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AZADIRACHTA INDICA (SEMAMBU) IN TRADITIONAL MALAY MEDICAL MANUSCRIPTS AND ASSESSMENT OF BIOACTIVE COMPOUNDS

(AZADIRACHTA INDICA (SEMAMBU) DALAM MANUSKRIP PERUBATAN MELAYU DAN PENILAIAN BERDASARKAN KANDUNGAN BIOAKTIF)

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Abstract

Azadirachta indica (neem) is an Asia native plant from Meliaceae family, which has been recognized for its pharmaceutical potential for ages and contain numerous bioactive phytochemicals with therapeutic potential. This research aims to examine the ethnomedicinal plants that have been recorded in transliterated manuscripts and to discover the bioactive compounds. For methodology, secondary data were obtained from five selected Malay medical books. The experimental design involved two extraction methods, namely water extraction and sequential alkaline extraction. The data was qualitatively and quantitatively analyzed by Total Phenolic Content (TPC), and High-Performance Liquid Chromatography (HPLC). The findings from the Malay medical manuscript indicate that most diseases were physical illnesses. The total phenolic content of A. indica was 779.89 ± 12.32 µg GAE/g DW, and the phenolic acids detected by HPLC in both extractions were Vanillic acid, trans-p-coumaric acid, and ferulic acid. In conclusion, this study demonstrates that A. indica has a huge potential for further exploration in pharmaceuticals.

Keywords: Azadirachta indica, Malay medical manuscript, ethnomedicine, phenolic compound, antibacterial activities, ethnoscience.

Abstrak

Azadirachta indica (neem) ialah tumbuhan asli Asia daripada keluarga Meliaceae, yang telah diiktiraf bagi potensi farmaseutikalnya sejak sekian lama dan mengandungi banyak fitokimia bioaktif dengan potensi terapeutik. Penyelidikan ini bertujuan untuk mengkaji tumbuhan etnoperubatan yang telah direkodkan dalam manuskrip secara transliterasi dan untuk menemui sebatian bioaktif. Bagi metodologi, data sekunder diperoleh daripada lima buah buku perubatan Melayu terpilih. Reka bentuk eksperimen melibatkan dua kaedah pengekstrakan iaitu pengekstrakan air dan pengekstrakan alkali berjujukan. Data telah dianalisis secara kualitatif dan kuantitatif oleh Jumlah Kandungan Fenolik (TPC), dan Kromatografi Cecair Berprestasi Tinggi (HPLC). Dapatan daripada manuskrip perubatan Melayu menunjukkan kebanyakan penyakit adalah penyakit fizikal. Jumlah kandungan fenolik A. indica ialah 779.89 ± 12.32 µg GAE/g DW, dan asid fenolik yang dikesan oleh HPLC dalam kedua-dua pengekstrakan ialah asid Vanilik, asid trans-p-kuumarik, dan asid ferulik. Kesimpulannya, kajian ini menunjukkan bahawa A. indica mempunyai potensi besar untuk penerokaan lanjut dalam farmaseutikal.

Kata Kunci: *Azadirachta indica, manuskrip perubatan Melayu, etnoperubatan, sebatian fenolik, aktiviti antibaktaria, etnosains*

INTRODUCTION

The Azadirachta indica from Meliaceae family is known as the neem tree or "semambu," "mambu," "laksamana," "mambul," "nim," and "sadu" in Malay. The plant originates from India and Java; the botanical name of "semambu," Azadirachta indica, is derived from the Persian, where Azad means "free," dirachta means "tree," I-Hindi means "of Indian Origin" (Quraishi et al. 2018). A. indica is an evergreen tree that can reach 16 m tall. The bark is smooth-grey or red brown within 1.2 cm wide, white, and fragrant flowers. This tree is revered in Hindu rituals, thereby bearing significance beyond its fragrant flowers and sacred leaves. The flowers are bisexual and slender axillary bunched, white and fragrant; while the leaves are spirally arranged and simple pinnate compound (Othman et al. 2016). Its optimal growth conditions includes a soil pH within the range of 6.2 to 7.0 (Bhowmik et al. 2010). It is situated at an altitude of approximately 1500 m, experiencing rainfall between 450 to 1150 mm. Neem trees flourish in prolonged dry weather conditions, even in low-quality soils (Saxena & Kumar 2009). Various components of the neem plant have exhibited diverse pharmacological effects for addressing various conditions, including the management of diseases, such as antimicrobial infections, diabetes, neurological disorders, and cancer (Alzohairy 2016). Azadirachta indica, commonly known as neem, is among the highly beneficial medicinal plants with preventive capabilities against a broad spectrum of diseases. Extracts derived from various components of the neem tree, including leaves, flowers, seeds, bark, and roots, contain numerous bioactive phytochemicals recognized for their significant therapeutic potential (Sarkar et al. 2021). It holds great significance as a medicinal plant, utilized for treating various ailments in the Unani System of Medicine, as well as in traditional systems, such as Ayurveda, Homeopathy, and the Chinese and European "Materia Medica" (Quraishi et al. 2018). Despite its medicinal importance, there remains a noticeable gap in documentation within Malay medical books. To demonstrate the applicability and validity of this particular assertion in light of contemporary techniques and paradigms, scientific investigation must be conducted.

