Original Research Article

Chromatographic Profiling of *Ziziphus mauritiana* Lam. Extracts for Phytochemical Characterization

Nurul Adlina Nadhirah Zamani[1], Ibtisam Abdul Wahab[1] and Hannis Fadzillah Mohsin[1]

[1]Pharmacology and Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor Kampus Puncak Alam, Bandar Puncak Alam, Selangor 42300, Malaysia

ABSTRACT

Ziziphus mauritiana, commonly known as Chinese date, Ber, and Indian jujube, belongs to the Rhamnaceae family and is native to Southeast Asia. Although it has been naturalized in tropical regions from South Africa to the Middle East and the Indian subcontinent, its phytochemical profile remains underexplored. A comprehensive phytochemical profiling and standardization of extract can be achieved by TLC and HPLC analysis. The study involves the development of improved chromatographic methods for the analysis of local *Z. mauritiana* leaves, including selection of the mobile phase type, gradient elution method and temperature, which are all potential HPLC parameters that may affect the result of analysis. The leaves were subjected to extraction through maceration utilizing methanol and hexane. The results indicated a percentage yield of 7.28% for hexane and 18.98% for methanol. TLC investigations were conducted using the crude extracts obtained, employing a dual mobile phase system (ethyl acetate: chloroform = 6:4) for the methanol extract and hexane:acetone (6:4) for the hexane extract, respectively. Both systems exhibited successful separation of phytochemicals. The methanol extract was subjected to HPLC profiling in accordance with the established protocols. Naringin was determined to be present through qualitative HPLC screening. Phytochemicals present in the leaves of *Z. mauritiana* are identified as a result of this research, which also sheds light on the optimal parameters for TLC and HPLC analyses. This research may also provide valuable insight regarding the importance of quality control and formulation of herbal medicines from the standardized extract.

Keywords: Extraction, TLC, HPLC, *Ziziphus mauritiana*

Corresponding author:
Hannis Fadzillah Mohsin
Department of Pharmacology and Pharmaceutical Chemistry,
Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Puncak Alam Campus,
42300 Bandar Puncak Alam, Selangor, Malaysia
Email: hannis@uitm.edu.my

Received: 10 Nov 2023; accepted: 15 March 2024
Available online: 01 April 2024
http://doi.org/10.24191/IJPNaCS.v7i1.04

1.0 Introduction

Indian jujube, Ber, Bidara, and Epal Siam (Fig. 1) are one of the few names for *Ziziphus mauritiana* Lam., belonging to the family of Rhamnaceae. This plant is categorized as a small, thorny, deciduous tree or shrub that can reach as high as 3-25m, and its leaves are wide and shiny. This species is found primarily in Central Asia, North Africa, India, South China, South East Asia, and tropical and subtropical regions (1). *Z. mauritiana* is a multipurpose tree because it bears fruit and its bark, leaves, and roots are of great use. Both fruit and leaves are edible. In addition, the leaves, fruits, and bark are commonly used in medicinal formulations.

Figure 1: Picture of *Z. mauritiana* leaves taken in Pekan, Pahang, Malaysia.

Z. mauritiana is commonly used in Islamic medicine because it is believed to be able to keep away evil elements and remove negative things from the body (2). In addition to being utilized in Islamic practice, *Z. mauritiana* has attracted the interest of researchers and product developers because of its many generally overlooked benefits. The fruit of *Z. mauritiana*, also called jujube, is an effective herbal medication that helps weight gain, improves muscular strength, and increases stamina. In Chinese medicinal practice, it is said to act as a natural remedy to strengthen liver function. The leaves of this plant carry astringent and febrifuge properties that help heal diarrhoea and reduce fever, respectively, and are also identified as boosting hair growth. In addition, leaves are also used as traditional remedies that are beneficial for fever, asthma, and liver problems. The fruits can be dried and carry medicinal benefits, including analgesics, anticancer, pectoral, cooling, sedatives, stomachache, styptic, and tonic. They are believed to promote healthy digestion and blood purification, plus, when taken internally, they can treat hysteria, anaemia, loss of appetite, and chronic exhaustion. The seed has soothing, stomachache-relieving, hypnotic, and narcotic properties and can also be taken internally to treat excessive sweating, palpitations, sleeplessness, nervous weariness, and nocturnal sweats. The root is used to treat dyspepsia or indigestion. The root has been used as a decoction to cure fever. In addition, to treat old wounds and ulcers, the root is ground to a powder and applied to the affected area. The herb is a folk treatment for neurological disorders, nephritis, hypertonia, and anemia (3).

