation and stability issues is circumvented by the acidic pH and, therefore, can increase stability throughout the shelf-life (Ayesu et al., 2022; Oppong et al., 2016). At all the concentrations and weeks, the pH of ACP was significantly lower than the standard which could be accounted for by the acid utilised in the extraction.

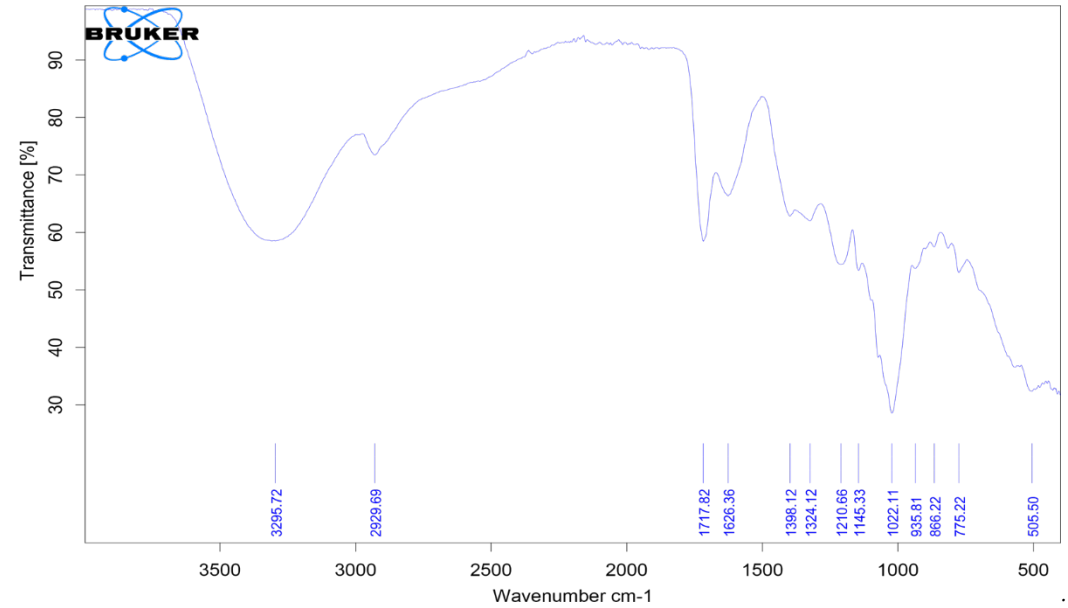
Table 4: pH determination of ACP, ALP, and Acacia as suspending agents

WEEK	1% ACACIA	1% ACP	1% ALP	2% ACACIA	2% ACP	2% AL
Week 1	3.58±0.03	3.05 ± 0.02^a	3.54 ± 0.04	3.70±0.03	2.86 ± 0.02^a	3.56±0.
week 2	2.67±0.04	2.27 ± 0.03^a	2.89 ± 0.02^b	2.91±0.03	2.33 ± 0.03^a	3.02±0.
week 3	3.06±0.01	2.61 ± 0.02^a	3.46 ± 0.03^a	3.22±0.02	2.46 ± 0.01^a	3.21±0.
week 4	3.14±0.01	2.83 ± 0.01^a	3.42 ± 0.01^a	3.39±0.02	2.75 ± 0.03^a	3.14±0.

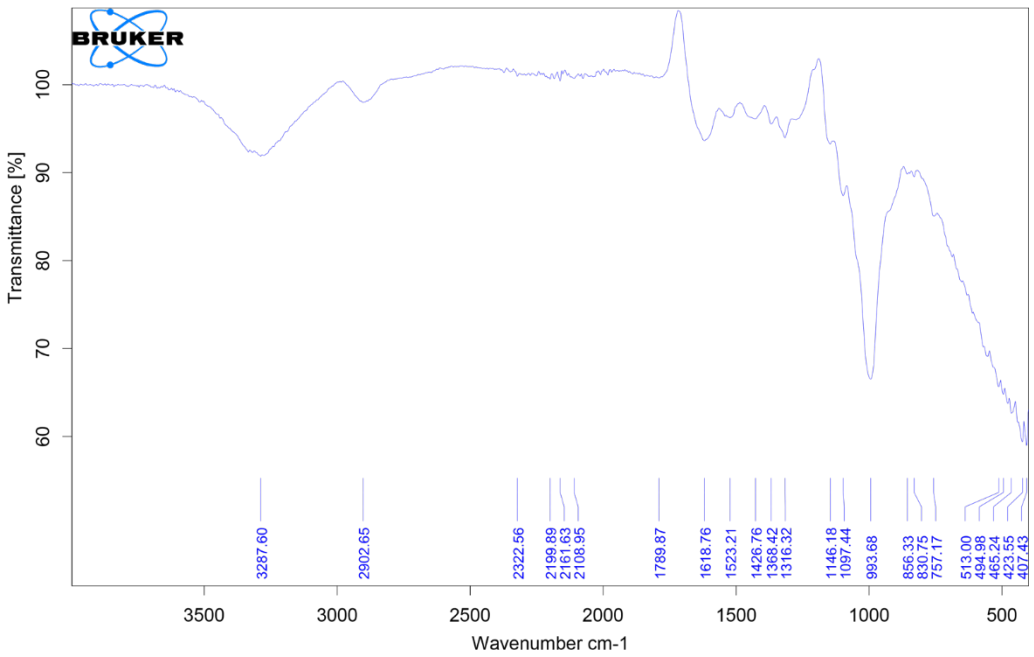
The results are presented as a mean ± SD (n=3). ****P <0.0001 (a), **P ≤ 0.01 (b), and *p < 0.05 (c), statistical difference between pH of acacia and pectin suspensions using two-way Analysis of variance (ANOVA) followed by Turkey's multiple comparisons.

Ease of Re-dispersibility of Suspensions

The number of cycles necessary to rotate the sediment through 180° and achieve homogenous



(a)



(b)

Fig. 2: FTIR spectra of ACP (a) and ALP (b).

suspension determines how easily it can be redispersed. The quality of suspension and ease of redispersion are improved with fewer cycles (Allen & Ansel, 2013; Owusu et al., 2021). During the 4 week evaluation period, both ACP and ALP demonstrated significant differences ($p<0.05$) compared to acacia at all concentrations except for ALP 2% during the 3rd week (figure 3). This implies that the pectin requires less agitation to re-disperse and ensure that uniform doses are obtained.

A comparison of the re-dispersibility of ALP and the ACP suspensions alone demonstrated non-significant differences generally except for the 2% concentrations during the second week. It may be concluded that the extraction technique has no impact on the transformation of sediments into a homogeneous suspension (Figure 4).

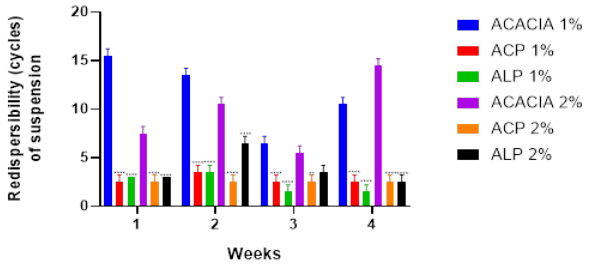


Fig. 3: Redispersibility of acacia and pectin suspensions; ****p <0.0001, **p ≤ 0.01, statistical difference between redispersibility of acacia (standard) and pectin suspensions using two-way ANOVA followed by Turkey's multiple comparisons.

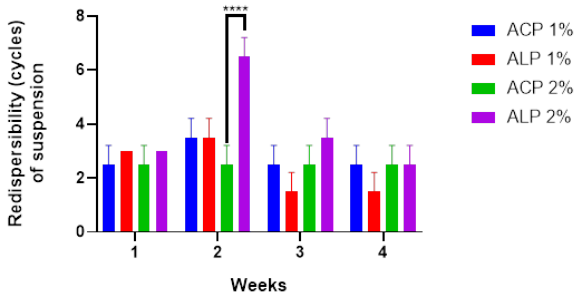


Fig. 4: Redispersibility of ALP and ACP suspensions; ****P <0.0001 statistical difference between redispersibility pectin suspensions using two-way ANOVA followed by Turkey's multiple comparisons.

Flow rate of suspensions

Apparent viscosity is an essential property of suspensions as it impacts stability and ensures that the dispersed phase in suspensions settles slowly for a sufficient time to guarantee correct dosing. This occurs as a result of the breakdown of the flocculated structure, which must be restored to create a good suspension (thixotropic suspensions) and avoid the product's long-term caking (Asantewaa et al., 2011; Ayesu et al., 2022). The apparent viscosity relates directly to the concentration and inversely to the flow rate which was observed for all suspending agents when the concentration increased from 1% to 2% as shown in Figures 5 and 6 (Bamigbola et al., 2017).

According to Ngwuluka *et al.*, autocatalytic hydrolysis accounts for the natural tendency of natural polymers such as gums and pectin to decline in flow rates with time as was observed during the 4-week evaluation period (Figure 5) (Ngwuluka et al., 2012). Nevertheless, the phenomenon had no detrimental effect on the formulation stability.

Furthermore, the flow rates of both ALP and ACP were significantly higher ($P \leq 0.01$) than the acacia during the 2nd week at 1% concentration; however, only 1% ACP demonstrated significantly higher flow rates compared to 1% acacia during the 1st and 3rd weeks (Figure 5). This supposes that both ALP and ACP were comparable to acacia gum, particularly at higher concentrations and extended storage periods. Similar observations were made when ALP and ACP were compared suggesting a non-significant impact of extraction methodology on flow rates of pectin suspensions (Figure 6).

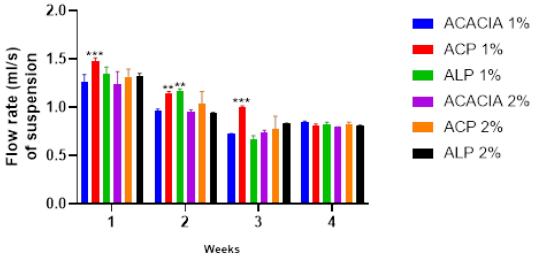


Fig. 5: Flow rate of Acacia and pectin suspensions; ****P <0.0001, ***P < 0.001, and **P ≤ 0.01, statistical difference between the flow rate of acacia (standard) and pectin suspensions using two-way ANOVA followed by Turkey's multiple comparisons.

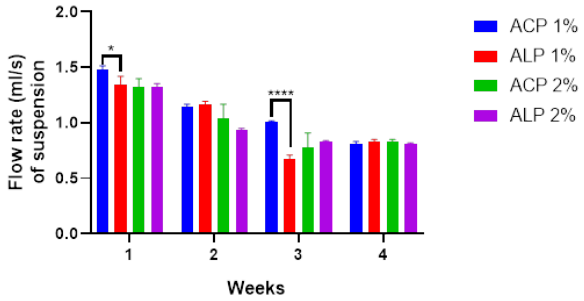


Fig. 6: Flow rate of ALP and ACP suspensions; ****P <0.0001, and *P ≤ 0.05, statistical difference between the flow rate of pectin suspensions using two-way ANOVA followed by Turkey's multiple comparisons.

Sedimentation Volume (SV) and Sedimentation Rate of Suspensions

The SV values typically range between 0 and 1 with values closer to 1 being associated with superior suspending qualities (Larsson et al., 2011; Owusu et al., 2021). Generally, except for the 2nd week, the ALP and ACP demonstrated a variable significance difference ($p<0.05$) between the pectin and Acacia (Figure 7). It can be inferred that pectin demonstrates better suspending properties when compared to acacia at 1% and 2% concentrations. This has been reported by Owusu et al. (2024) to be accounted for by the branching, monosaccharide compositions and molecular weights of the polymers which impacts the gelling properties.

A further comparison of the SV of ALP and ACP highlights variations in significant differences across the weeks. For instance, during the 1st week, there was a non-significance difference at 2% and a significant difference was observed at 1%. The opposite was observed during the 2nd week (Figure 8). The sedimentation volume only provides a qualitative appreciation of how the suspension sediments, however, lacks any practical reference point, therefore, the sedimentation rate is a more significant parameter to analyse (Ayesu et al., 2022).

The quality of suspension formulation is inversely related to the rate of sedimentation; as such, a decreased rate of sedimentation implies a better-suspending quality (Ayesu et al., 2022). A general decrease in sedimentation rate was observed when the concentration increased from 1% to 2% for all the suspensions. This supports the hypothesis that sedimentation is concentration-dependent (Owusu et al., 2022). ALP and ACP at all concentrations significantly had slower sedimentation rates when compared to acacia ($P < 0.05$) except for the 4th week where only ALP 2% showed a significant difference ($P < 0.0001$). This confirms the earlier results that the pectin exhibited better-suspending activity than the acacia.

When ACP and ALP were compared, at all concentration levels, significant differences were observed between ALP except for the 1% concentrations in the 4th week (Figure 10). This unambiguously suggests that though ALP and ACP are better than Acacia, the alkaline extraction technique yields pectin with superior suspending properties. This may be accounted for by the significant variations observed in the equivalent weight, degree of esterification and anhydrouronic acid contents. These parameters have been reported to impact the rheological behaviour and gelling properties of pectins (Chan et al., 2017; Nascimento et al., 2016).

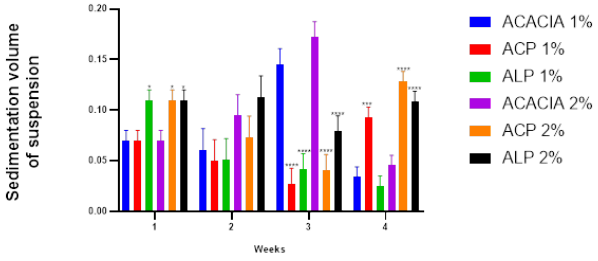


Fig. 7: Sedimentation volume of Acacia and pectin suspensions; ****P <0.0001, ***P < 0.001 and *P ≤ 0.05, statistical difference between sedimentation volume of acacia (standard) and pectin suspensions using two-way ANOVA followed by Turkey's multiple comparisons.

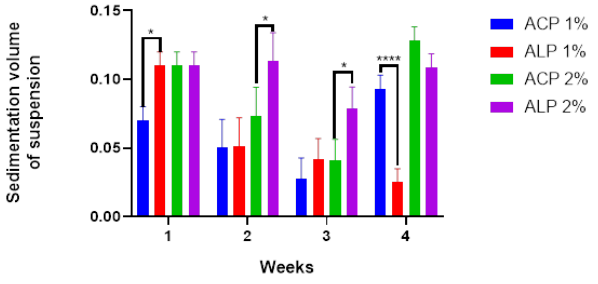


Fig. 8: Sedimentation volume of ALP and ACP suspensions; ****P <0.0001, and *P ≤ 0.05, statistical difference between sedimentation volume of pectin suspensions using two-way ANOVA followed by Turkey's multiple comparisons.

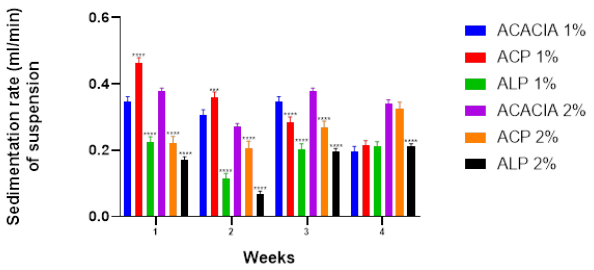


Fig. 9: Sedimentation rate of Acacia and pectin suspensions; ****P <0.0001, and ***P ≤ 0.001, statistical difference between sedimentation rate of acacia (standard) and pectin suspensions using two-way ANOVA followed by Turkey's multiple comparisons using two-way ANOVA followed by Turkey's multiple comparisons.

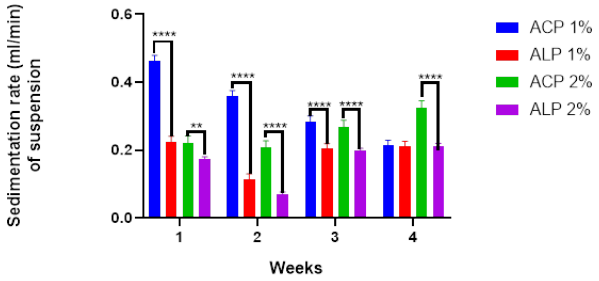


Fig. 10: Sedimentation rate of ALP and ACP suspensions; ****P <0.0001, and **P < 0.01, statistical difference between sedimentation rate of pectin suspensions using two-way ANOVA followed by Turkey's multiple comparisons. two-way ANOVA followed by Turkey's multiple comparisons.

Conclusion

This study highlights that both alkaline and acid extraction techniques yield high-quality pectins. Furthermore, at concentrations of 1% and 2%, Ipomoea batatas pectin extracted via alkaline or acid extraction possesses suspending properties that were comparable to Acacia gum which was evidenced by the significant differences for all quality control parameters during the evaluation period. Moreover, alkaline-extracted pectins produce suspensions with better sedimentation rates when compared to acid-extracted pectins.

Authors contributions

"Conceptualization, F.W.A.O. and M.E.B.G.; methodology, P.G.J.A, D.A.B.O, S.N, and P.O.A.; formal analysis, R.J, F.W.A.O, and P.G.J.A.; writing—original draft preparation, P.G.J.A, D.A.B.O, S.N, and P.O.A; writing—review and editing, F.W.A.O., M.E.B.G., M.T.B., S.N., D.A.B.O.; visualization, R.J, F.W.A.O, and P.G.J.A.; supervision, R.J., M.T.B., M.E.B.G. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The technical help provided by the Department of Pharmaceutics at Kwame Nkrumah University of Science and Technology is appreciated by the authors.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Declaration of generative AI and AI-assisted technologies in the writing process

Grammarly was used to enhance the readability and language of the work.

References

Adebisi, Y. A., Nwogu, I. B., Alaran, A. J., Badmos, A. O., Bamgboye, A. O., Rufai, B. O., Okonji, O. C., Malik, M. O., Teibo, J. O., Abdalla, S. F., Lucero-Prisno, D. E., Samai, M., & Akande-Sholabi, W. (2022). Revisiting the issue of access to medicines in Africa: Challenges and recommendations. *Public Health Challenges*, *1*(2), e9. https://doi.org/10.1002/puh2.9

Aina, V. O., Barau, M. M., Mamman, O. A., Zakari, A., Haruna, H., Umar, M. S. H., & Abba, Y. B. (2012). Extraction and characterization of pectin from peels of lemon (Citrus limon), grape fruit (Citrus paradisi) and sweet orange (Citrus sinensis). *British Journal of Pharmacology and Toxicology*, *3*(6), 259–262.

Alam, M. K. (2021). A comprehensive review of sweet potato (Ipomoea batatas [L.] Lam): Revisiting the associated health benefits. *Trends in Food Science & Technology*, *115*(April), 512–529. https://doi.org/10.1016/j.tifs.2021.07.001

Allen, L., & Ansel, H. C. (2013). *Ansel's pharmaceutical dosage forms and drug delivery systems*. Lippincott Williams & Wilkins.

Arachchige, M. P. M., Mu, T., & Ma, M. (2020). Structural, physicochemical and emulsifying properties of sweet potato pectin treated by high hydrostatic pressure and/or pectinase: a comparative study. *Journal of the Science of Food and Agriculture*, *100*(13), 4911–4920.

Asantewaa, Y., Ofori-Kwakye, K., Kipo, S. L., Boamah, V. E., & Johnson, R. (2011). Investigation of the emulsifying and suspending potential of cashew tree gum in pharmaceutical formulations. *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(4), 215–219.

Ayesu, H. D., Kuntworbe, N., Sekyere, M., Johnson, R., Owusu, W. A. F., Entsie, P., Amankwah, F., & Ofori-Kwakye, K. (2022). Investigation of the physicochemical properties of freeze-dried fruit pulp of Telfairia occidentalis and its potential use as suspending agent. *Heliyon*, *8*(7), e09997. https://doi.org/10.1016/j.heliyon.2022.e09997

Azad, A. K. M., Ali, M. A., Akter, M. S., Rahman, M. J., & Ahmed, M. (2014). Isolation and characterization of pectin extracted from lemon pomace during ripening. *Journal of Food and Nutrition Sciences*, *2*(2), 30–35. https://www.researchgate.net/publication/325859830_Isolation_and_characterization_of_pectin_extracted_from_lemon_pomace_during_ripening

Bamigbola, E. A., Olorode, O. A., & Uzim, D. A. (2017). Evaluation Of The Suspending Properties Of Cola Acuminata Gum On Calamine Suspension. *Journal of Phytomedicine and Therapeutics*, *16*(2), 96–113.

Boakye-Gyasi, M. El, Owusu, F. W. A., Entsie, P., Agbenorhevi, J. K., Banful, B. K. B., & Bayor, M. T. (2021). Pectin from Okra (Abelmoschus esculentus L.) Has Potential as a Drug Release Modifier in Matrix Tablets. *The Scientific World Journal*, *2021*(1), 1–10. https://doi.org/10.1155/2021/6672277

British Pharmacopoeia. (2018). *General Monographs*. The Stationary Office.

Chan, S. Y., Choo, W. S., Young, D. J., & Loh, X. J. (2017). Pectin as a rheology modifier: Origin, structure, commercial production and rheology. *Carbohydrate Polymers*, *161*, 118–139. https://doi.org/10.1016/j.carbpol.2016.12.033

Chandel, V., Biswas, D., Roy, S., Vaidya, D., Verma, A., & Gupta, A. (2022). Current Advancements in Pectin: Extraction, Properties and Multifunctional Applications. *Foods*, *11*(17), 2683. https://doi.org/10.3390/foods11172683

Chen, H., Qiu, S., Gan, J., Liu, Y., Zhu, Q., & Yin, L. (2016). New insights into the functionality of protein to the emulsifying properties of sugar beet pectin. *Food Hydrocolloids*, *57*, 262–270. https://doi.org/10.1016/j.foodhyd.2016.02.005

Chen, Y., Zhang, J. G., Sun, H. J., & Wei, Z. J. (2014). Pectin from Abelmoschus esculentus: Optimization of extraction and rheological properties. *International Journal of Biological Macromolecules*, *70*, 498–505. https://doi.org/10.1016/j.ijbiomac.2014.07.024

Conway, M., Holt, T., Sabow, A., & Sun, I. Y. (2019). *Should sub-Saharan Africa make its own drugs*. McKinsey and Company. https://www.mckinsey.com/industries/public-sector/our-insights/should-sub-saharan-africa-make-its-own-drugs

Darko, C., Yeboah, S., Amoah, A., Opoku, A., & Berchie, J. N. (2020). Productivity of sweet potato (Ipomoea batatas (L) Lam) as influenced by fertilizer application in different agro-ecologies in Ghana. *Scientific African*, *10*, e00560.

Edeoga, H. O., Omosun, G., & Uche, L. C. (2006). Chemical composition of Hyptis suaveolens and Ocimum gratissimum hybrids from Nigeria. *African Journal of Biotechnology*, *5*(10), 892–895.

Evans, W. C. (2009). Trease and Evans' pharmacognosy. In *Elsevier Health Sciences.* https://doi.org/10.4103/0970-4388.180371

Funami, T., Zhang, G., Hiroe, M., Noda, S., Nakauma, M., Asai, I., Cowman, M. K., Al-Assaf, S., & Phillips, G. O. (2007). Effects of the proteinaceous moiety on the emulsifying properties of sugar beet pectin. *Food Hydrocolloids*, *21*(8), 1319–1329. https://doi.org/10.1016/j.foodhyd.2006.10.009

Garna, H., Mabon, N., Wathelet, B., & Paquot, M. (2004). New method for a two-step hydrolysis and chromatographic analysis of pectin neutral sugar chains. *Journal of Agricultural and Food Chemistry*, *52*(15), 4652–4659. https://doi.org/10.1021/jf049647j

Hamidon, N. H., Abang Zaidel, D. N., & Mohd Jusoh, Y. M. (2020). Optimization of Pectin Extraction from Sweet Potato Peels Using Citric Acid and its Emulsifying Properties. *Recent Patents on Food, Nutrition & Agriculture*, *11*(3), 202–210. https://doi.org/10.2174/2212798411666200207102051

Hamidon, N. H., & Zaidel, D. N. A. (2017). Effect of extraction conditions on pectin yield extracted from sweet potato peels residues using hydrochloric acid. *Chemical Engineering Transactions*, *56*, 979–984. https://doi.org/https://doi.org/10.3303/CET1756164

Ismail, N. S. M., Ramli, N., Hani, N. M., & Meon, Z. (2012). Extraction and characterization of pectin from dragon fruit ([i]Hylocereus polyrhizus[/i]) using various extraction conditions. *Sains Malaysiana*, *41*(1), 41–45.

Joel, J. M., Barminas, J. T., Riki, E. Y., Yelwa, J. M., & Edeh, F. (2018). Extraction and characterization of hydrocolloid pectin from goron tula (Azanza garckeana) fruit. *World Scientific News*, *101*, 157–171. https://www.researchgate.net/publication/327883731_Extraction_and_Characterization_of_Hydrocolloid_Pectin_from_Goron_Tula_Azanza_garckeana_fruit#:~:text=This study

Kliemann, E., De Simas, K. N., Amante, E. R., Prudêncio, E. S., Teófilo, R. F., Ferreira, M. M. C., & Amboni, R. D. M. C. (2009). Optimisation of pectin acid extraction from passion fruit peel (Passiflora edulis flavicarpa) using response surface methodology. *International Journal of Food Science and Technology*, *44*(3), 476–483. https://doi.org/10.1111/j.1365-2621.2008.01753.x

Larsson, M., Hill, A., & Duffy, J. (2011). Suspension Stability; Why Particle Size, Zeta Potential and Rheology are Important. *Annual Transactions of the Nordic Rheology Society*, *20*, 1–35.

Li, D. Q., Du, G. M., Jing, W. W., Li, J. F., Yan, J. Y., & Liu, Z. Y. (2015). Combined effects of independent variables on yield and protein content of pectin extracted from sugar beet pulp by citric acid. *Carbohydrate Polymers*, *129*, 108–114. https://doi.org/10.1016/j.carbpol.2015.04.058

Mada, T., Duraisamy, R., & Guesh, F. (2022). Optimization and characterization of pectin extracted from banana and papaya mixed peels using response surface methodology. *Food Science and Nutrition*, *10*(4), 1222–1238. https://doi.org/10.1002/fsn3.2754

Mei, X., Mu, T. H., & Han, J. J. (2010). Composition and physicochemical properties of dietary fiber extracted from residues of 10 varieties of sweet potato by a sieving method. *Journal of Agricultural and Food Chemistry*, *58*(12), 7305–7310. https://doi.org/10.1021/jf101021s

Nascimento, G. E. do, Simas-Tosin, F. F., Iacomini, M., Gorin, P. A. J., & Cordeiro, L. M. C. (2016). Rheological behavior of high methoxyl pectin from the pulp of tamarillo fruit (Solanum betaceum). *Carbohydrate Polymers*, *139*, 125–130. https://doi.org/10.1016/j.carbpol.2015.11.067

Ngwuluka, N. C., Akanbi, M., Agboyo, I., & Uwaezuoke, O. J. (2012). Characterization of gum from Sesamum indicum leaves as a

suspending agent in a pediatric pharmaceutical suspension. *World Journal of Pharmaceutical Research*, *1*(4), 909–924.

Nussinovitch, A. (2009). *Plant Gum Exudates of the World*. CRC Press. https://doi.org/10.1201/9781420052244

Ocloo, F., Otoo, G., & Nkansah, R. (2011). Functional and Physicochemical Characteristics of Starch Obtained from Gamma-Irradiated Sweet Potato (Ipomea batatas L.). *Journal of Agriculture …*, *1*(7), 116–122.

Ofori, G., Oduro, I., Ellis, W. O., & Dapaah, K. H. (2009). Assessment of vitamin A content and sensory attributes of new sweet potato (Ipomoea batatas) genotypes in Ghana. *African Journal of Food Science*, *3*(7), 184–192.

Ogutu, F. O., & Mu, T.-H. (2017). Ultrasonic degradation of sweet potato pectin and its antioxidant activity. *Ultrasonics Sonochemistry*, *38*, 726–734.

Oluyori, A. P., Shaw, A. K., Olatunji, G. A., Rastogi, P., Meena, S., Datta, D., Arora, A., Reddy, S., & Puli, S. (2016). Sweet Potato Peels and Cancer Prevention. *Nutrition and Cancer*, *68*(8), 1330–1337. https://doi.org/10.1080/01635581.2016.1225107

Oppong, E. E., Osei-Asare, C., & Klu, M. W. (2016). Evaluation of the suspending properties of shea tree gum. *International Journal of Pharmacy and Pharmaceutical Sciences*, *8*(7), 409–413.

Otu, D. A. B., Owusu, F. W. A., Acquah, P. G. J., Boakye-Gyasi, M. El, Edzor-Agbo, Y., Johnson, R., Bayor, M. T., & Archer, M.-A. (2024). Effect of Ripening and Extraction Method on the Physicochemical Properties of Pectin Extracted from Peels of Apem and Apantu Plantain Cultivars in Ghana. *Journal of Chemistry*, *2024*(1), 1–19. https://doi.org/10.1155/2024/6677179

Owusu, F. W. A., Acquah, P. G. J., Boakye-Gyasi, M. E. L., Johnson, R., Yeboah, G. N., Archer, M.-A., Antwi, M. B., & Asare, S. O. (2023). Pharmaceutical Assessment of the Impact of the Method of Extraction on the Suitability of Pectin from Plantain (Musa paradisiaca) Peels as a Suspending Agent in Oral Liquid Formulations. *The Scientific World Journal*, *2023*(1), 1–11. https://doi.org/10.1155/2023/8898045

Owusu, F. W. A., Asare, C. O., Enstie, P., Adi-Dako, O., Yeboah, G. N., Kumadoh, D., Tetteh-Annor, A., Amenuke, E. M., & Karen, M. (2021). Formulation and in Vitro Evaluation of Oral Capsules and Suspension from the Ethanolic Extract of [i]Cola nitida[/i] Seeds for the Treatment of Diarrhea. *BioMed Research International*, *2021*. https://doi.org/10.1155/2021/6630449

Owusu, F. W. A., El Boakye-Gyasi, M., Bayor, M. T., Ofori-Kwakye, K., Acquah, P. G. J., Quarcoo, E. A., Asare, J., Anokye, B. A., & Tandoh, P. K. (2024). Evaluation of Okra Pectin from Different Genotypes as Effective Suspending Agents in Pharmaceutical Formulations. *Journal of Pharmacy*, *4*(1), 33–50. https://doi.org/https://doi.org/10.31436/jop.v4i1.251

Owusu, F. W. A., El Boakye-Gyasi, M., Bayor, M. T., Osei-Asare, C., Johnson, R., Osei, Y. A., Asare, V. A., Mensah, K. A., Acquah, P. G., Otu, D. A. B., & Asante, R. (2022). Pharmaceutical Assessment of Watermelon Rind Pectin as a Suspending Agent in Oral Liquid Dosage Forms. *BioMed Research International*, *2022*(1), 1–9. https://doi.org/10.1155/2022/9526404

Pacheco, M. T., Villamiel, M., Moreno, R., & Moreno, F. J. (2019). Structural and Rheological Properties of Pectins Extracted from Industrial Sugar Beet By-Products. *Molecules*, *24*(3), 392. https://doi.org/10.3390/molecules24030392

Ranganna, S. (1986). *Handbook of analysis and quality control for fruit and vegetable products*. Tata McGraw-Hill Education.

Sáez-Plaza, P., Navas, M. J., Wybraniec, S., Michałowski, T., & Asuero, A. G. (2013). An Overview of the Kjeldahl Method of Nitrogen Determination. Part II. Sample Preparation, Working Scale, Instrumental

Finish, and Quality Control. *Critical Reviews in Analytical Chemistry*, *43*(4), 224–272. https://doi.org/10.1080/10408347.2012.751787

Sato, M. de F., Rigoni, D. C., Canteri, M. H. G., Petkowicz, C. L. de O., Nogueira, A., & Wosiacki, G. (2011). Caracterização química e instrumental de pectinas isoladas de bagaço de 11 cultivares de maçã. *Acta Scientiarum - Agronomy*, *33*(3), 383–389. https://doi.org/10.4025/actasciagron.v33i3.7125

Schieber, A., Hilt, P., Endreß, H. U., Rentschler, C., & Carle, R. (2003). A new process for the combined recovery of pectin and phenolic compounds from apple pomace. *Innovative Food Science and Emerging Technologies*, *4*(1), 99–107. https://doi.org/10.1016/S1466-8564(02)00087-5

Shaari, N., Shamsudin, R., Nor, M. Z. M., & Hashim, N. (2021). Quality attributes of Malaysia purple-fleshed sweet potato at different peel condition. *Agronomy*, *11*(5), 5. https://doi.org/10.3390/agronomy11050872

Shamsudin, R., Shaari, N., Mohd Noor, M. Z., Azmi, N. S., & Hashim, N. (2022). Evaluation of Phytochemical and Mineral Composition of Malaysia's Purple-Flesh Sweet Potato. *Pertanika Journal of Science & Technology*, *30*(4).

Sugri, I., Maalekuu, B. K., Gaveh, E., & Kusi, F. (2017). Sweet potato value chain analysis reveals opportunities for increased income and food security in Northern Ghana. *Advances in Agriculture*, *2017*.

Teye, E., & Abano, E. E. (2012). Physical properties of two varieties of sweet potato grown in the coastal savannah zone of Ghana. *International Journal of Science and Nature*, *3*(1), 105–109.

The United States Pharmacopeial Convention. (2021). *United States Pharmacopeia 44-NF 39 : General Chapter Identification of Articles of Botanical Origin*.

Torres, M. D., & Domínguez, H. (2020). Valorisation of potato wastes. *International Journal of Food Science and Technology*, *55*(6), 2296–2304. https://doi.org/10.1111/ijfs.14228

Twinomuhwezi, H., Godswill, A. C., & Kahunde, D. (2020). Extraction and Characterization of Pectin from Orange (Citrus sinensis), Lemon (Citrus limon) and Tangerine (Citrus tangerina). *American Journal of Physical Sciences*, *1*(1), 17–30. https://doi.org/10.47604/ajps.1049

Wandee, Y., Uttapap, D., & Mischnick, P. (2019). Yield and structural composition of pomelo peel pectins extracted under acidic and alkaline conditions. *Food Hydrocolloids*, *87*, 237–244. https://doi.org/10.1016/j.foodhyd.2018.08.017

Willats, W. G. T., Knox, J. P., & Mikkelsen, J. D. (2006). Pectin: New insights into an old polymer are starting to gel. *Trends in Food Science and Technology*, *17*(3), 97–104. https://doi.org/10.1016/j.tifs.2005.10.008

Yapo, B. M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chemistry*, *100*(4), 1356–1364. https://doi.org/10.1016/j.foodchem.2005.12.012

Zaidel, D., Zainudin, N., Jusoh, Y., & Muhamad, I. (2015). Extraction and characterisation of pectin from sweet potato (Ipomoea batatas) pulp. *Journal of Engineering Science and Technology Special Issue on SOMCHE*, 22–29.

Zhang, C., & Mu, T. (2011). Optimisation of pectin extraction from sweet potato (Ipomoea batatas, Convolvulaceae) residues with disodium phosphate solution by response surface method. *International Journal of Food Science and Technology*, *46*(11), 2274–2280. https://doi.org/10.1111/j.1365-2621.2011.02746.x

Journal of Pharmacy

Home Medication Review among Patients with Chronic Diseases in Rural Villages of Pahang, Malaysia: A Case Report

Norny Syafinaz Ab Rahman[1,2*], Nur Alyaa Anisa Mat Harom[1], and Nor Ilyani Mohamed Nazar[1,2]

[1]*Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[2]*Sustainable Initiative towards Healthy Living and Environment for Sejahtera Community (SITEC) Flagship, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

Abstract

Introduction: Home Medication Review (HMR) is crucial for optimising medication use and improving patient's outcomes. Despite its potential benefits, HMR implementation in rural areas faces challenges. This study evaluates the impact of HMR programs on patients with chronic diseases in a rural setting, addressing challenges and proposing strategies for improvement. **Case Presentations:** This case report presents two cases involving patients with chronic diseases residing in a rural area of Pahang, Malaysia, who participated in pharmacist-led HMR visits. The first case describes a 62-year-old single Malay female with multiple chronic conditions, including Type 2 diabetes mellitus (T2DM), hypertension, dyslipidemia, and bronchial asthma, managed with eight prescribed medications. The second case involves a 34-year-old married Malay female diagnosed with hypertension and T2DM, receiving nine prescribed medications. Both patients underwent a follow-up HMR visit six months after the initial consultation. During each visit, assessments included blood pressure (BP), pulse rate (PR), and blood glucose tests. HMR activities encompassed medication reconciliation, review, patient interviews, evaluation of medication knowledge and adherence, inspection of medication storage, and patient counseling. Identified drug-related problems (DRPs) were addressed through appropriate interventions. **Results:** The study reveals improved patient understanding post-HMR and emphasising the role of pharmacists in addressing medication-related issues. Challenges like non-adherence, uncontrolled disease conditions, and incorrect insulin storage persist. The discussion explores these challenges, citing the need for patient education, regular follow-ups, multidisciplinary collaboration, and technology integration to enhance patient outcomes. **Summary:** These cases underscore the challenges of managing chronic diseases in resource-limited rural settings and highlight the critical role of pharmacist-led HMR in optimizing medication use and improving patient outcomes. The findings highlight the importance of regular follow-up, tailored interventions to address drug-related problems, and collaborative care approaches. They also advocate for integrating technology, enhancing patient education, and addressing social determinants to improve medication understanding and overall health outcomes in underserved populations.

Article history:

Received: 6 June 2024
Accepted: 21 January 2025
Published: 31 January 2025

Keywords:

Home medication review
Medication management therapy
Medication adherence
Chronic diseases
Rural areas

doi: 10.31436/jop.v5i1.326

*Corresponding author's email: norny@iium.edu.my; nornysyafinaz@gmail.com

Introduction

Home Medication Review (HMR) is a patient-centered approach involving collaboration among clinicians, pharmacists, and patients to optimize the quality use of medicines, improve patients' understanding of their medications, and enhance health outcomes by ensuring continuity of care in community settings (Gudi *et al*., 2019). The primary aim of the HMR program is to reduce drug-related problems (DRPs) resulting from improper medication use, thereby improving patients' compliance with their prescribed regimens (Dhillon *et al.,* 2015). One significant advantage of the HMR model is that assessments occur in the patient's home environment, allowing for a comprehensive evaluation of how medications are managed. While HMR consultations typically occur at home, patients may choose alternate locations based on their preferences, cultural beliefs, and socioeconomic circumstances (Patounas *et al.,* 2021).

The delivery of HMR services in Malaysia is currently limited to public hospitals and health clinics (Sundus *et al.,* 2022; 2024). Appointments for HMR are typically conducted at these facilities; however, the availability of trained staff and resources restricts the frequency of geriatric HMR services to a maximum of one visit per week or four visits per month. HMR services are often provided as a one-time intervention without follow-up visits, with urgent clinic appointments arranged only in cases of emergencies. The lack of dedicated personnel further limits the expansion of HMR services and the provision of consistent follow-up care (Sundus *et al.,* 2024). Moreover, significant disparities exist in the delivery of HMR services, particularly in rural areas, due to insufficient resources and infrastructure (Rahman *et al.,* 2020).

In the current study, HMR was initiated during a community engagement program in rural villages in Pahang by a multidisciplinary team comprising pharmacists, nurses, doctors, and non-medical volunteers under a university flagship initiative. Follow-up HMR visits were integrated into a university core course. The objectives of this program were to reconcile and review patients' medication regimens, assess medication adherence, identify pharmaceutical care issues, and provide education to patients and caregivers on disease management and proper medication storage.

This case report highlights the experiences of patients with chronic diseases who participated in the HMR program and examines the impact of HMR on their understanding, medication management, and adherence. The manuscript aims to provide insights into the potential benefits of HMR services in rural areas, the challenges encountered in their implementation, and their role in improving medication management and health outcomes in community settings. The following cases illustrate key findings from the HMR services provided.

Case Presentation

Case 1

History

A 62-year-old Malay female with multiple chronic conditions, including type 2 diabetes mellitus (T2DM), hypertension, dyslipidemia, and bronchial asthma, participated in an HMR program. The patient was single and lived with her mother. The initial HMR visit was conducted at the patient's home by a multidisciplinary team comprising pharmacists, nurses, doctors, and non-medical volunteers. Functional assessments revealed normal vision, hearing, speech, swallowing, cognition, insight, mobility, and no issues with language or literacy. She did not utilize dose administration aids (e.g., pillboxes or alarms) or monitoring devices such as blood pressure and blood glucose monitors.

The patient's prescribed medications included subcutaneous Insulatard 20U ON, metformin 500 mg (2 tablets BD), gliclazide 80 mg (2 tablets BD), perindopril 4 mg (2 tablets OD), amlodipine 10 mg (1 tablet OD), atorvastatin 20 mg (½ tablet ON), Flixotide Evohaler 125 mcg (2 puffs BD), and salbutamol MDI (2 puffs PRN). Health screenings revealed elevated blood pressure (184/101 mmHg) and pulse rate (119 bpm), while random blood glucose (RBG) was 5.6 mmol/L. Her HbA1C from medical records indicated suboptimal glycemic control (7.2%). Although she reported no alarming symptoms related to her elevated blood pressure or heart rate, she exhibited mild shortness of breath, likely attributed to asthma, and denied experiencing medication-related side effects.

Six months later, a follow-up HMR visit was conducted under a university program involving healthcare professionals and students from International Islamic University Malaysia (IIUM) Kuantan. The patient's prescribed medications remained unchanged, but clinical parameters showed persistent poor control. Her blood pressure

(189/113 mmHg) and pulse rate (104 bpm) remained elevated, while RBG increased to 8.4 mmol/L. Additionally, the patient reported a persistent cough.

Intervention and Outcome

During the initial HMR visit, the healthcare team conducted medication reconciliation, reviewed prescribed medications (name, dosage, frequency, and administration route), and assessed for allergies and adverse reactions. The patient's technique for administering medications, including the use of inhalers, was evaluated, and proper techniques were reinforced. The team also examined medication storage practices, including insulin storage, and assessed the patient's understanding of medication use using the Drug's Dose, Frequency, Indication, and Time (DFIT) assessment. The patient scored 100% on the DFIT assessment, indicating good knowledge and competence in self-administering her medications. However, her blood pressure remained uncontrolled (184/101 mmHg), and she reported that her Flixotide Evohaler and salbutamol MDI were ineffective despite correct usage. During the HMR visit, the patients were requested to show how the MDI and Evohaler were used. Any incorrect technique was properly corrected and informed to the patient. We communicated and presented the issue to the doctor for further action.

During the follow-up visit, the patient's medications were reviewed. Flixotide Evohaler was replaced with MDI Fluticasone (2 puffs BD), and atorvastatin 20 mg (½ tablet ON) was switched to simvastatin 20 mg (1 tablet ON). Although the patient retained good knowledge of her medications (100% DFIT score), issues with insulin injection technique and improper insulin storage persisted. The pharmacist provided counseling on proper insulin injection techniques and emphasized correct storage practices. This includes guidance on storing the unopened insulin inside the refrigerator without freezing it, keeping an open insulin pen with a cartridge at room temperature for four to six weeks after the first use (Richter *et al.*, 2023), and emphasising the importance of regularly checking expiration dates. The patient was advised to store other medications in a dry place away from direct sunlight, heat, and humidity.

Additionally, the patient's uncontrolled blood pressure (189/113 mmHg) and persistent cough were discussed with the doctor, who suspected the cough might be a side effect of perindopril. According to Brugts *et al.*, (2014), ACE inhibitors may induce a dry, irritating cough in 4% to 35% of individuals. This cough can commence at any point during ACE inhibitor treatment, either after the initial dose or over several weeks or months. The cough is attributed to the accumulation of bradykinin and substance P in the lungs, causing constriction of smooth muscle and subsequent coughing (Yilmaz, 2019). Factors that potentially elevate the risk include older age, female gender, non-smoking status, and conditions like asthma or chronic obstructive pulmonary disease (COPD) leading to airway hyperreactivity (Nasser *et al.*, 2018). Hence, the concern was noted in the patient's record for the doctor's attention. Educational interventions on asthma and diabetes management were also provided.

Case 1

History

A 34-year-old Malay female living in a rural village in Pahang was included in the HMR program. She lived with her family, was married, and had a history of hypertension and T2DM. Functional assessments indicated that she was independent in activities of daily living (ADL). The patient did not use dose administration aids or home monitoring devices. Her prescribed medications included subcutaneous insulin (Insugen), perindopril 4 mg (2 tablets OD), amlodipine 10 mg (1 tablet OD), bisoprolol (1 tablet OD), hydrochlorothiazide 50 mg (1 tablet OD), metformin 500 mg (2 tablets OD), vildagliptin 50 mg (1 tablet BD), and gliclazide 80 mg (2 tablets BD). During the initial visit, her blood pressure (161/116 mmHg), pulse rate (134 bpm), and RBG (10.5 mmol/L) were elevated. She did not complain any side effects of her prescribed medications.

During the follow-up visit, gliclazide MR 30 mg was newly prescribed. Despite counseling, her clinical parameters remained poorly controlled, with blood pressure at 153/120 mmHg and RBG at 10.5 mmol/L.

Intervention and Outcome

Medication reconciliation, DFIT assessment, and evaluation of medication administration and storage were conducted. The patient scored 95% on the DFIT assessment during the initial visit. It was

found that the patient stored the unopened insulin at room temperature and did not keep it at a cool temperature due to the broken refrigerator. Hence, the improper insulin storage due to a broken refrigerator was addressed by coordinating with health clinic personnel to supply smaller insulin quantities.

Duplication of therapy with gliclazide 80 mg and gliclazide MR 30 mg was identified, and the patient was educated on the risks of overdose, with the discontinued medication retrieved for disposal. Sulfonylurea (gliclazide) overdose might cause severe hypoglycaemia, which may necessitate urgent intravenous glucose administration and close monitoring (Megarbene *et al.,* 2022). Symptoms of hypoglycaemia, such as sweating, shakiness, increased hunger, nervousness, fatigue, and loss of consciousness, were explained to the patient. Unfortunately, her RBG was 10.7 mmol/L indicating that a home blood glucose monitoring device was deemed necessary to enable regular monitoring of her RBG levels.

Despite counselling and educational efforts, the patient's adherence to medications remained poor, as indicated by unused medications, including insulin.

During the follow-up visit, the patient's knowledge of her medications improved to 100% on the DFIT assessment. However, issues with insulin storage and medication non-adherence persisted, contributing to uncontrolled clinical parameters. The pharmacist referred the patient to a doctor for further evaluation and management.

Discussion

This study demonstrated that patients exhibited improved understanding of medication use following HMR visits, as evidenced by the DFIT assessment score. This finding highlights the positive impact of pharmacist-led HMR programs on enhancing patients' comprehension of their treatment regimens. Proper understanding and adherence to prescribed medications are critical for effective disease management (Saqib *et al.,* 2018). Insufficient knowledge about medications can hinder adherence, potentially compromising therapeutic outcomes (Tan *et al.,* 2019). During HMR visits, pharmacists provided essential information on medication indications, dosages, and safe storage practices (Sundus *et al.,* 2022). Notably, these interventions significantly improved patients' understanding of medication indications and appropriate dosages. Enhanced comprehension of drug dosages mitigates the risks of underdosing or overdosing, thereby optimizing therapeutic efficacy and minimizing toxicity (Tan *et al.,* 2019). Similarly, understanding the indications of medications aligns treatment objectives with therapeutic goals, improving effectiveness and patient outcomes.

The study also revealed that HMR programs enable healthcare professionals to identify and address medication-related issues. Issues resolved during HMR visits included ineffective medications and medication duplication, consistent with findings from prior studies (Newman *et al.,* 2020; Sundus *et al.,* 2022). Addressing such issues during HMR visits enhances adherence, reduces adverse drug reactions, and prevents hospitalizations (Goh *et al.,* 2014; Ravindra & Kaushik, 2022). However, persistent challenges remain, including medication non-adherence, improper insulin storage, incorrect insulin injection techniques, uncontrolled BP, and high blood glucose levels. These findings underscore the need for healthcare teams to implement targeted strategies to address these issues and improve the quality of life for patients with chronic diseases (Gudi *et al.,* 2019).

The World Health Organization (WHO) recognized medication non-compliance as a significant challenge in managing chronic illnesses (Brown *et al.*, 2011). Approximately 50% of patients with chronic illnesses do not adhere to their prescribed medication regimens (Sabaté, 2003; Lee *et al.,* 2006). Patients with diabetes mellitus, in particular, face difficulties adhering to complex treatment regimens and managing medication side effects (Sari *et al.,* 2022). Diabetes management involves tasks such as dietary adjustments, regular exercise, and blood glucose monitoring, which can disrupt patients' daily lives. Lack of dose administration aids, such as pillboxes or alarms, further exacerbates adherence issues, impacting health outcomes. A study by Fitria *et al.* (2023) found that the use of pillboxes during HMR visits improved adherence in Indonesian hypertension patients. These aids facilitate timely and accurate medication intake, enhancing therapeutic efficacy and reducing missed doses. Medication non-adherence in diabetes mellitus patients is associated with unfavourable clinical outcomes, increased complications, and reduced quality of life (Tampa'i *et al.,* 2021). Complications include cardiovascular disease, neuropathy, retinopathy, cerebrovascular

disease, nephropathy, and peripheral vascular diseases (Sari *et al.,* 2022). Education, motivation, continuous monitoring, and assessment are pivotal in improving adherence and health outcomes.

Pharmacists play a critical role in patient education during HMR visits, complemented by consistent monitoring and assessment. However, rural areas present unique challenges due to geographical distances from healthcare facilities, leading to prolonged gaps between HMR visits. These delays disrupt continuity of care, limiting opportunities for medication adjustments, monitoring, and preventative measures. This can result in deteriorating health conditions, increased hospitalizations, and higher healthcare costs. Telehealth services or telemedicine including telemonitoring and teleconsultation, offer a viable solution to overcome geographical barriers and ensure continuity of care. Telemedicine effectively addresses logistical, financial, sociocultural, and infrastructural challenges, enhancing healthcare accessibility (Anawade *et al.,* 2024). Transportation barriers in rural areas further limit access to healthcare facilities, compounding these challenges (Cochran *et al.,* 2022).