The Malay manuscript represents the practice of composing traditional Malay literature. In a tangible aspect, this is distinguished through writing on paper using ink in a script derived from Arabic (Yahya & Jones 2021). The exploration of Malay medical manuscripts (MMM) becomes imperative in unveiling the rich traditional knowledge embedded in these writings. Dating back to approximately 1400 AD, these manuscripts are a testament to the deep-rooted understanding of medicine among Malay communities, even preceding Western colonization. The oldest MMM was originally written by Sheikh Shafiyuddin al-'Abbasi (Shaharir 2021). To date, there has only been a single record of discovery of one Jawi-Malay inscription, specifically the Terengganu inscription dated either 1303 AD or 1308 AD (Shaharir 2021).

Around 1% or roughly 40 manuscripts are focused on medicinal topics. However, the examination of their content is relatively limited. Some of these manuscripts are delicate and approaching a state of degradation where readability may soon be compromised. The insistence to preserve these fragile documents, some dating back to the 1920s, arises as a critical task to safeguard the scientific civilization of Malays (Nadzirin 2021). In this work, the researchers have delved into the cradle of MMMs, revealing that only approximately 12 of these valuable resources have undergone transliteration and modern publication (Mohd Shafri 2019), this implies that Malay traditional medicine has yet to gain widespread acceptance as a thoroughly practiced complementary discipline. Within the Malay Manuscript Centre of the National Library of Malaysia, there are over 40 manuscripts classified as medical manuscripts, but a considerable number of them were either written or translated later, as the earliest date back to the 1920s (Harun 2019). Malay philologists were responsible for transliterating Malay classical Jawi texts into Malay Romanized texts. This was done to aid non-Jawi readers or researchers in accessing information and conducting research at more advanced levels (Din et al. 2021). As mentioned by Mohd Affendi (2020), the project to map manuscripts can be traced to three primary locations within the Malay world, namely, Aceh, Riau,

and the East Coast of the Malay Peninsula, all of which have been identified as the main centers for manuscript production. Malay manuscripts are also regarded as a strength in information keeping, where they played important roles in recording, reporting, teaching, disseminating and channeling knowledge about histories, laws, religion (especially about Islam), nationalism, traditional medicine, farming, construction, and so forth (Eizah et al. 2019). Kitab Tibb, regarded as ancient Malay manuscripts of medical and health sciences recorded various ways to cure and heal illness/diseases by using natural ingredients which include flora, fauna, spices, and roots, was believed (and found) to be effective (Eizah et al. 2019). The Kitab Tibb collection located at the National Library of Malaysia are coded with MSS as the initial, followed by numbers such as 1292, 759, 1754, 2219, and so on. Meanwhile, the Kitab Tibb collection in the Departemen Pendidikan dan Keguruan Jakarta are coded as M1.832 and M1.833 (Eizah et al. 2019), while Kitab Tibb in the Netherlands are coded as Cod. OR. 6558, 1769, 4818(b), 8704, 8216, 1268B, 8733, and 7230 (Wan Mamat 1985). The presence of medical knowledge and practitioners in Malay can be traced back to the 7th century, as illustrated in two inscriptions, namely, the Talang Tuwo and Kota Kapur inscriptions in Palembang, Sumatera. During that period, diseases in Malay were referred to as "wyadhi." These manuscripts provide insights into traditional healing practices utilizing natural ingredients.

The contents of Malay medical manuscripts cover medical philosophy, procedures for diagnosing diseases, care practices, health, and procedures for treating physical and non-physical diseases, including the use of prayers and treatment ingredients. It covers plants, animals, minerals and changes in time and weather (Mohd Shafri 2019). Traditional communities had verbally inherited old advice although living among younger and modernized generations who can write; these younger generations will then record all information received from the older generations. Common practices which were influenced by Malay philosophy and belief were closely related to the philosophy of nature revolved around the traditional Malay living environment (Eizah et al. 2019). Local knowledge is referred to as all forms of knowledge, beliefs, understanding, determination, vision, customs or ethics which influence human life among an ecological society (Keraf 2010). Besides that, local knowledge is an explicit knowledge that emerges from a long period and evolve with the community and their environment in the shared local systems.

Ethnomedicine falls under the category of ethnoscience, and ethnobotany is described as a traditional medical approach based on the beliefs and practices of medicine within diverse ethnic groups (Calderon et al. 2019). The use of traditional medicine has been recognized at the level of the Malaysian Ministry of Health, which is in line with the World Health Organization (WHO). In Malaysia, traditional and complementary medicine acts were first introduced in 2016 (Mohd Shafri 2019). The challenge in plant identification constitutes the main factor leading to the neglect and exclusion of historical ethnobotanical records. These records are often preserved alongside folklore and local history collections in archives but are not frequently included in contemporary scientific discussions (Sõukand & Kalle 2022). Hence, it demonstrates that there is an essential demand recognition for scientific research on herbs among researchers, emphasizing the practical necessity rather than possessing solely theoretical knowledge. Therefore, the present study endeavors to analyze and compile ethnomedicinal plants documented in Malay transliterated manuscripts, with a specific focus on A. indica (semambu). This botanical exploration aligns with the Malaysian Ministry of Health's Blueprint 2018–2027 on Traditional and Complementary Medicine, acknowledging the importance of integrating traditional practices into contemporary healthcare.