Z. mauritiana has phytochemicals that are non-nutritive bioactive compounds with protective or disease-prevention qualities (1). Phytochemicals are important because they play a role in the exertion of the medicinal properties of *Z. mauritiana*, such as an anti-inflammatory, antimicrobial, antioxidant, anti-tumour, and liver protective agent and as an immune system stimulant (4). In addition, this species also exerts effects such as antispasmodic, antimalarial, antihypertensive, antidepressive, hypocholesterolemia, detoxification, and many other potentials (1). The phytochemicals can be identified through several tests, but the most prominent is thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). TLC is a much simpler and easier chromatographic approach than HPLC. It is widely used in pharmaceutical, industrial chemistry, clinical analysis, and

several other related fields (5). This separation technique is used to separate the nonvolatile mixture. On the contrary, the TLC method was usually performed on a planar and inert substrate such as glass or aluminium foil (stationary phase). This method works by placing the sample on a plate and allowing a solvent, called the mobile phase, to move up the plate through a capillary action. Different compounds in the sample have varying affinities for the mobile and stationary phases. They would migrate at different rates on the TLC plate, thus leading to their individual separation.

HPLC is one of the methods in column chromatography that drives samples at high pressure, which are initially dissolved in a solvent. This solvent will act as a mobile phase that transports the analyte along the stationary phase, the column, with an immobilized chromatographic material. The HPLC principle is based on the properties of the solvent, sample, and stationary phase. These properties determine the retention time (tR), which is the time it takes for the sample to pass through the column. Separation of analytes occurs based on their different retention times. Analytes less attracted to the stationary phase will elute more quickly, resulting in a shorter retention time. However, analytes that strongly interact with the stationary phase will take longer to pass through the column, resulting in a longer retention time (6). Both methods are optimal and helpful in detecting the biochemicals of *Z. mauritiana*, specifically its phytochemicals that exert medicinal properties. With newer studies and information, promoting more products containing *Z. mauritiana* on the market is possible. Therefore, this study aims to educate consumers and producers about the benefits and functions of *Z. mauritiana*.

Z. mauritiana is a traditional plant that has been used for generations. However, in this modern society, the commercial value of this species is still not as popular as dates, grapefruit, and several other commercialized fruits. The benefits and importance of this species on its leaves in the medical field are being forgotten due to the lack of research articles that discuss the bioactive compounds of *Z. mauritiana* that could support its medicinal use for the development of health products. Additionally, the establishment of research on phytochemicals for *Z. mauritiana* of local origin is still inadequate. This study aims to extract the phytochemical components of *Z. mauritiana* using methanol as a solvent and to analyze the TLC and HPLC profiles of the methanolic leaves extract of *Z. mauritiana*. Manufacturers may get information from this research on the feasibility of incorporating *Z. mauritiana* into their products in appropriate quantities or dosages. Furthermore, this work provides a guide for researchers in increasing their understanding and knowledge of the optimization of appropriate HPLC parameters for phytochemical screening.

2.0 Materials and Methods

2.1 Plant collection

The leaves of *Z. mauritiana* were collected directly from its tree, picking the leaves located in Pekan, Pahang Darul Makmur, Malaysia (3°32'19.2"N 103°23'02.0"E). The leaves were washed with distilled water and wiped with a clean cloth. The cleaned leaves were stored in a closed container for the analysis.

2.2 Equipment and chemicals

Equipment includes precoated silica gel 60 F_{254} sheets (Merck, Darmstadt, Germany), TLC development glass chambers, Whatman filter paper number 1, Cole-Parmer ultraviolet (UV) viewing

cabinet, and Bosch GHG 630 DCE heat gun. The reagents are HPLC grade acetonitrile, laboratory analytical reagents including methanol, hexane, ethyl acetate, chloroform, acetone, distilled water, Dragendorff's reagent, anisaldehyde, and acetic acid. The standard chemicals for HPLC consisted of quercetin, cinnamic acid, and naringin and were obtained from Sigma.

