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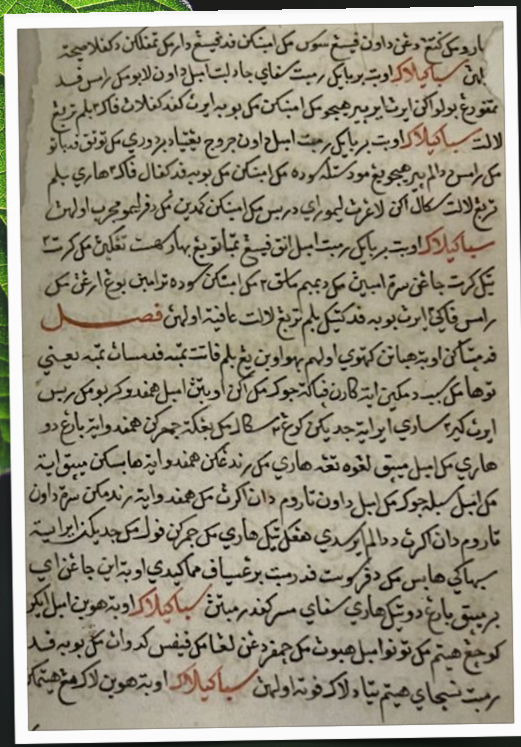

MSS 1292 PNM p.28

5-6 September 2023
via Online Platform

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4TH INTERNATIONAL CONFERENCE ON MALAY MEDICAL MANUSCRIPTS 2023

"TOWARDS MAINSTREAMING MALAY MEDICAL MANUSCRIPTS"

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ICOMMM 5-6 SEPT 2023

FOREWORD BY THE RECTOR OF IIUM

PROFESSOR EMERITUS TAN SRI DATO DZULKIFLI ABDUL RAZAK

In the name of Allah, the Most Compassionate, the Most Merciful.

Over the years, IIUM has made a tremendous effort to realise its vision for the betterment of human life and civilisation. We continuously look for new concepts, and approaches and support every initiative that leads to the realisation of the vision.

Islamisation of knowledge requires the first step of decolonisation. The principles of each disciple need to be constructed anew where necessary or revived from our own history, culture and philosophy. In terms of medicine and health sciences, while there is already a huge amount of materials on Islamic medicine the contribution of Malay Muslims in the field has been largely overlooked.

We at IIUM take the initiative to establish what their contributions were, and what could be benefited from it by the present globalised world. Since its first installation in 2015, the International Conference on Malay Medical Manuscript has provided a strong foundation and platform to gather scholars, researchers and historians to assess the Malay or Nusantara medical ideas and approaches to be applied in the diverse scientific backgrounds.

On behalf of the university, I would like to congratulate and thank the organising committees and everyone who has been working directly and indirectly to ensure the success of this conference. I wish you all the best and I hope your presence in this virtual conference will be a memorable one. Thank you and wassalam.

FOREWORD BY THE DEAN OF THE KULLIYYAH OF ALLIED HEALTH SCIENCES

PROFESSOR AHMAD AIDIL ARAFAT DZULKARNAIN

In the name of Allah, the Most Gracious Most Merciful.

All praises to Allah, Lord of the Worlds, and to His Messenger, Prophet Muhammad, peace and blessings be upon him, his family and companions. There is no power nor strength except through Allah.

Greetings and Welcome to the International Conference on Malay Medical Manuscript 2023 with the theme of "Towards Mainstreaming Malay Medical Manuscript."

As the Dean of the Faculty of Allied Health Sciences at the International Islamic University Malaysia (IIUM), it is both an honour and a privilege to extend a warm and cordial welcome to all the distinguished delegates, researchers, academicians, and participants from around the world joining us virtually for this significant event.

The theme of this conference, "Towards Mainstreaming Malay Medical Manuscript," reflects the growing recognition of the invaluable contributions of Malay traditional medicine to the field of healthcare. Malaysia boasts a rich heritage of traditional healing practices rooted in Malay culture, encompassing a wealth of knowledge encapsulated within ancient manuscripts. These manuscripts are a treasure trove of traditional medicinal wisdom that has been passed down through generations. This conference is an acknowledgment of the importance of preserving and integrating this knowledge into contemporary healthcare practices.

In the last 20 years ago, there has been a concerted effort by the Ministry of Health Malaysia to establish a dedicated division for Traditional and Complementary Medicine (T&CM). This initiative underscores the government's commitment to recognising the significance of traditional healing methods within the healthcare system. By organizing this conference, we hope to foster a closer collaboration between academia, healthcare practitioners, and policymakers to effectively integrate Malay traditional medicine into mainstream healthcare.

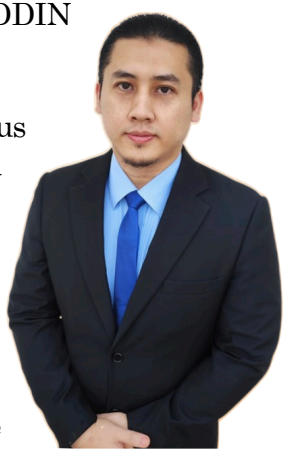
The COVID-19 pandemic has underscored the importance of holistic healthcare approaches, including traditional and complementary medicine. Many individuals have turned to alternative methods to boost their immunity, alleviate stress, and enhance overall well-being during these challenging times. The knowledge embedded in Malay medical manuscripts offers a unique perspective on preventive and therapeutic interventions, making it increasingly relevant in the post-COVID era.

As we gather virtually for this conference, I am filled with hope and anticipation. I hope that this platform will serve as a catalyst for fruitful discussions, innovative research, and collaborations that will not only enhance our understanding of Malay traditional medicine but also contribute to its integration into the broader healthcare landscape. Through your collective efforts, we can make substantial strides towards promoting the mainstreaming of Malay medical manuscript knowledge, ensuring its rightful place in contemporary healthcare practices.

In conclusion, I extend my sincere gratitude to the organizing committee, presenters, and participants for your dedication and commitment to advancing the field of Malay traditional medicine. Let this conference be a testament to our shared vision of a future where the wisdom contained in these manuscripts is cherished, respected, and harness-ed to improve the health and well-being of our communities. pave the way towards a healthier and more holistic approach to healthcare, rooted in our rich Malay heritage.

WELCOMING REMARKS BY THE PROGRAMME MANAGER OF ICOMMM 2023

ASSISTANT PROFESSOR DR IZZUDDIN AHMAD NADZIRIN



In the name of Allah, the Most Gracious Most Merciful. All praises to Allah, Lord of the Worlds, and to His Messenger, Prophet Muhammad, peace and blessings be upon him, his family and companions. There is no power nor strength except through Allah.

Please allow me to welcome all delegates of the International Conference on Malay Medical Manuscripts (ICOMMM) 2023! This year's theme is Towards Mainstreaming Malay Medical Manuscript.

Malay medical manuscripts harbour a lot of medicinal information that have been preserved since hundreds of years ago. They serve as the documented records of how Malay traditional medicine was practised in the past. The notion saying that Malay traditional medicine is primarily based on superstition is widely known especially among the Malaysian citizens.

However, the findings from the studies on Malay medical manuscripts have proven otherwise. The work on Malay Medical Manuscripts has been started more than 10 years ago. Since then, numerous findings and information have been unearthed. Most of medicinal content preserved in the Malay medical manuscripts actually have scientific bases, which are not known to many. The superstitious content is only minimum and definitely does not define Malay traditional medicine. Nevertheless, most of the works stay on paper and the public have yet to benefit much of these works.

Therefore, we as the main players in this field need to put bludgeoned effort to ensure that the content of Malay medical manuscripts can be mainstreamed at par with other traditional and complementary medicines such as Chinese and Indian traditional medicine, homeopathy and chiropractic. Though Malay traditional medicine is also listed in the Traditional and Complementary Medicine Division, Ministry of Health Malaysia, but it is more confined to Malay massage.

Mainstreaming this requires a concerted effort from various parties including researchers, medical doctors, Malay traditional practitioner, policy makers and also public. This conference is one way to disseminate the discoveries that have been made in the field of Malay medical manuscript especially the findings since the last ICOMMM in 2020. Pursuant to this conference, we hope there will be more collaborations happening among all parties to increase the visibility and uplift the dignity of Malay medical manuscripts.

Finally, this event could not have taken place without the support from our honourable IIUM Rector, Prof. Emeritus Tan Sri Dato' Dzulkifli Abdul Razak, Flagship leader Assoc. Prof. Dr. Mohd Affendi Mohd Shafri and the Dean of Faculty of Allied Health Sciences, Prof. Ahmad Aidil Arafat bin Dzulkarnain. I am sure that all delegates cannot wait to consume new knowledge shared by our colleagues. Hopefully we will all find this ICOMMM captivating .

INTRODUCTION

Within the Malay World's context, Islamisation of medical science requires the existence of a continuous, integrated study on Islamic-Malay works within medical science and related fields – medicine, pharmacy, food science, botany, zoology, etc.

The works of Malay Muslim intellectuals in previous centuries that are fully Islamic and rich in humanity would reveal the character of Islamic science which are scientific and pragmatic, yet remain vigorously tawhidic in its nature, principles and visions.

Their experiences and interpretations of science within the framework of tawhid are to be reclaimed, reintroduced and shared in formats such as annotated and non-annotated transliterations, publication of anthologies of manuscripts, and catalogues of works at remaining scriptoriums.

THEME

Conference main theme: Abstracts should be focusing on the study of Malay medical manuscripts.

All abstracts and/or papers submitted for presentation and/or publications in ICOMMM2023 will be peer-reviewed. Abstracts and/or papers are evaluated and accepted for presentation and/or publication in special issue of IJAHS is based on the conference theme and sub-themes as follow:Themes is specific to Malay medicine and will include but not limited to:

1. Theme 1: Manuscript study
   Any philology work, transliteration and critical appraisal of Malay medical manuscripts or old documents with information in Malay medicine.

2. Theme 2: Social history of Malay medicine – gender, economics, politics and religion-related issues
   Studies which are based on manuscripts or old documents, with relevance to Malay medicine or healthcare system in the Malay Archipelago

3. Theme 3: Databasing natural resources from Malay medical manuscripts
   Studies which are based on manuscripts or old documents, with relevance to Malay medicine or healthcare system in the Malay Archipelago

4. Theme 4: Incorporation of Malay traditional medicine into mainstream practice

Any work that deals with the effort of mainstreaming Malay medicine in the contemporary healthcare

5. Theme 5: Policy making – Act 775, an act on Traditional and Complementary Medicine in Malaysia Research, comments and analysis on Act 775

6. Theme 6: Modern research, tools and ideas to innovate the Malay medicine studies, research or innovations which are founded based on ideas, philosophy, principles or methodology from the Malay medical manuscripts; such as empirical studies that relate remedies (natural products) with their respective diseases as mentioned in manuscript(s), or optimisation of medical formulations as described in the Malay manuscript(s).

TENTATIVE: DAY 1

8.30am : Registration

9.00am : Quran and Doa recitation
Mohd Arsyam Daslam
Mohammad Nabhan Lutfi

9.10am : Welcoming remarks
Asst. Prof. Dr.Izzuddin Ahmad Nadzirin
ICOMMM 2023 Program Manager

9.20am : Officiating Ceremony
Prof. Emeritus Tan Sri Dato' Dzulkifli Abdul Razak
IIUM Rector

9.35am : Keynote Speaker I
Dr Goh Cheng Soon
Director Traditional and Complementary Medicine Division, Ministry of Health Malaysia

10.00am : Break

10.15am : Keynote Speaker II
Assoc. Prof. Dr. Pravit Akarasereenont
Head Center of Applied Thai Traditional Medicine (CATTM), Mahidol University, Thailand

11.15am : Oral Presentation Session I

1.00pm : Break

2.30pm : Oral Presentation Session II

4.30am. : End of first day

TENTATIVE: DAY 2

9.00am : Quran and Doa recitation
Mohd Arsyam Daslam
Mohammad Nabhan Lutfi

9.15am : Oral Presentation Session III

11.15am : Break

11.30am : Keynote Speaker III
Dr Intan Azura Shahdan
Senior Lecturer
Kulliyyah of Allied Health Sciences.

12.30pm : Break

2.00pm : Keynote Speaker IV
Dr. Viswajanani J. Sattigeri
Head CSIR-Traditional Knowlede Digital Library (CSIR-TKDL) Unit, New Delhi

3.00pm : Closing remark and disperse

ORAL PRESENTATION SESSION I

OP1: AP DR. MOHD AFFENDI MOHD SHAFRI
Cacar in Pontianak: the history of a smallpox pandemic from the perspective of Tabib Umar bin Harun in MSS3789

OP2: FARAH NAZIHAH ZULKIFLI
Shingles treatment from Malay Medical Manuscript MSS 3048: a descriptive and in silico study between the plant active ingredients and P2X4 receptor for antinociception

OP3: NORFARIHAH AHMAD RADZAUDIN
Shingles remedies based on analysis of accordion-folded Malay Medical Manuscript MSS 3048

OP4: PRIYO JOKO PURNOMO
Malay and Javanese perspective on cough treatment based on 19th century medical manuscripts

OP5: SYASYA SYAFIQAH MAHDI
Digestive problems in transliterated Malay Medical Manuscripts: ancient remedies and their pharmacological effects

ORAL PRESENTATION SESSION II

OP6: DR NURUL WAHIDAH FAUZI
Etika pemakanan berdasarkan konsep nutrigenomik: analisis terhadap sumber hadith dan manuskrip perubatan Melayu terpilih

OP7: DR IZZUDDIN AHMAD NADZIRIN
Unveiling medicinal content from Malay Medical Manuscript MSS 4016: a descriptive study

OP8: DR NORHASNIRA IBRAHIM
Systematic literature review on traditional Malay medicine in Malay Medical Manuscripts

OP9: DR MARDHIAH MOHAMMAD
'Resdung' treatment among the Malay local healers in Kuantan, Pahang and in selected Malay Medical manuscripts

ORAL PRESENTATION SESSION III

OP12: DR SUHANA MAMAT
A scoping review on medicinal properties of piper betle (sirih) based on Malay Medical Manuscripts and scientific literatures

OP13: NUR 'ATIFAH BATRISYIA MOHAMMAD NADZRI HISHAM
The medical use and medicinal potential of selected limau (Citrus spp. and Triphasia sp.) as cure for diseases in selected Malay Medical Manuscripts

OP14: WARDINI ABDULLAH SANI
Data mining on fats and oils used in selected Malay Medical Manuscripts

OP15: AISYAH ZAWAWI
Analysis of abstract characteristic of materia medica from Malay Medical Manuscript MSS 4016

OP16: FARIYA PANDIT
Assessment of the hair treatment formulations from selected Malay Medical Manuscripts

AN OVERVIEW OF THE MEDICINAL USES OF FATS AND OILS FROM MALAY MEDICAL MANUSCRIPTS

Intan Azura Shahdan[1,2]* and Wardini Abdullah Sani[2]

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ABSTRACT

Introduction: Malay medical manuscripts (MMM) are documentations of Malay medical knowledge, composed of different types of disease together with their treatment which had been practiced in the past centuries. The medicinal values of fats and oils had also been mentioned in the manuscripts. Medicinal oils are widely distributed and applied within Southeast Asian nations, primarily driven by their adherence to traditional and therapeutic assertions. Despite the rampant uses of so-called traditional Malay oils, studies on the origin and efficacy of these Malay medicine are lacking. Thus, this study focused on the collection of information regarding the use of fats and oils as a treatment for the diseases described in Malay medical manuscripts. **Methods:** Malay medical manuscripts were selected based on their contents on fats and oils. Data on fats and oils were tabulated and sorted based on the types of fats and oils, types of diseases and modes of delivery. The International Classification of Diseases 11th Revision (ICD-11) was used as a guideline and reference in categorising medical conditions and symptoms in the MMM. **Results:** Based on six MMM which dated between the 17th and 19th centuries, 28 types of fats and oils, consisted of plants, animals and minerals have been identified. The total number of formulations using fats and oils was 232. *Minyak sapi, minyak lenga* and *minyak zaytun* were the most frequently mentioned oils whilst *lemak sapi* was the most frequently mentioned fat. By using the ICD-11 as a guideline, medical conditions which used fats and oils in their treatments can be divided into 15 categories. The foremost medical conditions in traditional Malay medicine that necessitate the utilisation of fats and oils include disorders of the digestive system, infections and parasitic infections, and musculoskeletal and connective tissues disorders. **Conclusion:** Undoubtedly, the Malay medical manuscripts are replete with extensive information regarding the usage and properties of medicated fats and oils. To mainstream the medical conditions described in the MMM with the current modern morbidity and mortality, ICD-11 tool has been adopted. A health data collection that is comparable to health data at the international level highlights the relevance of traditional medicine in the current healthcare setting.

Key words: essential oil, ethnomedicine, ghee, ICD-11, olive, sesame oil

INTRODUCTION

Oil-based medications are now becoming more popular than ever. People are turning to traditional and indigenous ways to improve their health, resorting to natural remedies rather than medication. Traditional medicinal oils are produced in Malaysia by a variety of manufacturers, from established pharmaceutical companies to smaller, traditional drug manufacturers. These include medicinal oils based on traditional Chinese and Malay medicine (Figure 1). Despite the widespread use of "traditional Malay" claims on the labels of these healing oils, studies on traditional Malay oil medicines are scarce. Studying ancient medical manuscripts, which contain extensive information on historical practices for treating diseases, is one approach to validating the traditional claims of such medicine.

Figure 1 Oil-based medicines sold in one of the largest retail hypermarket chains in Southeast Asia. All except the white packaged Nutmeg Oil Plus (labelled as TCM, traditional Chinese medicine) are claimed as traditional Malay healing oils.

Malay medical manuscripts (MMM), also known as Kitab Tib, contain traditional medical knowledge coded in Jawi-Malay language. There are hundreds of copies of the MMM in the world, and only about 20 of them have been studied and transliterated into romanised Malay books. The use of natural ingredients such as plants, animals, minerals, and spices to treat various types of diseases is evident in the MMM. Information about the exact parts of the plants and animals used as the remedies, the scales and measurements of the *materia medica*, the dos and don'ts (known as *petua* or *pantang larang* in Malay, respectively), the administration of medications as well as the concept of balance and homeostasis according to the Malay people is available in these MMM. The current review presented a compilation of medicinal fats and oils extracted from the MMM.

METHODOLOGY

Sources

Transliterated MMMs which are available in the IIUM Kuantan library and from the collection of members from the Department of Biomedical Sciences, Kulliyyah of Allied Health Sciences, IIUM were reviewed to extract information on fats and oils. Online dictionaries (e.g., Pusat Rujukan Persuratan Melayu; https://prpm.dbp.gov.my), glossaries of MMMs and available Malay dictionaries were used to define a list of the Malay terms related to fats and oils. Details on scientific, vernacular and family name of the plants

and animals were obtained from qualified sources like Forestry Image (https://www.forestryimages.org), National Parks Flora and Fauna (https://www.nparks.gov.sg/florafaunaweb) as well as Plants for a Future (https://pfaf.org).

Study selection and data analysis

The research employed a data mining approach to analyse selected transliterated MMM. The evaluation of these manuscripts was conducted using the Scientific Analysis of Kitab Tib Index (SAKTI-iMS), a tool developed by Mohd Shafri (2021a). Screening process was carried out based on four major criteria including author's profile, legibility and integrity of the text as well as amount of medical content in the manuscripts (Mohd Shafri, 2021a). Additionally, the presence of information on fats and oils was a key criterion for manuscript selection. Key words used include "*minyak*" (oil) and "*lemak*" (fats).

Types of medical conditions and morbidity were categorised into broader categories in accordance with the International Classification of Diseases, Eleventh Revision (ICD-11) by the World Health Organization (https://icd.who.int/browse11/l-m/en), with some modifications. For example, instead of categorising the morbidity based on Category 26: Supplementary Chapter Traditional Medicine Conditions, the symptoms and morbidity were classified according to various disorders and the body system (Category 1 to 20) of the ICD-11 (Figure 2). Certain illnesses that do not fit into a certain system, like fever or *sakit pusat* (belly button pain), were placed in the "Not classified signs and symptoms" category.

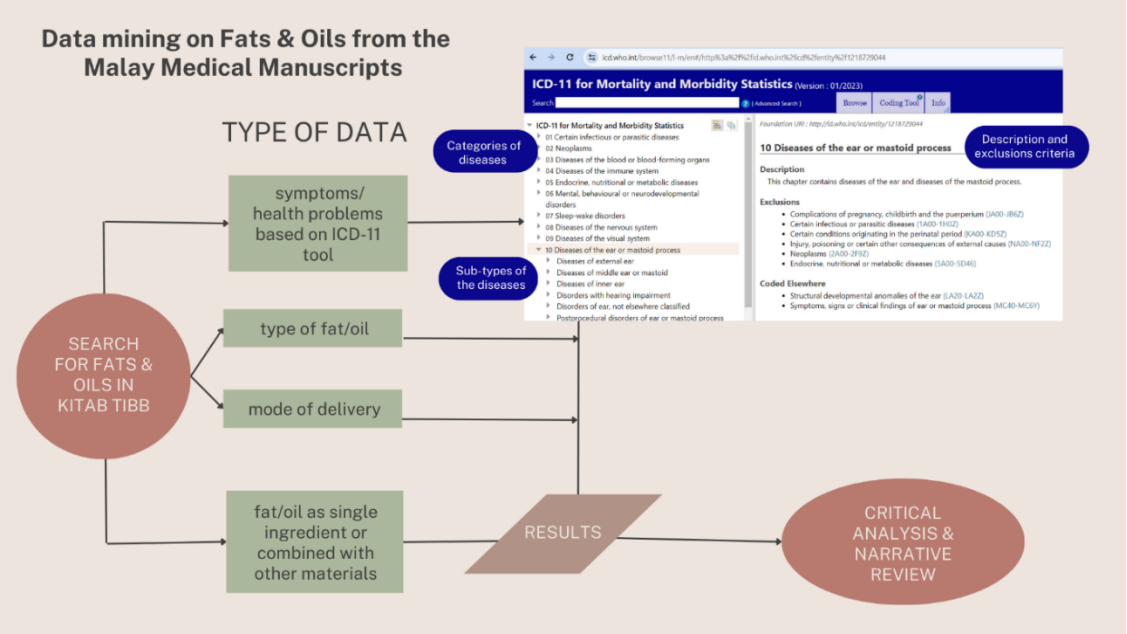

Figure 2 A flowchart on the data mining approach used in this study, using the International Categorisation of Diseases-11 (ICD-11) as a guide to group the medical conditions.

RESULTS

Overview of the Malay medical manuscripts

The study is an elaborated finding from the Kitab Perubatan Melayu Rumah Ubat di Pulau Penyengat book by Dr. Mohd. Affendi (Mohd Shafri, 2018a). In the final chapter of the book, a list of six Malay medical texts with the respective oils were mentioned (see page 60 and 61 of the book). The book compiles medicinal formulations using fats and oils from six MMM namely MSS 2999 (Hussain, 2015), Tayyib al-Ihsan fi Tibb al-Insan (Mohd. Shafri, 2018a), Al-Rahmah fi al-Tibb wa al-Hikmah (Mohd. Shafri & Muhammad Yahya,

2017), MSS 2515 (Mat Piah & Baba, 2013), MS 1988.400 and Rumah Ubat (Mohd. Shafri, 2018b) itself. Due to the unavailability of some of the texts, MSS 2515 and MS 1988.400 were not included in our narrative review. Instead, we have added medicated fats and oils from two other transliterated works which are Sari Segala Ubat (Mohd. Shafri, 2019) and Kebun Segala Raja-Raja Bab VII, Bustan Al-Salatin (Mohd. Shafri & Muhammad Yahya, 2022).

All six MMM in this study were rated as category A, signifying a high priority for research in fats and oils study. These manuscripts were qualified based on certain criteria, including having legible and intact text with over 80% completeness (Table 1). They also scored the highest '3' in terms of medical content, indicating that over 80% of manuscript's sections contained listed remedies. Additionally, a fifth criterion was introduced in this study, which assessed the presence of fats or oils as ingredients in the medicinal formulations. A score of '6' and more indicates that these manuscripts contained extensive information about fats and oils, and highly recommended for further analysis.

Table 1 Analysis of six transliterated Malay medical manuscripts using Index of Manuscripts Selection (SAKTI-iMS) based on Mohd Shafri (2021a)

Manuscripts±	Author' profile (x=score)	Text integrity (x=score)	Text legibility (x=score)	Medical content (physical treatment) (x=score)	Fats and Oils Content (x=score)	x Score	Grade	Priority for research
	Unknown (0); Known (1)	Incomplete (0); Complete >80% (1)	Illegible (0); Legibility >80% (1)	<5% (0); 5-50% (1); 50-80% (2); >80% (3)	None (0); Yes (1)			
TITI	1	1	1	3	1	7	A	High
ARFAH	1	1	1	3	1	7	A	High
SSU	1	1	1	3	1	7	A	High
MSS2999	0	1	1	3	1	6	A	High
RUPP	1	1	1	3	1	7	A	High
KSRR	1	1	1	3	1	7	A	High

± TITI=Kitab Tayyib Al-Ihsan, ARFAH=Kitab Al-Rahmah, SSU=Kitab Sari Segala Ubat, MSS 2999=MSS 2999 Kitab Tib,RUPP=Kitab Rumah Ubat Pulau Penyengat, KSRR=Kitab Kebun Segala Raja-Raja.

Although only seven fats and oils were used, Kitab Rumah Ubat contains the greatest number of formulations on fats and oils (n=98; 42%), followed by Tayyib al-Ihsan (n=59; 26%) and Al-Rahmah (n=50; 22%) (Table 2). Tayyib al-Ihsan and Al-Rahmah both contained 12 and 9 different fats and oils respectively. Kitab Kebun Segala Raja-Raja on the other hand, has only 5 formulations and therefore the least number of formulations using fats or oils. Only *minyak zaytun* and *sapi* were mentioned in this text. In total, 232 formulations using 27 known fats and oils were discovered in the six MMM used in this study (Figure 3).

Table 2 List of fats and oils used in the MMM. The numbers in the right-hand columns represent the frequency of medical benefits for each fat/oil.

No.	Family	Scientific Name	Oil	Vernacular Name	Malay Medical Manuscripts△ KSRR	TITI	ARFAH	SSU	MSS 2999	RUPP
	Plant-based oils±									
1	Amaryllidaceae	*Allium sativum*	*Bawang putih*	Garlic						17
2	Arecaceae	*Cocos nucifera**	*Kelambir*	Coconut			2	1		

			Nyiur tuha							
3	Asteraceae	*Anacyclus pyrethrum*	*Ud al-qarh*	Lungwort		2				
4	Caprifoliaceae	*Sambucus nigra*	*Balsan*	Black elder		2				
5	Cucurbitaceae	*Lagenaria spp.*	*Labu air*	Water gourd		3				
6	Dipterocarpaceae	*Dryobalonops aromaticum**	*Kapur*	Camphor tree			1			
7	Euphorbiaceae	*Ricinus communis*	*Jarak Burma*	Castor Bean		3				
8	Illiciaceae	*Illicium verum**	*Bunga lawang*	Star anise						8
9	Malvaceae	*Grewia laevigata**	*Sempelas*	*Akar sekapu, Akar sempelas*				1		
10	Myrtaceae	*Melaleuca leucadendra**	*Kayu putih*	Weeping paper bank			2			
11	Oleaceae	*Olea europaea*	*Zaytun*	Olive	2	8	10			
12	Pedaliaceae	*Sesamum indicum*	*Lenga*	Sesame		5	14	4	4	
13	Rosaceae	*Prunus dulcis*	*Lawz*	Almond		8				
14	Rosaceae	*Rosa spp.*	*Ward*	Rose		8				
15	Salicaceae	*Flacourtia rukam Zoll.**	*Ganda rukam*							3
16	Santalaceae	*Santalum album L.**	*Sandal*	Sandalwood			1			
17	Styracaceae	*Styrax benzoin**	*Kemenyan*	Balsamic resin						13
18	Violaceae	*Viola glabella, Viola Odorata*	*Banafsaj*	Sweet *violet*		6				
19	Zingiberaceae	*Zingiber officinale*	*Halia*	Ginger						30
	Animal-based oils									
20	Bovidae	*Ovis aries*	*Ekor kibas/ lemak ekor biri-biri*	Goat/ sheep			1	1		
21	Bovidae	*Bos taurus*	*Lembu/ sapi*	Ghee	3	17	19	4	3	
22	[Unknown]	[Unknown species]	*Summun*	Cooking butter		1				
	Mineral-based and miscellaneous oils									
23		-	*Belerang*	Sulphur						10
24		-	*Bulung*	Tar, asphalt, bitumen		2				
25		Sodium chloride, NaCl	*Garam*	Salt						17
26		Acetic acid, CH_3COOH	*Cuka*	Vinegar oil			1			

27	-	*Mekasar/ Mengkasar*	-	1	
28	-	*Bahu/bau*	-		1

±Native plants in the Malay Archipelago are marked in asterisks (*).
ΔThe number represents the frequency of formulations. KSRR=Kitab Kebun Segala Raja-Raja, TITI=Kitab Tayyib Al-Ihsan, ARFAH=Kitab Al-Rahmah fi al-Tibb wa al-Hikmah, SSU=Kitab Sari Segala Ubat, MSS 2999=MSS 2999 Kitab Tib,RUPP=Kitab Rumah Ubat Pulau Penyengat.