The following sections detail our methodology, encompassing both historical manuscript analysis and contemporary scientific techniques, such as Gas Chromatography–Time-of-Flight Mass Spectrometry (GCTOF-MS) and High-Performance Liquid Chromatography (HPLC). This study seeks to shed light on the untapped potential of A. indica in pharmaceutical applications, thereby bridging the gap between traditional wisdom and modern scientific exploration.

MATERIALS AND METHODS

Selection of manuscripts

The search on Azadirachta indica was selected in five Malay medical manuscripts, which were transcribed medical manuscripts from the Pusat Penyelidikan Manuskrip Alam Melayu (PPMAM), Perpustakaan Alam dan Tamadun Melayu (PATMA), and Institut Alam dan Tamadun Melayu (ATMA) from Universiti Kebangsaan Malaysia's collection, which are as follows:

1. Tamam Kitab Taj Al-Mulk;
2. Kitab Tib Muzium Terengganu;
3. Kitab Tib MSS 2515 Perpustakaan Negara Malaysia: Kajian Teks & Suntingan;
4. MSS 2999 Kitab Tib: Pandangan dan Tafsiran Perubatan Moden Terhadap Manuskrip Perubatan Melayu; and
5. MSS B15 Kitab Perubatan Melayu Sari Segala Ubat.

The criteria for selecting manuscripts included: (a) accessibility; and (b) transcriptions from Jawi writing (a classical Malay writing style influenced by the Arabic language, particularly from the Quran), into Roman Malay. All phrases and keywords related to Azadirachta indica, specifically "mambu," were identified and extracted. A research officer from PPAM, familiar with the terminologies and specifically trained for information extraction, performed the data extraction. Subsequently, formulations related to "mambu" were systematically reviewed to extract information guided by several elements, including: (a) the type of diseases; (b) sources of materials used; (c) processing and dosing terms in the formulations; and (d) the method of treatments, as described by (Mohd Shafri 2019).

Plant material and sample preparation

The leaves of Azadirachta indica were collected at International Islamic University Malaysia. Then, the plant species was authenticated at the Herbarium Unit, Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia. Table 1 and Figure 1 show the taxonomic description of A. indica. The leaves were chosen for extraction due to their established pharmacological significance

Table 1. Taxonomic description of *A. indica* (Sarkar et al. 2021)

Kingdom	**Plantae**
Division	Magnoliophyta
Order	Rutales
Family	Meliaceae
Genus	Azadirachta
Genus	Indica

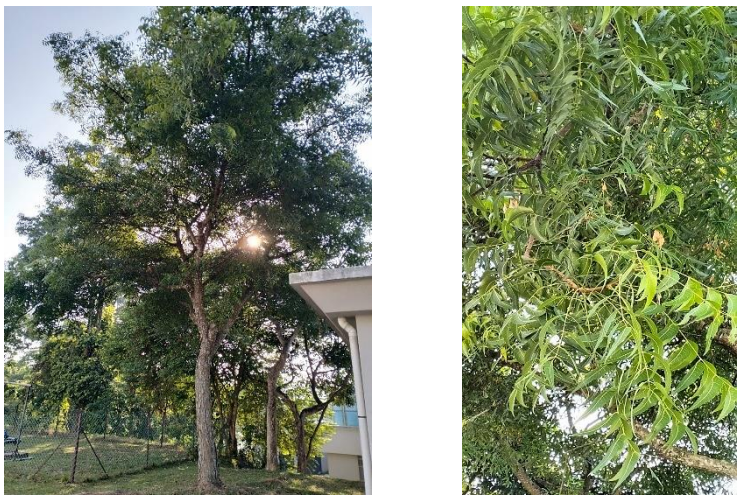

Figure 1. Azadirachta indica (semambu) tree and leaves

Maceration extractions

Two extraction techniques were employed: water extraction and sequential alkaline extraction. *Water extraction*. Ten grams of freeze-dried powdered leaves were mixed with 100 mL of distilled water and shaken for 30 minutes at room temperature. The mixture was then incubated at 60°C for 30 minutes and left to stand overnight in darkness at room temperature. The following day, the sample was filtered and re-extracted using hexane, ethyl acetate, and butanol, according to a specified procedure (Tsatsaroni 1998).

Sequential alkaline extraction. The sequential alkaline extraction process involves exposing phenolic compounds to sodium hydroxide (NaOH) for 12 hours at 60°C. The extract is then adjusted to pH 2 with hydrochloric acid (HCl), centrifuged, and the supernatant is collected. This supernatant is subsequently re-extracted using hexane, ethyl acetate, and butanol (Aarabi et al. 2016).

Next, the crude extract was carried out from the maceration extracts of *A. indica* using a specified method (Bertin et al. 2003). The crude extraction is represented by the clear supernatant, which underwent extraction using hexane, ethyl acetate, and butanol through a funnel separator. For further examination, the final concentration was reconstituted with 5 mL of methanol in tubes, sealed with parafilm to prevent oxygen exposure, and promptly stored at –20°C until subsequent analysis. A volume of 50 µL from the re-dissolved sample was then transferred to a vial for further analysis using Gas Chromatography–Time-of-Flight Mass Spectrometry (GCTOF-MS), Total Phenolic Content (TPC), and High-Performance Liquid Chromatography (HPLC).