2.3 Sample preparation

The leaves were weighed and dried in an oven at 40°C. After two days, the leaves were removed to weigh and the values were compared with the initial weight. The leaves were placed again in the oven and left for 24 hours. After that, the leaves were weighed again to ensure the value was constant, indicating that all moisture had disappeared. The final weight was recorded, and the leaves were grounded manually with mortar and pestle. The maceration method was employed for extraction. About 50 g of oven-dried leaves of *Z. mauritiana* powder was soaked in hexane in a stopper container for 72 hours at room temperature (25^{o}C). The extraction was then strained directly in a conical flask using filter paper. The marc from the mixture was pressed to excrete the excess liquid. The hexane extract was transferred to a round bottom flask and the solvent was evaporated using a rotary evaporator. This evaporation led to the hexane crude extract of *Z. mauritiana,* which was transferred to a vial. The remaining marc was then used again by soaking in methanol for another round of maceration. The macerate was left at room temperature for 48 hours. After 48 hours, the crude methanol extract was yielded by repeating the evaporation step.

2.4 Thin-Layer Chromatography (TLC)

Two lines were drawn on a TLC silica plate about a few cm apart (solvent front distance), with the sample starting point at the bottom line. A mobile phase was prepared by adding ethyl acetate and chloroform with a drop of acetic acid in a ratio of 6: 4 (v/v). The prepared mobile phase was placed in the TLC tank. A capillary tube was used to spot the sample on the TLC plate. The TLC plate was introduced into the TLC tank to allow the mobile phase to travel on the plate until it reached the front line. The TLC plate was allowed to dry at room temperature. Then, it was visualized under UV light at short (λ=254 nm) and long (λ=366 nm) wavelengths. The TLC plates were also sprayed with reagents like Dragendorff's reagent and sulfuric anisaldehyde.

2.5 High-Performance Liquid Chromatography (HPLC)

The crude methanol extract of *Z. mauritiana* was diluted with HPLC grade methanol (1 mg/ml) and filtered through a 0.45 μm PTFE filter into an HPLC vial. Then, the HPLC parameter was set according to the procedure (Table 1). The reverse-phase HPLC system (Agilent 1200) was used. The mobile phase was prepared using a gradient separation mode, comprising water and 0.1% formic acid (90%) combined with acetonitrile (10%). 10 μL of the sample was injected, and the system was run at a flow rate of 1 mL/min. The temperature of the column was set at 30° C. Another 10 μL of 1 mg/mL quercetin, naringin, and cinnamic acid standards were run together with the sample for about 72 minutes. The detector was the diode array detector (DAD), and the wavelengths (λ) were set at 260 nm. The data were analyzed using ChemStation software. The sample chromatograms and the standards were

compared to detect the presence of quercetin, naringin, and cinnamic acid.

Table 1: HPLC parameter and gradient elution setup for the methanol extract of *Z. mauritiana*.

(A) HPLC parameter setup

Detector	Diode array detector (DAD)
Flow Rate	1 ml/min
Injection volume	10 µl
Column temperature	30 °C
Concentration of extract	1 mg/ml
Column detail	4.6 x 250 mm, 5 µm
Wavelength	260 nm
Run time	72 min
Mobile phase	Water + 0.1% formic acid (Solvent A), acetonitrile (Solvent B)

(B) Gradient elution setup for mobile phase

Time (min)	A%	B%
0.0	90.0	10.0
3.0	90.0	10.0
25.0	79.0	21.0
45.0	55.0	45.0
55.0	40.0	60.0
60.0	5.0	95.0
65.0	5.0	95.0
72.0	90.0	10.0

3.0 Results and Discussion

3.1 Extraction of samples

The sample extraction produced two outcomes following the involvement of two organic solvents of different polarities: hexane and methanol. The yields were calculated, and the results were recorded, as shown in Table 2. Hexane yielded 7.28% of the extract, while methanol provided 18.98% of the crude extract. Based on the results, the methanol extract produced a higher percentage of yield compared to the hexane extract (Table 2). This indicates that the extracts from the leaves may have more polar compounds compared to nonpolar compounds, as methanol, being more polar than hexane, was used in the extraction process.

3.2 Thin Layer Chromatography (TLC)

When the crude extract of the sample was obtained, TLC was carried out to observe the separation of phytochemicals. The eluents employed are ethyl acetate and chloroform (6:4) for the methanol extract and hexane and acetone (6:4) for the hexane extract. Eluent is deemed good when it can separate compounds in sufficient amounts to cause the formation of spots (7). The retention factor (Rf) for each stain or spot was calculated by the distance travelled by the sample from the original position, divided by the distance travelled by the solvent from the original position (8)(9). The farther the spot moves, the greater the Rf value, meaning that these spots have a higher affinity for the mobile phase than for the stationary phase (10). Table 3 shows the Rf values of the spots in the methanol and hexane extracts.