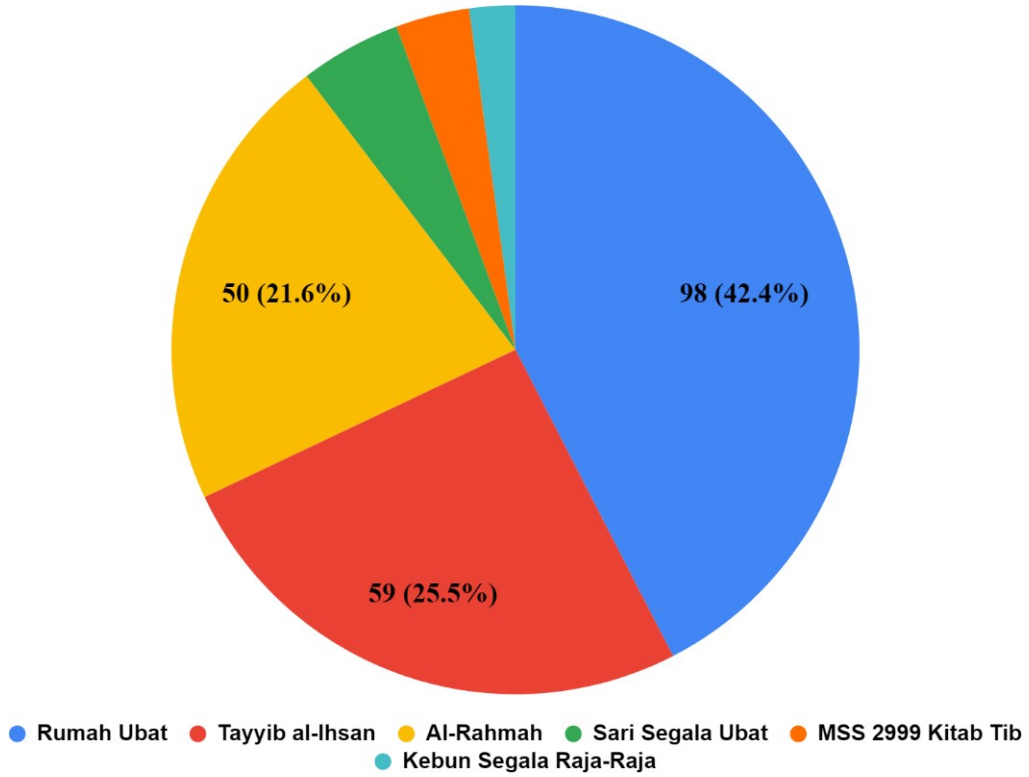

Figure 3 Distribution of medicinal formulations using fats and oils from selected MMMs

Types of fats and oils mentioned in the Malay medical manuscripts

The MMM use fats and oils derived from plants (70%), animals (11%) and other sources (19%). Plant-based oils come from 18 different plant families. Out of these, eight are known to be native plants of the Malay Archipelago (Table 2). One of these native plants, *Dryobalanops aromaticum* (kapur) is considered as a near threatened species whilst *Santalum album* L. (sandal) is a vulnerable species according to the Malaysia Biodiversity Information System (Chua et al., 2010). The vulnerable and endangered species are of interest because they are critical indicator of the world biodiversity. Butter and ox tail fats exemplify animal-derived fats, while mineral-based oils sourced from salt, asphalt, and sulfur represent miscellaneous fats and oils. Among the oils mentioned, only *minyak bahu* or *bau*, the latter denoting "scented" in Malay, remains unidentified. Notably, most medicated fats and oils are unique to specific MMM. For instance, despite its inclusion in 30 formulations for various conditions, *minyak halia* is exclusively prescribed in Kitab Rumah Ubat. Interestingly, all seven oils listed in Kitab Rumah Ubat, including *minyak bawang putih*, *bunga lawang*, *ganda rukam, kemenyan, belerang, cuka* and *halia* are unique to this text and absent in other MMM (Table 2). This exclusivity is particularly intriguing considering that Kitab Rumah Ubat, authored by the

renowned royal doctor Raja Haji Ahmad, is one of the most recent MMM originating from the era of the Riau Sultanate, where Raja Haji Ahmad was esteemed for his expertise in crafting medicated oils.

Despite being authored by individuals residing in diverse locations and eras over a period of 200 years, certain common medicated fats and oils are evident across the MMM. This could suggest some reliability in the oils used, and the familiarness of the co-ingredients as they were used for the same conditions. *Minyak kelambir (nyiur), zaytun, lenga as* well as *lemak ekor kibas* and *sapi* are fats and oils which can be found in two or more MMM (Table 2). Except from Kitab Rumah Ubat, *minyak/lemak sapi* emerged as the most frequently cited oil across all other MMM. Kitab Tayyib Al-Ihsan, Kitab Al-Rahmah and Kitab Sari Segala Ubat also mentioned rare oils such as *minyak cuka* (vinegar oil), *minyak ekor kibas* (sheep's tail oil), *minyak sempelas*, and *minyak sandal* (sandalwood oil).

Fats and oils based on their uses in various medical conditions

The majority of MMMs provide a succinct description of the illnesses and the appropriate therapies. Malay medical writings frequently employ a system-based or symptom-based methodology when describing the traditional remedies in the manuscripts. Categorising the formulations based on the ICD-11 revealed that the disorders of digestive system (n=36) have the greatest number of formulations using medicated fats and oils in the MMM, followed by the infections and parasitic diseases (n=32) and musculoskeletal and connective tissues disorders (n=27). Morbidity categorisation is arranged in the order of medical conditions with the greatest to the least number of formulations using fats and oils (Figure 3). Symptoms and medical conditions which do not match with any of the categories, are labelled as "Not classified signs and symptoms" (n=29). The following section describes each category and the associated medicated fats and oils prescribed in the MMM.

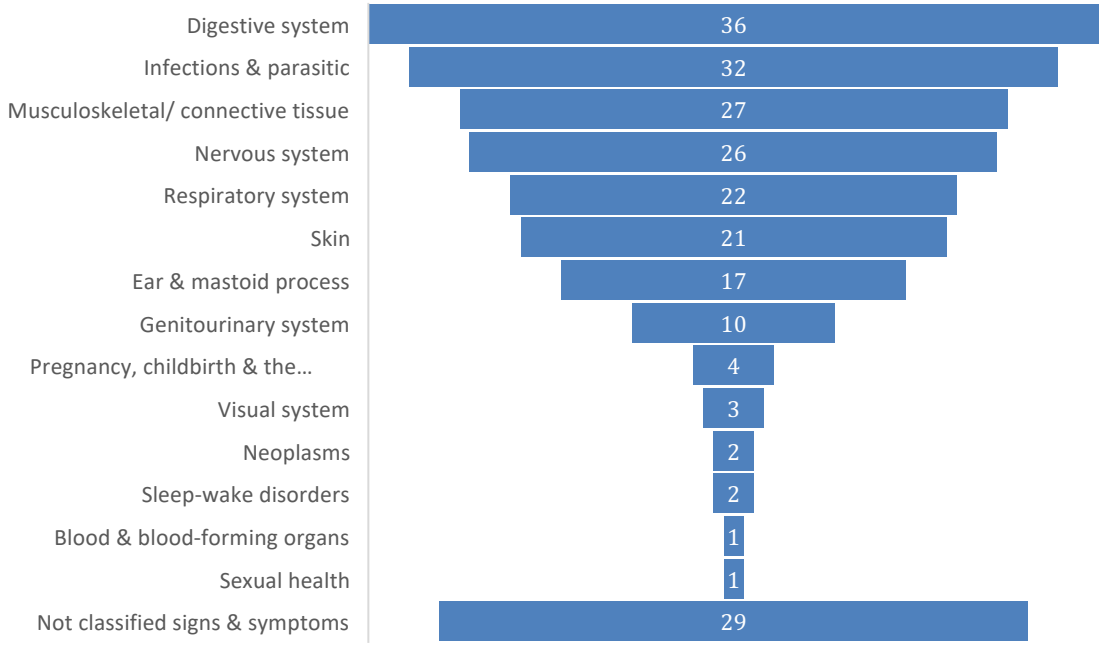

Figure 3 Frequency of formulations using medicated fats and oils in the MMM based on the medical conditions.

Medical conditions of the digestive system have the highest number of prescriptions using fats and oils in the MMM. Problems in the digestive system can be divided into mouth, upper gastrointestinal tract (GIT), lower GIT and hepatobiliary system (Table 4). *Minyak sapi* is the most common fat used and is prescribed

across all sub-groups of the digestive system. Many literatures recognised the use of *minyak sapi* or ghee in traditional Ayurvedic medicine (Kataria & Singh, 2024; Sharma et al., 2010). *Minyak halia, bawang putih* and *lenga* are also used commonly for the treatment of digestive problems, and in particular, for upper GIT conditions. Ethnomedicinal studies also show that these plants help in digestive problems (Okoro et al., 2023; Verma et al., 2023; Tesfaye, 2021; Khodaie et al., 2015; Ooi et al., 2022; Andargie et al., 2021). Haemorrhoids, a disorder of the lower GIT, is a common gastrointestinal disorder in MMM. Kebun Segala Raja-Raja, Tayyib al-Ihsan and Al-Rahmah have prescriptions for haemorrhoids using *minyak sapi* to make topical ointment. The fat is said to soothe pain when applied around the anal area. A narrative review on digestive problems and the respective traditional remedies from the MMM was presented as a conference presentation at the 4th International Conference on Malay Medical Manuscripts (Mahdi & Shahdan, 2023).

The second category in the list of medical conditions with the most formulations using fats and oils is the category of infections and parasitic infectious diseases. The communicable diseases are sub-grouped into viral, bacterial, parasitic infections as well as other causative agents (Table 4). In this category, *minyak sapi* has been found to be the common fat in treating all sub-groups of infectious diseases. The Malays often use common terms like *kurap, kudis* and *bisul* interchangeably to describe various skin problems. The terms are likely referred to bacterial, fungal infections or worm infestations. A total of 9 different fats and oils have been prescribed to treat these infections. Medications using fats and oils are also prescribed for ancient diseases such as leprosy, malaria and yaws in the MMM. For example, *minyak sapi* is one of the ingredients used in a remedy to treat ulceration of bone due to tertiary yaws (Abdul Halim et al., 2021).

The category with the third greatest number of formulations using fats and oils in the MMM is the musculoskeletal and connective tissues. Conditions of the musculoskeletal and connective tissues include signs and symptoms related to bone, knee, muscle sprain, backpain, joint pain as well as gait problems. The greatest number of fats and oils are the ones used to treat backpain, joint and knee pain (Table 4). Besides applying the fats and oils as ointment for massage, some of the formulations are also prescribed as decoction medicine, such as the ones for sprain, knee pain, joint pain and back pain. *Minyak garam* is the most used oil in this category and is used either orally or topically applied to painful body parts. The other medicated fats and oils are either prescribed once or twice in each condition (Table 4).

The fourth on the list is the nervous system. MMM contain numerous formulations to treat various disorders of the nervous system including epilepsy, stroke, paralysis, Bell's palsy, tremor, forgetfulness and numbness. *Minyak sapi, lenga* and *zaytun* are the top three medicated oils for treating nervous system-related conditions. Hemiplegia and general paralysis can be treated with single-ingredient or as mixtures of fats and oils – using different types of fats and oils (*minyak sapi, zaytun, lenga, halia* dan *garam*).

The respiratory system is the fifth most common medical conditions using fats and oils as their therapeutic agents. Coughs and respiratory-related illnesses have been described in many MMM. Raja Perdaus et al. (2021) produced a comprehensive review on the Malay medicine for cough and cough related problems and found 62 formulations comprised of 93 plant species, 6 animals and 2 minerals available in the MMM. Here, we compiled the diseases ranging from nasopharyngeal problems, cough, dyspnoea and chest pain, and their respective therapies using fats and oils. We found five different oils are used in the prescription to treat dry cough alone (Table 4). *Minyak lenga, lawz, balsan, banafsaj* and *sapi* are all prescribed for dry cough. Dry cough is usually treated with antitussive, decongestant, antihistamine and sometimes sedations if the patient is unable to sleep at night. Interestingly, *minyak lenga* is also used to treat phlegmy (productive) cough.

The skin came sixth place in the category of medical conditions using fats and oils. Skin problems are also considered as a common health condition in the MMM. This is evident in the book titled "*Dermatologi dalam Kitab Tib Muzium Terengganu*" by Hussain (2019) which gave extensive details about various formulations for skin problems, including skin infections. Here, we categorised skin infections in the category of infectious diseases and parasitic infections. Traditional formulations using fats and oils for

hair and scalp conditions, and body and face problems can also be found in the MMM. *Minyak belerang* is found to be the most common oil in treating itchy and urticaria-like symptoms. *Minyak belerang* is prescribed as a single ingredient and for topical use only. In Kitab Al-Rahmah, *minyak cuka, kapur* and *sandal* are also prescribed for boils, eczema and dry hair respectively.

Ear and mastoid process system is the seventh category which use fats and oils as part of their remedies. Ear conditions are divided into otitis media, hearing impairments and other ear problems. *Minyak bunga lawang* which is used as a single ingredient, is the most used medicated oil to treat all types of ear problems including foul-smelling and watery ear, otalgia and deafness. Rumah Ubat di Pulau Penyengat is the only Kitab Tib which used *minyak bunga lawang* as eardrops. In general, all oil medications are delivered as eardrops. Some oil medications are used with other co-ingredients. For example, in Tayyib al-Ihsan, otalgia with ringing sound can be treated using an eardrop made of *minyak lenga* with garlic, black pepper, *mustaki* and cloves. The procedure involves initially applying eardrops to the affected ear, followed by covering the ear canal with cotton before bedtime, leaving it in place overnight until the following morning.

The next in the category is the genitourinary system. In this study, genitourinary problems include urinary tract, female and male diseases. *Minyak sapi* is prescribed orally to treat urinating problems whilst *minyak bawang putih* is prescribed topically for the condition. For menorrhoea problems, formulations using *minyak bawang putih* and *halia* are prescribed. Scrotal swelling is treated with *minyak belerang* and *ud al-qarh*.

Traditional Malay medicine is popularly associated with the care and treatment for pregnancy, childbirth and puerperium. We found only a few formulations using fats and oils to treat medical conditions in this category. A study by the Malaysian Ministry of Health showed that women prefer to hire traditional masseur after giving birth, up to day 45th of postnatal. Most traditional masseurs would use their own home-made oil for the massage. The study found that some traditional masseurs use cold-pressed coconut oil (Akhiar, 2015). In this study, *minyak garam, sapi, halia* and *lenga* are prescribed for miscarriage, medications for postnatal care and for postpartum disorder. In addition, we also found a single formulation using a mixture of *minyak lenga* to treat postpartum disorder – a medical condition known as *meroyan* in Malay.

The tenth category of medical conditions is the eye system. Studies on the treatment for eye disorders in the MMM are published elsewhere (Mohd. Shafri, 2021b; Nadzirin, 2021). In this study, we found that eyesight can be improved with medicine using *minyak sapi*. Black cataract can be treated by mixing *minyak ward* and *banafsaj* together to make *celak* (Malay: eyeliner).

The eleventh category, which is the neoplasm system, is defined as medical conditions whereby an abnormal or uncontrolled cellular proliferation took place and is not coordinated with an organism's requirements for normal tissue growth, replacement or repair. This can be of benign or cancerous form of abnormal growth. *Barah* is a Malay term that is associated with tumour or cancerous condition. Interestingly, *minyak kemenyan* has been prescribed as a single ingredient to treat fungating tumour whilst *minyak lembu/sapi* is prescribed for scrofulosis (Table 4). Presumably both fat and oil are considered warm and hot in nature. Perhaps the principles and mechanisms of actions in treating *barah* are to use warm and hot-natured medicine. In fact, the traditional Chinese medicine has been using heat-clearing and detoxicating herbs as anticancer agents (Zhang et al., 2017).

Sleep-wake syndrome was previously categorised under the mental and behavioural disorders in the ICD-10. The increasing cases of insomnia and other sleep-related physiological and behavioural disorders might suggest WHO to nominate a special category in the ICD-11 for the sleep-wake syndrome. Tayyib al-Ihsan describes two formulations using ghee for "*penyakit kering kepala iaitu sedikit*

tidur", which can be literally transliterated as a 'sleepless empty-headed sickness'. The first formulation is prepared by mixing honey, sugar and rose water with ghee. The second formulation is prepared by adding yellow egg and sugar to ghee. Both mixtures are made by heating them gently, to form a pudding-like structure, known as *halwa*-a confectionary originated from the Middle East. It is believed that insomnia can be improved when taking the medicine before sleep.

In ICD-11, conditions which are related to blood and blood-forming organs are grouped as a distinguished category. The only one formulation extracted for this category is an anticoagulant remedy, a prescription which uses *minyak sapi* as one of the ingredients. In the remedy, cumin seeds (*jira putih*) are cooked with *minyak sapi*. Cumin seeds have been shown to inhibit platelet aggregation (Srivastava, 1989). Ghee contains high concentrations of monounsaturated omega-3 and this essential polyunsaturated fatty acid has been shown to have antiplatelet effects (Li et al., 2022), which could suggest the mechanism of ghee as an anticoagulant.

Another category with only one formulation using oil from the MMM is the category for conditions related to sexual health. Information and knowledge on sexual health is contained in substantial number of pages of many MMM. The information is not only limited to prescriptions of plants and herbs for the treatment, but also incantations and practical aspects of sexual intercourse. Sexual health is considered an important aspect of life in maintaining family institution in the Malay community, in accordance with the Islamic teachings (Mat Piah & Sobri, 2019). The only oil prescribed for this disorder is *minyak halia*. Men suffering with impotence is advised to drink *minyak halia* as well as apply the oil all over the body. Earlier studies supported the use of ginger in improving sexual dysfunction or erectile dysfunction (Akbari et al., 2017; Ferrini et al., 2015).

The last in this list is the not classified signs and symptoms category. Conditions which are less well-defined and symptoms that, without the necessary study of the case to establish a final diagnosis, could be designated 'not otherwise specified', 'unknown aetiology' or 'transient' are all grouped under this category. Fevers, oedema, body swelling, malaise and fatigue are some of the conditions that are considered in this group. Moreover, other injuries due to external consequences such as burn and trauma are also included. *Minyak sempelas* is prescribed as a supplement for a healthy body. *Minyak labu air, lawz, garam* and *halia* are ingredients to make various medicines for fever. *Minyak bawang putih, halia* and *kemenyan* are prescribed for general body swelling, malaise and fatigue. *Minyak zaytun* is used to treat burns and *minyak garam* is prescribed orally and topically to treat injuries due to trauma (Table 4).

Table 4 List of fats and oils prescribed in the MMM for various conditions and diseases.

Disease/Disorder	Malay term for disease/disorder	Fat/oil	Administration±
Digestive System			
Mouth			
Toothache (molar pain)	*Ubat sakit gigi geraham*	*Zaytun*	M, T
Swollen gum	*Bengkak gigi*	*Garam*	S, OT
Bleeding gum	*Ubat berdarah gusi dan bengkaknya dan binasa dagingnya*	*Lembu/sapi* *Lenga*	M, G M, G
Mouth ulcer	*Sakit Seriawan*	*Lembu/sapi*	M, T
Upper gastrointestinal tract			
Heartburn	*Bisa hati*	*Bawang putih* *Halia*	S, OT S, OT
Indigestion	*Menghancurkan makanan dalam perut*	*Halia*	S, OT
Dyspepsia, indigestion or heartburn	*Sakit hati/ sakit ulu hati*	*Lenga*	M, O
Stomach ache Stomach cramps Abdominal colic	*Bisa perut* *Sakit menggulung dalam perut* *Ubat bernyala-nyala panas dalam perut* *Rejan* *Sakit perut mulas*	*Bawang putih* *Halia* *Lembu/sapi*	S, T S, OT M, O
Bloated	*Kembung perut*	*Bawang putih* *Halia*	S, T S, OT
Nausea	*Mual* *Hendak muntah*	*Bawang putih* *Bawang putih*	S, T S, T
Fullness	*Senak* *Senuh hati (senak perut kerana kenyang atau penyakit)* *Qawlanj (senak perut)*	*Garam* *Jarak burma* *Lembu/sapi*	S, OT S, O M, NS
Make someone feel full	*Mengembangkan mi'dah*	*Lenga*	M, O
Lower gastrointestinal tract			
Diarrhoea (bloody)	*Sakit memulas yang lama dan berdarah*	*Lawz*	M, O

Diarrhoea	*Itlaq al-butuni (penyakit cirit perut)*	*Zaytun*	M, O
Haemorrhoids	*Buasir/ al-bawasir*	*Lembu/sapi*	S, M, T
Prolapsed rectum	*Ubat penyakit keluar dubur*	*Zaytun* *Ud al-qarh*	M, O S, B
Constipation/ tenesmus	*Ubat tiada buang besar* *Al-Zahir*	*Lembu/sapi*	M, O
Hepatobiliary system			
Jaundice	*Yurqan*	*Lembu/sapi*	M, O
Miscellaneous	*Sejuk hati*	*Halia*	S, OT
Certain infections & parasitic diseases			
Viral infection			
Shingles	*Kayap*	*Garam*	S, OT
Bacterial infection			
Leprosy	*Badam/ Al-Judhamah* *Kusta* *Budok*	*Ekor kibas* *Lembu/sapi* *Zaytun* *Ward*	M, O M, O M, O M, O
Scrofulosis	*Khanazir*	*Summon* *Ward*	M, T M, T
A highly contagious disease and fatal; might be caused by *Yersinia pestis*	*Sampar*	*Lenga*	M, NS
Syphillis	*Puru peringgi*	*Lenga*	M, NS
Parasitic infection			
Malaria	*Demam kura*	*Halia*	S, OT
Malaria-like intermittent fever	*Humma al-rib'*	*Lembu/sapi* *Lenga*	M, O S, O
Elephantiasis	*Da'u al-fil*	*Bulung*	S, T
Endemic	*Waba'*	*Banafsaj* *Lenga*	S, NS M, NS
Other causative agents			

Other skin infections	*Kudil/kudis/al-jarb*	*Lenga*	M, T
	Kuman pada tubuh	*Kemenyan*	S, T
Scabies-like, an itchy rash	*Kurap*	*Bulung*	M, T
	Tokak	*Lembu/sapi*	M, OT
Boils	*Bisul*	*Belerang*	S, T
		Cuka	M, T
		Gandarukam	S, T
		Kelambir	M, T
		Zaytun	M, T
Musculoskeletal & Connective Tissue			
Bone			
Bone pain	*Bisa di dalam tulang*	*Bawang putih*	S, T
Broken bone	*Patah tulang*	*Garam*	S, OT
		Halia	S, OT
Sprain	*Salah urat*	*Bawang putih*	S, T
		Halia	S, OT
		Garam	S, OT
		Kemenyan	S, T
Knee pain	*Ubat sakit lutut dan sendi*	*Lembu/sapi*	M, O
		Zaytun	M, T
		Lenga	M, T
Buttock pain	*Sakit papan punggung*	*Zaytun*	M, T
Backpain	*Sakit pinggang*	*Bawang putih*	S, T
		Halia	S, OT
		Kayu putih	S, T
		Kemenyan	S, T
		Garam	S, OT
Pain at the joints including backpain, knee pain	*Penyakit mufasil, segala sendi seperti sakit pinggang dan lutut (sejuk), berat sendi-sendi*	*Garam*	S, OT
		Jarak burma	M, O
		Kemenyan	S, T
		Ward	M, OT
		Zaytun	M, T
Joint pain	*Sakit urat*	*Kemenyan*	S, T
Difficulty in walking	*Berat kaki* *Tidak boleh berjalan jauh*	*Halia*	S, OT
Stabbing pain (in the limbs and/or ribs)	*Sakit mencucuk pada anggota* *Sakit menikam pada rusuk*	*Halia*	S, OT
Nervous system			
Epilepsy	*Sawan*	*Banafsaj*	M, O
	Al-Sira'	*Labu air*	M, T
Childhood epilepsy	*Umm al-sibyan*	*Zaytun*	S, O
		Lenga	S, O

Stroke	*Al–Saktah* *Angin ahmar*	*Lenga* *Zaytun*	M, T M, T
Hemiplegia	*Falij*	*Ekor kibas* *Lembu/sapi* *Zaytun* *Lenga*	M, NS M, T M, T M, T
Paralysis	*Lumpuh*	*Halia* *Garam*	S, OT S, OT
Bell's palsy	*Lawqah* *Lumpuh sebelah muka*	*Lawz*	S, OS
Tremor in elderly	*Kebuyutan*	*Halia*	S, OT
(Hot-headed) Headache	*Sakit kepala yang panas*	*Ward*	M, T
Light-headed	*Ringan kepala*	*Lembu/sapi*	M, O
Forgetfulness	*Ubat terlupa*	*Lembu/sapi* *Zaytun* *Kemenyan*	S, O M, T S, T
(Possibly) Vertigo	*Al-Dawran*	*Lenga* *Zaytun*	S, T S, T
Pain and numbness	*Ubat ngilu dan kebas*	*Nyiur tuha* *Bawang putih* *Halia*	M, T S, T S, OT
Respiratory system			
Nasopharynx Sinusitis	*Resdung*	*Belerang* *Lembu/sapi*	S, T M, T
Flu	*Al-Zukkam*	*Lenga*	M, O
Cough Dry cough	*Batuk kering* *Al - Su 'al-Yabis*	*Balsan* *Banafsaj* *Lawz* *Lembu/sapi* *Lenga*	S, O M, OT M, OT M, O M, NS
Cough due to cold weather	*Al-Su 'al al-Rihi*	*Lenga*	M, O
Cough with phlegm	*Ubat balgham (kahak)*	*Lenga*	M, O
Dyspnoea	*Rabw (lelah dan penyakit picik bernafas)* *Senak/sesak nafas* *Semput nafas*	*Balsan* *Bawang putih* *Halia*	S, OT S, T S, OT
Chest pain	*Ubat sakit dalam dada*	*Kayu putih*	M, OT

Skin			
Hair/Scalp			
Dry hair	*Ubat rambut (kering)*	*Lenga*	S, NS
		Zaytun	M, T
		Sandal	M, T
Wet hair	*Ubat rambut basah*	*Lenga*	M, T
		Zaytun	M, T
Pilar cyst	*Al-Damamil*[Δ]	*Kelambir*	M, T
		Lenga	M, T
Face & Body			
Itchy skin/ pruritis	*Gatal-gatal*	*Belerang*	S, T
		Garam	S, OT
		Kemenyan	S, T
		Lembu/sapi	M, NS
Hives/ urticaria	*Gelegata*	*Belerang*	S, T
Eczema	*Quba'*	*Kapur*	M, O
		Lembu/sapi	M, O
Cyst/Polyp/Boil	*Ubat kembung - kembung bisul*	*Lembu/sapi*	M, T
Acne/ pimples	*Jerawat*	*Lembu/sapi*	M, NS
Miscellaneous[¥]	*Keruk-keruk* *Kerat-kerat*	*Belerang*	S, T
Ear & Mastoid Process			
Otitis media			
Acute/chronic suppurative otitis media	*Sakit telinga bernanah*	*Bahu/bau* (unidentified)	S, E
Foul-smelling ear	*Telinga busuk*	*Bunga lawang*	S, E
Watery ear	*Telinga berair*		
Suppurative ear	*Telinga bernanah*		
Ear pain, with pus, infection and hearing impairment	*Sakit telinga dan bernanahnya dan ulatnya dan tulinya*	*Jarak Burma*	M, E
		Ward	M, E
		Lenga	M, E
Antimicrobial for ear infection	*Mati ulatnya*	*Zaytun*	M, E
Other ear problems			
Swollen ear	*Telinga bengkak*	*Bunga lawang*	S, E
Otalgia	*Bisa telinga*	*Bunga lawang*	S, E
	Waj' al - udhun	*Lenga*	M, E
Bleeding ear	*Telinga berdarah*	*Bunga lawang*	S, E
		Lenga	M, E
Hearing impairment			
Deafness	*Berat pendengaran*	*Bunga lawang*	S, E
		Lenga	M, E
	Tiada mendengar	*Zaytun*	M, E

Otalgia with hearing impairment (ringing sound)	*Ubat sakit telinga dan beratnya dan tulinya dan berdengungnya*	*Lenga*	M, E
Genitourinary System			
Urinary tract			
Increased frequency in voiding/ urinating	*Kerap kencing*	*Bawang putih*	S, T
Urinary continence	*Hasr al - Bawl*	*Lembu/sapi*	M, O
Difficulty to urinate	*Ubat payah kencing*	*Lembu/sapi*	M, O
Female diseases			
Oligomenorrhoea	*Perempuan tiada daras membawa adat*	*Bawang putih* *Halia*	S, T S, OT
Dysmenorrhoea	*Sakit senggugut*	*Bawang putih*	S, T
Pain in lower abdomen	*Bisa ari-ari*	*Garam*	S, OT
Pain in the upper part of the female groin area	*Sakit di bahagian atas kemaluan perempuan*	*Lawz*	S, OT
Male disease			
Scrotal swelling	*Bengkak-bengkak dhakar* *Patik*	*Belerang* *Ud al - qarh*	S, T S, OT
Pregnancy, Childbirth & Puerperium			
Miscarriage	*Perempuan keguguran anak*	*Garam*	S, OT
Postnatal medication	*Perempuan lepas beranak*	*Lembu/sapi* *Halia*	M, O S, OT
Postpartum disorder	*Meroyan*	*Lenga*	M, O
Sleep-wake Disorders			
Visual System			
Black cataract	*Kumnah*	*Banafsaj* *Ward*	M, T M, T
Short-sightedness/myopia	*Menambah jawhat penglihatan*	*Lembu/Sapi*	M, O
Neoplasms			
Fungating tumour	*Barah yang telah memecah*	*Kemenyan*	S, T
Scrofulosis	*Al-khanazir* *Barah*	*Lembu/sapi*	M, O

Insomnia	*Penyakit kering kepala iaitu sedikit tidur*	*Sapi*	M, O
Blood & Blood-Forming Organs			
To treat bleeding (anticoagulant)	*Mencair darah beku*	*Sapi*	M, NS
Sexual health			
Impotence	*Laki-laki lemah syahwat*	*Halia*	S, OT
Unclassified Signs & Symptoms and Injuries due to External Consequences			
Supplement to stay healthy	*Badan yang sihat*	*Sempelas*	M, O
Cold hand and feet	*Sejuk kaki tangan*	*Bawang putih* *Halia* *Kemenyan*	S, T S, OT S, T
Oedema	*Basal*	*Bawang putih* *Halia*	S, T S, OT
Swelling	*Bengkak-bengkak* *Sembab-sembab*	*Bawang putih* *Halia* *Kemenyan*	S, T S, OT S, T
Malaise, fatigue	*Tiada sedap badan* *Lemah tubuh* *Penat* *Berat badan* *Sakit tubuh*	*Garam* *Kemenyan* *Lenga*	S, OT S, T M, O
Fever Hectic fever Chill fever	*Demam* *Humma al-diq'* *Demam gigil*	*Labu air* *Lawz* *Garam* *Halia*	M, I M, I S, OT S, OT
Boil	*Bisul*	*Kemenyan*	S, T
Bellybutton pain	*Ubat sakit pusat*	*Lembu/sapi*	M, O
Wandering aimlessly (someone with mental disturbances)	*Orang yang berjalan berdiwana (jalan tanpa tujuan - gila)*	*Zaytun*	S, T
External injury Burn	*Terhangus api*	*Zaytun*	M, T
Crushed by heavy thing Beaten traumatic injury	*Terkena timpa satu benda berat* *Terkena pukul*	*Garam*	S, OT

[±]Administration and delivery method of the medicine. Fats and oils are administered as a single (S) ingredient or combined in a mixture (M). Delivery methods: B, blow; E, eardrop; G, gargle; I, inhalation; O, oral; OS, oral and sniffing/smelling; OT, oral and topical; T, topical. Formulation with no delivery method mentioned is depicted as NS (not specified).