Determination of total phenolic content

The determination of Total Phenolic Content (TPC) using the Folin–Ciocalteu assay, as described by (Singleton & Rossi 1965), was used with slight modification. Quantification was carried out on hydrolyzed samples, and the outcomes were conveyed in terms of gallic acid equivalence (GAE) per gram of dry weight sample via a Tecan microplate reader.

Determination of phenolic acid by HPLC

Analysis for the identification and quantification of phenolic acids in *A. indica* extracts was performed through High-Performance Liquid Chromatography (HPLC) using the Agilent 1200 series (Agilent Technologies, Palo Alto, CA, USA). The system was equipped with a binary pump, an autosampler, and a diode array detector (DAD). The HPLC method employed a reverse-phase column, ZORBAX SB-C18 (Eclipse 100 × 2.1 mm, 1.8 µm), where the column temperature was maintained at 25°C for the analysis. Two mobile phases were employed: Phase A consisting of 1% of formic acid in

water/acetonitrile (90:10 v/v), and Phase B comprising acetonitrile. The solvent gradients conform to the following patterns: 0%–40% of Solvent B (0–20 min), 40%–60% of Solvent B (20–25 min); 60%–100% (25–25.1 min), 100% of Solvent B (25.1–35 min), and 100%–0% of Solvent B (35–35.1 min). Meanwhile, the flow rate was set at 0.4 mL/min in which detection was observed at 280 nm throughout the gradient (Zhao et al. 2008). The identification of phenolic acid content was accomplished by matching their retention times and UV spectra with standards. Phenolic acid standards, such as ferulic acid, caffeic acid, *trans-p*-coumaric acid, 2-coumaric acid, 3-coumaric acid, and 4-hydrobenzoic acid, were selected due to their relevance to the chemical compositions of the plant. These standards were procured from Sigma Aldrich (USA).

RESULTS

Analysis of Malay Medical Manuscript

The study analyzed five medical manuscripts from the Pusat Penyelidikan Manuskrip Alam Melayu (PPMAM), Perpustakaan Alam dan Tamadun Melayu (PATMA), and Institut Alam dan Tamadun Melayu (ATMA) from the Universiti Kebangsaan Malaysia's collection. Extractions and analyses were conducted for each prescription containing the term "*mambu*" representing *A. indica* (neem). The chosen manuscripts are as follows:

1. *Tamam Kitab Taj Al-Mulk* is one of the Malay medical series books that are widely discussed through classic works (*karya klasik*), such as *Hikayat Melayu*, *Hikayat Hang Tuah*, *Kitab Tibb Al-Insan*, and others, which are more synonymous with the name *Tajul Muluk*. This book is an adaptation of a book that originated from Persia and has immersed through the incorporation of elements of Islamic teachings; it was then re-translated during the time of the Aceh Sultanate by Sultan Mansur Bilal Shah Ibni Sultan Jauhar Alam Shah (Khuwaidumullah Anwar 2021).
2. *Kitab Tib Muzium Terengganu* has the acquisition number of MS 1988.40 and goes by the name of *Ubat Tradisyinal* in the Muzium Negeri Terengganu's collection. The manuscript is similar to other Malay Kitab Tibs, including types of diseases, treatments, and medicines, processes and material usage, a system of measure and scale, taboos, and others (Harun 2017).
3. *Kitab Tib MSS 2515 Perpustakaan Negara Malaysia* is one of the manuscripts studied by Dr. Harun Mat Piah by introducing a deep understanding of manuscript transliterations, a comprehensive glossary, and an index for reference. This manuscript contains 232 pages, completed from the beginning until the end, and is without a colophon. The original MSS 2515 was copied by Ibrahim bin Muhammad Fabian in Kampung Punjut, Patani, in the year 1829 (Harun & Zawiyah 2014).
4. *MSS 2999 Kitab Tib: Pandangan dan Tafsiran Perubatan Moden Terhadap Manuskrip Perubatan Melayu* (Abdul Ghani 2015). This manuscript was the result of a combination of transliterations from the classical Jawi to Rumi book, which contains an analysis of the diseases and its prescription. It also describes on medicinal material, such as botanical name, taste, part of usage and pharmacological effect.
5. *MSS B15 Kitab Perubatan Melayu Sari Segala Ubat* is one of the earliest medicinal Malay manuscripts that exist in the late 18th or early 19th century. It consists of the medical practices of the Royal physicians of the Sultanate of Pontianak, West Kalimantan, on the island of Borneo. It encompasses the practice of science and technology among the Malay community of Borneo in the past. There are 51 health complications mentioned in this manuscript, 18 prescriptions that use a single ingredient, 85 prescriptions that use more than one ingredient mixture, and 28 prescriptions that involve spiritual elements (Mohd Shafri 2019).