Effective management of blood glucose and blood pressure (<130/80 mm Hg) significantly reduces cardiovascular complications in individuals with type 2 DM (Hanley *et al.,* 2015). However, this study found no significant changes in BP or random blood glucose levels between baseline and six-month follow-up visits. This may be attributed to prolonged intervals between visits, the absence of interim interventions, and unmonitored lifestyle changes such as diet and stress levels. Additionally, the lack of home monitoring devices, such as blood glucose and BP monitors, limits patients' ability to manage their conditions effectively. Regular monitoring is essential for informed decision-making and proactive healthcare management.

Improper insulin storage and injection techniques were also identified as barriers to effective diabetes management. Poor storage conditions, such as exposure to extreme temperatures, can compromise insulin efficacy (DDRC, n.d). Incorrect injection techniques can lead to complications, including hypo- and hyperglycemia, glycemic fluctuations, and diabetic ketoacidosis (Trief *et al.,* 2016). Disease management programs tailored for cardiovascular conditions, including diabetes, have demonstrated improved clinical outcomes (Ofman *et al.,* 2004). Frequent HMR visits, coupled with telehealth interventions, can enhance medication adherence, patient engagement, and clinical outcomes (Rothwell & Hogan, 2015; Rosli *et al.,* 2021).

This study underscores the importance of adherence to medication regimens, lifestyle modifications, and patient education on proper insulin storage and injection techniques. Shorter follow-up intervals, such as monthly home visits and weekly telephonic updates, could further enhance outcomes. Telehealth solutions offer a promising avenue for improving long-term disease management by fostering patient engagement and reducing hospital admissions (Hanley *et al.,* 2015).

Strategies to address issues and to improve patient's outcomes

Tailored assessments, multidisciplinary collaboration and effective communication with patients

Implementing individualized home medication reviews that consider each patient's medical history, obtaining patient's details, challenges, preferences, and lifestyle can effectively identify and address drug-related issues, optimize medication use, and enhance overall patient outcomes. Collaboration between pharmacists, physicians, and other healthcare providers during HMR sessions ensures comprehensive medication management. For instance, drug-related problems identified during HMR visits can be discussed with physicians, allowing for the development of a tailored management plan. This plan is subsequently shared with the patient and caregiver, ensuring its effective implementation with necessary modifications (Chandrasekhar *et al.,* 2019).

Gudi *et al.* (2019) demonstrated that collaborative efforts between general practitioners and pharmacists during HMR visits resolved approximately 85% of drug-related problems. Effective communication is equally essential in improving patient outcomes. The Agency for Healthcare Research and Quality (2018) recommends communication strategies such as teach-back, warm handoffs, and medication reviews, which involve discussing the patient's complete medication regimen. Additionally, engaging with patients outside their homes,

including follow-up calls, can enhance patient satisfaction and adherence to treatment plans.

Frequent and comprehensive HMR visits

More frequent and comprehensive HMR visits are crucial for addressing medication-related challenges and improving outcomes for patients with chronic diseases. Regular reviews facilitate early identification of drug-related problems and support personalized care for optimized disease management. Scheduling HMR visits every 2–3 months can ensure consistent follow-up, particularly for patients in remote locations. Rosli *et al.* (2021) suggested shorter follow-up intervals to enhance the interaction between pharmacists and patients.

Monthly HMR visits supplemented with weekly telephonic updates on drug-related problems (DRPs) and clinical progress may significantly improve medication adherence and patient quality of life. Sundus *et al.* (2022) found that participants valued follow-up HMR visits to monitor progress and resolve medication-related issues, providing reassurance during the waiting period for the next clinic appointment. Personalized and in-depth discussions during HMR sessions improve patient understanding of medication indications and administration techniques, fostering adherence and better health outcomes.

Enhancing patient education on medications and storage

Healthcare professionals should prioritize educating patients about their medications, including proper administration, potential adverse effects, and the importance of adherence. Proper medication storage, particularly for insulin, is critical. According to the Diabetes Disaster Response Coalition (n.d), insulin should be stored in a refrigerator at 2–6°C (36–43°F) and never frozen. Patients should be informed about the expiration date of insulin and advised to store it in a cool, dry place away from direct heat and sunlight when refrigeration is unavailable. Ice packs and insulated containers may also be utilized for storage (Jacob *et al.,* 2023).

Richter *et al.* (2023) highlighted that opened insulin can be stored safely at room temperature for 4–6 weeks. Patients should also be educated on proper injection techniques, including site selection, needle length, and injection angle, to ensure effective absorption and avoid complications. Visual materials, such as pamphlets or posters, and mobile applications can provide instructional content and reminders on proper insulin administration. Collaborating with local health clinics for hands-on demonstrations and utilizing telehealth services for real-time guidance can further support patients, particularly in rural settings.

Leveraging technology for telehealth and telemedicine

The adoption of telehealth technologies has expanded significantly, particularly during the COVID-19 pandemic, providing a means to deliver HMR services to patients in rural and remote areas. Telehealth platforms enable patients to access healthcare services and records through smartphones, facilitating communication with healthcare teams and improving care navigation (Hanjani *et al.,* 2020).

Telemedicine allows remote consultations and periodic follow-ups via communication tools like SMS, WhatsApp, or phone calls (Ang *et al.,* 2022). These technologies can monitor vital signs at home, reducing hospital readmissions and improving the quality of life for chronically ill patients (Guideway care, 2023). For example, hospitals in Australia have utilized video conferencing to conduct medication reviews, benefiting patients in rural and remote regions (Hanjani *et al.,* 2020).

Medication adherence can be supported through smartphone apps, smart pill dispensers, and electronic packaging, which remind patients to take their medications and alert healthcare providers of adherence issues. In areas with limited internet access, alternative strategies like phone-based consultations, SMS reminders, or local telehealth hubs equipped with internet access can bridge connectivity gaps. These hubs, located in community centers or clinics, provide patients with a centralized location to access telehealth services. Evidence suggests that telehealth-delivered medication reviews improve clinical outcomes, reduce costs, and are well-accepted by patients,

offering a sustainable solution for chronic disease management (Hanjani *et al.*, 2020).

Conclusion

This study highlights the significance of HMR programs in rural areas, emphasizing their impact on patients' understanding, medication management, and adherence. The findings demonstrate the positive influence of HMRs in improving patients' knowledge of their medications, particularly regarding indications and proper dosage. However, challenges such as medication non-adherence, uncontrolled blood pressure, and elevated blood glucose levels persist, underscoring the complexity of chronic disease management in these settings.

The study identifies several critical issues, including the use of ineffective medications, inappropriate insulin storage, and incorrect injection techniques, which highlight the vital role of pharmacists in resolving medication-related problems during HMR visits. To address these challenges, the research emphasizes the need for personalized assessments, multidisciplinary collaboration, and enhanced communication among healthcare providers to optimize patient care.

Furthermore, the study underscores the importance of regular follow-ups, community-based educational initiatives, and the integration of technological interventions, such as telehealth, to improve patient outcomes. Patient education is particularly crucial, with a focus on proper medication storage especially for insulin and correct administration techniques. Recognizing the influence of social determinants, such as education levels, on medication understanding, the study advocates for a comprehensive approach that combines enhancing patients' knowledge with addressing broader systemic factors.

In conclusion, this research demonstrates the positive impact of HMR programs in rural areas while emphasizing the need for ongoing efforts to address persistent challenges. Collaborative care models, regular follow-ups, and the integration of technology are essential to improving the overall health outcomes of patients with chronic diseases in rural settings.

Authors contributions

Conceptualization, NSAR & NIMN; methodology, NSAR, NIMN, AA.; software, AA.; validation, NSAR and NIMN.; formal analysis, AA.; investigation, NSAR and NIMN.; resources, NSAR and NIMN; data curation, AA; writing—original draft preparation, AA; writing—review and editing, NSAR; supervision, NSAR. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The author would like to express gratitude to all patients, local communities and collaborators that involved in the HMR programmes conducted at the rural area of Pahang, Malaysia.

Ethical approval statement

This study protocol was approved by the IIUM Research Ethics Committee (IREC) of International Islamic University Malaysia (ID No: IREC 2023-171 on 24th October 2023).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Conflict of interest

The authors declare that there is no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work ChatGPT was used to improve readability and language. The author reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

Agency for Healthcare Research and Quality. (2018). Guide to Improving Patient Safety in Primary Care Settings by Engaging Patients and Families. Content last reviewed April 2018., Rockville, MD. https://www.ahrq.gov/patient-safety/reports/engage/strategies.html

Anawade, P. A., Sharma, D., Gahane, S. (2024) A Comprehensive Review on Exploring the Impact of Telemedicine on Healthcare Accessibility. Cureus, 16(3): e55996. DOI 10.7759/cureus.55996.

Ang, W.C., Lah, J. C., Zulkepli, N., Syukri, N., & Rosedi, A. (2022). Carers' Perspectives on Home Medication Review by a State Hospital in Malaysia, International Journal of Care Scholars, 5(1),49-55. https://journals.iium.edu.my/ijcs/index.php/ijcs/article/view/236

Bingham, J. M., Black, M., Anderson, E. J., Li, Y., Toselli, N., Fox, S., Martin, J. R., Axon, D. R., Silva-Almodóvar, A. (2021). Impact of Telehealth Interventions on Medication Adherence for Patients with Type 2 Diabetes, Hypertension, and/or Dyslipidemia: A Systematic Review. Annals Pharmacotherapy, 55(5), 637-649. doi: 10.1177/1060028020950726. Epub 2020 Aug 20. PMID: 32815400.

Brown, M. T., & Bussell, J. K. (2011). Medication adherence: WHO cares? Mayo Clin Proc, 86(4), 304-14. doi: 10.4065/mcp.2010.0575. Epub 2011 Mar 9. PMID: 21389250; PMCID: PMC3068890.

Brugts, J. J., Arima, H., Remme, W., Bertrand, M., Ferrari, R., Fox, K., DiNicolantonio, J., MacMahon, S., Chalmers, J., Zijlstra, F., Caliskan, K., Simoons, M. L., Mourad, J. J., Boersma, E., & Akkerhuis, K. M. (2014). The incidence and clinical predictors of ACE-inhibitor induced dry cough by perindopril in 27,492 patients with vascular disease. International Journal of Cardiology, 176(3), 718–723. https://doi.org/10.1016/j.ijcard.2014.07.108

Chandrasekhar, D., Joseph, E., Ghaffoor, F. A., & Thomas, H. M. (2019). Role of pharmacist led home medication review in community setting and the preparation of medication list. Clinical Epidemiology and Global Health, 7(1), 66–70. https://doi.org/10.1016/j.cegh.2018.01.002

Cochran, A. L., McDonald, N. C., Prunkl, L., Vinella-Brusher, E., Wang, J., Oluyede, L., Wolfe, M. (2022). Transportation barriers to care among frequent health care users during the COVID pandemic. BMC Public Health, 22(1), 1783. doi: 10.1186/s12889-022-14149-x. PMID: 36127650; PMCID: PMC9486769.

Diabetes Disaster Response Coalition. [Last accessed on 2025 Jan 14]. Available from: https://diabetes.org/sites/default/files/2023-10/ddrc-storing-insulin-2018.pdf

Dhillon, A. K., Hattingh, H. L., Stafford, A., & Hoti, K. (2015). General practitioners' perceptions on home medicines reviews: a qualitative analysis. BMC Family Practice, 16(1). https://doi.org/10.1186/s12875-015-0227-8

Fitria, N., Lailaturrahmi, L., Sari, Y. O., Anata, F. T., & Husnia, K. (2023). Adherence Assessment on Hypertension Therapy Using the Pill Count Method In Lubuk Kilangan Health Center, Indonesia. *Jurnal Sains Farmasi & Klinis*, *10*(1), 28–34. https://doi.org/10.25077/jsfk.10.1.28-34.2023

Goh, B. Q., Tay, A. H. P., Khoo, R. S. Y., Goh, B. K., Lo, P. F. L., & Lim, C. J. F. (2014). Effectiveness of Medication Review in Improving Medication Knowledge and Adherence in Primary Care Patients. Proceedings of Singapore Healthcare, 23(2), 134–141. https://doi.org/10.1177/201010581402300207

Gudi, S. K., Kashyap, A., Chhabra, M., Rashid, M., & Tiwari, K. K. (2019). Impact of pharmacist-led home medicines review services on drug-related problems among the elderly population: a systematic review. Epidemiology and Health, 41, e2019020. https://doi.org/10.4178/epih.e2019020

Guideway Care. (2023, November 8). *Guideway Care ranked number 83 fastest-growing company in North America on the 2023 Deloitte Technology Fast 500™*. BusinessWire. https://www.businesswire.com/news/home/20231108916913/en/Guideway-Care-Ranked-Number-83-Fastest-Growing-Company-in-North-America-on-the-2023-

Deloitte-Technology-Fast-500%E2%84%A2

Hanjani, L. S., Bell, J. S., & Freeman, C. (2020). Undertaking medication review by telehealth. Australian Journal of General Practice, 49(12), 826–831. https://doi.org/10.31128/ajgp-06-20-5461

Hanley, J., Fairbrother, P., McCloughan, L., et al. (2015). Qualitative study of telemonitoring of blood glucose and blood pressure in type 2 diabetes. BMJ Open, 5:e008896. doi:10.1136/bmjopen-2015008896

Jacob, J. J. (2023). Insulin Storage Guidance for Patients with Diabetes Using Insulin. Indian J Endocrinol Metab, 27(2), 93-95. doi: 10.4103/2230-8210.374161. Epub 2023 Apr 14. PMID: 37292077; PMCID: PMC10245305.

Lee, J. K., Grace, K. A., Taylor, A. J. (2006). Effect of a pharmacy care program on medication adherence and persistence, blood pressure, and low-density lipoprotein cholesterol: a randomized controlled trial. JAMA, 6, 296(21), 2563-71. doi: 10.1001/jama.296.21.joc60162. Epub 2006 Nov 13. PMID: 17101639.

Mégarbane, B., Chevillard, L., Nihel Khoudour, & Declèves, X. (2021). Gliclazide disposition in overdose – a case report with pharmacokinetic modeling. Clinical Toxicology, 60(4), 541–542. https://doi.org/10.1080/15563650.2021.1993245

Nasser, J., Al Aradi, K., Ebrahim, K. S., Omran, A. (2018). High incidence of cough users of Angiotensin-Converting Enzyme Inhibitors. Bahrain Med Bull, 40(2), 97-99.

Newman, T. V., San-Juan-Rodriguez, A., Parekh, N., Swart, E. C. S., Klein-Fedyshin, M., Shrank, W. H., Hernandez, I. (2020). Impact of community pharmacist-led interventions in chronic disease management on clinical, utilization, and economic outcomes: An umbrella review. Res Social Adm Pharm, 16(9), 1155-1165. doi: 10.1016/j.sapharm.2019.12.016. Epub 2020 Jan 14. PMID: 31959565.

Ofman, J. J., Badamgarav, E., Henning, J. M., Knight, K., Gano, A. D., Levan, R. K., Gur-Arie, S., Richards, M., Hasselblad, V., & Weingarten, S. (2004). Does disease management improve clinical and economic outcomes in patients with chronic diseases? A systematic review. The American Journal of Medicine, 117(3), 182–192. https://doi.org/10.1016/j.amjmed.2004.03.018

Patounas, M., Lau, E. T., Chan, V., Rigby, D., Kyle, G. J., Khatri, J., Poudel, A., & Nissen, L. M. (2021). Home medicines reviews: a national survey of Australian accredited pharmacists' health service time investment. Pharmacy Practice, 19(3), 2376. https://doi.org/10.18549/pharmpract.2021.3.2376

Rahman, M., White, E. M., Thomas, K. S., & Jutkowitz, E. (2020). Assessment of Rural-Urban Differences in Health Care Use and Survival Among Medicare Beneficiaries With Alzheimer Disease and Related Dementia. JAMA Network Open, 3(10), e2022111. https://doi.org/10.1001/jamanetworkopen.2020.22111

Ravindra, B. N., & Kaushik, A. (2022). Effect of Pharmacy Home Medications Review Services On Drug-Related Issues Among The Elderly Population. International Journal of Advance and Applied Research, 10(1), 1306–1315. https://doi.org/10.5281/zenodo.8017181

Richter, B., Bongaerts, B., Metzendorf, M-I. (2023). Thermal stability and storage of human insulin. Cochrane Database of Systematic Reviews, Issue 11. Art. No.: CD015385. DOI: 10.1002/14651858.CD015385.pub2.

Rosli, M. R., Neoh, C. F., Wu, D., Hassan, N. W., Mahmud, M., Rahimi, A., & Karuppannan, M. (2021). The evaluation of home medication review for patients with type 2 diabetes mellitus by community pharmacists: A randomised controlled trial. Pharmacy Practice (Internet), 19(3), 2397.https://doi.org/10.18549/pharmpract.2021.3.2397

Rothwell, M., & Hogan, A. (2015). Clinical pharmacists connecting with patients in rural and remote towns via telehealth. 13th National Rural Health Conference, Darwin Convention Centre, NT, Australia. https://www.ruralhealth.org.au/13nrhc/images/paper_Rothwell,%20Michelle.pdf

Sabaté, E., ed. (2003). Adherence to Long-Term Therapies: Evidence for Action. Geneva, Switzerland: World Health Organization.

Saqib, A., Atif, M., Ikram, R., Riaz, F., Abubakar, M., & Scahill, S. (2018). Factors affecting patients' knowledge about dispensed medicines: A Qualitative study of healthcare professionals and patients in Pakistan. PLOS ONE, 13(6), e0197482. https://doi.org/10.1371/journal.pone.0197482

Sari, Y. O., Permatasari, D., Mariza, W., Fitria, N., & Lailiani, R. (2023). Application of Home Medication Review (HMR) on Patient Adherence in Type 2 Diabetes Mellitus (T2DM) Blood Sugar Management. JSFK (Jurnal Sains Farmasi & Klinis), 9(sup), 160–167. https://doi.org/10.25077/jsfk.9.sup.160-167.2022

Sundus, A., Pin, T., & Sellappans, R. (2022). What Do Older Adults and Their Caregivers in Malaysia Think of Home Medication Review? A Qualitative Inquiry. Malaysian Journal of Medicine and Health Sciences, 18(1), 2636–9346. https://expert.taylors.edu.my/file/rems/publication/108546_8462_2.pdf

Sundus, A., Sellappans, R., Pin, T. M. (2024). Informing policy makers in developing countries: Practices and limitations of geriatric home medication review in Malaysia-A qualitative inquiry. Asian Pac J Trop Med, 17(1), 21-29.

Tan, Y. M., Chong, C. P., & Cheah, Y. C. (2019). Impact of hospital pharmacist-led home medication review program for people with schizophrenia: A prospective study from Malaysia. Journal of Applied Pharmaceutical Science, 9(7), 034-041.

Tampa'i, R., Sumombo, J., Hariyadi, H., Lengkey, Y. (2021). Gambaran Drug Related Problems (DRPs) pada Pasien Diabetes Melitus Tipe 2 di Puskesmas Tuminting. Jurnal Kefarmasian Indonesia, 11(1), 49–55. https://doi.org/10.22435/jki.v11i1.3499

Trief, P. M., Cibula, D., Rodriguez, E., Akel, B., & Weinstock, R. S. (2016). Incorrect Insulin Administration: A Problem That Warrants Attention. Clinical Diabetes, 34(1), 25–33. https://doi.org/10.2337/diaclin.34.1.25

Yilmaz, I. (2019). Angiotensin-Converting Enzyme Inhibitors Induce Cough. Turkish Thoracic Journal, 20(1), 36–42. https://doi.org/10.5152/turkthoracj.2018.18014

Journal of Pharmacy

Measuring the Prevalence of Chronic Kidney Disease among Type 2 Diabetes Mellitus Patient in Hospital Jitra: A Population-Based Cohort Study

Norazila Abdul Ghani[1], Sharifah Nor Faiza Syed Hassan[1], Rusyidah Zainol Abidin[2], Zarmisha Zakaria[1], Fatin Farhana Ahmad Nasir[1] and Dr Ahmad Zulfitri Azmi[3]

[1]*Department of Pharmacy, Hospital Jitra.*
[2]*Department of Pharmacy, Hospital Sik.*
[3]*Department of Medical, Hospital Jitra*

Abstract

Introduction: Chronic kidney disease (CKD) develops in approximately 40% of patients with diabetes. The prevalence of CKD in Malaysia is 9.07% among adults. It is important to obtain accurate local data on CKD among patient with Type 2 Diabetes Mellitus (T2DM) patients to facilitate health-care planning including the review of health-care priorities, program activities, and allocation of resources. The objective of this study is to determine the prevalence of CKD among DM. We further explored the association between glycaemic control, eGFR and adverse outcomes (cardiovascular events, hospitalizations, and kidney failure) among these patients. **Materials and method:** This is a retrospective cohort study involving 91 DM patients who received treatment in Hospital Jitra, Kedah, Malaysia. Patients' demographic data, comorbidities, history of hospital admission, medications, complication, and laboratory test results were retrieved from their medical record. Descriptive and inferential statistics have been used to analyse the data. **Results:** 60.4% of the patients were female and 85.7% Malay with mean age of 62.15 years old. Hypertension, hypercholesterolemia and heart disease were the most common comorbidities among them; 92.3%, 78.0%, and 23.1%, respectively. The prevalence of CKD among DM in Hospital Jitra was 38.46%. 20.9% of the patients experienced diabetic complications and 49.5% showed a progression of kidney disease. The results indicate a high percentage of poor glycaemic control among DM in Hospital Jitra; 69%-100% of the patients had HbA1c >7% at each visit and this is associated with CKD (P=0.042). **Conclusion:** The prevalence of CKD among DM patients at Hospital Jitra was 38.46%. Higher HbA1c was associated with CKD in adults with type DM, suggesting that improving glycaemic control may reduce the risk of CKD.

Article history:

Received: 23 June 2024
Accepted: 9 January 2025
Published: 31 January 2025

Keywords:

Chronic Kidney Disease,
Diabetes Mellitus,
Prevalence,
Diabetes Complication

doi: 10.31436/jop.v5i1.331

*Corresponding author's email: ila80@yahoo.com

Introduction

Chronic kidney disease (CKD) is defined by abnormalities in kidney structure or function that have been present for more than 3 months with implications for health.

According to the Centers for disease control and prevention (CDC), about 10% of adults in the United States are estimated to have CKD. National studies reveal that CKD prevalence in Malaysia raised from 9.07% in 2011 to 15.48% in 2018 (Saminathan et al., 2020). Diabetes is the most common or leading single cause of CKD. Type 2 Diabetes mellitus (DM) and chronic kidney disease (CKD) are intricately intertwined. DM is the most common cause of CKD. Adequate control of DM is necessary for prevention of progression of CKD, while careful management of the metabolic abnormalities in CKD will assist in achieving better control of DM (Centers for Disease Control and Prevention, 2021).

Diabetic kidney disease (DKD) affects a significant portion of patients with DM, with estimates suggesting that around 40% of DM patients develop CKD over time (American Diabetes Association, 2021; National Kidney Foundation, 2022).

The relationship between diabetic kidney disease (DKD) and cardiovascular (CV) morbidity and death underscores the urgent need for comprehensive therapeutic strategies. Patients with DKD face a significantly higher risk of cardiovascular problems, as noted by Mann et al. (2018). These patients often die from cardiovascular disease before progressing to end-stage renal disease, highlighting the importance of early, proactive treatment of both renal and cardiovascular health in individuals with DM and DKD (American Diabetes Association, 2023; Pisters et al., 2017).

Modifications to the pharmacokinetics and pharmacodynamics of anti-hyperglycaemic medicines in people with impaired renal function complicate treatment optimization. Careful assessment of these elements is critical for assuring medication safety and efficacy in this patient population (Sinha et al., 2019). The multidimensional character of diabetic kidney disease (DKD) necessitates a comprehensive approach that includes renal protection, cardiovascular risk reduction, and individualized pharmaceutical therapies to optimize outcomes and improve quality of life for those affected by the condition (Zhang et al., 2021; KDIGO, 2020).

Taking renal function into consideration is crucial when prescribing antidiabetic medications due to the potential side effects. Patients with reduced renal function may be more susceptible to hypoglycaemia from insulin and sulfonylureas, and metformin may cause lactic acidosis, particularly in patients with advanced chronic kidney disease (CKD) (Sinha et al., 2019). The American Diabetes Association (ADA) (2023) and KDIGO (2020) guidelines recommend careful dose adjustments or alternative therapies for patients with impaired renal function to minimize the risk of serious adverse effects, such as hypoglycemia and lactic acidosis.

The reluctance to prescribe newer antidiabetic drugs, which have been shown to be safe and effective at differing degrees of chronic kidney disease (CKD), could be due to a variety of factors, including familiarity with traditional therapies, cost concerns, or a lack of understanding of newer alternatives (Lazarus et al., 2020). However, embracing new medications, such as SGLT-2 inhibitors and GLP-1 receptor agonists, may improve results for CKD patients, providing improved glucose control with fewer side effects and reduced cardiovascular risks (McMurray et al., 2019; Buse et al., 2020)

The combined effects of the two major chronic diseases, CKD and DM, lead to significant morbidity, mortality, and a striking economic burden (Khan et al., 2020). Patients with both conditions often present some of the most challenging cases for achieving adequate glycemic control, as management must be tailored to the specific patient's situation, with attention to the degree of CKD or, in the case of End-Stage Renal Disease (ESRD), the type of renal replacement therapy (Harrington et al., 2019). Improved guidelines are needed regarding the proper use of

the new emerging anti-diabetic agent classes, such as SGLT-2 inhibitors and GLP-1 receptor agonists, and safe targets for glycemic control in this complex patient population (American Diabetes Association, 2023; KDIGO, 2020).

It is important to obtain accurate local data on CKD among DM patients to facilitate healthcare planning, including review of healthcare priorities, program activities, and allocation of resources. Therefore, this study aims to determine the prevalence of CKD among DM patients and the relationship between their glycemic control and negative clinical outcomes. This holistic approach could lead to healthcare goals and strategies customized to the individual needs of DM patients with CKD.

Furthermore, there is a need for improved guidelines to navigate the use of emerging anti-diabetic agents and to establish safe targets for glycaemic control in this population. Accurate local data on the prevalence of CKD among DM patients is crucial for informing healthcare planning, including prioritizing health initiatives, program development, and resource allocation. By understanding the specific challenges and prevalence of CKD among DM patients in a given population, healthcare providers and policymakers can better address the needs of this vulnerable group and enhance overall patient outcomes.

Materials and methods

Study Design and Settings

This is a retrospective cohort study; conducted from April 2023 to December 2023, involving Type 2 Diabetes Mellitus patients; aged ≥ 18 years old receiving treatment in Hospital Jitra for more than 1 year. Hospital Jitra is a district hospital providing treatment to the population of the Kubang Pasu District. It is located in the northern part of Peninsular Malaysia and serves the largest town and administrative centre of the district. As of 2024, the estimated sub-urban population in Jitra stands at nearly 63,489 (Real Time World Statistics,2024).

Study Instrument & Data Collection

The data was collected by using the data collection form; that was developed via extensive review of available literature published. Patients' demographic data, comorbidities, history of hospital admission, medications, complications, and laboratory test results were retrieved from their medical records, the Pharmacy Information System (PHIS), and Lab Information System (LIS). The data for each sample was collected from the start of treatment in Hospital Jitra until the latest visit. Mean duration of follow-up was 10.00 years; SD= 4.97.The estimated glomerular filtration rate (eGFR) was estimated from calibrated serum creatinine using the CKD-EPI equation. This study followed CKD categorization based on the Clinical Practice Guideline of The Management of Chronic Kidney Disease in Adults 2018 classification recommendations (Malaysian Health Technology Assessment Section (MaHTAS), 2018). CKD stages 1–3a were categorised as mild to moderate, while stages 3b–5 was categorised as moderate to renal failure. In this study, CKD was defined as eGFR < 60 ml/min/1.73m2 (CKD Stage 3-5).

Study Participants

The inclusion criteria were Malaysians 18 years of age and above who were diagnosed with Type 2 Diabetes Mellitus for more than one year and had received treatment during the study period. The exclusion criteria were insufficient or illegible records.

Sample Size Calculation

Assuming that 40% of the subjects in the population have the factor of interest, and a population size of 2000, the study would require a sample size of 89 for estimating the expected proportion with 10% absolute precision and 95% confidence. (Dhand, N. K., & Khatkar, M. S. (2014).

Data Analysis

All analyses were performed using SPSS version 16. Descriptive statistics were used to determine the prevalence, frequency, mean and percentage of the data. Multiple logistic regression analysis was used to determine the associated

factors between glycaemic control (HbA1c), age, gender and duration of diabetes mellitus and adverse outcomes (mortality, CV events, hospitalizations, and kidney failure). Generalised Estimating Equations (Linear) were used to examine the trend of glycaemic control (HbA1c) and progression of kidney disease (eGFR). A P-value of less than 0.05 was considered to be statistically significant.

Results

A total of 91 adults with T2DM were included in the study. 60.4% of them were female and 85.7% Malay with mean age of 62.15 years old. Hypertension, hypercholesterolemia and heart disease were the most common comorbidities among them; 92.3%, 78.0%, and 23.1%, respectively.

The prevalence of CKD among diabetes mellitus patients in Hospital Jitra

Out of 91 patients, 26.37% had an eGFR >90 ml/min/1.73 m², indicating normal kidney function, while 35.16% had mildly reduced function with an eGFR of 60–89 ml/min/1.73 m². A further 23.08% had moderate kidney impairment (eGFR 30–59 ml/min/1.73 m²), 13.18% had severe impairment (eGFR 15–29 ml/min/1.73 m²), and 2.2% had end-stage kidney disease with an eGFR <15 ml/min/1.73 m² (Table 1).

Among these patients, 18.7% experienced cardiovascular events, 2.2% were newly diagnosed with end-stage renal disease (ESRD), and 49.5% showed progression of kidney disease, despite an overall increasing trend in eGFR values (Table 1).

The prevalence of chronic kidney disease (CKD) among patients with diabetes mellitus (DM) in Hospital Jitra was 38.46%. Additionally, 20.9% of the patients experienced diabetic complication; cardiovascular disease (CVD) (myocardial infarction/ stroke/ heart failure); newly diagnosed ESRD and 40.7% were hospitalized during the study period due to uncontrolled diabetes mellitus. 49.5% showed a progression of kidney disease (Table 1).

The trend of glycaemic control and progression of kidney disease

The analysis utilised Generalised Estimating Equations (Linear) to examine the trend of glycaemic control and the kidney disease as indicated by eGFR. The findings revealed a significant pattern of elevated HbA1c levels and declining eGFR values (P<0.001) across successive visits (Table 2).

The results indicate a high percentage of poor glycaemic control among type 2 diabetes in Hospital Jitra; 69%-100% of the patients had HbA1c >7% at each visit (Table 2). A correlation analysis was conducted between glycemic control (HbA1c) and eGFR, revealing a Pearson correlation coefficient (r) of 0.113, which indicates a very weak positive correlation between the two variables. Despite this modest correlation, a statistically significant relationship was found between HbA1c levels and eGFR levels (P=0.035).

The association between glycaemic control and adverse outcomes

Four major adverse outcomes diabetes patients; cardiovascular events, newly diagnosed end-stage renal disease (ESRD), the progression of kidney disease and hospitalisation due to uncontrolled diabetes were explored using logistic regression with potential predictors of each adverse outcomes, including glycaemic control, gender, age, and duration of diabetes. The results showed a substantial correlation between all predictors and the progression of kidney disease, but no significant correlations between this factor and cardiovascular events, newly diagnosed ESRD, or hospitalisation for uncontrolled diabetes. The comprehensive results of the logistic regression analysis are shown in Table 3.

Table 1: Demographic, Clinical Characteristics, The Prevalence of CKD and The Complication of DM

Parameters	Mean values (±SD) / Number of patients (%) (N=91)
Age, years	62.15 (11.99)
Gender	
• Male	36 (39.6%)
• Female	55 (60.4%)
Ethnicity	
• Malay	78 (85.7%)
• Chinese	7 (7.7%)
• Indian	6 (6.6%)
DOD (Duration of Diabetes, years)	10.00 (4.97)
Haemoglobin A1c (HbA1c); %	9.01 (2.33)
Serum Creatinine (mg/dL)	183.56 (55.26)
eGFR(ml/min)*	76.81 (2.57)
Comorbidity	
• Hypertension	84 (92.3%)
• Hypercholesterolemia	71 (78.0%)
• Heart disease	21 (23.1%)
Stages of CKD; Estimated GFR* (ml/min per 1.73m2)	
• Stage 1; eGFR >90 (Normal or high)	24 (26.37%)
• Stage 2; eGFR 60-89 (Mildly decreased)	32 (35.16%)
• Stage 3; eGFR 30-59 (Moderately decreased)	21 (23.08%)
• Stage 4; eGFR 15-29 (Severely decreased)	12 (13.18%)
• Stage 5; eGFR <15 (Renal failure)	2 (2.20%)
Complication of DM	
• Cardiovascular event (myocardial infarction/ stroke/ heart failure)	17 (18.7%)
• Newly diagnosed ESRD	2 (2.2%)
• Progression of kidney disease (increasing trend of eGFR)	45 (49.5%)
• Hospitalisation due to uncontrolled of diabetes mellitus	37 (40.7%)
Risk factors of CKD in patients with DM (OR, CI)#	OR=2.96, 95% CI = 0.60-14.67#

*eGFR, estimated glomerular filtration rate in ml/min per 1.73 m2 using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

OR=Odd ratio, CI= Confidence Interval

Table 2: The trend of glycaemic control (HbA1c) and progression of kidney disease (eGFR)

Visit	HbA1c level (%)		eGFR level (mg/dl)		HbA1c[bc]> 7.0%	HbA1c[bc]	eGFR[d] (ml/min per 1.73m2)
	Mean (SE)	*p*-value[a]	Mean	*p*-value[a]	N (%)	Mean (SD)	Mean (SD)
1	8.9 (0.23)	<0.001	79.4(2.75)	<0.001	73 (80.2)	8.86 (2.19)	71.77(28.30)
2	8.5 (0.22)		77.7(2.72)		57 (68.7)	8.54 (2.02)	71.85 (28.24)
3	9.0 (0.28)		72.7(2.97)		52 (77.6)	9.06 (2.36)	68.52 (28.42)
4	9.4(0.34)		71.2 (3.04)		39 (75.0)	9.42 (2.68)	70.00 (26.17)
5	8.9(0.36)		67.8 (3.14)		31 (89.0)	9.41 (2.50)	65.36 (25.37)
6	9.2(0.59)		68.4 (3.18)		16 (88.9)	9.69 (2.85)	65.97 (27.78)
7	8.4(0.39)		65.0 (3.35)		8 (72.7)	9.30 (1.99)	77.49 (32.60)
8	11.8(0.85)		66.2 (3.65)		4 (100)	9.52 (2.23)	68.40 (18.20)
9			75.1 (6.58)				77.49 (32.60)
10			70.8(4.65)				68.63 (17.28)
11			45.2(5.71)				68.40 (18.20)

[a]Generalized Estimating Equations (Linear).

[b]Target HbA1c is individualised; ≤6.5% for those young, uncomplicated, with short duration of disease; while <7.0% would be appropriate for most other adult DM individuals. (Ministry of Health; Malaysia (2020) "Clinical Practice Guidelines. Management of Type 2 Diabetes Mellitus (6th Edition) p.43."

[c]Laboratory investigations for HbA1c were done 6-monthly/yearly.

[d]Laboratory investigations for Creatinine/BUSE + eGFR at every visit (3-6 monthly)

Table 3: Association Between Predictors and Adverse Outcomes in Diabetes: Logistic Regression Results

Predictor	Cardiovascular event (OR, p-value)	Newly diagnosed ESRD (OR, p-value)	Progression of kidney disease (OR, p-value)	Hospitalisation due to uncontrolled of diabetes (OR, p-value)
HbA1c (Poor Control)	1.34 (p = 0.63)	0.76 (p = 0.71)	0.26 (p = 0.01)	0.54 (p = 0.22)
Gender (Male)	3.09 (p = 0.53)	1.63 (p = 0.45)	2.63 (p = 0.04)	1.14 (p = 0.77)
Age (per year)	1.04 (p = 0.16)	1.01 (p = 0.65)	1.05 (p = 0.02)	1.00 (p = 0.74)
Duration of Diabetes	1.01 (p = 0.86)	0.99 (p = 0.92)	0.95 (p = 0.24)	0.99 (p = 0.77)

*Logistic regression

A lower chance of kidney disease progression was substantially correlated with poor glycaemic controlled (HbA1c > 7%), according to the logistic regression analysis (OR = 0.26, p = 0.01). In comparison to females, males had 2.63 times the odds of progress (OR = 2.63, p = 0.04). Age also had a significant impact with the risks increased by 5% for every year of age (OR = 1.05, p = 0.02).

Discussions

To our knowledge, this is the first to investigate the incidence of CKD among DM patients in a Malaysian secondary healthcare context. This study is unique and important to obtain accurate local data on the prevalence of CKD among DM patients which can facilitate health-care planning including review of health-care priorities, program activities and allocation of resources. This study evaluated the relationship between glycemic management, eGFR, and unfavourable outcomes such cardiovascular events, hospitalizations, and renal failure in patients.

Patients with DM at Hospital Jitra had a 38.46% prevalence of CKD. This finding was somewhat similar with previous study (Mohd Zuki & Rodi Isa, 2022) that found that the prevalence of CKD, in all phases, was 38.6%, That prevalence shown significant increased of CKD patient based on increased of DM patient.

The patients' mean age was 62.15 years. Kidneys alter morphologically and functionally as we age. As a result, there is an increased risk of chronic kidney disease (CKD) progression with age. Additionally, kidney disease has been found to be more prevalent in individuals over 60 compared to the general population (Nitta et al., 2014).

HbA1c is the key figure used worldwide to evaluate diabetes control. The findings of this study revealed that HbA1c values continuously surpassed the limits provided in the Clinical Practice Guidelines (CPG) for diabetes care in Malaysia, suggesting poor glycemic control that varied from 69% to 100% at each visit. Patients with higher HbA1c levels in this study showed progression of kidney disease (OR=2.96, 95% CI = 0.60-14.67). These results were in line with a study by Jitraknatee, et al. (2020), which found significant results of uncontrolled HbA1c levels with HbA1c ≥ 7% related to developing CKD (OR = 3.32, 95% CI = 2.20–5.01) Renal dysfunction is a notable a risk factor for uncontrolled diabetes, as the kidneys are vital for both gluconeogenesis and drug metabolism (Kong et al., 2014). This also contributed to the high hospitalization rate observed among the patients in this study (40.7%). Chronic kidney disease (CKD) can result from high blood sugar because it damages the kidneys' small blood capillaries (Kurzhagen et al., 2020). In order to avoid consequences including nerve damage, kidney disease, and cardiovascular disease, diabetics must keep their blood glucose levels within a specific range (Advani, 2020).

The findings showed that the number of people suffering from diabetes-related complications, such as heart failure, myocardial infarction, and stroke, has significantly increased. Our observed prevalence of 18.7% is lower in comparison to global data, where approximately 32.2% of individuals with type 2 diabetes mellitus are affected by cardiovascular complication. Importantly, CVD constitutes a leading cause of mortality among people with type 2 diabetes mellitus, accounting for approximately half of all deaths during the study period (Einarson et al., 2018). Due to the higher risk of cardiovascular complication among diabetes patient, treatments that lower this risk by a similar percentage can have a bigger impact.

The results showed cardiovascular events did not show significant associations with glycaemic control. The ACCORD Study Group (2011) found that intensive glycaemic control did not reduce the risk of cardiovascular events in people with type 2 diabetes, and in fact, intensive therapy was associated with an increased risk of mortality in some subgroups. The study suggests that other factors like blood pressure, lipid levels, and smoking might be more important in preventing cardiovascular events than glycaemic control alone.

Newly diagnosed ESRD also did not significantly associated with glycaemic control in this study. One study that supported up the findings revealed that while glycaemic control (HbA1c) improved microvascular outcomes in diabetes, it did not significantly alter the risk of ESRD in patients who had already developed chronic kidney disease (CKD). The results suggest that for individuals with advanced CKD, other factors like blood pressure control and proteinuria may play a more crucial role in determining the risk of progressing to ESRD (Chalmers et al., 2010).

This study found glycaemic control significantly correlated with the progression of kidney disease. In line with our findings, few clinical trials have consistently shown that maintaining HbA1c levels below 7% is linked to a reduced risk of both clinical and structural manifestations of diabetic nephropathy in diabetes patients. For example, the Diabetes Control and Complications Trial (DCCT) demonstrated that intensive diabetes management led to a 39% reduction in the incidence of microalbuminuria

(Gross et al., 2005).

This study suggests that poor glycaemic control, as indicated by elevated HbA1c levels, is a key factor significantly associated with CKD in diabetic patients. Improving glycaemic control may therefore help to reduce the risk of CKD and other diabetic complications, highlighting the importance of early intervention and continuous management of blood glucose levels in patients with diabetes to prevent further kidney damage.

On the other hand, this study also has several limitations. First, the study involved only 91 patients from a single hospital in Kedah, Malaysia. This relatively small and localized sample may limit the generalizability of the findings to the broader population of Malaysia, especially since different regions may have varying healthcare access, ethnic distributions, and lifestyle factors. Secondly, this study was conducted retrospectively, hence the data is reliant on existing medical records, which could have inconsistencies, incomplete information, or variability in documentation practices. This may affect the accuracy and comprehensiveness of the findings. Thirdly, despite statistical adjustments, there may still be unmeasured confounding variables, such as genetic predispositions or undetected comorbidities, that could influence the association between HbA1c, CKD, and adverse outcomes.

Conclusion

Our study has shown that higher HbA1c was robustly associated with the risk of CKD in adults with type 2 diabetes, suggesting that improving glycaemic control may also reduce the risk of CKD. This study provides compelling evidence that current local practice may not adequately address the complexities inherent in the use of these novel therapies in diabetic populations. Therefore, revising and updating existing guidelines based on current practice is crucial to ensure the safe and efficacious management of diabetes, particularly in light of evolving therapeutic options and patient-specific considerations.

Future studies will be crucial to reassess the selection of antidiabetic therapies and the prevalence of chronic kidney disease (CKD), aiming to evaluate the effectiveness of current management of diabetes mellitus in the facility.

Authors Contributions

Conception and design: NAG, SNFSH; Analysis and interpretation of the data: NAG; Drafting of the article: NAG, FFAN; intellectual content: NAG, FFAN, AZA; Final approval of the article: NAG, RZA; Provision of study materials or patients: NAG, SNFSH; Statistical expertise: NAG; Administrative, technical, or logistic support: NAG, FFAN; Collection and assembly of data: FFAN, ZZ, RZA

Acknowledgements

The authors wish to extend their gratitude to the Head of the Pharmacy Unit and Hospital Director for giving full support and assistance in this research, and to all respondents who participated in this study.

Special thanks to the Director General of Health, Ministry of Health, Malaysia and all the individuals who had contributed to make this study a success.

Ethical Approval Statement

The study was conducted in compliance with ethical principles outlined in the Declaration of Helsinki and Malaysian Good Clinical Practice Guideline. All study materials and procedures, including data collection form underwent MREC review and approval.

Ethics approval for the study was obtained from the Medical Research Ethic Committee (NMRR ID-23-00546-IWT).

Informed Consent Statement

This is a retrospective study that was conducted using medical records. No direct interaction with the patient during the study period. Hence Informed consent is not applicable.

Conflict of interest

None.

Funds

None.

Declaration of generative AI and AI-assisted technologies in the writing process

I hereby declare that, during the process of creating this work, I have utilized generative AI technologies for improving clarity and refining grammar. The use of these tools was intended to enhance the quality and efficiency of the writing process, while maintaining the integrity and originality of the content. Any external input generated by AI tools has been thoroughly reviewed, revised, and integrated in a manner that aligns with my own creative and academic intentions.

References

The ACCORD Study Group. (2011). Effects of intensive glucose lowering in type 2 diabetes. New England Journal of Medicine, 364(9), 818–828. https://doi:10.1016/S0084-3741(09)79280-7

Advani A. (2020). Positioning time in range in diabetes management. Diabetologia, 63(2), 242–252. https://doi.org/10.1007/s00125-019-05027-0

American Diabetes Association. (2021). Diabetic kidney disease (DKD). Diabetes Care, 44(Supplement 1), S163–S184. https://doi.org/10.2337/dci21-0014

American Diabetes Association. (2023). Diabetes and Kidney Disease. Diabetes Care, 46(Supplement 1), S171–S180. https://doi.org/10.2337/dci22-0034

American Diabetes Association. (2023). Cardiovascular Disease and Risk Management. Diabetes Care, 46(Supplement 1), S158-S168. https://doi.org/10.2337/dci22-0067

American Diabetes Association. (2023). Pharmacologic approaches to glycemic treatment: Standards of medical care in diabetes—2023. Diabetes Care, 46(Supplement 1), S111-S124. https://doi.org/10.2337/dc23-S011

Buse, J. B., Wexler, D. J., et al. (2020). Treatment of type 2 diabetes with GLP-1 receptor agonists and insulin. Diabetes Care, 43(2), 241-250. https://doi.org/10.2337/dci19-0065

Centers for Disease Control and Prevention. (2021). Chronic Kidney Disease in the United States, 2021. U.S. Department of Health and Human Services. Available at: https://www.cdc.gov/kidneydisease/publications-resources/ckd-national-facts.html

Chalmers, J., et al. (2010). The effects of intensive glucose lowering on kidney disease progression in patients with type 2 diabetes. Journal of the American Society of Nephrology, 21(3), 507–514. https://doi.org/10.1681/ASN.2009090974

Dhand, N. K., & Khatkar, M. S. (2014). Statulator: an online statistical calculator. Sample size calculator for estimating a single proportion. [Internet]. Accessed 27 November 2022 at http://statulator.com/SampleSize/ss1P.html

Einarson, T. R., Acs, A., Ludwig, C., & Panton, U. H. (2018). Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007–2017. Cardiovascular diabetology, 17, 1-19. https://doi.org/10.1186/s12933-018-0728-6

Garla, V., Yanes-Cardozo, L., & Lien, L. F. (2017). Current therapeutic approaches in the management of hyperglycemia in chronic renal disease. Reviews in Endocrine and Metabolic Disorders, 18(1), 5–19. https://doi.org/10.1007/s11154-017-9416-1

Gross, J. L., De Azevedo, M. J., Silveiro, S. P., Canani, L. H., Caramori, M. L., & Zelmanovitz, T. (2005). Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes care, 28(1), 164-176. https://doi.org/10.2337/diacare.28.1.164

Harrington, J. E., & Ward, C. (2019). Challenges in managing diabetes in patients with chronic kidney disease. American Journal of Kidney Diseases, 74(4), 442-448. https://doi.org/10.1053/j.ajkd.2019.02.341

Jitraknatee, J., Ruengorn, C., & Nochaiwong, S. (2020). Prevalence and risk factors of chronic kidney disease among type 2 diabetes patients: a cross-sectional study in primary care practice. Scientific reports,

10(1), 6205. https://doi.org/10.1038/s41598-020-63443-4

Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. (2020). KDIGO 2020 clinical practice guideline for diabetes management in chronic kidney disease. Kidney International, 98(4), S1-S115. https://doi.org/10.1016/j.kint.2020.05.032

Khan, N., Huxley, R. R., & Bakris, G. L. (2020). The global burden of chronic kidney disease and diabetes: Implications for the economic and healthcare system. Clinical Journal of the American Society of Nephrology, 15(5), 654-661. https://doi.org/10.2215/CJN.04550420

Kong, A. P., Yang, X., Luk, A., Ma, R. C., So, W. Y., Ozaki, R., ... & Chan, J. C. (2014). Severe hypoglycemia identifies vulnerable patients with type 2 diabetes at risk for premature death and all-site cancer: the Hong Kong diabetes registry. Diabetes Care, 37(4), 1024-1031. https://doi.org/10.2337/dc13-2507

Kurzhagen, J. T., Dellepiane, S., Cantaluppi, V., & Rabb, H. (2020). AKI: an increasingly recognized risk factor for CKD development and progression. Journal of Nephrology, 33(6), 1171-1187. https://doi.org/10.1007/s40620-020-00793-2

Lazarus, B., & Wu, A. W. (2020). Barriers to the use of newer therapies in diabetes management. Journal of Clinical Endocrinology & Metabolism, 105(4), 1367-1375. https://doi.org/10.1210/clinem/dgz227

Mann, J. F. E., Fonseca, V., Mosenzon, O., Raz, I., Goldman, B., Idorn, T., Von Scholten, B. J., & Poulter, N. R. (2018). Effects of liraglutide versus placebo on cardiovascular events in patients with type 2 diabetes mellitus and chronic kidney disease: Results from the LEADER trial. Circulation, 138(25), 2908–2918. https://doi.org/10.1161/CIRCULATIONAHA.118.036418

McMurray, J. J. V., Solomon, S. D., et al. (2019). Dapagliflozin in patients with heart failure and reduced ejection fraction. New England Journal of Medicine, 381(21), 1995-2008. https://doi.org/10.1056/NEJMoa1911303

Malaysian Health Technology Assessment Section (MaHTAS), Ministry of Health Malaysia. Clinical Practice Guidelines on the Management of Chronic Kidney Disease (2nd ed. 2018). https://www.moh.gov.my/moh/resources/penerbitan/CPG/CPG%20Management%20of%20Chronic%20Kidney%20%20Disease%20(Second%20Edition).pdf

Mohd Zuki, A. H., & Isa, M. R. (2022). Chronic Kidney Disease Staging Among Type II Diabetes Mellitus Patients in Kedah, Malaysia: Prevalence and its Factor Associated. J Pub Health Issue Pract, 6(1), 198. J Pub Health Issue Pract, 6(1): 198 https://doi.org/10.33790/jphip1100198

National Kidney Foundation. (2022). Diabetic Kidney Disease: A Global Epidemic. Retrieved from https://www.kidney.org/atoz/content/diabetic-kidney-disease

Nitta, K., Okada, K., Yanai, M., & Takahashi, S. (2014). Aging and chronic kidney disease. Kidney and Blood Pressure Research, 38(1), 109-120. https://doi.org/10.1159/000355760

Pisters, R., van der Auwera, J., & Peters, W. (2017). Diabetic Kidney Disease and Cardiovascular Risk: A Systematic Review. Clinical Kidney Journal, 10(5), 685-691. https://doi.org/10.1093/ckj/sfx049

Real Time World Statistics. Malaysia population [Internet]; 2024 [Retrieved 2024 April 23]. Available at: https://www.worldometers.info/ world-population/malaysia-population/

Sinha, N., & Bansal, S. (2019). Pharmacokinetics and pharmacodynamics of anti-hyperglycemic drugs in patients with chronic kidney disease. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 13(2), 1135-1144. https://doi.org/10.1016/j.dsx.2019.02.002

Saminathan, T. A., Hooi, L. S., Mohd Yusoff, M. F., Ong, L. M., Bavanandan, S., Rodzlan Hasani, W. S., ... & Aris, T. (2020). Prevalence of chronic kidney disease and its associated factors in Malaysia; findings from a nationwide population-based cross-sectional study. BMC nephrology, 21, 1-11.