The variety in modes of delivery would also be an interesting aspect to study. As mono-ingredients, *minyak sapi, minyak halia* and *minyak lenga* each can be used orally as well as for topical use. As mono-ingredients, *minyak bunga lawang* and *minyak bahu/bau* are the only ones prescribed as eardrops. *Minyak labu air* and *minyak lawz* on their own, can be used by inhalation. Gargling has been suggested as a method of delivery to treat bleeding gums, whilst blowpipe (Malay: *sumpit*) is used to treat prolapsed rectum. In the latter, a traditional remedy is inserted into the rectum by blowing it gently through the blowpipe. Some remedies can be taken through inhalation methods, such as the ones seen to treat fever (Table 4).

Mono-ingredient use of these fats and oils might be interesting for commercialisation. Some unusual oils, such as *minyak garam, minyak halia* or *minyak bawang putih*, might need further study to understand how the Malay people prepared them and categorised them as oils. Some prescriptions provide quantity and dosage for the oil usage, but others lack this information. Hence, it is possible that although it appears that most of the medicated oils listed in Rumah Ubat derived from single ingredients, these oils are merely the designated names given and were made using other spices and herbs that are common during the time (Mohd. Shafri, 2018a). Additionally, concerning medicated fats and oils, Kitab Rumah Ubat was discovered to be more comprehensive compared to other Malay medical manuscripts, focusing detailed formulations of oils. In Sari Segala Ubat, one particular oil called *minyak Mekasar,* was mentioned in detail in the book. According to the transliterated author, *minyak Mekasar (or Mengkasar)* is a famous medicated oil made by Raja Haji Ahmad Tabib, the Royal Doctor of the Riau-Lingga Sultanate in the 19th century. Yet, no details were available regarding the utilisation of the oil. *Minyak Mekasar* is concocted by blending and grinding six spices and herbs in a vessel, then interring the vessel underground for a period of time (see page 65 of the book, Mohd. Shafri, 2019).

CONCLUSION

Fats and oils have become one of the most vital parts in modern healthcare treatment. In this study, six Malay medical manuscripts, which dated between the 17th and 19th century, were found to have records on the medical use of fats and oils in Malay Archipelago. Collectively, 28 types of fats and oils and 232 formulations were identified throughout the findings. The three most common oils which are *minyak sapi* (ghee), *minyak lenga* (sesame oil) and *minyak zaytun* (olive oil) are mentioned 40, 27 and 21 times respectively in five out of six transliterated manuscripts. The integration of medical conditions outlined in MMM with contemporary morbidity and mortality data has been facilitated through the adoption of the ICD-11 tool. This enables the collection of health data that is internationally comparable, emphasising the significance of traditional medicine within the current healthcare landscape. Rather than aligning conditions solely with the ICD-11's Supplementary Chapter for Traditional Medicine, incorporating ancient medical conditions into present-day morbidity profiles underscores the continued relevance of traditional remedies.

While abundant literature exists regarding the medical claims of fats and oils when considered individually, the pharmacological efficacy of a singular substance might be restricted, failing to fully capture its therapeutic potential compared to when combined with adjuvants that augment its properties. Consequently, numerous traditional remedies are meticulously formulated as blends, incorporating a few ingredients alongside the primary active component to optimise therapeutic effects. This study can be useful to initiate more research and interests as well as strengthen the use of oils, particularly those which have been used over centuries as part of traditional knowledge. A better understanding of their medical benefits can be obtained by studying the pharmacokinetics and pharmacodynamics of the ancient remedies, not only in their extracted or single forms, but synergistically when various ingredients are formulated and blended as remedy mixtures, based on their prescriptions in the MMM. More studies need to be done to ascertain whether the fats and oils used are the active compound, stabiliser, lubricant or adjuvant compound in the formulations. In fact, having a complete understanding of the benefits and

drawbacks of these items will ensure that traditional medicine is still relevant, important and effective in the healthcare system towards achieving the universal healthcare coverage goals.

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THE MEDICAL USE AND MEDICINAL POTENTIAL OF SELECTED LIMAU (CITRUS SPP. AND TRIPHASIA SP.) AS CURE FOR DISEASES IN SELECTED MALAY MEDICAL MANUSCRIPTS

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ABSTRACT

Malay medicine has historically been one of the most prominent fields of study documented by Malay physicians. The Malay medical literature covers the materia medica and treatment recommendations for numerous illnesses. Citrus fruits, also known as *limau*, are one of the ingredients that are frequently mentioned in the texts. They are grown all over the world and are used extensively for cooking as well as medicinal purposes. This study was conducted to discover the medical use of citrus fruits in six Malay medical manuscripts namely MSS 2999 Kitab Tib, Sari Segala Ubat, Tayyib al-Ihsan fi tibb al-Insan, Kitab Tib MSS 1292, Kitab Tib MSS 2515, and Khazinat al-Insan as well as to identify its medicinal potential based on existing scientific studies. Data were tabulated according to the species of *limau*, sources of data, and diseases. The pharmacological properties of each *limau* were then identified via literature search in several databases namely PubMed, ScienceDirect, Scopus and Google Scholar. As a result, there are 165 prescriptions involving varieties of *limau*, which are *limau nipis* (key lime), *limau purut* (kaffir lime), *limau raya* (pomelo), *limau kerbau* (citron) and *limau kerisik* (limeberry) found in these manuscripts. Overall, *limau* was mentioned in prescriptions for diseases related to ear, eye, gastrointestinal, hair, men's health, musculoskeletal, nose, renal, respiratory, skin, teeth and women's health, including common illnesses like headache and fever. *Limau* was reported to possess pharmacological properties such as antidiarrheal, antimicrobial, anti-inflammatory, and anti-melanogenic, and provides analgesic effects and is used as a treatment for typhoid fever.

KEYWORDS: Malay medical manuscript, *limau nipis, limau purut, limau raya,* citrus fruits, Malay traditional medicine

INTRODUCTION

Malay medicine has traditionally been one of the most prominent subjects discussed by Malay physicians (Mohd Shafri, 2020). It demonstrates how medical science has emerged as an essential branch of knowledge during the past century, studied and practised by the Malay people in the past. Generally, Malay medical literature addresses the materia medica and treatment prescriptions for various ailments. According to Mat Piah and Mustapha (2019), there are illnesses about men's and women's health, as well as the eyes and ears referenced in the manuscripts. In addition, treatment prescriptions also include the use of flora and fauna as medicinal ingredients in treating diseases (Mat Piah, 2017).

Today, *limau*, commonly referred to as citrus fruits, is widely cultivated and utilized across the globe. Citrus plants have long been incorporated into traditional remedies worldwide, including in ancient medical practices. Traditional Chinese medicine has frequently used citrus as a core component in various treatment protocols. According to Karp and Hu (2018), citrus was thought to help balance *qi*, or life energy, in Traditional Chinese medicine. Besides, in the Malay civilization, Jelani and Noor Muhammad (2021) stated that *limau nipis* is the most used plant to treat diseases such as eye pain, hernia, and cough. In addition, citrus is rich in various vitamins and bioactive compounds that have numerous health benefits for the human body, such as vitamin C and flavonoids such as hesperidin,

narirutin and naringin (Miles & Calder, 2021). These components are antioxidants that aid in the reduction of oxidative stress in diseases such as obesity and cardiovascular disease.

Various publications, including magazines, newspapers, and online sources have suggested that traditional remedies used by the Malay community often incorporate *limau* as a common component for treating ailments. However, none of these sources cited any documented records related to Malay medicine. Therefore, this study is essential for providing a scientific examination of the Malay Medical Manuscript, aiming to counter unfounded claims and connect contemporary and historical treatments. Additionally, this research acknowledges *limau* as a potentially significant *materia medica* in Malay medical practice, aiming to assess its usage in selected manuscripts and its medicinal value as reported in scientific literature.

METHODOLOGY

Selection of manuscripts

Seven transliterated Malay medical manuscripts gathered for this study were Rumah Ubat di Pulau Penyengat (Mohd Shafri, 2018), Tayyib Al-Ihsan Fi Tibb Al-Insan (Mohd Shafri, 2018), Sari Segala Ubat (Mohd Shafri, 2019), MSS 2999 Kitab Tib (Hussain, 2015), Kitab Tib MSS 2515 (Mat Piah & Baba, 2013), Khazinat Al-Insan (Abdullah, 2017), and Kitab Tib MSS 1292 PNM (Mat Piah & Mustapha, 2019). However, Rumah Ubat di Pulau Penyengat was excluded from the study because it did not contain the keywords '*limau*' that was required for this study, Therefore, only six Malay medical manuscripts were reviewed and analysed.

Search strategy

The online dictionaries of *Pusat Rujukan Persuratan Melayu* (PRPM: http://www.prpm.dbp.gov.my/) and online tropical plants databases such as Malaysia Biodiversity Information System (MyBIS) (https://www.mybis.gov.my) and National Parks Board of Singapore (https://www.nparks.gov.sg), as well as glossaries from the published transliterated Malay medical manuscripts were used as sources of references in finding the existed vernacular names for the *limau*. Then, the vernacular names of "*limau nipis*"," *limau kapas*", "*limau raya*", "*limau kedangsa*", "*limau bali*", "*limau kerisik*" and "*limau purut*" were recognised as keywords to extract data from the selected manuscripts. The availability of the keywords was examined and noted. Any *limau* with more than one vernacular name was distinguished to avoid confusion during data analysis. Then, scientific literature was conducted to determine the pharmacological properties of each *limau* in in databases such as pubmed, ScienceDirect, Scopus and Google scholar. Only reports on experimental or clinical studies were chosen.

Data Extraction

The keywords obtained were searched in the index of the transliterated manuscripts and if they were unavailable, each page of the manuscript was examined for the related keywords. Thus, any prescriptions in the transliterated Malay medical manuscripts that uses *limau*, *limau nipis*, *limau purut* and *limau kasturi* as the *materia medica* were recognised. Then, the *limau* were classified according to the type, sources of data, prescription and treatment of disease. The scientific name for each type of *limau* was checked against the tropical plant databases during the sorting process.

Analysis of data

The prescriptions and treatment of diseases using *limau* as *materia medica* were identified. The frequency of each type of *limau* used in the manuscripts was examined and tabulated. Then, each of these prescriptions was categorised as a single ingredient or multiple ingredient formulation.

RESULTS

Distribution and Medical Uses of *limau* in the Malay Medical Manuscripts

The study found 165 treatment prescriptions in the six selected Malay medical manuscripts. Five distinct *limau* recognised within these manuscripts were *limau nipis* (key lime), *limau purut* (kaffir lime), *limau raya* (pomelo), *limau kerbau* (citron) and *limau kerisik* (limeberry) (Figure 1). Limau nipis was commonly used materia medica which was mentioned in 124 prescriptions compared to *limau purut, limau raya and limau kerbau*, and *limau kerisik* with 24, 8, 7, and 1 prescription respectively (Table 1). There were several *limau* that cannot be identified. These *limau* were written as *limau*, *limau mengkar*, *limau hantu*, *limau jalung*, *limau ningrat* and *limau keling* in the manuscripts.

In total, *limau* appears as a *materia medica* in the treatment prescriptions for 13 diseases, including some uncategorized conditions. The highest prescription counts using *limau* werefor eye-related and gastrointestinal conditions (n=28 and n=25, respectively), with hair-related ailments having only one recorded prescription. The type and frequency of limau in treating specific disorders are illustrated in Table 2. Notably, 3% (5/165) of prescriptions involved only a single *materia medica* (one type of *limau*), while 97% (160/165) were multi-compound formulations. Most of the prescriptions uttilised the citrus's juice but some uses the leaf.

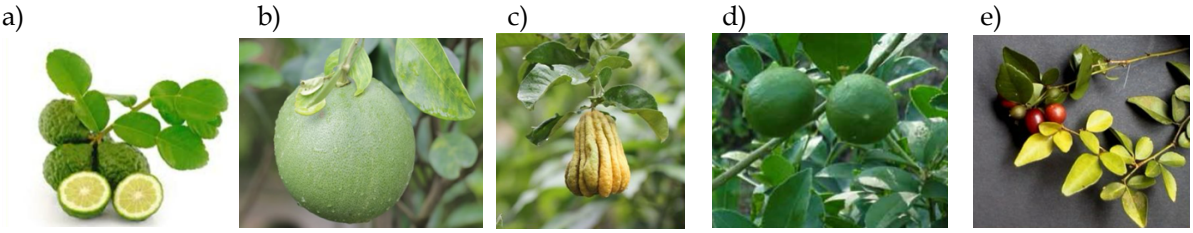

Figure 1 Species of citrus fruits mentioned in the Malay Medical manuscripts (Suresh et al., 2020; National Park of Singapore, 2024; Shamsudin, 2017: Alessandrello et al., 2021,)

Table 1 Frequencies of *limau* (*limau nipis, limau purut, limau raya, limau kerbau* and unidentified *limau*) mentioned in six selected Malay medical manuscripts

Type of *limau*	Malay Medical Manuscripts						Total
	MSS 2999 Kitab Tib	**Sari Segala Ubat**	**Tayyib al-Ihsan fi tibb al-Insan**	**Kitab Tib MSS 1292**	**Kitab Tib MSS 2515**	**Khazinat Al-Insan**	
Limau nipis *(Citrus aurantifolia)*	7	8	2	37	68	2	124
Limau purut *(Citrus hystrix)*	0	3	0	5	16	0	24
Limau raya *(Citrus grandis)*	0	2	0	4	1	1	8
Limau kerbau *(Citrus medica)*	0	0	0	3	4	0	7
Limau kerisik *(Triphasia trifolia)*	0	0	0	1	0	0	1
Unidentified	0	0	0	1	5	1	7

Table 2 Frequencies of *limau* (*limau nipis, limau purut, limau raya, limau kerbau* and unidentified *limau*) according to diseases

Diseases (Organ/System)	**Type of *Limau***					
	Limau Nipis *(Citrus aurantifolia)*	***Limau Purut*** *(Citrus hystrix)*	***Limau Raya*** *(Citrus grandis)*	***Limau Kerbau*** *(Citrus medica)*	***Limau Kerisik*** *(Triphasia trifolia)*	**Unidentified**
Men's health-related disease	1	1	1	0	0	0
Ear-related disease	3	0	0	0	0	0
Eye-related disease	25	0	2	0	0	1
Gastrointestinal-related disease	21	3	0	0	1	1
Hair-related disease	0	0	1	0	0	0
Renal-related disease	2	1	0	0	0	0
Musculoskeletal-related disease	6	0	0	1	0	0
Nose-related disease	17	1	0	0	0	1
Women's health-related disease	4	0	0	0	0	0
Respiratory-related disease	10	2	2	3	0	2
Skin-related disease	13	7	0	0	0	1
Teeth-related disease	3	2	0	0	0	0
Uncategorised illnesses	19	7	2	3	0	1

Table 3 Diseases that can be cured by *limau* (*limau nipis, limau purut, limau raya, limau kerbau, limau kerisik*) in the manuscripts and their medicinal potential in scientific publications.

Type of limau	Disease	Scientific evidence/ report	References
Limau nipis (*Citrus aurantifolia)*	Men's health-related disease	No information	-
	Ear-related disease	No information	-
	Eye-related disease	No information	-
	Gastrointestinal-related disease	Antidiarrheal agent Exhibit significant anti-diarrhoea activity when administered in low, medium and high dose on castor oil-induced diarrhoea in Wistar rats	Irisima and Uahomo (2023)
	Renal-related disease	No information	-
	Musculoskeletal-related disease	Analgesic effects Penetration of *Citrus aurantifolia* essential oil after massage provide great effect on reducing lactic acid and pain intensity	Harahap et al. (2023)
	Nose-related disease	No information	-
	Women's health-related disease	No information	-

	Respiratory-related disease	Antimycobacterial agent Hexane extract of *Citrus aurantifolia* showed a potent antimycobacterial effect against the drug-resistant variants of *M. tuberculosis* H37Rv.	Camacho-Corona et al. (2007)
	Skin-related disease	No information	-
	Teeth-related disease	Antimicrobial agent *Citrus aurantifolia* exhibits greater antibacterial activity than gentamycin on seven bacteria isolated from carious teeth	Owhe-Ureghe et al. (2010)
	Uncategorised illnesses		
	Fever	Treatment of typhoid fever *Citrus aurantifolia* juice inhibit the activity of *Salmonella typhi* when mixed with 'Kanwa' (Locally mined alkaline salt in Nigeria)	Evans et al. (2002)
	Headache, Syncope, Skin aging, Scar, Poison	No information	-
Limau purut	Men's health related disease	No information	-

(trus hystrix)	Gastrointestinal-related disease	The liver mitochondrial structure and function, blood lipid levels, histological abnormalities including steatohepatitis, oxidation, and inflammation were all improved by the extract of kaffir lime leaves together with the rhizomes of galangal and lemongrass	Tirawanchai et al. (2020)
	Renal-related disease	No information	-
	Nose-related disease	No information	-
	Respiratory-related disease	Antibacterial agent *Citrus hystrix* fruit peel oil and leaf oil exhibit antibacterial effect on three respiratory tract pathogens which are *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, and *Haemophilus influenzae* ATCC 49247	Srifuengfung et al. (2019)
	Skin-related disease	Antimelanogenic Essential oil of Citrus hystrix inhibit the activity of intracellular melanin and reduce the melanin content of the B16F10 mouse melanocytes	Aumeeruddy-Elalfi et al. (2018)
	Teeth-related disease	No information	-
	Uncategorised illnesses Poison,Fever, Syncope, Shivering	No information	-
Limau raya *(Citrus grandis)*	Men's health-related disease	No information	-
	Eye-related disease	No information	-

	Hair-related disease	No information	-
	Respiratory-related disease	Anti-inflammatory Polysaccharide of Citrus grandis together with luteolin suppressed phosphorylation of IKK, inhibit the NF-κB activation and reduce the number of rabbits with chronic pharyngitis	Chen et al. (2018)
	Uncategorised illness (Headache)	No information	-
Limau kerbau *(Citrus medica)*	Musculoskeletal-related disease	No information	-
	Respiratory-related disease	Possible antiviral potential In silico research suggest compound from Citrus medica could inactivate the pathogenesis of SARS-CoV-2 by binding to the spike protein and ACE-2 receptor	Haridas et al. (2021)
	Uncategorised illnesses Syncope Headache	 No information on syncope Citrus medica syrup lessens the severity and length of attacks of migraine headache	 Jafarpour et al. (2016)
Limau kerisik *(Triphasia trifolia)*	Gastrointestinal-related disease	No information	-

DISCUSSION

Five types of *limau* were identified from six selected Malay medical manuscripts: *limau nipis* (*Citrus aurantifolia*), known as key lime; *limau purut* (*Citrus hystrix*), or kaffir lime; *limau raya* (*Citrus grandis*), known as pomelo; *limau kerbau* (*Citrus medica*), or citron; and *limau kerisik* (*Triphasia trifolia*), known as limeberry. *Citrus aurantifolia* was cited 124 times in the treatment prescriptions, more than any other plant, followed by *Citrus hystrix* 24 times.

*Limau nipis (Citrus aurantifolia) c*ontains various active compounds such as flavonoids that are beneficial to human health. It exhibits antioxidant properties that include hydrogen-donating and radical-scavenging capabilities, which help to reduce oxidative stress (Mulvihill et al., 2016)). As a result, flavonoids inhibit the production of reactive oxygen species (ROS) and shield cells from the resulting harm. Besides, flavonoids act as an anti-inflammatory due to their inherent antioxidant properties (Mahmoud et al., 2019). Apart from flavonoids, *Citrus aurantifolia* contains linalool that poses analgesic and anti-inflammatory qualities (Lemes et al., 2018). Linalool may inhibit pro-inflammatory cytokines like TNF-α and IL-6, which lowers pain sensation (Huo et al., 2013). *Citrus aurantifolia* also acts as an antimycobacterial agent, inhibiting the growth of the drug-resistant variants of M. tuberculosis H37Rv in a study (Camacho-Corona et al., 2007). *Citrus aurantifolia* could acts as an antidiarrhoea agent that could reduce the frequency of defecation (Irisima & Uahomo, 2023). Nevertheless, *limau nipis* (*Citrus aurantifolia)* was prescribed in the Malay Medical manuscripts to treat 12 different ailments, including respiratory and gastrointestinal disorders.

Additionally, it was not only widely used in Malay traditional medicine but is also frequently utilized in other traditional treatments across various global populationsAjibesin et al. (2008) stated that indigenous people from Akwa Ibom state in Nigeria used *Citrus aurantifolia* in their treatment formulation for treating stomachache. An ethnopharmacological study in southern Ghana reported that *Citrus aurantifolia* was traditionally used as one of the *materia medica* in their antimalarial treatments. This formulation was administered through drinking and bathing to alleviate malaria symptoms. However, limited data exist on the effectiveness of this prescription in the locally practiced antimalarial treatments. (Asase et al., 2012). In addition, Xavier et al. (2015) found that *Citrus aurantifolia* is used by Malayali tribes as a cooling agent and skin emollient, as well as for treating asthma, circulatory problems, and fungal infections. In Rajasthan's traditional medicine, a daily spoonful of a *Citrus aurantifolia* and cowrie mixture was consumed to treat asthma (Upadhyay et al., 2010). It was also used to treat coughs in the Bulamogi community in Uganda (Tabuti et al., 2003)."

Other variants of *limau* are also effective, and their use in in treating respiratory disorders has been acknowledged. For example, Srifuengfung et al. (2019) reported that *limau purut* (*Citrus hystrix)* acts as an antibacterial agent that could inhibit respiratory tract pathogens such as *Haemophilus influenzae* and *Streptococcus pneumoniae*. Interestingly cough, a symptom of respiratory diseases, is treated using *Citrus hystrix* fruit by locals in Tipang village, North Sumatera. (Siahaan et al., 2021). According to a study by Chen et al. (2018), *limau raya* (*Citrus grandis*) exhibits anti-inflammatory properties by suppressing the phosphorylation of IkappaB kinase (IKK), an enzyme involved in the inflammatory response. By inhibiting this enzyme, the authors reported a reduction in NF-κB activation, leading to a decrease in the number of rabbits with chronic pharyngitis in the study. Also, an in-silico study showed that *Limau kerbau* (*Citrus medica)* exhibited antiviral potential against SARS-CoV-2 (Haridas et al., 2021). Although *limau kerisik* (*Triphasia trifolia*) is mentioned in the manuscripts as treatment for gastrointestinal diseases, no scientific studies have been found regarding its pharmacological properties. In fact, there is a lack of study on *Triphasia trifolia* for treating specific disorders."

This study finds seven unidentified *limau* as one of the *materia medica* in the prescription from the six selected Malay medical texts. There is no scientific nomenclature for the citrus fruits found in the plant database that corresponds to the local name of these *limau*, making it impossible to be identified. It is possible that the authors of these Malay medical texts used specific terms to refer to *limau*. For instance, *limau ningrat*, an unidentified type of *limau* found in a manuscript, may indicate a variety reserved for the aristocracy, as *ningrat* refers to noble or bourgeois classes (Dewan Bahasa dan Pustaka, 2013). Several times, the term '*limau*' is used without further description, suggesting that the community

of the time understood the specific type being referenced. This implies that *limau* was commonly used in daily life during that period.

This study also finds that *limau* was most used to treat illnesses that could not be categorized into specific organ or system. These ilnesses refer to symptoms not associated with any specific organ or system, such as *demam* (fever), *peningkepala* (headache), *warangan*, *pitam* (fainting), *ketar* (shivering), *tuaanpada muka* (wrinkles), and *parut* (scars). Among these, *demam* and *pening kepala* were the most frequent conditions treated with *limau*.

In the manuscripts, *demam* is like fever, while *pening kepala* closely corresponds to headaches. Fever is the rise in a person's body temperature that goes beyond a "set point" controlled by the hypothalamus. It indicates that the body undergoes a physiological reaction caused by viral or non-infectious factors, including inflammation, cancer, or autoimmune processes (Balli et al., 2022). Meanwhile, headaches usually result from the stimulation of nociceptors in the vicinity, which include blood vessels, meninges, muscle fibres, facial muscles, and cranial or spinal nerves (Baraness & Baker, 2022). These two sources demonstrated that fever and headaches are not particular symptoms for any organs or physiological systems. Overall, this condition shows that treating symptoms with *limau* is a component of the therapeutic approach for pain alleviation utilised by the Malays in the past, though these symptoms are not associated with any specific organ or system. Therefore, it is not surprising that *limau* is often referred to simply as '*limau*' in Malay medical manuscripts, as it has become a widely used *materia medica* in the treatment of uncategorized illnesses, including fever and headaches.

Besides, in Malay medical manuscripts, *limau* was used either as a single ingredient in single-ingredient therapy formulations or in multiple-compound formulations when combined with other *materia medica*. When used alone, *limau* may possess significant pharmacological properties capable of combating various diseases. Sah et al. (2011) demonstrated that parts of *the Citrus medica* plant, specifically the juice, could inhibit the growth of nine different microbes. This study demonstrated that *Citrus medica* alone can exhibit significant antimicrobial activity, without the need for additional *materia medica*. Meanwhile, the addition of other ingredients to the therapy formulations involving *limau* in multiple compound formulations may enhance the citrus fruit's potential for therapeutic benefits. Evans et al. (2002) reported that *Citrus aurantifolia* juice could inhibit *Salmonella typhi* only when *Kanwa* (Nigerian salt) was added to the mixture. This study showed that the combination of *limau* with other materia medica is also significant in improving the effectiveness of the treatment prescriptions.

CONCLUSION

In conclusion, this study is significant in preserving Malay medical manuscripts as primary, permanent sources of traditional medicine. The conservation of these manuscripts ensures their accessibility to future generations. Moreover, the treatment formulations within them are reliable, as they were written by *Tabib* (Malay physicians) and other medical practitioners, whose expertise is difficult to challenge. This contrasts with traditional tips or *petua* passed orally, which can be altered over time through verbal transmission. Preserving and acknowledging the use of various *limau* variants in traditional Malay medicine is crucial for safeguarding this cultural heritage. These variants, such as *limau nipis*, *limau purut*, and others, play vital roles in both historical and contemporary medical practices. Recognizing their medicinal value not only ensures the continuity of traditional knowledge but also highlights the importance of these plants in contemporary therapeutic contexts. Such efforts contribute to the conservation of local wisdom and promote the integration of these practices into modern health care system.