Disease treatment found in selected manuscripts

From the findings, all the medical manuscripts in Malay encompass traditional remedies for addressing various diseases and illnesses, presented through specific formulations and diverse processes and treatments except in *MSS 2999 Kitab Tib: Pandangan dan Tafsiran Perubatan Moden Terhadap Manuskrip Perubatan Melayu* (Table 2). Most of the diseases described in Malay manuscripts concerned physical illnesses, such as:

1. seizure (*ubat sawan*);
2. stomach-related illnesses (*penyakit perut*), such as gastric and diarrhea;
3. dental care (*ilmu kukuh gigi*); and
4. several types of fever, including typhoid fever (*demam kepialu*).

There were similarities found in *Tamam Kitab Taj-Al-Mulk* and *Kitab Tib Muzium Terengganu*, which described the remedy of seizure or *ubat sawan.* However, the findings showed a different ingredient or formulation to treat the disease. In *Tamam Kitab Taj-Al-Mulk*, three types of herbs were used as formulation, which were *daun saranag manang*, *daun jempelia*, and *mambu* (*A. indica*). Those herbs were pounded before being put on the forehead and have its water drunk in the morning (three days). Meanwhile, in *Kitab Tib Muzium Terengganu,* the ingredients mentioned were the leaves of *mambu* (*A. indica*) and *daun tulang hitam*, which were squeezed with hot water and then put on the forehead or on the chest. The mentioned remedy showed that there were differences from those techniques used. The first technique mentioned that the *mambu* and other ingredients must be pounded before it is pasted on the forehead and have its water drunk for a certain period (three days in the morning). However, for the second technique, the leaves were squeezed and pasted directly on the forehead and no period was mentioned.

The analysis showed a similarity of mambu (*A. indica*) to treat stomach-related illness in Tamam Kitab Taj-Al-Mulk and Kitab Tib MSS 2515 Perpustakaan Negara Malaysia. However, the formulations for stomach aches were different. Tamam Kitab Taj-Al-Mulk formulated herbal drinks from herbs such as jendawan ara, sirih pinang, mambu (*A. indica*). Meanwhile, the herbal formulation in Kitab Tib MSS 2515 Perpustakaan Negara Malaysia were mata kunyit, mambu's leaves, asam jawa's root, and remunggai's root, where the pills are then to be consumed in the morning, as detailed in Table 2. No description of A. indica (mambu) was found in MSS 2999 Kitab Tib: Pandangan dan Tafsiran Perubatan Moden Terhadap Manuskrip Perubatan Melayu.

Table 2. Type of disease and formulation of treatment from *A. indica* (mambu) in Malay medical

Book name/ physical illnesses	Seizure (*ubat sawan*);	Stomach-related illnesses (*penyakit perut*), such as gastric and diarrhea;	Dental care (*ilmu kukuh gigi*);	Several types of fever, including typhoid fever (*demam kepialu*).	Deworming (*ubat cacing*),
Tamam Kitab Taj Al-Mulk (Khuwaidumullah Anwar 2021)	*Ambil daun saranag manang dan daun jempelia dan daun mambu, campurkan ketiganya, dipipis lunak-lunak, maka ditempelkan pada kepalanya, maka airnya diminum 3 pagi.*	*Ambil jendawan ara, mamah dengan sirih pinang. Maka jangkakan akan kanak-kanak itu rupa cendawan itu seperti kelakuan batangnya hitam. (sebagai lagi) ambil akar mambu, ertinya rotan besar. Maka tukul pecah-pecah, maka direndam pada air itu, diminumnya. Maka jikalau diasahkan pun baik.*		No description	
Kitab Tib Muzium Terengganu (Harun & Zawiyah 2014)	*Ubat budak jangan menangis. Ambil daun mambung yang luruh maka ramas dan daun tulang hitam, maka ramas dengan air didih maka pupukkan pada tubuh atau pada dada atau pada dabinya afiat.*	No description	*Ubat gigi ambil kapur barus berat sekupang dan jintan putih berat dua kupang dan kecu berat seemas dan getah mambu berat dua emas, maka pipis lumat-lumat; akan airnya minyak kapur maka gosokkan pada giginya itu. Jikalau tiada minyak kapur maka giling dengan air, kelik besar-besar lada sulah maka kulum afiat.*	*Ambil halia dan cabai dan ketumbar dan mata mambu merah dan akar sempang, buah Melaka dan kayu manis dan cekur dan akar rumput manis sama banyak. Maka giling lumat-lumat beburahkan, adapun ubat ini dimakan pun baik juga*	No description
Kitab Tib MSS 2515 Perpustakaan Negara Malaysia (Harun 2017)	No description	*Sebagai lagi ubat sakit perut yang tidak boleh sembuh dengan ubat lain, maka ambil mata kunyit dan daun mambu dan akar asam jawa dan akar remunggai kerat. Ambil mata kunyit maka kerat maka rendangkan semuanya itu*		No description	

	bingga kering, tutuk lumat-lumat bubuh air madu maka gelek sembilan biji maka telan tiga pagi atau tujuh pagi, afiat.	
MSS 2999 Kitab Tib: Pandangan dan Tafsiran Perubatan Moden Terhadap Manuskrip Perubatan Melayu (Abdul Ghani 2015)	No description	
MSS B15 Kitab Perubatan Melayu Sari Segala Ubat (Mohd Shafri 2019)	No description	*sebagai ubat cacing, ambil akar mambu maka tutuk (ketuk atau lumat) pecah-pecah, maka rendam pada air maka beri air itu diminumnya, jika demah (menekan berturut-turut pada bahagian sakit dengan suhu sederhana) pon baik*