Zhang, Y., Li, X., & Liu, Z. (2021). Management of diabetic kidney disease: A comprehensive approach to optimize renal and cardiovascular outcomes. Journal of Diabetes Research, 2021, 1-12. https://doi.org/10.1155/2021/8994087

Journal of Pharmacy

JOP
Journal of Pharmacy

Circadian Variation of Zinc, Copper, Selenium, and Bromine in Human Milk

Nor Hidayah Mohd Taufek[1*], Hana' Nahlaa Hafiz Majdi[1], Aina Zafirah Azhar[1] Abdul Rahman Fata Nahas[1], Awis Sukarni Mohmad Sabere[2], and Joseph Bidai[3]

[1]*Department of Pharmacy Practice, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[2]*Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[3]*South China Sea Repository & Reference Center, Institute of Oceanography and Environment (INOS), University Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia.*

Abstract

Introduction: Human milk undergoes dynamic modifications throughout lactation to optimally meet the dietary and immunological requirements of the growing infant, with variations of its composition also occurring throughout the day. Circadian variation in some bioactive components helps the development of the biological clock by passing on chronobiological information from mother to infant. This study aimed to identify the circadian variation of zinc, copper, selenium, and bromine in human milk during the postpartum period. **Methods:** Human milk samples were collected from a postpartum mother who was taking zinc and copper supplements. Milk samples were analysed using inductively coupled plasma mass spectrometry. Data on zinc, copper, selenium, and bromine concentrations were analysed using Microsoft Excel 2021 and reported descriptively to determine circadian variation. **Results:** The concentration of all four trace elements declined throughout the six months postpartum period with consistent fluctuations for bromine. Zinc, copper, and bromine possess the most similar circadian variation throughout the day with a 'V' shaped pattern, but selenium showed inconsistent circadian pattern over the first six months postpartum. The 'V' shaped pattern generally disappeared in the fourth, fifth, and sixth months. **Conclusion:** Circadian rhythms for zinc, copper, selenium, and bromine varied during the first six months postpartum. This may indicate a functional circadian clock regulating infants' biological development. Future studies should explore the factors influencing the development of fully functional circadian rhythmicity in infants.

Article history:

Received: 1 October 2024
Accepted: 9 October 2024
Published: 31 January 2025

Keywords:

Zinc
Copper
Selenium
Bromine
Circadian variation

doi: 10.31436/jop.v5i1.355

*Corresponding author's email: hidayahtaufek@iium.edu.my

Introduction

Human milk contains nutritional components, antibodies, and hormones as important bioactive components for infant development. These components exhibit circadian patterns which aid the transfer of information from the mother to her newborn during the day and night. This aspect of knowledge of human milk has been referred to as "chrononutrition." The importance of chrononutrition of human milk includes the physiological impact on the establishment of regulation of the biological clock in infants. The variation between the time of expression and feeding may affect how well an infant's circadian clock and sleep homeostasis develop when they are breastfed (Italianer et al., 2020). The circadian rhythm of infants concerning metabolic clocks influences feeding behaviour such as time-restricted feeding as well as energy distribution throughout the day, which could also be therapeutic for metabolism including insulin responsiveness (Flanagan et al., 2021). The circadian cue from the mother is transferred to infants and involves hormones such as glucocorticoids and melatonin which play vital roles in metabolism, immunity, and sleep regulation (Caba-Flores et al., 2022). The homeostasis of glucocorticoids in stress response for example, particularly by cortisol exposure is linked with the development of autoimmune disease (Ramamoorthy & Cidlowski, 2016). Other components including fat, amino acids, and endogenous cannabinoids also exhibit diurnal variation (Caba-Flores et al., 2022). The optimal exposure to specific quality and quantity of nutritive and non-nutritive components in human milk corresponding to the time of the day may have biological significance in human biological clock coordination. Nevertheless, its long-term impact from early developmental exposure is unclear.

The transition from the womb to birth involves changes in the circadian rhythms of infants since the foetus in the womb is exposed to the physiological and metabolic rhythms of the mother. It was suggested that maternal milk after birth substitutes the exposure to nutrition and other bioactive components to regulate the circadian rhythms. For example, the optimal neurodevelopment in infants is partly attributable to the sensitivity to breast milk melatonin which highlights the role and impact of breastfeeding (Häusler et al., 2024). Breastfeeding practices are always superior to formula feeding despite the low breastfeeding rate worldwide. Globally, the costs of not breastfeeding are significant including 595,379 childhood deaths annually, and 98,243 maternal comorbidities and mortality from chronic diseases with the total estimated global economic losses between US$257 billion and US$341 billion (Walters, Phan, & Mathisen, 2019). In adults, it was suggested that chrononutrition promotes cardiometabolic health, but many knowledge gaps remain to be addressed in developing approaches for chronic disease prevention (Raji et al., 2024). Therefore, there was limited knowledge about chrononutrition in adults and infants which requires further research.

There are concerns about the adverse external circadian signals including mistimed exposure to hormones in breast milk which cause misalignment of temporal sequence which leads to susceptibility to poor infant development. The maturation of the biological clock in humans contributes to the development of synchronicity of cognitive and physical function in response to the demands of the environment (Wong et al., 2022). It is measured by patterns of hormone secretion and sleep pattern and the ability to adjust to external time cues, of which this aspect is poorly understood in the first year of life (Wong et al., 2022). The presence of trace elements in human milk as part of the optimal nutrition in infants particularly in the first six months after birth indicates the important requirement for healthy development. Nevertheless, the variation in its concentration is less understood. Zinc, copper, and selenium have been reported to vary in human milk over the postpartum period (Li et al., 2016). Bromine plays an important role in supporting the formation of the cell membrane, and its quantities in human milk are comparable to zinc but its variation over the postpartum period is unclear (McCall et al., 2014; Mohd-Taufek et al., 2016; Mohd-Taufek et al., 2024). Little is known regarding the circadian variation of these minerals and any changes over the

postpartum period. This study aimed to investigate the circadian pattern of zinc, copper, selenium, and bromine in human milk throughout six months postpartum.

Materials and methods

In the present study, a total of 196 milk samples were collected from one postpartum mother as a case study. She consented to the study and collected the samples at her convenience throughout the six months postpartum, from June until November 2023. The samples were expressed using breast pumps and kept in breast milk plastic packaging, labelled with the date and time of the day, then stored in the freezer at -21˚C. The volume of milk samples in each pack was between 90mL to 150mL. The number of samples for each date selected varied depending on the availability, and only the days with samples from three sessions of morning, evening, and night were included. All milk samples were labelled once collected at different times of the day and were divided into three time intervals which were 4:00 to 11:59, 12:00 to 19:59, and 20:00 to 3:59. The time range was selected for a similar range of samples between each interval for morning, afternoon, and night sessions. The days with incomplete samples or labels, or with missing at least one session were excluded. The day postpartum included were randomly selected to represent each week of the month. A data collection form was completed by the participant for demographic information.

The analyses of milk samples have been conducted following the protocol described in the previously published article (Mohd Taufek et al., 2024). The details regarding instruments, procedure, and analysis were described. The developed and validated method has been used to analyse and report zinc, copper, selenium, and bromine concentration in human milk, and was analysed using the acid digestion method by inductively coupled plasma mass spectrometry at the Institute of Oceanography and Environment (INOS), University Malaysia Terengganu.

Results were analysed for descriptive analysis using Microsoft Excel version 2021, and reported as a case study. This study received ethics approval from the International Islamic University Malaysia (IIUM) Research Ethics Committee (IREC) (ID No.: IREC 2021-053).

Results and discussion

The participant in the case study was a healthy 39-year-old mother, who had neither medical illnesses nor chronic medications, practiced mild exercise three times a week, and took nutritional supplements of zinc and copper. Her height was 157 cm, with a weight from pre-pregnancy of 72kg, pre-delivery of 89kg, and post-delivery of 80kg. It was her first pregnancy and delivered a healthy male infant at 38 weeks of gestation via the caesarean method. She followed a normal diet per the Malaysian dietary guidelines for pregnant mothers.

A total of 196 expressed breast milk samples were collected from one participant as a case study. They were analysed and reported for zinc, copper, selenium, and bromine. As shown in Table 1, all trace elements exhibited the highest concentration in the first month compared to the later months. The mean concentration of zinc over the first six months of lactation dropped from 10,353 to 2,157 mcg/l. Generally, zinc showed the highest concentration in human milk compared to copper, selenium, and bromine. For copper, the mean concentration ranged from 309 to 999 mcg/l, which showed relatively small differences over the six months duration. The mean selenium concentration decreased gradually from 85 mcg/l to 27 mcg/l. The bromine concentration fluctuated across the six months with 1803 mcg/l in the first month and 1004 mcg/l in the sixth month.

Table 1: Concentration of Zn, Cu, Se, and Br over six months postpartum.

Trace element		Month postpartum					
		1	2	3	4	5	6
	No. of milk samples	18	55	45	26	31	21
Zn (μg/l)	mean ± SD	10353 ± 4353	3311 ± 975	3898 ± 758	2477 ± 922	2182 ± 611	2157 ± 781
	median (range)	8730 (4540-18300)	3080 (1670-6110)	3870 (1820-5280)	2590 (627-5450)	2100 (1190-3550)	2100 (1020-3580)
Cu (μg/l)	mean ± SD	999 ± 367	413 ± 126	505 ± 125	392 ± 232	309 ± 67	351 ± 131
	Median (range)	952 (482-1690)	384 (201-814)	503 (268-780)	351 (90.7-1360)	300 (184-461)	347 (152-610)
Se (μg/l)	mean ± SD	85 ± 58	27 ± 20	39 ± 21	16 ± 8	23 ± 16	27 ± 15
	Median (range)	72 (24-196)	20 (6-78)	45 (10-82)	16 (10-27)	17 (4-47)	22 (8-50)
Br (μg/l)	mean ± SD	1803 ± 640	764 ± 196	1075 ± 231	864 ± 228	666 ± 164	1004 ± 360
	Median (range)	1800 (926-2970)	716 (444-1350)	1070 (507-1640	847 (362-1360)	667 (363-1000)	999 (462-1880)

The time of the day influenced the concentration of zinc, copper, selenium, and bromine in human milk as shown in Figures 1, 2, 3, and 4. Diurnal variations were noticeable for all elements particularly in the first three months postpartum, indicated by the 'V' shaped pattern of the graphs. The concentration of these elements rose in the morning, dropped in the afternoon, and returned to a higher level at night. Nevertheless, the diurnal pattern was inconsistent and mostly disappeared from the fourth until the sixth month.

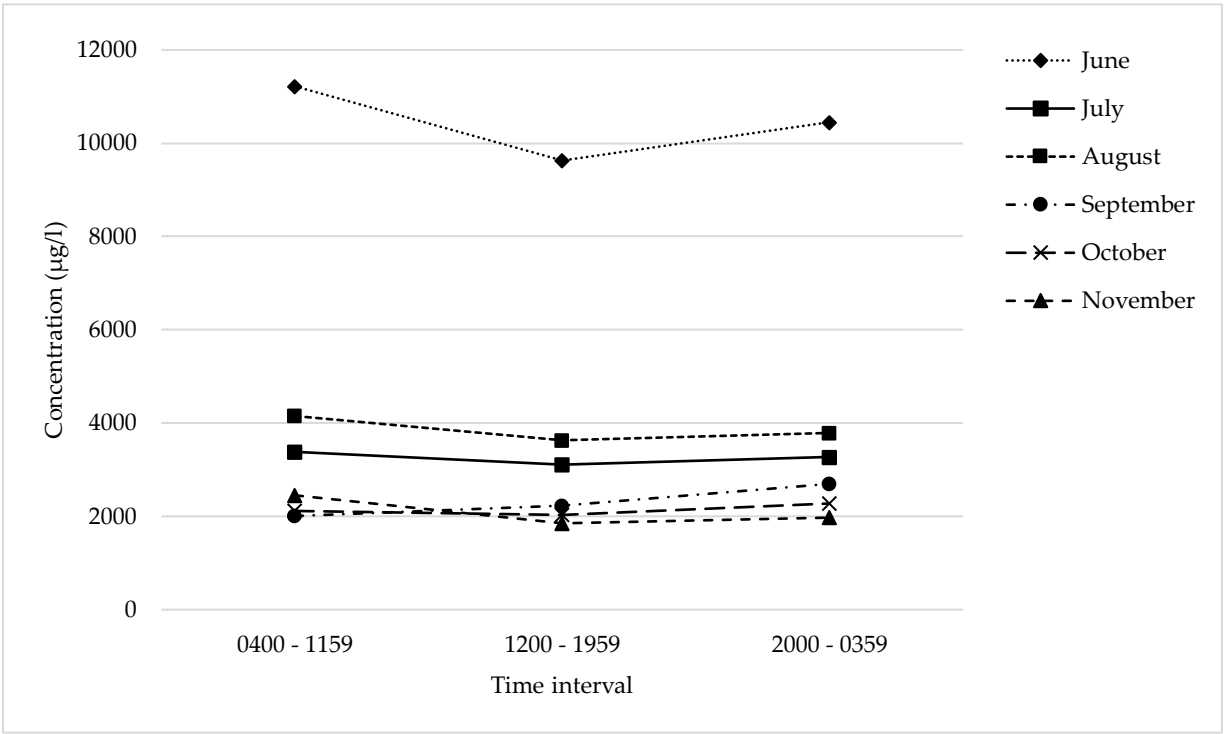


Fig. 1: Mean zinc concentration at three-time intervals during the 24 hours over six months postpartum.

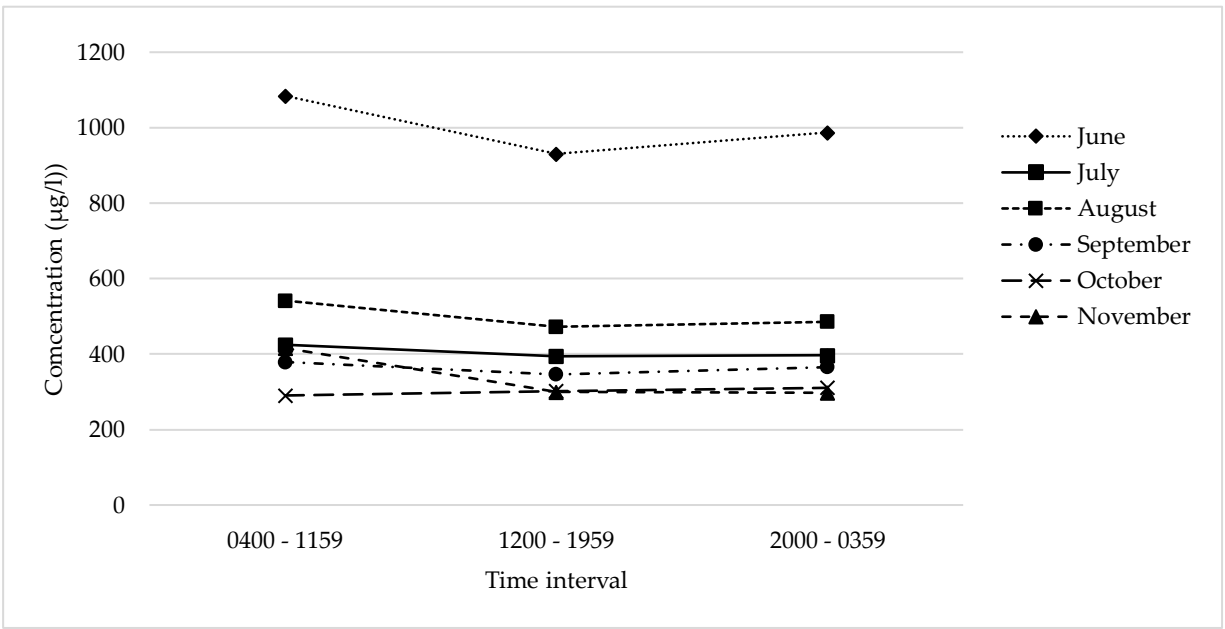


Fig. 2: Mean copper concentration at three-time intervals during the 24 hours over six months postpartum.

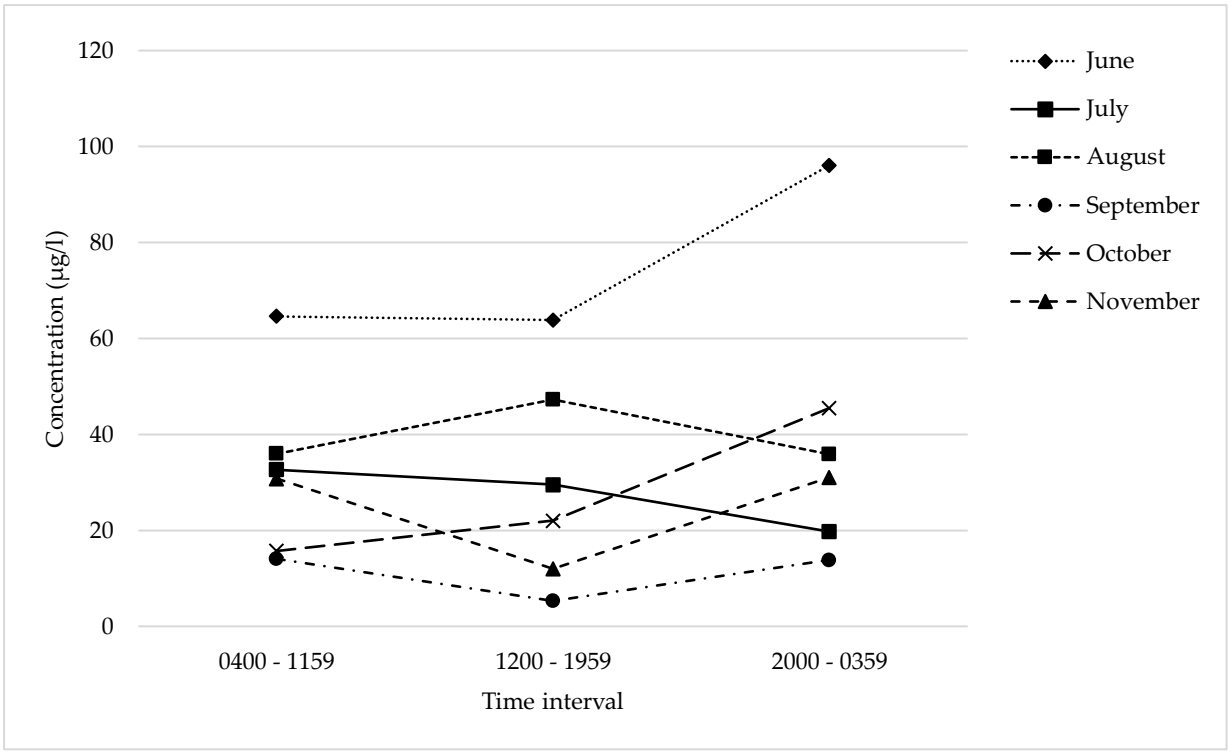


Fig. 3: Mean selenium concentration at three-time intervals during the 24 hours over six months postpartum.

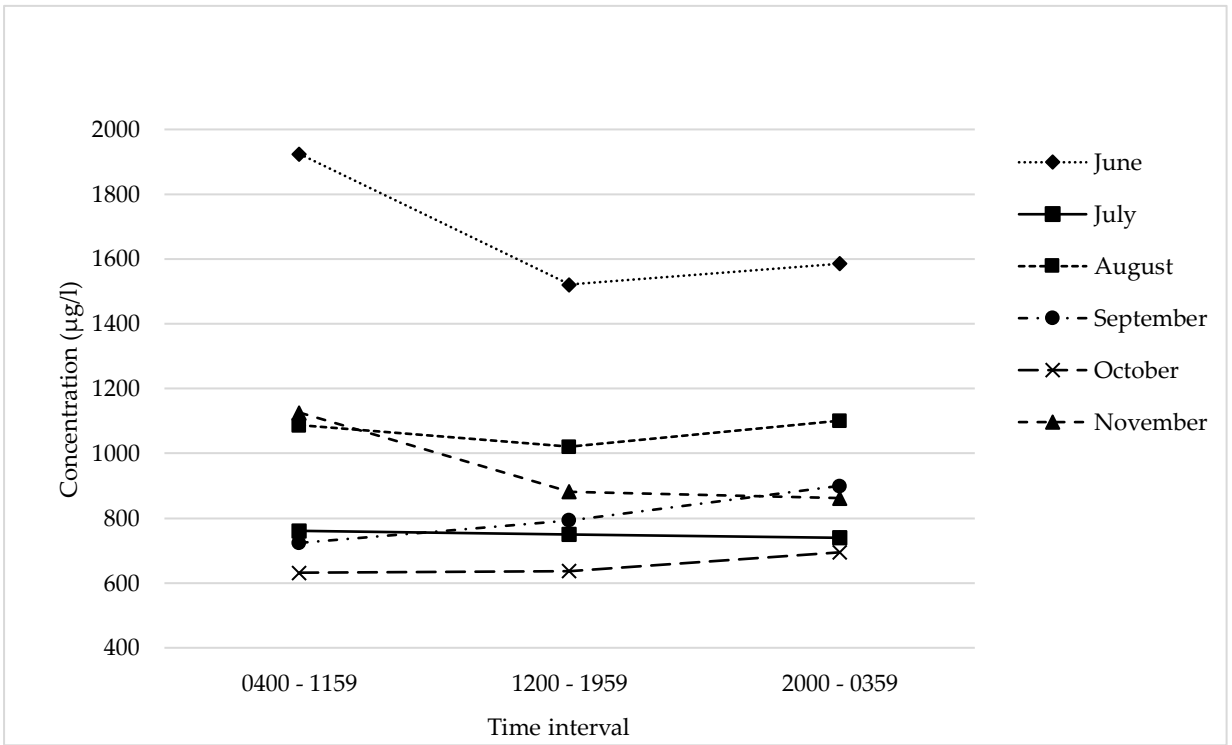


Fig. 4: Mean bromine concentration at three-time intervals during the 24 hours over six months postpartum.

The present study reported that variation of zinc, copper, selenium, and bromine from a case study exhibited a decreasing trend in concentrations over a six-month postpartum period. It has been reported worldwide that these minerals gradually decreased and fluctuated over the postpartum period (Hunt & Nielsen, 2009; Kim et al., 2012; Mohd-Taufek et al., 2017; Sabatier et al., 2019; Mandia et al., 2021). The decreased concentration could be due to the decrease in the levels of proteins including casein and whey in human milk that serve as ligands for zinc and copper (Fransson & Lönnerdal, 1983). Nevertheless, zinc has been reported to decline despite increased total protein in human milk in the second year postpartum (Perrin et al., 2017). It is unclear whether the specific protein changes are different in the first year as compared to the second year postpartum, which requires further investigation.

All minerals in the current study showed similar changes and dropped by about half of the initial concentration from the first month to the second month, then gradually decreased in later months. Zinc showed the highest concentrations in the first month postpartum ranging from 4,540 to 18,300 mcg/l. The results in the current study showed very high levels of zinc in the first month compared to other studies which reported the range of 400 to 11,900 mcg/L in term milk of 34 mothers (Sabatier et al., 2019), 900-7,800 mcg/L in preterm milk of 67 mothers (Kim et al., 2012), and 1,419-6,303 mcg/L in term milk of nine mothers (Mohd-Taufek, 2017). Copper concentration ranged from 201 to 1,690 mcg/L in the first and second months, which was higher than reported in other studies 111-926 mcg/L (Mohd-Taufek, 2017), and 130-1,000 mcg/L (Sabatier et al., 2019). This could be due to the supplements containing zinc and copper taken by the participant in our study.

Selenium despite showing the highest level in the first month (24-196 mcg/L) dropped to 6-28 mcg/L (second month) and then fluctuated consistently across the lactation month. Nevertheless, the concentration was relatively higher than those reported previously in the literature 10-22 mcg/L (first month) and 7-14 mcg/L (second month) (Mohd-Taufek et al., 2017), 6-24 (first and second month) (Sabatier et al., 2019). Selenium was found to be lower than recommended in the milk of mothers who delivered low birth-weight infants (Mandia et al., 2021). Nevertheless, our case study involved full-term milk. In Malaysia, the recommended intake of selenium in infants aged 0-5 months is 6 μg/day (Ministry of Health Malaysia, 2017). Our findings showed bromine concentrations of 926-2970 mcg/l (first month) and dropped to about half in the sixth month (462-1880 mcg/l). It demonstrated that relatively stable bromine concentrations are maintained throughout the six months of lactation. These concentrations are comparable to the previous studies which reported concentrations ranging from 834 to 1443 mcg/l of 16 donor milk collected at random lactation stages (Mohd-Taufek et al., 2016), and of 661-1026 mcg/l (first month) and 868-1387 mcg/l (second and third month) of preterm milk (Mohd-Taufek, 2017). Limited data are available regarding the biological importance of bromine in humans, but our findings indicated that bromine concentration is relatively high in human milk and is comparable to other minerals. It exhibits circadian rhythm which could further be investigated for its role in early infant growth and development. Bromine has been reported to be abundant in humans and is required for cell metabolism (McCall et al., 2014). The updated nutrient composition data of human milk is still greatly needed as it is paramount for the management of infant feeding, assessment of infant and maternal nutritional and health needs, and as a reference for infant formula development (Mandiá et al., 2021). Future studies should explore the specific role of bromine and its reference range to prevent deficiency and toxicity.

The circadian changes were present in daily concentrations of zinc, copper, selenium, and bromine in human milk. Zinc concentration demonstrated the 'V' shaped pattern with higher concentration in the morning and at night for the first, second, and third months postpartum (Figure 1). Copper showed a similar trend in concentration in the first three months of postpartum compared to the later months (Figure 2). A previous study reported that diurnal variation demonstrated no significant differences between the concentrations of zinc and copper in the morning and at night (Silvestre et al., 2000). For selenium, the 'V' shaped pattern was noticeable in the first, fourth, and sixth months, despite there being only slight differences in its concentrations (Figure 3). Bromine showed a slightly 'V' shaped pattern in the first and third month postpartum with no obvious pattern for other months (Figure 4). For selenium and bromine, the inconsistent circadian pattern may suggest that the time of day may not be a major factor in influencing biological rhythm in infants. Overall, we

speculate that beyond three months postpartum, the time of day may not be considered as the major factor for zinc, copper, selenium, and bromine in human milk to regulate the circadian pattern physiologically due to a better maturation of the infant body system. These aspects may be related to the knowledge of the respective biological roles such as nutritional and immunological as part of chronological development in humans.

Trace elements may influence circadian nutrition adaptation, although the mechanisms are not clearly understood, unlike melatonin and other hormones which affect sleep patterns and metabolism during the day and night. Other bioactive components that exhibited significant circadian variation include tryptophan, fats, triacylglycerol, cholesterol, iron, cortisol, and cortisone which may also influence their respective role in the biological clock (Italianer et al., 2020). Trace elements which are also involved in the metabolisms of cells and tissues may affect the growth and development of infants, particularly during the first year of life. Zinc deficiency is associated with abnormalities in humoral and cell-mediated immunity that increase the risk of infection and liver disease (Tuerk & Fazel, 2009). Copper is involved in all organs and systems, such as the hematologic and neurologic systems, cardiovascular, cutaneous, and immune systems, and its deficiencies are more common than previously reported (Altarelli et al., 2019). Selenium is associated with cardiovascular disease, of which selenoproteins are involved in oxidative stress, redox regulation, thyroid hormone metabolism, calcium flux, and microRNA regulation (Shimada, Alfulaij & Seale, 2021). Bromine plays a crucial role in brain metabolism and tissue development (Canavese et al., 2006; McCall et al., 2014). The mechanisms of these elements involved in the circadian rhythm of physiological and biological processes during the day and night are poorly understood. We propose that to a certain extent, these elements could influence the regulation of circadian-related activities including body temperature, hormonal secretion, sleep-wake cycle, food intake, mood, and cognitive and physical performance in infants (Montaruli et al., 2021; Wong et al., 2022). For example, this aspect could lead to dietary approaches for addressing persistent cardiometabolic health inequities by designing and implementing flexible and feasible interventions in real-world settings (Raji et al., 2024). The knowledge regarding the chrononutrition dimension on health will be the key to quantifying the true impact of the infant's temporal eating pattern on health.

Our findings supported the suggestion that human milk is optimally formulated to communicate the time of the day with infants through chrononutrition. However, modern practices of pumped and stored milk (e.g. chilled or frozen) may cause infants to ingest mistimed milk, affecting circadian rhythm development (Hahn-Holbrook et al., 2019). It is important to explore whether the expressed breast milk practice may influence sleep problems and physiological adaptation of infants to the environment as well as any adverse health outcomes resulting from disruption of circadian-matching milk. Similarly, infants receiving formula milk may be affected differently by the absence of chrononutrition in formula as compared to human milk mistimed milk. Nonetheless, the essential roles of bioactive components in human milk, although pumped and stored are superior to formula for healthy development of infants. Little is known about circadian-match stored milk, of different weeks and months postpartum which meets the needs of infants' development.

Alterations in circadian rhythm can lead to chronic pathologies and are influenced by a complex phenotype. The concept of chronotypes has been raised based on the bi-directional influences of the rest-activity circadian rhythm and sleep-wake cycle in chronic pathologies and disorders based on the interindividual differences in adults (e.g. morning, neither, and evening types) (Montaruli et al., 2021). However, the maturation in circadian rhythm in adults is different and adjustments could be easier to regulate the physiological processes compared to infants. Early life dysregulation of circadian rhythm has been linked to adult-onset cardiovascular disease, musculoskeletal issues, predisposition to diabetes, and mood disorders (Wong et al., 2022). Sleep disruption during infancy and childhood is common and causes psychosocial and behavioural issues. It has been proposed that the circadian clocks occur at the molecular level, with a set of clock genes organised in a system of interlocked transcriptional–translational feedback loops that coordinate physiological functions throughout the day that impact the cardiovascular system, energy metabolism, immunity, hormone secretion, reproduction and other systems (Pilorz, Helfrich-Förster & Oster, 2018). Therefore, further research is required to understand the association of early human development of circadian rhythm, the role of breastfeeding, and chrononutrition.

Our findings are not applicable to be generalised in the population since the limitation is only one participant was included in a case study. A bigger number of participants is recommended to reflect the population data on these elements and explore whether concentration is correlated with certain health parameters. The participant in our study was taking zinc and copper supplements during lactation, which may have altered the concentrations; thus, the data must be interpreted carefully. However, our findings can act as a reference for future studies to identify the importance of circadian changes in trace element concentration and how they reflect the health outcomes in the Malaysian population. It also serves as the basis to investigate the factors regulating trace elements circadian pattern, and the differences of pattern occurring after three months of lactation.

Conclusion

The trace elements zinc, copper, selenium, and bromine concentration in human milk exhibited a 'V' shaped pattern indicating the presence of functional circadian rhythm, particularly in the first three months postpartum. The gradual decrease in zinc, copper, selenium, and bromine concentration in the first six months postpartum may influence the circadian variation of these minerals. The role of chrononutrition of these minerals in the early lactation stages requires further investigation to confirm its developmental importance in infants.

Authors contributions

NHMT, ASMS & JB designed the study and collected samples and data. HNHM, AZA, and ARFN analysed the samples and data. All authors wrote and reviewed the manuscript.

Acknowledgements

The authors wish to thank the Institute of Oceanography and Environment (INOS), University Malaysia Terengganu for providing the facilities and expertise to complete the sample analysis. NHMT received research funding from the Research Management Centre International Islamic University Malaysia (ID: RMCG20-046-0046).

Ethical approval statement (if applicable)

This study received ethics approval from the International Islamic University Malaysia (IIUM) Research Ethics Committee (IREC) (ID No.: IREC 2021-053).

Informed consent statement (If applicable)

Informed consent was obtained from the participant included in the study.

Conflict of interest

NHMT, HNHM, ARFN, AZA, ASMS & JB declare that there is no competing interest in this research.

Declaration of generative AI and AI-assisted technologies in the writing process

Not applicable.

References

Altarelli, M., Ben-Hamouda, N., Schneider, A., & Berger, M. M. (2019). Copper deficiency: causes, manifestations, and treatment. Nutrition in Clinical Practice, 34(4), 504-513.

Caba-Flores, M. D., Ramos-Ligonio, A., Camacho-Morales, A., Martínez-Valenzuela, C., Viveros-Contreras, R., & Caba, M. (2022). Breast Milk and the Importance of Chrononutrition. Frontiers in Nutrition, 9, 867507. https://doi.org/10.3389/fnut.2022.867507

Canavese, C., De Costanzi, E., Stratta, P., & Sabbioni, E. (2006). A role for bromine deficiency in sleep disturbances of long-term dialysis patients. American Journal of Kidney Diseases, 48(6), 1018-1019.

Flanagan, A., Bechtold, D. A., Pot, G. K., & Johnston, J. D. (2021). Chrono-nutrition: From molecular and neuronal mechanisms to human epidemiology and timed feeding patterns. Journal of Neurochemistry, 157(1), 53–72. https://doi.org/10.1111/jnc.15246

Fransson, G. B., & Lönnerdal, B. O. (1983). Distribution of trace elements and minerals in human and cow's milk. Pediatric Research, 17(11), 912-915.

Hahn-Holbrook, J., Saxbe, D., Bixby, C., Steele, C., & Glynn, L. (2019). Human milk as "chrononutrition": implications for child health and development. Pediatric Research, 85(7), 936-942.

Häusler, S., Lanzinger, E., Sams, E., Fazelnia, C., Allmer, K., Binder, C., ... & Felder, T. K. (2024). Melatonin in Human Breast Milk and Its Potential Role in Circadian Entrainment: A Nod towards Chrononutrition?. Nutrients, 16(10), 1422.

Hunt, C. D., & Nielsen, F. H. (2009). Nutritional aspects of minerals in bovine and human milks. In Advanced Dairy Chemistry: Volume 3: Lactose, Water, Salts and Minor Constituents (pp. 391-456). New York, NY: Springer New York.

Italianer, M. F., Naninck, E. F., Roelants, J. A., van der Horst, G. T., Reiss, I. K., Goudoever, J. B. V., ... & Vermeulen, M. J. (2020). Circadian variation in human milk composition, a systematic review. Nutrients, 12(8), 2328.

Kim, S. Y., Park, J. H., Kim, E. A. R., & Lee-Kim, Y. C. (2012). Longitudinal study on trace mineral compositions (selenium, zinc, copper, manganese) in Korean human preterm milk. Journal of Korean Medical Science, 27(5), 532.

Li, C., Solomons, N. W., Scott, M. E., & Koski, K. G. (2016). Minerals and trace elements in human breast milk are associated with Guatemalan infant anthropometric outcomes within the first 6 months. The Journal of Nutrition, 146(10), 2067-2074.

Mandiá, N., Bermejo-Barrera, P., Herbello, P., López-Suárez, O., Fraga, J. M., Fernández-Pérez, C., & Couce, M. L. (2021). Human milk concentrations of minerals, essential and toxic trace elements and association with selective medical, social, demographic and environmental factors. Nutrients, 13(6), 1885.

McCall, A. S., Cummings, C. F., Bhave, G., Vanacore, R., Page-McCaw, A., & Hudson, B. G. (2014). Bromine is an essential trace element for assembly of collagen IV scaffolds in tissue development and architecture. Cell, 157(6), 1380-1392.

Ministry of Health Malaysia (2017). Recommended Nutritional Intake for Malaysia. https://hq.moh.gov.my/nutrition/wp-content/uploads/2023/12/FA-Buku-RNI.pdf

Mohd Taufek, N.H. (2017). Essential trace element analysis of human breast milk. The University of Queensland. https://doi.org/10.14264/uql.2017.477

Mohd-Taufek, N., Cartwright, D., Davies, M., Hewavitharana, A.K., Koorts, P., Mcconachy, H., Shaw, P.N., Sumner, R., & Whitfield, K. (2016). The effect of pasteurization on trace elements in donor breast milk. J Perinatol, 36, 897–900. https://doi.org/10.1038/jp.2016.88

Mohd Taufek, N. H., Mohmad Sabere, A. S., Mohamad Jamahari, U. S., Amran, N. B., Fata Nahas, A. R., & Bidai, J. (2024). Variation of bromine concentration as an essential trace element in human milk over lactation stages. Journal of Pharmacy, 4(1), 68–73. https://doi.org/10.31436/jop.v4i1.257

Montaruli, A., Castelli, L., Mulè, A., Scurati, R., Esposito, F., Galasso, L., & Roveda, E. (2021). Biological rhythm and chronotype: new perspectives in health. Biomolecules, 11(4), 487.

Perrin, M. T., Fogleman, A. D., Newburg, D. S., & Allen, J. C. (2017). A longitudinal study of human milk composition in the second year postpartum: implications for human milk banking. Maternal & Child Nutrition, 13(1), e12239.

Pilorz, V., Helfrich-Förster, C., & Oster, H. (2018). The role of the circadian clock system in physiology. Pflügers Archiv-European Journal of Physiology, 470, 227-239.

Raji, O. E., Kyeremah, E. B., Sears, D. D., St-Onge, M. P., & Makarem, N. (2024). Chrononutrition and Cardiometabolic Health: An Overview of Epidemiological Evidence and Key Future Research Directions. Nutrients, 16(14), 2332. https://doi.org/10.3390/nu16142332

Ramamoorthy, S., & Cidlowski, J. A. (2016). Corticosteroids: mechanisms of action in health and disease. Rheumatic Disease

Clinics, 42(1), 15-31.

Sabatier, M., Garcia-Rodenas, C. L., De Castro, C. A., Kastenmayer, P., Vigo, M., Dubascoux, S., ... & Affolter, M. (2019). Longitudinal changes of mineral concentrations in preterm and term human milk from lactating Swiss women. Nutrients, 11(8), 1855.

Shimada, B. K., Alfulaij, N., & Seale, L. A. (2021). The impact of selenium deficiency on cardiovascular function. International Journal of Molecular Sciences, 22(19), 10713.

Silvestre, M. D., Lagarda, M. J., Farré, R., Martínez-Costa, C., Brines, J., Molina, A., & Clemente, G. (2000). A study of factors that may influence the determination of copper, iron, and zinc in human milk during sampling and in sample individuals. Biological trace element research, 76, 217-227.

Tuerk, M. J., & Fazel, N. (2009). Zinc deficiency. Current Opinion in Gastroenterology, 25(2), 136-143.

Walters, D. D., Phan, L. T., & Mathisen, R. (2019). The cost of not breastfeeding: global results from a new tool. Health Policy and Planning, 34(6), 407-417.

Wong, S. D., Wright Jr, K. P., Spencer, R. L., Vetter, C., Hicks, L. M., Jenni, O. G., & LeBourgeois, M. K. (2022). Development of the circadian system in early life: maternal and environmental factors. Journal of physiological anthropology, 41(1), 22.

Journal of Pharmacy

Pharmacy Students' Views on Pilot Interprofessional Learning at a Teaching Hospital in Pahang, Malaysia

Nor Hidayah Mohd Taufek[1*], Norny Syafinaz Ab Rahman[1], Tengku Karmila Tengku Mohd Kamil[1] Mohamad Haniki Nik Mohamed[1], Nur Amni 'Afifah Mohd Faisal[1] and Christopher John Turner[2]

[1]*Department of Pharmacy Practice, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

[2]*Retired but formerly with Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA.*

Abstract

Introduction: Interprofessional learning (IPL) has been practiced in pharmacy curricula worldwide. The unique approach and design for IPL must be tailored within the context of practice to be effective. This study aimed to explore the reflection of experiences by pharmacy students regarding a pilot interprofessional learning activity at a teaching hospital in Malaysia. **Methods:** Data were collected from 21 pharmacy students using reflective diaries to explore actual experiences, perceived challenges, and learning. All students completed a four-week pilot IPL activity in a clinical setting at a teaching hospital. Data were extracted, analysed sorted, and coded using Atlas.ti® version 9 and subjected to thematic analysis. **Results:** Four themes emerged from the data: 1) Professional development and interdisciplinary skills 2) Favourable interprofessional engagement, 3) Roles identification and development, and 4) Operational flaws in IPL implementation. **Conclusion:** The pilot IPL has become a useful platform for pharmacy students to develop metacognitive skills during interprofessional experiences with students, healthcare professionals, and patients. Improvement in its implementation requires strategies tailored to contextual needs. Future studies are required to ensure continuous assessments of improvised IPL components to prepare competent pharmacists for patient-centred care.

Article history:

Received: 7 October 2024
Accepted: 22 January 2025
Published: 31 January 2025

Keywords:

Interprofessional learning
Pharmacy students
Reflective diary
Teaching hospital
Views

doi: 10.31436/jop.v5i1.359

*Corresponding author's email: hidayahtaufek@iium.edu.my

Introduction

The coordination and delivery of high-quality healthcare require reliable teamwork and collaboration within, and across organisational, disciplinary, technical, and cultural boundaries (Rosen et al., 2018). Interprofessional learning (IPL) is one of the measures known to improve these aspects (Murray, 2021). It occurs when two or more healthcare practitioners (including student practitioners) from different disciplines learn from, about, and alongside each other to improve teamwork and healthcare.

Health educators are responsible for preparing students for collaborative clinical decision-making between professionals to ensure students' exposure to these collaborative models of learning. Students may develop their skills and competency through collaboration which leads to their preparedness for future interprofessional work environments (Semple & Currie, 2022). Many accreditation agencies require students to participate in IPL experiences in various programmes. Early training in IPL is a vital component to ensure that graduates of healthcare professional programmes are qualified to practice in such settings (Nagge et. al., 2017). Multi-faceted strategies could enrich the training of IPL to form an effective and high-quality healthcare workforce.

Reflective-thinking skills are important for IPL and need to be emphasised for graduates to be able to assess clinical problems from many aspects and obtain fresh ideas and perspectives (Tsingos et al., 2015). Reflective writing about students' experiences and observations is an effective tool to develop reflective thinking and metacognitive skills (Mann, Gordon, & MacLeod, 2009). This study sought to explore the experiences and observations reflected by pharmacy students regarding a pilot interprofessional learning activity at a teaching hospital in Malaysia.

Materials and methods

The study received ethical approval from the IIUM Research Ethics Committee (ID no. IREC 2022-237). This qualitative study used a phenomenological approach, conducted retrospectively using purposive sampling from 21 reflective diaries written by the third-year pharmacy students. The data of the reflective diaries were retrieved from students who completed the pilot IPL activity during their Hospital Attachment course (PPPP 3423) at the Sultan Ahmad Shah Medical Centre teaching hospital in Pahang. A total of 28 out of 113 students in the class were involved in the pilot IPL. Pharmacy students received instructions and briefings from the course coordinator one week before pilot IPL activities. The learning objectives and outcomes were developing teamwork, communication, interprofessional interaction, and sharing of knowledge between students of different professions during the ward round.

The activities were conducted for four weeks in August 2022. Pharmacy students were paired and assigned to IPL groups. In each group, there were two pharmacy students (third year), four to five medical students (fifth year), and one to two nursing students (second year). The students were grouped to conduct different activities throughout the one-month period which included ward rounds, bedside teaching, case discussions, and clinical pathological conferences. A total of 90 students (medical, pharmacy and nursing) were involved in the pilot IPL to accommodate the slots available for ward rounds which were conducted for two or three times a week based on the availability of medical specialists in the respective wards. A total of 14 student groups were formed throughout the four weeks of hospital attachment for the purpose of pilot IPL. There were specific timetables prepared by medical lecturers/instructors for surgical and medical ward round groups for medical students, of which the pharmacy and nursing students were included in the respective groups. The medical and pharmacy lecturers were available during the ward round and students were expected to consult the respective lecturers after clerking the cases.

Following the completion of IPL activities, students wrote self-reflective diaries regarding their learning experiences, observations, and feelings. The diaries were submitted to the course coordinator and excluded as part of the course assessment of student performance. The diaries

were written in English and submitted for documentation in September 2022. The diary consisted of six sections: 1) activities 2) significant experience/observation, 3) learning from experience/observation, 4) additional learning needs and plan to achieve learning 5) summary of learning, and 6) meaningful interaction with the team (peers, participants, residents, preceptors). The reflective diaries were de-identified from participant information.

The class was contacted via email for informed consent purposes. They were informed that their reflective diaries would be used for the purpose of this study and a total of 21 students consented to the study. Sample size of 21 was sufficient to be representative of the 28 students involved in IPL activities in qualitative research. After receiving informed consent, personal details were removed from reflective diaries and de-identified using number e.g., S1. Then the reflective diaries were used for data analysis conducted by two investigators. Students who consented to this study did not receive any honorarium.

Data analysis

Data about accounts of experiences and learning were analysed using thematic content analysis for components of learning. Two researchers conducted the analysis and theme coding. The data from reflective diaries were analysed qualitatively by identifying the themes related to learning. The data were sorted for the meanings of accounts identified, and a list of code was constructed for major themes following repeated and close reading of the individual reflective diaries. Two researchers independently constructed, compared, and cross-checked data to produce a final list themes code. The coding decisions were agreed upon and the coded data were aggregated into code files. The summary of the data was done through manually scrutinising and generalising the data sets by one researcher. Another researcher coded the findings using Atlas.ti® version 9. A protocol was used to guide the analysis to four categories from the reflective diaries which were habitual action/non-reflection, understanding, reflection, and critical reflection (Kember et al., 2008), together with the six steps of Braun and Clarke (2006) theme analysis technique. The thematic analysis process was conducted through reflexivity by comparing the documented coding and themes, interpretations, and reasoning between the two researchers and compared their analyses.

Results and discussion

The analysis of the 21 reflective diaries identified four themes and 12 codes (Table 1). All 21 students were female, with Malay ethnicity, with an average age of 23 years old. We achieved theme saturation after the analysis of the fifteenth reflective diary. The following themes emerged from the reflective diaries:

1. Professional development and interdisciplinary skills

Students recognised that there were opportunities to learn about different aspects of medications and disease management during their IPL activities. It allowed them to identify and take responsibility for addressing gaps in their existing knowledge and perceptions. They realised the need to take proactive steps to ensure an understanding of the patient care process. This finding demonstrated that patient-centred care underlies all patient care activities and initiates independent learning skills among students. The importance of communication skills within healthcare teams and between team members and patients (and their family members) was recognised. Communication skill is developed from training and experience of which IPL is an invaluable platform to shape the quality of future health care professionals.

"I learned about correct case presentation. I learned about the current management of certain diseases. For example, empagliflozin can be used to treat heart disease as well as diabetes. I learned the importance of knowing the medication regimens of patients prior to hospital admission to help avoid hospital-related medication errors"(S15)

"From this experience, I recognised the need to understand the treatment of disease as practiced by the doctors and how they decide on such treatment. To

achieve that, I need to be more proactive and ask the doctors for explanations. Be more confident and always try to clarify my understanding" (S21)

"I also realised that ward round activities require good communication skills, especially skills in talking to patients. On top of that, professional communication skills are crucial to have effective discussions with medical students and doctors" (S2)

2. Favourable interprofessional engagement

The students indicated that they benefited from team discussions, particularly in clarifying unfamiliar jargon. They reported pleasant encounters with patients and overall positive involvement with members of other professions. They appreciated the effort taken by physicians and medical students to discuss medications with them. The appreciation of positive interaction indicates that IPL activities could provide an opportunity for interdisciplinary interaction, however small in scale, and could encourage the team of students and practitioners to appreciate each other's presence and initiate engagement.

"The medical students were helpful and approachable. When I asked questions, they would answer them clearly. There were many medical terms I could not understand during team discussions, but I managed to understand the terms once I had the chance to ask questions. It was easy to contact my team members during the IPL and they did not leave out my partner and me. It seemed like they were glad to have us join the discussion" (S14)

"Also, these experiences changed my perception towards patients, as I worried before to ask/disturb patients. In turn, all patients that we interacted with were too kind and even made some jokes to us" (S1)

"Mostly, the doctor questioned the medical students about patients' conditions and there was not much to say about drugs. However, since it was an interprofessional activity, the doctor asked us about drugs including insulin and how we should conduct an enquiry about the drugs taken by patients" (S18)

3. Roles identification and development

The pharmacy students were able to identify the role of different professions during IPL experience in terms of interactions with patients and health care professionals and their roles as part of IPL teams in contributing information about medications. They appreciated the importance of IPL in applying best practices by focusing on patients' specific circumstances and medication needs. It encouraged them to evaluate the information required to solve issues raised in patient management and made them aware of the pharmacy profession's responsibility in contributing to improved patient healthcare outcomes.

"I was able to see how the medical student used layman's terms to interact with the patient" (S15)

"From this experience, I observed that the medical team, or in this case medical students, were not well-versed in terms of medications. From what I could see, they were only taught the common management of the disease and some of the mechanisms of the drugs. They would sometimes ask us, why the patient was given one medication in preference to another medication. We then had to search for the answer" (S8)

"I observed that the medical students and physician did not include any issues regarding the medications during the history taking process. This reflects the importance of pharmacists to ensure that the treatment given is fully optimized for the patient, by investigating any issues related to the medications. It is also important to assess the patients' medication adherence and to investigate any drug related problems described by patients" (S11)

4. Operational flaws in IPL implementation

Some students reported that the IPL activities did not meet their expectations due to the passive role they had during the activities. These included limited interactions with patients and other IPL team members and priority associated with the needs of students from other disciplines. The students' negative experiences could be due to students' passive behaviour and/or lack of training of health practitioners.