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OBSERVATION OF pH, COLOUR CHANGES AND MICROBIOLOGICAL OCCURANCE IN HAIR TREATMENT FORMULATIONS FROM SELECTED MALAY MEDICAL MANUSCRIPTS

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ABSTRACT

Introduction: Hair being an integral part of the integumentary system, has several physical and chemical properties that differ individually. Most of the hair conditions are often treated using traditional or commercial products. However, this knowledge stems primarily from historical experiences, lack scientific validation and currently, there are no commercially available formulations for hair treatment derived from the Malay Medical Manuscripts (MMMs). **Aim:** This study focuses on development of hair treatment formulations in the MMMs and explores its potential in addressing hair problems. **Method:** Three available MMMs, namely, MSS 1292, MSS 2905 and IAMM1998.1.3370 were reviewed for hair treatments prescriptions. The prescriptions were analysed against the Scientific Analysis of Kitab Tib Index (SAKTI) tool, whereby two formulations were chosen before carefully being prepared in the laboratory and parameters such as pH, colour, and microbiological changes of the formulations were assessed. **Results:** The first formulation used *Citrus maxima* (pomelo) whilst the second formulation used *Musa acuminata* (banana) as their main ingredients respectively. For each formulation, yellow and green fruits were used. Samples were labelled as PomY and PomG for yellow and green pomelo, as well as BanY and BanG for yellow and green bananas respectively. Most samples showed consistent pH values between pH 5.0 - 5.4 at room temperature (23℃ - 27℃) as well as at cool temperature (4℃) indicating a mildly acidic nature. However, a few samples show a slightly more acidic pH around 4.0, while others are closer to neutral with a pH of 6.0. Overall, the discolouration of the samples was not noticeable for formulations using pomelo. However, discolouration was more apparent in BanY on day 6 and BanG on day 3. The microbiology test showed growth of Gram-positive as well as Gram-negative bacteria in all samples except PomY within 24 to 48 h of storage. **Conclusion:** These findings are preliminary observation for homemade prescriptions and provide valuable insights into the potential of Malay medicine and natural ingredients for hair treatment as well as paving the way for the integration of traditional medicine into contemporary healthcare practices.

KEYWORDS: Hair problems, traditional medicine, pomelo, natural products, citrus, pharmaceutical codex

INTRODUCTION

An essential element of the integumentary system, hair is a filament consisting of a protein called keratin growing from the dermis layer of the skin and has its unique physical and chemical activities. Hair comes in different shapes, lengths, thicknesses, textures, and colours, which vary for everyone. Thus, different people would have different hair types (oily, dry, balanced) with different textures. However, regardless of the type of hair a person has, almost everyone deals with hair problems at some point in their lives (Watson, 2010). The problems include scalp seborrheic dermatitis (dandruff), alopecia (hair loss), dry hair, and trichoptilosis (split ends) (Brandon, 2014).

Traditional or home remedies are mostly sought in treating hair problems in many parts of the world. For many years, ancient people of previous civilisations relied on traditional herbal medicine because of their preference for natural products Ethnopharmacological survey of home remedies for treatment of hair and scalp by Zaid et al. (2017) mentioned that 80% of rural populations in developing countries consider herbal remedies significant and important. Nevertheless, natural product use has increased in both developed and developing countries in recent years. Furthermore, natural products are being used as hair treatment in traditional medicine practised by the Chinese, Indian (Ayurvedic), Malay, Japanese (Kampo), Korean and Unani. According to Yuan et al., (2016), even if the traditional methods have certain flaws in their many forms, they nonetheless serve as an important archive of human knowledge. Counterintuitively, some of the traditional medical practices lack safety empirical and guidelines for use.

For centuries, traditional medicines have been utilised to address various ailments, including hair-related issues. These treatments, devoid of complex synthetic chemicals, may exhibit efficacy in managing hair problems. The knowledge surrounding traditional medicine primarily stems from historical experiential accounts and lacks scientific validation. Nevertheless, the integration of traditional medicinal knowledge with advanced understanding and technologies holds promising prospects for the treatment of numerous diseases in the future.

In the past, the traditional knowledge was often stored in the form of manuscripts which are handwritten pieces of text or typed documents created prior to the advent of the printing press. They encompass a wide range of written materials, such as literary works, historical records, scientific papers, religious scriptures, as well as medical texts - emphasising their varied formats and their significance in history. As for the traditional Malay medicine, the knowledge and practices of health, diseases, and their treatments, which are unique to the Malay culture have been passed down several generations through oral traditions, traditional practices as well as written manuscripts. The latter, which are medical texts written and practised by the Ancient Malay people are collectively known as the Malay Medical Manuscripts (MMMs). These manuscripts contain a multitude of traditional remedies for various ailments, including hair problems that afflicted the local population of the Malay Archipelago in the past, as well as in contemporary times (Nadzirin, 2021). MMMs also include diagnosis and treatment of various illnesses, the use of herbal remedies, and the importance of diet and lifestyle in maintaining good health. They also provide insights into traditional medical practices such as cupping, massage, and acupuncture, which were widely used in Southeast Asia long before they became popular in the Western world.

Due to the lack of scientific backing, Malay medicine has fallen behind, keeping the Malay scholars from studying these writings (Mohd Shafri, 2021). Nonetheless, MMMs are an indispensable part of Malaysian culture and heritage, thus provide a window into the country's unique traditional medical practices and beliefs. Currently, there is very little experimental study available on the possible cure of maladies mentioned in the manuscripts, especially regarding the treatment of hair problems. Presently, however, there are no commercially available formulated products for hair treatment derived from the MMMs. Furthermore, the existing traditional medical practices lack sufficient empirical safety data and usage guidelines. Therefore, the objectives of this study were to identify prospective formulations for hair treatment according to selected prescriptions in the MMMs as well as to assess these prepared formulations for pH, colour, and presence of microbiological changes.

METHODS

Selection of the Manuscripts and Formulations

This study utilised the Scientific Analysis of Kitab Tib Index (SAKTI) tool for selection of manuscripts and formulations. SAKTI, developed by Mohd Shafri (2021), serves as a tool which is comparable to Pharmaceutical Codex for evaluating the efficacy of MMMs. This index assesses the potency and viability of these manuscripts for scientific study. By applying this index, researchers can prioritise formulations, identifying those with the highest potential for development into effective treatments. The tool ensures the validation and systematic evaluation of both existing and newly discovered MMMs, particularly for treatments related to hair ailments. The selection of relevant MMMs with contents on hair care and treatment was made based on the quality of the MMMs, the hair formulations and the prospects of commercialising the hair formulations.

The SAKTI-iMS or Index of Manuscript Selection is designed to facilitate a systematic evaluation of a manuscript before it is selected for further study. SAKTI-iMS incorporates four primary criteria: author's profile, completeness of the text, legibility of the text, and the amount of medical content present in the manuscript. Among these criteria, the amount of medical content holds the most significant weight in determining manuscript selection. In addition to the criteria outlined by Mohd. Shafri (2021), manuscripts in this study were selected based on information for hair care and hair treatment prescriptions, which was identified using relevant words such as *rambut* (hair), *ubat rambut* (hair treatment), *rambut gugur* ("hair loss)", *rambut lebat* ("hair growth)", and *kemumur/kelumumur* ("dandruff).

After selecting MMMs based on the SAKTI-iMS assessment, relevant hair treatment formulations from those manuscripts that satisfied the SAKTI-iMS criteria, were subjected to analysis using the SAKTI-iPharmaprospect index (Mohd Shafri, 2021). SAKTI-iPharmaprospect assigns scores based on various criteria, including the origin of materials, formulation complexity involving the use of single or mixed materials, ease of preparation, and ease of use or delivery route, such as local application or systemic administration (oral). The selected formulations were then used for laboratory experiments (see Results, Figure 1).

Laboratory Experiments

Preparation of Formulation One

The first formulation was prepared in accordance with the prescription from KITAB TIB MSS 1292 PNM employing *Citrus maxima* (pomelo) or *limau raya* and water as primary ingredients (Piah & Mustapha, 2019, p.61). The prescription in the manuscript stated, "*Sebagai lagi akan langirnya limau raya direbus maka embunkan, kemudian maka diperlimau, mujarab olehnya*" which suggests boiling the pomelo in water to form a paste.

The formulations were prepared on a standard laboratory bench, with standard aseptic techniques to mimic as much as possible, the homemade preparation. All glassware and working bench used during the laboratory work were sterilised and sanitised respectively. Two types of pomelos were used: yellow coloured (PomY) and green coloured (PomG) pomelos. Four samples were prepared according to the following pomelo to water ratio: 1:7 for PomY1 and PomG1 and 1:9 for PomY2 and PomG2. To prepare the sample, a pomelo fruit, weighing approximately 220 g, was washed and diced, with equal amounts of peel and fruit weighed, mixed with distilled water, and boiled until the pomelo reached a mashed consistency, a process taking approximately 1.5 to 2 hours. Half of the mash was blended and combined with the remaining material, and the resulting paste was allowed to cool to room temperature. The samples were then stored at cool temperature (4℃) as well as room temperature (23℃ - 27℃) for further analysis within four bottles prepared for each sample.

Preparation of Formulation Two

The second formulation was prepared following the guidance from PETUA KITAB TIB MSS 2905, using *Musa acuminata* (Cavendish banana) or *pisang*, *Cocos nucifera* (young coconut) or *kelapa*, and water as core ingredients (Raja Perdaus, 2021). The prescription stated, "*Sebagai lagi ubat rambut juga supaya lebat maka ambil ubi pisang mengkal tumbuklah lumat-lumat maka ambil airnya dan ambil kelapa yang dimakan tupai yang lagi ada isinya kukur ambil patinya campur maka dijarangkan/dijaringkan pakai-pakai lebat olehnya*" which suggests mashing bananas and extracting coconut flesh from freshly grated coconut and ultimately mixing it together into a paste.
The formulations were prepared on a standard laboratory bench, with standard aseptic techniques to mimic as much as possible, the homemade preparation. All glassware and working bench used during the laboratory work were sterilised and sanitised respectively. Two types of bananas were used, the yellow coloured (BanY) and green coloured (BanG) bananas. The latter type is also called pisang *lemak manis*, which is smaller than the yellow bananas with a hard pulp. Four samples were prepared according to the following banana: coconut milk: distilled water ratio: 15:3:5 for BanY1 and BanG1 and 15:5:5 (or 3:1:1) for BanY2 and BanG2. To prepare the sample, 150 g of bananas was diced, mashed, and mixed with distilled water and coconut milk. The samples were then kept in a chiller at 4℃ and at room temperature (23℃ - 27℃) for further analysis.

pH Assessment

The pH assessment was conducted using a pH electrode (ThermoScientific™ Eutech Expert pH meter, Massachusetts). pH readings were recorded three times per sample and the average pH reading was taken for analysis. pH readings were performed for all samples over six days.

Colour Changes

Colour changes of the samples were assessed manually in a controlled laboratory environment. Each sample was placed against a white-coloured background under consistent lighting conditions. Observations were made regarding any colour changes, such as fading, darkening, or the emergence of new hues, over six days.

Microbiological Assessment

In this study, the microbial quality of hair treatment formulations was maintained by aseptically sterilising glassware and environmental surfaces prior to use. Culture media and water underwent autoclaving, and controls were prepared to validate aseptic techniques. However, all ingredients used in the formulations were not sterilised to preserve their therapeutic properties. Microbiology assessment involved streaking samples on nutrient agar plates to check for bacterial growth and subsequent Gram staining of microbial colonies on day 1.

Briefly, samples were kept at different storage temperatures for 3 to 4 hours before they were individually cultured in duplicates, on fresh Nutrient agar plates using a sterilised inoculating loop. The four-quadrant streaking technique was adopted to visualise single colonies (if any). Inoculated plates were checked for any microbial growth. Colonies were observed for size, shape, and colour and subsequently were microscopically assessed using Gram staining.

RESULTS

SAKTI as a Tool in Selecting Manuscripts and Formulations

The selection of manuscripts for hair treatment formulations was guided by the SAKTI tool, which assessed the medical content and author profiles of five chosen manuscripts: Kitāb Ṭibb ʻAbd Allah Wan Besar IAMM1998.1.3370 (KTAWB) (possibly Fathani), MSS 1292 PNM (Piah & Mustapha, 2019), MSS 2905 (Raja Perdaus, 2020), MSS 3048 (Ahmad Radzaudin, 2021) and Mujarrabāt al-Fawāʼid (Terengganu).

The scores obtained from SAKTI-iMS were as follows: IAMM1998.1.3370 received the highest score of 8 (Grade A), followed by MSS 1292 (score 7, Grade A), MSS 2905 (score-7; Grade A), MSS 3048 (score-

6, Grade B) and Mujarrabāt al-Fawā'id (score 3, Grade C). Based on the SAKTI-iMS, KTAWB, MSS 1292, and MSS 2905 were identified as high-priority manuscripts for inclusion in the hair treatment study (Figure 1).

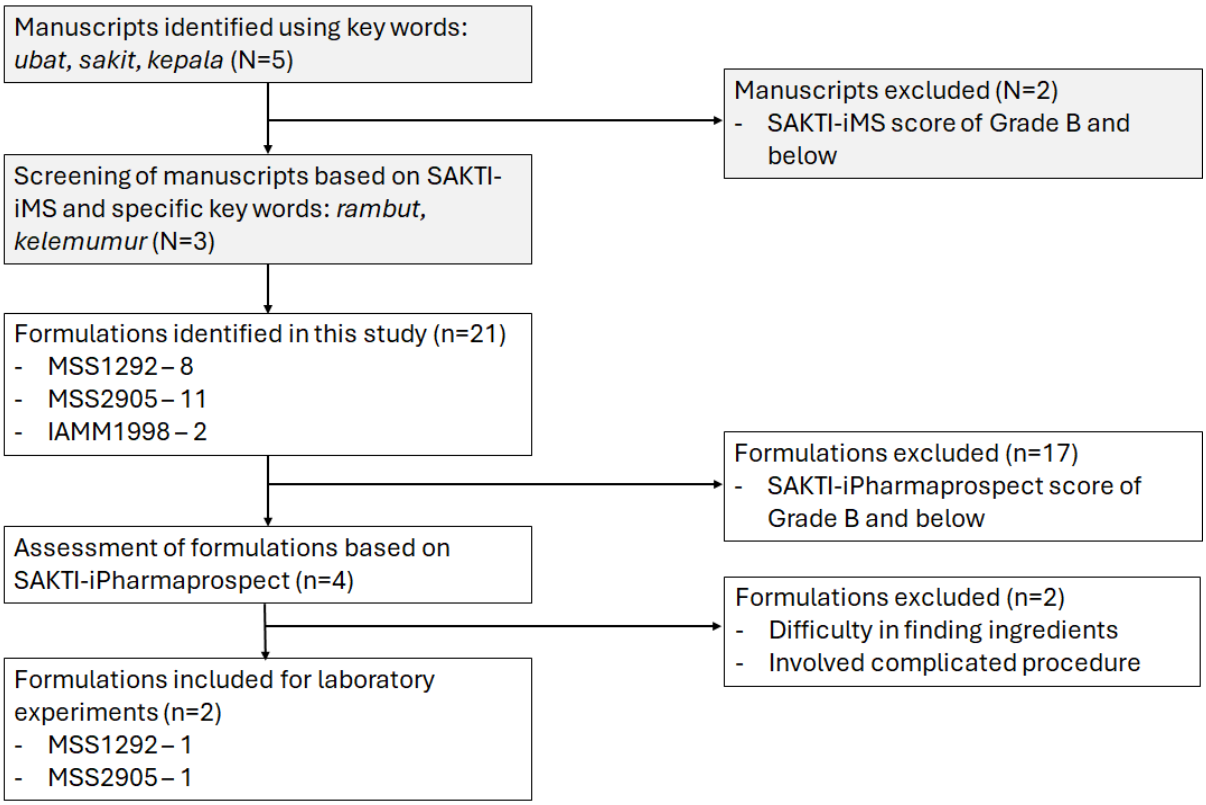

Figure 1 The flow chart of the criteria and selection guideline for included Malay medical manuscripts (grey boxes) and formulations (white boxes) in this study. N, number of manuscripts; n, number of formulations.

Data mining of the three selected manuscripts revealed 21 hair treatment formulations. Out of these 21 formulations, four were identified as Grade A based on the SAKTI-iPharmaprospect assessment (Figure 1). After further scrutiny, two formulations had to be excluded due to the difficulty in finding the main ingredients which were shrubby herbs and black goat respectively. Hence, for the laboratory experiments were proceeded with only two formulations. The first formulation was retrieved from MSS 1292 (designated as Formulation 1292.7), with the highest score of 12 for its simplicity, easiness of preparation and requirement of only a single ingredient (i.e. pomelo). The second formulation, designated as Formulation 2905.4 from MSS 2905 with a score of 10, was also selected due to its easy-to-find ingredients (i.e. bananas and coconut milk).

Storage Temperature and Fruit Type Affect pH

The pH analysis results offer valuable insights into the stability of the formulations under varying temperature conditions. In general, yellow pomelo (PomY) samples showed a slightly decreasing pH trend throughout the six days period whilst the green pomelo (PomG) samples displayed an increasing pH trend.

Yellow pomelo samples displayed a slight decrease to almost no changes in pH over the six days period at both cool and room temperatures. Conversely, PomY2 samples exhibited a slight and insignificant decreased of pH at cool storage, and an increase of pH at room temperature. The lowest pH was seen

for cool PomY2 sample on day 6 and the highest pH was for PomY2 sample on day 6, kept at room temperature (Figure 2a).

Overall, the green pomelo samples shown an increasing trend in pH over the six days period, although the trends seen for green pomelos kept at room temperature seemed to be stable throughout the six days period (Figure 2b). A big increase in alkalinity was seen in each cool PomG1 and PomG2 sample, between day 1 and day 3. The lowest pH recorded was for cool PomG2 on day 1 whilst the highest pH recorded was for PomG1 sample on day 6, at cool temperature (Figure 2b).

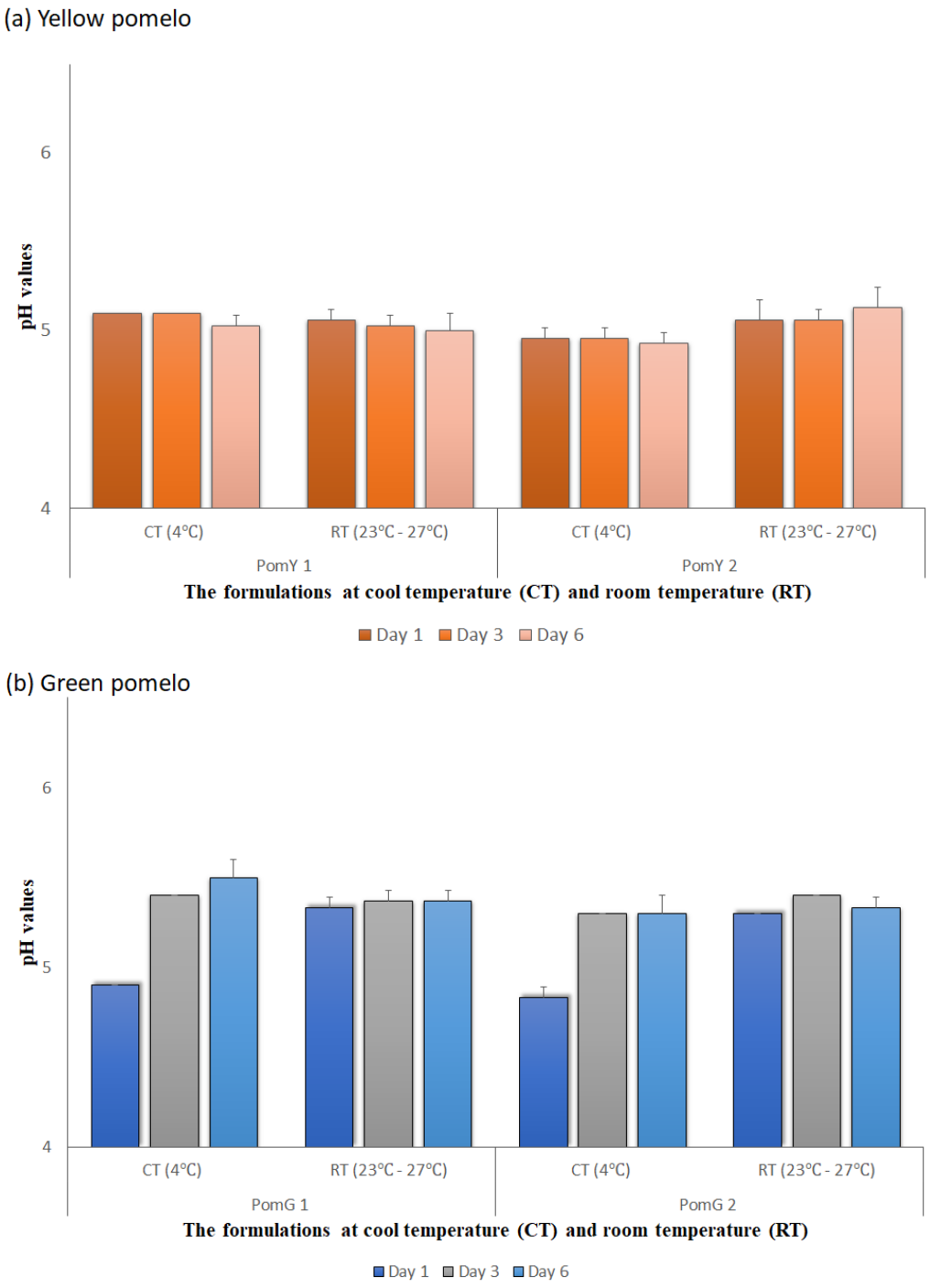

Figure 2 pH values of formulations (a) yellow and (b) green pomelos on days 1, 3 and 6 at cool temperature (4°C) and room temperature (23°C - 27°C).

Like yellow pomelos, yellow banana (BanY) samples also showed a decreasing pH trend (Figure 3a). However, green banana (BanG) samples showed an increasing pH trend when the samples were kept in cool temperature, and a decreasing trend when samples were kept at room temperature (Figure 3b). For yellow bananas, the lowest pH was seen in BanY2 samples on day 6 at room temperature. The highest pH was recorded for BanY2 samples on day 1 at room temperature. For green bananas, the lowest pH was recorded for BanG2 on day 6 at room temperature, whilst the highest pH was seen for BanG1 on day 6 at cool temperature (Figure 3b). A significant decrease in pH was also seen in BanY2 and BanG2 samples respectively, at room temperature, and over the six days period.

On day 1, the mean pH for yellow pomelo (pH 5.05), green pomelo (pH 5.09) and yellow banana (pH 5.08) was rather consistent at pH 5. However, green banana recorded the highest read for the mean which was pH 5.99 on day 1. Overall, these results highlight the temperature-dependent variations in the pH values of all the samples with different trends observed for yellow and green ingredients.

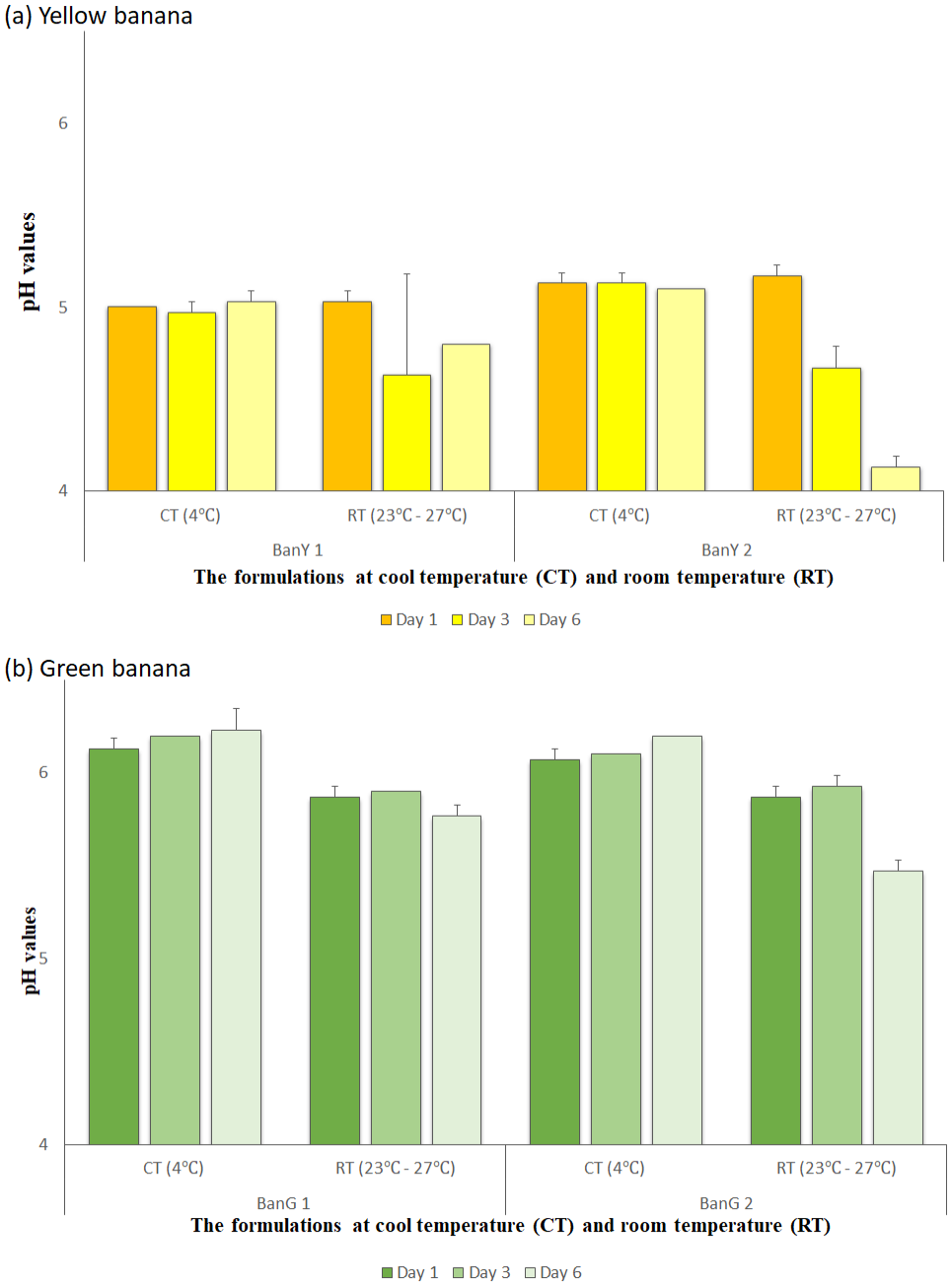

Figure 3 pH values of formulations (a) yellow and (b) green bananas on days 1, 3 and 6 at cool temperature (4°C) and room temperature (23°C - 27°C).

Colour Changes in the Prepared Formulations

For PomY 1 and PomY 2 exhibited a consistent yellowish colour, which could be attributed to the yellow peel used in their preparation (Figure 4a). Similarly, green pomelo (PomG samples) maintained a greenish-yellow colour, possible due to the green peel (Figure 4b). In contrast, yellow bananas (BanY samples) appeared beige-like coloured paste with a brown ring on the paste's top on day 1, which then turned into brownish beige on day 3 and eventually becoming entirely brown on day 6 (Figure 5a). Green bananas (BanG samples) kept in cool temperature displayed creamish white paste with a grey ring on day 1 which then changed into a greyish paste on day 6 (Figure 5b). However, no such colour change was seen for green bananas kept at room temperature (Figure 5b). Overall, the changes in colour were not strikingly apparent for the formulations with pomelos kept at different temperatures, over the six days period. Except for the formation of an upper clear liquid layer on day 6, no significant change in colour was also observed for green banana (BanY) samples kept at room temperature. Interestingly, green banana samples kept at cool temperature displayed obvious colour changes over the six days period (Figure 5b).

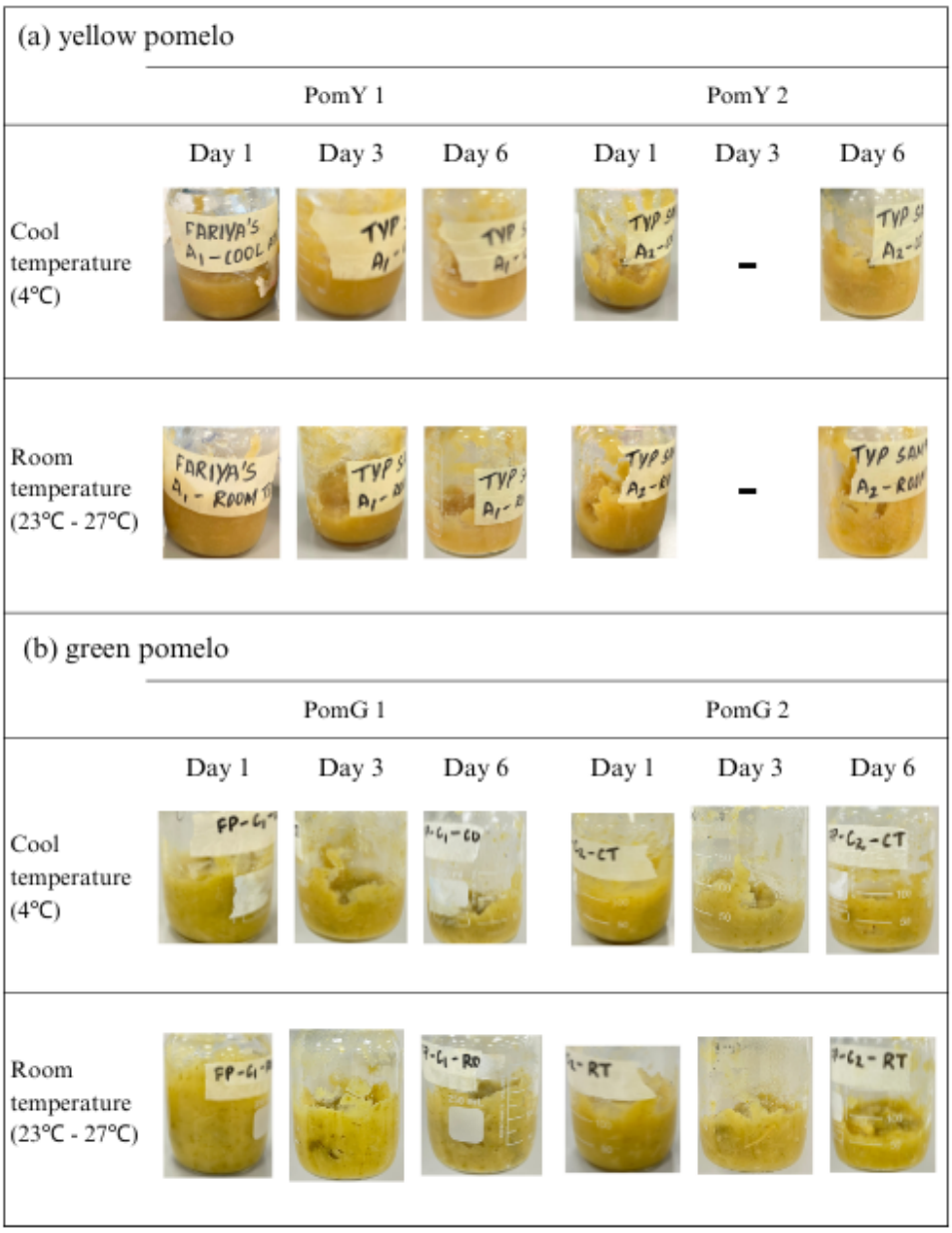

Figure 4 Colour changes observed in (a) yellow and (b) green pomelos on days 1, 3, and 6 at cool (4°C) and room temperatures (23°C - 27°C).