The phytochemical spots were detected at both short (254 nm) and long (366 nm) waves using an ultraviolet lamp. Ten spots can be observed in the methanol extract, with 7 spots observed in the short wave, while 6 spots can be observed in the long wave (Fig. 2). On the other hand, the hexane extract showed the presence of 9 spots, with all spots seen in short waves, while about 5 spots can also be detected in long waves (Fig. 2). Natural compounds with aromatic rings and conjugated double bonds could be identified by the UV short wavelength at 254 nm. Furthermore, plant pigments and fluorescent compounds, such as riboflavin and quinines, were easily detected at long wavelength UV at 366 nm (1). The methanol extract shows more distributed separation with more

chromophores that can be detected under long-wave ultraviolet light. The most significant spots were numbers 4, 5, and 6 (Table 3). Spot 4 can be seen under short waves and even with the naked eye. Spot 5 and 6 exerted the brightest and most obvious colour as chromophores. The most common chromophore found in plant leaves is chlorophyll and other compounds, such as quercetin (11, 12). On the other hand, the combinations of hexane and acetone as mobile phase showed that many nonpolar compounds were obtained from the hexane extract, which exhibits a high Rf value on TLC. The highest Rf value was 0.96 cm, and the lowest Rf value was 0.47 cm. In contrast to the other spots, Spot 6 exhibited a distinctive orange colour under long waves.

The results of this observation indicate that the solvent systems of hexane and acetone (6:4) are more favorable for the separation of compounds in hexane extract than ethyl acetate and chloroform (6:4) are for the separation of compounds in the methanol extract. Identification procedures were conducted during the TLC studies with Dragendorff and anisaldehyde reagents, respectively, to determine the presence of of the alkaloid and terpenoid. When Dragendorf's reagent interacts with alkaloids, the spots turn orange, while anisaldehyde produces a purple colour in spots containing terpenoids (13). However, no notable changes occurred when the TLC plates were treated with both reagents, indicating the absence of both types of compounds.

Table 2: Results of leaves extraction.

Solvent	Dry leaves (g)	Crude extract (g)	Yield (%)
Hexane	147.0	10.7	7.2
Methanol	147.0	27.9	18.9

Table 3: TLC Rf values of the methanol and hexane extracts under UV (366 nm).

Spot	Methanol extract		Hexane extract	
	Rf value	Colour (366 nm)	Rf value	Colour (366 nm)
1	0.94	Orange	0.96	-
2	0.92	Red	0.91	-
3	0.83	-	0.85	Red
4	0.73	-	0.70	Bluish green
5	0.67	Orange	0.60	-
6	0.60	Blue	0.57	Bright orange
7	0.44	-	0.55	Orange
8	0.35	Orange	0.51	Orange
9	0.30	-	0.47	-
10	0.25	Red	-	-

3.3 High-Performance Liquid Chromatography (HPLC)

The chromatogram peaks and separation of the methanol extract from *Z. mauritiana* were investigated using HPLC analysis in 72 minutes with a flow rate of 1 ml/min. A satisfactory separation was achieved with the gradient elution comprising the mobile phase of water with 0.1 % formic acid in water and acetonitrile in gradient elution. Standard quercetin, cinnamic acid, and naringin were included in the HPLC analysis as external standards to compare the presence of those compounds in the extract (Fig. 3). The presence of several peaks in the chromatogram indicates various significant polar compounds (Fig. 4). The chromatogram shows a peak comparable to the retention time of standard naringin, which was detected at 24.76 minutes. Naringin, an important flavanone, was reported with several health-related benefits, including antioxidant, inhibiting microsomal triglyceride that transfers protein and acetyl-coenzyme A acetyltransferase, and in the regulation of cytochrome P450 enzymes (14). The chromatogram shows no similar peaks to the quercetin and cinnamic acid at tR = 35.746 minutes and tR = 34.862