Analysis of Total Phenolic Content and Individual phenolic acids content of Azadirachta indica leaf extract

The total phenolic content of *A. indica* leaf extract was assessed using the Folin–Ciocalteu method. The determination of total phenolic content was 779.89 ± 12.32 μg GAE/g DW. The HPLC analysis identified the phenolic acid content from both alkaline and water extractions with different solvent extractions of *A. indica* leaves, as shown in Figure 2. The most abundant phenolic acids in both extractions were vanillic acid, *trans-p*-coumaric acid, and ferulic acid. Meanwhile, four phenolic acids were detected in the alkaline extraction, which were caffeic acid (2.38–14.63 μg/g DW), vanillic acid (1.96–9.38 μg/g DW), *trans-p*-coumaric acid (1.50–9.83 μg/g DW), and ferulic acid (1.68–9.53 μg/g DW). On the other hand, five phenolic acids were detected in water extraction: 4-hydroxybenzoic acid (0.22–0.23 μg/g DW), vanillic acid (0.11–0.61 μg/g DW), *trans-p*-coumaric acid (0.43–0.80 μg/g DW), ferulic acid (0.24–0.28 μg/g DW), and 3-coumaric acid (0.24 μg/g DW).

In comparison of the types of solvents in both extraction methods, phenolic acid was detected in all solvents, except for hexane in water extraction. Similarly, both extractions showed no presence of 2-coumaric acid in each solvent extract.

DISCUSSION

Azadirachta indica (Mambu) as a Diversified Resource

Azadirachta indica, commonly known as *mambu*, emerges as valuable good alternative with multifaceted applications, ranging from wood and medicinal uses to cosmetic, veterinary care, fertilization, and insecticides. The economic potential of *A. indica* is significant, contributing to income generation. However, caution is warranted, particularly with the oral administration of aqueous neem wood extracts or neem oil. Studies confirmed that prolong usage that exceeds seven days may induce contraceptive effects in mammals, affecting sperm shape and motility (Auta & Hassan 2016). Traditional Malay communities have long capitalized on natural resources for maintaining health, treating illnesses, and adopting healthy lifestyles. The medicinal benefits of *A. indica* in Malay culture span fever, diabetes, gastric, diarrhea, low blood pressure, malaria, hepatitis, measles, rheumatic, even cancer (Siti Fauziah 2013).

This aligns with the research findings of this study in the Malay medical manuscripts, where *A. indica* formulations were applied for diverse treatments, showcasing its versatility. Specifically, within MSS 3136, a comprehensive list of 13 diseases affecting various body systems and organs was identified. The manuscript contained a total of 45 formulations addressing these 13 diseases, with three of them specifically targeting eye diseases through six formulations. Among these formulations, nine incorporated plant-based ingredients, and modern studies have confirmed the pharmacological activities of five of them, particularly related to treating eye diseases. Meanwhile, *MSS B15 Kitab Perubatan Melayu Sari Segala Ubat* exclusively employed *A. indica* root for deworming (*ubat cacing*),

distinguishing itself from other manuscripts utilizing a combination of medicinal plants. Such distinctions highlight the specificity and varied applications of *A. indica* across different traditional medical practices.

Azadirachta indica in Ancient and Developed Civilizations

The incorporation of *A. indica* in both surgical and phytochemical procedures echoes its historical use in some the world's oldest and most advanced civilizations (Kumar & Navaratnam 2013). For instance, the global pursuit of effective treatments for COVID-19 has led scientists to explore natural products and traditional medicines, acknowledging their potential in regions with limited access to modern drugs. *A. indica* surfaces as a subject of interest, with its possible contributions to the development of new therapeutic solutions (Raja Nurhanin et al. 2020). The potential application of *A. indica* may be beneficial for symptomatic COVID-19 patients who commonly exhibit symptoms, such as a dry cough, breathlessness, or respiratory difficulty. Another study by Bezerra et al. (2021) reported that *A. indica* was highlighted for its efficacy in pest control. The utilization of microencapsulated bioinsecticides derived from *A. indica* not only reduces the amount of product utilized but also minimizes environmental impact and potential health risks to humans.

Bioactive Compounds and Biomedical Applications

Plant secondary metabolites either from plant extract or essential oil are studied for their bioactivities and have been observed to possess antibacterial, insecticidal and antifungal activities (Zeng et al. 2012). Modern analytical methods, such as HPLC–MS, LC–MS, GC–MS, NMR, and infrared ray spectroscopy, have unveiled the bioactive compounds in *A. indica* (Atawodi & Atawodi 2009). Maintaining the antioxidant activity of various parts of the neem plant is crucial, and this is closely tied to the total polyphenolic content. The phenolic levels in neem may vary depending on geographical locations and other abiotic factors (Ghimeray et al. 2009). Similar to this study, HPLC analysis has shown that neem oil comprises phenolic compounds, including hydroxytyrosol, tyrosol, vanillic acid, caffeic acid, vanillin, *p*-coumaric acid, vitamin D, vitamin E, ferulic acid, luteolin, pin-retinol, oleuropein aglycon, and ligstroside aglycon (Gossé et al. 2005). The presence of *A. indica* in alkaloids, steroids, flavonoids, terpenoids, fatty acids, and carbohydrates contributes to its pharmaceutical potential. The fungicidal properties of the tree, attributed to azadirachtin and nimbin, add further value, particularly in the context of environmental sustainability and human health. (Saleem et al. 2018). In conclusion, *A. indica* stands as a versatile resource with economic, medicinal, and environmental implications. Its historical significance and modern biomedical applications underscore its relevance across diverse domains, warranting continued exploration and utilization.