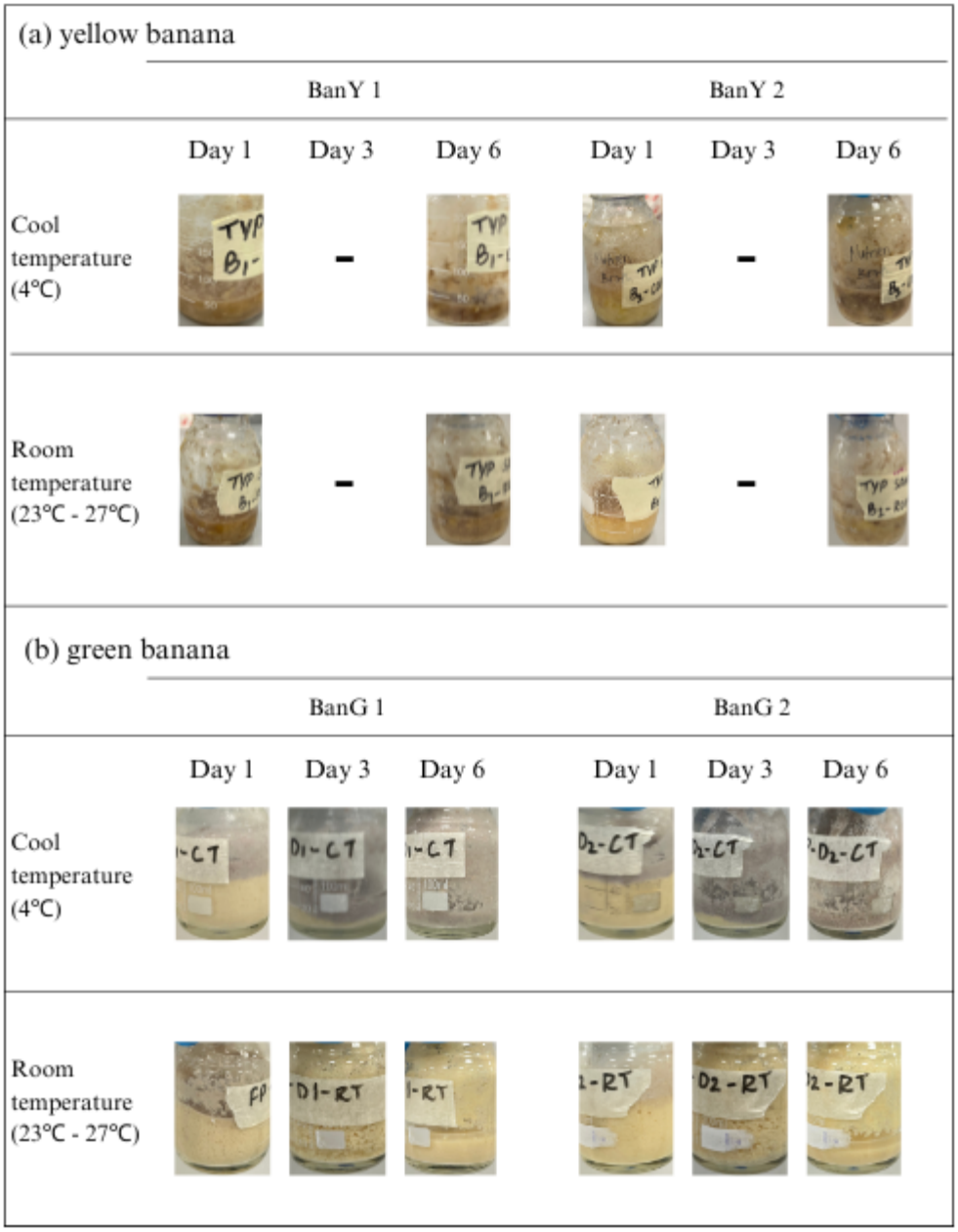

Figure 5 Colour changes observed in (a) yellow and (b) green bananas on days 1, 3, and 6 at cool (4°C) and room temperatures (23°C - 27°C).

Microbiology Assessment and Observations of the Prepared Formulations

All samples were subjected to microbiological assessment on day 1, to ascertain the microbiological quality of the samples during the preparation process. Each sample was done in duplicates. All nutrient agar plates inoculated with yellow pomelos (PomY) samples showed no microbial growth except for one of the duplicates for PomY2 sample, kept at room temperature (Figure 6a). Gram staining microscopy reveals Gram-positive bacilli-shaped bacteria (Figure 6b).

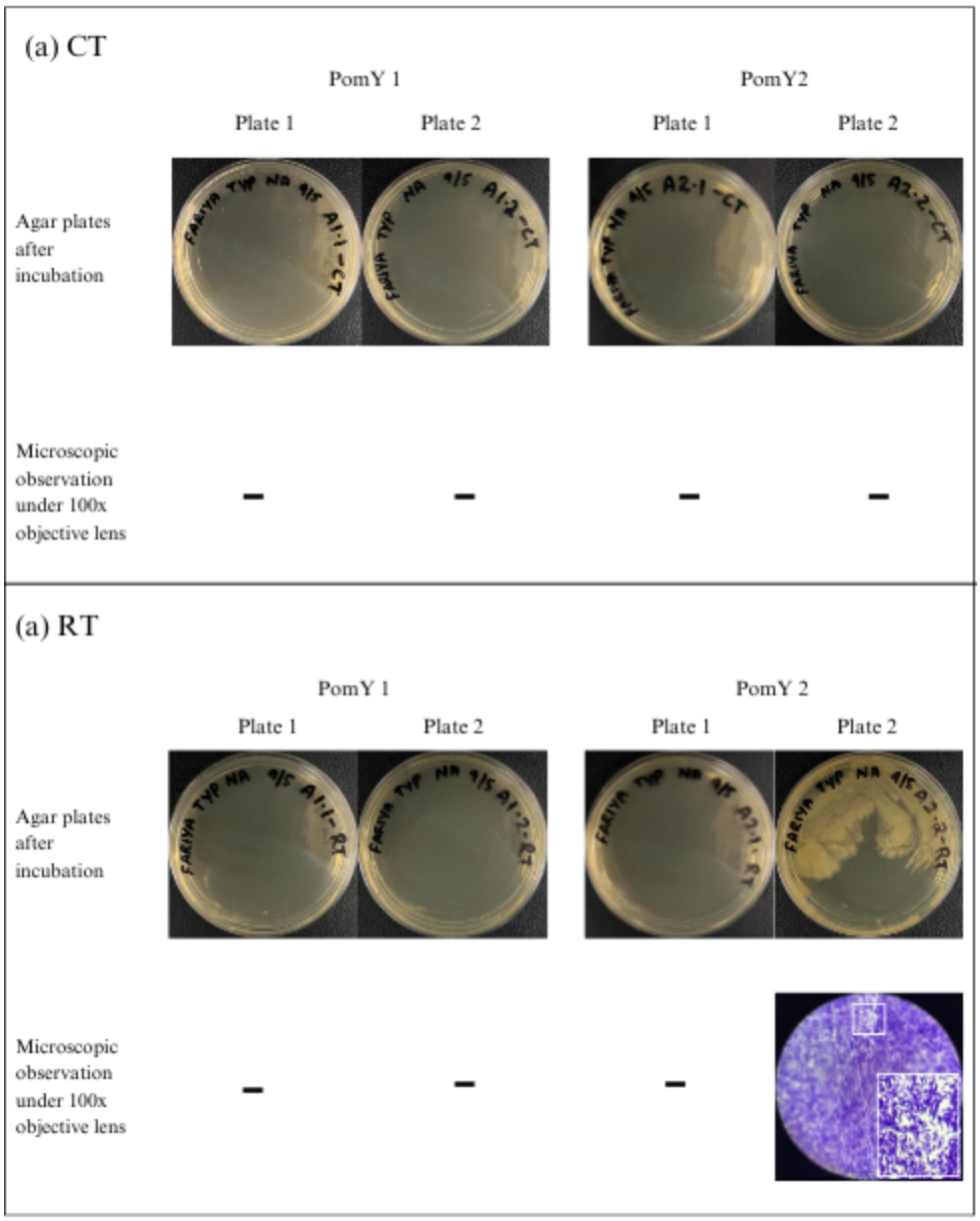

Figure 6 Formulations using yellow pomelos stored at (a) cool temperature (4℃) and (b) room temperature showed no microbial growth on nutrient agar plates, except for in one of the duplicates for PomY2 at RT.

Green pomelos have microbial growth on all nutrient agar plates kept at cool and room temperatures. The samples which were kept at room temperature had more microbial cultures grown on them, than those kept at cool temperature (Figure 7). Microscopic observation of PomG1 kept at cool temperature revealed predominantly Gram-positive cocci grown on the plates. Whilst plates inoculated with cool PomG2 samples revealed Gram-negative bacilli (Figure 7a). At room temperature, all samples showed substantial microbial growth. Microscopic observation showed PomG2 plates were inoculated with a mixture of Gram-positive and Gram-negative bacilli (Figure 7b).

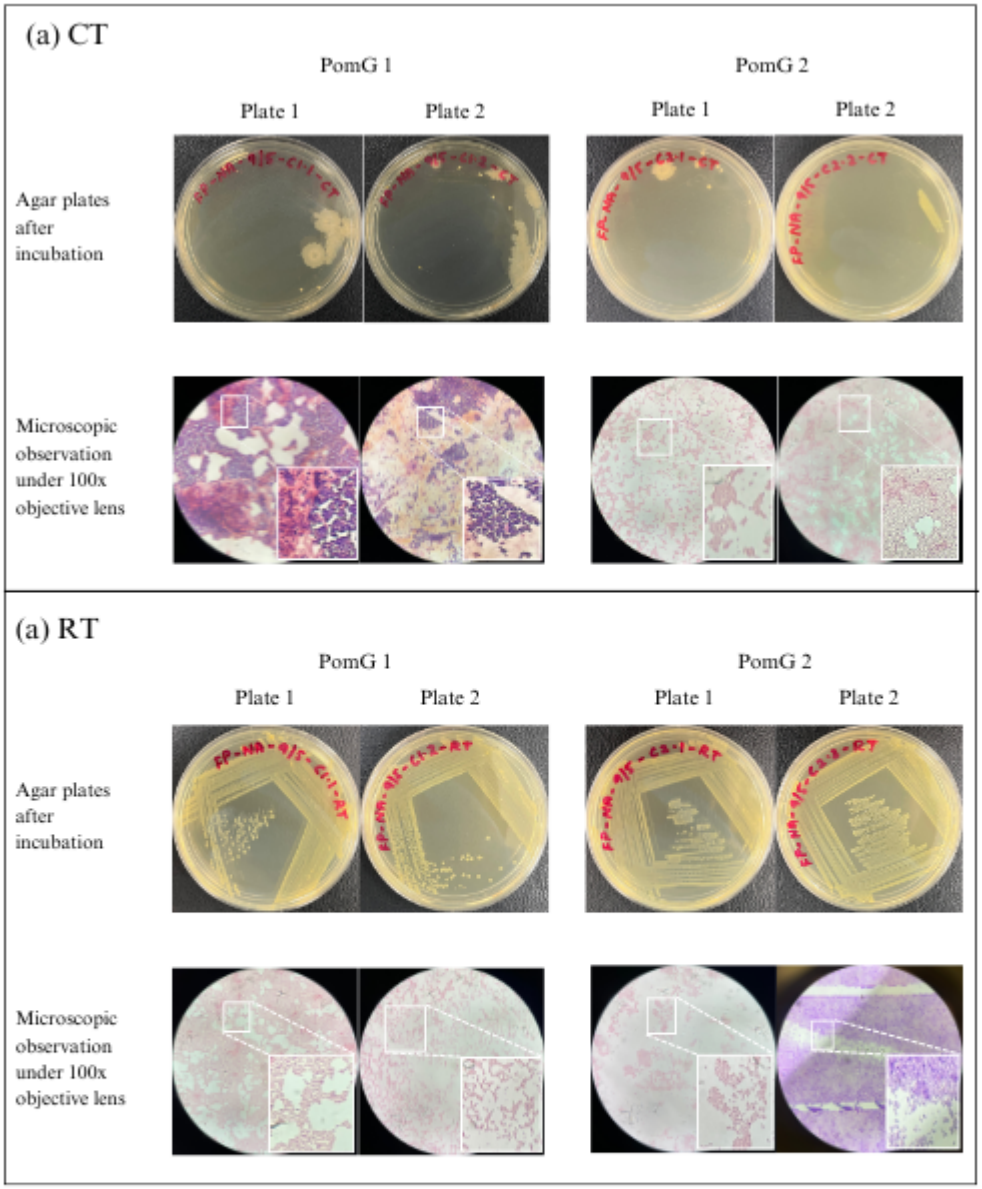

Figure 7 Formulations using green pomelos stored at (a) cool temperature (4°C) and (b) room temperature showed microbial growth on all nutrient agar plates. Gram staining revealed a mixture of Gram-positive and Gram-negative microorganisms.

All nutrient agar plates of yellow banana samples showed substantial microbial growth. Microscopic observation revealed a mixture of Gram-negative bacilli and Gram-positive bacilli on all plates (Figure 8).

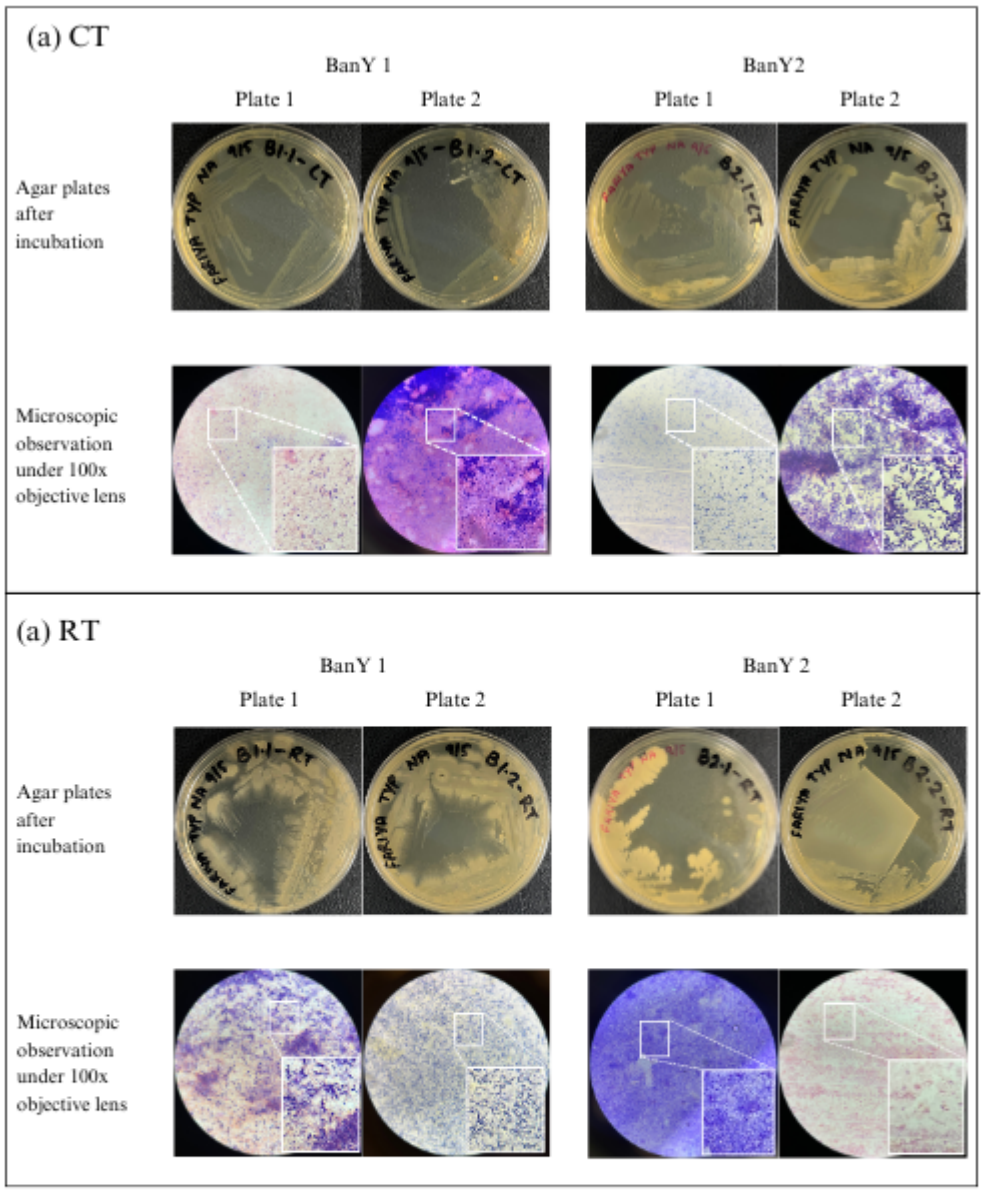

Figure 8 Formulations using yellow bananas stored at (a) cool temperature (4℃) and (b) room temperature showed microbial growth on all nutrient agar plates. Gram staining revealed predominantly Gram-positive bacilli.

Microbial growths were seen in all nutrient agar plates, inoculated with green bananas. Plates which were kept at cool temperature displayed less microbial growth (Figure 9a), than those kept at room temperature (Figure 9b). Microscopic observation revealed Gram-negative bacilli in cool BanG1 plates and Gram-positive staphylococci-like organisms in cool BanG2 plates. At room temperature, the microbial growth in all plates were predominantly Gram-negative rods.

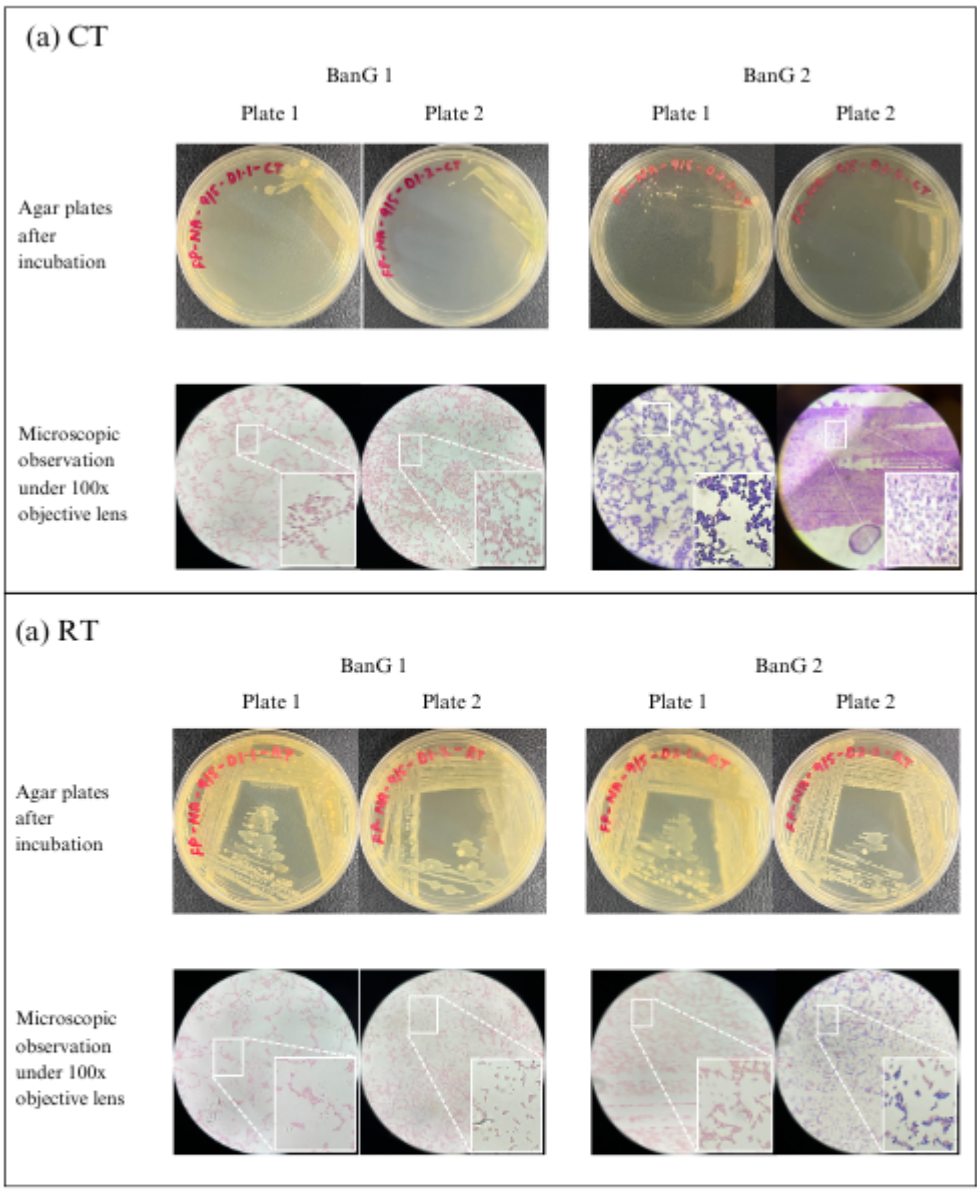

Figure 9 Formulations using green bananas stored at (a) cool temperature (4℃) and (b) room temperature showed microbial growth on all nutrient agar plates. Gram staining revealed predominantly Gram-positive microorganisms.

DISCUSSION

Manuscript and Formulation Selection for Hair Treatment from the MMMs

The selection of manuscripts for this study relied on the SAKTI Index (Mohd Shafri, 2021), a robust tool that ensures the quality and authenticity of MMMs. It ensures standardisation, quality assessment, authenticity, and credibility of the selected Malay manuscripts while also remaining true to its contents, the formulations, and prescriptions (Mohd Shafri, 2021). Within the SAKTI framework, the Index of Manuscript Selection (SAKTI-iMS) played a vital role by focusing on the selection process to identify high-quality and relevant manuscripts. To tailor the selection for this study, the iMS index was modified to include a hair treatment content criterion, leading to the selection of three manuscripts: KITAB IAMM 1998, KITAB TIB MSS 1292 PNM, and PETUA KITAB TIB MSS 2905. These manuscripts met criteria such as 80% or higher medical content, legibility, and containing multiple hair treatment formulations. From these manuscripts, 21 hair treatment formulations were identified and

subsequently translated from Malay into English, with a note on potential translation limitations. The Jawi language as well as the old Malay language, both have unique linguistic features, cultural expressions, and certain technical terms which can be challenging to accurately translate into English. One such example is from the formulation PETUA KITAB TIB MSS 2905.4 which states the following:

> "*Sebagai lagi ubat rambut juga supaya lebat maka ambil ubi pisang mengkal tumbuklah lumat-lumat maka ambil airnya dan ambil kelapa yang dimakan tupai yang lagi ada isinya kukur ambil patinya campur maka dijarangkan/dijaringkan pakai-pakai lebat olehnya*."

The literal translation of this formulation is "Also as a hair remedy for thickness (of hair), take a banana tuber, mash the pulp, then take the water and take a coconut which has been hole-punched by a squirrel, with some leftover of its content. Scrap the coconut flesh, mix it, then apply it thickly on hair." The initial part of the translation is understood quite well; however, the second part of the translation can cause confusion to the readers as well as researchers. Here, the coconut that is "hole-punched by a squirrel" does not literally mean to use a coconut which has already been partially eaten by a squirrel. This phrase is used metaphorically to point out the pulp of the coconut which is still young and fresh. From this example, it can be said that the nuances, idiomatic expressions, and the context-specific meanings may not be fully captured into the translation, potentially leading to misinterpretations or loss of information from the translations.

Additionally, regions like countries in Southeast Asia, which have rich cultures and diversities tend to have their native cultural and historical contexts embedded in their manuscripts, which convey certain concepts, beliefs and practices deeply rooted in their culture. Their translations into English may not express their relevance. MMMs often contain specialised medical or technical terminologies which do not necessarily have direct equivalents in English. Translation of such terms requires careful considerations and may result in ambiguous translations which can affect the understanding of the formulations.

When choosing the right formulations for laboratory experiments or commercialisation, SAKTI-Pharmaprospect gives a good indication for quality formulations. The index is easy to use and serves as a suitable guideline for selecting the best possible formulation for pharmaceutical development. In this study, the final selection for laboratory experiment of only two out of 21 formulations was straightforward and was performed with great precision.

Changes in pH Values Observed in the Prepared Formulations

Changes in pH values of the prepared formulations have provided valuable insight into the stability and changes in acidity of the formulation over the span of six days. A healthy human hair strand exhibits an acidic pH ranging from 3.5 to 4.5, while the normal pH of the human scalp averages around 5.5, indicating a slightly lower acidity compared to the hair strand alone (Gavazzoni Dias et al., 2014). It has been recommended by Rathi and D'Souza (2015) that hair treatment products, including shampoos and other formulations, should not have a higher pH value than the scalp's pH of 5.5. When the formulations were prepared fresh, the mean pH for yellow pomelo (pH 5.05), green pomelo (pH 5.09) and yellow banana (pH 5.08) were slightly lower than the recommended pH for scalp. Green banana recorded the highest read for the mean which was pH 5.99 on day 1. Maintaining the pH close to the natural pH of the scalp is important for natural balance and preservation of the hair and the scalp. This observation suggests that the formulations were developed in consistence with the scalp's pH and are in harmony with its natural acidic environment of the human scalp.

Yellow fruits (both pomelo and banana) tend to have stable pH over the six days period, when kept in cold temperature. However, yellow banana recorded fluctuation when kept at room temperature. Green fruits also recorded fluctuation in pH during the six-day duration. Overall, cooler temperature generally helps to maintain the stability of the samples, while storage at room temperature leads to fluctuations in the pH over time. Though the fluctuations were relatively small, they indicate a certain degree of instability in the formulations. This instability can affect the efficacy and longevity of hair treatment products.

Moreover, in the absence of preservatives, microbial activity can exert an influence on the pH of the formulations over time. Microorganisms, whether present in the ingredients as part of their microbiota or introduced during formulation preparation, can modify certain components, thereby causing variations in pH. Additionally, chemical reactions occurring within the formulations can also impact pH levels. Interactions between different components of the ingredients, such as acid-base reactions or enzymatic reactions, can induce alterations in acidity or alkalinity (Rathi and D'Souza, 2015). Considering these factors, it is reasonable to assume that the deviations in pH trends observed in formulations green pomelos and bananas are the combined result of inherent ingredient variations, microbial activity, and chemical reactions within the formulations.

Changes in Colour Observed in the Prepared Formulations

Overall, the changes in colour were not strikingly apparent for the formulations with pomelos kept at different temperatures, over the six days period. This can be attributed to the natural ripening process of pomelo, which occurs gradually over time. Sirisomboon and Lapchareonsuk (2012) observed that the colour of the pomelos did not change much for at least a period of 45 days of storage and could be due to the natural biochemical processes that occur inside the fruit. This suggests that the ripening process may not have been significantly affected by the temperature variations used in the study This could be because the temperatures used were not too extreme to induce the rapid ripening process or colour changes in pomelo.

Except for the formation of an upper clear liquid layer on day 6, no significant change in colour was also observed for green banana (BanY) samples kept at room temperature. Interestingly, green banana samples kept at cool temperature displayed obvious colour changes over the six days period (Figure 5b). Banana, being a tropical fruit, can be sensitive to temperatures as low as 4℃ and exposure to such chilling stress can cause a chilling injury in the fruit (Herppich & Zsom, 2021). Chilling injury, a physiological disorder in plants, can cause discoloration due to oxidation and polymerisation of phenols present in the banana (Murata, 1969) that contribute to their respective colorations. According to Rodrigo et al., (2013) and Chen et al., (2021), fruits, especially citrus fruits contain a variety of pigments. In the case of citrus fruits, the pigment chlorophyll is mainly responsible for green colour, while the pigment, carotenoid is mainly responsible for the yellow colour. The pigments, namely, lycopene, lutein, zeaxanthin, α-carotene, and β-carotene are various other pigments found in the composition of the pomelo fruit (Zhao et al., 2021). So, when the pomelo peel and pulp were boiled in water, these pigments may have drained out into the paste, imparting the characteristic yellow and greenish yellow colours to the respective formulations. The stability of these pigments over the observation period of six days suggests that they were retained in the formulations without significant degradation or colour fading. Overall, the observed colour changes in the formulations can be attributed to the inherent pigments present in the pomelo peel, resulting in distinct colours corresponding to the peel's natural coloration. However, it is important to note that colour stability alone does not necessarily guarantee the overall stability and safety of these prepared pastes for hair treatment.