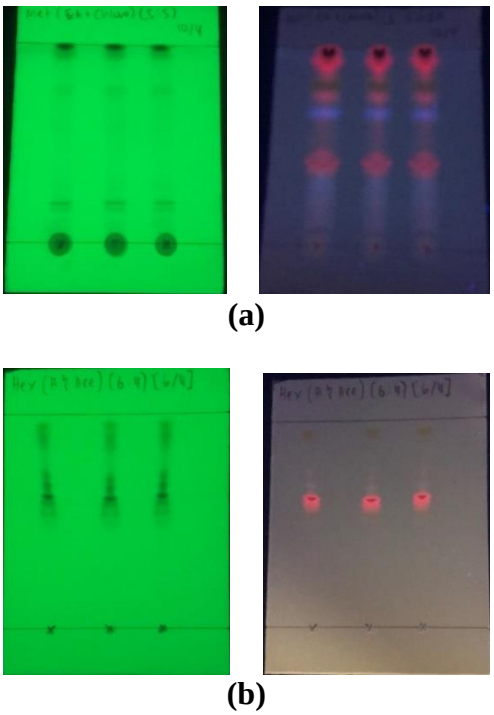
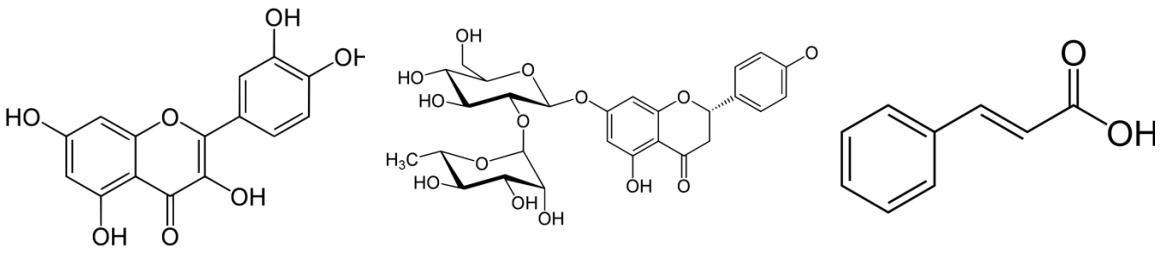


Figure 2: (a) TLC of the methanol extract under UV 254 nm (left) and UV 366 nm (right); **(b)** TLC of the hexane extract under UV 254 nm (left) and UV 366 nm (right).

minutes, respectively. The absence of these two compounds may be due to the low concentration during the preparation of the samples, thus insufficient to be detected by the HPLC detector. According to Butt *et al.* (15), *Z. mauritiana* leaves contain proteins, amino acids, alkaloids, terpenoids, fibres, flavonoids, tannins, glycosides, and phenolic compounds. In n-hexane, chloroform, and methanol extracts, leaves prominently showed the presence of linolenic acid, palmitic acid, and methyl stearate (14). Another study by Naaz *et al.* (16) stated that the chemical constituents of *Z. mauritiana* leaves are alkaloids, glycosides, saponins, fibres, and phenolic compounds. Yahia *et al.* (17) reported the presence of *trans*-cinnamic acid and quercetin in the leaves.

Understanding the phytochemical components of plants is useful not only for developing novel therapeutic treatments but also for discovering new commercial resources. Natural herbs are high in pharmacologically active phytochemicals and phytomedicine, making them an important resource in the search for more effective and safer medicines (17). Naringin, which was detected by HPLC, has medicinal benefits that were usually ignored by the medical field. The pharmacological actions of naringin are numerous and include antioxidant, anti-inflammatory, antiapoptotic, anti-ulcer, and anti-osteoporosis properties. Its therapeutic effects have been observed in a variety of clinical settings, including rheumatic disorders, diabetes, neurodegenerative diseases, and atherosclerosis (18).