CONCLUSIONS

The findings derived from the analysis of five Malay medical manuscripts, namely, *Tamam Kitab Taj Al-Mulk, Kitab Tib Muzium Terengganu, Kitab Tib MSS 2515, MSS 2999 Kitab Tib,* and *MSS B15*, revealed a predominant focus on physical ailments, such as seizure (ubat sawan), stomach-related illness, such as gastric and diarrhea (penyakit perut), dental care (ilmu kukuh gigi), and several types of fever, including typhoid fever (demam kepialu). The total phenolic content of *A. indica* was measured at 779.89 ± 12.32 μg GAE/g DW, with consistent identification of individual phenolic acids detected by HPLC, including vanillic acid, ferulic acid, and *trans-p*-coumaric acid. Most of the traditional claims associated with *A. indica*'s therapeutic properties have been validated by scientific studies. However, further and more rigorous clinical investigations are warranted for a comprehensive understanding of the plant's therapeutic potential and its integration into mainstream medical practices.

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REFERENCES

Aarabi, A., Mizani, M., Honarvar, M., Faghihian, H. & Gerami, A. 2016. Extraction of ferulic acid from sugar beet pulp by alkaline hydrolysis and organic solvent methods. *Journal of Food Measurement and Characterization* 10(1): 42–47.

Abdul Ghani, H. 2015. *MSS 299 Kitab Tib: Pandangan dan Tafsiran Perubatan Moden Terhadap Manuskrip Perubatan Melayu*. Kuala Lumpur: Institut Terjemahan dan Buku Malaysia.

Alzohairy, M.A. 2016. Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. *Evidence-Based Complementary and Alternative Medicine 2016*.

Atawodi, S.E. & Atawodi, J.C. 2009. Azadirachta indica (neem): a plant of multiple biological and pharmacological activities. *Phytochemistry reviews* 8: 601–620.

Auta, T. & Hassan, A.T. 2016. Reproductive toxicity of aqueous wood-ash extract of Azadirachta indica (neem) on male albino mice. *Asian Pacific Journal of Reproduction* 5(2): 111–115.

Bertin, C., Yang, X. & Weston, L.A. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil* 256(1): 67–83.

Bezerra, D.G., Andrade, I.R. de, Santos, H.L.V., Xavier, M.D. da S., Fernandes, P.Í., Devilla, I.A., Nascimento, T.L., Borges, L.L., Conceição, E.C. da & Paula, J.A.M. de. 2021. Azadirachta indica A. Juss (Meliaceae) microencapsulated bioinsecticide: Spray drying technique optimization, characterization, in vitro release, and degradation kinetics. *Powder Technology* 382: 144–161.

Bhowmik, D., Chiranjib, Y.J., Tripathi, K.K. & Kumar, K.S. 2010. Herbal remedies of Azadirachta indica and its medicinal application. *Journal of Chemical and Pharmaceutical Research* 2(1): 62–72.

Calderon, A.R., Munoz, J.A., Moreno, D. & Celis, M. 2019. Describing and diffusing the ethnobotanical knowledge of Bogota D.C (Colombia) through an online tool focused on common names of plants. *Acta Botanica Brasilica* 33(2): 303–314.

Din, W.M., Zakaria, R.M.A. & Rahman, P.N. 2021. Discourse on ethnomedicinal plants in ancient malay medical manuscript for gastrointestinal diseases. *Jurnal Pengajian Umum Asia Tenggara* 22(1): 75–85.

Eizah, M.H., Zahir, A., Tengku Intan Marlina, T.M.A., Nur Yuhanis, M.N. & Nurhamizah, H. 2019. Old advices/tips and healthcare practice as a Malay local knowledgable based on manuscirpt MSS3140 Kitab Tibb. *Proceedings of the 28th International Conference on Literature: "Literature as a Source of Wisdom",* hlm. 86–95. Universitas Syiah Kuala, Banda Acheh, Indonesia.

Ghimeray, A.K., Jin, C.W., Ghimire, B.K. & Cho, D.H. 2009. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in Azadirachta indica A. Juss grown in foothills of Nepal. *African Journal of Biotechnology* 8(13): 2009.

Gossé, B., Amissa, A.A., Anoh Adjé, F., Bobélé Niamké, F., Ollivier, D. & Ito, Y. 2005. Analysis of components of neem (Azadirachta indica) oil by diverse chromatographic techniques. *Journal of liquid chromatography & related technologies* 28(14): 2225–2233.

Harun, M.P. 2017. *Kitab Tib Muzium Terengganu*. Batu Caves, Selangor: Johan Interlink Sdn Bhd.