According to Pereira and Maraschin (2015) and Amini Khoozani (2019), the banana peel and pulp contains various phytochemical compounds such as phenolics, carotenoids, flavonoids, biogenic amines, and phytosterols. They are also rich in starch and cellulose (Singh et al. 2016). The initial colour changes from beige to brown and cream to purplish grey in formulation BanY and BanG respectively, suggest enzymatic reactions and possible degradation of the ingredients. This enzymatic reaction is called enzymatic browning, and it occurs due to oxidation caused by polyphenol oxidase which changes phenolic compounds present in bananas into quinones (Lohner, 2019). Following this, the quinones polymerise to form polyphenols which cause the browning of the fruits and vegetables in general and in this case, bananas (Aimi Azira et al., 2021). In formulation BanG, however, the changes in colour and the fermentation observed are not necessarily only due to the enzymatic reaction that occurred, it strongly indicates microbial growth which could be caused by the microbiota of the used ingredients or due to potential contamination of the preparation in the laboratory. These observations

raise concerns about the stability and safety of these formulations for hair treatment prepared without any additives and preservatives.

Microbiology Assessment and Microscopic Observations of the Prepared Formulations

Natural products are expected to have higher microbial contamination (bioburden) than chemically synthesised active substances (EMA, 2015). Hence, the purpose of microbial assessment in the hair care formulations in this study is to provide a baseline on the presence of microbiological quality. It is noteworthy that the microbial quality of natural products should be generally depended on the nature of the product (ingredient) as well as method of preparation. For instance, boiling was used in preparing the formulations using pomelos, thus the microbial quality of the samples is expected to be low. Findings in this study are limited to screening of microorganisms grown on nutrient agar alone, and no identification of the bacterial species were performed due to time constraint. Nevertheless, the findings suggested that certain bacteria are more predominant than others, when the formulations were stored at different temperatures. Overall, microbial assessment and microscopic observation of the samples revealed visible growth in all the plated samples except for yellow pomelo samples. Despite of the same boiling treatment for all samples containing pomelos, formulations with green pomelos shown a significant growth of microorganisms. PomG samples at cool temperatures contained predominantly Gram-positive staphylococci, whereas PomG samples at room temperature were mainly grown with Gram-negative short rods and bacilli. The presence of the bacteria could also be due to the natural microbiota of the fruits used in the samples. Gram-negative bacteria are prevalence in raw fruits and vegetables with *Bacteroidetes spp.,* a Gram-negative bacillus, are often found in citrus fruits (Ruimy et al., 2010; Xu et al., 2018).

Microbial cultures in banana samples were more diversified with the least growth was detected in formulations using green bananas stored at cool temperature. Mixtures of Gram-positive and Gram-negative short rods and bacilli were seen in all banana samples. Köberl et al. (2022) in their study investigated the microbiome of the banana fruit which consisted of *Pseudomonas spp.* (22–44%), unidentified *Enterobacteriaceae* (23–36%), *Acinetobacter* (10–23%), and *Enterobacter* (5–8%). According to Diskin et al. (2017) and Zhang et al. (2020), these group of bacteria, especially the *Enterobacteriaceae spp,* are native to several fruits such as mangoes and strawberries, even though are potentially harmful to humans, they have specific functions as the microbiota of these fruits. Studies on coconut fruit (flesh) however, are limited. Coconut shells were said to contain bacteria such as *Acinetobacter, Enterobacteriaceae, Flavobacterium, Microbacterium,* and *Micrococcus* species (Kajs et al., 1976). Coconut milk in the shells may be contaminated if the integrity of the coconut shell is compromised, such as broken shells, or unsuitable storage conditions, that could allow microorganisms from the shell to infiltrate the inner hard flesh. The presence of the observed bacteria in the formulations could be collectively due to the above-mentioned reasons.

Considering all the stability assessment results, it can be tentatively concluded that the prescribed formulations from the MMMs may be suitable for immediate use in hair treatment but may have a limited shelf life, as the MMMs do not explicitly mention the duration and the temperatures at which these formulations can be stored. In terms of stability, it can be suggested that the formulation using the yellow pomelo is the most stable formulation and the one using green banana is the least stable in this study. In a nutshell, microbial assessment of the prepared formulations suggested diversity of microorganisms in each formulation. Future study may want to investigate the compatibility of these bioburden of the formulations, with the microbiota of hair and scalp. Identification of the species may also help to understand the interaction between the bioburden of the traditional medical formulations with the microbiota on hair and scalp.

Limitations of the Study

This study has encountered several limitations. To begin with, only a handful of manuscripts were analysed for finding hair treatment formulations due to the unavailability of many transliterated works. Despite the transliteration, the exact details for the preparation of the formulations were not provided in the manuscripts and were left to the interpretation of the text by the readers. This may have somehow influenced the understanding of the ingredients used and methods for preparation of the formulations.

When assessing the microbial quality of traditional medicine or natural products, one must allow a wider acceptance of microbial findings, due to the presence of natural bioburden in the formulations. No identification of microorganisms was carried out in this study, which could contribute to the baseline microbial data for the traditional medicine.

CONCLUSION

In conclusion, this study represents a pioneering effort in both the analysis and experimental preparation of hair treatment formulations sourced from Malay Medical Manuscripts (MMMs). When selecting manuscripts and traditional medical formulations for further study, SAKTI tool provides a simple and reliable assessment. Analysis and grading with the help of the SAKTI indices, helped to narrow down the selection of formulations, and optimise time and resources. In this study, we demonstrated two formulations: (i) pomelo as the main ingredient, and (ii) a mixture of bananas and coconut milk; have high potentials as promising hair care products. In terms of their formulation stability, both products showed steady pH over six days period and minimum changes in terms of the colour (except for formulations using green banana) when kept at cool and room temperatures. Microbial assessment suggested that bioburden of these formulations include predominantly both Gram-positive and Gram-negative bacilli. These preliminary findings can be helpful for further studies in analysis of the treatment formulations present in the MMMs as well as help in potentially developing such formulations as commercial pharmaceutical products.

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SHINGLES REMEDIES BASED ON ANALYSIS OF ACCORDION-FOLDED MALAY MEDICAL MANUSCRIPT MSS 3048

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ABSTRACT

Shingles or herpes zoster (HZ) is a painful disease caused by reactivation of latent varicella zoster virus (VZV) in the sensory dorsal root ganglion cells of human body after the primary infection, chickenpox. Currently, there is no drug that can cure it besides for symptoms relief. Therefore, traditional remedies from credible sources such as the Malay medical manuscript MSS 3048 was referred to for getting various valuable medicinal information regarding cure and treatment of shingles disease. A transliterated Malay medical manuscript (MMM) containing remedies on shingles disease was selected, followed by data extraction and tabulation on its formulations and ingredients used. Subsequently, ingredients from the formulations were comparatively analysed against contemporary studies to discover scientific evidence and pharmacological properties that has high potential to be developed as new pharmaceutical products. After evaluation using SAKTI-iPharmaprospect index, the highly scored-formulations were assessed based on comparative analysis to determine the best formulations that can be recommended for further studies. In MSS 3048, 19 formulations were discovered for shingles disease. Data analysis and comparative study on shingles provided information on pharmacological properties of each plant and there were a few plants having high scientific evidences including *Annona* spp., *Piper betel* Linn, *Schima wallichii*, *Dipterocarpus* spp., *Areca catechu*, *Euphorbia hirta* and *Quercus infectoria*. The use of SAKTI-iPharmaprospect discovered eight Grade A formulations and after assessment with contemporary scientific evidence, three formulations which were F2, F4 and F15 were selected as the best formulations. In conclusion, grade A formulations, F2, F4 and F15 have high potential to be selected for further studies and developed into new pharmaceutical products. The findings from this study may contribute to future laboratory works and research for development of pharmaceutical products for shingles treatment and Traditional and Complementary Medicine (T&CM) in Malaysia.

KEYWORDS

Shingles, Herpes zoster, *kayap*, Varicella-zoster virus (VZV), MSS 3048, Traditional Malay medicine

INTRODUCTION

Shingles or herpes zoster (HZ) is a painful viral infection caused by reactivation of varicella zoster virus (VZV), a type of herpesvirus from family herpesviridae that able to induce persistent infection causing chickenpox and shingles (Freer, 2018). Signs and symptoms of shingles are burning or tingling pain in the skin, rash of fluid-filled blisters, numbness, itching, fever, fatigue and headache. Shingles can be treated using antiviral drugs such as acyclovir, valacyclovir or famycyclovir along with other symptom-based treatments.

Many current modern treatments are known to help alleviate shingles symptoms. However, they cannot effectively cure the patients after primary infection subsided because the virus stayed latent in the sensory dorsal root ganglion cells. Other than that, shingles also cause complications such as long-term nerve pain called postherpetic neuralgia (PHN), eye complications, hearing or balance problem and meningitis (National Institute of Neurological Disorders and Stroke, 2015). Therefore, traditional remedies against shingles are very interesting and beneficial to be studied as they might possess different treatments for the disease and its symptoms. To find credible source from traditional

remedies, a Malay medical manuscript was referred to as it contained valuable medical information that has been preserved from more than 100 years ago.

Malay medical manuscripts (MMMs), handwritten in *Jawi* scripts were produced in the early 16th century until the early 20th century in Malay language (National Library of Malaysia, 2018) and recorded more than 80% of medicinal contents (Mohd Shafri, 2021a) such as illnesses or health issues, formulations for treatment, materia medica, and various healing methods in their texts. In this study, manuscript MSS 3048 containing traditional remedies for shingles or '*kayap*' was obtained from the collection of National Centre for Malay Manuscripts (PKMM) of the National Library of Malaysia.

This study is important in conserving and analysing the medicinal information, their uses and benefits which may contribute to the new discovery and development of pharmaceutical products from naturally occurring ingredients. Additionally, these findings align with the establishment of Traditional and Complementary Medicine (T&CM), and could be beneficial for research and development of Malay medicine for T&CM, as well as modern medicine as supported by the Ministry of Health, Malaysia (MOH).

MATERIALS & METHODS

Manuscript Selection and Data Extraction

An accordion-folded MMM, MSS 3048 that have been transliterated from *Jawi* scripts into Roman alphabets containing formulations for treatment of shingles or '*kayap*' was selected to be studied based on Scientific Analysis of Kitab Tib Index- Index of Manuscript Selection (SAKTI-iMS) (Mohd. Shafri, 2021b). To enable a systematic and objective evaluation for the manuscript selection of MSS 3048, SAKTI-iMS used four major criteria which were: (i) author's profile, (ii) completeness of text, (iii) legibility of text and (iv) amount of medical content in the manuscript. Finally, the sum of scores given according to the four criteria were be graded accordingly. Manuscript with final score (Σx = 5-6) was graded A which indicates a strong candidate, or of high priority, B (Σx =3-4) indicates medium or intermediate priority, C (Σx = 1-2) indicates low priority and D (Σx=0) denotes very low priority for manuscript selection.

Next, numbering system were generated for each medical formulation found in the manuscript. FXX system was used where F refers to 'formulation' and XX were given numbers that denoted the sequence of formulations for shingles remedies. FXX(a) or FXX(b) were designated to indicate the same formulation having two different drug delivery routes. Data from the formulations were extracted and tabulated.

Comparative Analysis and Assessment of Ingredients with Contemporary Scientific Evidence

Comparative analysis was performed on all identified plant-based ingredients mentioned in the shingles formulations to find scientific evidence of their pharmacological properties that could be used for further research on treatment and prevention of shingles disease. Comparative study on the identified plants were conducted using online database Google Scholar and PubMed where related articles were searched using keywords such as "scientific name of plant", "disease", "medicinal importance" and "pharmacological properties", with relevant Boolean operators. Review papers of a particular plant were referred to for getting some ideas on the experimented therapeutic or pharmacological properties of the plant that related to the VZV infection or symptoms of shingles. The articles were selected based on a few criteria. The inclusion criteria include (i) English or Malay language, (ii) time ranging between 1980 and present, (iii) in-vivo, in-vitro, ex-vivo or clinical trial studies (v) related pharmacological action and (vi) full articles. Next, the exclusion criteria were articles of (i) different languages other than English and Malay language, (ii) not full paper (containing abstract only) and (iii) unrelated pharmacological action.

Evaluation of Formulations Using SAKTI- iPharmaprospect

Lastly, systematic search bioprospect using Scientific Analysis of Kitab Tib Index- Index of Pharmaceutical Prospectivity (SAKTI-iPharmaprospect) (Mohd. Shafri, 2021b) was performed to discover the potential and useful application of a formulation. Several components including material

provenance, complexity of formulation, ease of use or drug delivery system and ease of preparation were summed up to get the final score between ∑x = 4 and Σx = 12. The component material provenance consisted of local material, foreign material and mix material from foreign and local source were scored 3, 2 and 1 respectively. Next, the type of formulation consisted of single ingredients was given score 2 and mixed ingredients, was given score 1. Drug delivery system by topical or external application was given the highest score, 3, followed by ear or eye drop, score 2 and oral route, score 1. As for ease of preparation, the formulation that could be immediately used was given the highest score, 4. Other than that, formulation that need one step in a day was scored 3 and formulation with more than 2 steps in a day was scored 2. Table 4 showed the final score graded between A (Σx = 10-12), B (Σx =7-9), and C (Σx = 4-6). Finally, comprehensive assessment of ingredients along with contemporary scientific evidence were performed across all formulations, focusing particularly on Grade A formulations to determine the best formulation with highest potential to be selected for further research.

RESULTS

Manuscript Selection and Evaluation Using SAKTI-iMS Index.

MSS 3048 was selected and graded based on SAKTI-iMS evaluation (Mohd Shafri, 2021b). The amount of medical content of MSS 3048 was evaluated and it was found that the manuscript had more than 80% amount of medical content. Next, evaluation of completeness and legibility of text was conducted. The text in MSS 3048 was legible and considered complete as it has more than 80% of the pages that are able to be read. Lastly, the author's profile was evaluated. There was no author's profile found from this manuscript. Table 1 recorded the evaluation of manuscript MSS 3048 based on the four major selection criteria.

Table 1 Evaluation of MSS 3028 using Index of Manuscript Selection (SAKTI-IMS)

Name of manuscript	Author's profile (x=score)		Integrity of text (x=score)		Legibility of text (x=score)		Medical content (physical treatment) (x=score)					
	Unknown (0)	Known (1)	Incomplete (0)	Complete >80% (1)	Illegible (0)	Legibility >80% (1)	<5% (0)	5-50% (1)	50-80% (2)	>80% (3)	Σx Score	Grade
MSS 3048	✓			✓		✓				✓	5	A

As grading for MSS 3048 was carried out, the score was (∑x = 0+1+1+3) where the final score Σx = 5. Thus, MSS 3048 was graded as Grade A which indicates a strong candidate for manuscript selection and was selected for this study.

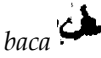
Formulation and data extraction

MSS 3048 containing traditional remedies on shingles or *'kayap'* was found to have 19 formulations in the transliterated text. The formulations were described for different types of *'kayap'* including *'ubat kayap'*, *'ubat kayap ular dan kayap api'*, *'ubat kayap di dalam perut busuk bangar baunya lagi memulas perutnya'* and *'ubat kayap atau cercak'*. The formulations were presented in Table 2.

Table 2: Formulations related to shingles and *'kayap'* in MSS 3048.

Formulation number	Formulations	Ingredients/ Vernacular name	Scientific name
F01 *Ubat kayap*	*Bab ini ubat kayap ambil daun keruing, hujung lemukut, mata kunyit giling lumat-lumat bubuhkan. Jikalau pecah ambil daun benalu mayang dan daun cengkilan, hujung lemukut, mata kunyit giling lumat-lumat bubuhkan pada kayap itu.*	*Daun Keruing*	*Dipterocarpus* spp.
		Hujung Lemukut/ Rice	*Oryza sativa L.*
		Mata Kunyit/ Turmeric	*Curcuma longa*
		Daun Benalu Mayang	-
		Daun Cengkilan/ Purging Croton	*Croton tiglium*
F02 (b) *Ubat kayap di dalam perut*/ Eruption of epithelial surface in gut lining	*Bab ini ubat kayap di dalam perut busuk baunya dan atau putus perutnya oleh sebab penyakit itu, maka ambil daun keremak betina dan daun lonang dan daun nyarang songsang dan sekaliannya itu ambil patinya minum*	*Daun Keremak Betina*/ False Daisy	*Eclipta alba hassk*
		Daun Lonang/ Sugar Apple	*Annona* spp.
F02 (a)	*dan hampasnya bedakkan pada penyakit itu ā'fiyāt.*	*Daun Nyarang Songsang*/ Chaff-Flower	*Achyranthes aspera*
F03 *Ubat membantutkan kayap*	*Bab ini ubat membantutkan kayap maka ambil lada dan kulit tengar maka mamah-mamah semburkan dengan sirih pinang ā'fiyāt.*	*Lada*	*Capsicum*
		Kulit Tengar/ Spurred Mangrove	*Ceriops tagal*
		Sirih/ Betel	*Piper betel Linn*

		Pinang/ Betel Nut	*Areca catechu*
F04 *Ubat membantutkan kayap*	*Sebagai lagi ubat membantutkan kayap maka ambil sirih yang masak tiga helai atau yang kuning maka semburkan pada kayap.*	*Sirih*/ Betel	*Piper betel Linn*
F05 *Ubat kayap*	*Sebagai lagi ubat kayap maka ambil akar lemah-lemah dengan daki buyung bubuh pada kayap itu mujarāb.*	*Akar Lemah-lemah*	-
		Daki Buyung	-
F06 (b) *Ubat kayap di dalam perut*/ Eruption of epithelial surface in gut lining	*Bab ini ubat kayap di dalam perut busuk bangar baunya lagi memulas perutnya. Maka daun tulang-tulang dan daun keremak betina, maka pipis ambil patinya minum*	*Daun Tulang-tulang*/ Indian Tree Spurge	*Euphorbia tirucalli*
F06 (a)	*dan hampasnya bedakkan sekalian tubuhnya*	*Daun Keremak Betina*/ False Daisy	*Eclipta alba hassk*
F07 *Ubat kayap atau cercak*	*Sebagai lagi ubat kayap atau cercak. Maka ambil karang dan beras dan akar cemperai dan ara tanah merah dan kunyit maka giling lumat-lumat tampalkan pada penyakit itu, basuh pagi dan petang. Sudah giling tutup, jangan kena angin kalau-kalau tawar bisanya.*	*Karang*	*Hedyotis phillippensis*
		Beras/ Asian Rice Paddy	*Oryza sativa*
		Akar Cemperai/ False Olive	*Champereia griffithii, Champereia manillana*
		Ara Tanah Merah/ Hairy Spurge	*Euphorbia hirta*
		Kunyit/ Turmeric	*Curcuma domestica, Curcuma longa*
F08		*Akar* [illegible]	-

Ubat kayap atau cercak di mata	*Sebagai lagi ubat kayap atau cercak di mata, keluar nanah dari mata* *. Titis di dalam mata, ambil akar*  *yang menghadap matahari naik asah dengan air bermalam bubuh ke dalam mata itu ā'fiyāt.*	*Air Bermalam/* Day-old water	-
F09 *Ubat kayap*	*Sebagai lagi ubat kayap ambil akar*  *dan daun delima dan pucuk kemudu dan bunga*  *, lada tiga butir semburkan pada kayap itu ā'fiyāt.*	*Akar Melidang*	-
		Daun Delima/ Pomegranate	*Punica granatum*
		Pucuk Kemudu/ Lettuce Tree	*Pisonia grandis*
		Bunga Memeri	-
		Lada	*Capsicum*
F10 *Ubat kayap*	*Sebagai lagi ubat kayap maka ambil pucuk nipah, basuh. Maka kikis kulitnya jangan kena kulit daunnya. Maka ambil batangnya pipis, masuk, hujung lemukut, mata kunyit giling lumat-lumat hancurkan dengan air bermalam maka bubuh pada kayap itu maka baca*  *hingga akhirnya tiga kali senafas, bubuh dengan bulu ayam pada tempat yang sakit itu ā'fiyāt.*	*Pucuk Nipah/* Nipah Palm	*Nypa fruticans Wurmb*
		Hujung Lemukut / Rice	*Oryza sativa L.*
		Mata Kunyit/ Turmeric	*Curcuma domestica, Curcuma longa*
		Air bermalam/ Day-old water	-
		Bulu Ayam/ Chicken	*Gallus gallus domesticus*
F11 *Ubat kayap*	*Sebagai lagi ubat yang diminum dalamnya maka ambil akar lemah-lemah dan akar terung kemar dan buah pala dan manjakani dan cendana canggi asah beri minum ā'fiyāt.*	*Akar Lemah-lemah*	-
		Akar Terung Kemar	*Cyclea laxiflora*

		Buah Pala/ Nutmeg	*Myristica fragrans*
		Manjakani/ Aleppo Oak	*Quercus infectoria*
		Cendana Canggi	*Pterocarpus santalinus Blanco*
F12 *Ubat kayap*	*Sebagai lagi ubat kayap maka ambil daun delima dan daun cucuran atap dan lada maka semburkan pada kayap itu ā'fiyāt.*	*Daun Delima*/ Pomegranate	*Punica granatum*
		Daun Cucuran Atap/ False Ru	*Baeckea frutescens*
		Lada	*Capsicum*
F13 *Ubat kayap*	*Sebagai lagi ubat sakit kayap maka ambil sabuk dan sepah dan rambu tikar maka ambil abunya bubuh dengan air pinang ā'fiyāt.*	*Sabuk*	-
		Sepah	-
		Rambu Tikar	-
		Air Pinang/ Betel Nut	*Areca catechu*
F14 *Ubat kayap*	*Sebagai lagi ubat kayap maka ambil butir macang dan lada semburkan pada kayap itu ā'fiyāt.*	*Butir Macang*/ Horse Mango	*Mangifera foetida*
		Lada	*Capsicum*
F15 *Ubat kayap*	*Ini ubat bedaknya maka ambil daun cangkuk manis dan beras kunyit giling lumat-lumat bedakkan ā'fiyāt.*	*Daun Cangkuk Manis*/ Needlewood	*Schima wallichii*
		Beras Kunyit/ Rice	*Oryza sativa*
F16 *Ubat kayap*		*Isi* طاکیت	-

	Sebagai lagi ubat kayap maka ambil isi طالكيت *dan akar rotan tawar semburkan pada kayap itu ā'fiyāt.*	*Akar Rotan Tawar*/ Rattan	*Calamus aquatilis*
F17 *Ubat kayap*	*Fasal ini pada menyatakan ubat kayap. Sebermula kayap itu tiga pagi, pertamanya kayap api dan keduanya kayap ular, ketiganya kayap air. Adapun kayap api itu maka ambil timah hitam, asah bubuhkan. Kemudian maka ambil bawang dan daun kayu yang lendir dan yang sejuk maka giling dengan hujung lemukut, mata kunyit maka bubuh pada kayap itu.*	*Timah Hitam*	*Lead*
		Bawang	*Allium sp.*
		Daun Kayu yang Lendir dan Sejuk (Slime, From Plants)	-
		Hujung Lemukut/ Rice	*Oryza sativa L.*
		Mata Kunyit/ Turmeric	*Curcuma longa*
F18 *Ubat kayap*	*Sebagai lagi ubat kayap maka ambil sekalian perkara wangi dan jangan yang gatal tiada baik, maka bubuh pada kayap itu ā'fiyāt.*	*Perkara Wangi* (Aromatic)	-
F19 *Ubat kayap ular dan kayap api*	*Dan adapun kayap ular dan kayap api itu sangat-sangat mintak tolong orang tawar bisanya akan hal ubatnya kayap ular dan kayap api itu sekalian barang yang lendir insyā Allāh tawarlah bisanya.*	*Barang Yang Lendir* (Slime, might be from Plants or Animals)	-

Comparative analysis against contemporary scientific evidence

To compare the identified plants in MSS 3048 against contemporary published studies, the pharmacological properties of each plant was presented in Table 3. Pharmacological actions found related to shingles were antiviral or antiherpetic, anti-inflammatory, wound healing, analgesic, antibacterial, and anti-neuroinflammatory properties.

Keruing found in F01 possess antibacterial, antiviral, anti-inflammatory and wound healing properties. Next, *keremak betina* mentioned in F02 and F06, *lada* or *Capsicum* spp. in F03, F09, F12 and F14, *nipah* in F10, *buah pala* or nutmeg in F11, *cendana canggi* in F11, and *macang* or horse mango in F14 possess antibacterial, analgesic and anti-inflammatory pharmacological properties. Other than that, analgesic, antibacterial, wound healing and anti-inflammatory properties were found for *lonang* in F02, *sirih* or betel leaves in F03 and F04, *pinang* or betel nut in F03 and F13, *ara tanah merah* in F07 and *manjakani* in F11. As for *delima* or pomegranate cited in F09 and F12, it comprises of anti-inflammatory, antibacterial, and antiviral properties while *cangkuk manis* noted in F15 held anti-inflammatory, analgesic, antipyretic, and antibacterial properties. As for *mata kunyit* or turmeric in F01, F07, F10 and F17, *kemudu in F09 and bawang or Allium* spp. *in F17*, they held anti-inflammatory, wound healing, and antibacterial properties. Wound healing and antibacterial properties were identified for *nyarang songsang* in F02 while antiviral and antibacterial properties was identified for *tulang-tulang* in F06. On the other hand, *cengkilan* in F01 had the analgesic and anti-neuroinflammatory properties that could reduce pain caused by shingles lesion and helped in the injury to peripheral as well as central nervous system caused by PHN complication. *Rotan tawar* written in F16 was observed to have anti-inflammatory and antibacterial properties. *Karang* in F07 and *Oryza sativa* mentioned in MSS 3048 as *hujung lemukut, beras,* and *beras kunyit* found in F01, F07, F10, F15 and F17 only had anti-inflammatory properties. Finally, *terung kemar* cited in F11 and *cucuran atap* mentioned in F12 consisted of antibacterial and wound healing properties respectively.

From the analysis of ingredients against contemporary published studies depicted in Table 4, the findings demonstrated that *keruing* (*Dipterocarpus* spp.), *lonang* (*Annona* spp.), *sirih (Piper betel Linn), pinang (Areca catechu), ara tanah merah (Euphorbia hirta), manjakani (Quercus infectoria)* and *cangkuk manis (Schima wallichii)* exhibited the highest number of pharmacological properties. Each ingredients possess a minimum of four distinct pharmacological properties and were most frequently cited in published articles in relation to treatments for symptoms associated with shingles infections.

Table 3 Analysis and contemporary published articles for ingredients in shingles formulation.