Quercetin Naringin Cinnamic acid

Figure 3: The chemical structures of quercetin, naringin, and cinnamic acid

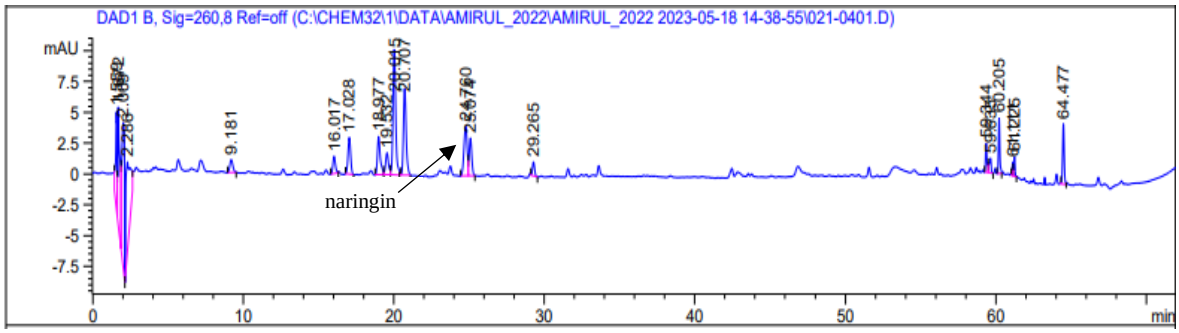


Figure 4: HPLC chromatogram of *Z. mauritiana* methanol extract, recorded at λ = 260 nm.

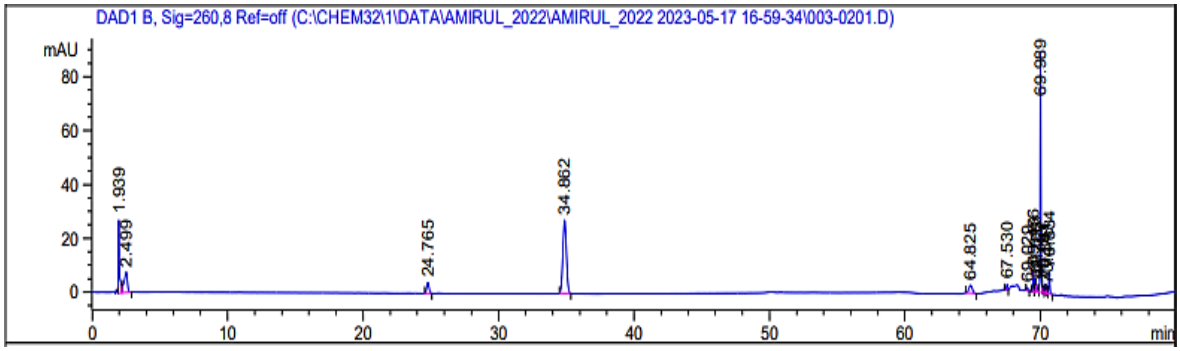


Figure 5: HPLC chromatogram of a standard mixture of cinnamic acid and naringin (λ = 260 nm).

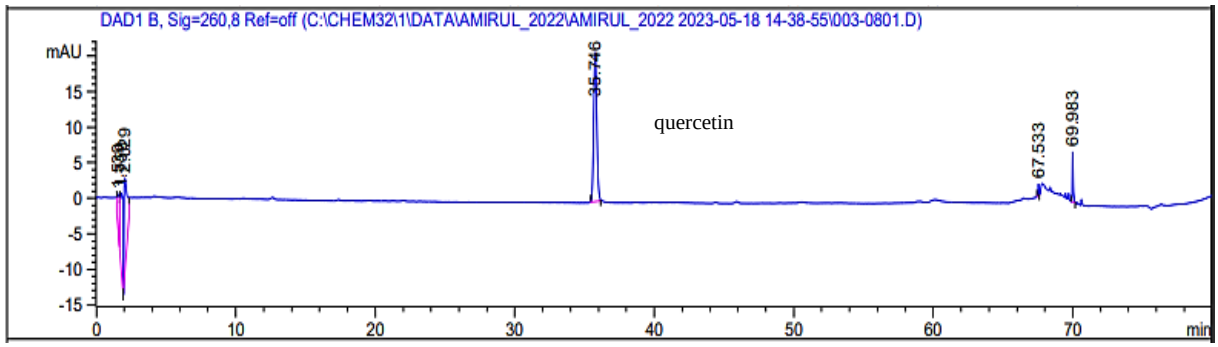


Figure 6: HPLC chromatogram of standard quercetin (λ = 260 nm).