"They checked the heart rate and asked how the patient was feeling today. but I was not able to ask the patient any questions about her medication" (S15)

"My expectation was that we as a pharmacist student can learn something about our job scopes but this session, we only learned about medical terminology. In addition, due to feeling that I got nothing from my IPL experience, I

joined my friend's surgical ward round" (S21).

"I experienced a lack of interaction with the specialist. The specialist prioritised the medical students, but I understood that he needed to prepare them for their examination. The specialist asked the question generally to us and we were not able to give the answer because the specialist wanted the answer from the medical students" (S14)

We report the reflections of pharmacy students' from a pilot IPL activity involving pharmacy, medical, and nursing students in their third, fifth, and second year of the respective programme. In our study, the five themes that emerged from the reflective diaries of pharmacy students were: 1) Professional development and interdisciplinary skills, 2) Favourable interprofessional engagement, 3) Roles identification and development, and 4) Operational flaws in IPL implementation.

For the theme of Professional Development and Interdisciplinary skills, students highlighted the need for improving their overall interactions with both health care professionals, patients, as well as their fellow students from other disciplines. This component was identified as crucial to obtain new skills and knowledge from other disciplines during clinical ward rounds and integrating them into their own to provide the best services to patients. The ability to realise self-insufficiency through engagement with other disciplines towards achieving the treatment goal for patients indicated the involvement of metacognitive skills that developed from IPL. This outcome is vital to empowering lifelong learners. Students with metacognition skills possess the ability to monitor and regulate reasoning, comprehension, and problem-solving (Medina, Castleberry & Persky, 2017). A previous study has reported that simulated IPL improved the efficiency of pharmacy students in working with other healthcare professionals through communicating and prioritising tasks (Hamilton et al., 2021). It has also been highlighted that cultural competency through conceptualised knowledge-based, skill-based, and behaviour-based components could be developed from IPL (Jarrar et al., 2024). However, these outcomes have not been assessed in our pilot study. It is important that future studies investigate these components to improve students' competencies through IPL activities. Nevertheless, the pilot IPL was beneficial in exposing pharmacy students to these learning components directly and indirectly.

Another theme that emerged from the reflective diaries was Favourable Interprofessional Engagement. Pharmacy students perceived the positive experiences with the patient, health professionals, instructors, and students. The discussion of medical jargon used in case discussion, pleasant responses from the patient, and the effort of instructors to engage with all students from different disciplines have been reported. Favourable experiential learning from IPL is a positive outcome that may indicate readiness for full implementation of IPL provided the feedback is addressed appropriately. Our study did not compare the responses between students from other disciplines and solely focused on pharmacy students' views. It is also important to investigate the differences, associations, and correlation of factors influencing IPL activity among students of different disciplines to ensure the effectiveness of shared learning. For example, the readiness for shared learning was identified as high among female students, those with advanced degrees, and those with healthcare experience prior to enrolment in health professional school, whereas nursing students scored significantly higher than physician associates and medical students (Talwalkar et al., 2016). In New Zealand, significant differences were reported between final-year pharmacy, medical, and nursing students' responses regarding the prioritisation of care, systematic assessment of patients, and communication strategies although pharmacy students had less favourable responses regarding the IPL experience than medical and nursing students (Curley et al., 2019). The positive experiences of students of different health professions are crucial to providing conducive IPL engagement and achieving its objective.

For the theme Roles Identification and Development, students discovered that other professions practice differently in patient care, and they understood the process and its importance. This indicates the ability to recognise the respective

role and responsibility which is important to function as a health care team. In our study, students noticed their role as pharmacists to complement the history taking to support the medical students and practitioners who need to focus on other components in patient management. They also had to initiate searching for new information to answer the questions or support the clinical team. It was common that pharmacy students reported feeling underprepared for and underutilised during patient care scenarios (Curley et al., 2019). The principle of adult learning is applicable in IPL activities involving learning from others in the groups as well as from others (Black, Balatti & Falk, 2013). This concept is holistic to IPL which involves patients, health care practitioners, education instructors, and students that promote the development of unique contextual strategies that benefit students learning.

The theme of Operational Flaws in IPL Implementation revealed that there was ambiguity in terms of understanding IPL purposes and processes, which led to limited interaction between professionals, students, and patients on certain occasions. This aspect has commonly been reported in the early implementation of IPL. For example, the limited understanding of health professionals' roles may constrain the effectiveness of IPL interventions delivered in allied health programmes (Olson & Bialocerkowski, 2014). In this respect, the development and delivery of IPL through educator mechanisms and curricular mechanisms should address the practice and clarify the role of students, health educators, and other staff involved in the setting. The former should include components such as academic staff training, institutional support, managerial commitment, and learning outcomes, whereas the latter involves logistics and scheduling, programme content, compulsory attendance, shared objectives, adult learning principles, and contextual learning (Gilbert, Yan & Hoffman, 2010). The development of detailed guidelines and simulations may assist the students and staff to achieve the IPL objectives by improving interaction and equity between professions. An understanding of the complexities of these processes, however, would improve attitudes towards interprofessional interaction and teamwork in clinical settings (Olson & Bialocerkowski, 2014). Additionally, the role of instructors in IPL activities is important and could be enhanced by intentional questioning, modelling techniques, and reflection (Medina, Castleberry & Persky, 2017). Undeniably, the mix and power dynamics across health professions during IPL activity may also be significant. For example, physiotherapy and medical students were described as dominant within patient scenario activities, undermining IPL effectiveness (Olson & Bialocerkowski, 2014). Also, a single IPL activity carried out in a dentistry clinic mostly concentrated on role clarification and seemed to have little impact on other collaborative learning outcomes (Dresser et al., 2021). Alternatively, innovative IPL activity using a team-based learning format was effective in addressing students' knowledge and attitudes focusing on the roles and responsibilities (Wheeler et. al., 2019). A variety of strategies should be adopted and evolved to improve IPL effectiveness across disciplines that are unique to each clinical setting.

Internationally, the scope of practice of health professions differs considerably and thus local practice assessments must be considered in improving IPL implementation. It has also suggested that the maturity (age) of the participants, or perceived power imbalances between professions and other factors be considered when planning, delivering, and evaluating a programme (Olson & Bialocerkowski, 2014). While undergraduate students of different health professions need to be trained to work in multidisciplinary teams, it is important to implement IPL unique to the setting to allow effective university-based IPL methods that are valid for local practice. In the US, the IPL was embedded in the Introductory Pharmacy Practice Experience (IPPE) in the earlier years (first and second year), whereas the Advanced Pharmacy Practice Experiences (APPEs) were included in the latter years of the programme in most colleges and schools using both subjective and objective measures to assess students' competencies and curriculum improvement (Jones et al., 2012). Nevertheless, IPL models used in different contexts (courses/curricula, settings, demographics) should

not be assumed to be transferable into other contexts, due to the complexity of health professions differences, variation in service delivery models, and pedagogical approaches to education (Olson & Bialocerkowski, 2014). Therefore, it is important to explore the unique issues underlying each setting to identify tailored strategies for improvement.

This study has a limitation in that a sample size of 21 students is small compared to the class size of 113 students and, accordingly, the findings are not generalisable to the whole class. Nevertheless, only 28 students were involved with pilot IPL activities considering the available resources and thus representative of the cohort involved. The findings provide the grounds for addressing IPL implementation among pharmacy students in our setting. These data will assist in adapting the intervention to suit the local context and demands to correlate with the need to improve curriculum structure. Nonetheless, since the diary was required to be written at the end of the IPL activities, there was a possibility of potential recall bias. Future studies may consider the diary to be written on the day of IPL activities to improve the findings. We also acknowledge that medical and nursing students involved in the IPL activities were not included in the present study, because their inclusion might alter the findings and discussion which aimed to explore pharmacy students' perspective. We did not conduct data triangulation with students after finalising the themes, and this could be improved in the future.

Table 1: Themes and codes identified by thematic analysis.

Themes	Codes
Professional development and interdisciplinary skills	• Reflection on areas for improvement • Acquisition of new knowledge • Recognition of communication skills
Favourable interprofessional engagement	• Positive interaction with professionals • Positive interaction with students of other profession • Positive patient interaction
Roles identification and development	• Observation of practice by other professions • Recognition of responsibility • Sharing of knowledge
Operational flaws in IPL implementation	• Lack of understanding on IPL purpose and process • Lack of interprofessional interaction • Lack of patient interaction

Conclusion

Pharmacy students benefited from the pilot IPL by learning professional and interdisciplinary skills, interprofessional engagement, identifying their roles in IPL, and identifying operational flaws that could be improved for future implementation. They developed metacognitive skills during interprofessional experiences with other students, healthcare professionals, and patients. Future studies may explore continuous assessments of IPL components to ensure effectiveness in patient-centred care.

Authors contributions

N.H.M.T., N.S.A.R., T.K.T.M.K., and M.H.N.M. performed the concepts and design of the study and was involved in the project administration, N.A.A.M.F. performed data analysis, C.J.T. involved in supervision and editing of the manuscript, N.H.M.T., and N.A.A.M.F. wrote the original draft of the manuscript. All authors reviewed the manuscript and agreed to the published version of the manuscript.

Acknowledgements

The authors wish to thank the staff involved in the pilot interprofessional learning activities from the Kulliyyah of Medicine, International Islamic University Malaysia, and from Sultan Ahmad Shah Medical Centre, Pahang, Malaysia.

Ethical approval statement

The study protocol was approved by the International Islamic University Malaysia Research Ethics Committee (ID no. IREC 2022-237)."

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Conflict of interest

The authors declare that they have no conflict of interests.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare they have not used any generative AI and AI-assisted technologies in writing the manuscript.

References

Black, S., Balatti, J., & Falk, I. (2013). Health literacy and social capital: what role for adult literacy partnerships and pedagogy? Studies in Continuing Education, 35(2), 146–164. https://doi.org/10.1080/0158037X.2012.712038

Braun, V., & Clarke, V. (2006). Using thematic analysis in psychology. Qualitative Research in Psychology, 3(2), 77–101.

Curley, L. E., Jensen, M., McNabb, C., Ram, S., Torrie, J., Jowsey, T., & McDonald, M. (2019). Pharmacy Students' Perspectives on Interprofessional Learning in a Simulated Patient Care Ward Environment. American Journal of Pharmaceutical Education, 83(6), 6848. https://doi.org/10.5688/ajpe6848

Dresser, J., Barazanchi, A., Meldrum, A., Marra, C., & Wilby, K. J. (2021). Identifying perceptions and themed learning outcomes between pharmacy and dentistry students through interprofessional education and collaboration in the dental clinic. Currents in Pharmacy Teaching and Learning, 13(7), 843–847. https://doi.org/10.1016/j.cptl.2021.03.012

Gilbert, J. H., Yan, J., & Hoffman, S. J. (2010). A WHO report: framework for action on interprofessional education and collaborative practice. Journal of Allied Health, 39 Suppl 1, 196–197.

Hamilton, P., Coey-Niebel, C., McCaig, J., Zlotos, L., Power, A., Craig, G., ... & Paton, C. (2021). Evaluation of Inter-Professional Education (IPE) with medical, nursing and pharmacy students through a simulated IPL Educational Intervention. International Journal of Clinical Practice, 75(11), e14725.

Jarrar, R., Lim, R., Richardson, C. L., Naqvi, A. A., Rathbone, A. P., & Lau, W. M. (2024). How cultural competence is conceptualised, developed and delivered

in pharmacy education: a systematic review. International Journal of Clinical Pharmacy, 46(1), 40-55.

Jones, K. M., Blumenthal, D. K., Burke, J. M., Condren, M., Hansen, R., Holiday-Goodman, M., & Peterson, C. D. (2012). Interprofessional education in introductory pharmacy practice experiences at US colleges and schools of pharmacy. American Journal of Pharmaceutical Education, 76(5), 80.

Kember, D., McKay, J., Sinclair, K., & Wong, F.K.Y. (2008). A four-category scheme for coding and assessing the level of reflection in written work, Assessment & Evaluation in Higher Education, 33(4): 369-379. doi: 10.1080/02602930701293355

Mann, K., Gordon, J., & MacLeod, A. (2009). Reflection and reflective practice in health professions education: A systematic review. Advances in Health Sciences Education, 14(4), 595–621. https://doi.org/10.1007/s10459-007-9090-2

Medina, M. S., Castleberry, A. N., & Persky, A. M. (2017). Strategies for Improving Learner Metacognition in Health Professional Education. American journal of pharmaceutical education, 81(4), 78. https://doi.org/10.5688/ajpe81478

Murray, M. (2021). The impact of interprofessional simulation on readiness for interprofessional learning in health professions students. Teaching and Learning in Nursing, 16(3), 199–204. https://doi.org/10.1016/j.teln.2021.03.004

Nagge, J. J., Lee-poy, M. F., & Richard, C. L. (2017). Evaluation of a Unique Interprofessional Education Program Involving Medical and Pharmacy Students. 81(10). https://doi.org/10.5688/ajpe6140

Olson, R., & Bialocerkowski, A. (2014). Interprofessional education in allied health: a systematic review. Medical education, 48(3), 236–246. https://doi.org/10.1111/medu.12290

Rosen, M. A., DiazGranados, D., Dietz, A. S., Benishek, L. E., Thompson, D., Pronovost, P. J., & Weaver, S. J. (2018). Teamwork in healthcare: Key discoveries enabling safer, high-quality care. The American psychologist, 73(4), 433–450. https://doi.org/10.1037/amp0000298

Semple, L., & Currie, G. (2022). "It opened up a whole new world": An innovative interprofessional learning activity for students caring for children and families. International Journal of Educational Research Open, 3(December 2021), 100106. https://doi.org/10.1016/j.ijedro.2021.100106

Talwalkar, J. S., Fahs, D. B., Kayingo, G., Wong, R., Jeon, S., & Honan, L. (2016). Readiness for interprofessional learning among healthcare professional students. International journal of medical education, 7, 144–148. https://doi.org/10.5116/ijme.570d.7bd8

Tsingos, C., Bosnic-Anticevich, S., Lonie, J. M., & Smith, L. (2015). A model for assessing reflective practices in pharmacy education. American Journal of Pharmaceutical

Wheeler, S., Valentino, A. S., Liston, B. W., Li, J., & McAuley, J. W. (2019). A team-based learning approach to interprofessional education of medical and pharmacy students. Currents in Pharmacy Teaching and Learning, 11(11), 1190–1195. https://doi.org/10.1016/j.cptl.2019.07.010

Journal of Pharmacy

Journal of Pharmacy

Mobile Application Intervention Effectiveness in Improving Hypertensive Patients Medication Adherence: A Systematic Review

Nur Hannan Najihah binti Mohamad Tawpik[1], Nur Adlina binti Ruslan[1], Syahrir bin Zaini[1] and Hussam Abdeljabar Ahmad Mizher[1]*

[1]*Kulliyyah of Pharmacy, International Islamic University Malaysia.*

Abstract

Introduction: Hypertension and its complications are massive global health issues. Major limitations in hypertensive patients' treatment include suboptimal blood pressure control and nonadherence to medication. The popularity of telemedicine has risen in recent years. Mobile phone applications intervention in particular, provides features including medication-taking, refilling reminders, and biometric results tracker resulting in better health outcomes and improved medication adherence. This review aims to assess the effectiveness of mobile application in improving adherence in hypertensive patients. **Methods:** PubMed, Scopus, and Cochrane Library were searched with filters applied for studies published between 2013 and 2023 and content published in English with the keywords; telemedicine, mobile apps, medication adherence, and hypertension. These keywords were joined using Boolean operators for an effective search. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement was followed for this systematic review. The Mixed Method Appraisal Tool was used to assess the quality of the included studies. The data was extracted by the authors and validated by another for accuracy and completeness. **Results:** Twelve studies ranging from moderate to high quality were included in this review. A total of 10 studies showed a statistically significant improvement ($p < 0.001$) in medication adherence with mobile apps intervention. The combined apps features from the mobile apps empower patients to be more adherent, involved and informed about their treatment progress. **Conclusion:** Hypertensive patients' medication adherence improved with mobile apps intervention. However, the heterogeneity of adherence measurement methods and apps functionality in the included studies calls for further studies to determine the effectiveness of specific mobile apps feature as well as the standardisation of the adherence measurement method used.

Article history:

Received: 18 February 2024
Accepted: 30 December 2024
Published: 31 January 2025

Keywords:

Telemedicine
Hypertension
Mobile Apps

doi: 10.31436/jop.v5i1.286

*Corresponding author's email: hussam@iium.edu.my

Introduction

Hypertension, also known as high blood pressure (BP), and its related complications stand as prominent contributors to both morbidity and mortality. (Mills et al., 2017). Among the major problems in managing hypertensive patients include suboptimal blood pressure control and poor treatment adherence. Despite the availability and access to effective and safe medication, at least half of hypertensive patients fail to achieve their target blood pressure goals (Michalakeas et al., 2020).

Telemedicine is a form of supplement effort to achieve health objectives using wireless technologies such as mobile phones (Beleigoli et al., 2019). Given lower costs and greater patient engagement, telemedicine interventions such as mobile phone applications and wireless devices have been introduced for chronic disease management, including hypertension. (Morrissey et al., 2018). As effective management of hypertension is crucial for preventing cardiovascular disease, all possible tools should be used to help achieve target blood pressure levels, both for individuals and the population as a whole. This includes leveraging mobile phones, smartphones, and their various applications as approximately 85% of the world's population has mobile phone coverage (Cowie et al., 2016).

To address nonadherence, a few of the mobile applications' functions include medication-taking reminder, refilling reminders and biometric results trackers (Kumar et al., 2015). Recent studies regarding mobile applications intervention in managing hypertension and medication adherence improvement were found to have positive impacts (Ma et al., 2019). However, outcomes are inconsistent, with some studies showing no significant impact on blood pressure control despite better adherence (Moore, Neher et al. 2011, Rupert and Mounsey 2022). Factors influencing effectiveness include patient motivation, ease of use, and health literacy. These apps are particularly relevant for hypertension management due to the chronic nature of the condition, the need for remote monitoring, and the ability to provide personalized care plans.

Despite these advancements, there is a lack of systematic review evaluating the effectiveness of mobile applications intervention and the adherence measurement methods used. This paper aims to bridge the gaps in the existing literature, highlighting the potential of mobile apps in managing hypertension and improving patient outcomes.

Methodology

The Preferred Reporting Items for Systematic Reviews and Meta Analyses Protocols (PRISMA-P) was followed for this systematic review.

Eligibility criteria

Study Design: Both randomised and non-randomised control trial studies related to mobile applications intervention impact on the medication adherence in hypertensive patients were included in this paper.

Inclusion and exclusion criteria

The research focused on articles reporting studies about hypertensive patients of above 18 years and older, with mobile application intervention for medication adherence. Only full-text studies in English from 2013 to 2023 are included, excluding other types of telemedicine interventions.

Information Sources

The principal source of literature is from electronic bibliographic databases using a comprehensive search strategy via PubMed, Scopus, and Cochrane Library.

Search Strategy

The search strategy is developed to target three key domains: Medication Adherence, Hypertension, and Telemedicine. To ensure the search strategy used is effective, these three domains were joined together using the Boolean operator "AND" while the groups of keywords were joined with the Boolean operator "OR". The group of keywords for the domains are, (1) Medication Adherence; "Patient Compliance", "Drug Adherence", "Drug

Compliance", "Medication Noncompliance", "Medication Nonadherence", "Medication Compliance", "Patient Adherence", "Treatment Compliance", "Therapeutic Compliance", (2) Hypertension; "High Blood Pressure", "Hypertensive Patients", "Elevated Blood Pressure", and (3) Telemedicine; "Mobile Health", "mHealth", "Telehealth", "eHealth", "mobile applications", "mobile application", "mobile apps" and "mobile app".

Data management

All citations of the selected literature and the duplicate records were managed using Mendeley.

Selection process

Two authors conducted the screening process based on the inclusion and exclusion criteria. The selected articles were then downloaded in full text for inclusion in the systematic review. HN, HA initially evaluated the titles and abstracts by enlisting the search results using Microsoft Excel to exclude irrelevant studies. Following this, HN, HA and AR examined the full-text articles of the remaining studies based on the eligibility criteria. Discourse between the authors was done to reach a consensus on which articles be included in the paper.

Quality assessment

Two authors independently conducted a quality assessment of the chosen journals, engaging in discussions to achieve consensus. The evaluation of bias risk adhered to the guidelines outlined in the Mixed Methods Appraisal Tool (MMAT) version 2018. This tool can appraise quantitative non-randomised and randomised controlled trials by assessing five criteria for methodological quality.

Three response options "Yes," "No," and "Can't tell" (see Appendix) were used to indicate whether the criteria are fulfilled, not met, or if there is insufficient information in the paper. "Yes" responses to ≤2, 3, and ≥4 of the questions were classified as low, moderate and high quality respectively. An overall summary was created utilising Risk-of-Bias Visualisation (robvis) (McGuinness & Higgins, 2021).

Data extraction

HN and AR independently carried out data extraction, covering study characteristics and outcomes of interest. HA validated the extracted data for accuracy and completeness, ensuring the absence of errors or crucial omissions during the extraction. The data were then tabulated covering author, year of publication, location and duration of the study, study design; sample size and mobile application used, adherence measurement method and the outcomes.

Results

Search result and study selection

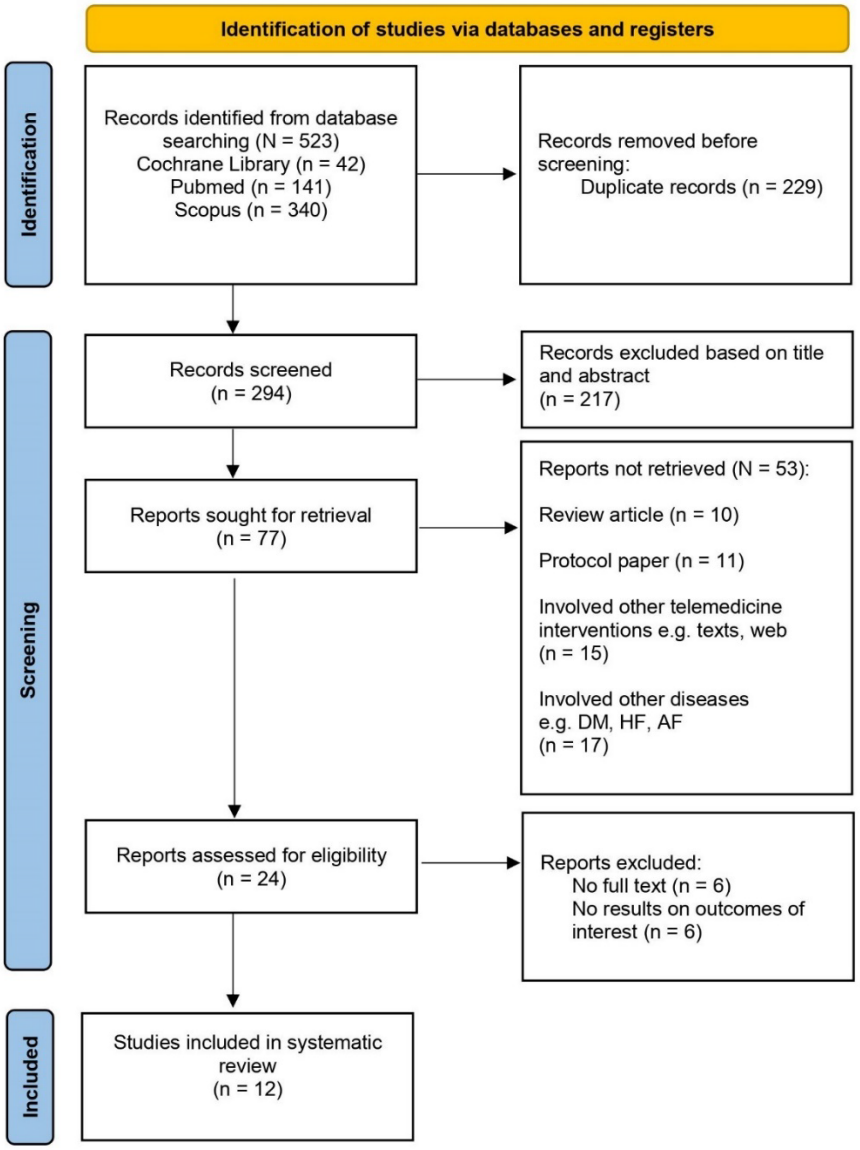
A total of 523 articles were found from the database search, as depicted in Fig. 1. Following the removal of 229 duplicates, 294 records underwent screening based on titles and abstracts. Fifty-three studies were not retrieved for reasons outlined in Fig. 1, and 77 were actively sought through manual searching. Subsequently, 24 articles underwent further assessment for eligibility, with 12 articles ultimately selected following the eligibility criteria. Twelve articles not included were due to unavailability of full text (n=6) and lack of primary results on any relevant outcome (n=6).

Risk of bias and quality assessment

All the studies incorporated in the analysis exhibited moderate to high quality, and the majority demonstrated a low risk of bias, as depicted in Tables 1 and 2.

Characteristics and design of included studies

Table 3 provides an overview of the characteristics of the studies incorporated in this analysis. All studies were published within the past decade from 2013 to 2023. The research was conducted in seven different countries, with almost half of the studies reporting data from the USA (Chandler et al., 2019; Morawski et al., 2018; Patel et al., 2013; Persell et al., 2020; Zha et al., 2020). Five studies originated from Palestine (Abu-El-Noor et al., 2021), Brazil (Volpi et al., 2021), Georgia (Manigault et al., 2020), Kazakhstan (Nurakysh et al., 2022) and Spain (Márquez Contreras et al., 2019), respectively and



*DM: diabetes mellitus; HF: heart failure; AF: atrial fibrillation

Fig. 1: Article Review Process

Table 1: Risk of bias for RCT

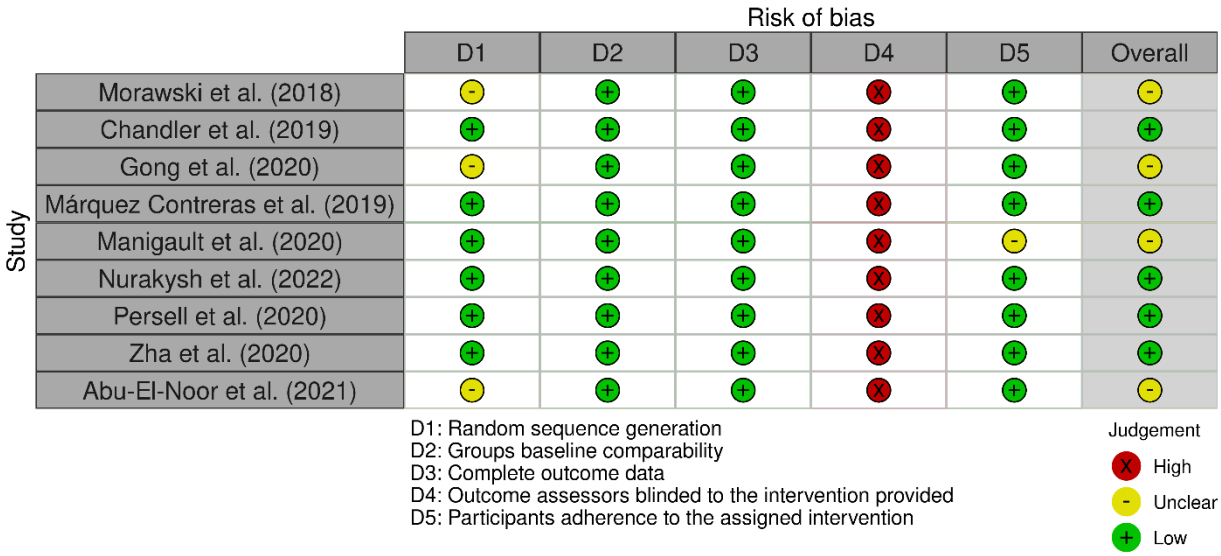
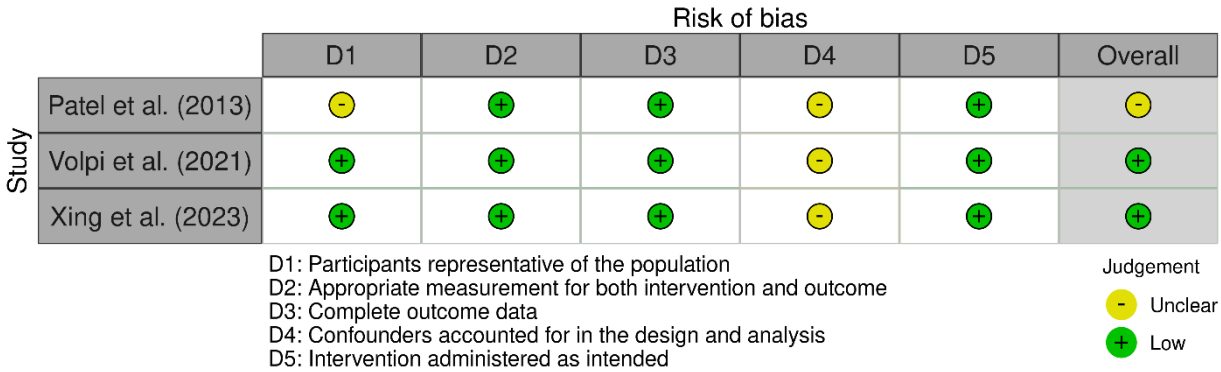


Table 2: Risk of bias for non-RCT



two studies were conducted in China (Gong et al., 2020; Xing et al., 2023). All but three studies are randomised controlled trials.

Participant Characteristics

The collective sample size of the studies involved 8,154 individuals diagnosed with hypertension, ranging from 30 to 5,937 participants in a study. Participants' mean age ranged from 44 (Manigault et al., 2020) to 62 years (Nurakysh et al., 2022). Six studies included the participants' educational backgrounds ranging from high school graduates or lesser to college graduates or more.

Medication Adherence Assessments

Table 3 illustrates the variability in adherence measures across the studies. The majority used questionnaires, such as Morisky Medication Adherence Scale (Chandler et al., 2019b; Gong et al., 2020; Morawski et al., 2018; Patel et al., 2013), Lebanese Medication Adherence Scale (Nurakysh et al., 2022), Patient Medication Adherence Questionnaire (Persell et al., 2020), Martín-Bayarre-Grau Questionnaire (Volpi et al., 2021), Hill-Bone Compliance to High Blood Pressure Therapy Scale (Abu-El-Noor et al., 2021) and Medication Adherence Self-efficacy Scale (Zha et al., 2020). Other methods include Medication Event Monitoring System (Márquez Contreras et al., 2019), refill history (Manigault et al., 2020) and random forest algorithm (Xing et al., 2023).

Effects on Medication Adherence and Clinical Outcomes

Ten studies demonstrated a statistically significant enhancement in medication adherence within the intervention group (Abu-El-Noor et al., 2021; Chandler et al., 2019; Gong et al., 2020; Márquez Contreras et al., 2019; Morawski et al., 2018; Nurakysh et al., 2022; Patel et al., 2013; Volpi et al., 2021; Xing et al., 2023; Zha et al., 2020). However, there was a notable high level of methodological heterogeneity, as different adherence assessment methods and study durations varied among the studies. Of the nine trials that evaluated health-related outcomes, specifically blood pressure readings (both systolic and diastolic), seven reported significant results, indicating an overall improvement with the intervention in blood pressure control (Chandler et al., 2019; Gong et al., 2020; Márquez Contreras et al., 2019; Morawski et al., 2018; Patel et al., 2013; Xing et al., 2023; Zha et al., 2020).

Characteristics of Mobile App

Table 4 outlines the features of the mobile applications employed. All apps features promote medication adherence as their primary function, through medication reminders, blood pressure tracker, patient education and reminders for appointments and refills. Patients' adherence data and BP readings were stored on the cloud storage as health records for ease of access to associated healthcare providers (Gong et al., 2020; Volpi et al., 2021; Xing et al., 2023; Zha et al., 2020). Six studies had healthcare providers interactive features on the mobile apps interface (Abu-El-Noor et al., 2021; Gong et al., 2020; Manigault et al., 2020; Márquez Contreras et al., 2019; Volpi et al., 2021; Xing et al., 2023).

Discussion

Hypertension continues to pose a global public health challenge, impacting an estimated 80 million adults in the USA and exhibiting a high prevalence in Asia over the past decade (Kim et al., 2016; Mahmood et al., 2021). Following the use of mobile applications to curb this challenge, this review compiles the current literature, highlighting 12 studies from the past decade (2013–2023), to examine the impact of mobile application interventions on medication adherence.

A preferable approach to monitor adherence should be dependable, feasible, straightforward, and reasonably cost-effective. As such, no single method fulfils all these criteria, as each type of drug adherence measurement has advantages and disadvantages (Hamdidouche et al., 2017). Although questionnaires are easy to administer, they often suffer from inaccuracy attributable to patients' behavioural biases. Regardless, its use is effective in large populations and allows clinicians to further counsel patients based on the

Table 3: Characteristics of included studies

Study (Country, study setting, duration, mobile app)	*Participants' characteristics*	*Intervention group (mobile apps features)*	*Control group*	*Method of adherence measurement*	*Main outcomes (significance)*	
					Medication Adherence	*BP measurement (mmHg)*
Patel et al. (2013) ***USA*** ***Pilot study*** ***3-month*** ***Pill Phone App***	Mean age: 53 Gender Male: 31% Female: 69% Race African-American: 96% Education General education: 79% College graduate: 17% N = 48	- Dose reminder - Pill-taking history record - Dose intake verification - Potential side effects and drug interactions information	Usual care	MMAS-8	Baseline= 2.0 Post= 3.2 p <0.001	Baseline: 144/89 Post: 135/85 p = 0.006
Morawski et al. (2018) ***USA*** ***RCT*** ***3-month*** ***MedISAFE-BP***	Mean age: 52 Gender Female: 60% Race White: 71.3% Black: 20.6% Other: 8.1%	- Dose reminder - Drug interaction checker - BP tracker	Usual care	MMAS-8	IG: 6.3 CG: 5.7 p=0.001	SBP IG: 140.8 CG: 141.2 p= 0.97

Study (Country, study setting, duration, mobile app)	*Participants' characteristics*	*Intervention group (mobile apps features)*	*Control group*	*Method of adherence measurement*	*Main outcomes (significance)* *Medication Adherence*	*BP measurement (mmHg)*
	Education High school or less: 14% College grad: 86% N = 411					
Chandler et al. (2019) ***USA*** ***RCT*** ***9-month*** ***SMASH App***	Mean age: IG: 44.4 ± 7.2 CG: 46.8 ± 8.1 Education High school or less: 71% Partial college grad: 29% Gender Male: 61% Race: Hispanic N = 54	- BP progress log - Daily dose reminder	Usual care	MMAS-8	IG: 9.81 ± 1.31 CG: 6.84 ± 1.52 p<0.001	SBP IG: 121.8 CG: 145.7 p < 0.01
Gong et al. (2020) ***China*** ***RCT*** ***6-month*** ***Yan Fu app***	Mean age: IG: 58.20±7.479 CG: 59.27±7.439	- BP tracker - Medicine and exercise reminder - BP limit alarms - Remote consultations with GP	Usual care	MMAS-8	IG: 3.5% CG: 1.83% p= 0.004	SBP IG: 131.52 CG: 135.27 p< 0.05 DBP

Study (Country, study setting, duration, mobile app)	*Participants' characteristics*	*Intervention group (mobile apps features)*	*Control group*	*Method of adherence measurement*	*Main outcomes (significance)* *Medication Adherence*	*BP measurement (mmHg)*
	Gender Male: 53% N = 480	- One-click emergency call				IG: 76.86 CG: 78.44 p< 0.05
Márquez Contreras et al. (2019) ***Spain*** ***RCT*** ***12-month*** ***ALERHTA***	Mean age: 57.5 ± 9.9. Gender Male: 47.9% Female: 52% N = 154	- BP goals - Doctor's advice recorder - Appointments reminder - BP tracker	Usual care	MEMS	IG: 89.4 % CG: 81.31% p <0.01	SBP IG: 132.2 ± 12 CG: 134.4 ± 11 p < 0.001 DBP IG: 78.5 ± 7 CG: 81.4 ± 9 p < 0.01
Zha et al. (2020) ***USA*** ***RCT*** ***6-month*** ***iHealth MyVitals***	Mean age: IG: 48.9 ± 8.0 CG: 55.5 ± 5.20 Gender Female: 83% Race Black: 99% White: 1% N = 30	- BP tracker - Instant feedback feature - BP data cloud storage	Usual care	MASES	IG: 69.17 ± 7.77 CG: 61.00 ± 13.08 p = 0.06	SBP IG: 137.38 ± 4.86 p < 0.05. CG: 140.88 ± 5.01 p = 0.17 DBP IG: 88.08 ± 7.45 CG: 88.10 ± 9.41 p = 0.6

Study (Country, study setting, duration, mobile app)	*Participants' characteristics*	*Intervention group (mobile apps features)*	*Control group*	*Method of adherence measurement*	*Main outcomes (significance)* *Medication Adherence*	*BP measurement (mmHg)*
Abu-El-Noor et al. (2021) ***Palestine*** ***RCT*** ***3-month*** ***Self-developed***	Mean age: IG: 55.4 ±10.9 CG: 57.5 ±11.9 Gender: Male: 36.1% Female 63.9% Education High school or less: 86% Partial college grad: 14% N = 191	- Dose and follow-up appointment reminders - Daily education short messages - BP tracker - Short instruction video	Usual care	Hill–Bone CHBPTS	IG: 11.73 CG: 13.98 p< 0.01	Not mentioned
Manigault et al. (2020) ***Georgia*** ***RCT*** ***3-month*** ***BP-n-Me App***	Mean age: IG: 44.4 ± 7.2 CG: 46.8 ± 8.1 Gender Male: 59% Race African-American: 38% Caucasian: 47% Other: 15% N = 78	- Daily dose reminder - "Call your Pharmacy" button - BP tracker - Health tips tailored to patient's lifestyle	Usual care	Refill history	IG: 0.20 CG: 0.20 p = 0.83	SBP IG: 128 CG: 141 p = .001 DBP IG: 78 p = 0.009 CG: 79 p= .0004

Study (Country, study setting, duration, mobile app)	*Participants' characteristics*	*Intervention group (mobile apps features)*	*Control group*	*Method of adherence measurement*	*Main outcomes (significance)* *Medication Adherence*	*BP measurement (mmHg)*
Persell et al. (2020) ***USA*** ***RCT*** ***6-month*** ***HBPM***	Mean age: 59.6 ± 12.4 Gender Female: 61.3% Race Asian: 5.9% Black: 30.7% White: 52.3% Other: 11.1% Education High school or less: 9% Partial college grad: 91% N = 297	- Daily dose reminder - Adherence checker	Usual care	PMAQ	Baseline (78.7) Result (80.6) p=0.99	SBP Result:132.3 ± 15.0 Baseline: 140.6 ± 12.2 p = 0.16 DBP Result: 85.1 ± 9.6 Baseline: 89.4 ± 8.7 p=0.61
Volpi et al. (2021) ***Brazil*** ***Non RCT*** ***3-month*** ***Self-developed***	Mean age: IG: 57.2 ± 7.1 CG: 60.4 ± 10.4 Gender Female: 40% Male: 60%	- BP and BMI record - Risk assessment - Recommendations, alerts, and medication reminders - Cloud storage for remote healthcare monitoring.	Usual care	MBGQ	IG:92% adherent, 8% partially adherent ($p < 0.001$). CG: remained virtually the same ($p \geq 0.999$).	Not mentioned

Study (Country, study setting, duration, mobile app)	*Participants' characteristics*	*Intervention group (mobile apps features)*	*Control group*	*Method of adherence measurement*	*Main outcomes (significance)* *Medication Adherence*	*BP measurement (mmHg)*
	Education High school or less: 84% College grad: 16% N = 49					
Nurakysh et al. (2022) ***Kazakhstan*** ***RCT*** ***12-month*** ***MyTherapy***	Mean age: 62.4 ± 3.9 Gender Female: 29.9% Male: 70.1% N = 425	- Customisable medication intake schedule - Tutorial video and text	Usual care	LMAS-14	IG: 40.3 ± 1.3 CG: 33.6 ± 1.9 p ≤ 0.001	Not mentioned
Xing et al. (2023) ***China*** ***Cohort study*** ***Jan 2014 to December 2021.*** ***Self-developed***	Mean age: 66.2±10.8 Gender Male: 47.6% Female: 52.4%% N = 5937	- Medical record BP level and body weight - Interactive family doctor feature	Usual care	Random forest algorithm	IG: 85.8 p<0.001)	p<0.001

RCT: Randomised controlled trial; IG: Intervention Group; CG: Control Group; Hill–Bone CHBPTS: Hill-Bone Compliance to High Blood Pressure Therapy Scale; MMAS-8: 8-item Morisky Medication Adherence Scale; LMAS-14: 14-item Lebanese Medication Adherence Scale; PMAQ: Patient Medication Adherence Questionnaire; MBGQ: Martín-Bayarre-Grau Questionnaire; MASES: Medication Adherence Self-Efficacy Scale; MEMS: Medication event monitoring systems; SBP: Systolic blood pressure; DBP: Diastolic blood pressure

questionnaires (Pandey et al., 2015). Employing an electronic monitoring approach, enhances participants' awareness of their involvement in a study, potentially influencing adherence behaviour (Márquez Contreras et al., 2019).

The assessment of refill history involved a prospective examination of patients' antihypertensive medication refill records and electronic health records. While this method assumes possession of the medication by the patient, it does not necessarily guarantee actual drug intake (Hamdidouche et al., 2017). The usage of models such as the random forest algorithm performed the best in terms of classification accuracy in medication adherence measurements. Nevertheless, the specificity of the algorithm in question would need careful investigation to avoid overprediction of medication nonadherence, consequently wasting resources for preventative measures (Bohlmann et al., 2021).

The utilised mobile apps exhibited varied functionality, incorporating reminders, educational features, or a combination of both, to enhance medication adherence. While the combined features facilitate the patients toward adherence, it is impractical to attribute the effectiveness of specific apps components or characteristics from these interventions. The use of reminders primarily targets individuals who unintentionally forget to take their medication, aiming to address "unintentional nonadherence."

Five studies found that education level did not affect the outcomes as the majority of the participants were high school graduates or of lesser education background (Abu-El-Noor et al., 2021; Chandler et al., 2019; Morawski et al., 2018; Patel et al., 2013; Volpi et al., 2021). One study consisted of participants from higher education background but had no significant difference in the outcomes ($p > 0.05$) (Persell et al., 2020). This finding shows that participants' adoption and e-skills literacy in using mobile apps are effective irrespective of their literacy levels.

This review indicated that the engagement of healthcare providers (HCPs) in-apps interventions for hypertension care primarily included physicians (Abu-El-Noor et al., 2021; Gong et al., 2020; Márquez Contreras et al., 2019; Volpi et al., 2021; Xing et al., 2023), with one trial reporting pharmacist involvement (Manigault et al., 2020). The lesser involvement of pharmacists in the studies is unexpected as pharmacists' roles in clinical patient-facing within primary care are evolving and widening due to their effectiveness in hypertension management and improvement in hypertensive patients' medication adherence (Khaira et al., 2020). Incorporating HCPs alongside the concurrent use of apps in interventions demands careful consideration, as it could potentially lead to an escalation in HCP workload. However, there is inadequate information to determine whether the associated involvement costs outweigh the observed benefits. Although all applications with HCP involvement demonstrated improvement in medication adherence, no cost-benefit analysis was done.

Strengths and Limitations

This systematic review provides insight into mobile apps interventions' effects on medication adherence in hypertensive patients. It was conducted following an extensive literature search, employing MeSH terms and a consistent eligibility criterion to enhance the likelihood of identifying all pertinent studies. The inclusion of both randomised controlled trials (RCTs) and non-RCTs is noteworthy, and despite study quality not being a basis for exclusion, all included studies were moderate to high quality. This suggests that the review encompassed the most reliable evidence available on the subject.

The inclusion of English language publications only, may lead to the exclusion of other language studies with relevant information. Additionally, the conclusions in this review are drawn from a limited dataset of only 12 studies. The heterogeneity in trial methodologies, apps, and adherence assessment methods made it impractical to calculate precise adherence rates and assess the efficacy of individual components' functionality in mobile apps. Despite the limitations, adhering to established guidelines for synthesis afforded us the optimal chance to derive meaningful insights from the available literature. Consequently, this review offers valuable perspectives that can contribute to the enhancement of hypertension care and management.

Conclusion

Mobile apps intervention enhanced medication adherence in hypertensive patients. However, the impact of specific apps intervention features and their effectiveness remains uncertain, which calls for in-depth analysis of the features involved. Further research is required to investigate the involvement of HCPs in mobile apps interventions regarding cost-effectiveness. Lastly, it is recommended that

future studies adopt a standardised and validated approach for measuring medication adherence, facilitating the comparison of results.

Authors contributions

HN and HA autonomously reviewed titles and abstracts and evaluated the complete texts of all eligible studies. HN and AR independently extracted the data, with HA confirming the accuracy and completeness. HN assessed the risk of bias in each included study. All authors have read and approved of the final manuscript.

Acknowledgements

The authors express gratitude to the institution and all contributors involved in this research for their original studies that served as the foundation for this review.

Conflict of interest

All authors declare no conflict of interest for this paper.

Declaration of generative AI and AI-assisted technologies in the writing process

In the creation of this work, generative AI technologies were utilised to enhance clarity and refine grammar. The employment of these tools was intended to improve the quality and efficiency of the writing process while preserving the integrity and originality of the content. All AI-generated output was carefully reviewed, revised, and integrated to ensure alignment with our creative and academic objectives.

References

Abu-El-Noor, N. I. I., Aljeesh, Y. I. I., Bottcher, B., & Abu-El-Noor, M. K. K. (2021). Impact of a mobile phone app on adherence to treatment regimens among hypertensive patients: A randomised clinical trial study. European Journal of Cardiovascular Nursing, 20(5), 428–435. https://doi.org/10.1177/1474515120938235

Beleigoli, A. M., Andrade, A. Q., Cançado, A. G., Paulo, M. N. L., Diniz, M. D. F. H., & Ribeiro, A. L. (2019). Web-based digital health interventions for weight loss and lifestyle habit changes in overweight and obese adults: Systematic review and meta-analysis. Journal of Medical Internet Research, 21(1), 1–10. https://doi.org/10.2196/jmir.9609

Bohlmann, A., Mostafa, J., & Kumar, M. (2021). Machine Learning and Medication Adherence: Scoping Review. JMIRx Med, 2(4), e26993. https://doi.org/10.2196/26993

Chandler, J., Sox, L., Kellam, K., Feder, L., Nemeth, L., & Treiber, F. (2019a). Impact of a culturally tailored mhealth medication regimen self-management program upon blood pressure among hypertensive hispanic adults. International Journal of Environmental Research and Public Health, 16(7). https://doi.org/10.3390/ijerph16071226

Chandler, J., Sox, L., Kellam, K., Feder, L., Nemeth, L., & Treiber, F. (2019b). Impact of a Culturally Tailored mHealth Medication Regimen Self-Management Program upon Blood Pressure among Hypertensive Hispanic Adults. International Journal of Environmental Research and Public Health, 16(7), 1226. https://doi.org/10.3390/ijerph16071226

Cowie MR, Bax J, Bruining N, Cleland JG, Koehler F, Malik M, Pinto F, Van Der Velde E, Vardas P. E-Health: a position statement of the European Society of Cardiology. Eur Heart J. 2016; 37:63–66

Gong, K., Yan, Y.-L., Li, Y., Du, J., Wang, J., Han, Y., Zou, Y., Zou, X.-Y., Huang, H., She, Q., & Roever, L. (2020). Mobile health applications for the management of primary hypertension: A multicenter, randomised, controlled trial. Medicine (United States), 99(16), E19715. https://doi.org/10.1097/MD.0000000000019715

Hamdidouche, I., Jullien, V., Boutouyrie, P., Billaud, E., Azizi, M., & Laurent, S. (2017). Drug adherence in hypertension:

Frommethodological issues to cardiovascular outcomes. Journal of Hypertension, 35(6), 1133–1144. https://doi.org/10.1097/HJH.0000000000001299

Khaira, M., Mathers, A., Benny Gerard, N., & Dolovich, L. (2020). The Evolving Role and Impact of Integrating Pharmacists into Primary Care Teams: Experience from Ontario, Canada. Pharmacy, 8(4), 234. https://doi.org/10.3390/pharmacy8040234

Kim, J. Y., Wineinger, N. E., & Steinhubl, S. R. (2016). The Influence of Wireless Self-Monitoring Program on the Relationship Between Patient Activation and Health Behaviors, Medication Adherence, and Blood Pressure Levels in Hypertensive Patients: A Substudy of a RandomisedControlled Trial. Journal of Medical Internet Research, 18(6), e116. https://doi.org/10.2196/jmir.5429

Kumar, N., Khunger, M., Gupta, A., & Garg, N. (2015). A content analysis of smartphone-based applications for hypertension management. Journal of the American Society of Hypertension, 9(2), 130–136. https://doi.org/10.1016/j.jash.2014.12.001

Ma, Y., Cheng, H. Y., Cheng, L., & Sit, J. W. H. (2019). The effectiveness of electronic health interventions on blood pressure control, self-care behavioural outcomes and psychosocial well-being in patients with hypertension: A systematic review and meta-analysis. International Journal of Nursing Studies, 92, 27–46. https://doi.org/10.1016/j.ijnurstu.2018.11.007

Mahmood, S., Jalal, Z., Hadi, M. A., Khan, T. M., Haque, M. S., & Shah, K. U. (2021). Prevalence of nonadherence to antihypertensive medication in Asia: a systematic review and meta-analysis. International Journal of Clinical Pharmacy, 43(3), 486–501. https://doi.org/10.1007/s11096-021-01236-z

Manigault, K. R., McKinley, D., Patel, S., Truong, C., Nguyen, S., Akil, A., Newsom, L., Murnane, K. S., & Thurston, M. M. (2020). The impact of a <scp>pharmacist-designed</scp> mobile application on blood pressure control and medication adherence in patients with hypertension. JACCP: Journal of The American College of Clinical Pharmacy, 3(7), 1286–1295. https://doi.org/10.1002/jac5.1296

Márquez Contreras, E., Márquez Rivero, S., Rodríguez García, E., López-García-Ramos, L., Carlos Pastoriza Vilas, J., Baldonedo Suárez, A., Gracia Diez, C., Gil Guillén, V., & Martell Claros, N. (2019). Specific hypertension smartphone application to improve medication adherence in hypertension: a cluster-randomised trial. Current Medical Research and Opinion, 35(1), 167–173. https://doi.org/10.1080/03007995.2018.1549026

McGuinness, L. A., & Higgins, J. P. T. (2021). Risk-of-bias VISualisation (robvis): An R package and Shiny web app for visualising risk-of-bias assessments. Research Synthesis Methods, 12(1), 55–61. https://doi.org/https://doi.org/10.1002/jrsm.1411

Mills, K. T., Bundy, J. D., Kelly, T. N., Reed, J., Kearney, P., Reynolds, K., Chen, J., & He, J. (2017). Global disparities of hypertension prevalence and Control: A systematic analysis of population-based studies from 90 countries. Physiology & Behavior, 176(3), 139–148. https://doi.org/10.1161/CIRCULATIONAHA.115.018912.Global

Michalakeas, C., Katsi, V., Soulaidopoulos, S., Dilaveris, P., Vrachatis, D., Lekakis, I., Vlachopoulos, C., Tsioufis, K., & Tousoulis, D. (2020). Mobile phones and applications in the management of patients with arterial hypertension. American journal of cardiovascular disease, 10(4), 419–431.