Scientific name/ Vernacular name	Formulation number	Properties of ingredients	References	Types of study
Dipterocarpus spp./ *Keruing*	F01	Antibacterial	(Samad & Silva, 2021)	in vitro
			(Le et al., 2021)	in vitro
		Antiviral	(Shen et al., 2017)	in vitro
		Anti-inflammatory	(Yang et al., 2013)	in vivo
			(Fernandes & Maharani, 2019)	in vitro
		Wound healing	(Biswas et al., 2004)	in vivo
Oryza sativa/ *Hujung lemukut, Beras, Beras kunyit*	F01 F07 F10 F15 F17	Anti-inflammatory	(Limtrakul et al., 2016)	in vitro

Curcuma longa/ *Mata kunyit*	F01 F07 F10 F17	Anti-inflammatory	(Motterlini et al., 2000) (Ramsewak et al., 2000)	in vitro in vitro
		Wound healing	(Kundu et al., 2014)	in vivo
		Antibacterial	(Singh et al., 2002)	in vitro
Croton tiglium/ *Cengkilan*	F01	Analgesic	(Liu et al., 2016)	in vivo & in vitro
		Anti-neuroinflammatory	(Gupta et al., 2020)	in vitro
Eclipta alba hassk/ *Keremak betina*	F02 F06	Antibacterial	(Pandey et al., 2011)	in vitro
		Analgesic	(Sawant et al., 2004)	in vivo
		Anti-inflammatory	(Leal et al., 2000)	in vivo
Annona spp./ *Lonang*	F02	Analgesic	(Abd Hamid et al., 2012)	in vivo
		Antibacterial	(Padhi et al., 2011)	in vitro
		Wound healing	(Moghadamtousi et al., 2015)	in vivo
		Anti-inflammatory	(De Sousa et al., 2010)	in vivo
Achyranthes aspera/ *Nyarang songsang*	F02	Wound healing	(Edwin et al., 2008)	in vivo
		Antibacterial	(Abi Beaulah et al., 2011)	in vitro
Capsicum spp./ *Lada*	F03 F09 F12 F14	Analgesic	(Jolayemi & Ojewole, 2014)	in vivo
		Anti-inflammatory	(Zimmer et al., 2012)	in vivo
		Antibacterial	(Nascimento et al., 2014)	in vitro
Piper betel Linn/ *Sirih*	F03 F04	Antibacterial	(Akter et al., 2014)	in vivo
		Analgesic	(Reddy et al., 2016) (Alam et al., 2021)	in vivo in vivo
		Anti-inflammatory	(reddy et al., 2016) (Alam et al., 2021)	in vivo in vivo
		Wound healing	(Nilugal et al., 2014)	in vivo
Areca catechu/ *Pinang*	F03 F13	Anti-inflammatory	(Khan et al., 2011)	in vivo
		Wound healing	(Dewi & Fatonah, 2019)	in vivo
		Analgesic	(Bhandare et al., 2010)	in vivo
		Antibacterial	(Negi & Dave, 2010) (Anupama et al., 2021)	in vitro in vitro

Euphorbia tirucalli/ Tulang-tulang	F06	Antiviral	(Son et al., 2013)	in vitro
			(Betancur-Galvis, 2002)	in vitro
		Antibacterial	(Upadhyay et al., 2013)	in vitro
Hedyotis phillippensis/ Karang	F07	Anti-inflammatory	(Lin et al., 2002)	in vivo
			(Chen et al., 2015)	in vitro
Euphorbia hirta/ Ara tanah merah	F07	Antibacterial	(Enerva et al., 2015)	in vitro
		Wound healing	(Upadhyay et al., 2014)	in vivo
		Anti-inflammatory	(Gunjan et al., 2021)	in vivo
		Analgesic	(Lanhers et al., 1990)	in vivo
Punica granatum/ Delima	F09 F12	Anti-inflammatory	(Lee et al., 2010)	in vitro & in vivo
		Antibacterial	(Duman et al., 2009)	in vitro
		Antiviral	(Angamuthu, 2019)	in vitro
Pisonia grandis/ Kemudu	F09	Anti-inflammatory	(Radha, 2008)	in vivo
		Antibacterial	(Prabu, 2015)	in vivo
		Wound healing	(Prabu, 2015)	in vivo
Nypa fruticans Wurmb/ *Nipah*	F10	Antibacterial	(Lovly & Marlee, 2018)	in vitro
		Anti-inflammatory	(Kang & Hyun, 2020)	in vivo
		Analgesic	(Reza et al., 2011)	in vivo
Cyclea laxiflora/ Terung kemar	F11	Antibacterial	(George et al., 2017)	in vitro
			(Raja et al. 2011)	in vitro
Myristica fragrans/ Buah pala	F11	Anti-inflammatory	(Jin et al., 2012)	in vitro
		Antibacterial	(Ibrahim et al., 2011)	in vitro
		Analgesic	(Hayfaa et al., 2013)	in vivo
Quercus infectoria/ Manjakani	F11	Anti-inflammatory	(Kaur et al., 2004)	in vivo & in vitro
		Wound healing	(Jalalpure et al., 2002)	in vivo
			(Umachigi et al., 2008)	in vivo
		Analgesic	(Fan et al., 2014)	in vivo
		Antibacterial	(Basri & Fan, 2004)	in vitro
Pterocarpus santalinus Blanco/ *Cendana canggi*	F11	Analgesic	(Tippani et al., 2010)	in vivo
				in vivo

			(Sikdar et al., 2013)	
		Anti-inflammatory	(Wu et al., 2011)	in vitro
			(Mohammed Usman, 2012)	in vitro
		Antibacterial	(Gayathri & Kannabiran, 2009)	in vitro
			(Donga et al., 2017)	in vitro
Baeckea frutescens/ Cucuran atap	F12	Wound healing	(Kamarazaman et al., 2022)	In vitro
Mangifera foetida/ Macang	F14	Analgesic	(Garrido et al., 2001)	in vivo
		Anti-inflammatory	(Garrido et al., 2001)	in vivo
			(Garrido et al., 2004)	in vivo & in vitro
		Antibacterial	(Engels et al., 2011)	in vitro
Schima wallichii/ Cangkuk manis	F15	Anti-inflammatory	(Dewanjee et al., 2009)	in vivo
			(Dewanjee et al., 2011)	in vivo & in vitro
		Analgesic	(Dewanjee et al., 2009)	in vivo
		Antipyretic	(Dewanjee et al., 2009)	in vivo
		Antibacterial	(Barma et al., 2015)	in vitro
Calamus aquatilis/ Rotan tawar	F16	Anti-inflammatory	(Chang et al., 2010)	in vitro
		Antibacterial	(Borah et al., 2015)	in vitro
Allium spp./ *Bawang*	F17	Wound healing	(Jalali et al., 2009)	in vivo
		Anti-inflammatory	(Jayanthi & Dhar, 2011)	in vivo
		Antibacterial	(Daka, 2011)	in vitro

Evaluation of formulations using SAKTI- iPharmaprospect

Subsequently, SAKTI-iPharmaprospect (Mohd Shafri, 2021b) was performed for grading of shingles formulations along with assessment of their contemporary scientific evidence to evaluate the best formulation that have high potential to be selected for further studies. The scoring and grading of the formulations were shown in Table 4. Grade A, which was regarded as easy formulations was given for F4 with final score ($\Sigma x = 12$) while F2(a), F3, F4, F7, F13, F15, F18 and F19 with ($\Sigma x = 11$). For grade B or intermediate formulations, the final score ($\Sigma x = 9$) was given for F2(b), F6(a), F10, F12, F14, and F17. Final score ($\Sigma x = 8$) was given for F1, F5, F9 and F16 while F6(b) scored ($\Sigma x = 7$)**.** Lastly, F8 ($\Sigma x = 5$) and F11 ($\Sigma x = 6$) that were graded as grade C observed as difficult formulations. F2(a) and F2(b) consisted of same material provenance, type of formulation and ease of preparation but different drug delivery system which had affected the grading of the formulations. F2(a) used the oral drug delivery system and graded as A while F2(b) utilized topical application and graded as B. As for F6(a) and F6(b), they also used different drug delivery system but were graded as B.

Table 4 Scoring and grading of shingles formulations in MSS 3048 using SAKTI iPharmaprospect.

FORMULATION NUMBER, F(Y)	Material Provenance			Type of Formulation		Drug Delivery System			Ease of Preparation				Σx Score	GRADE: Easy: A: 10-12 Intermediate: B: 7-9 Difficult: C: 4-6
	Local material	Foreign material	Mix	Single ingredient	Mixed ingredients	Topical /external	Ear / Eye drop	Oral	Immediate use	1 step, in a day	>2 steps, in a day	>1 day		
SCORE	3	2	1	2	1	3	2	1	4	3	2	1		
F1			-		✓	✓			✓				8	B
F2 (a)	✓				✓	✓			✓				11	A
F2 (b)	✓				✓			✓	✓				9	B
F3	✓				✓	✓			✓				11	A
F4	✓			✓		✓			✓				12	A
F5			-		✓	✓			✓				8	B
F6 (a)			✓		✓	✓			✓				9	B
F6 (b)			✓		✓			✓	✓				7	B
F7	✓				✓	✓			✓				11	A
F8			-	-			✓			✓			5	C
F9			-		✓	✓			✓				8	B
F10	✓				✓	✓					✓		9	B
F11			-		✓			✓	✓				6	C
F12			✓		✓	✓			✓				9	B
F13	✓				✓	✓			✓				11	A
F14			✓		✓	✓			✓				9	B
F15	✓				✓	✓			✓				11	A
F16			-		✓	✓			✓				8	B
F17			✓		✓	✓			✓				9	B
F18	✓				✓	✓			✓				11	A
F19	✓				✓	✓			✓				11	A

DISCUSSION

Shingles treatment in Grade A MSS 3048 mentioned 19 formulations consisting of single and compounded ingredients. From the 44 ingredients mentioned, 29 plants were identified and analysed against contemporary published studies. From analysis of Table 3, three plants had been tested in vitro for their antiviral or antiherpetic properties against herpesviridae family. In a study by Betancur-Galvis et al. (2002), the capacity of *Euphorbia tirucalli* extract to inhibit herpes simplex virus type 2 (HSV-2) lytic activity was evaluated using end-point-titration technique and MTT antiviral colorimetric assay. The findings showed that *Euphorbia tirucalli* water-methanol extract exhibited antiherpetic action with highest therapeutic index >7.1 compared to other plants from genus Euphorbia and shows no cytotoxicity.

As shingles was caused by VZV, studies on plants that were conducted on other herpesviruses such as HSV-1 and HSV-2 might have potential to exhibit same antiherpetic effect against the virus because all three viruses had been categorised as alphaherpesviruses (Baines & Pellett, 2007) and neurotropic that infected nervous system tissue (Roizman & Thayer, 2001). These viruses had same characteristics of unique four-layered structure which were a core with large, double-stranded DNA genome, enclosed by icosapentahedral capsid composed of capsomers and capsid surrounded by tegument amorphous protein coat. It was encased in a glycoprotein-bearing lipid bilayer envelope (Whitley, 1996). They also had 120 to 230 kbp length, base composition ranging from 31% to 75% G+C content and contained 60 to 120 genes (Roizman & Thayer, 2001). These common characteristics among the viruses might have potential for the plants to express same antiviral effect against them.

Shingles disease associated with pain, skin lesion, inflammation, and acute neuritis that causes burning or tingling sensation of the skin (Bolton et al., 2021). From Table 3, 13 plants possessed analgesic pharmacological properties and one of them was *Nypa fruticans* Wurmb. According to Reza et al. (2011), an in-vivo study for analgesic activity was performed using methanolic extract of leaf and stem of *Nypa fruticans* Wurmb(MENF) in experimental animals. The experiment used acetic acid induced writhing test in mice model to detect central and peripheral analgesia where they were treated with MENF of different doses and standard drug, aspirin. The mice were injected with acetic acid to cause pain by releasing endogenous mediators and production of prostaglandin, mainly prostacyclines (PGI2) and prostaglandin-E (PG-E) that was liable for pain sensation. Analgesic properties in the plant inhibited endogenous mediator prostaglandin synthesis observed through the results on maximum inhibition of writhing. The findings demonstrated significant analgesic activity which was better than the result obtained with aspirin indicating MENF have high potential to be developed as analgesia derived from local products.

Next, 20 plants had been studied in vivo and in vitro for their anti-inflammatory properties. One of the plants, *Myristica fragrans* mentioned by Jin et al. (2012) contained myrislignan, a compound that could attenuate lipopolysaccharide-induced inflammation in macrophages. As inflammation could occur due to inflammatory mediators, myrislignan were able to exhibit anti-inflammatory properties by inhibiting nitric oxide production in a dose-dependent manner, suppressed mRNA expression and inhibited the release of interleukin-6 (IL-6) and tumour necrosis factor-a (TNF-a). Myrislignan also decreased the cytoplasmic loss of inhibitor kB-α (IkB-α) protein and the translocation of NF-kB from cytoplasm to the nucleus, hence exhibited anti-inflammatory effects in LPS-stimulated macrophages cells by inhibiting the NF-kB signalling pathway activation.

Wound healing property of plants also important for healing of skin lesion after infection. 11 plants mentioned in the formulations possessed this property. An in vitro study of *Baeckea frutescens* by Kamarazaman et al. (2022) evaluated wound healing property by observing the increased rate of cytotoxicity proliferation and migration rate on keratinocytes and fibroblasts that were parts of complex wound healing process. In this study, cells viability of immortalized human keratinocyte and human dermal fibroblast against *Baeckea frutescens* leaves extract (BFLE) were performed using MTT assay. It was discovered that migratory effect BFLE on both fibroblast and keratinocyte displayed good response on speeding the proliferative phase of wound healing and toward the closing of wound gaps during wound contraction. Other than that, BFLE contained condensed tannins, flavonoids, steroids and saponins that also contributed to wound healing.

According to Bassukas and Kiorpelidou (2006), another complication of shingles was secondary invasive cutaneous and extracutaneous bacterial superinfection commonly caused by *Staphylococcus aureus* and Group A β hemolytic Streptococcus. Based on Table 3, in vitro studies for antibacterial properties were found on 21 plants. Anupama et al. (2021) demonstrated in vitro antibacterial study for evaluation of antibacterial properties of *Areca catechu* against two common bacterial pathogens, *Staphylococcus aureus* and *Escherichia coli*. The bacterial strains were inoculated into nutrient broth and incubated overnight with increasing concentration of *Areca catechu* extract. The absorbance at 530nm were recorded and bacterial growth inhibition were calculated. It was discovered that aqueous extract of *Areca catechu* exhibited antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli* being tested. In conclusion, the plant that exhibited antibacterial action against *Staphylococcus aureus* could potentially treat and prevent bacterial superinfection due to VZV infection.

Other pharmacological property found was anti-neuroinflammatory property of *Croton tiglium*. As infection of VZV causing shingles would lead to unilateral, painful vesicular rash at a single dermatome due to reactivation of virus at the sensory root ganglion cells, it might cause injury to peripheral and central nervous system (Koshy et al., 2018). With anti-neuroinflammatory property, the consequences and complications of the infection could be prevented. Gupta et al. (2020) described an investigation on neuroprotective and anti-inflammatory effect of *Croton tiglium* extract (CTE). It was found that CTE significantly suppressed neurotoxic inflammatory factors production and increased neurotrophic factor and microglia production. In addition, CTE improved protection of neurons against neurotoxic factors released from LPS-stimulated microglia. This indicated the neuroprotective effect of CTE and had high potential as anti-neuroinflammatory agent.

MSS 3048 demonstrated 19 formulations for shingles remedies with 4 formulations consisting of single ingredients and 15 formulations encompassing mixed ingredients. Individual formulations were evaluated by utilizing SAKTI-iPharmaprospect, hence determined the grade of each formulation. In addition, as this evaluation was formulation-centred, the SAKTI-iPharmaprospect scores must also be assessed together with results from the comparative analysis. The combination of SAKTI-iPharmaprospect and scientific evidence from contemporary studies on plants found during the analysis further helped and contributed in evaluation and selection of the best formulation to treat this disease. In this study, SAKTI-iPharmaprospect was used to identify the formulations with the highest final score, especially those graded as A as these formulations had highest potential to be utilised for new treatments and development of drugs. Next, the re-assessment of contemporary scientific evidence from the former methodology was performed on ingredients used in the Grade A formulations to finally determine the best formulation that can be recommended for further study.
From Table 4, Grade A formulations including F2(a), F3, F4, F7, F13, F15, F18 and F19 used combination of two or more ingredients from local source, employed external drug delivery system and could be used immediately. Subsequently, the contemporary scientific evidence on the ingredients mentioned in Grade A formulations were assessed and the best formulations were described by the author as below:

F02:
"*Bab ini ubat kayap di dalam perut busuk baunya dan atau putus perutnya oleh sebab penyakit itu, maka ambil daun keremak betina dan daun lonang dan daun nyarang songsang dan sekaliannya itu ambil patinya minum dan hampasnya bedakkan pada penyakit itu ā'fiyāt.*"

Meaning: This chapter is a remedy for eruption of epithelial surface in gut lining, the smell is bad, and or the stomach breaks due to the disease. Take *keremak betina* leaves and *lonang* leaves and *nyarang songsang* leaves, take their extract and drink and grind (any leftover) the residues finely. Apply it on the disease. It will heal.

F02(a), a Grade A formulation with final score of ($\Sigma x = 11$) cited the usage of mixture of three ingredients and all the ingredients which were *keremak betina* (*Eclipta alba* hassk), *lonang (Annona* spp.) and *nyarang songsang* (*Achyranthes aspera)* showed scientific evidence of pharmacological properties including antibacterial, analgesic, anti-inflammatory, and wound healing. These ingredients were local ingredients that could be easily found and the formulation could be immediately used by applying it topically on the disease.

F04:
"*Sebagai lagi ubat membantutkan kayap maka ambil sirih yang masak tiga helai atau yang kuning maka semburkan pada kayap.*"

Meaning: Also, remedy to curb shingles. Take three ripe betel leaves or the yellow one, then spray on the shingles.

Grade A formulation, F04 had the highest final score of ($\Sigma x = 12$). It used only one ingredient, *betel* leaves (*Piper betel Linn)* that was be easily found and could be sourced locally. The *Piper betel* Linn presented evidence on antibacterial, analgesic, anti-inflammatory and wound healing properties. The

formulation was also easy to prepare, could be immediately used and the delivery system of the drug was also less complex as it can be applied externally. This indicated the benefit of using one local ingredient with many pharmacological properties that has potential for further studies to be developed as treatment for shingles infection.

F15: elaborate
"*Ini ubat bedaknya, maka ambil daun cangkuk manis dan beras kunyit giling lumat-lumat bedakkan ā'fiyāt.*"

Meaning: This is the powder medicine. Take *cangkuk manis* leaves and turmeric rice, grind them finely, apply. It will heal.

Lastly, grade A formulation F15 with final score of ($\Sigma x = 11$) containing *cangkuk manis* (*Schima wallichii*) and *beras kunyit (Oryza sativa)* were revealed to be having anti-inflammatory, analgesic, antipyretic and antibacterial effects based on the contemporary scientific evidence. These mixed ingredients could be easily sourced locally, utilized external application and immediately used after preparation. The formulation suggested its potential to be considered for further studies related to shingles infection.

Meanwhile, for other Grade A formulations, scientific evidence on their pharmacological properties against contemporary studies on some of the ingredients were not found. This could be attributed to the decreased usage of ingredients, stemming from scarcity of the plants, which lead to challenges in their acquisition. Consequently, there was a decrease or no research conducted on these ingredients.

Following assessment and evaluation using both SAKTI-iPharmaprospect and contemporary scientific evidence criteria, F2, F4 and F15 which exhibited various important pharmacological properties essential for treatment of shingles could be shortlisted for further laboratory works and studies. With proper research supported by modern scientific empirical data, these formulations utilizing available local resources from the Malay Archipelago would demonstrate significant potential to be developed into pharmaceutical products for treatment of shingles diseases.

CONCLUSIONS

MSS 3048 is a unique accordion-folded manuscript, handwritten in *Jawi* scripts that recorded 19 formulations for shingles remedies mentioning 44 ingredients with 29 plants identified with their scientific names. The plants had various pharmacological properties related to shingles where 3 plants possessed antiviral properties against the alphaherpesviruses, 13 plants showed analgesic pharmacological properties, 20 plants with anti-inflammatory properties and 1 plant exhibited anti-neuroinflammatory properties. Other than that, 21 plants possessed antibacterial properties that could fight against shingles complication of bacterial superinfection and 11 plants presented wound healing properties that could be used to heal the wound due to lesion during infection. In addition, evaluation of shingles formulations using SAKTI-iPharmaprospect had found that three Grade A formulations, F2, F4 and F15 could be selected as the best formulations using assessment based on contemporary scientific evidence. These findings revealed high potential of formulations to be developed as commercially valuable and viable pharmaceutical products that able to contribute to further research and laboratory works for treatment and prevention of shingles disease other than help in the development of Traditional and Complementary Medicine (T&CM) in Malaysia.

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PLANTS WITH ANTINOCICEPTIVE ACTIVITY FROM MSS 3048 FOR SHINGLES TREATMENT: A MOLECULAR DOCKING STUDY OF THE ACTIVE COMPOUNDS WITH P2X4 RECEPTOR

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ABSTRACT

Malay medical manuscripts (MMM) discuss diseases and remedies according to Malay traditional medicine. One of them, MSS 3048, compiles a variety of diseases including shingles; a viral disease that manifests itself with neuropathic pain. One of the mechanisms that cause neuropathic pain is through the activation of P2X4, an ionotropic receptor. Hence, one of the strategies for treating neuropathic pain is by inhibiting the receptor. However, current antinociceptive drug can cause serious side effects like respiratory depression. Thus, the goal is to identify potential compounds that can block P2X4 based on the information from MSS 3048 and *in silico* study. Firstly, the content of MSS 3048 was analysed to find the plants used for shingles. Then, the active compounds possessed by the plants were studied and the ones that had been shown to have antinociceptive effect based on literature studies were selected. Then the 3D structures of the plant's active ingredients were docked on the human P2X4 homology model using Autodock Vina software. The software produced results that showed the binding affinity of the active ingredients towards P2X4. The inhibitors' affinity was compared to known ligands which are 5-(3-Bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one(5-BDBD),adenosine triphosphate (ATP), cytidine triphosphate (CTP) and α,β-methylene ATP (α,β-meATP) as a reference. The result shows that the top six compounds with high affinity towards P2X4 are dioscin, ampelopsin F, corilagin, ellagic acid pentoside, punicalin and proanthocyanidins. Then the top six compounds were analysed by looking at the chemical interaction. The compound that showed the highest potential was dioscin, one of the compounds from nipah palm, with binding affinity of -9.87 kcal/mol. This compound may have the potential to block P2X4 receptor and could be studied in *in vitro* and *in vivo* study before being used as treatment for shingles.

Keywords: Malay medical manuscript, shingles, P2X4 receptor, *in silico* study, molecular docking

INTRODUCTION

Shingles, or known as *kayap* in Malay, is caused by Varicella-zoster virus (VZV). People with previous infections to chickenpox are more prone to develop shingles as it is caused by the same virus. Peate (2010) stated that this virus infects the nerves and manifests itself with a painful rash on the side supplied by the infected nerves. Wilson and Wilson (2021) explained that the symptoms include pain before and during the rash, fever, malaise, and headache, which can last up to four weeks. This type of pain is called neuropathic pain as it arises from nerve damage. One of the treatments is to manage the pain with the use of painkillers. Ibuprofen and acetaminophen have fewer side effects but are not strong enough to relieve the pain. On the other hand, Tylenol and codeine are among the stronger painkillers but with more serious side effects. Other than painkillers, antiviral medicine is one of the treatments for shingles. Center for Disease Control (CDC) (2023) stated that the administration of antiviral is to shorten the length and severity of the pain, not eliminating the virus fully. Hence, herbal medicine becomes an option when modern medicine is ineffective in the treatment of disease or due to concern about the adverse effects.

The treatment for shingles had been discussed in many Malay medical manuscripts including MSS 3048. The manuscript is divided into two sections, part A, Pelbagai Petua and Part B, Kitab Tib. *Katalog Manuskrip Melayu Koleksi Perpustakaan Negara Malaysia Tambahan Keempat* (2006) added that among the contents covered in part B includes traditional remedies for seizures, coughs, stomach aches, eye

diseases, shingles, urinary stones, and amenorrhea. This manuscript also describes a few plants, herbs, and food that are used to treat the previously mentioned diseases. Besides that, MSS 3048 also contains non-medical information such as amulets and advice for the community's daily lives at the time.

One of the receptors involved in pain signalling is P2X4 in which its activation, induced by binding of adenosine triphosphate (ATP), results in the propagation of nociception. Natural ingredients in plants are being investigated to develop new antinociceptive drugs with potential therapeutic effects in pain management. Makoto et al. (2012) stated that the P2X4 receptor is involved in the pathogenesis of neuropathic pain when expressed on microglia. The activated glia elicits responses such as the release of cytokines and neurotrophic factor, resulting in the hyperexcitability of dorsal horn neurons and neuropathic pain. Thus, blocking the P2X4 receptor will reverse the injury and is said to be a potential therapeutic target for neuropathic pain (Tsuda et al., 2013). It serves as the basis for this research to find compounds that can bind to P2X4 receptors in which the compounds are tested for antinociceptive activity.

Since the conventional drug development process is time-consuming, *in silico* method such as computer-aided drug design (CADD) are introduced. In CADD, drug targets are identified, chemical compounds are screened and optimized for drug candidates, and toxicity is assessed. All these are done using computational methods. These strategies can reduce the number of chemical compounds that need to be tested experimentally while increasing the success rate by eliminating ineffective and harmful chemical compounds from consideration (Shaker et al., 2021). Hence, *in silico* study help in reducing time and cost in identifying active ingredients that have high affinity to P2X4 receptor.

MATERIALS & METHODS

Materials

All the molecular docking and modelling jobs were performed using a laptop with processor of Intel® core ™i5-10210U CPU@1.60GHz 2.11GHz with Windows 11 operating system. The molecular docking was conducted using Autodock Vina version 1.1.2, The Scripps Research Institute, United States of America. (Trott & Olson, 2010).

Identification of P2X4 and Ligands

P2X4 was chosen as the receptor of interest in this study. Since the crystal structure of P2X4 in Protein Data Bank (PDB) is only available for zebra fish, the homology model of human P2X4 was obtained from Zayuri (2020).

Ligands are the active ingredients of the plants and were obtained from the transliteration of MSS 3048 by Ahmad Radzaudin (2021). A total of 19 formulations were listed for shingles. From the formulations, a total of 22 plants were listed. The active ingredients of each plant were identified through literature search. The inclusion criteria for the literature were articles from year 2000 until present, written in Malay or English, and in full paper. The database used was Google Scholar, using the keywords such as "scientific name of plants" "antinociceptive" "analgesic" "phytochemical analysis" together with the use of Boolean operators, AND and OR.

A total of 112 compounds were found altogether through literature search. These compounds were filtered based on Lipinksi's Rule using an online tool (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp), which is useful for identifying molecules that are drug-like and those that are not.

Receptor and Ligands Preparation

The 3D structure of the P2X4 receptor was obtained from Zayuri (2020) in *.pdb format. The structure was modified to be used for molecular docking by removing water molecules, adding hydrogen atoms, and merging polar and non-polar hydrogens, using a software called AutoDockTools, The Scripps Research Institute, United States of America. (Sanner et al., 1999). The structure then was converted to *.pdbqt format.

The structures for active ingredients were obtained from the PubChem Open Chemistry database. The 3D structure of the compounds was downloaded in *.sdf format. Certain compounds only have 2D structure available in the database, so Avogadro software (open-source tool) was used to generate the 3D structure. The 3D structure was optimized in Avogadro using Generalized Amber Force Field (GAFF), a type of force field. Then, Open Babel software (SourceForge, United States of America) was used to convert *.sdf to *.mol2 format and hydrogen had been added to polar atoms only. The conversion of *.mol2 to *.pdbqt format was done in AutoDockTools. This process started by detecting the root of the torsion tree. Then, the root was chosen, and torsion was added to that root.

Docking Parameter and Control Docking

AutoDock Vina software (The Scripps Research Institute, United States of America) was used for docking. The parameter for docking needs were set to search for a binding site. The amino acid residues were Lys67, Lys69, Thr186, Lys190, Ile218, Asn293, Arg295, and Lys313 (Ahmad, 2018). These residues acted as binding sites for the ligands in the target structure. The receptor grid box size and coordinates are the important parameters in AutoDock Vina. The size of the grid box was set to 32.0 Å by 36.0 Å by 28.0 Å with 1.0 Å spacing, centered at coordinates x=69.393, y=-24.195, z=-8.315 and the exhaustiveness was set to twelve.

Control docking was done using AutoDock Vina to optimize the correct parameters and to make sure it is reliable for the receptor. It was done on a known agonist which are, adenosine triphosphate (ATP), cytidine triphosphate (CTP) and α, β-methylene ATP (α, β-meATP) and the antagonist which is 5-(3-Bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one (5-BDBD). This step was repeated three times, and the average binding energy for each agonist was recorded and compared to the known P2X4 agonist rank of EC_{50} (Ahmad, 2018). After the parameter was verified, the prepared ligands were docked with the P2X4 receptor in triplicate, and the average binding affinity was then reported. Control docking was performed, and the outcome shows the reliability of the binding site based on the ranking of affinities.

Docking and Analysis

For Autodock Vina, several files were required after downloading and preparing the ligands that bind to the P2X4 receptor. The files required are receptor files with residue information, ligand files, and configuration files with docking parameter information. The docking was carried out using the Windows command prompt (cmd). The docking process was done in triplicate to get the average for better confirmation results. Vina produced 9 conformations for each docking in a text document (.txt) file and .pdbqt file format.

The Ligplot+ software (European Bioinformatics Institute, United Kingdom) was used to analyse hydrogen bonding and hydrophobic interaction between ligands and P2X4. The PDB file for the complex of P2X4 and the docked compound was created. Types of interactions between ligands and P2X4 were examined and important residues that made up the binding were recorded.

RESULTS

Malay medical manuscript MSS 3048 was referred to find the plants associated with shingles treatment. A total of 22 plants were listed from 19 formulations related to shingles. Then, its active compounds were searched through literature using the inclusion criteria. The articles include review and experimentation articles. A total of 112 compounds with antinociceptive activity were found. The full result of the plants' scientific names and its active compounds is listed in Appendix A.

Control Docking

The results of binding between P2X4 with its antagonist and agonists are shown in Table 1. The results were consistent with the known EC_{50} level that ATP is higher than CTP and $\alpha\beta$-meATP (Ahmad, 2018).

Table 1 Average binding affinity of P2X4 antagonist and agonists

Compounds	Average binding affinity (kcal/mol) (σ=0.46)
5-BDBD	-8.50
ATP	-7.57
CTP	-7.50
$\alpha\beta$-meATP	-7.30

Molecular Docking

Table 2 shows the binding affinity of the top six hit compounds produced by Vina. Binding affinity is the strength of interaction between a protein and a ligand. The highest binding affinity towards P2X4 was produced by dioscin then followed by ampelopsin F, corilagin, ellagic acid pentoside, punicalin, and proanthocyanidins. The result for binding affinity of all 112 compounds is listed in Appendix B.

Table 2 Binding affinity of top six hit compounds produced by Vina.

Compounds	Average binding affinity (kcal/mol) (σ=0.21)
Dioscin	-9.87
Ampelopsin F	-9.47
Corilagin	-9.43
Ellagic acid pentoside	-9.43
Punicalin	-9.27
Proanthocyanidins	-9.20

Analysis of Protein-Ligand Complexes

The analysis of protein-ligands complexes was presented in Figure 1. Figure 1(a) shows the interaction between P2X4 and dioscin. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. Two hydrogen bonds were formed between oxygen atom of Asn287 with 14th and 15th oxygen of dioscin with length 2.95 Å and 2.91Å respectively. Hydrophobic interactions occurred between the 15th oxygen of dioscin and residue Thr211. Another hydrophobic interaction was formed between 43rd carbon of dioscin and residues Pro290, His172 and Glu249.