The preliminary phytochemical screening of *Z. mauritiana* carried out by Egbe *et al.* (19) showed the presence of saponins, tannins, alkaloids, phenolics, terpenoids, and flavonoids, with methanol extract having a high content of total flavonoid. Individual components, such as saponins, are widely used in various fields, such as commercial, food, and cosmetic applications, as an alternative to a natural approach (20). In addition to saponins, flavonoid and phenol are also the major components that are being extracted. Interestingly, flavonoids are very beneficial to humans, especially in health and well-being. Quercetin, for example, exerts vasoprotective activity and an anticarcinogenic effect, making it a great alternative in medicine. This compound is believed to have protective activity against diabetes mellitus (DM), with the intake of 500 mg/day of flavonoids associated with a 5% reduction in the risk of DM (21). Phenolic compounds, such as cinnamic acid, are bioactive substances that, when included in a regular diet, are typically linked to protective actions that help preserve good health. These substances have demonstrated inhibitory activity against the development of several major diseases, including diabetes, Alzheimer's, and cancer. These positive effects have been primarily linked to antioxidant and radical scavenging properties that can postpone or prevent DNA, protein, and lipid oxidation (22).

HPLC fingerprinting has been shown to be a reliable, exact, and accurate approach to identifying the components of natural products. It may also be used to authenticate and characterize the plant, which is crucial for medicine. The manufacturer would benefit from the HPLC fingerprints created to ensure quality assurance and standardisation of herbal products. In parallel with this investigation, the phytochemicals and biological activities of *Z. mauritiana* were recently published (23, 24).

4.0 Conclusion

The organic solvent extraction provided the methanolic extract, with a higher yield than the hexanoic extract, indicating that the leaves of *Z. mauritiana* might contain more polar compounds. The separation of the phytochemicals in the extracts was demonstrated by TLC analysis using the appropriate eluents. A more evenly distributed separation was observed in the methanol extract, and many notable spots, particularly spots 4, 5, and 6, indicated the presence of chromophore chemicals. Numerous nonpolar molecules with high Rf values were produced by hexane extract, and spot 6 under long-wave UV illumination displayed a vivid orange colour. The key polar components of the methanol extract were discovered using HPLC, including the presence of naringin, which has several therapeutic advantages. The presence of naringin in the methanol extract draws attention to its possible medical benefits, which include antioxidant, anti-inflammatory, antiapoptotic, anti-ulcer, and anti-osteoporosis activities. Quercetin and cinnamic acid were not detected by HPLC analysis, in this research specimen. It is possible that the sample's concentration of these substances was below the UV spectrometer detection threshold. Saponins, tannins, alkaloids, phenolics, terpenoids, and flavonoids were also discovered during the preliminary phytochemical screening, with the methanol extract having the highest level of total flavonoid. HPLC analysis serves as a valuable tool for identifying and characterizing the chemical constituents of plants. It can be applied to quality control, the standardization of herbal goods, and the taxonomic grouping of plants. The pharmaceutical sector needs a reliable and precise method to identify and certify herbal items, and HPLC fingerprinting meets this need. In summary, the objectives of this study

were achieved and the hypothesis could be supported. The findings of this study would provide additional evidence for future researchers to experiment with similar species. Furthermore, the liquid chromatography investigation can provide valuable insights for product developers interested in incorporating this plant into their products, indirectly increasing the species' market value.

A**uthorship contribution statement**

NANZ: Experimental, writing the original draft, data analysis and reviews. **IAW:** Conceptualization, literature search, methodology, experimental evaluations and reviews. **HFM**: Conceptualization, methodology, data analysis, project administration, reviews and supervision.

Acknowledgement

The authors thank the Faculty of Pharmacy, UiTM, Puncak Alam Campus, for providing the laboratory facilities.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. Aqilah S, Yusof M, Saat R. Phytochemical Analysis and Bioactivity Studies of Ziziphus Mauritiana (Twigs and Leaves). J Acad UiTM Negeri Sembilan. 2017;5:17–26.

2. Mohd Yusoff AN, Abdul Aziz MH, Magiman MM. Kajian Tematik Buah-buahan dalam Al-Quran dan Penggunaannya untuk Rawatan Perubatan Islam. ANP J Soc Sci Humanit. 2022;3(2022):10–6.

3. Palejkar CJ, Palejkar JH, Patel AJ, Patel MA. A Plant Review on Ziziphus mauritiana. Int J Univers Pharm Life Sci. 2012;2(2):202–11.

4. Dureja AG, Dhiman K. Free radical scavenging potential and total phenolic and flavonoid content of Ziziphus mauritiana and Ziziphus nummularia fruit extracts. Int J Green Pharm. 2012;6(3):187–92.