Morawski, K., Ghazinouri, R., Krumme, A., Lauffenburger, J. C. C., Lu, Z., Durfee, E., Oley, L., Lee, J., Mohta, N., Haff, N., Juusola, J. L. L., & Choudhry, N. K. K. (2018). Association of a smartphone application with medication adherence

and blood pressure control: The MedISAFE-BP randomisedclinical trial. JAMA Internal Medicine, 178(6), 802–809. https://doi.org/10.1001/jamainternmed.2018.0447

Moore, T., J. O. Neher and S. J. A. F. P. Safranek (2011). "Improving medication adherence in patients with comorbidities." 84(7): online-online.

Morrissey, E. C., Casey, M., Glynn, L. G., Walsh, J. C., & Molloy, G. J. (2018). Smartphone apps for improving medication adherence in hypertension: patients' perspectives. Patient Preference and Adherence, Volume 12, 813–822. https://doi.org/10.2147/PPA.S145647

Nurakysh, S., Kurakbayev, K., Kosherbaeva, L., Tazhiyeva, A., Aimakhanova, A., Kulkaeva, G., Asykbaeva, L., Ainabekov, M., Fakhradiyev, I., & Tanabayeva, S. (2022). Evaluation of the Effectiveness of the Mobile Application on Adherence of Patients with Arterial Hypertension. Acta Informatica Medica, 30(1), 18. https://doi.org/10.5455/aim.2022.30.18-24

Pandey, A., Raza, F., Velasco, A., Brinker, S., Ayers, C., Das, S. R., Morisky, D. E., Halm, E. A., & Vongpatanasin, W. (2015). Comparison of Morisky Medication Adherence Scale with therapeutic drug monitoring in apparent treatment–resistant hypertension. Journal of the American Society of Hypertension, 9(6), 420-426.e2. https://doi.org/10.1016/J.JASH.2015.04.004

Patel, S., Jacobus-Kantor, L., Marshall, L., Ritchie, C., Kaplinski, M., Khurana, P. S., & Katz, R. J. (2013). Mobilising your medications: An automated medication reminder application for mobile phones and hypertension medication adherence in a high-risk urban population. Journal of Diabetes Science and Technology, 7(3), 630–639. https://doi.org/10.1177/193229681300700307

Persell, S. D., Peprah, Y. A., Lipiszko, D., Lee, J. Y., Li, J. J., Ciolino, J. D., Karmali, K. N., & Sato, H. (2020). Effect of Home Blood Pressure Monitoring via a Smartphone Hypertension Coaching Application or Tracking Application on Adults with Uncontrolled Hypertension: A Randomised Clinical Trial. JAMA Network Open, 3(3), e200255. https://doi.org/10.1001/jamanetworkopen.2020.0255

Rupert, J. and A. J. A. F. P. Mounsey (2022). "Can Mobile Phone–Based Interventions Improve Adherence to Medication for Primary Prevention of Cardiovascular Disease in Adults?" 105(3): 236-237.

Volpi, S. S., Biduski, D., Bellei, E. A., Tefili, D., McCleary, L., Alves, A. L. S., & De Marchi, A. C. B. (2021). Using a mobile health app to improve patients' adherence to hypertension treatment: a non-randomised clinical trial. PeerJ, 9, e11491. https://doi.org/10.7717/peerj.11491

Xing, F., Guo, Y., Xia, N., Zhang, S., Yin, J., Qin, L., Zhu, C., Gao, Q., Jia, J., Zhao, Y., Qi, Y., & Li, W. (2023). Mobile APP-assisted family physician program for improving blood pressure outcome in hypertensive patients. BMC Primary Care, 24(1). https://doi.org/10.1186/s12875-023-01965-2

Zha, P., Qureshi, R., Porter, S., Chao, Y.-Y., Pacquiao, D., Chase, S., & O'Brien-Richardson, P. (2020). Utilizing a Mobile Health Intervention to Manage Hypertension in an Underserved Community. Western Journal of Nursing Research, 42(3), 201–209. https://doi.org/10.1177/0193945919847937

Appendix A

Table A1: Mixed Methods Appraisal Tool (MMAT), version 2018

Category of study designs	Methodological quality criteria	Responses		
		Yes	*No*	*Can't tell*
Quantitative randomised controlled trials	Is randomisation appropriately performed?			
	Are the groups comparable at baseline?			
	Are there complete outcome data?			
	Are outcome assessors blinded to the intervention provided?			
	Did the participants adhere to the assigned intervention?			
Quantitative non-randomised	Are the participants representative of the target population?			
	Are measurements appropriate regarding both the outcome and intervention?			
	Are there complete outcome data?			
	Are the confounders accounted for in the design and analysis?			
	During the study period, is the intervention administered as intended?			

Journal of Pharmacy

Journal of Pharmacy

The Effects of *Nigella Sativa* (Black Seed) in Rhinosinusitis Subjects: A Systematic Review

Muhamad Najmi Muhamad Najemudin[1], Muhamad Zafran Anas Mahmood[1], Syahrir Zaini[2] and Nurul Asyiqin Yusof[3*]

[1]*Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[2]*Department of Pharmacy Practice, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*
[3]*Department of Basic Medical Sciences, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

Abstract

Introduction: Allergic rhinitis is a persistent inflammatory nasal condition triggered by an exaggerated immune response to allergens. Its primary complication is sinusitis which progresses to rhinosinusitis. *Nigella Sativa* known for its anti-inflammatory effects, has shown promising efficacy in treating rhinosinusitis. While various studies have reported *Nigella Sativa's* effects on rhinosinusitis, there is paucity in specifically addressing the optimal therapeutic dose and efficacy compared to conventional anti-histamine or anti-allergic drugs. Thus, this study aims to systematically review the effects of *Nigella Sativa* on rhinosinusitis in both human and animal subjects. **Method**: This systematic review followed the guidelines of PRISMA. A systematic literature search was performed through searches in PubMed, Scopus, Cochrane Library, and Google Scholar, along with the application of the snowball technique. Two authors independently evaluated the identified articles at various stages, including title, abstract, and full text, against predefined eligibility criteria. The assessment of potential bias in the studies incorporated the Joanna Briggs Institute (JBI) checklist for critical appraisal of human studies and the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool for assessing bias in animal studies. **Results**: Twelve studies were included in this study. Six studies were found to exhibit a low risk of bias, whereas three studies were categorized with a moderate risk of bias, and an additional three studies were identified as having a high risk of bias. Seven studies recorded significant symptom reduction while other studies showed better histological changes and chemical parameters compared with conventional medication. **Conclusion**: *Nigella Sativa* demonstrates anti-inflammatory, anti-histaminic, and antimicrobial properties, aiding in alleviating symptoms of allergic rhinitis and rhinosinusitis. 10 to 100 mg/kg/day *Nigella Sativa* is proposed to be considered as the optimum dose range as an alternative to conventional drugs such as montelukast, mometasone furoate, and dexamethasone due to its minimal side effects.

Article history:

Received: 18 February 2024
Accepted: 1 October 2024
Published: 31 January 2025

Keywords:

allergic rhinitis
rhinosinusitis
Nigella Sativa
thymoquinone
anti-inflammatory

doi: 10.31436/jop.v5i1.288

*Corresponding author's email: drnurul@iium.edu.my

Introduction

Allergic rhinitis (AR) is a prevalent, persistent inflammatory condition affecting the nasal passages, triggered by an exaggerated immune system response to allergens. According to the International Consensus Statement on Allergy and Rhinology (ICAR) 2023, it is primarily associated with a type 1 hypersensitivity reaction mediated by immunoglobulin E (IgE) (Sarah et al., 2023). It happens when allergens, like dust mites or pollen, bind to the nasal mucosa, triggering an IgE response that swiftly releases histamine. This process leads to allergic rhinitis symptoms such as sneezing, nasal congestion, and rhinorrhoea (Mims, 2014). There are two classifications of AR: (1) seasonal AR, linked to outdoor allergens and (2) perennial AR, associated with year-round indoor allergens. Additionally, allergic rhinitis has two sub-categories: (i) Intermittent AR, marked by symptoms less than four days per week or lasting less than four consecutive weeks and (ii) Persistent AR, characterized by symptoms occurring more than four days per week for at least one month (ICAR, 2023). The most common complication in AR is sinusitis, which is defined as inflammation of the paranasal sinuses caused by infection (Li et al., 2021). There are four classifications of sinusitis: (1) acute sinusitis, associated with the duration of symptoms between 10 days until less than 4 weeks, (2) sub-acute sinusitis, linked with duration of symptoms between 4 and 12 weeks (3) chronic sinusitis, similar to asthma with duration more than 12 weeks (Mahboubi, 2018) and (4) recurrent sinusitis, related to four or more episodes of sinusitis (Sharma et al., 2023). These two conditions often overlap which leads to rhinosinusitis (Helman et al., 2020). The existence of two or more primary symptoms such as blockage, drainage (anterior or posterior) and facial pain or pressure lasting for a minimum of 12 weeks, are validated through sinus endoscopy or computed tomography (CT) scan (Vlaminck et al., 2021).

AR affects 5 to 52% of the global population in 2020 (Oliveira et al., 2020). These figures may be greater in places with high allergen levels such as thick vegetation and increased exposure to environmental triggers. The prevalence of AR in the United States in 2020 was approximately 10% to 30% of adults and up to 40% of children (Dykewicz et al., 2020). It has become more common in several industrialised nations during the last few decades. The main causes of this rise are unknown, however, they might be ascribed to variables such as changes in environmental exposure, lifestyle, and urbanisation. In Singapore, the general prevalence of allergic rhinitis is estimated to range from 5.5% to 13%. Given the equatorial climate characterized by year-round warmth and humidity, persistent allergic rhinitis is the prevailing disease pattern (Liu et al., 2020). On the other hand, AR prevalence varied from 8.14 to 9.23% in the pre-pandemic period and from 1.83 to 6.40% in the post-pandemic phase in Malaysia (Chew et al., 2023). Malaysia's tropical setting and diverse plantations create a variety of allergens like pollen and mould spores, triggering allergic rhinitis symptoms in susceptible individuals. The increasing prevalence of this condition may also be influenced by lifestyle shifts and urbanisation (Chew et al., 2023). In Europe, passive smokers face a higher risk of allergic rhinitis compared to primary smokers, potentially worsening symptoms by heightening sensitivity to airborne allergens (Wu et al., 2021). On the other hand, rhinosinusitis affects 5% to 12% of the global population (Mullol et al., 2022) and 5% to 22% in the United States (Helman et al., 2020). In CPG Management of Rhinosinusitis in Adolescents and Adults (2016), it affects 8% in China and 2.7% in Singapore.

Nigella Sativa (NS) known as black seed or black cumin, belongs to the Ranunculaceae family and is native to regions in the eastern Mediterranean, northern Africa, the Indian subcontinent, and Southwest Asia (Hannan et al., 2021). It is a herbaceous annual plant that produces seeds that

are called black cumin or black seed, although, in old Latin, it was known as "*Panacea*" which means "cure-all", meanwhile in Arabic generally known as "*Habbat-uL-Sauda*", or "*Habbat el Baraka*" which means 'Seeds of blessings' (Nyemb et al., 2022). The botanical characteristics of NS consist of flowers that exhibit grace, primarily in shades of white, yellow, pink, light blue, or lavender colour boasting 5-10 petals each. Moreover, the fruits manifest as sizable, inflated capsules housing abundant black coats and white-content seeds possessing both aromatic and bitter flavours (Nyemb et al., 2022). The long-standing historical use of *Nigella Sativa* oil (NSO) and seeds in Indian and Arabic cultures spans various culinary and therapeutic applications. Traditionally, it has been employed to address conditions like asthma and hypertension, leveraging its potential health benefits including anti-inflammatory and immune-modulating properties, which could be beneficial in managing symptoms of allergic rhinitis (Mahboubi, 2018). The main bioactive compound in this plant is Thymoquinone (TQ) which exhibits anti-inflammatory effects and was found to be effective in suppressing histamine release from mast cells (Ikhsan et al., 2018). Meanwhile, it also demonstrated robust antimicrobial effects against both Gram-negative and Gram-positive bacteria, displaying minimum inhibitory concentration (MIC) values ranging from 8 to 512 μg/ml (Mahboubi, 2018). This evidence suggests that the anti-inflammatory and antimicrobial properties may help alleviate nasal inflammation and symptoms associated with allergic rhinitis and rhinosinusitis.

Currently, many strategies are being practised for managing allergic rhinitis patients, pharmacologically and non-pharmacologically. According to the Korean Academy of Asthma, Allergy and Clinical Immunology (KAAACI), the recommendation of AR pharmacotherapy: (1) intranasal corticosteroid (INCS)/intranasal antihistamine (INAH) combination therapy, (2) oral antihistamine/INCS combination therapy, (3) leukotriene receptor antagonist (Yang et al., 2023). Besides, non-pharmacological interventions such as avoidance of triggers or allergens and environmental control.

To date, many studies have been conducted using herbs in treating allergic rhinitis and rhinosinusitis. The most recent systematic review was done regarding herbal medicine in allergic rhinitis in the year 2021 however, the review includes all available herbs that have the potential to treat allergic rhinitis such as *Zingiber Officinale, Psidium Guajava* and *Curcuma Longa* (Koshak, 2021). Despite many studies being done on the effects of NS on rhinosinusitis, there is paucity in the systematic analysis between these studies to draw a firm conclusion on the effects of NS towards rhinosinusitis in terms of optimum therapeutic dose and efficacy compared to anti-histamine or anti-allergic drugs. Moreover, many NS studies showed non-toxic properties and low side effects profile, especially on mast cells (Ikhsan et al., 2018). Hence, the current study was conducted to collect, systematically analyse and conclude the relevant studies on NS effects on rhinosinusitis in humans and animals.

Methods

Protocol

The current systematic review adhered to the statement and overall principles outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021).

Eligibility criteria

This systematic review examined the effects of NS on treating the rhinosinusitis of human and animal subjects in terms of optimum therapeutic dose, efficacy and safety profile compared to anti-histamine or anti-allergic drugs.

Human model

The study subjects were aged between 18 to 80 years and diagnosed with allergic rhinitis or sinusitis. According to the World Allergy Organization (WAO) 2023, the diagnosis of allergic rhinitis or sinusitis can be determined by the history which is the symptoms and the physical examination such as

skin prick as a confirmatory test. The patients with the symptoms of allergic rhinitis such as sneezing, nasal congestion, rhinorrhoea and positive skin-prick test will specify allergen triggers will be included in this review (Small et al., 2018). The study design included randomized controlled trials (RCT), cohort studies, case-control, case series and quasi-experimental studies are also included in the systematic review. The comparator of the studies was pharmacological interventions such as leukotriene receptor antagonist (LTRA), and montelukast. Full-text original articles published from 2010 until 2023 in the English language were included. However, this systematic review excluded studies that involved pregnant or suspected pregnant women, smokers and alcohol drinkers.

Animal model

On the other hand, the animal inclusion involved in this review includes mammal models that consist of mice, rats and rabbits regardless of gender, age, species or strain. The comparator of the studies was pharmacological interventions such as corticosteroids: mometasone fuorate. Meanwhile, the exclusion criteria included of non-mammal models such as zebrafish, and mammal models that were diagnosed with multiple diseases such as allergic rhinitis with diabetes mellitus rats or diseases besides allergic rhinitis or sinusitis.

Outcome

The outcome in this review was allergic rhinitis symptoms reduction or resolvents such as sneezing, nasal congestion and rhinorrhoea including the safety profile of NS. Moreover, the histological changes or the changes in chemical parameters such as IgE levels, histamine levels and interleukin levels will be included in this systematic review.

Search strategy

Studies or articles were searched from four databases which include Scopus, Cochrane Library, PubMed, and Google Scholar. The search strategy included a combination of domain and sub-domain that were combined with Boolean operators "OR" and "AND". Keywords within the same domain were connected using the Boolean operator "OR" while "AND" operator was used to connect keywords between other domains. The search terms included four main domains from the title of this study in alignment with the inclusion and exclusion criteria which were: (1) effects: list of all keywords that are synonyms to effect such as benefits and advantages; (2) NS and its associated terms; (3) allergic rhinitis: a term describing patients who have been diagnosed with AR or sinusitis or rhinosinusitis; and (4) subjects: human or animal models. The list of keywords that were used for each domain are listed in Table 1. Besides, the snowball technique which is a technique to search for articles from the reference list of articles retrieved from the databases, was also used to add more articles for selection.

Table 1: The keywords used in search of articles from databases

Keywords	Effects	*Nigella Sativa*	Rhinosinusitis	Subjects	
Synonym 1	Effect	*N. Sativa*	AR	Animals	Adults
Synonym 2	Benefit	NS	Allergic patients	Rats	Human
Synonym 3	Benefits	Black seed	Hay fever	Experimental trial	People
Synonym 4	Positive effect	Thymoquinone	Sinusitis	Preclinical trial	Individuals
Synonym 5	Positive effects	Black cumin	Allergic rhinitis	Rabbits	Clinical trial
Synonym 6	Positive impact	Habbatus Sauda	-	Mice	-
Synonym 7	Positive impacts	Habbat-uL-Sauda	-	Rodent	-
Synonym 8	Advantage	-	-	-	-
Synonym 9	Advantages	-	-	-	-

Study records

Data management

All identified studies from the search strategy were retrieved from the databases and kept in separate folders according to each database in the library of the Mendeley reference manager. There was a folder that placed all the identified articles for duplicate checking. Duplicate studies were removed from the folder. Subsequently, the remaining studies were exported as a BibTeX file and converted to an Excel file using a BibTeX format converter for the selection process.

Study selection

Two authors (MNM & MZA) independently examined the studies against predetermined eligibility criteria in the stages of title, abstract, and full-text study selection. None of the

screening authors were blinded to any details of the studies. Two independent authors independently reviewed study titles and abstracts until convergence was reached. In instances of discrepancies, the two authors engaged in discussion, and if consensus was not reached, the disagreements were further deliberated with the involvement of the third author (NAY). Criteria were adjusted as needed during this collaborative process. Articles meeting the inclusion criteria underwent a thorough full-text assessment. If necessary, the authors were contacted to address missing or insufficient information for determining study eligibility. Reasons for article exclusion were documented throughout the study selection process. Microsoft Excel was employed for study selection, and a consolidated list of articles for data extraction was created and stored in Mendeley and Microsoft Excel.

Data collection/extraction

Qualified studies underwent independent review, and pertinent data were extracted. The gathered information encompassed authors, language, publication year, study region, objectives, study design, duration, total participants recruited, intervention, and outcomes: symptom reduction and safety profiles. Data from each study were entered into a table in Microsoft Excel to ease the comparison process between the included studies. Numerical data were also extracted and expressed as mean ± standard deviation, or mean (SEM), and p-values, and the differences were considered statistically significant when $p < 0.05$.

Quality appraisal and the risk of bias assessment

Two authors (MNM & MZA) independently assessed the risk of bias for all included human studies by assessing their methodological quality using the Joanna Briggs Institute (JBI) critical appraisal checklists (Joanna Briggs Institute, 2017). A score of '1' was given if the studies fulfilled the stated criteria of the checklist and '0' if not. After that, the total score was calculated and converted into a percentage. Studies with a percentage of <50% were considered as having a high risk of bias, while moderate risk, and high risk of bias if the percentage were between 50% - 69% and ≥70%, respectively (Franco et al., 2020). The risk of bias results for each study type was visually represented using traffic-light plots through the utilization of Risk-of-bias VISualization (robvis) (McGuinness & Higgins, 2021).

Meanwhile, the quality assessment of all selected animal intervention studies was conducted using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE's) risk of bias tool. It comprised of 10 domains encompassing six types of biases: sequence generation, baseline characteristics, allocation concealment, random housing, researcher blinding, random outcome assessment, outcome assessor blinding, incomplete outcome data, selective outcome reporting, and "other sources of bias".

The risk of bias assessments for both human and animal studies was performed by two independent reviewers (MNM & MZA). When there is a disagreement, the reviewers debate it and reach a resolution based on mutual agreement or appoint a third reviewer (NAY) to resolve that particular disagreement.

Data synthesis

A narrative synthesis was used in this systematic review to describe the qualitative data from each included study. The narrative synthesis provided a written summary of the study's characteristics, study design, population, models of prediabetes that were used, interventions, and comparison studies. The framework method was employed for all parts to examine patterns and organize data according to the subthemes (Bauer, 2013). This method generated a new structure for the data, which made it easier to summarise the data in a manner that can support the research question in this study (Bauer, 2013). Results by subtheme were presented in narrative form to describe the findings of the synthesis.

Results

Study selection

Initially, a total of 360 studies were identified within the databases (PubMed, Scopus, Cochrane Library and Google Scholar). PubMed located 31 studies, Scopus located 124 studies, and Cochrane Library located 173 studies which provided a total of 328 studies. An additional 32 studies were discovered through Google Scholar.

From 360 articles, 41 studies were excluded due to being duplicates, leaving 319 studies to be screened. After manually screening the title and abstract, 298 studies were excluded as (i) they did not have the target outcome which is resolving or reducing allergic rhinitis symptoms (n = 221), (ii) recruited the wrong study population such as children or participants under the of 18 years old (n = 60), and (iii) review articles (n = 17). The remaining study was 21.

Furthermore, 10 out of the remaining 21 studies were excluded as no full-text was available. Eventually, 11 studies were assessed for eligibility and one study was excluded due to recruiting all types of allergic subjects such as skin allergies. Finally, only 10 studies were selected.

In addition, the snowball technique was also employed, resulting in an additional 13 studies. This involved searching for related studies within the references of the previously identified articles in the databases. Three studies were removed as there was no access to the full-text articles, leaving 10 studies. From this, five studies were excluded due to wrong outcomes such as in determining the efficacy and safety of olopatadine/mometasone nasal spray for the treatment of seasonal allergic rhinitis and three studies had the wrong study population which was rhino-conjunctivitis patients, leaving only two studies to be included via the snowball technique.

Consequently, this search approach yielded a total of 12 studies meeting the inclusion criteria and deemed eligible for inclusion in this systematic review, with ten sourced from databases and two from the snowball technique. Figure 1 displays a PRISMA flow diagram illustrating the results from all databases and the process of article selection.

Risk of bias within studies

Risk of bias of the 12 studies are divided into seven human studies (Alsamarai et al., 2014; Ansari et al., 2010; Isik et al., 2010; Nemati et al., 2021; Nikakhlagh et al., 2011; Oysu et al., 2014; Rezaeian and Amoushahi Khouzani, 2018) and five animal studies (Gul et al., 2022; Gunel, 2017; Liao et al., 2021; Yoruk et al., 2017; Yurttas et al., 2016). The seven human studies' risk of bias was assessed using the JBI checklist for Randomized Controlled Trials (RCT). On the other hand, SYRCLE risk of bias tools were adopted for the animal studies.

Based on Figures 2 and 3, six out of 12 human and animal studies (Alsamarai et al., 2014; Ansari et al., 2010; Isik et al., 2010; Nemati et al., 2021; Oysu et al., 2014; Rezaein and Amoushahi Khouzani, 2018) demonstrated low risk of bias (showing high percentage of positive answers to the questions of JBI tool). This is because randomized controlled clinical trials (RCTs) serve as the gold standard for establishing the effectiveness and safety of a treatment. They can illustrate a new treatment's superiority over an established standard treatment or a placebo (Kabisch et al., 2011). Three articles comprised of one human study (Nikakhlagh et al., 2011) and two animal studies (Gunel, 2017; Liao et al., 2021) had a moderate risk of bias. Finally, the three remaining articles (Gul et al., 2022; Yoruk et al., 2017; Yurttas et al., 2016) show a high risk of bias comprising animal studies.

Based on Figure 2 of human studies, there was a high risk of bias for domains four, five and seven (Ansari et al., 2010; Isik et al., 2010; Nikakhlagh et al., 2011; Oysu et al., 2014), due to those delivering treatment and the outcomes assessors were not blinded to the treatment assignment. Initial text deleted – as commented by the reviewer.

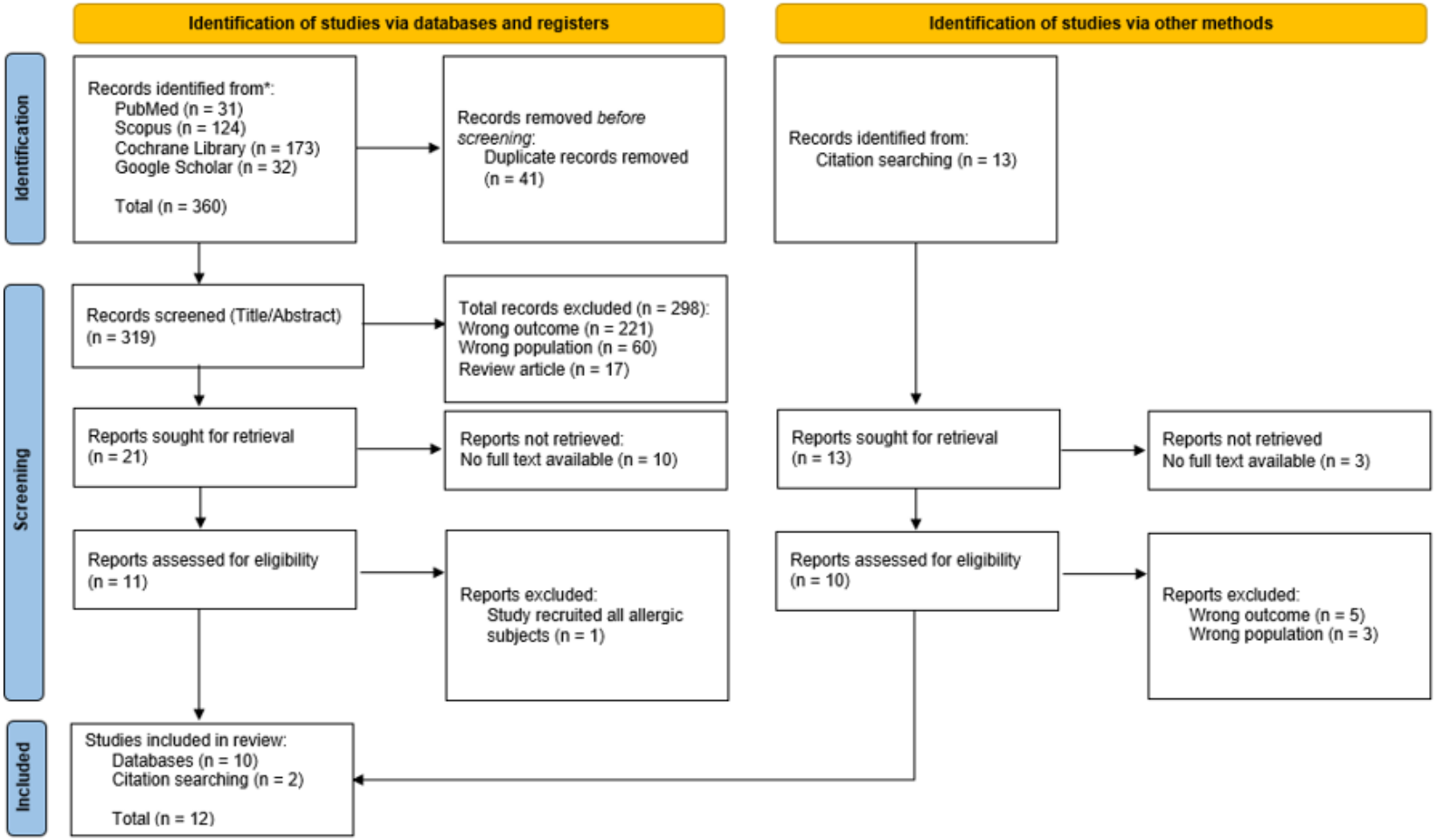


Fig. 1: PRISMA flowchart of the study selection process.

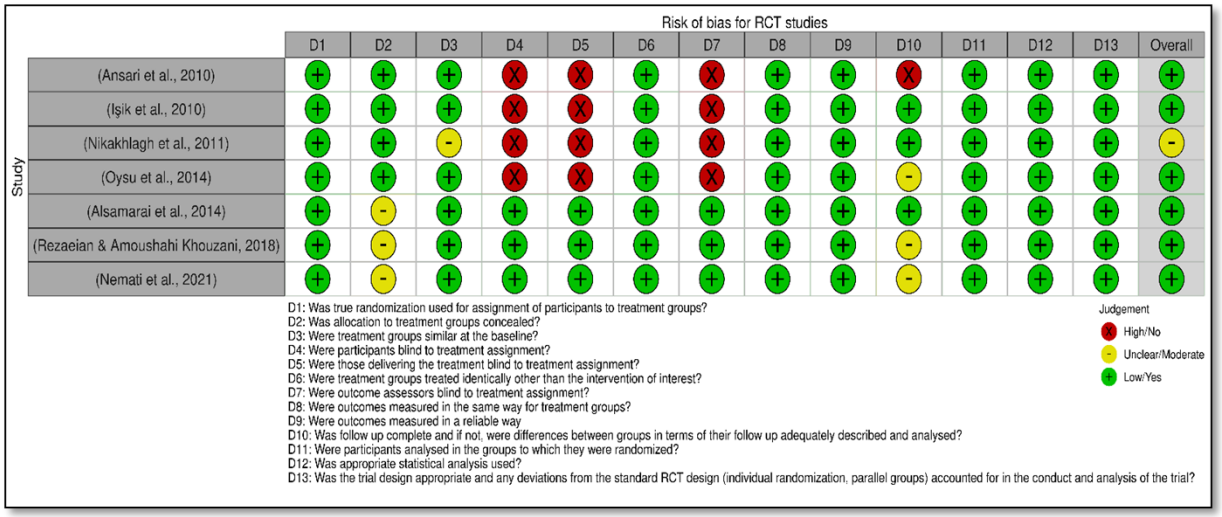


Fig. 2: Risk of bias for RCT studies.

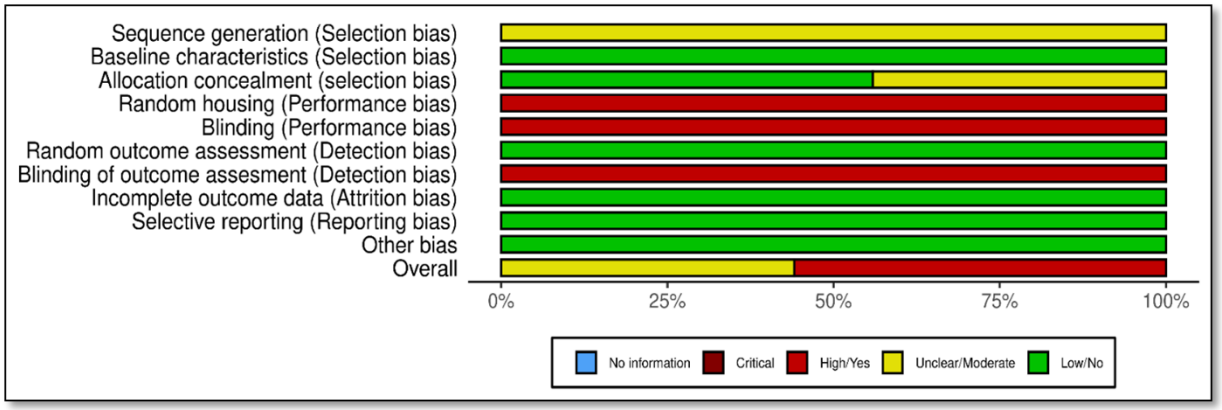


Fig. 3: PRISMA flowchart of the study selection process.

Table 2: Summary of the main characteristics of the seven human studies.

Author, year	Study design	Study region	Participants & Study Duration	Method/ Parameter	Results
(Ansari et al., 2010)	RCT	Nawabshah, Pakistan	**Participants**: 47 seasonal allergic rhinitis patients **Duration**: 2 weeks	**Route of administration**: Oral Comparison of Montelukast with NS (250mg/day) for the treatment of seasonal allergic rhinitis symptoms	• Montelukast and NS reduced the **day-time** and **ophthalmic symptoms** ($P < 0.001$) • NS group reduced in **nocturnal symptoms** at day-7 ($P = 0.001$)
(Işik et al., 2010)	RCT	Istanbul, Turkey	**Participants**: 31 allergic rhinitis patients **Duration**: 2 months	**Route of administration**: Oral Impact of NS seed (2g/day) on symptom levels, peripheral blood polymorphonuclear leukocyte (PMN) functions, and lymphocyte subsets in individuals with allergic rhinitis	• **Phagocytic and intracellular killing activities** of specific immunotherapy (SIT) with NS showed significant increases after one month compared to pre-SIT levels ($P < 0.01$). • **CD8 counts** of patients receiving SIT plus NS significantly increased compared to patients receiving only SIT
(Nikakhlagh et al., 2011)	RCT	Ahwaz, Iran	**Participants**: 66 allergic rhinitis patients **Duration**: 1 month	**Route of administration**: Oral Anti-inflammatory effects of NS oil (0.5mls) in individuals experiencing allergic rhinitis symptoms	**Itching**: • Statistically significant ($P = 0.0014$) between Day 0 & 15 • Statistically significant ($P = 0.001$) between Day 0 & 30 **Nasal congestion**: • Statistically significant ($P = 0.0012$) between Day 0 & 15 • Statistically significant ($P = 0.001$) between Day 0 & 30 **Sneezing**: • Statistically significant ($P = 0.001$) between Day 0 & 15 • Statistically significant ($P = 0.001$) between Day 0 & 30

					Runny nose: • Statistically significant (P = 0.0019) between Day 0 & 15 • Statistically significant (P = 0.001) between Day 0 & 30 **Turbinate hypertrophy and mucosal pallor**: • Statistically significant (P = 0.0012) between Day 0 & 15 • Statistically significant (P = 0.001) between Day 0 & 30 **Mean IgE**: • NS group: 3.31 • Placebo: 15.38 **Mean peripheral blood eosinophil percentage**: • NS group: 0.086 • Placebo: 0.350
(Oysu et al., 2014)	RCT	Istanbul, Turkey	**Participants**: 42 geriatric patients with nasal dryness and associated symptoms **Duration**: 1 month	**Route of administration**: Nasal Impact of topical NS oil on symptoms associated with aging, including nasal dryness, stuffiness, itching, crusting, and burning.	**Mucociliary clearance**: • No significant difference (P > 0.05) **Sinonasal symptoms**: • No significant difference (P>0.01) **Adverse effects**: • 2 patients complained regarding taste and odour of NS oil
(Alsamarai et al., 2014)	RCT	Tikrit, Iraq	**Participants**: 188 patients presenting with mild, moderate and severe allergic rhinitis **Duration**: 6 weeks	**Route of administration**: Nasal Efficacy of NS oil topical application as a treatment patients with allergic rhinitis	**Mild active group**: • Improve at 3rd weeks as 80% (P=0.01) • Improve 100% at 6 weeks (P=0.01) **Moderate active group**: • Improve at the 3rd week (P=0.008), 68.7% • 93.7% at 6 weeks (P=0.002) **Severe active group**: • Improve at 3rd weeks as 58.3% • 83.4% at 6 weeks (P=0.009)

(Rezaeian & Amoushahi Khouzani, 2018)	RCT	Isfahan, Iran	**Participants**: 65 patients diagnosed with rhinosinusitis, clinical symptoms, endoscopic evaluation and CT scan **Duration**: 3 months	**Route of administration**: Nasal Comparison of NS nasal spray and sodium chloride nasal spray in the management of patients with Chronic Rhinosinusitis Without a Nasal Polyp (CRSsNP)	• 8 weeks of interventions, the Lund–McKay, Modified Lund Kennedy, and SNOT-22 scores in the intervention group were significantly lower compared to the placebo group (P < 0.0001)
(Nemati et al., 2021)	RCT	Tehran, Iran	Participants: 50 chronic rhinosinusitis patients Duration: 28 days	**Route of administration**: Nasal Impact of Nigella seed oil on chronic rhinosinusitis (CRS) symptoms	**Mean SNOT-22 score**: • NS group: 19.08 ± 13.21 • Placebo group: 37.15 ± 21.47 (P = 0.001) **Major symptoms**: • Pain or pressure in the face (P = 0.011) • Congestion or fullness in the face (P = 0.028) • Congestion or nasal obstruction (P = 0.025) • Pus or nasal discharge (P = 0.032), between days 0 and 28 in the drug and placebo groups **Endoscopic nasal examination**: **NS group**: • Mild inflammation/muco-purulent discharge in 9 cases (37.5%) • Grade 1 polyposis in 6 patients (25%) **Placebo group**: • Inflammation/muco-purulent discharge in 14 subjects (53.8%) • Grade 1 nasal polyposis in 12 cases (46.1%) **No adverse effects**

Table 3: Summary of the main characteristics of the five animal studies.

Author, year	Study design	Study region	Type, total subjects & study duration	Method/ Parameter	Results
(Gul et al., 2022)	Case control	Istanbul, Turkey	**Animal type**: Rat **Total subjects**: 28 Wistar Hannover rats weighing 250-350 g, two to four months old **Duration**: 28 days	**Route of administration**: Nasal Comparison of mometasone furoate and NS oil in the prevention of disease symptoms in a rat AR model	Number of **sneezing**: • Day 14 (P = 0.000) than Day 1 • Day 17 (P = 0.012) than Day 14 • Day 20 (P = 0.001) than Day 14 • Day 23 (P = 0.001) than Day 14 • Day 26 (P = 0.001) than Day 14 • Day 28 (P = 0.001) than Day 14 **Nose scratching** frequency: • Day 14 (P = 0.001) than Day 1 • Day 17 (P = 0.001) than Day 1 • Day 20 (P = 0.001) than Day 1 • Day 23 (P = 0.002) than Day 1 • Day 26 (P = 0.002) than Day 1 • Day 28 (P = 0.001) than Day 1 Histology Evaluation**:** **Mild inflammation**: • 14.3% of the control group, AR group and NS oil group • 28.6% in mometasone furoate group **Loss cilia**: • NS group: 71.4% • Control % mometasone furoate group: No loss **Increase Goblet cells**: • Control group: 71.4%

					• Mometasone furoate group: 42.9% • NS group: 14.3% and did not have vascular proliferation
(Günel, 2017)	Case control	Aydin, Turkey	**Animal type**: Rat **Total subjects**: 42 of 12-15 month-old female Wistar rats **Duration**: 2 weeks	**Route of administration**: IV Effect of TQ on airway inflammation in a rat model of AR	**OVA-specific IgE levels**: • TQ10+AR group: (P = 0.038) • CS+AR groups: (P = 0.048) **IL-4 levels**: • TQ at dose of 3 mg/kg and 10 mg/kg: (both, P = 0.013) **IFN-g**: • Not differ significantly among the AR, control and treatment groups, its production tended to decrease in treatment groups **IL-10**: • 3 mg/kg TQ group (P = 0.033) • 10 mg/kg TQ group (P = 0.002) **Eosinophil count**: • TQ3+AR group: (P = 0.002) • TQ10+AR group: (P = 0.013) **Edema**: • TQ at doses of 3 mg/kg and 10 mg/kg: (both, P < 0.001) **TNF-a**: • CS+AR group: (P = 0.05) • TQ3+AR group: (P = 0.0015) • TQ10+AR groups: (P = 0.004) **IL-1b**: • CS+AR group: (P < 0.001) • TQ10+AR groups (P = 0.006)

					• TQ at dose of 3 mg/kg had no effect (P = 0.072)
(Yurttaş et al., 2016)	Case control	Bolu, Turkey	**Animal type**: Rabbit **Total subjects**: 24 male New Zealand rabbits (2,000–3,000 g each; 20–24 weeks **Duration**: 7 months	**Route of administration**: Nasal Histopathological effects of TQ treatment of allergic rhinitis rabbit model compared to nasal mometasone furoate	**Nasal mucosa**: • Control group: Normal • Ovalbumin (OVA) sensitization group: Elevated counts of inflammatory cells. Intraepithelial goblet cell hypertrophy and hyperplasia. Vascular congestion and ciliary loss • TQ group: The counts of intraepithelial and submucosal inflammatory cells were markedly lower than in the OVA group (P < 0.001). Reduction in the number and intensity of hypertrophic goblet cells • Mometasone furoate group: histological structure was slight irregularities, there were significant improvements compared to the OVA group. • Inflammation status of the mometasone furoate and TQ groups, the counts of intraepithelial and submucosal inflammatory cells were similar (P = 0.608) **TUNEL assay**: • Not differ between the mometasone furoate and TQ groups • TUNEL-positive cell counts similar

(Liao et al., 2021)	Case control	Shanghai, China	**Animal type**: Mice **Total subjects**: 72 specific pathogen-free five-week-old BALB/c male mice **Duration**: 28 days	**Route of administration**: Oral Anti-allergenic effects of the aqueous extract of BLAB (in a specific proportion) on the ovalbumin (OVA)-induced allergic rhinitis (AR) model.	**Nasal symptoms**: • Aqueous extract of BLAB group: Reduced the nasal symptoms compared to the OVA group • Dex group: Significant inhibition of nasal symptoms **Inflammatory cells**: • Decreased in nasal lavage fluid (NALF) in the BLAB and BLAB 200 and BLAB 400 group **Thickness of nasal mucosa**: • Aqueous extract of BLAB and Dex: Reduced the infiltration of inflammatory cells into the nasal mucosa **Levels of histamine, total IgE and OVA-specific immunoglobulins in serum**: • Administration of the aqueous extract of BLAB decreased the levels of histamine, total IgE, OVA-specific IgE, and OVA-specific IgG1 in serum, especially at a dose of 400 mg/kg
(Yoruk et al., 2017)	Case control	Erzurum, Turkey	**Animal type**: Rabbit **Total subjects**: 30 adult male albino rabbits weighing an average of 3 kg **Duration**: 1 week	**Route of administration**: Oral Effects of three different doses of oral administration of NS in a rabbit model of rhinosinusitis, compared with cephalexin treatment	**Histopathological lesions:** **Numerical density of neutrophils**: • Control group: 0.000351/µm3 • Cephalexin group: 0.000020/µm3 • NS 50 mg/kg/d group: 0.000072/µm3 • NS 100 mg/kg/d group: 0.000058/µm3

					• NS 200 mg/kg/d: 0.000068/µm3 **Nitric oxide (NO) level**: • Control group: Increased in the saline treated group in comparison to the cephalexin 20 mg/kg/d, NS 50 mg/kg/d, NS 100 mg/kg/d and NS200 mg/kg/d groups. **Mean tissue total NO levels**: • Significantly ($P < 0.001$) higher in the control group and correlated well with disease acuteness • Cephalexin group: 163.31 µmol/ ml • NS 50 mg/kg/d: 166.40 µmol/ml • NS 100 mg/kg/d: 165.20 µmol/ml • NS 200 mg/kg/d: 166.43 µmol/ml • Control group: 184.17 µmol/ml

On the other hand, three out of five animal studies had a high risk of bias meanwhile, the other two showed moderate risk of bias. All animal studies included in this review show a high risk of performance bias and detection bias (blinding outcome assessment), the individuals administering treatment and evaluating study outcomes were aware of the assigned treatments. For random housing, rats (Gul et al., 2022; Gunel, 2017), rabbits (Yurttas et al., 2016) and mice (Liao et al., 2021) were kept in room temperature conditions for 12 hours in light/dark cycle conditions.

Study characteristics

All studies were published between the years 2010 and 2022 and comprised seven studies involving the human population that consisted of RCT (n = 7) and five studies involving the animal population that consisting of rats (n = 2), mice (n = 1) and rabbits (n = 2).

For human studies, Table 2 shows three studies conducted in Iran (Nemati et al., 2021; Nikakhlagh et al., 2011; Razaeian and Amoushahi Khouzani, 2018), two in Turkey (Isik et al., 2010; Oysu et al., 2014), one in Pakistan (Ansari et al., 2010) and one in Iraq (Alsamarai et al., 2014). Five studies recruited patients diagnosed with allergic rhinitis (Alsamarai et al., 2014; Ansari et al., 2010; Isik et al., 2010; Nikakhlagh et al., 2011; Oysu et al., 2014) meanwhile two studies recruited patients with chronic rhinosinusitis regardless of genders (Nemati et al., 2021; Razaeian and Amoushahi Khouzani, 2018). The study conducted by Ansari et al. (2010) mainly focuses on the comparison of the Leukotriene receptor antagonist, Montelukast with NS in resolving or reduction of allergic rhinitis symptoms. On the other hand, four studies mainly focus on the efficacy of NS in reducing symptoms without comparing it to other medications or interventions (Alsamarai et al., 2014; Işik et al., 2010; Nikakhlagh et al., 2011; Oysu et al., 2014). Furthermore, the study conducted by Alsamarai et al. (2014) divided the participants into stages of allergic rhinitis symptoms such as mild, moderate and severe groups. Hence, the optimum efficacy of NS can be identified in which stage of allergic rhinitis.

Moreover, two studies focus on chronic rhinosinusitis (CR) that represent their data with Sinonasal Outcome Test 22 (SNOT-22) (Nemati et al., 2021; Rezaeian & Amoushahi Khouzani, 2018). The SNOT-22 serves as a validated self-administered survey designed for evaluating individuals with chronic rhinosinusitis (CRS). Comprising 22 items, respondents rate each item on a scale from 0 ('no problem at all') to 5 ('worst possible symptom'). Total scores on the SNOT-22 can vary from 0 to 110, with elevated scores reflecting more severe symptoms.

Subsequently, the results of animal studies are presented in Table 3. There were four studies conducted in Turkey (Gul et al., 2022; Gunel, 2017; Yoruk et al., 2017; Yurttas et al., 2016) and one in China (Liao et al., 2021). Out of these five animal studies, three mainly focus on the comparison of corticosteroids with NS. Two of the studies used topical corticosteroid, which was mometasone furoate (Gut et al., 2022; Yurttas et al., 2016) and one study used IV dexamethasone as an intervention (Günel, 2017). Finally, the remaining two studies utilized different doses of NS preparation without comparing them with any medication (Gunel, 2017; Yoruk et al., 2017). Hence, these two studies proposed the suggested dose which would eventually lead to the optimum doses in treating rhinosinusitis symptoms.

Individual outcomes of the studies

The targeted outcomes for this review are allergic rhinitis symptoms reduction or resolvent which include sneezing, nasal congestion and rhinorrhoea. In addition, the safety profile of NS was included if stated in the study. Further, the histological changes or the changes in chemical parameters such as IgE levels, histamine levels and interleukin levels were also included especially in animal studies.

Symptom reduction

Based on Tables 2 and 3, seven out of 12 studies emphasized the symptom reduction of allergic rhinitis (Alsamarai et al., 2014; Ansari et al., 2010; Gul et al., 2022; Liao et al., 2021; Nikakhlagh et al., 2011; Oysu et al., 2014) and chronic rhinosinusitis (CRS) (Nemati et al., 2021).

Six studies show positive effects in terms of symptom reduction such as sneezing (Gul et al., 2022; Liao et al., 2021; Nikakhlagh et al., 2011), day-time symptoms (Ansari et al., 2010) and major symptoms of CRS such as pressure in the face or nasal obstruction (Nemati et al., 2021). Moreover, NS could completely resolve mild allergic rhinitis symptoms ($P = 0.01$) and reduce as much as 83.4% after 6 weeks of treatment ($P = 0.009$) (Alsamarai et al., 2014). However, a study by Oysu et al. (2014) revealed that although the symptoms were reduced at the individual level, but when compared between

the NS group and the ISCS (isotonic sodium chloride solution) group, there was no significant difference in the nasal mucociliary clearance ($P > 0.05$) and sinonasal symptoms ($P > 0.01$).

Histological changes

Based on Table 3, mild inflammation was detected in 14.3% of rats within the control group, the allergic rhinitis (AR) group, and the NS oil group. In contrast, the mometasone furoate group exhibited a higher incidence of mild inflammation, with 28.6% of rats showing such levels (Gul et al., 2022). In addition, the hypertrophic goblet cells exhibited a notable reduction in intensity and quantity in the TQ group (Yurttaş et al., 2016). Plus, the numerical density of neutrophils in NS groups also exhibits better results than the control group as the dose of 100 mg/kg/d NS shows 0.000058/μm3 and 200 mg/kg/d NS shows 0.000068/μm3 (Yoruk et al., 2017).

Chemical parameter

The CS+AR, TQ3+AR, and TQ10+AR groups showed a significant decrease in TNF-α expression ($p=0.05$, $p=0.015$, $p=0.004$, respectively). Conversely, OVA (ovalbumin-induced AR) sensitization led to a notable increase in both TNF-α and IL-1β expression levels (both, $p<0.001$) (Günel, 2017). Furthermore, administering the aqueous extract of BLAB (black cumin seeds, liquorice, anise seeds, black tea) orally resulted in a significant dose-dependent decrease in IL-4, IL-5, and IL-13 levels in both nasal mucosa and serum compared to the OVA group. Conversely, there was a noteworthy and dose-independent rise in IFN-γ, IL-12, and IL-10 levels in both nasal mucosa and serum of mice in the BLAB group, as opposed to the OVA group (Liao et al., 2021).