Harun, M.P. 2019. *Kitab Tib: Ilmu Perubatan Melayu*. Kuala Lumpur, Malaysia: Perpustakaan Negara Malaysia.

Harun, M.P. & Zawiyah, B. 2014. *Kitab Tib MSS 2515 Perpustakaan Negara Malaysia: Kajian Teks & Suntingan*. Bangi, Selangor: Penerbit Universiti Kebangsaan Malaysia.

Keraf, A.S. 2010. *Etika Lingkungan hidup* (Environmental Ethics live). Jakarta, Indonesia: Penerbit Buku Kompas.

Khuwaidumullah Anwar, M. 2021. *Terjemahan Tamam Kitab Taj Al-Mulk*. Kota Bharu, Kelantan: Sidratul Muntaha Enterprise.

Kumar, V.S. & Navaratnam, V. 2013. Prehistory to contemporary medicinal uses to humankind. *Asian Pacific Journal of Tropical Biomedicine* 3(7): 505–514.

Mohd Affendi, M.S. 2020. Jaringan Ke'ilmuwan Tabib Melayu Satu Pemetaan Awwal Berdasarkan Beberapa Manuskrip Perubatan Melayu. Dlm. Mohd Affendi, M.S. (pnyt). *Manuskrip Perubatan Melayu Warisan Kebitaraan Melayu*, hlm. 1–14. Kuala Lumpur, Malaysia: Akademi Jawi Malaysia Sdn Bhd.

Mohd Shafri, M.A. 2019. *Kitab Perubatan Melayu Sari Segala Ubat*. Kuala Lumpur: Akadmei Jawi Malaysia.

Nadzirin, I.A. 2021. Analysis of Malay medical manuscript MSS 3136 and remedies for eye-related disease. *International Journal of Allied Health Science* 5(5): 2307–2415.

Othman, R., Ali, Q.A.M., Hatta, F.A.M., Ramya, R. & Yazid, A.F.A. 2016. *Landscape Plantarum in Islamic Built Environment* (December 2016): 231.

Quraishi, H.A., Islam, N., Iqbal, A., Bhat, S.A., Ahmed, J., Ashraf, S.S. & Khan, Q.A. 2018. Therapeutical and medicinal properties of Neem (Azadirachta indica) in context of Unani system of medicine: a review study. *Journal of Drug Delivery and Therapeutics* 8(6-s): 394–399.

Raja Nurhanin, R.P., Mohd Affendi, M.S. & Intan Azura, S. 2020. Ancient remedies for cough and cough-related symptoms: analysis from Malay medical manuscripts. *International Journal of allied Health Sciences* 5(5): 2425–2461.

Saleem, S., Muhammad, G., Hussain, M.A. & Bukhari, S.N.A. 2018. A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachta indica. *Phytotherapy Research* 32(7): 1241–1272.

Sarkar, S., Singh, R.P. & Bhattacharya, G. 2021. Exploring the role of Azadirachta indica (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. *3 Biotech* 11(4): 1–12.

Saxena, N. & Kumar, Y. 2009. Chemistry of azadirachtin and other bioactive isoprenoids from Neem (Azadirachta indica A. Juss). Dlm. Singh, K.K., Phogat, S., Dhillon, R.S. & Tomar, A. (pnyt). *Neem, a Treatise*, hlm. 208–230. New Delhi, India: International Publishing House Pvt. Ltd.

Shaharir, M.Z. 2021. The nature and international standing of jawi-Malay medical manuscripts before 1900. *International Journal of Allied Health Sciences* 5(5): 2322-2350.

Singleton, V.L. & Rossi, J. 1965. Colorimetry of total phenolics with phospho-molybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144–158.

Siti Fauziah, Y. 2013. *Ensiklopedia tumbuhan ubatan Malaysia*. Batu Caves, Selangor: A-Risalah Product Sdn Bhd.

Sõukand, R. & Kalle, R. 2022. The Appeal of Ethnobotanical Folklore Records: Medicinal Plant Use in Setomaa, Räpina and Vastseliina Parishes, Estonia (1888–1996). *Plants* 11(20): 2698.

Tsatsaroni, E. 1998. Comparative Study of dyeing Properties of Two Yellow Natural Pigments-Effect of Enzymes and Proteins 37(4): 307–315.

Wan Mamat, W.A. 1985. *Katalog Manuskrip Melayu di Belanda*. Kualar Lumpur, Malaysia: Perpustakaan Negara Malaysia.

Yahya, F. & Jones, R. 2021. Malay manuscripts: a guide to paper and watermarks. The collected works of Russell Jones 1972–2015. *Indonesia and the Malay World* 49(144): 139–394.

Zeng, W.C., He, Q., Sun, Q., Zhong, K. & Gao, H. 2012. Antibacterial activity of water-soluble extract from pine needles of Cedrus deodara. *International Journal of Food Microbiology* 153(1–2): 78–84.

Zhao, H., Sun, J., Fan, M., Fan, L., Zhou, L., Li, Z. & Guo, D. 2008. Analysis of phenolic compounds in Epimedium plants using liquid chromatography coupled with electrospray ionization mass spectrometry. *J. Chromatogr.* 1190: 157–181.

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