5. Santiago M, Strobel S. Thin layer chromatography. In: Methods in Enzymology. Methods Enzymol; 2013. p. 303–24.

6. Petrova OE, Sauer K. High-performance liquid chromatography (HPLC)-based detection and quantitation of cellular c-di-GMP. In: Methods in Molecular Biology. NIH Public Access; 2017. p. 33–43.

7. Adiga V, P SC, Kikkeri VV, Praveen C, Senapati S. Developing a Novel Solvent System to Separate Polar and Nonpolar Leaf Pigments of Copperleaf (Acalypha Wilkesiana) Using Thin Layer Chromatography. Qeios. 2023 Jul; 1-16 12

8. Swamy V AL. Analysis of few crude compounds and separation of leaf pigments in few medicinal plant species. Int Res J Plant Sci. 2021;12(2):1–7.

9. Thin Layer Chromatography. BYJU'S. Retrieved December 15, 2022, from https://byjus.com/chemistry/thin-layer-chromatography/ (January 2020).

10. Kharb S. General Lab Techniques. Mind Maps Clin Chem (Part II). Bentham Science Publisher, 2021;47–56.

11. Mutakin M, Juwita T, Megantara S, Puspitasari IM, Levita J. Determination of quercetin and rutin in the ethyl acetate fraction of Etlingera Elatior (Jack) R.M. Smith flower by reversed-phase liquid chromatography-mass spectroscopy. Rasayan J Chem. 2020;13(3):1379–85.

12. Zhong S, Bird A, Kopec RE. The Metabolism and Potential Bioactivity of Chlorophyll and Metallo-chlorophyll Derivatives in the Gastrointestinal Tract. Mol Nutr Food Res. 2021 Apr 1;65(7):2000761.

13. Raal A, Meos A, Hinrikus T, Heinämäki J, Romāne E, Gudienė V, et al. Dragendorff's reagent: Historical perspectives and current status of a versatile reagent introduced over

150 years ago at the University of Dorpat, Tartu, Estonia. Pharmazie [Internet]. 2020 Jul 1;75(7):299–306.

14. Clayden J, Greeves N, Warren S. Organic Chemistry Organic Chemistry. Org Chem Front. 2012;58(6):1261.

15. Butt SZ, Hussain S, Shahzad Munawar K. Phytochemistry of Ziziphus mauritiana: An Overview of its Nutritional and Pharmaceutical Potential. Sci Inquiry Rev., 2021;5(2), 1-15.

16. Naaz F, Agari N, Singh A. Medicinal Properties of Ziziphus mauritiana: A Review Article introduction. Int J Pharm Pharm Res. 2020;19(1):218–30.

17. Yahia Y, Benabderrahim MA, Tlili N, Bagues M, Nagaz K. Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two Ziziphus Mill. species. PLoS One. 2020 May 1;15(5):e0232599.

18. Srivastava A, Srivastava P, Pandey A, Khanna VK, Pant AB. Phytomedicine: A Potential Alternative Medicine in Controlling Neurological Disorders. New Look to Phytomedicine Adv Herb Prod as Nov Drug Leads. 2019 Oct 26;625–55.

19. Wang Y, Li X, Lv H, Sun L, Liu B, Zhang X, et al. Therapeutic potential of naringin in improving the survival rate of skin flap: A review. Front Pharmacol. 2023 Mar 2;14:1128147.

20. Eucharia Egbe N, Garba S, Adamu A, Aliyu F. Phytochemical Screening, In Vitro Antioxidant And Gc-Ms Analysis of Ziziphus Mauritiana Leaves Extract. BimaJ Sci Technol. 2022;6(03):80–90.

21. Roopashree KM, Naik D. Saponins : Properties, Applications and as Insecticides: A Review. Trends Biosci. 2019;12(1):1–14.

22. Rahaman ST, Mondal S. Flavonoids: A vital resource in healthcare and medicine. Pharm Pharmacol Int J. 2020;8(2):91–104.

23. Parveen S, Bukhari N, Nazir M, Qureshi WA, Yaqoob A, Shahid M. Phytochemical analysis, in-vitro biological activities and Pearson correlation of total polyphenolic content with antioxidant activities of Ziziphus mauritiana fruit pulp and seed during different ripening stages. South African Journal of Botany, 2023; 157:346-354.

24. Sakna ST, El-Maghraby Y, Abdelfattah MS, Farag MA. Phytochemical diversity and pharmacological effects of triterpenes from genus Ziziphus: a comprehensive review, Phytochem Rev, 2023; 22(2):1611–1636.