Comparison with anti-allergic medication

The statistical analysis indicated that the impact of both montelukast and NS in reducing daytime symptoms was highly significant ($P < 0.001$) when compared with the baseline pre-treatment phase of the study (Ansari et al., 2010). However, upon comparing the inflammation status between the mometasone furoate, and the TQ group which is the main active ingredient of NS, it was observed that the intraepithelial and submucosal inflammatory cell numbers were similar ($P = 0.608$) (Yurttaş et al., 2016).

Discussion

This study was conducted to systematically review the effects of NS (black seed) on rhinosinusitis in both humans and animals. To comprehend the effects of NS in rhinosinusitis, understanding the pathophysiology of allergic rhinitis and rhinosinusitis is crucial. Many researchers have performed experimental studies on NS, recognizing it as a potent herb for treating various ailments. In recent decades, there has been significant exploration of NS's pharmacological actions, particularly for rhinosinusitis treatment.

NS demonstrates positive effects on reducing symptoms of allergic rhinitis and rhinosinusitis, with itching and sneezing serving as a benchmark for efficacy in allergic rhinitis. In one clinical study, the frequency of sneezing significantly decreased between Day 0 and Day 30 ($P = 0.001$) for the treatment group consuming oral 0.5ml NS oil in 66 allergic rhinitis patients within a one-month duration (Nikakhlagh et al., 2011). An earlier study by Ansari et al. (2010) in line with these findings, noted a significant reduction in daytime symptoms, including sneezing, between Day 0 and Day 14 ($P < 0.001$) in 47 seasonal allergic rhinitis patients. However, the categorisation of symptoms between these two studies is different, where Nikakhlagh et al. (2011), categorised symptom severity into stages (mild, moderate, severe), while Ansari et al. (2010), provided a general assessment of symptom severity.

However, in geriatric patients, there was no significant difference in the reduction of sinonasal symptoms such as itching ($P = 0.083$). This could be due to the process of ageing, which leads to the gradual replacement of lymphatic tissue in the nasal mucosa with connective tissue, causing fibrosis and interfering with mucosal secretory function (Oysu et al., 2014). Furthermore, this phenomenon may be attributed to the impact of osmolarity and tonicity which influence ciliary beat frequency. Research has demonstrated that isotonic and hypotonic solutions do not induce ciliary slowing (Oysu et al., 2014). Hence, a hypertonic solution of NS might be employed for administration in the elderly. Moreover, mucociliary transport rates tend to decline with ageing (Bailey, 2022). As a result, the optimised effectiveness of NS may be compromised. Furthermore, this study did not specifically elucidate the effects of NS on allergic rhinitis in geriatric patients. While NS demonstrates promising potential in reducing symptoms through

its anti-inflammatory effects, it is a noteworthy candidate for the treatment of allergic rhinitis and rhinosinusitis and, thus needs further exploration in geriatric populations.

Recent studies on rhinosinusitis conducted in Iran (Nemati et al., 2021; Rezaeian & Amoushahi Khouzani, 2018) revealed promising results on the NS within one-month to 3 months durations. In 50 adult chronic rhinosinusitis patients, there is a notable clinical improvement in nasal obstruction ($P = 0.025$), nasal discharge ($P = 0.032$) and pain or pressure on the face ($P = 0.011$) between days 0 and 28 in the NS nasal drop compared to placebo groups (Nemati et al., 2021). This suggests the anti-inflammatory and analgesic properties of NS exhibiting its effects. In addition, the mean SNOT-22 score of the NS group was 19.08 ± 13.21 significantly lower ($P = 0.001$) compared to the placebo group (37.15 ± 21.47). In another study, 65 adult chronic rhinosinusitis patients showed a very significant reduction in Lund-McKay, Modified Lund Kennedy and SNOT-22 scores in the NS nasal spray group compared to the placebo group ($P < 0.0001$) (Rezaeian & Amoushahi Khouzani, 2018). Although both were using SNOT-22 scores, the latter study revealed a more significant outcome, probably due to the study population that excluded rhinosinusitis patients with nasal polyps compared to Nemati et al. (2021) subjects with nasal polyposis stage 1. This could be due to alterations in the anatomical structure of the nasal passages that may impede the optimal efficacy of intra-nasal NS in treating chronic rhinosinusitis.

In animal studies, the symptoms of allergic rhinitis in NS-treated rats exhibit a significant reduction of sneezing ($P = 0.001$) up to Day 28 compared to the allergic rhinitis (AR) group (Gul et al., 2022). Moreover, Liao et al. (2021) emphasized a significant reduction of nasal symptoms such as sneezing and rubbing compared to the ovalbumin (OVA)-induced AR model of the mice group. However, Gul et al. (2022) demonstrated more significant outcomes with nasal administration compared to Liao et al. (2021) which utilised oral administration of NS ($P < 0.01$). Nasal absorption is faster than oral administration since the turbinates of the nasal have extensive surface area and thin membranes, expediting swift absorption into the bloodstream upon contact with a drug (Ehrick et al., 2013).

Histologically, a substantial decrease in eosinophil infiltration, cilia loss, chondrocyte hypertrophy, vascular proliferation, and an elevation in goblet cells was observed in both the mometasone furoate and NS groups compared to the AR group (Gul et al., 2022). There was a statistically significant decrease in neutrophil numbers in both treatment groups when compared to the saline-treated group in 30 adult male rabbits (Yoruk et al., 2017). This study conducted a comparison between NS and cephalexin, with the latter aiming to eliminate infection and mitigate inflammation. It is also worth noting that the positive outcomes of both NS-treated and medication-treated (mometasone furoate and cephalexin) groups are comparable. These studies exhibit the efficacy of NS in terms of its anti-inflammatory, antioxidant and anti-microbial properties.

Subsequently, when analysing the effects of NS on chemical parameters, 10 mg/kg of TQ administered to allergic rhinitis rats had a greater impact on eosinophil count ($P = 0.013$), oedema ($P < 0.001$), and IL-4 ($P = 0.013$) level compared to corticosteroids and 3 mg/kg TQ (Günel, 2017). Moreover, in allergic rhinitis rabbits, 100 mg/kg/day of NS shows a slightly better reduction of nitric oxide (NO) level compared to 50 mg/kg/d and 200 mg/kg/d NS, although all posed significant results compared with the control (Yoruk et al., 2017). Therefore, it is suggested the optimal dosage range for allergic rhinitis and rhinosinusitis treatment, is proposed to be between 10 - 100 mg/kg/day in animal studies. Subsequently, further clinical trials are essential for translational study, as the existing studies have a limited number of RCTs to date, with different study designs and parameters evaluated, with the oral administration of NS ranging from 0.5mls to 250mg to 2g per day. In comparison, there has been a notable p-value significance in many recent animal studies, weighing more on animal research than human studies.

Finally, the extracts from NS seeds and their bioactive components are generally recognised as substances with low toxicity, demonstrating a broad margin of safety (Mashayekhi-Sardoo et al., 2020). Numerous clinical trials exploring NS and TQ have reported the safety of these agents (Mekhemar et al., 2020). There was no significant difference observed in the activities of liver markers (ALT, AST, ALP) and kidney function markers (serum creatinine, serum urea) in healthy adults consuming NS oil formulation containing 5.2% TQ in 200mg per day for 90 days (Thomas et al., 2022). However, it is noteworthy that some adverse effects, including bloating,

nausea, and a burning sensation, were observed in functionally dyspeptic patients treated with NS oil (Tavakkoli et al., 2017). Therefore, it is proposed that NS exhibits a favourable safety profile compared to other herbal plants such as kratom, which can lead to withdrawal and dependence by affecting the central nervous system (CNS) (Prevete et al., 2022).

Conclusion

NS has demonstrated benefits in subjects with rhinosinusitis, including both humans and animals. With the comprehensive data searching and collection, the study has successfully achieved its objective by demonstrating that NS exhibits anti-inflammatory, anti-histaminic, and antimicrobial effects, contributing to the resolution or reduction of symptoms in allergic rhinitis and rhinosinusitis. Furthermore, NS could serve as an alternative treatment to conventional medications like montelukast, mometasone furoate, and dexamethasone since it exhibits a low side effects profile. The recommended optimal dose for the treatment of allergic rhinitis is suggested to be within the range of 10 to 100 mg/kg/day of NS. However, it is important to note that this suggestion is currently limited to animal studies. For human studies, the authors have not concluded the optimum dosage of NS due to different study settings in design and parameters in the clinical trials available. Further investigations are necessary for larger human studies in a standardised method to determine the precise optimum therapeutic dose for the treatment of rhinosinusitis. Finally, the efficacy of NS is proposed to be tested in the rhinosinusitis Malaysian population as part of the SEA area, as there is paucity in the studies conducted in this specific demographic location.

Authors contributions

Conceptualization, N.A. and S.Z.; introduction, M.N. and N.A.; methodology, M.N. and S.Z.; results, M.N., M.Z., N.A. and S.Z.; discussion, M.N. and N.A.; conclusion, M.N. and N.A. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no financial interests or commercial associations.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly and ChatGPT to improve readability and language.

References (APA 7th style)

Abd El Aziz, A. E., El Sayed, N. S., & Mahran, L. G. (2011). Anti-asthmatic and anti-allergic effects of thymoquinone on airway-induced hypersensitivity in experimental animals. Journal of Applied Pharmaceutical Science, 1(8), 109–117.

Alqahtani, J. M. (2020). Atopy and allergic diseases among Saudi young adults: A cross-sectional study. Journal of International Medical Research, 48(1). https://doi.org/10.1177/0300060519899760

Alsamarai, A. M., Abdulsatar, M., & Ahmed Alobaidi, A. H. (2014). Evaluation of topical black seed oil in the treatment of allergic rhinitis. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry, 13(1), 75–82. https://doi.org/10.2174/18715230113129990014

Ansari, M. A., Ansari, N. A., & Junejo, S. A. (2010). Montelukast versus nigella sativa for management of seasonal allergic rhinitis: A single blind comparative clinical trial. Pakistan Journal of Medical Sciences, 26(2), 249–254.

Ayşenur Koç, Nazan Güven, Sıdıka Büyükhelvacıgil Öztürk, Burçak Deniz Dedeoğlu, Hale Feyza Büyükhelvacigil, Ramazan Büyükhelvacıgil, & Beril Koparal. (2019). Therapeutic Trials of Nigella sativa. Journal of Pharmacy and Pharmacology, 7(11), 564–577. https://doi.org/10.17265/2328-2150/2019.11.002

Bailey, K. L. (2022). Aging Diminishes Mucociliary Clearance of the Lung. Advances in Geriatric Medicine and Research, 4(2), 1–11.

https://doi.org/10.20900/agmr20220005

Bauer, J. (2013). Význam průkazu RAS mutací pro anti-EGFR protilátky v léčbě 1. linie metastazujícího kolorektálního karcinomu. Onkologie (Switzerland), 7(5), 260–261.

Boskabady, M. H., Keyhanmanesh, R., Khamneh, S., & Ebrahimi, M. A. (2011). The effect of Nigella sativa extract on tracheal responsiveness and lung inflammation in valbuminsensitized guinea pigs. Clinics, 66(5), 879–887. https://doi.org/10.1590/S1807-59322011000500027

Cagatay Oysu, Ahmet Tosun, Huseyin Baki Yilmaz, Asli Sahin-Yilmaz, Deniz Korkmaz, A. K. (2014). Topical Nigella sativa for nasal symptoms in elderly. Auris Nasus Larynx, 41(3), 269–272. https://doi.org/10.1016/j.anl.2013.12.002

Chew, C. C., Lim, X. J., Narayanan, M. S., Letchumanan, P., Rajan, P., & Chong, C. P. (2023). The Trend of the Burden of Allergic Rhinitis Pre- and Post-COVID-19 Pandemic in the Urban Population of Perak, Malaysia. Indian Journal of Otolaryngology and Head and Neck Surgery. https://doi.org/10.1007/s12070-023-03896-x

Dykewicz, M. S., Wallace, D. V., Amrol, D. J., Baroody, F. M., Bernstein, J. A., Craig, T. J., Dinakar, C., Ellis, A. K., Finegold, I., Golden, D. B. K., Greenhawt, M. J., Hagan, J. B., Horner, C. C., Khan, D. A., Lang, D. M., Larenas-Linnemann, D. E. S., Lieberman, J. A., Meltzer, E. O., Oppenheimer, J. J., … Wang, J. (2020). Rhinitis 2020: A practice parameter update. Journal of Allergy and Clinical Immunology, 146(4), 721–767. https://doi.org/10.1016/j.jaci.2020.07.007

Ehrick, J. D., Shah, S. A., Shaw, C., Kulkarni, V. S., Coowanitwong, I., De, S., & Suman, J. D. (2013). Considerations for the Development of Nasal Dosage Forms. https://doi.org/10.1007/978-1-4614-7978-9_5

Elango, A., Rao, L. N., Sugumar, P., & Radhakrishnan, A. (2022). Evaluation of Clinical Efficacy and Safety of Nigella Sativa Seed Oil added to Standard Treatment in Uncomplicated Respiratory Infection - A Randomised, Open Labelled, and Parallel Arm Study. Journal of Communicable Diseases, 2022(91), 91–97. https://doi.org/10.24321/0019.5138.202214

Franco, A., Vidigal, M. T. C., Oliveira, M. N. de, Nascimento, C. T. de J. S., Silva, R. F. da, & Paranhos, L. R. (2020). Evidence-based mapping of third molar techniques for age estimation applied to Brazilian adolescents – a systematic review. Research, Society and Development, 9(10), e9339109395. https://doi.org/10.33448/rsd-v9i10.9395

Gul, R., Tansuker, H. D., Cengiz, A. B., Gul, M., Tabaru, A., Emre, F., & Oktay, M. F. (2022). Effects of nigella sativa oil on allergic rhinitis: an experimental animal study. Brazilian Journal of Otorhinolaryngology, 88, S148–S155. https://doi.org/10.1016/j.bjorl.2022.09.003

Günel, C. (2017). The anti-inflammatory effects of thymoquinone in a rat model of allergic rhinitis. The Turkish Journal of Ear Nose and Throat, 226–232. https://doi.org/10.5606/kbbihtisas.2017.58997

Hannan, M. A., Rahman, M. A., Sohag, A. A. M., Uddin, M. J., Dash, R., Sikder, M. H., Rahman, M. S., Timalsina, B., Munni, Y. A., Sarker, P. P., Alam, M., Mohibbullah, M., Haque, M. N., Jahan, I., Hossain, M. T., Afrin, T., Rahman, M. M., Tahjib-Ul-arif, M., Mitra, S., … Kim, B. (2021). Black cumin (Nigella sativa l.): A comprehensive review on phytochemistry, health benefits, molecular pharmacology, and safety. Nutrients, 13(6). https://doi.org/10.3390/nu13061784

Helman, S. N., Barrow, E., Edwards, T., DelGaudio, J. M., Levy, J. M., & Wise, S. K. (2020). The Role of Allergic Rhinitis in Chronic Rhinosinusitis. Immunology and Allergy Clinics of North America, 40(2), 201–214. https://doi.org/10.1016/j.iac.2019.12.010

Ikhsan, M., Hiedayati, N., Maeyama, K., & Nurwidya, F. (2018). Nigella sativa as an anti-inflammatory agent in asthma. *BMC research notes*, *11*, 1-5.

Işik, H., Çevikbaş, A., Gürer, Ü. S., Kiran, B., Üresin, Y., Rayaman, P., Rayaman, E., Gürbüz, B., & Büyüköztürk, S. (2010). Potential adjuvant effects of nigella sativa seeds to improve specific immunotherapy in allergic rhinitis patients. Medical Principles and Practice, 19(3), 206–211. https://doi.org/10.1159/000285289

Kabisch, M., Ruckes, C., Seibert-Grafe, M., & Blettner, M. (2011). Randomisierte kontrollierte studien: Teil 17 der serie zur bewertung wissenschaftlicher publikationen. Deutsches Arzteblatt, 108(39), 663–668. https://doi.org/10.3238/arztebl.2011.0663

Koshak, A. E. (2019). Prevalence of herbal medicines in patients with chronic allergic disorders in western Saudi Arabia. Saudi Medical Journal, 40(4), 391–396. https://doi.org/10.15537/smj.2019.4.24006

Koshak, A. E. (2021). Attitudes and Beliefs towards Herbal Medicines in Patients with Allergic Diseases: A pilot survey study in Western Saudi Arabia. Journal of Herbal Medicine, 25(November 2020), 100413. https://doi.org/10.1016/j.hermed.2020.100413

Li, S., Zhao, C. J., Hua, H. L., Deng, Y. Q., & Tao, Z. Z. (2021). The association between allergy and sinusitis: a cross-sectional study based on NHANES 2005–2006. Allergy, Asthma and Clinical Immunology, 17(1), 1–9. https://doi.org/10.1186/s13223-021-00642-2

Liao, C., Han, Y., Chen, Z., & Baigude, H. (2021). The extract of black cumin, licorice, anise, and black tea alleviates OVA-induced allergic rhinitis in mouse via balancing activity of helper T cells in lung. Allergy, Asthma and Clinical Immunology, 17(1), 1–14. https://doi.org/10.1186/s13223-021-00587-6

Liu, X., Wang, D. Y., Charn, T. C., Koh, L. T., Teo, N. W., Ong, Y. K., Thong, M. K., Bachert, C., Pfaar, O., Schünemann, H. J., Bedbrook, A., Czarlewski, W., & Bousquet, J. (2020). Next-Generation Allergic Rhinitis Care in Singapore: 2019 ARIA Care Pathways. Annals of the Academy of Medicine, Singapore, 49(11), 885–896. https://doi.org/10.47102/annals-acadmedsg.202076

Mahboubi, M. (2018). Natural therapeutic approach of Nigella sativa (Black seed) fixed oil in management of Sinusitis. Integrative Medicine Research, 7(1), 27–32. https://doi.org/10.1016/j.imr.2018.01.005

Mashayekhi-Sardoo, H., Rezaee, R., & Karimi, G. (2020). Nigella sativa (black seed) safety: An overview. Asian Biomedicine, 14(4), 127–137. https://doi.org/10.1515/abm-2020-0020

McGuinness, L. A., & Higgins, J. P. T. (2021). Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. Research Synthesis Methods, 12(1), 55–61. https://doi.org/10.1002/jrsm.1411

Mekhemar, M., Hassan, Y., & Dörfer, C. (2020). Nigella sativa and thymoquinone: A natural blessing for periodontal therapy. Antioxidants, 9(12), 1–19. https://doi.org/10.3390/antiox9121260

Mims, J. W. (2014). Epidemiology of allergic rhinitis. International Forum of Allergy and Rhinology, 4(SUPPL.2), 18–20. https://doi.org/10.1002/alr.21385

Mullol, J., Azar, A., Buchheit, K. M., Hopkins, C., & Bernstein, J. A. (2022). Chronic Rhinosinusitis With Nasal Polyps: Quality of Life in the Biologics Era. Journal of Allergy and Clinical Immunology: In Practice, 10(6), 1434-1453.e9. https://doi.org/10.1016/j.jaip.2022.03.002

Nemati, S., Masroorchehr, M., Elahi, H., Kamalinejad, M., Ebrahimi, S. M., & Akbari, M. (2021). Effects of Nigella sativa Extract on Chronic Rhinosinusitis: A Randomized Double Blind Study. Indian Journal of Otolaryngology and Head and Neck Surgery, 73(4), 455–460. https://doi.org/10.1007/s12070-020-02296-9

Nikakhlagh, S., Rahim, F., Aryani, F. H. N., Syahpoush, A., Brougerdnya, M. G., & Saki, N. (2011). Herbal treatment of allergic rhinitis: The use of Nigella sativa. American Journal of Otolaryngology - Head and Neck Medicine and Surgery, 32(5), 402–407. https://doi.org/10.1016/j.amjoto.2010.07.019

Nyemb, J. N., Shaheen, H., Wasef, L., Nyamota, R., Segueni, N., & Batiha, G. E. S. (2022). Black cumin: a review of its pharmacological effects and its main active constituent. *Pharmacognosy Reviews*, *16*(32), 107-125.

Oliveira, T. B., Persigo, A. L. K., Ferrazza, C. C., Ferreira, E. N. N., & Veiga, A. B. G. (2020). Prevalence of asthma, allergic rhinitis and pollinosis in a city of Brazil: A monitoring study. Allergologia et Immunopathologia, 48(6), 537–544. https://doi.org/10.1016/j.aller.2020.03.010

Park, D. Y., Lee, Y. J., Kim, D. K., Kim, S. W., Yang, H. J., Kim, D. H., Jun, Y. J., Park, S. C., Kim, B. S., Yang, S. I., Lee, I. H., Kim, M., Ryu, G., Kang, S. Y., Kim, M. A., Lee, S. M., Kim, H. J., Choi, G. S., Chung, S. J., … Choi, J. H. (2023). KAAACI Allergic Rhinitis Guidelines: Part 2. Update in Non-pharmacological Management. Allergy, Asthma and Immunology Research, 15(2), 145–159. https://doi.org/10.4168/aair.2023.15.2.145

Prevete, E., Kuypers, K. P. C., Theunissen, E. L., Corazza, O., Bersani, G., & Ramaekers, J. G. (2022). A systematic review of (pre)clinical studies on the therapeutic potential and safety profile of kratom in humans. Human Psychopharmacology, 37(1). https://doi.org/10.1002/hup.2805

Rezaeian, A., & Amoushahi Khouzani, S. (2018). Effect of Nigella sativa Nasal Spray on the Treatment of Chronic Rhinosinusitis Without a Nasal Polyp . Allergy & Rhinology, 9, 215265671880005. https://doi.org/10.1177/2152656718800059

Sarah, K., Lauren, T., Joshua, M., Ahmad, R., Tim, F., Mahoney, E., John, M., & Anne, K. (2023). International Consensus Statement on Allergy and Rhinology_ Allergic Rhinitis _ Enhanced Reader.

Sharma, G. K., Lofgren, D. H., & Taliaferro, H. G. (2023). Recurrent Acute Rhinosinusitis.

Sm, J., Kb, C., Ry, P., & Bushra, S. (2013). Assessment of Nigella Sativa Induced Adverse Drug Reactions. 6(2), 3–6.

Small, P., Keith, P. K., & Kim, H. (2018). Allergic rhinitis. Allergy, Asthma and Clinical Immunology, 14(s2), 1–11. https://doi.org/10.1186/s13223-018-0280-7

Tavakkoli, A., Mahdian, V., Razavi, B. M., & Hosseinzadeh, H. (2017). Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone. Journal of Pharmacopuncture, 20(3), 179–193. https://doi.org/10.3831/kpi.2017.20.021

Thomas, J. V, Mohan, M. E., Prabhakaran, P., Das S, S., Maliakel, B., & Krishnakumar, I. M. (2022). A phase I clinical trial to evaluate the safety of thymoquinone-rich black cumin oil (BlaQmax®) on healthy subjects: Randomized, double-blinded, placebo-controlled prospective study. Toxicology Reports, 9, 999–1007. https://doi.org/10.1016/j.toxrep.2022.04.020

Vlaminck, S., Acke, F., Scadding, G. K., Lambrecht, B. N., & Gevaert, P. (2021). Pathophysiological and Clinical Aspects of Chronic Rhinosinusitis: Current Concepts. Frontiers in Allergy, 2(October), 1–15. https://doi.org/10.3389/falgy.2021.741788

Wu, A. C., Dahlin, A., & Wang, A. L. (2021). The role of environmental risk factors on the development of childhood allergic rhinitis. Children, 8(8). https://doi.org/10.3390/children8080708

Yang, S. I., Lee, I. H., Kim, M., Ryu, G., Kang, S. Y., Kim, M. A., Lee, S. M., Kim, H. J., Park, D. Y., Lee, Y. J., Kim, D. K., Kim, S. W., Kim, D. H., Jun, Y. J., Park, S. C., Kim, B. S., Chung, S. J., Lee, H. J., Kim, H. Bin, … Yang, H. J. (2023). KAAACI Allergic Rhinitis Guidelines: Part 1. Update in

Pharmacotherapy. Allergy, Asthma and Immunology Research, 15(1), 19–31. https://doi.org/10.4168/aair.2023.15.1.19

Yarnell, E., & Abascal, K. (2011). Nigella sativa: Holy herb of the middle east. Alternative and Complementary Therapies, 17(2), 99–105. https://doi.org/10.1089/act.2011.17203

Yoruk, A., Tatar, A., Keles, O. N., & Cakir, A. (2017). Potenziale della Nigella sativa nel trattamento della rinosinusite indotta in setting sperimentale. Acta Otorhinolaryngologica Italica, 37(1), 32–37. https://doi.org/10.14639/0392-100X-1143

Yurttaş, V., Şereflican, M., Erkoçoğlu, M., Dagli, M., Terzi, E. H., Flrat, T., & Seyhan, S. (2016). Comparison of histopathological effects of thymoquinone and local nasal corticosteroids in allergic rhinitis in a rabbit model. Orl, 78(1), 55–60. https://doi.org/10.1159/000442353

Journal of Pharmacy

JOP
Journal of Pharmacy

Ethnopharmacology of *Psychotria*: Potential Use of *P. malayana* Jack Leaves as Antidiabetic Agent

Sharifah Nurul Akilah Binti Syed Mohamad[1], Alfi Khatib[1,2*], Siti Zaiton Mat So'ad[1], Qamar Uddin Ahmed[1], and Zalikha Ibrahim[1]

[1]*Pharmacognosy Research Group, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

[2] *Faculty of Pharmacy, Airlangga University, Surabaya, 60155, Indonesia*

Abstract

This review provides a comprehensive exploration of the *Psychotria* species, a genus of plants known for their medicinal properties and traditional uses. The focus is on the species' ethnomedicinal applications, their potential as an antidiabetic agent, the pharmacologically active antidiabetic compounds possessed, and their toxicological profiles. The escalating global prevalence of diabetes underscores the need for alternative therapeutic agents. The *Psychotria* species, with their antidiabetic properties, present a promising area of research. The traditional medicinal uses of the *Psychotria* species across various cultures are examined, providing valuable insights for the development of novel treatments. This review delves into the mechanisms through which these species exert their antidiabetic effects especially *Psychotria malayana*. The review discusses the pharmacologically active compounds unique to these species, which are of considerable interest for drug development in diabetes treatment. A summary of these studies and their implications is presented. Finally, the review addresses the toxicological studies on the *Psychotria* species, assessing their safety as therapeutic agents.

Article history:

Received: 29 July 2024
Accepted: 23 August 2024
Published: 31 January 2025

Keywords:

Psychotria
antidiabetic
ethnomedicinal
phytochemistry
toxicity

doi: 10.31436/jop.v5i1.341

*Corresponding author's email: alfikhatib@iium.edu.my

Introduction

The species of *Psychotria*, belonging to the *Rubiaceae* family, encompasses over 2000 species. These species are typically found in the understory of forests, preferring areas with limited sunlight and moist soil. *Psychotria* is mostly distributed in subtropical and tropical regions and comprises a variety of forms, including shrubs, herbs, and treelets (Moraes *et al*., 2011b). The species belonging to the subfamily *Rubioideae* and the tribe *Psychotriae*, has been documented as the largest species observed in three distinct tropical regions: Neotropical, Africa, Asia, and Oceania as shown in Fig. 1. (Bremer, 2009; Hamilton, 1989).

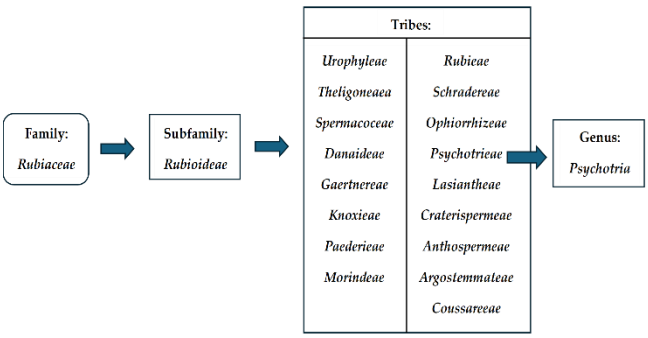


Fig. 1: Subfamily, tribes, and species/genus of *Psychotria* in the *Rubiaceae* family (Bremer, 2009)

The *Rubiaceae* family is characterized by some anatomical features, including hypostomatic leaves, paracytic stomata, dorsiventral mesophyll, and collateral bundles (Britannica, 2020). Furthermore, an additional characteristic commonly observed in the *Rubiaceae* family is the presence of domatia, which are primarily found along the secondary veins on the lower surface of the leaf and occasionally exist along the tertiary veins of the leaf blade. The diagnostic criteria for *Psychotria* include the identification of alkaloids and styloid crystals inside the mesophyll (Moraes *et al*., 2011a; Robbrecht, 1988). The taxonomic complexity of defining the delimited morphology of this species persists despite the absence of sufficient morphological data to accurately characterize its stated features (Nepokroeff *et al*., 1999).

Ethnomedicinal Uses of Psychotria

The *Psychotria* species has been extensively used as medicinal plants by our predecessors across diverse cultures worldwide (Table A2) in supplementary data. For example, the communities residing in the Uttara Kanada district of India have been used the root of *P. dalzellii* to address scorpion bites. This was achieved by grinding the root with lime juice and administering it as both a topical and oral medication (Bhat *et al*., 2012). Similarly, the infusion of the root of *P. flavida* has been employed for the treatment of snakebites (Bhat *et al*., 2012; Kshirsagar & Singh, 2001). The Kani tribes residing in Agasthiayamalai, located in South India, have traditionally incorporated the consumption of leaves and tender fruits from the *P. ophioxyloides*, along with milk, as a remedy for stomach discomfort (Britto & Mahesh, 2007). In the Chittagong Hill Tract region of Bangladesh, individuals have traditionally used the root of *P. adenophylla* for the treatment of mouth sores and rheumatism. Interestingly, the same therapeutic applications of the specific plant parts are also observed among the population of Assam in the Eastern Himalaya (Biswas *et al*., 2010; Choudhury *et al*., 2012). Furthermore, the inhabitants of Assam have traditionally employed the root of *P. denticulata* for the purpose of alleviating toothache, while the leaves of *P. montana* have been employed to mitigate discomfort and colitis (Choudhury *et al*., 2012).

In eastern Nicaragua, the indigenous population known as Rama traditionally employed many *Psychotria* species, including *P. elata* and *P. ipecacuanha*, for the treatment of diarrhea. Additionally, *P. poeppigiana* was used specifically for the management of amoebic dysentery (Coe, 2008). Furthermore, *P. ipecacuanha* has been used by indigenous populations in South America for the treatment of diarrhea and dysentery (Fisher, 1973). This plant also exhibits similar medicinal applications among the inhabitants of Costa Rica and Nicaragua (Fisher, 1973; Ocampo & Balick, 2009). In addition, it is worth noting that this plant, which possesses rhizomes and roots, is used in the form of syrup and powder as stimulants and diaphoretics, respectively (Ocampo & Balick, 2009).

Certain species of *Psychotria*, such as *P. nudiflora* and *P. nilgiriensis*, have been identified as having similar therapeutic properties for the treatment of rheumatism, as traditionally given by the Kanikkar tribes residing in the Agasthiarmalai region of India. In order to alleviate rheumatism, individuals ingested either the leaves and flowers of *P. nudiflora* or the tender fruits of *P. nilgiriensis* along with honey. Another method involved creating a paste using the leaves and flowers of *P. nudiflora* or the tender fruits of *P. nilgiriensis*, which was also consumed with honey (Rani *et al*., 2011; Sutha *et al*.,

2010; Devadoss *et al*., 2013; Iniyavan *et al*., 2012).

P. viridis, a member of the *Psychotria* species, has been historically employed in the preparation of ayahuasca, a psychoactive beverage originating from the Amazon rainforest. This plant species is recognised for its hallucinogenic property and its significance in religious rituals (Choffnes, 2017; Gambelunghe *et al*., 2008; Schultes & Hofmann, 1980). The beverage has been made through the process of extracting the substance of *P. viridis* leaves and incorporating them into the beverage (Gambelunghe *et al*., 2008).

The inhabitants of Andaman and Nicobar Islands, India, use *P. montana* as a remedy for constipation and *P. sarmentosa* for the treatment of itching and soreness. Similarly, herbalists from Rivercess County, Liberia, employ the leaves of *P. peduncularis* to alleviate stomach soreness (Kamble *et al*., 2008; Lebbie *et al*., 2017). Certain species of *Psychotria* have been used by the Temiar people in Lojing Highland, Kelantan, Malaysia as well as in Madagascar, for the synthesis of an oral antipyretic (fever-reducing) through decoction. These species include *P. malayana*, *P. obtusifalia*, and *P. bulata* (Rao *et al*., 2016; Rasoanaivo *et al*., 1992).

Furthermore, the *Psychotria* species has been historically recognized for its potential in treating injuries and promoting wound healing since ancient times. As an illustration, the Valaya tribes employ the paste derived from *P. octosulcata* leaves for the purpose of treating muscular fractures, while *P. henryiis* finds application in traditional Chinese medicine to stimulate the spleen and alleviate pain (Rajendran *et al*., 2002; Liu *et al*., 2013). *P. colorata* was reportedly used by Amazonian caboclos as a means of alleviating pain. Similarly, tribes residing in southern India employed a dried powder derived from the root of *P. flavida*, which was combined with coconut oil to treat wounds (Elisabetsky *et al*., 1995).

Psychotria as Antidiabetic Agent

Traditional remedies have been extensively used for several centuries and continue to hold significant relevance in the realm of healthcare, particularly in the regions of Asia and Africa (Lezotre, 2014). As per the findings of Pan *et al*. (2014), the World Health Organization reports that a significant proportion, over 75%, of the global population relies on plants as a primary means of obtaining therapeutic substances to fulfil their fundamental healthcare requirements. The use of the *Psychotria* genera in traditional medicinal practices is employed for the alleviation of symptoms and the treatment of specific ailments. To date, researchers from various regions have conducted studies on roughly 41 species of *Psychotria*, resulting in the successful isolation of over 160 phytochemicals (Yang *et al*., 2016). The chemical constituents encompass phenols, alkaloids, terpenoids, steroids, and phenolic compounds that demonstrate antibacterial, antiparasitic, and antiviral properties. Certain components of the *Psychotria* species, namely leaves, rhizomes, and roots, have been employed in traditional medicine for the purpose of alleviating symptoms associated with cough, bronchitis, and ulcers (Calixto *et al*., 2016). The utilization of this plant for the management of stomach pain, gastrointestinal issues, and female reproductive system infections has been reported among populations in Brazil, India, and Indonesia (Benchoula *et al*., 2019). Several studies have demonstrated the potential therapeutic benefits of this plant in the treatment of diabetes mellitus, as it displays antidiabetic properties. Additional investigation is warranted to enhance comprehension of the underlying bioactivity. The objective of this overview is to collate a comprehensive inventory of the phytochemical constituents present in the *Psychotria* species, specifically focusing on those with demonstrated antidiabetic activities.

Recent studies have provided evidence suggesting that different species of *Psychotria* possess characteristics that may be effective in managing diabetes. The antidiabetic effect of these plants can be attributed to the presence of numerous important compounds. Recent studies have generated considerable interest in the possible therapeutic applications of *Psychotria* and its phytochemical constituents for diabetes mellitus (DM), due to their notable antidiabetic properties. This review offers a complete examination of the *Psychotria* species, spanning multiple species and their corresponding phytochemicals that have exhibited antidiabetic activities. A thorough search was carried out across four web-based databases, leading to the discovery of a total of fifteen papers related to different species of *Psychotria*. Based on the data provided in Table A1 (Supplementary data), it can be observed that a cumulative number of 13 unique species of *Psychotria* have been effectively gathered through several research endeavors. The species included in this list are *P. malayana*, *P. leiocarpa*, *P. dalzellii*, *P. viridiflora*, *P.*

calocarpa, *P. carthagenensis*, *P. capillacea*, *P. camptopus*, *P. deflexa*, *P. ipecacuanha*, *P. microphylla*, *P. nilgiriensis*, and *P. insularum*. The phytochemical compounds that demonstrate antidiabetic properties exhibit variability across different species, while there are specific molecules that are commonly found among them.

The identification and evaluation of antidiabetic activities were conducted on the plants listed in Tables A1. Six out of the fifteen studies included in the analysis provide a comprehensive description of the mechanism of action for the antidiabetic effect. These processes were recognized as alpha-glucosidase and alpha-amylase inhibitions. Enzymes like alpha-glucosidase and alpha-amylase play a crucial role in the process of carbohydrate hydrolysis, which subsequently results in elevated levels of glucose after a meal. According to Poovitha and Parani (2016), research has demonstrated that the suppression of these two enzymes is an effective approach in the management of postprandial hyperglycaemia and the mitigation of the risk of diabetes. The predominant mode of action for the *Psychotria* species in the reduction of blood glucose levels, as suggested by most studies, is the inhibition of alpha-glucosidase. The process of absorption in the small intestine was hindered due to the occurrence of competitive inhibition of the alpha-glucosidase enzymes. Isomaltase, sucrase, glucoamylase, and maltase are enzymatic catalysts that are essential for the hydrolysis of complex non-absorbable carbohydrates, facilitating their transformation into readily absorbable simple carbs (Tannous *et al*., 2023). As a result, the inhibition of alpha-glucosidase activities will hinder the absorption of carbohydrates, hence limiting the postprandial rise in blood glucose levels (Bhatnagar & Mishra, 2022). Previous research conducted by Abhishek *et al*. (2019) and Chen *et al*. (2021) has revealed that *P. dalzellii* and *P. viridiflora* have the capacity to block alpha-amylase, hence potentially aiding in the modulation of blood glucose levels. The compounds inhibit the activity of alpha-amylase enzymes, which play a crucial role in the initial stage of starch hydrolysis, namely the conversion of starch into maltose. Following this, maltose undergoes additional enzymatic hydrolysis by alpha-glucosidase, resulting in the conversion of maltose into glucose. Therefore, the crucial role of inhibiting alpha-amylase activity to slow down the process of starch hydrolysis is evident in its ability to mitigate the increase in glucose levels that occurs during postprandial hyperglycaemia (Dandekar *et al*., 2021).

Based on the reported findings, it is reasonable to consider *Psychotria* as a potentially efficacious therapeutic approach for the development of a novel antidiabetic medicine soon, characterized by a diminished occurrence of undesirable effects. However, it is crucial to recognize and address the several limitations present. This review study examines the underlying mechanisms that contribute to the antidiabetic characteristics displayed by various species of *Psychotria*. Nevertheless, a considerable proportion of the research (9 out of 15) has not been subjected to a thorough examination of the fundamental mechanisms that contribute to their antidiabetic properties. Additional research is necessary to clarify the complex characteristics of phytochemical compounds and their fundamental mechanisms of action to acquire a more complete comprehension of their potential medicinal properties. Moreover, it has been noted that there exist certain knowledge gaps regarding the contradictory data on phytochemical compounds present in *Psychotria*, which have been recognized for their antidiabetic activities. This suggests that additional research is necessary to provide evidence for the presence of phytochemical constituents in *Psychotria*. In summary, there exists a scarcity of research on *in vivo* evaluations of medicinal plants, despite the abundance of *in vitro* studies focusing on the antidiabetic attributes of these plan.

Phytochemistry of Psychotria

In recent times, there has been a significant surge in interest in the domain of natural products. There is a need for more research on *Psychotria* species due to their significant presence of natural ingredients, including alkaloids (the primary type of compound), flavonoids, coumarins, and terpenoids.All of these compounds were responsible towards antidiabetic activity possessed by *Psychotria.*

Alkaloids

Numerous classes of alkaloids derived from *Psychotria* species have been documented in the scientific literature, with a limited number currently undergoing further investigation. However only one alkaloids (5′-hydroxymethyl-1′-(1, 2, 3, 9-tetrahydro-pyrrolo (2, 1-b) quinazolin-1-yl)-heptan-1′-one) was reported by Nipun et al. (2020b) corresponding towards antidiabetic activity which presented in a tabulated table (Table A3) in

supplementary data.,

Flavonoids

Flavonoids exhibit distribution patterns among various species within the *Psychotria* species. The flavonoids obtained from several species of *Psychotria* have been compiled and presented in a tabular format (Table A4) in supplementary data. All flavonoids present in *Psychotria* species are classified into few types including flavonoid glycoside (, luteolin-7-O-rutinoside)and flavonol (isorhamnetin, , quercetin). All these flavonoids demonstrate antidiabetic activities.

Terpenoids

This literature presents a comprehensive compilation of terpenoids that have been derived from several species of *Psychotria*. The terpenoids that were discovered and documentedwere assembled and presented in Table A5 (Supplementary data). All terpenoids (β-sitosterol, stigmasterol, ursolic acid) present in *Psychotria* species are classified under triterpenoids group.

Coumarins

Coumarin possesses a structural arrangement with a 2H-chromen-2-one (also referred to as 1,2-benzopyrone or 2H-1-benzopyran-2-one) oxa-heterocycle. This compound has garnered significant attention in scientific research due to its prevalence in numerous biologically active substances (Stefanachi *et al*., 2018). The coumarin discovered and documentedwere presented in Table A6 (Supplementary data). All coumarins present in *Psychotria* species are classified into few types including simple coumarins (1,2-benzopyrones, scopoletin), and angular furanocoumarin (umbelliferone).

Toxicology of Psychotria

The lack of comprehensive data on plant toxicity has limitations on the feasibility of prolonged utilization in the context of chronic illnesses. Therefore, the primary objective of this review was to examine the toxicity studies conducted on several species of *Psychotria*.

The biochemical parameters affected by diabetes, such as serum glucose, urea, uric acid, SGOT, total cholesterol, alkaline phosphatase, creatinine, and others, show that the methanol extract of *P. dalzelli* (MEPD) controls diabetes as well as glibenclamide. Biochemical parameters increased by ~3–10 folds, and protein levels were normalised by standard antidiabetic drugs, and MEPD, though less effective than glibenclamide, showed marked improvement in parameters, suggesting potential antidiabetic properties. MEPD reduced blood glucose levels at 200 mg/kg b.w. compared to DM control, showing anti-hyperglycaemic action. The changes in these biochemical markers suggest the toxic effect of these on diabetic animals' organs, including kidney and liver toxicity (Pecoits-Filho et al., 2016). Thus, MEPD are safer than synthetic medications and can prevent such problems, making them suitable for long-term usage as diabetes treatments (Abhishek et al., 2019).

The researchers performed the fish embryo acute toxicity (FET) test in accordance with the rules set by the Organization for Economic Cooperation and Development (OECD) on *Psychotria* species. The LC_{50} values of the methanol and water extracts of *P. malayana* leaf extract were exceeded their therapeutic concentrations, specifically 37.50 and 252.45 μg/mL, respectively. The results indicated that both the water and methanol extracts exhibited potential antidiabetic effects and are considered safe for use. Nevertheless, the water extract exhibits a higher degree of favourability owing to its significantly larger therapeutic index (LC_{50}/therapeutic concentration) in comparison to the methanol extract (Nipun *et al*., 2021a).

Besides that, further investigation of FET test was done on optimized extract of *P. malayana*. The optimised extract (OE) exhibited an LC_{50} value of 224.29 μg/mL, exceeding its therapeutic index of 111.03. Additionally, it displayed the most potent alpha-glucosidase inhibitory activity, with an IC_{50} value of 2.02 μg/mL (Syed Mohamad et al., 2023). According to this finding, the therapeutic index of OE was higher than that of the methanol extract (13.84) in previous art. These findings indicate that OE has a reduced level of toxicity, giving it a safer option for use, and is expected to be highly effective in its ability to treat diabetes.

Furthermore, Benchoula et al., (2019) were observed that the *P. malayana* extract did not induce any alterations in the hepatic morphology of the zebrafish specimens that were in a healthy state.

P. malayana Jack Leaves as Antidiabetic Agent

Psychotria malayana Jack (as shown in Fig. 2) is a member of the *Rubiaceae* family, which is recognised as the largest family within the *Plantae* kingdom. It exhibits a significant level of species richness, with around 1600 distinct species. In Malaysia, *P.*

malayana is referred to as "salung" and among the Lombok people, it is known as "lolon jarum." The botanical specimen in question exhibits vertical growth, reaching a range of 1 to 4 metres in height. *P. malayana* is naturally found in Andaman Island, Borneo, Jawa, Lesser Sunda Island, Malaysia, Sulawesi, Sumatera, and Thailand. However, its distribution is primarily concentrated within the western region of the Indonesian archipelago. It is a woody plant that often thrives in the moist tropical ecosystem. *P. malayana* was taxonomically classified within the kingdom *Plantae*, phylum *Streptophyta*, class *Equisetopsida*, subclass *Magnoliidae*, order *Gentianales*, family *Rubiaceae*, and *Psychotria* species. *P. malayana* has synonyms categorized as homotypic and heterotypic synonyms. The homotypic synonyms for this species include *Grumilea aurantiaca* Miq., *P. aurantiaca* Wall., and *Uragoga malayana* (Jack) Kuntze. While the heterotypic synonyms are *P. aurantiaca* var. lanceolata Miq., *P. odorata* Blume ex Miq., *P. stipulacea* Wall., *P. stipulacea* var. grandifolia Craib, and *Uragoga stipulacea* (Wall.) Kuntze (Plant of The World Online, 2024).

Fig. 2: *P. malayana* Jack leaves photographed at Cermin Nan Gedang, Sarolangun, Jambi, Indonesia

Psychotria species plants have been utilised in traditional medicinal practices to address many medical conditions, including diabetes (Situmorang *et al*., 2015), pain management (Anvar & Haneef, 2015), fever, and splenomegaly (Koch *et al*., 2015). Traditionally, *P. malayana* has been employed in the region of Sumatra (specifically Jambi) for the treatment of diabetes.

According to our research findings (Table 1), *P. malayana* has recently become the subject of a study looking into its potential therapeutic effects especially in the management of DM. A lot of research has been done on the antidiabetic effects of *P. malayana* leaf extract by Benchoula *et al.* (2019), Nipun *et al*. (2020a, 2020b, 2021a, 2021b), Fairuz *et al*. (2020) and Syed Mohamad et al., (2023). The results of this study indicated that the extract has potential as a therapeutic intervention for diabetes mellitus (DM). The bioactive compounds exhibiting antidiabetic activity in this study exhibit dissimilarities from those elucidated in other investigations, with several compounds being reported for the first time. An exemplification of compounds such as 4-hydroxyphenylpyruvic acid, glutamine, and 5′-hydroxymethyl-1'-(1, 2, 3, 9-tetrahydro-pyrrolo (2, 1-b) quinazolin-1-yl) can be furnished.

According to the initial research results (Benchoula *et al*., 2019), it was observed that the administration of water extract derived from *P. malayana* leaves at doses of 1, 2, and 3 g/kg resulted in a significant decrease in blood glucose levels in a zebrafish model with diabetes.

In recent investigations conducted by Nipun *et al*. (2020b, 2021b), unique phytochemical substances, including heptan-1'-one, α-terpinyl-β-glucoside, and machaeridiol-A, were identified in this plant. However, it has been discovered that 4-hydroxyphenylpyruvic acid, a phenolic molecule, lacks antidiabetic effects. Nevertheless, it exhibits considerable promise for numerous additional biological activities, namely in terms of its antibacterial and antioxidant properties. The antidiabetic properties of the following three bioactive compounds (5′-hydroxymethyl-1'-(1, 2, 3, 9-tetrahydro-pyrrolo (2, 1-b) quinazolin-1-yl), α-terpinyl-β-glucoside, machaeridiol-A) were confirmed through molecular analyses conducted *in silico* by Nipun *et al*. (2020b). The present experiments employed computational methodologies to predict the interaction, binding, and mechanism of action of these drugs, together with their precise binding sites on the enzyme.

Fairuz *et al*. (2020) examined blood glucose and pancreatic cells in induced type 1 diabetic rats. This study used alloxan to induce six rat cohorts. All treatment groups showed a considerable drop in blood glucose levels, with the highest reduction at 1000 mg/kg BW (49.76%).

Additional study has been conducted to optimise the extraction of *P. malayana* leaf extract in order to increase its inhibitory effect against α-glucosidase linked to diabetes. The optimised extract (OE) had a notable inhibitory effect on alpha-glucosidase, with an IC_{50} value of 2.02 μg/mL, as reported by Syed Mohamad et al. (2023). In comparison, the methanol extract, as stated by

Table 1: Literature Review Matrix of *P. malayana* Jack as Antidiabetic Agent

References	Part	Medicinal Value / Activity	Profiled Metabolites / Active Compounds
(Benchoula *et al*, 2019)	Leaves	Antidiabetic activity (Type 1 diabetes) * using diabetes zebrafish model	Phytosterols, Sugar alcohols, Sugar acid, Free fatty acids, Cyclitols, Phenolics, Alkaloid
(Fairuz *et al*., 2020)	Leaves	Antidiabetic activity (Type 1 diabetes) * using diabetes rat model	Chimonanthus, (+) Chimonanthus, Meso-Chimonanthus, Calychanthine, Hodgkinsine, 2-ethyl-6-methylpyrazine, 3-methyl-1,2,3,4-tetrahydro-gamma- carboline
(Nipun *et al*., 2020b; Nipun *et al*., 2021b)	Leaves	Antidiabetic activity (alpha-glucosidase inhibition)	5′-hydroxymethyl-1'-(1, 2, 3, 9-tetrahydro-pyrrolo (2, 1-b) quinazolin-1-yl), α-terpinyl-β-glucoside, Machaeridiol-A, 1,3,5-benzenetriol, Palmitic acid, Cholesta-7,9(11)-diene-3-ol, 1-monopalmitin, β-tocopherol, α-tocopherol, 24-epicampesterol, stigmast-5-ene, 4-hydroxyphenylpyruvic acid, Glutamine
Syed Mohamad et al., 2023	Leaves	Antidiabetic activity (alpha-glucosidase inhibition)	Propanoic acid, Succinic acid, D-tagatose, Myo-inositol, Isorhamnetin, Moracin M-3′-O-β-D-glucopyranoside, Procyanidin B3, and Leucopelargonidin

Nipun et al. (2021a), had an IC_{50} value of 2.71 µg/mL. Various substances, including propanoic acid, succinic acid, D-tagatose, myo-inositol, isorhamnetin, moracin M-3′-O-β-D-glucopyranoside, procyanidin B3, and leucopelargonidin, have been identified as possessing antidiabetic properties (Syed Mohamad et al., 2023). This discovery has significant possibilities for future investigations in the field of diabetes therapy.