Figure 1(b) shows the interaction between P2X4 and ampelopsin F. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the fifth oxygen of ampelopsin F and nitrogen of Tyr299 with distance of 2.82Å. The second hydrogen bond is between the third oxygen of ampelopsin F and oxygen of Ala297(A) with length of 2.69Å. Another hydrogen bond occurred between the fourth oxygen of ampelopsin F and oxygen of Ala297(B) with distance of 2.98Å. Hydrophobic interaction occurred between the first oxygen of ampelopsin F and residue Ala9(A), second oxygen of ampelopsin F and residues Ala93(C) and Pro92. Another hydrophobic interaction formed between the third, fourth and fifth oxygen of ampelopsin F and residues Lys298(A), Phe296(B) and Lys298(C) respectively. Hydrophobic interaction also occurred between the 21st and 22nd carbon of ampelopsin F and residue Ala297. Another hydrophobic interaction was between the 27th carbon of ampelopsin F and residues Ala87 and Phe296(C).

Figure 1(c) shows the interaction between P2X4 and corilagin. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the eighth oxygen of corilagin with the nitrogen and oxygen of Ala297 and oxygen of Ala87(B) with distance of 2.94Å, 3.13Å and 3.10Å respectively. The second hydrogen bond is between the 12th oxygen of P2X4 with the

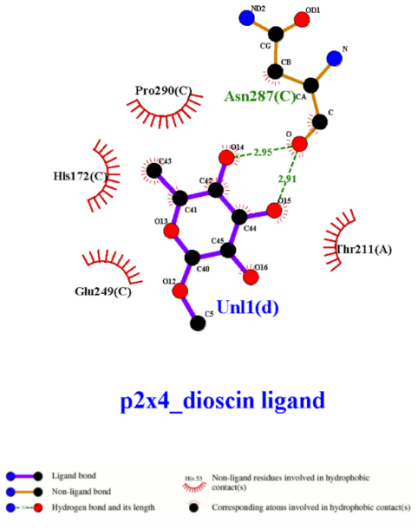
nitrogen of Ala93 with distance of 2.81Å. Another hydrogen bond is between the 16th oxygen of corilagin with oxygen atom of Val90(C) and Ala87(C) with length 2.76Å and 2.72Å respectively. The 18th oxygen of corilagin also formed hydrogen bond with oxygen atom of Ala87(C) with distance 2.93Å. The next hydrogen bond is between the eleventh oxygen of corilagin with oxygen atom of Val90(A) with length 3.2Å. Another hydrogen bond is between the ninth oxygen of corilagin with oxygen of Ala87(B) and Val90(B) with length 2.7Å and 3.31Å respectively. The tenth oxygen of corilagin also formed hydrogen bond with the oxygen of Val90(B) with distance 3.16Å. The 18th oxygen of corilagin formed hydrophobic interaction with residues Ile91(C), Asp88(C) and Tyr299(B). The 13th oxygen of corilagin formed hydrophobic interaction with residues Lys298(B) and Ala297(B). The fourth, eighth, tenth, and 15th oxygen of corilagin formed hydrophobic interaction with residues Phe296(C), Tyr299(A), Ile91(B) and Lys298(C) respectively. The next hydrophobic interaction is between eleventh oxygen of corilagin with residues Pro92(A) and Ile91(A). Another hydrophobic interaction is between 13th carbon of corilagin with residues Phe296(A), Pro92(B) and Ala93(B).

Figure 1(d) shows the interaction between P2X4 and ellagic acid pentoside. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the 11th oxygen of ellagic acid pentoside with nitrogen of Ala297 (B) and Ala87(C) with distance 3.12Å and 3.06Å respectively. The next hydrogen bond is between the 7th oxygen of ellagic acid pentoside with nitrogen and oxygen of Ala297(A) and oxygen of Ala87(B) with distance of 2.93Å, 2.94Å and 3.26Å respectively. The 12th oxygen of ellagic acid pentoside also formed hydrogen bond with hydroxide of Tyr299(B) and oxygen of Ala87(C) with length 3.22Å and 2.82Å. The hydrogen bond is formed between the eight oxygen of ellagic acid pentoside and oxygen of Ala87(B) with distance of 2.95Å. Another hydrogen bond is between the ninth oxygen atom of ellagic acid pentoside with oxygen of Val90(B) with distance 2.73Å. The hydrophobic interaction occurred between the ninth oxygen of ellagic acid pentoside with residues Ile91(B) and Pro92(B). The fourth oxygen of ellagic acid pentoside also shows hydrophobic interaction with residues Phe296(C) and Ile91(C). Another hydrophobic interaction is with the third and fifth oxygen and 17th carbon of ellagic acid pentoside with residues Phe296(B), Ala93(B) and Tyr299(A) respectively.

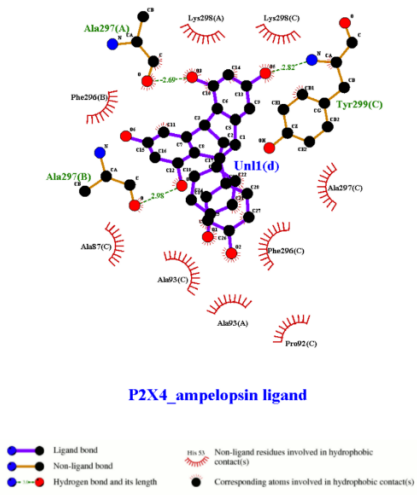
Figure 1(e) shows the interaction between P2X4 and punicalin. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the 16th oxygen of punicalin with nitrogen of Asn293 (C) with length 3.03Å. The second hydrogen bond is between the fourth oxygen of punicalin with nitrogen of Lys67 (A) with distance of 3.28Å. Another hydrogen bond is between the 21st oxygen of punicalin with the second nitrogen of His172 (C) with distance of 3.12Å. The 12th oxygen of punicalin shows hydrogen bond with oxygen of His140(C) with distance 2.94Å. The hydrogen bond between the 14th oxygen of punicalin and oxygen of Thr186(A) has 3.03Å. The 19th oxygen of punicalin also shows hydrogen bond with the oxygen of Thr171(C) with length 3.12Å. Another hydrogen bond is between the 21st oxygen of punicalin with the nitrogen of His172(C) with length 3.13Å. Hydrophobic interaction occurred between the 19th oxygen of punicalin with residue Glu249(C). Hydrophobic interaction also occurred between the 30th carbon of punicalin with residue Lys313(C). The 13th oxygen of punicalin shows hydrophobic interaction with residues Leu188(A) and Ile229(A). Other hydrophobic interaction is between the eight oxygen of punicalin with residue Lys69(A), the 14th oxygen of punicalin with residue Thr139(C) and the 15th oxygen of punicalin with residue Lys215(A).

Figure 1(f) shows the interaction between P2X4 and proanthocyanidins. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the 13th oxygen of proanthocyanidins with nitrogen of Ala297 (B) with distance 2.80Å. The fourth oxygen of proanthocyanidins formed hydrogen bond with the oxygen of Ala87(A) and Val90(A) with length 2.73Å and 3.13Å respectively. The fifth oxygen of proanthocyanidins formed hydrogen bond with the hydroxide of Tyr299(C) with distance 3.07Å. Another hydrogen bond is formed between the seventh

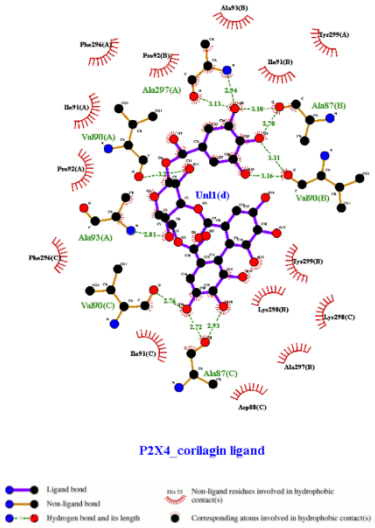
oxygen of proanthocyanidins with the oxygen of Ala87(B) with distance 2.77Å. Hydrophobic interaction occurred between the fifth oxygen of proanthocyanidins with residue Asp88(A). The eight oxygen of proanthocyanidins show hydrophobic interaction with residues Pro92(B) and Ile91(B). The 12th oxygen of proanthocyanidins also shows hydrophobic interaction with residues Phe296(C) and Ile91(C). Another hydrophobic interaction is between the eighth carbon of proanthocyanidins with residues Tyr299(B) and Ala87(C). The 17th carbon of proanthocyanidins shows hydrophobic interaction with residues Val90(B) and Ala93(B). Other hydrophobic interaction is with the ninth carbon of proanthocyanidins with residue Phe296(B), 14th carbon of proanthocyanidins with residue Phe296(A), 20th carbon of proanthocyanidins with residue Ile91(A) and the 30th carbon of proanthocyanidins with residue Ala297(C).



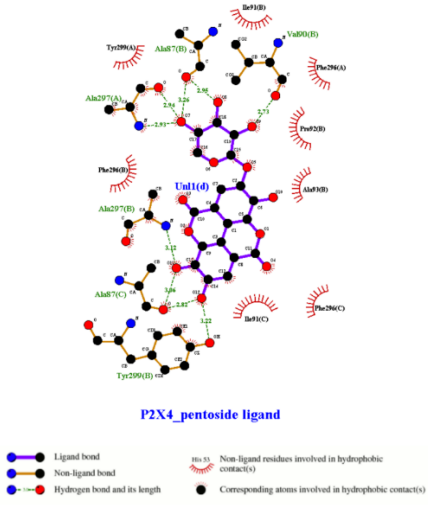
(a) Dioscin-P2X4 complex



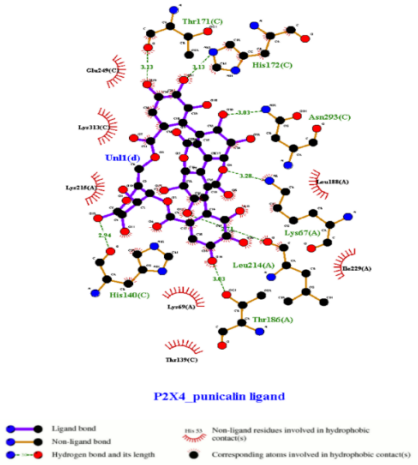
(b) Ampelopsin F-P2X4 complex



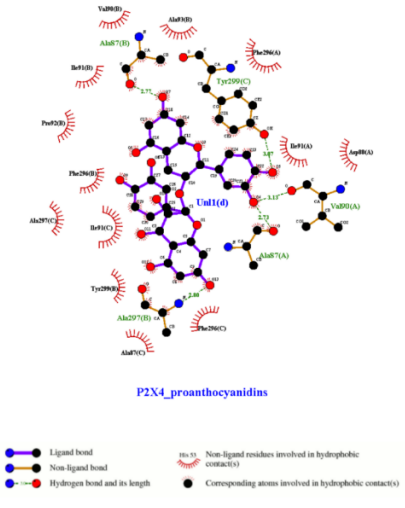
(c) Corilagin-P2X4 complex



(d) Ellagic acid pentoside-P2X4 complex



(e) Punicalin-P2X4 complex



(f) Proanthocyanidins-P2X4 complex

Figure 1 Analysis of protein-ligands complexes

DISCUSSION

The extraction of information from Malay medical manuscript MSS 3048 is a continuation of previous study by Ahmad Radzaudin (2021). MSS 3048 contains traditional remedies for diseases including shingles. This study focuses on shingles treatment since the use of the existing drugs may cause unwanted side effects and may not be fully effective. Since there is a long list of remedies listed, a virtual screening needs to be done to identify which ingredients possess the desired pharmacological action. *In silico* method is used to aid the drug development since it can save time and cost compared to the conventional drug design. This includes molecular docking, that can predict the binding of ligand to the target's binding site, and then is scored based on the strength of binding affinity (Batool et al., 2019).

Control docking was done beforehand to make sure the parameter is correct and reliable for the subsequent docking tests. This was done by docking 5-BDBD, ATP, CTP and α, β-meATP. The result shows that the binding site is correctly validated as it follows the characteristics of P2X4 receptor as described in earlier study (Ahmad, 2018). A total of 112 compounds were docked with P2X4 using the binding parameters set. The compound with the highest binding affinity is dioscin at -9.87 kcal/mol.

Literature search of *Nypa fruticans* or nipah palm reveal that it has 10 active ingredients. Studies from Reza et al (2011) stated that the extract of the plants inhibits prostaglandin synthesis and downregulates the expression of transient receptor potential vanilloid 1 (TRPV1). The most important active ingredient is dioscin with binding affinity at -9.87 kcal/mol indicating high interaction towards P2X4 receptor. Dioscin is a steroidal saponin that possesses anti-inflammatory properties by inhibiting toll-like receptor 2 (TLR2) signalling pathway in mice and rats (Zhao et al., 2018). Furthermore, dioscin also reduce inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expressions (Marahatha et al., 2021). Dioscin potential as anti-inflammatory may contribute to antinociceptive effects.

Ligplot+ shows the binding of the compounds to P2X4 is based on hydrogen bond and hydrophobic interactions. Dioscin has two hydrogen bonds and four hydrophobic interactions. This compound is a potential lead compound as it has strong interaction with P2X4, shown by the binding affinity. From the screening through Lipinski's rule, this compound has low molecular weight at 312 Dalton, low lipophilicity (log P) at -0.053 and low hydrogen-bonding capacity which helps in absorption and permeability (Benet et al., 2016).

CONCLUSION

The objective of this study, which is to identify the plants used for shingles treatment in MSS 3048, was achieved. Overall, 19 formulations related to shingles were listed and a total of 22 plants were identified from the manuscript such as *Nypa fruticans*, *Punica granatum* and *Capsicum*. Next, the active compounds of the plants that showed antinociceptive properties were also identified through literature review with a total of 112 compounds. The last objective, which is to identify the active ingredients that have high affinity to the P2X4 receptor was achieved. All 112 active compounds were docked using Autodock Vina and the compounds with high affinity towards P2X4 were identified. The top six compounds that show high affinity to P2X4 are dioscin, ampelopsin F, corilagin, ellagic acid pentoside, punicalin and proanthocyanidins. The compound that showed the highest potential based on its binding affinities is dioscin, with binding affinity of -9.87 kcal/mol. Further study in *in vivo* and *in vitro* method is recommended for validation as there is few studies on dioscin as antinociception.

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APPENDIX A

Scientific name of plants and its active compounds obtained from literature.

No.	Scientific names	Active compounds	Mechanism of Action	References
1.	*Allium cepa*	Allicin	The compounds prevent the formation of pro-inflammatory messengers and inhibit COX-2 enzyme and LOX (lipoxygenase)	Tigu et al., 2021, Ranjan et al., 2014
		Alliin		
		β-sitosterol		
		Cycloartenol		
		Quercetin		
2.	*Areca catechu*	Arecaidine	*A. catechu* possesses significant anti-nociceptive effects. Its effect is closely related to suppression of COX-2 expression	Ghelardini et al., 2001, Ansari et al., 2021
		Arecaine		
		Arecoline		
		Choline		
		Gallic acid		
		Guvacine		
		Guvacoline		
3.	*Baeckea frutescens*	1,8-Cineole	Possess anti-inflammatory activity against lipopolysaccharide (LPS)-induced nitric oxide production in macrophage	Saad et al., 2021, Chen et al., 2008, Hou et al., 2017
		Baeckeol		
		Caryophyllene		
		Ethyl gallate		
		Oleanolic acid		
		P-cymene		
4.	*Capsicum*	Capsaicin	Reduced oedema generated by the carrageenan in rat paw	Da Silva Antonio et al., 2018, Hernandez-Ortega et al., 2012
		Capsiate		
		Dihydrocapsaicin		
		Nonivamide		

5.	*Ceriops tagal*	2-methyl-4-(3′ -phenylpropyl)	Inhibits the release of inflammatory mediators	Biswas et al., 2023
		5-nitro-2-aminobenzophenone thiosemicarbazone		
		α-guaiene		
		β-sitosterol		
		Betulinic acid		
		Lupeol		
		Tagalsin P		
6.	*Champeira*	β-carotene	Inhibit nuclear factor kappa light chain enhancer of activated B cells (NF-κB)	Ragasa et al., 2015
		Lutein		
		Phytol		
		Squalene		
7.	*Croton tiglium*	2-(furan-2-yl)-5-(2,3,4-trihydroxy-butyl)-1,4- diazine	Inhibit the acetic acid-induced abdominal writhing in mice	Liu et al., 2012), (Wu et al., 2007, Dey et al., 2018
8.	*Curcuma longa*	Curcumin	Curcumin selectively inhibits lipoxygenase, phospholipase A2 and COX-2, but not COX-1	Eke-Okoro et al., 2018 Kalirajan et al., 2020
		Cyclocurcumin		
9.	*Cyclea laxiflora*	Dicentrine	Inhibit nociceptive response of transient receptor potential ankyrin 1 (TRPA1)	Lemmens & Horsten, 2022, Montrucchio et al., 2013
10.	*Dipterocarpus*	Ampelopsin F	Inhibit the production of inflammatory cytokines	Wan Mohd Zain et al., 2021, Toan Phan et al., 2015, Aslam et al., 2015
		Davidiol A		
		E-viniferin		
		Laevifonol		
		Myricetin		
		Myricitrin		
		Scopoletin		

		Stenophyllol B		
		Tiliroside		
11.	*Eurphobia hirta*	Afzelin	Reduce inflammation in rat's ear	Al-Snafi, 2017, Vallisuta & Olimat, 2012, Kumar et al., 2010
		β-sitosterol		
		Campesterol		
		Cycloartenol		
		Euphorbin E		
		Stigmasterol		
12.	*Hedyotis phillipensis*	Asperuloside	Studies have shown this compound possesses anti-inflammatory activity. A study in treating rheumatoid arthritis done by Li et al. has found that the compound exhibited an inhibitory effect on the release of TNF	Mahbob et al., 2014
13.	*Mangifera foetida*	Daidzein	Inhibit acetic-acid writhing test in mice	Khoo et al., 2016, Fitmawati et al., 2019), Chang et al., 2020
		Genistein		
		Mangiferin		
14.	*Myristica fragrans*	Myristicin	Inhibit the release of interleukin-1β and interleukin-8 by resident peritoneal cells or to suppression of prostaglandins and bradykinin	Hayfaa et al., 2013, Santos et al., 2013
		Nordihydroguaiaretic acid		
		Trimyristin		
15.	*Nypa fruticans*	β-sitostenone	The extract inhibits prostaglandin synthesis and downregulate the expression of transient receptor potential vanilloid 1 (TRPV1)	Kang & Hyun, 2020,Reza et al., 2011
		β-sitosterol		
		Chlorogenic acid		
		Daucosterol		
		Dioscin		
		Diosgenin		
		Kaempferol		
		Protocatechuic acid		
		Stigmasta-4, 22- dien-3-one		

		Stigmasterol		
16.	*Oryza sativa*	Catechin	Rice bran oil reduces the inflammation by interfering the arachidonate metabolites	Tyagi et al., 2022, Ghasemzadeh et al., 2018, Ashraf et al., 2019
		Cinnnamic acid		
		GABA		
		Tocotrienol		
		Myricetin		
		Oryzanol		
		P-coumaric acid		
		Quercetin		
17.	*Piper betel*	4- allyl phenyl acetate	inhibit the increase of the intracellular Ca2+ through transient receptor potential ankyrin 1 (TRPA1)	Alam et al., 2013, Sakinah et al., 2020, Chan & Wong, 2014
		4-allylphenol		
		β-sitosterol		
		Cepharadione		
		Chavibetol		
		Eugenol		
		Hydroxychavicol		
		Piperine		
18.	*Pisonia grandis*	α-spinasterol	Inhibit the release of inflammatory mediators	(Radha et al., 2008) (Shubashini et al., 2011)
		Allantoin		
		β-sitosterol		
		β-sitosterolglucoside		
		Dulcitol		
		Pinitol		
19.	*Pterocarpus*	Calocedrin	Inhibit production of inflammatory mediators like histamine, cyclooxygenase, and arachidonic acid metabolites	Bulle et al., 2016, Navada & Vittal, 2014, Kumar, 2011
		Pterocarpol		
		Pterolinus L		
		Santalin A		

		Santalin B		
		Savinin		
20.	*Punica granatum*	Anthocyanins	Ellagic acid's antinociceptive effect could be due to COX inhibition. Anthocyanin inhibits prostaglandin synthesis and suppresses COX2 transcription.	Saad et al., 2014 Guerrero-Solano et al., 2020, Yu et al., 2021
		Corilagin		
		Ellagic acid hexoside		
		Ellagic acid pentoside		
		Gallic acid		
		Proanthocyanidins		
		Punicalagin		
		Punicalin		
		Punicic acid		
21.	*Quercus infectoria*	Ellagic acid	Increase reaction time of rats towards stimulus	Fan et al., 2014, Elham et al., 2021
		Gallic acid		
		Syringic acid		
22.	*Schima wallichii*	Coumarin	Inhibit the release of inflammatory mediators	Banjara et al., 2022, Dewanjee et al., 2009
		Terpenoid		

APPENDIX B

Binding affinity arranged from highest to lowest.

Number	Compounds	Binding affinity (kcal/mol)			
		Run 1	Run 2	Run 3	Average
1	Dioscin	-9.7	-9.9	-10.9	-9.87
2	Ampelopsin F	-9.7	-9.3	-9.4	-9.47
3	Corilagin	-8.7	-11.0	-8.6	-9.43
4	Ellagic acid pentoside	-9.1	-9.6	-9.6	-9.43
5	Punicalin	-9.3	-9.3	-9.2	-9.27
6	Proanthocyanidins	-10.3	-9.4	-7.9	-9.20
7	Tiliroside	-8.9	-9.3	-9.3	-9.17
8	Cepharadione	-9.0	-9.0	-9.0	-9.00
9	Ellagic acid hexoside	-9.0	-9.0	-8.8	-8.93
10	Stenophyllol B	-9.2	-8.3	-9.0	-8.83
11	Punicalagin	-8.7	-8.9	-8.9	-8.83
12	Oryzanol	-8.8	-8.7	-8.6	-8.70
13	Cycloartenol	-8.7	-8.7	-8.7	-8.70
14	Cycloartenol	-8.7	-8.7	-8.7	-8.70
15	β-sitosterol	-8.9	-8.3	-8.5	-8.57
16	β-sitosterol	-8.9	-8.3	-8.5	-8.57
17	β-sitosterol	-8.9	-8.3	-8.5	-8.57
18	Stigmasterol	-8.6	-8.5	-8.6	-8.57
19	β-sitosterol	-8.9	-8.3	-8.5	-8.57
20	Stigamasterol	-8.6	-8.5	-8.6	-8.57
21	β-sitosterol	-8.9	-8.3	-8.5	-8.57
22	β-sitosterol	-8.9	-8.3	-8.5	-8.57
23	Calocedrin	-8.6	-8.2	-8.7	-8.50
24	Diosgenin	-8.5	-8.5	-8.4	-8.47
25	Savinin	-8.5	-8.5	-8.4	-8.47
26	Betulinic acid	-8.4	-8.4	-8.4	-8.40
27	E-viniferin	-8.2	-8.5	-8.5	-8.40
28	Laevifonol	-8.4	-8.3	-8.5	-8.40
29	Campesterol	-8.4	-8.4	-8.4	-8.40
30	Stigmasta-4, 22- dien-3-one	-7.7	-8.9	-8.6	-8.40
31	Lupeol	-8.4	-8.3	-8.4	-8.37
32	Santalin A	-8.4	-8.4	-8.3	-8.37
33	Santalin B	-8.4	-8.4	-8.3	-8.37
34	α-spinasterol	-8.3	-8.1	-8.6	-8.30
35	Davidiol A	-8.1	-8.4	-8.4	-8.30
36	Myricitrin	-8.4	-8.1	-7.9	-8.13

37	Cyclocurcumin	-8.5	-8.0	-7.8	-8.10
38	β-sitostenone	-7.8	-8.6	-7.9	-8.10
39	Tocotrienol	-8.0	-8.3	-8.0	-8.10
40	Dicentrine	-8.4	-7.9	-7.9	-8.07
41	Afzelin	-8.0	-8.1	-8.1	-8.07
42	Oleanolic acid	-8.0	-7.9	-8.0	-7.97
43	Tagalsin P	-7.9	-7.9	-7.9	-7.90
44	Asperuloside	-8.2	-7.6	-7.7	-7.83
45	β-sitosterolglucoside	-8.3	-7.3	-7.7	-7.77
46	Caryophyllene	-7.7	-7.7	-7.7	-7.70
47	Ellagic acid	-7.9	-7.9	-7.3	-7.70
48	Daucosterol	-7.7	-7.7	-7.6	-7.67
49	Euphorbin e	-7.4	-8.2	-7.2	-7.60
50	Mangiferin	-7.7	-7.6	-7.5	-7.60
51	Daidzein	-7.6	-7.6	-7.6	-7.60
52	Anthocyanins	-7.6	-7.6	-7.6	-7.60
53	Chlorogenic acid	-7.6	-7.5	-7.6	-7.57
54	Terpenoid	-7.7	-7.2	-7.8	-7.57
55	Nordihydroguaiaretic acid	-7.3	-7.8	-7.4	-7.50
56	Curcumin	-7.4	-7.5	-7.5	-7.47
57	Kaempferol	-7.4	-7.8	-7.5	-7.47
58	Quercetin	-7.1	-7.6	-7.6	-7.43
59	Genistein	-7.5	-7.4	-7.4	-7.43
60	Quercetin	-7.1	-7.6	-7.6	-7.43
61	Myricetin	-7.6	-7.0	-7.6	-7.40
62	Myricetin	-7.6	-7.0	-7.6	-7.40
63	Catechin	-7.3	-7.5	-7.3	-7.37
64	β-carotene	-7.3	-7.6	-7.2	-7.34
65	α-guaiene	-7.3	-7.3	-7.3	-7.30
66	Piperine	-7.1	-7.2	-7.1	-7.13
67	5-nitro-2-aminobenzophenone thiosemicarbazone	-7.0	-7.1	-7.0	-7.03
68	Pterocarpol	-7.1	-6.9	-7.1	-7.03
69	Pterolinus L	-6.9	-7.0	-6.9	-6.93
70	Squalene	-6.6	-7.1	-6.9	-6.87
71	Capsiate	-6.8	-6.7	-6.4	-6.63
72	2-(furan-2-yl)-5-(2,3,4-trihydroxy-butyl)-1,4- diazine	-6.8	-6.5	-6.5	-6.60
73	Scopoletin	-6.6	-6.6	-6.6	-6.60
74	Capsaicin	-6.4	-6.5	-6.6	-6.50
75	Dihydrocapsaicin	-6.5	-6.5	-6.5	-6.50
76	Allantoin	-6.5	-6.5	-6.5	-6.50
78	Nonivamide	-6.7	-6.4	-6.3	-6.47

79	2-methyl-4-(3′ -phenylpropyl)	-6.4	-6.4	-6.4	-6.40
80	Gallic acid	-6.3	-6.3	-6.3	-6.30
81	Ethyl gallate	-6.3	-6.3	-6.3	-6.30
82	Hydroxychavicol	-6.3	-6.3	-6.3	-6.30
83	Gallic acid	-6.3	-6.3	-6.3	-6.30
84	Gallic acid	-6.3	-6.3	-6.3	-6.30
85	Baeckeol	-6.2	-6.2	-6.3	-6.23
86	Phytol	-6.4	-6.1	-6.1	-6.20
87	Chavibetol	-6.2	-6.2	-6.2	-6.20
88	Trimyristin	-6.6	-5.4	-6.5	-6.17
89	Cinnnamic acid	-6.1	-6.1	-6.1	-6.10
90	4- allyl phenyl acetate	-6.1	-6.1	-6.1	-6.10
91	Eugenol	-6.1	-6.1	-6.1	-6.10
92	Pinitol	-6.1	-6.1	-6.1	-6.10
93	Myristicin	-6.0	-6.0	-6.0	-6.00
94	Protocatechuic acid	-6.0	-6.0	-6.0	-6.00
95	P-coumaric acid	-6.0	-6.0	-6.0	-6.00
96	Coumarin	-5.9	-5.9	-5.9	-5.90
97	Punicic acid	-6.0	-5.7	-6.0	-5.90
98	P-cymene	-5.8	-5.8	-5.8	-5.80
99	4-allylphenol	-5.8	-5.8	-5.8	-5.80
100	Syringic acid	-5.8	-5.8	-5.8	-5.80
101	1,8-Cineole	-5.7	-5.7	-5.7	-5.70
102	Dulcitol	-5.5	-5.6	-5.4	-5.50
103	Arecaine	-5.4	-5.5	-5.5	-5.47
104	Guvacine	-5.3	-5.3	-5.3	-5.30
105	Arecaidine	-5.2	-5.2	-5.2	-5.20
106	Arecoline	-5.1	-5.1	-5.1	-5.10
107	Guvacoline	-5.1	-5.1	-5.1	-5.10
108	Alliin	-4.9	-5.1	-5.1	-5.03
109	Lutein	-0.9	-6.0	-6.4	-4.43
110	Allicin	-4.3	-4.3	-4.3	-4.30
111	GABA	-4.1	-4.1	-4.1	-4.10
112	Choline	-3.6	-3.6	-3.6	-3.60