Conclusion

Our studies have shown that there has been a growing interest in the *Psycothria* genus in recent years, as seen by a rising number of publications. This interest is primarily driven by the genus' traditional applications and pharmacological properties. This review provides an overview of the primary conventional applications, as well as the pharmacological characteristics, phytochemistry, and chemotaxonomy, associated with the subject. The genus *Psychotria* has an intricate taxonomy, and its phytochemical analysis has proven to be a valuable tool for comprehending and establishing chemotaxonomy. This genus is known to contain several types of natural products such as alkaloids, flavonoids, and terpenoids.

Authors contributions

Conceptualization, S.N.A.S.M. and A.K.; methodology, S.N.A.S.M. and A.K.; software, S.N.A.S.M.; validation, S.N.A.S.M. and A.K.; formal analysis, S.N.A.S.M. and A.K.; investigation, S.N.A.S.M. and A.K.; resources, S.N.A.S.M., and A.K.; data curation, S.N.A.S.M. and A.K.; writing—original draft preparation, S.N.A.S.M.; writing—review and editing, S.N.A.S.M., A.K., S.Z.M.S., Q.U.A., Z.I., and T.S.N.; visualization, S.N.A.S.M. and A.K.; supervision, A.K.; project administration, S.N.A.S.M. and A.K.; funding acquisition, S.N.A.S.M. and A.K. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors express their gratitude to the Ministry of Higher Education of Malaysia (Grant No FRGS/1/2024/WAS12/UIAM/01/2), and Kulliyyah of Pharmacy for their assistance and provision of research facilities.

Conflict of interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors used AI-assisted technologies to enhance readability and clarity. The author reviewed, edited, and verified the content to maintain accuracy and integrity, with full responsibility for the final publication.

References

Abhishek, M., Somashekaraiah, B. V., & Dharmesh, S. M. (2019). *In vivo* antidiabetic and antioxidant potential of *Psychotria dalzellii* in streptozotocin-induced diabetic rats. South African Journal of Botany, 121, 494–499. https://doi.org/10.1016/J.SAJB.2018.12.006

Anvar, K., & Haneef, J. (2015). Ethnobotanical plants used for postnatal care by traditional practitioners from Kozhikode District, Kerala, India. International Journal of Research in Pharmacy and Chemistry, 5(4), 570-581.

Ayyanar, M., & Ignacimuthu, S. (2009). Herbal medicines for wound healing among tribal people in southern India: Ethnobotanical and scientific evidences. International Journal of Applied Research in Natural Products, 3(3), 29-42.

Babu, S., & Jayaraman, S. (2020). An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomedicine & Pharmacotherapy, 131, 110702. https://doi.org/10.1016/j.biopha.2020.110702

Bakrim, S., Benkhaira, N., Bourais, I., Benali, T., Lee, L. H., El Omari, N., Sheikh, R. A., Goh, K. W., Ming, L. C., & Bouyahya, A. (2022). Health benefits and pharmacological properties of stigmasterol. Antioxidants, 11(10), 1912. https://doi.org/10.3390/antiox11101912

Benchoula, K., Khatib, A., Quzwain, F. M. C., Che Mohamad, C. A., Wan Sulaiman, W. M. A., Abdul Wahab, R., Ahmed, Q. U., Abdul Ghaffar, M., Saiman, M. Z., Alajmi, M. F., & El-Seedi, H. (2019). Optimisation of hyperglycaemic induction in zebrafish and evaluation of its blood glucose level and metabolite fingerprint treated with *Psychotria* malayana Jack leaf extract. Molecules, 24(8), 1506. https://doi.org/10.3390/molecules24081506

Benevides, P. J. C., Young, M. C. M., & Bolzani, V. D. S. (2005). Biological activities of constituents from *Psychotria spectabilis*. Pharmaceutical Biology, 42(8), 565-569. https://doi.org/10.1080/13880200490901780

Bhat, P., Hegde, G., & Hegde, G. R. (2012). Ethnomedicinal practices in different communities of Uttara Kannada district of Karnataka for treatment of wounds. Journal of Ethnopharmacology, 143(2), 501–514. https://doi.org/10.1016/j.jep.2012.07.003

Bhatnagar, A., & Mishra, A. (2022). Alpha-Glucosidase inhibitors for diabetes/blood sugar regulation. In V. L. Maheshwari, & R. H. Patil (Eds.), Natural products as enzyme inhibitors. Springer.

Biswas, A., Bari, M. A., Roy, M., & Bhadra, S. K. (2010). Inherited folk pharmaceutical knowledge of tribal people in the Chittagong Hill tracts, Bangladesh. Indian Journal of Traditional Knowledge, 9(1), 77-89.

Bolton, J. L., & Dunlap, T. (2017). Formation and biological targets of quinones: Cytotoxic versus cytoprotective effects. Chemical Research in Toxicology, 30(1), 13–37. https://doi.org/10.1021/acs.chemrestox.6b00256

Bremer, B. (2009). A review of molecular phylogenetic studies of Rubiaceae. Annals of the Missouri Botanical Garden, 96(1), 4-26. http://dx.doi.org/10.3417/2006197

Bristy, T. A., Barua, N., Montakim Tareq, A., Sakib, S. A., Etu, S. T., Chowdhury, K. H., Jyoti, M. A., Aziz, M. A., Reza, A. S. M. A., Caiazzo, E., Romano, B., Tareq, S. M., Emran, T. B., & Capasso, R. (2020). Deciphering the pharmacological properties of methanol extract of *Psychotria calocarpa* leaves by *in vivo*, *in vitro* and *in silico* approaches. Pharmaceuticals, 13(8), 183. https://doi.org/10.3390/ph13080183

Britannica, T. (2020, February 10). Rubiaceae. Encyclopedia Britannica. https://www.britannica.com/plant/Rubiaceae

Britto, J. D., & Mahesh, R. (2007). Evolutionary medicine of Kani tribal's botanical knowledge in agasthiayamalai biosphere reserve, south India. Ethnobotanical Leaflets, 31(1), 280-290.

Calixto, N. O., Pinto, M. E. F., Ramalho, S. D., Burger, M. C. M., Bobey, A. F., Young, M. C. M., Bolzani, V. S., & Pinto, A. C. (2016). The genus *Psychotria*: Phytochemistry, chemotaxonomy, ethnopharmacology and biological properties. Journal of the Brazilian Chemical Society, 27(8), 1355-1378. http://dx.doi.org/10.5935/0103-5053.20160149

Cao, J., Yang, J.-N., Zhou, X.-Q., Zhang, Y.-Y., Zhu, X.-Y., Yue, R.-M., & Hui, Y. (2020). Chemical constituents of *Psychotria hainanensis*. Chemistry of Natural Compounds, 56(3). http://dx.doi.org/10.1007/s10600-020-03081-4

Chen, Q., Toy, J. Y. H., Seta, C., Yeo, T. C., & Huang, D. (2021). Inhibition effect of extract of *Psychotria viridiflora* stem on alpha-amylase and alpha-glucosidase and its application in lowering the digestibility of noodles. Frontiers in Nutrition, 8, 701114. https://doi.org/10.3389/fnut.2021.701114

Choffnes, D. (2017). Chapter 4. The actions of medicinal plants on the nervous system. Nature's pharmacopeia: A world of medicinal plants (pp. 62-79). Columbia University Press.

Choudhury, K. D., Choudhury, M. D., Paul, S., & Baruah, M. K. (2012). Bioactivities of some ethnomedicinal rubiaceous plants available from assam – a review. East Himalayan Society for Spermatophyte Taxonomy, 6(1), 56 - 65.

Coe, F. G. (2008). Ethnomedicine of the rama of southeastern Nicaragua. Journal of Ethnobiology, 28(1), 1–38. https://doi.org/10.2993/0278-0771_2008_28_1_eotros_2.0.co_2

Dandekar, P. D., Kotmale, A. S., Chavan, S. R., Kadlag, P. P., Sawant, S. V., Dhavale, D. D., & RaviKumar, A. (2021). Insights into the inhibition mechanism of human pancreatic alpha-amylase, a type 2 diabetes target, by dehydrodieugenol B isolated from Ocimum tenuiflorum. ACS Omega, 6(3), 1780–1786. https://doi.org/10.1021/acsomega.0c00617

Devadoss, S., Murugaiyan, I., Rajan, M., & Thangaraj, P. (2013). Evaluation of phytochemical, antioxidant, and antimicrobial properties of ethnomedicinal plant *Psychotria Nilgiriensis* Deb. & Gang. International Journal of Pharmacy and Pharmaceutical Sciences, 5(3), 417–422.

Elisabetsky, E., Amador, T. A., Albuquerque, R. R., Nunes, D. S., & Carvalho, A. doC. (1995). Analgesic activity of *Psychotria colorata* (Willd. ex R. & S.) Muell. Arg. alkaloids. Journal of Ethnopharmacology,

48(2), 77–83. https://doi.org/10.1016/0378-8741(95)01287-n

Fisher, H. H. (1973). Origin and uses of ipecac. Economic Botany, 27(2), 231–234. https://doi.org/10.1007/BF02872992

Focho, D. A., Tacham, W. N., & Fonge, B. A. (2009). Medicinal plants of Aguambu – Bamumbu in the Lebialem highlands, southwest province of Cameroon. African Journal of Pharmacy and Pharmacology, 3(1), 001–013.

Fokoua, A. R., Ndjenda, M. K., 2nd, Kaptué Wuyt, A., Tatsinkou Bomba, F. D., Dongmo, A. K., Chouna, R., Nkeng-Efouet, P. A., & Nguelefack, T. B. (2021). Anticonvulsant effects of the aqueous and methanol extracts from the stem bark of *Psychotria camptopus* Verdc. (Rubiacaea) in rats. Journal of Ethnopharmacology, 272, 113955. https://doi.org/10.1016/j.jep.2021.113955

Formagio, A. S. N., Volobuff, C. R. F., Kassuya, C. A. L., Cardoso, C. A. L., do Carmo Vieira, M., Pereira, Z. V., Bagatin, M. C., & de Freitas Gauze, G. (2019). *Psychotria leiocarpa* extract and vincosamide reduce chemically-induced inflammation in mice and inhibit the acetylcholinesterase activity. Inflammation, 42(5), 1561–1574. https://doi.org/10.1007/s10753-019-01018-w

Formagio, A. S., Volobuff, C. R., Santiago, M., Cardoso, C. A., Vieira, M. doC., & Valdevina Pereira, Z. (2014). Evaluation of antioxidant activity, total flavonoids, tannins and phenolic compounds in *Psychotria* leaf extracts. Antioxidants, 3(4), 745–757. https://doi.org/10.3390/antiox3040745

Frankova, A., Vistejnova, L., Merinas-Amo, T., Leheckova, Z., Doskocil, I., Wong Soon, J., Kudera, T., Laupua, F., Alonso-Moraga, A., & Kokoska, L. (2021). *In vitro* antibacterial activity of extracts from Samoan medicinal plants and their effect on proliferation and migration of human fibroblasts. Journal of Ethnopharmacology, 264, 113220. https://doi.org/10.1016/j.jep.2020.113220

Gambelunghe, C., Aroni, K., Rossi, R., Moretti, L., & Bacci, M. (2008). Identification of N, N-dimethyltryptamine and beta-carbolines in psychotropic ayahuasca beverage. Biomedical Chromatography, 22(10), 1056–1059. https://doi.org/10.1002/bmc.1023

Hamilton, C. W. (1989). A revision of mesoamerican *Psychotria* subgenus *Psychotria* (Rubiaceae), Part I: Introduction and species 1-16. Annals of the Missouri Botanical Garden, 76(1), 67–111. https://doi.org/10.2307/2399343

Hoult, J. R., & Payá, M. (1996). Pharmacological and biochemical actions of simple coumarins: Natural products with therapeutic potential. General Pharmacology, 27(4), 713–722. https://doi.org/10.1016/0306-3623(95)02112-4

Iniyavan, M., Sangeetha, D., Saravanan, S., & Parimelazhagan, T. (2012). Evaluation of antioxidant and pharmacological properties of *Psychotria nilgiriensis* Deb & Gang. Food Science and Biotechnology, 21(5), 1421–1431. https://doi.org/10.1007/S10068-012-0187-X

Jang, J. H., Park, J. E., & Han, J. S. (2018). Scopoletin inhibits alpha-glucosidase *in vitro* and alleviates postprandial hyperglycemia in mice with diabetes. European Journal of Pharmacology, 834, 152–156. https://doi.org/10.1016/j.ejphar.2018.07.032

Kalai, F. Z., Boulaaba, M., Ferdousi, F., & Isoda, H. (2022). Effects of isorhamnetin on diabetes and its associated complications: A review of *in vitro* and *in vivo* studies and a post hoc transcriptome analysis of involved molecular pathways. International Journal of Molecular Sciences, 23(2), 704. https://doi.org/10.3390/ijms23020704

Kamble, M. Y., Mane, S. S., Murugan, C., & Jaisankar, I. (2008). Chapter 3 - Diversity of ethno-medicinal plants of tropical islands – with special reference to Andaman and

Nicobar Islands. In C. Sivaperuman, A. Velmurugan, A. K. Singh, & I. Jaisankar (Eds.), Biodiversity and climate change adaptation in tropical islands (pp. 55-103). Elsevier Inc.

Koch A. K., Silva, P. C., & Silva, C. (2010). Reproductive biology of *Psychotria carthagenensis* (Rubiaceae), a distylous species of riparian forest fragments, West Central Brazil. Rodriguésia, 61(3). https://doi.org/10.1590/2175-7860201061314

Kshirsagar, R. D., & Singh, N. P. (2001). Some less known ethnomedicinal uses from Mysore and Coorg districts, Karnataka state, India. Journal of Ethnopharmacology, 75(2-3), 231–238. https://doi.org/10.1016/s0378-8741(01)00199-4

Lebbie, A., Kouamé, F., & Kouassi, E. (2017). Specialization in ethnomedicinal plant knowledge among herbalists in the forest region of Rivercess County, Liberia. Journal of Medicinal Plants Research, 11(14), 264–274. http://dx.doi.org/10.5897/JMPR2017.6329

Lee, K. H., Lin, Y. M., Wu, T. S., Zhang, D. C., Yamagishi, T., Hayashi, T., Hall, I. H., Chang, J. J., Wu, R. Y., & Yang, T. H. (1988). The cytotoxic principles of Prunella vulgaris, *Psychotria serpens*, and *Hyptis capitata*: Ursolic acid and related derivatives. Planta Medica, 54(4), 308–311. https://doi.org/10.1055/s-2006-962441

Lezotre, P. L. (2014). State of play and review of major cooperation initiatives. International Cooperation, Convergence and Harmonization of Pharmaceutical Regulations, 7–170. https://doi.org/10.1016/B978-0-12-800053-3.00002-102.3

Liu, Y., Wang, J. S., Wang, X. B., & Kong, L. Y. (2013). Two novel dimeric indole alkaloids from the leaves and twigs of *Psychotria henryi*. Fitoterapia, 86, 178–182. https://doi.org/10.1016/j.fitote.2013.03.013

Mazimba, O. (2017). Umbelliferone: Sources, chemistry, and bioactivities review. Bulletin of Faculty of Pharmacy, Cairo University, 55(2), 223-232. https://doi.org/10.1016/j.bfopcu.2017.05.001

Mlala, S., Oyedeji, A. O., Gondwe, M., & Oyedeji, O. O. (2019). Ursolic acid and its derivatives as bioactive agents. Molecules, 24(15), 2751. https://doi.org/10.3390/molecules24152751

Moraes, T. M. d. S., de Araújo, M. H., Bernardes, N. R., de Oliveira, D. B., Lasunskaia, E. B., Muzitano, M. F., & Da Cunha, M. (2011a). Antimycobacterial activity and alkaloid prospection of *Psychotria* species (Rubiaceae) from the Brazilian Atlantic Rainforest. Planta Medica, 77(9), 964–970. https://doi.org/10.1055/s-0030-1250656

Moraes, T. M. d. S., Rabelo, G. R., Alexandrino, C. R., Neto, S. J. d. S., and Cunha, M. D. (2011b). Comparative leaf anatomy and micromorphology of *Psychotria* species (Rubiaceae) from the Atlantic Rainforest. Acta Botanica Brasilica, 25(1), 178-190. http://dx.doi.org/10.1590/S0102-33062011000100021

Nepokroeff, M., Bremer, B., & Sytsma, K. J. (1999). Reorganization of the genus *Psychotria* and tribe Psychotrieae (Rubiaceae) inferred from ITS and rbcL sequence data. Systematic Botany, 24(1), 5–27. https://doi.org/10.2307/2419383

Nipun, T. S., Khatib, A., Ahmed, Q. U., Nasir, M. H. M., Supandi, F., Taher, M., & Saiman, M. Z. (2021a). Preliminary phytochemical screening, *in vitro* antidiabetic, antioxidant activities, and toxicity of leaf extracts of *Psychotria malayana* Jack. Plants, 10(12), 2688. https://doi.org/10.3390/plants10122688

Nipun, T. S., Khatib, A., Ahmed, Q. U., Redzwan, I. E., Ibrahim, Z., Khan, A. Y. F., Primaharinastiti, R., Khalifa, S. A. M., & El-Seedi, H. R. (2020a). Alpha-glucosidase inhibitory effect of *Psychotria malayana* Jack Leaf: A rapid analysis using infrared fingerprinting. Molecules, 25(18), 4161. https://doi.org/10.3390/molecules25184161

Nipun, T. S., Khatib, A., Ibrahim, Z., Ahmed, Q. U., Redzwan, I. E., Saiman, M. Z., Supandi, F., Primaharinastiti, R., & El-Seedi, H. R. (2020b). Characterization of alpha-glucosidase inhibitors from *Psychotria malayana* Jack leaves extract using LC-MS-based multivariate data analysis and *in silico* molecular docking. Molecules, 25(24), 5885. https://doi.org/10.3390/molecules25245885

Nipun, T. S., Khatib, A., Ibrahim, Z., Ahmed, Q. U., Redzwan, I. E., Primaharinastiti, R., Saiman, M. Z., Fairuza, R., Widyaningsih, T. D., AlAjmi, M. F., Khalifa, S. A. M., & El-Seedi, H. R. (2021b). GC-MS- and NMR-based metabolomics and molecular docking reveal the potential alpha-glucosidase inhibitors from *Psychotria malayana* Jack leaves. Pharmaceuticals, 14(10), 978. https://doi.org/10.3390/ph14100978

Nualkaew, S., Padee, P., & Talubmook, C. (2015). Hypoglycemic activity in diabetic rats of stigmasterol and sitosterol-3-O-D-glucopyranoside isolated from *Pseuderanthemum palatiferum* (Nees) Radlk. leaf extract. Journal of Medicinal Plants Research, 9, 629-635. http://dx.doi.org/10.5897/JMPR2014.5722

Ocampo, R., & Balick, M. J. (2009). Plants of semillas sagradas: An ethnomedicinal garden in Costa Rica. Revista Cubana de Plantas Medicinales, 14(3), 61-62.

Orji, O. U., Awoke, J. N., Harbor, C., Igwenyi, I. O., Obasi, O. D., Ezeani, N. N., & Aloke, C. (2020). Ethanol leaf extract of *Psychotria microphylla* rich in quercetin restores heavy metal induced redox imbalance in rats. Heliyon, 6(9), e04999. https://doi.org/10.1016/j.heliyon.2020.e04999

Orji, O. U., Ibiam, U. A., Aja, P. M., Uraku, A. J., Ezeani, N., & Alum, E. U. (2015b). Hepatotoxic effects of aqueous extract of *Psychotria microphylla* leaves on Clarias giriepinus Juveniles. IOSR Journal of Pharmacy and Biological Sciences, 10(4), 60–68. http://dx.doi.org/10.9790/3008-10456068

Pan, S. Y., Zhou, S. F., Gao, S. H., Yu, Z. L., Zhang, S. F., Tang, M. K., Sun, J. N., Ma, D. L., Han, Y. F., Fong, W. F., & Ko, K. M. (2014). New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evidence-Based Complementary and Alternative Medicine, 2013, 627375. https://doi.org/10.1155/2013/627375

Pecoits-Filho, R., Abensur, H., Betônico, C. C., Machado, A. D., Parente, E. B., Queiroz, M., Salles, J. E., Titan, S., & Vencio, S. (2016). Interactions between kidney disease and diabetes: dangerous liaisons. Diabetology & Metabolic Syndrome, 8, 50. https://doi.org/10.1186/s13098-016-0159-z

Plants of The World Online (2024). *Psychotria malayana* Jack. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:762593-1

Poovitha, S., & Parani, M. (2016). *In vitro* and *in vivo* alpha-amylase and alpha-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC Complementary and Alternative Medicine, 16 (S1), 185. https://doi.org/10.1186/s12906-016-1085-1

Rajendran, S. M., Sekar, K. C., & Sundaresan, V. (2002). Ethnomedicinal lore of Valaya tribals in Seithur Hills of Virudunagar district, Tamil Nadu, India. Indian Journal of Traditional Knowledge, 1(1), 59-71.

Ramu, R., Shirahatti, P. S., Zameer, F., Ranganatha, L. V., & Prasad, M. N. N. (2014). Inhibitory effect of banana (Musa sp. var. Nanjangud rasa bale) flower extract and its constituents Umbelliferone and Lupeol on alpha-glucosidase, aldose reductase and glycation at multiple stages. South African Journal of Botany, 95, 54-63. https://doi.org/10.1016/j.sajb.2014.08.001

Rani, S. L., Devi, V. K., Soris, P. T., Maruthupandian, A., & Mohan, V. R. (2011). Ethnomedicinal plants used by Kanikkars of Agasthiarmalai biosphere reserve, Western Ghats. Journal of

Ecobiotechnology, 3(7), 16–25.

Rao, P. V., Huey, L. L., Mohamed, S., Rahayu, I., Wahab, A., & Soon, J. M. (2016). Ethnomedicinal knowledge of Temiar Ethnic Tribe of Lojing Highlands, Kelantan: A source for nutritional and antioxidant potential. 5th World Conference on Applied Sciences, Engineering & Technology.

Rasoanaivo, P., Petitjean, A., Ratsimamanga-Urverg, S., & Rakoto-Ratsimamanga, A. (1992). Medicinal plants used to treat malaria in Madagascar. Journal of Ethnopharmacology, 37(2), 117–127. https://doi.org/10.1016/0378-8741(92)90070-8

Robbrecht, E. (1988). Tropical woody Rubiaceae characteristic features and progressions contributions to a new subfamilial classification. Kew Bulletin, 1(3), 1-271. http://dx.doi.org/10.2307/4110534

Rosales-López, C., Muñoz-Arrieta, R., & Abdelnour-Esquivel, A. (2020). Emetine and cephaeline content in plants of *Psychotria ipecacuanha* in Costa Rica. Revista Colombiana de Química, 49(2), 18-22. https://doi.org/10.15446/rev.colomb.quim.v49n2.78347

Santos Junior, C. M., Silva, S. M. C., Sales, E. M., Velozo, E. D. S., Dos Santos, E. K. P., Canuto, G. A. B., Azeredo, F. J., Barros, T. F., & Biegelmeyer, R. (2023). Coumarins from Rutaceae: Chemical diversity and biological activities. Fitoterapia, 168, 105489. https://doi.org/10.1016/j.fitote.2023.105489

Schultes, R. E., & Hofmann, A. (1980). The botany and chemistry of hallucinogens. Alcohol and Alcoholism, 8(3), 122. https://doi.org/10.1093/oxfordjournals.alcalc.a046117

Selvaraj, G., & Jeyasankar, D.A. (2018). Larvicidal properties of *Psychotria octosulcata* (W. A. Talbot.) (Rubiaceae) crude extracts on human vector mosquitoes Aedes aegypti (Linn.), Culex quinquefasciatus (Say.) and Anopheles stephensi Liston. Journal of Entomology and Zoology Studies, 6(1), 1190-1195.

Sharifi-Rad, J., Cruz-Martins, N., López-Jornet, P., Lopez, E. P., Harun, N., Yeskaliyeva, B., Beyatli, A., Sytar, O., Shaheen, S., Sharopov, F., Taheri, Y., Docea, A. O., Calina, D., & Cho, W. C. (2021). Natural coumarins: Exploring the pharmacological complexity and underlying molecular mechanisms. Oxidative Medicine and Cellular Longevity, 2021, 6492346. https://doi.org/10.1155/2021/6492346

Situmorang, R. O. P., Harianja, A. H., & Silalahi, J. (2015). Karo's local wisdom: The use of woody plants for traditional diabetic medicines. Indonesian Journal of Forestry Research, 2(2), 121-131. https://doi.org/10.20886/ijfr.2015.2.2.121-130.

Stefanachi, A., Leonetti, F., Pisani, L., Catto, M., & Carotti, A. (2018). Coumarin: A natural, privileged and versatile scaffold for bioactive compounds. Molecules, 23(2), 250. http://dx.doi.org/10.3390/molecules23020250

Subash-Babu, P., Abdulaziz AlSedairy, S., Abdulaziz Binobead, M., & Alshatwi, A. A. (2023). Luteolin-7-O-rutinoside protects RIN-5F cells from high-glucose-induced toxicity, improves glucose homeostasis in L6 myotubes, and prevents onset of type 2 diabetes. Metabolites, 13(2), 269. https://doi.org/10.3390/metabo13020269

Sutha, S., Mohan, V. R., Kumaresan, S., Murugan, C., & Athiperumalsami, T. (2010). Ethnomedicinal plants used by the tribals of Kalakad-Mundanthurai tiger reserve (KMTR), Western Ghats, Tamil Nadu for the treatment of rheumatism. Indian Journal of Traditional Knowledge, 9(3), 502–509.

Syed Mohamad, S. N. A., Khatib, A., So'ad, S. Z. M., Ahmed, Q. U., Ibrahim, Z., Nipun, T. S., Humaryanto, H., AlAjmi, M. F., Khalifa, S. A. M., & El-Seedi, H. R. (2023). In Vitro Anti-Diabetic, Anti-Inflammatory, Antioxidant Activities and Toxicological

Study of Optimized Psychotria malayana Jack Leaves Extract. Pharmaceuticals, 16(12), 1692. https://doi.org/10.3390/ph16121692

Tabart, J., Kevers, C., Pincemail, J., Defraigne, J.-O., & Dommes, J. (2009). Comparative antioxidant capacities of phenolic compounds measured by various tests. Food Chemistry, 113(4), 1226–1233. https://doi.org/10.1016/j.foodchem.2008.08.013

Tannous, S., Stellbrinck, T., Hoter, A., & Naim, H. Y. (2023). Interaction between the alpha-glucosidases, sucrase-isomaltase and maltase-glucoamylase, in human intestinal brush border membranes and its potential impact on disaccharide digestion. Frontiers In Molecular Biosciences, 10, 1160860. https://doi.org/10.3389/fmolb.2023.1160860

Xu, L., Zhao, X.-Y., Wu, Y.-L., & Zhang, W. (2015). The study on biological and pharmacological activity of coumarins. Proceedings of the 2015 Asia-Pacific Energy Equipment Engineering Research Conference. https://doi.org/10.2991/ap3er-15.2015.33

Yang, D., Wang, T., Long, M., & Li, P. (2020). Quercetin: Its main pharmacological activity and potential application in clinical medicine. Oxidative Medicine and Cellular Longevity, 2020, 8825387. https://doi.org/10.1155/2020/8825387

Yang, H., Zhang, H., Yang, C., & Chen, Y. (2016). Chemical constituents of plants from the genus *Psychotria*. Chemistry and Biodiversity, 13(7), 807–820. https://doi.org/10.1002/cbdv.201500259

Zhou, B. D., Zhang, X. L., Niu, H. Y., Guan, C. Y., Liu, Y. P., & Fu, Y. H. (2018). Chemical constituents from stems and leaves of *Psychotria serpens*. China Journal of Chinese Materia Medica, 43(24), 4878–4883. https://doi.org/10.19540/j.cnki.cjcmm.20180912.003

Table A1. Data from published research papers on *Psychotria* species related to their antidiabetic property

References	Plant - parts & extraction method	Antidiabetic mechanism of action	Bioactive compounds	Identification method
(Nipun et al., 2020b)	○ *P. malayana* ○ Leaves ○ Sonication technique by using methanol at different ratio (0%, 25%, 50%, 75% & 100%)	Alpha-Glucosidase Inhibition	○ 5′-Hydroxymethyl-1' -(1, 2, 3, 9-tetrahydro-pyrrolo (2, 1-b) quinazolin-1-yl) -heptan-1' -one ○ *α*-Terpinyl-β-glucoside ○ Machaeridiol-A	○ LC-MS analysis ○ *In Silico* Molecular Docking
(Nipun et al., 2021b)	○ *P. malayana* ○ Leaves ○ Sonication technique by using methanol at different ratio (0%, 25%, 50%, 75% & 100%)	Alpha-Glucosidase Inhibition	○ 1,3,5-Benzenetriol ○ Palmitic acid ○ Cholesta-7,9(11)-diene-3-ol ○ 1-Monopalmitin ○ β-Tocopherol ○ *α*-Tocopherol ○ 24-Epicampesterol ○ Stigmast-5-ene ○ 4-Hydroxyphenylpyruvic acid ○ Glutamine	○ GC-MS analysis ○ H-NMR analysis
(Abhishek et al., 2019)	○ *P. dalzellii* ○ Leaves ○ Soxhlet extraction (1:3 W/ V) using methanol for 10 hours	○ Alpha-Glucosidase Inhibition ○ Alpha-Amylase Inhibition	NA	NA

References	Plant - parts & extraction method	Antidiabetic mechanism of action	Bioactive compounds	Identification method
(Benchoula et al., 2019)	○ *P. malayana* ○ Leaves ○ A mixture of 3 g of leaf powder and 30 mL of distilled water was boiled for 15 minutes until reduced to 20 mL. The extract was filtered, and the supernatant was stored at -80˚C prior to force-feeding into the zebrafish	Alpha-Glucosidase Inhibition	○ Stearic acid ○ Palmitic acid ○ Myo-inositol ○ Erythritol ○ Beta-sitosterol ○ Quinic acid ○ Shikimic acid ○ 1-Monopalmitin ○ Glycerol monostearate ○ α-Tocopherol	○ LC-MS analysis fingerprinting ○ GC-MS analysis
(Chen et al., 2021)	○ *P. viridiflora* ○ Stem ○ A total of 6.2 kg of powder was extracted with hexane, dichloromethane, ethyl acetate, methanol, and water.	○ Alpha-Glucosidase Inhibition ○ Alpha-Amylase Inhibition	○ Fortunellin ○ Proanthocyanidins	○ LC-MS analysis ○ HPLC-MS analysis
(Bristy et al., 2020)	○ *P. calocarpa* ○ Leaves ○ 500 g of powder was soaked in 2 L of methanol for 15 days with occasional shaking and stirring at 27 ± 2 ˚C, followed by filtration	NA	○ Alkaloid ○ Flavonoid	○ *In silico* molecular docking

References	Plant - parts & extraction method	Antidiabetic mechanism of action	Bioactive compounds	Identification method
(Formagio et al., 2014)	○ *P.carthagen-ensis, P. deflexa, P. leiocarpa,* and *P. capillacea* ○ Leaves ○ Maceration with methanol at room temperature	NA	○ *p*-Coumaric acid	○ High-Performance Liquid Chromatography (HPLC/PAD)
(Nipun et al., 2020a)	○ *P. malayana* ○ Leaves ○ Sonication technique by using methanol at different ratio (0%, 25%, 50%, 75% & 100%)	Alpha-Glucosidase Inhibition	○ Alkane ○ Alkene ○ Aldehyde ○ Aromatic	○ Fourier-Transform Infrared Spectroscopy (FTIR)
(Fokoua et al., 2021)	○ *P. camptopus* ○ Stem bark ○ Maceration in water and methanol	NA	○ Rutin ○ Butin ○ Psycotrianoside B ○ Bauerenone ○ 10-Hydroxy-antirhine ○ 10-Hydroxy-iso-deppeaninol ○ Emetine ○ Hodkinsine	○ Liquid Chromatography-Mass Spectroscopy (LC-MS)
(Rosales-López et al., 2020)	○ *P. ipecacuanha* ○ Leaves, Stems & Roots ○ Vortex agitation, maceration with agitation, maceration without agitation, and ultrasonic bath using 70 % ethanol, methanol, acetone, ethyl acetate, and hexane	NA	○ Emetine ○ Cephaeline	○ High-Performance Liquid Chromatography (HPLC)

References	Plant - parts & extraction method	Antidiabetic mechanism of action	Bioactive compounds	Identification method
(Orji et al., 2020)	○ *P. microphylla* ○ Leaves ○ Maceration in ethanol with intermittent shaking using water bath shaker	NA	○ Quercetin	○ High-Performance Liquid Chromatography (HPLC)
(Iniyavan et al., 2012)	○ *P. nilgiriensis* ○ Fruit, stem, and leaves ○ Soxhlet extraction using petroleum ether, chloroform, acetone, and methanol.	NA	○ Nakijiquinone B ○ 2-Hydroxy-2, N-dimethyloctanoic acid amide octanamide, 2-hydroxy-N,2-dimethyl ○ (Z)-2-Methylhex-4-en-3-yl Nphenylcarbamate ○ 9-Octadecenoic acid (Z) ○ 3,4-Epoxy-7-octen-2-one	○ Gas Chromatography-Mass Spectroscopy (GC-MS)
(Frankova et al., 2021)	○ *P. insularum* ○ Leaves ○ Maceration with 80% ethanol in an orbital shaker for 24 hr at room temperature	NA	NA	○ High-Performance Liquid Chromatography (HPLC)

References	Plant - parts & extraction method	Antidiabetic mechanism of action	Bioactive compounds	Identification method
(Situmorang et al., 2015)	○ *Psychotria* sp. ○ Leaves ○ A handful of dried leaves is combined with 2 liters of water, then heated until the water has been reduced to around one-third of its original volume. The rest of the liquid/water is taken twice a day.	NA	○ Alkaloid ○ Steroid ○ Flavonoid	○ Phytochemical screening
(Formagio et al., 2019)	○ *P. leiocarpa* ○ Leaves ○ Maceration with methanol	NA	○ Vincosamide	○ Isolation ○ LC-DAD

NA: Not available

Table A2. Conventional and contemporary applications of the *Psychotria* species

***Psychotria* species**	**Plant part used**	**Indications**	**Herbal preparation**	**Method of administration**	**References**
P. dalzellii	Root	Scorpion bite	Ground with lime juice to make a paste	Topical and oral	(Kshirsagar & Singh, 2001)
P. ophioxyloides	Leaves and tender fruits	Stomach indigestion	Consumed with milk	NAD	(Britto & Mahesh, 2007)
P. adenophylla	Root	Mouth sore, rheumatism	Powder	NAD	(Biswas et al., 2010)
P. adenophylla	Root	Mouth sore, rheumatoid, lung ailments	NAD	NAD	(Choudhury et al., 2012)
P. denticulata	Root	Toothache	NAD	NAD	
P. montana	Leaves	Pain, colitis	NAD	NAD	
P. poeppigiana	Leaves, root, and wood	Amebic dysentery	Decoctions	NAD	(Coe et al., 2008)
P. elata	Roots, leaves, and flowers	Ear infections, Diarrhea, Emetic (induce vomiting for poisoning), Fever, Respiratory & Pulmonary Disorders (cold, coughs, etc.)	Decoctions and/or poultices	NAD	
P. ipecacuanha	NAD	Diarrhea	NAD	NAD	
P. ipecacuanha	NAD	Diarrhea and dysentery	NAD	NAD	(Fisher, 1973
	Root	Amoebicide, emetic, expectorant	NAD	NAD	(Ocampo & Balick, 2009)
	Rhizomes and roots	Expectorant	Syrup form	NAD	

	Rhizomes and roots	Diaphoretic	Powdered form	NAD	
	Rhizomes and roots	Emetic	Syrup form	NAD	
P. nudiflora	Leaves and flowers	Rheumatism	Consumed along with honey	NAD	(Rani et al., 2011)
P. nudiflora	Leaves and flowers	Rheumatism	Paste	NAD	(Tabart et al., 2009)
P. nilgiriensis	Tender fruit	Rheumatism	Paste	NAD	
P. nilgiriensis	Tender fruits	Rheumatism	Consumed along with honey	NAD	(Rani et al., 2011)
P. nilgiriensis	Tender fruit	Rheumatism	Consumed with honey	NAD	(Devadoss et al., 2013)
P. nilgiriensis	Tender fruit	Rheumatism	Consumed along with honey	NAD	(Iniyavan et al., 2012)
P. viridis	NAD	Perception	NAD	Oral (beverages)	(Choffnes, 2017)
P. viridis	Leaves	Hallucinogen	Concoction	Oral	(Gambelunghe et al., 2008)
P. viridis	NAD	Hallucinations	NAD	NAD	(Schultes & Hofmann, 1980)
P. andamanica	Leaves	General health complaints	NAD	NAD	(Kamble et al., 2008)
P. platyneura	Leaves	General health complaints	NAD	NAD	
P. montana	Leaves	Constipation	NAD	NAD	
P. sarmentosa	Leaves	Itches and sores	NAD	NAD	
P. flavida	Root	Snakebite	Infusion	Oral	(Kshirsagar & Singh, 2001)
P. peduncularis	Leaf	Sore in stomach	NAD	NAD	(Lebbie et al., 2017)

Psychotria sp.	Bark	Hernia	NAD	NAD	
P. malayana	Whole plants	Fever	Decoction	Oral	(Rao et al., 2016)
P. obtusifalia	Aerial part	Febrifuge	Decoction	Oral	(Rasoanaivo et al., 1992)
P. bulata	Aerial part	Febrifuge	Decoction	Oral	
P. ipercacuanha	NAD	*Entameoba hystolitica* infection	NAD	NAD	(Selvaraj & Jeyasankar, 2018)
P. camptopus	Bark	Paralysis and activate nerves	Concoction	Orally and part used as an anal wash	(Focho et al., 2009)
P. henryiis	NAD	Revitalizing spleen and to reduce pain	NAD	NAD	(Liu et al., 2013)
P. octosulcata	Leaf	Muscle fracture	Paste	NAD	(Rajendran et al., 2002)
P. colorata	NAD	Pain killer	NAD	NAD	(Elisabetsky et al., 1995)
P. flavida	Root	Wound	Dried powdered and mixed with coconut oil	Topical	(Ayyanar & Ignacimuthu, 2009)

NAD: Not appropriately described

Table A3. Alkaloids of *Psychotria* species[a]

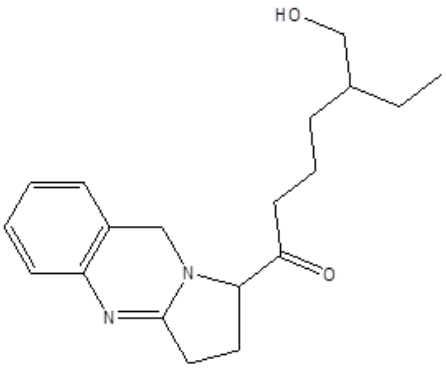
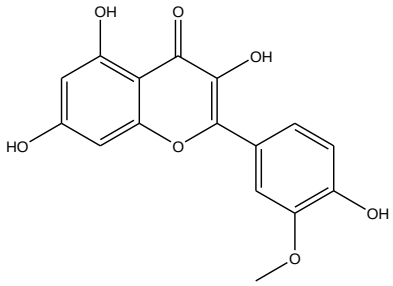
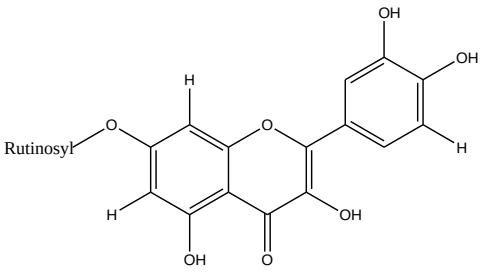
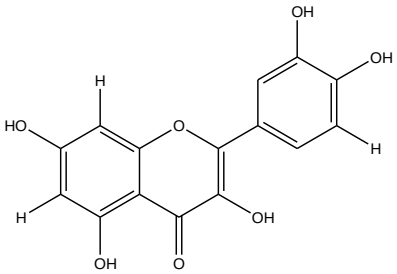
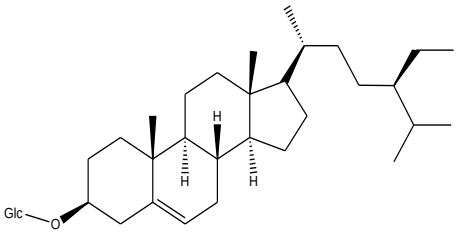
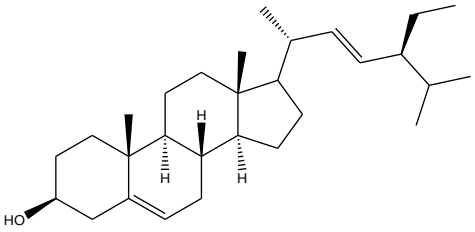
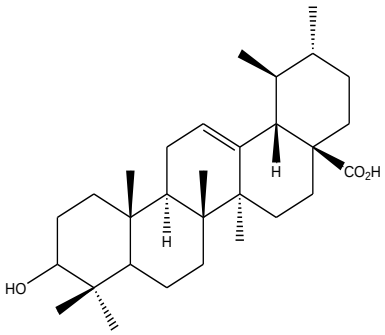
Compounds	Species	Chemical Structure	References
5′-hydroxymethyl-1′-(1, 2, 3, 9-tetrahydro-pyrrolo (2, 1-b) quinazolin-1-yl)-heptan-1′-one	*P. malayana*		Nipun et al., 2020b

Table A4. Flavonoids of *Psychotria* species[a] related to antidiabetic activity

Compounds	Species	Chemical Structure	References
Isorhamnetin	*P. serpens*		(Zhou et al., 2018; Kalai et al., 2022)
Luteolin-7-O-rutinoside	*P. rubra*		(Subash-Babu et al., 2023)
Quercetin	*P. haianensis* *P. serpens* *P. spectabilis*		(Benevides et al., 2005; Yang et al., 2020)

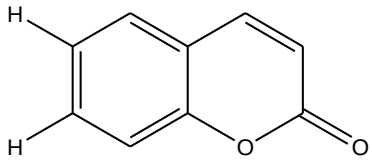
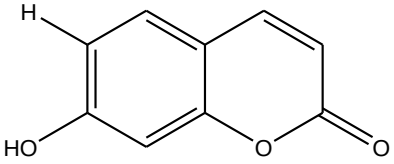
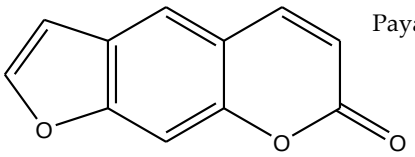
[a]Compounds are arranged according to their alphabetical order

Table A5. Terpenoids of *Psychotria* species[a]

Compounds	Species	Chemical Structure	References
β-sitosterol	*P. adenophylla* *P. haianensis* *P. mariniana*		(Cao et al., 2020; Babu & Jayaraman, 2020)
Stigmasterol	*P. vellosiana*		(Bakrim et al., 2022; Nualkaew et al., 2015)
Ursolic acid	*P. adenophylla* *P. mariniana* *P. serpens*		(Mlala et al., 2019)

[a]Compounds are arranged according to their alphabetical order

Table A6. Coumarins of *Psychotria* species[a]

Compounds	Species	Chemical Structure	References
1,2-benzopyrones	*P. spectabilis*		(Benevides et al., 2005; Xu et al., 2015; Sharifi-Rad et al., 2021; Santos Junior et al., 2023)
Scopoletin	*P. stachyoides* *P. vellosiana*		(Jang et al., 2018)
Umbelliferone	*P. spectabilis*		(Benevides et al., 2005; Mazimba, 2017; Hoult & Payá, 1996; Ramu et al., 2014)

[a]Compounds are arranged according to their alphabetical order

Journal of Pharmacy

Journal of Pharmacy

Solid-State Modification Strategies for Alpha-Mangostin Solubility Enhancement: A Review on Recent Progress

Nurin Syamimi Ahmad Izuren Shah[1], Muhammad Taher Bakhtiar[1], Syarifah Abd Rahim[2], Fatmawati Adam[2], Wan Hazman Danial[3], and Mohd Rushdi Abu Bakar[1, 4*]

[1]*Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

[2]*Faculty of Chemical and Process Engineering Technology, University Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang, 26300 Kuantan, Pahang, Malaysia.*

[3]*Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

[4]*IKOP Pharma (IKOP Sdn. Bhd.), Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

Abstract

Introduction: Enhancing the therapeutic efficacy of active pharmaceutical ingredients requires addressing the persistent challenge of improving their solubility. Alpha-mangostin (AM), a promising natural compound with various pharmacological properties, faces significant limitations due to its low aqueous solubility. This review focuses on evaluating solid-state modification (SSM) techniques developed to enhance AM solubility. It aims to identify the most effective SSM approaches, analyse their advantages, and provide insights for future research directions in addressing solubility challenges for poorly water-soluble compounds. **Methods:** This review article is based on a comprehensive analysis of the literature from databases like Scopus, Google Scholar, ScienceDirect, Springer, and PubChem, covering studies published in the past 15 years. Keywords such as "solubility," "alpha-mangostin," and "solubility improvement" were utilised, with a focus on scientific articles and reviews. **Results:** Several strategies, such as nanoparticle technology, particle size reduction, amorphous formation, and solid dispersion, have been used to enhance AM solubility. Solid dispersion with polyvinylpyrrolidone achieved the highest solubility (2743 µg/mL), while the AM-chitosan-oleic acid complex using nanotechnology improved AM solubility to 160 µg/mL, an 800-fold increase from 0.2 µg/mL. **Conclusion:** The reviewed methods have significantly enhanced the aqueous solubility of AM, with solid dispersion and nanotechnology showing the most promising results. These findings highlight the potential of solubility enhancement strategies to optimise AM's pharmaceutical applications and provide a framework for improving the bioavailability of other poorly water-soluble compounds. Future research should explore alternative methods, such as co-crystallisation and advanced nanotechnologies, to further enhance solubility and formulation efficiency.

Article history:

Received: 21 September 2024
Accepted: 28 January 2025
Published: 31 January 2025

Keywords:

Nanoparticle
Solid dispersion
Size reduction
Complex formation
Xanthone

doi: 10.31436/jop.v5i1.352

*Corresponding author's email: rushdi@iium.edu.my

Introduction

Solubility denotes the maximum quantity of a compound that can dissolve in a solvent at equilibrium, with solubility generally increasing with temperature (Savjani et al., 2012). In pharmaceuticals, solubility is essential for achieving effective active pharmaceutical ingredient (API) concentrations in plasma, as the API must be dissolved in a non-toxic solvent at the necessary concentration to ensure proper bioavailability. This ensures that the API reaches its target site and elicits the desired therapeutic effect (Savjani et al., 2012).

The solubility of APIs can be improved through solid-state modification (SSM), which involves altering the structure or form of the APIs, sometimes with the incorporation of excipients (Jain et al., 2005; Lavilla et al., 2013). SSM is commonly used to enhance the solubility, stability, and bioavailability of poorly water-soluble compounds. Methods such as solid dispersion, nanoparticle formulation, and amorphous formation are considered SSMs because they involve changes in the physical form of APIs while preserving their molecular structure. Unlike chemical modifications or liquid formulations, which alter the molecular structure and produce compounds with different properties, SSMs retain the original molecular structure. The focus on SSM is driven by its proven effectiveness in addressing the solubility challenges of APIs while offering additional advantages, such as cost-effectiveness, energy efficiency, and environmental sustainability.

Alpha-mangostin (AM) is a naturally occurring xanthone isolated from various parts of the mangosteen (*Garcinia mangostana Linn*) tree, including its pericarps (Ahmad Izuren Shah et al., 2025). The compound is attractive due to its potent pharmacological actions such as anticancer (Zhang et al., 2017), renal and hepatic protective, anti-diabetes (Tatiya-Aphiradee et al., 2019), antioxidant (Suthammarak et al., 2016; Akhmad et al., 2018), antibacterial and antifungal properties (Narasimhan et al., 2017), and a promising candidate for the treatment of obesity (Ardakanian et al., 2022; Taher et al., 2015), Alzheimer's disease, Parkinson's disease, and depression (Do & Cho 2020). However, the compound's poor aqueous solubility and low oral bioavailability are the major obstacles to its development. It is classified as a Class II substance in the Biopharmaceutical Classification System (BCS), characterised by low solubility and high permeability. The low aqueous solubility of 0.2 ± 0.2 μg/mL at room temperature, as reported by Aisha et al. (2012), impairs its dissolution in the upper gastric fluid (Savjani et al., 2012), which alters bioavailability and deters its therapeutic application. The low aqueous solubility of AM correlates with its molecular structure, as shown in Fig. 1. AM consists of an unsubstituted tricyclic xanthone core, three hydroxy groups at positions 1, 3, and 6, a methoxy group at position 7, a carbonyl group at position 9, and two isopentene groups at positions 2 and 8. The unsubstituted tricyclic rings lack polar functional groups capable of interacting with water molecules through hydrogen bonding or dipole-dipole interactions. In addition, the structure of AM lacks hydrogen bond donors and acceptors, restricting its ability to form favourable interactions with solvent molecules, resulting in poor solvation in water.

This review focuses on evaluating SSM techniques developed to enhance AM solubility. It aims to identify the most effective SSM approaches, analyse their advantages, and provide insights for future research directions in addressing solubility challenges for poorly water-soluble compounds. It is based on a comprehensive literature review of

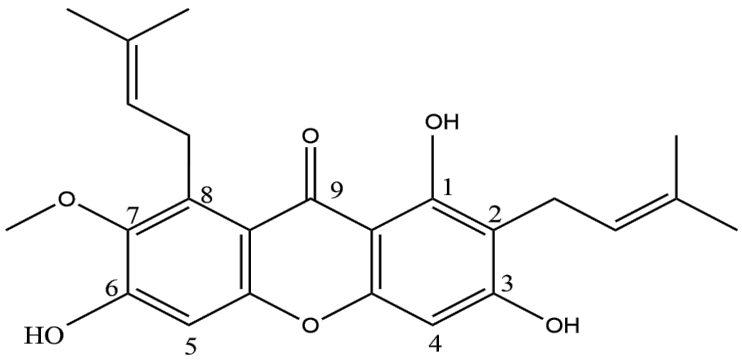


Fig. 1: The structural formula of alpha-mangostin (AM). (Reproduced from Ahmad Izuren Shah et al., 2025)

studies from databases like Scopus, Google Scholar, ScienceDirect, Springer, and PubChem, published in the past 15 years. Keywords such as "solubility," "alpha-mangostin," and "solubility improvement" were utilised, with a focus on scientific articles and reviews.

Factors affecting solubility.

The solubility of a compound is determined by both its intrinsic characteristics and the conditions of the solution. Key attributes such as particle size, crystalline order, and polarity influence how the compound interacts with various solvents (Kasimedua et al., 2015). Moreover, solution conditions, including pH, the presence of co-solvents, temperature, and pressure, play a significant role in affecting solubility (Kasimedua et al., 2015).

Particle size

Solubility enhancement is achieved through the reduction of particle size, which increases the surface area-to-volume ratio by providing more surface for interaction with the solvent. This principle is supported by the following equation (Chaudhary et al., 2012):

$$log\frac{S}{So} = \frac{2\gamma V}{2.303\ R.T.r} \qquad (1)$$

Where S_o = the solubility of large particles (mol/L), S = the solubility of fine particles (mol/L), V = the molar volume (L/mol), γ = the surface tension of the solid (N/m), r = the radius of the fine particles (m), R = gas constant (J/mol·K), and T = temperature (K).

This increase in surface area exposes more of the AM particles to the solvent, thereby facilitating improved solubilisation through enhanced solvent interaction. For AM, this is particularly significant due to its bulky xanthone core and limited aqueous solubility, as smaller particles can increase the likelihood of hydrogen bonding with solvents. Additionally, smaller particles present a higher number of high-energy sites, further augmenting their interaction with liquid solvents. The process of reducing particle size often introduces crystal defects or imperfections, which weaken AM's crystalline structure, characterised by strong intramolecular interactions, thereby promoting easier dissolution (Łuczak et al., 2023). Furthermore, smaller AM particles generally exhibit a lower melting point, leading to reduced intramolecular forces within the xanthone tricyclic ring system and promoting solubility (Alshora et al., 2016).

Crystalline order

Crystalline forms of APIs are generally the most stable, characterised by high density, high melting points, and minimal Gibbs free energy, which contribute to their low solubility (Peltonen & Strachan 2020). The well-ordered molecular arrangement in crystalline states limits interaction with solvent molecules, reducing solubility.

Although crystalline forms of APIs are typically the most stable, some compounds can be processed into an amorphous form to enhance their solubility. The amorphous state is generally the least stable, but it exhibits significantly higher solubility due to its disordered molecular arrangement (Babu et al., 2011). These high-entropy phases are produced by rapidly freezing molecular motion before it can organize into a crystalline lattice. Amorphous compounds, which can exist as solids or supercooled glassy states, lack the long-range order and periodicity of crystalline structures (Peltonen & Strachan, 2020). This structural disorder increases surface area and functional group exposure, allowing better solvent interaction and enhancing solubility. However, the metastable nature of amorphous compounds, combined with their susceptibility to recrystallisation during storage, poses challenges for drug development and necessitates stabilisation strategies to preserve the solubility advantage (Babu et al., 2011).

Polarity

The capacity of a compound and solvent to form hydrogen bonds is intrinsically linked to their polarity. Compounds and solvents with higher polarity typically exhibit a stronger tendency to function as hydrogen bond donors and acceptors (Mudalip et al., 2013; Hassan et al., 2018). AM, with its three hydroxyl groups and xanthone core, can form hydrogen bonds with polar solvents, enhancing its solubility. However, AM's limited

solubility in highly polar solvents, such as water, arises from its bulky, hydrophobic structure, which reduces compatibility with such solvents. Van der Waals interactions, though secondary to hydrogen bonding, play a more prominent role in AM's solubility in non-polar or semi-polar solvents, where intermolecular interactions better accommodate its unsaturated tricyclic xanthone ring (Guo et al., 2016).

Solution conditions

Solubility is also influenced by solution conditions such as pH, the presence of co-solvents, and temperature. For weakly ionisable APIs like AM, solubility can exhibit substantial variability with pH fluctuations (Taniguchi et al., 2014). AM, being a polyphenolic compound with weakly acidic properties, exhibits solubility dependence on pH, particularly in its crystalline form. Small changes in pH can lead to noticeable shifts in AM's solubility, similar to the behaviour of other BCS Class II substances, which are characterised by high permeability but low solubility. As pH decreases, AM's solubility increases due to its enhanced ionisation (Asasutjarit et al., 2019). In addition, an increase in pH can reduce AM particle size from 548 nm to 200 nm, with crystallisation occurring at lower pH levels and physical changes observed around pH 6 (Ahmad et al., 2013).

Co-solvents can enhance the solubility of low polarity compound, such as AM. The use of co-solvents reduces solvent-solvent interactions, thereby lowering surface tension and the dielectric constant, improving AM's solubility in polar solvents by reducing the incompatibility between its hydrophobic core and the solvent (Mantri et al., 2017). Temperature also plays a role in enhancing AM's solubility, especially when using endothermic processes. As temperature increases, the kinetic energy of molecules intensifies, leading to more frequent and energetic collisions between AM and solvent molecules (Hassan et al., 2018). Elevated temperatures may help overcome some of AM's inherent molecular structure, increasing AM solubility. Furthermore, temperature increases may lead to the formation of larger micelles, facilitating AM's dispersion in solution. Pressure typically has less of an impact on AM's solubility compared to gases, but variations in pressure can influence the solubility of AM in certain solvents, particularly under supercritical conditions (Lee et al., 2019).

To enhance solubility, various techniques can be used for AM, including physical modifications such as reducing particle size through micronisation or nanosuspension, and altering crystal forms. The use of co-solvents, surfactants, and other excipients can help to solvate AM more effectively (Lee et al., 2019). Moreover, chemical modifications, such as adjusting pH or derivatives formation, may improve AM's dissolution rate (Savjani et al., 2012).

Biopharmaceutics Classification System

The Biopharmaceutics Classification System (BCS) categorises substances into four classes based on their solubility and permeability characteristics, in accordance with the International Conference on Harmonisation (ICH) guidelines (2019), as depicted in Fig. 2. This system has become crucial in recent years for guiding the development of oral formulations and establishing bioavailability standards (Dahan et al., 2009).

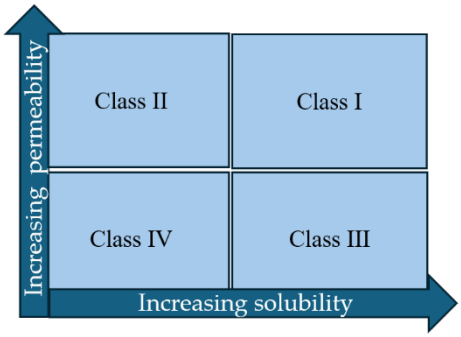


Fig. 2: API classification based on the Biopharmaceutics Classification System (BCS)

BCS Class II substances are characterised by high permeability but low solubility, which limits their bioavailability (Yasir et al., 2010). As of recent reports, 60–70 % of API candidates in development and 30 % of APIs that have reached the market belong to BCS Class II (Ting et al., 2018). AM is classified as a BCS Class II compound (Li et al., 2011). Its solubility in water at room temperature is 0.2 ± 0.2 μg/mL (Aisha et al., 2012). It is significantly

lower compared to ibuprofen (21 µg/mL) (National Center for Biotechnology Information, 2024) and atorvastatin (100 µg/mL) (Rodde et al., 2014), but comparable to curcumin (0.6 µg/mL) (Górnicka et al., 2023). In terms of permeability, AM shows high membrane permeability, similar to curcumin (apparent permeability (P_{app}) ≈ 0.07×10^{-6} cm/s) (Dempe et al., 2012) and atorvastatin (P_{app} ≈ 28.1×10^{-6} cm/s) (Li et al., 2011), though slightly lower than ibuprofen ($P_{app} \approx 1.8\times10^{-4}$ cm/s) (National Center for Biotechnology Information, 2024).

To enhance the solubility of BCS Class II substances, techniques are generally categorised into two main groups: physical modification and chemical modification. Physical modification involves particle and crystal engineering, along with amorphous systems, incorporating methods such as nanoparticle synthesis, particle size reduction, amorphous solid formation, and solid dispersion techniques (Jain & Chella, 2020). In contrast, chemical modification includes prodrug strategies and API derivatisation to modify physicochemical properties and biodistribution while preserving pharmacological activity, along with the formation of complex compounds using surfactants or co-solvents (Jain & Chella, 2020). To date, numerous attempts using various techniques have been employed to improve the solubility of AM.

Nanotechnology

While physical and chemical modification techniques offer various approaches for enhancing the solubility of BCS Class II substances, nanotechnology has emerged as a powerful strategy to further address these challenges. Nanotechnology is a key component of nanomedicine, enabling advancements in targeted treatments and enhancing therapeutic efficacy in clinical applications. Nanoparticles, which are around 10^{-9} meters in size, have a significantly larger surface area, leading to greater contact with solvents. In addressing the challenges of poor API solubility, nanoparticles increase the API surface area, thus improving dissolution rates and stabilising the API in a nanoscale form. Nanoparticle technology facilitates improved solubility through mechanisms such as solid dispersion formation and enhanced dissolution rates (Chen et al., 2011). By encapsulating the API in a nanoscale carrier, nanoparticles address solubility issues, making them an effective method for improving the solubility of poorly soluble APIs. Cellulose derivative polymers have the ability to form nanoreservoirs using particle technology (Pan-in et al., 2014; Kim et al., 2014). These enhancements are further amplified when coupled with polyethylene glycol-2000 (Sakpakdeejaroen et al., 2022). In addition, the combined application of letichin and propolis extract has demonstrated enhanced efficacy and solubility of AM (Suhandi et al., 2023).

The AM encapsulation procedure involved the use of a self-assembly technique (Pan-in et al., 2014) and a solvent displacement method, employing a mixture of ethylcellulose and methylcellulose polymers to form nanoparticles (Kim et al., 2014). The resulting nanoparticles were characterised to ascertain their loading capacity and encapsulation efficiency, in vitro release, cellular uptake, and susceptibility testing (Pan-in et al., 2014; Kim et al., 2014). The morphology of the nanoparticles encapsulated with AM showed their spherical form (Kim et al., 2014). The nanoparticles displayed favourable stability in suspension and excellent drug-loading and encapsulation efficiencies. The results demonstrate the effectiveness of cellulose-derivative nanoparticles in enclosing the hydrophobic AM, enhancing its solubility and bioavailability of AM, as well as successfully improving the delivery of AM into cancer cells (Kim et al., 2014).

Furthermore, nanotechnology enhances AM solubility through SSM to form proniosomes. The amphiphilic nature of proniosomes allows them to interact with both hydrophilic target molecules and the hydrophobic regions of APIs, thereby improving therapeutic agent delivery (Shah et al., 2021). Proniosomes are dehydrated powders made of nonionic surfactants and lipids (see Figure 3(b)). When these powders contact water, they transform into niosomes, encapsulating AM, a poorly soluble API, which improves its solubility and stability. Chin et al., (2016) used the coacervation phase separation method to prepare AM proniosomes, creating a stable system that enhances the transportation of lipophilic AM. The study reported that AM proniosomes had numerous significant advantages, including improved skin permeability, high entrapment efficiency, successful deposition in

the viable epidermis, better vesicle stability, and no agglomeration.

Pham et al. (2019) formulated AM-loaded fibroin nanoparticles using fibroin extracted from Bombyx mori silk, combined with carbodiimide and polyethylenimine. The resulting nanoparticles, averaging 300 nanometres in size with surface charges between −15 and +30 millivolts, exhibited a significantly enhanced solubility of 1.091 µg/mL, which is nearly a threefold increase compared to the 0.386 µg/mL solubility of free AM. In contrast, Yang et al. (2019) employed biodegradable monomethoxy poly(ethyleneglycol)-polycaprolactone copolymer nanomicelles. By re-suspending AM and the copolymer in methanol, followed by a self-assembly process in water, they fabricated nanomicelles with high encapsulation efficiency.

Micelles are colloidal structures formed by the self-assembly of amphiphilic molecules, as schematically shown in Fig. 3 (c). In an aqueous environment, the hydrophilic regions of these molecules' orient outward, interacting with the solvent, while the hydrophobic regions aggregate inward to form the particle's core (Ducheyne et al., 2017). The concentration at which micelles begin to form is referred to as the critical micelle

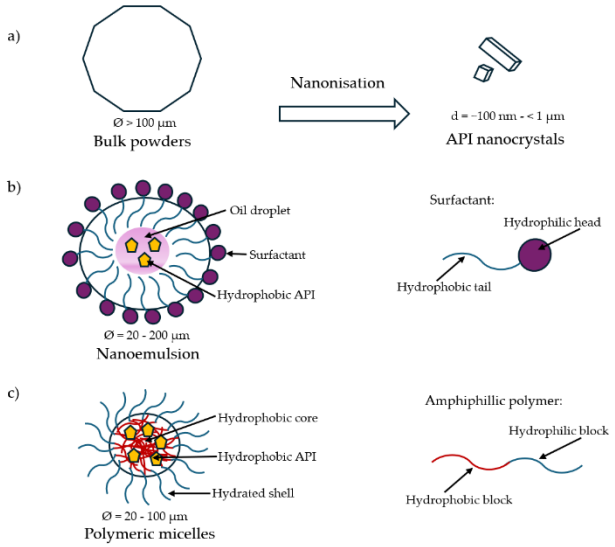


Fig. 3: Schematic overview of different nanotechnology techniques. (Modified from Chen et al., 2011)

concentration (CMC). A lower CMC is typically preferred in functional applications, as it indicates greater micelle stability, even under dilution (Meylina et al., 2021). These nanomicelles not only improved solubility but also demonstrated controlled drug release.

Both Samprasit et al., (2014) and Kalidason & Kuroiwa (2023) improved AM solubility using chitosan, albeit through different methods: thiolated chitosan polymer solutions and chitosan-oleic acid complexes, respectively. Samprasit et al., (2014) enhanced AM solubility by incorporating it into nanofiber mats using a polymer matrix. A composite solution of thiolated chitosan and polyvinyl alcohol was prepared to achieve a homogeneous dispersion of AM, followed by electrospinning to fabricate nanofibre mats. The resulting mats exhibited potent antibacterial activity and facilitated the rapid release of bioactive compounds. In contrast, Kalidason & Kuroiwa (2023) utilised nanoencapsulation technology to enhance the aqueous solubility of AM by preparing a chitosan solution from chitosan flakes dissolved in acetic acid and oleic acid, into which the AM extract was incorporated. Following stirring and centrifugation, a stable chitosan-oleic acid complex with a particle size of 830 nm was formed. The thiolated chitosan improved the prolonged release and efficacy of the mats when taken orally, while the chitosan-oleic acid complex exhibited excellent stability and efficient controlled delivery to the intestine, significantly increasing AM's solubility by over 800 times, from an initial 0.2 µg/mL. Nanoparticle drug delivery systems offer significant benefits, including enhanced solubility, controlled release, and targeted delivery, all of which improve the bioavailability and therapeutic effectiveness of AM. These systems also provide versatile formulations, reduce toxicity, and enhance pharmacokinetic properties (Wathoni et al., 2020). Nevertheless, scaling up nanoparticle formulations for industrial production is complex and costly. Stability, biocompatibility, and toxicity must be carefully managed, as environmental conditions and potential adverse effects can impact AM efficacy (Wathoni et al., 2020).

Size reduction

Nanotechnology uses the properties of nanoparticles, whereas size reduction techniques, such as high-pressure homogenisation, offer a more direct approach to improving AM's solubility. AM was dispersed in deionised water with stabilisers

such as sodium lauryl sulphate and poloxamer 188. The dispersion was sonicated to improve uniformity. High-pressure homogenisation was conducted at different pressures while maintaining temperature control to prevent excessive heating and reduce particle size (Limwikrant et al., 2019). The stability and efficacy of the particles were evaluated by monitoring their size and zeta potential. The study found that sodium lauryl sulfate resulted in the highest solubility for AM, with a value of 57.81 ± 0.21 μg/mL. This was attributed to its lower CMC compared to poloxamer 188 (Limwikrant et al., 2019).

Ball milling techniques were also used for the size reduction of poorly soluble APIs, with the potential to increase AM solubility. The API powders were weighed inside a glove box under a high-purity argon atmosphere (oxygen <25 ppm) to maintain inert conditions and prevent oxidation during milling. The powders were then loaded into hardened stainless-steel jars with stainless-steel balls, and stearic acid was added as a process-controlling agent. High-energy ball milling was performed at 280 and 350 rotations per minute, with a 30-minute pause after each hour of milling (Witharamage et al., 2021). The milled powders were subsequently cooled and compacted.

This technique effectively reduced particle size, increasing the surface area and active sites, which led to enhanced solubility. Reducing particle size offered significant advantages, such as improved emulsion stability and energy efficiency (Dumay et al., 2013). It also enhanced the functional properties and bioavailability of the API. While size reduction provided various benefits, it also required high operational costs, significant energy consumption, and potential temperature effects that could degrade the API. Additional challenges included scale-up difficulties, limited long-term data, microbial resistance, and the need for precise viscosity control, which complicated product formulation and consistency (Dumay et al., 2013).

Amorphous formation

The formation of amorphous solids is one of the SSM strategies used to enhance AM solubility. The amorphous form of a substance is characterised by its irregular structure and higher energy state compared to its crystalline counterpart, as shown in Fig. 4. This form generally enhances API dissolution, solubility, and bioavailability due to its less ordered lattice, which improves solvent contact and wetting (Iqbal et al., 2018). Amorphous solids exhibit elevated free energy, enthalpy, and entropy due to their disordered structure (Gurunath et al., 2013). A study indicated that rice husk ash mesoporous silica effectively facilitated the transformation of AM into its amorphous form, thereby enhancing its solubility (Iqbal et al., 2018). Rice husk was processed by washing, filtration, and rinsing, followed by calcination to produce rice husk ash. Mesoporous silica was synthesised from this ash using Pluronic 123, followed by additional calcination. The sol-gel method was then employed to load AM onto the rice husk ash mesoporous silica. Both AM and the mesoporous silica were dissolved in ethanol, concentrated, and dried (Iqbal et al., 2018). Amorphous AM enhances solubility, improves physical stability, and inhibits recrystallization. However, amorphous AM has limitations, such as high recrystallisation tendencies, agglomeration issues, and dependence on polymer choice and ratio. These challenges, along with storage difficulties and regulatory concerns, complicate its practical use in pharmaceutical formulations (Budiman et al., 2023).

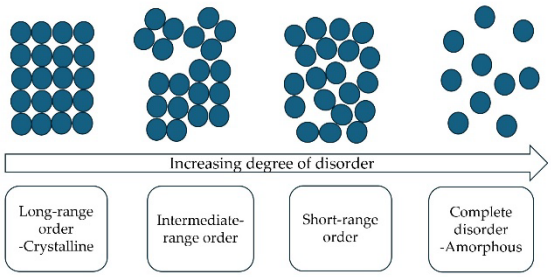


Fig. 4: The structural transition from crystalline to amorphous states. (Modified from Peltonen & Strachan 2020)

Solid dispersion

Solid dispersions, which are a method of enhancing the solubility of poorly soluble drugs by dispersing them in a solid matrix, are illustrated by the process flow shown in Fig. 5. They typically consist of a hydrophilic matrix and a hydrophobic API, with the matrix existing in either crystalline or amorphous states (Singh et al., 2013). This method

enhances API concentrations in gastrointestinal fluids by employing particle size reduction, improving wetting, and reducing agglomeration (Kumar et al., 2017). The inclusion of surfactants further minimises recrystallisation, improving both dissolution and stability (Chaudhari et al., 2017). Solid dispersions offer advantages over other systems by enhancing oral bioavailability without altering the API's active targets, often through salt formation or by incorporating polar or ionised groups (Kaur et al., 2012).

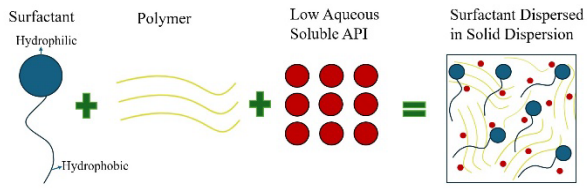


Fig. 5: Schematic representation of a technique for incorporating surfactant and polymer into solid dispersions.

The study by Aisha et al. (2012) employed solid dispersion techniques to enhance solubility and elucidate the underlying mechanisms. Solid dispersions were prepared through the solvent evaporation method using polyvinylpyrrolidone (PVP), resulting in a substantial increase in AM solubility from 0.2 ± 0.2 µg/mL to 2743 ± 11 µg/mL. Characterisation was performed using Fourier Transform Infrared (FTIR) spectroscopy to analyse API-polymer interactions and X-ray Diffraction (XRD) to verify the transition of AM to an amorphous state. Solubility studies involved dissolving the solid dispersions in phosphate-buffered saline. This study also confirmed the formation of spherical anionic nanomicelles with particle sizes ranging from 99 to 127 nm, which enhance tissue and cellular penetration. Solid dispersions provide versatility in AM formulation, stability for sensitive compounds, ease of manufacturing, and potential for combination therapies, while also reducing side effects by lowering required dosages. However, solid dispersions face challenges such as stability issues, limited drug loading, and complex formulation development, which can affect their efficacy and application (Tran et al., 2019).

Complex formation

Besides solid dispersions, complex formation with cyclodextrins provides another effective strategy for enhancing AM solubility. Cyclodextrins, cyclic oligosaccharides derived from starch, form non-covalent bonds with APIs, preserving their physicochemical properties. They possess primary and secondary hydroxyl groups that act as potential modification sites (Phunpee et al., 2018). Structurally, cyclodextrins resemble a truncated cone with a hydrophilic exterior and a hydrophobic interior, which facilitates the formation of inclusion complexes with non-polar molecules (Phunpee et al., 2018). This configuration, combined with the increased free energy and complex energy from such interactions, makes cyclodextrins effective carriers (Hotarat et al., 2020). Specifically, the glycerol ester group of dimethylcyclodextrin interacts with AM through hydrogen bonding, suggesting the permeation of the inclusion complex into the inner phospholipid membrane (Hotarat et al., 2020).

The phase solubility studies consisted of introducing AM into cyclodextrin solutions in a 1:1 stoichiometry ratio until the point of saturation was reached (Hotarat et al., 2019). Solutions were sonicated and incubated until equilibrium, then the supernatants were filtered and analysed. The experimental results showed that the formation of complexes with 2, 6-dimethyl-beta-cyclodextrin was the most favourable, as validated by molecular dynamics simulations. The simulations further demonstrated that AM could adopt two orientations inside cyclodextrin cavities, primarily stabilised by van der Waals forces (Hotarat et al., 2019). The results confirmed that 2, 6-dimethyl-beta-cyclodextrin significantly enhanced the solubility of AM, increasing its concentration from 1 µM in pure water to 104 µM, compared to 22 µM with beta-cyclodextrin and 28 µM with 2-hydroxypropyl-beta-cyclodextrin (Hotarat et al., 2019).

The enhanced AM solubility reached 2743 µg/mL using PVP through the solid dispersion method, which includes the solvent evaporation technique. FTIR and XRD analyses confirmed the formation of an amorphous AM-PVP solid dispersion. Moreover, the study observed no significant variation in pyrene fluorescence

intensity, indicating the physical stability of the AM-PVP complex and reinforcing the micellar stability. Complex formation techniques improved pharmacokinetics and reduced toxicity, making it safer for therapeutic use. However, limitations include the need for extensive in vivo and clinical trials, synthesis challenges, potential API interactions, and variability in individual responses. Regulatory hurdles, stability issues, and high production costs may delay approval and clinical use (Mardianingrum et al., 2024).

Summary

Nanotechnology using cellulose derivatives enhance AM's solubility and bioavailability but face challenges in preparation and stability. Proniosomes improve solubility, stability, and skin permeability, though phase separation can be problematic. Fibroin nanoparticles and nanomicelles offer biocompatibility and controlled release but are costly and hard to scale. Chitosan-based formulations are effective but affected by variability in quality. Size reduction techniques like high-pressure homogenisation and ball milling are simple and scalable but prone to aggregation and stability issues. Amorphous formation using mesoporous silica enhances solubility but is complex and costly. Solid dispersions improve wetting but face scaling and recrystallisation challenges, while cyclodextrin complexation significantly enhances solubility, with effectiveness depending on the cyclodextrin type. Each method improves AM's therapeutic potential but involves trade-offs in complexity, scalability, and stability.

Among the methods reviewed, as summarised in Table 1, Aisha et al., (2012) achieved the highest enhancement in AM solubility, reaching 2743 µg/mL using PVP via the solid dispersion method, which includes the solvent evaporation technique. FTIR and XRD analyses confirmed the formation of an amorphous AM-PVP solid dispersion. In

Table 1: Methods to enhance the aqueous solubility of AM.

Method	Free AM (µg/mL)	Enhanced AM (µg/mL)	Excipient	References
Solid dispersion, Amorphous formation	0.2	2743	Polyvinylpyrrolidone	Aisha et al., 2012
Nanoparticles	0.2	160	Chitosan-oleic acid complexes	Kalidason & Kuroiwa 2023
Particle size reduction	-	57.81	Sodium lauryl sulphate and poloxamer 188	Limwikrant et al., 2019
Complex formation	0.412	42.88	Beta cyclodextrin	Hotarat et al., 2019
Nanoparticles	0.386	1.091	Fibroin, carbodiimide, and polyethylenimine.	Pham et al., 2019
Nanoparticles (nanocarrier)	-	-	Ethylcellulose, methylcellulose (1:1)	Pin et al., 2014 Kim et al., 2014
Nanoparticles (nanocarrier)	-	-	Proniosomes	Chin et al., 2016
Nanoparticles (nanomicelles)	-	-	Monomethoxy poly(ethyleneglycol)-polycaprolactone	Yang et al., 2019
Nanoparticles (nanofiber)	-	-	Thiolated chitosan	Samprasit et al., (2014)
Amorphous formation			Rice husk, pluronic 123	Iqbal et al., 2018
Complex formation	-	-	Beta cyclodextrin	Phunpee et al., 2018

comparison, Kalidason & Kuroiwa (2023) obtained the second highest AM aqueous solubility enhancement, with 160 μg/mL, by developing an AM-chitosan-oleic acid complex. This complex exhibited excellent stability and controlled intestinal release, boosting AM's solubility over 800-fold from an initial 0.2 μg/mL.

Future strategies

In order to explore effective strategies for enhancing AM solubility, it is useful to examine similar approaches applied to other polyphenols, such as curcumin. Co-crystallisation of curcumin with resorcinol and pyrogallol has shown significant improvements in solubility and bioavailability (Sanphui et al., 2011). It was found that curcumin-resorcinol cocrystals have a solubility increase of 2.7 to 4.7 times, while curcumin-pyrogallol cocrystals show an increase of 6.7 to 11.8 times compared to the stable form of curcumin. These findings highlight the potential of using co-crystallisation to improve AM's solubility and therapeutic effectiveness.

Conclusion

This review examines both established and emerging SSM strategies for enhancing the solubility of AM, including nanoparticle technology, solid dispersions, particle size reduction, amorphous formation, and complexation. These strategies are discussed in the context of recent advancements and relevant research that highlight their effectiveness in improving AM solubility. As a BCS Class II substance, AM faces formulation challenges due to its low solubility and poor bioavailability, which limit its therapeutic effectiveness.

Solid dispersion (Aisha et al., 2012) and AM-chitosan-oleic acid complexation via nanotechnology (Kalidason & Kuroiwa, 2023) have demonstrated promising results, with AM solubility increasing by over 2700 μg/mL in the former and 160 μg/mL in the latter. These enhancements increase the potential for AM-based treatments for diseases like cancer, inflammation, and microbial infections, where AM shows significant bioactivity. Furthermore, scalable and reproducible solubility enhancement methods could facilitate the development of commercially viable AM formulations, making these therapies more accessible to patients.

Future research should focus on co-crystallisation, advanced nanotechnologies, lipid-based delivery systems, and long-term stability studies to ensure consistent solubility improvement. *In vivo* pharmacokinetic studies are also essential to assess absorption and therapeutic efficacy. Furthermore, exploring synergistic strategies for solubility enhancement and developing scalable, cost-effective formulations will be key to translating these findings into practical clinical applications

Authors contributions

Conceptualisation: M. R. A. B.; Writing—original draft preparation: N. S. A. I. S.; Writing—review and editing: M. R. A. B.; Visualisation: N. S. A. I. S.; Supervision: M. R. A. B., M. T. B, F. A., S. A. R. and W. H. D.; Project administration: M. R. A. B.; Funding acquisition: M. R. A. B. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

This work was supported by the Malaysian Ministry of Higher Education under Grant FRGS/1/2022/STG05/UIAM/02/1.

Conflict of interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that ChatGPT and QuillBot were used in order to improve readability and language. After employing this tool, the author meticulously assesses and modifies the text as necessary, taking full responsibility for the content of the publication.

References

Aisha, A. F. A., Ismail, Z., Abu-Salah, K. M., & Majid, A. M. (2012). Solid dispersions of α-mangostin improve its aqueous solubility through self-assembly of nanomicelles. *Journal of Pharmaceutical Sciences, 101(2),* 815–825. https://doi.org/10.1002/jps.22806

Ahmad Izuren Shah, N. S., Abu Bakar, M. R., Taher, M., Danial, W. H., Adam, F., & Abdul Rahim, S. (2025). Occurrence analysis of alpha-mangostin from different organs of Garcinia mangostana L. *Natural Product Research, 1–5.* https://doi.org/10.1080/14786419.2024.2449493

Ahmad M., Yamin B. M., Mat Lazim A. (2013). A study on dispersion and characterisation of α-mangostin loaded pH sensitive microgel systems. *Chemistry central journal, 7(1):*85.

Akhmad Husen, S., Khaleyla, F., Nur Muhammad Ansori, A., Kuncoroningrat Susilo, R. J., & Winarni, D. (2018). Antioxidant activity assay of alpha-Mangostin for amelioration of kidney structure and function in diabetic mice. *Atlantis Press, 84–88.*

Alpha mangostin. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/5281650. [Last accessed on 25 Jan 2025]

Alshora, D. H., Ibrahim, M. A., & Alanazi, F. K. (2016). Nanotechnology from particle size reduction to enhancing aqueous solubility. *In Surface Chemistry of Nanobiomaterials*, 163–191. https://doi.org/10.1016/b978-0-323-42861-3.00006-6

Ardakanian, A., Rahbardar, M. G., Omidkhoda, F., Razavi, B. M., & Hosseinzadeh, H. (2022). Effect of alpha-mangostin on olanzapine-induced metabolic disorders in rats. *Iranian Journal of Basic Medical Sciences, 25(2),* 198.

Asasutjarit, R., Meesomboon, T., Adulheem, P., Kittiwisut, S., Sookdee, P., Samosornsuk, W., & Fuongfuchat, A. (2019). Physicochemical properties of alpha-mangostin loaded nanoemulsions prepared by ultrasonication technique. *Heliyon, 5(9),* e02465. https://doi.org/10.1016/j.heliyon.2019.e02465

Babu, N. J., & Nangia, A. (2011). Solubility advantage of amorphous drugs and pharmaceutical cocrystals. *Crystal Growth & Design, 11(7),* 2662–2679. https://doi.org/10.1021/cg200492w

Budiman, A., Nurani, N. V., Laelasari, E., Muchtaridi, M., Sriwidodo, S., & Aulifa, D. L. (2023). Effect of drug–polymer interaction in amorphous solid dispersion on the physical stability and dissolution of drugs: The case of alpha-mangostin. *Polymers, 15(14),* 3034. https://doi.org/10.3390/polym15143034

Chaudhari, S. P., & Dugar, R. P. (2017). Application of surfactants in solid dispersion technology for improving solubility of poorly water-soluble drugs. *Journal of Drug Delivery and Science Technology, 41,* 68–77.

Chaudhary, A., Nagaich, U., Gulati, N., Sharma, V., & Khosa, R. (2012). Enhancement of solubilisation and bioavailability of poorly soluble drugs by physical and chemical modifications: A recent review. *Journal of Advanced Pharmacy Education & Research, 2(1),* 32–67.

Chen, H., Khemtong, C., Yang, X., Chang, X., & Gao, J. (2011). Nanonization strategies for poorly water-soluble drugs. *Drug Discovery Today, 16(7–8),* 354–360. https://doi.org/10.1016/j.drudis.2010.02.009

Chin, G. S., Todo, H., Kadhum, W. R., Hamid, M. A., & Sugibayashi, K. (2016). In vitro permeation and skin retention of α-mangostin proniosome. *Chemical and Pharmaceutical Bulletin, 64(12),* 1666–1673.

Dahan, A., Miller, J. M., & Amidon, G. L. (2009). Prediction of solubility and permeability class membership: Provisional BCS classification of the world's top oral drugs. *AAPS Journal, 11(4),* 740–746.

Dempe, J. S., Scheerle, R. K., Pfeiffer, E., & Metzler, M. (2012). Metabolism and permeability of curcumin in cultured Caco-2 cells. Molecular *Nutrition & Food Research, 57(9),* 1543–1549. https://doi.org/10.1002/mnfr.201200113

Ducheyne, P., Grainger, D. W., Healy, K. E., Hutmacher, D. W., & Kirkpatrick, C. J. (2017). *Comprehensive biomaterials II 3*, 384–386. Elsevier.

Dumay, E., Chevalier-Lucia, D., Picart-Palmade, L., Benzaria, A., Gràcia-Julià, A., & Blayo, C. (2013). Technological aspects and potential applications of (ultra) high-pressure homogenisation. *Trends in Food Science & Technology, 31(1),* 13–26. https://doi.org/10.1016/j.tifs.2012.03.005

Do, H. T. T., & Cho, J. (2020). Mangosteen pericarp and its bioactive xanthones: Potential therapeutic value in Alzheimer's disease, Parkinson's disease, and depression with pharmacokinetic and safety profiles. *International Journal of Molecular Sciences, 21(17),* 6211.

Górnicka, J., Mika, M., Wróblewska, O., Siudem, P., & Paradowska, K. (2023). Methods to improve the solubility of curcumin from turmeric. *Life, 13(1),* 207.

Guo, M., Wang, X., Lu, X., Wang, H., & Brodelius, P. E. (2016). α-Mangostin extraction from the native mangosteen (Garcinia mangostana L.) and the binding mechanisms of α-mangostin to HSA or TRF. *PLOS One, 11(9),* e0161566. https://doi.org/10.1371/journal.pone.0161566

Gurunath, S., Pradeep Kumar, S., Basavaraj, N. K., & Patil, P. A. (2013). Amorphous solid dispersion method for improving oral bioavailability of poorly water-soluble drugs. *Journal of Pharmacy Research, 6(4),* 476–480.

Hassan, S., Adam, F., Abu Bakar, M. R., & Abdul Mudalip, S. K. (2019). Evaluation of solvents' effect on solubility, intermolecular interaction energies and habit of ascorbic acid crystals. *Journal of Saudi Chemical Society, 23(2),* 239–248.

Hotarat, W., Phunpee, S., Rungnim, C., Wolschann, P., Kungwan, N., Ruktanonchai, U., Rungrotmongkol, T., & Hannongbua, S. (2019). Encapsulation of alpha-mangostin and hydrophilic beta-cyclodextrins revealed by all-atom molecular dynamics simulations. *Journal of Molecular Liquids, 288,* 110965. https://doi.org/10.1016/j.molliq.2019.110965

Hotarat, W., Nutho, B., Wolschann, P., Rungrotmongkol, T., & Hannongbua, S. (2020). Delivery of Alpha-Mangostin Using Cyclodextrins through a Biological Membrane: Molecular Dynamics Simulation. *Molecules, 25(11),* 2532. https://doi.org/10.3390/molecules25112532

International Conference on Harmonisation (ICH) Harmonised Guideline. (2019). Biopharmaceutics classification system-based biowaivers: Biopharmaceutics classification of the drug substance. International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Retrieved January 27, 2025, from https://database.ich.org/sites/default/files/M9_Guideline_Step4_2019_1116.pdf

Iqbal, A., Muhammad Shuib, N. A., Darnis, D. S., Miskam, M., Abdul Rahman, N. R., & Adam, F. (2018). Synthesis and characterisation of rice husk ash silica drug carrier for α-mangostin. *Journal of Physical Science, 29,* 95–107.

Jain, H., & Chella, N. (2020). Methods to improve the solubility of therapeutical natural products: A review. *Environmental Chemistry Letters.* https://doi.org/10.1007/s10311-020-01082

Jain, S., Goossens, H., Picchioni, F., Magusin, P., Mezari, B., & van Duin, M. (2005). Synthetic aspects and characterization of polypropylene–silica nanocomposites prepared via solid-state modification and sol–gel reactions. *Polymer, 46(17),* 6666–6681. https://doi.org/10.1016/j.polymer.2005.05.021

Kalidason, A., & Kuroiwa, T. (2023). Nanoencapsulation of α-mangostin using chitosan-oleic acid complexes: Evaluation of storage stability, in vitro release properties in simulated digestive environment, and bioaccessibility. *Lebensmittel-Wissenschaft & Technologie, 188,* 115406. https://doi.org/10.1016/j.lwt.2023.115406

Kasimedua, S., Thoppani, S. R., Pommalab, N., Orugonda, G., & Yelamanda, J. (2015). A review on solubility enhancement techniques. *Journal of Comprehensive Pharmacy, 2,* 36–41.

Kaur, J., Aggarwal, G., Singh, G., & Rana, A. C. (2012). Improvement of drug solubility using solid dispersion. *Journal of Pharmacological Sciences, 4,* 47-53.

Kim, A., Pan-in, P., Wanichwecharungruang, S., & Hanes, J. (2014). Cellular trafficking and anticancer activity of garcinia mangostana extract-encapsulated polymeric nanoparticles. *International Journal of Nanomedicine,* 3677. https://doi.org/10.2147/ijn.s66511

Kumar, B. (2017). Solid dispersion—a review. *PharmaTutor, 5,* 24–29.

Lavilla, C., Gubbels, E., Martínez de Ilarduya, A., Noordover, B. A. J., Koning, C. E., & Muñoz-Guerra, S. (2013). Solid-state modification of PBT with cyclic acetalized galactitol and d-mannitol: Influence of composition and chemical microstructure on thermal properties. *Macromolecules, 46(11),* 4335–4345. https://doi.org/10.1021/ma400760d

Lee, W. J., Ng, C. C., Ng, J. S., Smith, R. L., Kok, S. L., Hee, Y. Y., … Chong, G. H. (2019). Supercritical carbon dioxide extraction of α-mangostin from mangosteen pericarp with virgin coconut oil as co-extractant and in-vitro bio-accessibility measurement. *Process Biochemistry.* https://doi.org/10.1016/j.procbio.2019.09.009

Li, J., Volpe, D. A., Wang, Y., Zhang, W., Bode, C., Owen, A., & Hidalgo, I. J. (2011). Use of transporter knockdown Caco-2 cells to investigate the in vitro efflux of statin drugs. *Drug Metabolism and Disposition, 39(7), 1196–1202.* https://doi.org/10.1124/dmd.111.038075

Li, L., Brunner, I., Han, A. R., Hamburger, M., Kinghorn, A. D., & Frye, R. (2011). Pharmacokinetics of α-mangostin in rats after intravenous and oral application. *Molecular Nutrition & Food Research, 55 (1),* 67–74.

Limwikrant, W., Aung, T., Chooluck, K., Puttipipatkhachorn, S., & Yamamoto, K. (2019). Size reduction efficiency of alpha-mangostin suspension using high-pressure homogenization. Chemical and *Pharmaceutical Bulletin, 67(4),* 389–392. https://doi.org/10.1248/cpb.c18-00589

Łuczak, J., Kroczewska, M., Baluk, M., Sowik, J., Mazierski, P., & Zaleska-Medynska, A. (2023). Morphology control through the synthesis of metal-organic frameworks. *Advances in Colloid and Interface Science, 314,* 102864. https://doi.org/10.1016/j.cis.2023.102864

Mantri, R. V., Sanghvi, R., & Zhu, H. (2017). Solubility of Pharmaceutical Solids. *Developing Solid Oral Dosage Forms,* 3–22. https://doi.org/10.1016/b978-0-12-802447-8.00001-7

Mardianingrum, R., Endah, S. R., Daruwati, I., Muchtaridi, M., & Ruswanto, R. (2024). Synthesis and computational study of metal complex of α-mangostin as an anticancer candidate. *Journal of Pharmacy & Pharmacognosy Research, 12(3),* 423–438. https://doi.org/10.56499/jppres23.1827_12.3.423

Meylina, L., Muchtaridi, M., Joni, I. M., Mohammed, A. F., & Wathoni, N. (2021). Nanoformulations of α-mangostin for cancer drug delivery system. *Pharmaceutics, 13(12),* 1993. https://doi.org/10.3390/pharmaceutics13121993

Mudalip, S. K., Bakar, Mohd. R., Adam, F., & Jamal, P. (2013). Structures and hydrogen bonding recognition of mefenamic acid form I crystals in mefenamic acid/ethanol solution. *International Journal of Chemical Engineering and Applications,* 124–128.

Narasimhan, S., S. Maheshwaran, I. A. Abu-Yousef, A. F. Majdalawieh, J. Rethavathi, P. E. Das and Poltronieri. P. (2017). Anti-Bacterial and Anti-Fungal Activity of Xanthones Obtained via Semi-Synthetic Modification of alpha-Mangostin from *Garcinia mangostana*. *Molecules 22(2).*

National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 3672, Ibuprofen. Retrieved August 21, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Ibuprofen.

Pan-in, P., Tachapruetinun, A., Chaichanawongsaroj, N., Banlunara, W., Suksamrarn, S., & Wanichwecharungruang, S. (2014). Combating *Helicobacter pylori* infections with mucoadhesive nanoparticles loaded with *Garcinia mangostana* extract. *Nanomedicine, 9(3),* 457–468. https://doi.org/10.2217/nnm.13.30

Peltonen, L., & Strachan, C. J. (2020). Degrees of order: a comparison of nanocrystal and amorphous solids for poorly soluble drugs. *International Journal of Pharmaceutics*, 119492. doi:10.1016/j.ijpharm.2020.119492

Phunpee, S., Suktham, K., Surassmo, S., Jarussophon, S., Rungnim, C., & Soottitantawat, A. (2018). Controllable encapsulation of α-mangostin with quaternized β-cyclodextrin grafted chitosan using high shear mixing. *International Journal of Pharmacy, 538,* 21–29.

Pham, D. T., Saelim, N., & Tiyaboonchai, W. (2019). Alpha mangostin loaded crosslinked silk fibroin-based nanoparticles for cancer chemotherapy. *Colloids and Surfaces Biointerfaces, 181,* 705–713. doi:10.1016/j.colsurfb.2019.06.011

Pranjali, W. C., Tushar, A. S., & Anup, M. A. (2018). Effect of trimethoprim inclusion complexation with cyclodextrins on its antimicrobial activity. *Chemical Methodologies, 3,* 211–225.

Ramaiya, A., Li, G., M. Petiwala, S., & J. Johnson, J. (2012). Single-dose oral pharmacokinetic profile of α-mangostin in mice. *Current Drug Targets, 13,* 1698–1704.

Rodde, M. S., Divase, G. T., Devkar, T. B., Tekade, A. R. (2014). Solubility and bioavailability enhancement of poorly aqueous soluble atorvastatin: In vitro, ex vivo, and in vivo studies. *BioMed Research International,* 463895. https://doi.org/10.1155/2014/463895

Sakpakdeejaroen, I., Muanrit, P., Panthong, S., & Ruangnoo, S. (2022). Alpha-Mangostin-Loaded Transferrin-Conjugated Lipid-Polymer Hybrid Nanoparticles: Development and Characterization for Tumor-Targeted Delivery. *The Scientific World Journal, 2022,* 9217268. https://doi.org/10.1155/2022/9217268

Samprasit, W., Kaomongkolgit, R., Sukma, M., Rojanarata, T., Ngawhirunpat, T., & Opanasopit, P. (2014). Mucoadhesive electrospun chitosan-based nanofibre mats for dental caries prevention. *Carbohydrate Polymers, 117,* 933–940. https://doi.org/10.1016/j.carbpol.2014.10.026

Samprasit, W., Opanasopit, P., & Chamsai, B. (2021). Mucoadhesive chitosan and thiolated chitosan nanoparticles containing alpha mangostin for possible colon-targeted delivery. *Pharmaceutical Development and Technology, 26(3),* 362–372. doi:10.1080/10837450.2021.1873370

Sanphui, P., Goud, N. R., Khandavilli, U. B. R., & Nangia, A. (2011). Fast dissolving curcumin cocrystals. *Crystal Growth & Design, 11(9),* 4135–4145. doi:10.1021/cg200704s

Savjani, K. T., Gajjar, A. K., & Savjani, J. K. (2012). Drug solubility: importance and enhancement techniques. *ISRN Pharmacology,* 1–10. DOI: 10.5402/2012/195727.

Shah, H., Nair, A. B., Shah, J., Jacob, S., Bharadia, P., & Haroun, M. (2021). Proniosomal vesicles as an effective strategy to optimize naproxen transdermal delivery. *Journal of Drug Delivery Science and Technology, 63,* 102479. doi:10.1016/j.jddst.2021.102479

Singh, J., Walia, M., & Harikumar, S. L. (2013). Solubility enhancement by solid dispersion method: a review. *Journal of Drug Delivery and Therapeutics, 3,* 148–155.

Suhandi, C., Wilar, G., Lesmana, R., Zulhendri, F., Suharyani, I., Hasan, N., & Wathoni, N. (2023). Propolis-Based Nanostructured Lipid Carriers for α-Mangostin Delivery: Formulation, Characterization, and In Vitro Antioxidant Activity Evaluation. *Molecules*, *28(16),* 6057. https://doi.org/10.3390/molecules28166057

Suthammarak, W., Numpraphrut, P., Charoensakdi, R., Neungton, N., Tunrungruangtavee, V., Jaisupa, N., Charoensak, S., Moongkarndi, P., & Muangpaisan, W. (2016). Antioxidant-enhancing property of the polar fraction of mangosteen pericarp extract and evaluation of its safety in humans. *Oxidative Medicine and Cellular Longevity,* 1293036.

Taher, M., Mohamed Amiroudine, M. Z. A., Tengku Zakaria, T. M. F. S., Susanti, D., Ichwan, S. J. A., Kaderi, M. A., … Zakaria, Z. A. (2015). α-Mangostin improves glucose uptake and inhibits adipocytes differentiation in 3T3-L1 cells via PPARγ, GLUT4, and leptin expressions. *Evidence-Based Complementary and Alternative Medicine,* 1–9.

Tatiya-aphiradee, N., Chatuphonprasert, W., & Jarukamjorn, K. (2019). Anti-inflammatory effect of *Garcinia mangostana* Linn. pericarp extract in methicillin-resistant *Staphylococcus aureus*-induced superficial skin infection in mice. *Biomedicine & Pharmacotherapy, 111,* 705–713.

Taniguchi, C., Kawabata, Y., Wada, K., Yamada, S., & Onoue, S. (2014). Microenvironmental pH-modification to improve dissolution behavior and oral absorption for drugs with pH-dependent solubility. *Expert Opinion on Drug Delivery, 11(4),* 505–516. doi:10.1517/17425247.2014.881798

Ting, J. M., Porter, W. W., Mecca, J. M., Bates, F. S., & Reineke, T. M. (2018). Advances in Polymer Design for Enhancing Oral Drug Solubility and Delivery. Bioconjugate Chemistry, 29(4), 939–952. doi:10.1021/acs.bioconjchem.7b006

Tran, P., Pyo, Y.-C., Kim, D.-H., Lee, S.-E., Kim, J.-K., & Park, J.-S. (2019). Overview of the manufacturing methods of solid dispersion technology for improving the solubility of poorly water-soluble drugs and application to anticancer drugs. *Pharmaceutics, 11(3),* 132. https://doi.org/10.3390/pharmaceutics11030132

Vermeersdch, H. (2016). Solubility and permeation studies using soluplus® and HPMC with a BCS class II amorphous drug. *Journal of Pharmaceutical Research International, 18,* 1–40.

Wathoni, N., Rusdin, A., Motoyama, K., Joni, I. M., Lesmana, R., & Muchtaridi, M. (2020). Nanoparticle drug delivery systems for α-mangostin. *Nanotechnology, Science and Applications, 13,* 23–36. https://doi.org/10.2147/nsa.s243017

Witharamage, C. S., Christudasjustus, J., & Gupta, R. K. (2021). The effect of milling time and speed on solid solubility, grain size, and hardness of Al-V alloys. *Journal of Materials Engineering and Performance, 30(4),* 3144–3158. https://doi.org/10.1007/s11665-021-05663-x

Yang, S., Gao, X., He, Y., Hu, Y., Xu, B., Cheng, Z., Xiang, M., & Xie, Y. (2019). Applying an innovative biodegradable self-assembly nanomicelles to deliver α-mangostin for improving anti-melanoma activity. *Cell Death & Disease, 10(3).* https://doi.org/10.1038/s41419-019-1323-9

Yasir, M., Asif, M., Kumar, A., & Aggarval, A. (2010). Biopharmaceutical classification system: an account. International Journal of PharmTech Research, 2, 1681–1690.

Zhang, K. J., Gu, Q. L., Yang, K., Ming, X. J., & Wang, J. X. (2017). Anticarcinogenic effects of α-mangostin: A review. *Planta Medica, 83(3-4),* 188–20.

Journal of Pharmacy (JOP)

Published by Kulliyyah of Pharmacy,
International Islamic University Malaysia
(IIUM)

Scan to access our website