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Qualitative Phytochemical Screening and Antioxidant Properties of the Leaves of *Miconia crenata* (Vahl) Michelang. (Melastomataceae) from Ayer Hitam Utara Forest Reserve, Johor, Malaysia

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Abstract

Medicinal plants have long been recognised as valuable resources for traditional medicine and modern pharmaceutical research due to their rich reservoirs of bioactive compounds. This study aims to provide baseline data on the phytochemical content and antioxidant activity of a tropical medicinal shrub, *Miconia crenata,* from Ayer Hitam Utara Forest Reserve (AHUFR), Muar, Johor. Total flavonoid content was determined by using the chloride colorimetric method, while antioxidant activity was validated by the DPPH radical scavenging assay. Phytochemical screening of the methanol extract from the leaves of *M. crenata* revealed the presence of secondary metabolites, including phenols and flavonoids. The total flavonoid content was measured at 508.84 ± 154.42 mg/mg at a concentration of 0.02 mg/mL, while the extract demonstrated high antioxidant potential, with an IC50 value of 40.54 µg/mL. Further investigation into the phytochemical content of *M. crenata* may unlock valuable insights into its potential therapeutic uses and contribute to the development of novel pharmaceuticals and natural health products.

1. Introduction

Worldwide, medicinal plants are used by many people to treat illness, including people who reside in rural areas, such as those in West and East Malaysia [1]. Plants are known to be able to synthesise their own secondary metabolites that allows them to defend against herbivores, shielding against pathogens, and protection against harsh conditions such as water deficits and low temperatures [2]. Examples of secondary metabolites are alkaloid, flavonoid, phenol, terpenoid, and saponin. Phytochemicals found in plants exhibited protective and preventive properties against various diseases, which play an imperative role in plants' protection, including antibacterial, antiviral, antifungal, and insecticidal agents. For instance, flavonoid, which has 15-carbon atoms with two aromatic rings connected by a 3-carbon bridge, is a polyphenolic compound that helps to protect plants from pathogenic attacks and herbivory due to its deterrent effects by generating pigmentation in plant parts, acting as a shield against UV radiation and diseases [3]. This compound is usually found in the fruits' skin and on

the leaves' epidermis. Likewise, phenolic compounds that have at least one aromatic ring with hydroxyl groups have similar roles in plants' defense mechanisms against herbivores, pathogens, animals, and environmental stresses.

Miconia crenata (Vahl) Michelang. or Soapbush from the family Melastomataceae is a perennial shrub that grows primarily in the wet tropical biome. The native range of this species is Mexico to Tropical America though has been introduced to several parts of the world including Malaysia [4]. The species is characterized through its glossy, deeply veined leaves that are ovate to oblong with crenate leaf margin, have five major veins spreading from base to tip, inflorescence more or less pedicellate and laxly branched, small white flowers that are composed of 5-6 petals with rounded to truncate tips, as well as its fruits that is ellipsoid, pubescent with bluish-black berry colour [5]. The species was formerly known as the polyphyletic *Clidemia hirta* (L.) D.Don. It has been used in traditional and ethnomedicinal purposes, including treating infections, inflammation, and gastrointestinal issues. For example, in Malaysia, the crushed leaves are mixed with saliva as poultice on wounds to stop bleeding. In other parts of the world such as the South American region, the leaves are utilised for addressing ailments related to the nervous and digestive systems, intestinal blockage, stomach discomfort, diarrhea, gastritis, nausea, bloating, poor digestion, and congestion [6].

In addition, tea derived from the plant is utilized for alleviating heart palpitations, its flowers for treating kidney issues, and its fruits for addressing bladder problems and abnormal vaginal discharge. The ethanolic extract from *C. hirta* from Indonesia has been reported to demonstrate broad-spectrum antibacterial activity, including against *Salmonella typhi* and *Staphylococcus aureus*, by inhibiting their growth at all concentrations tested [7]. Moreover, through gas chromatography-mass spectrometry (GC-MS) analysis, Ismail et al. [8] reported the presence of ethyl acetate, nonanedioic acid, dibutyl ester, and methyl 7-octadecenoate as notable compounds from the species, with the major compound in the ethyl acetate extract identified as 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl, having a 50.05% peak area [8]. The identification of such compounds suggests that *M. crenata,* or the synonym *C. hirta,* could be a valuable source of natural bioactive agents for pharmaceutical and agricultural applications.

This paper aims to provide the first documentation of the phytochemical and antioxidant properties of one of the most abundant shrubs, *M. crenata,* from lowland vegetation in Ayer Hitam Utara Forest Reserve, Muar, Johor. Understanding the intricacies of the phytochemical composition of *M. crenata* is crucial for harnessing its full potential in the field of medicine and healthcare.

2. Materials and Methods

2.1 Plants Sampling and Documentation

Samples of *M. crenata* were collected from the lowland forest area of Ayer Hitam Utara Forest Reserve, Muar (GPS coordinates 2°3'3"N 102°49'39"E), in November 2021. Ayer Hitam Utara Forest Reserve is home to valuable and last remaining peat forest ecosystems in Peninsular Malaysia, besides the main lowland mixed dipterocarp vegetation. These ecosystems are ecologically significant for their valuable biodiversity, carbon storage, and role in local communities' livelihoods. Identification of species was made based on the morphology of vegetative and reproductive characters and further verified by comparing with herbarium specimens at herbaria (online repository of K and L) and protologues. Herbarium specimens were prepared accordingly and deposited at herbarium of Universiti Tun Hussein Onn Malaysia (Pagoh Campus).

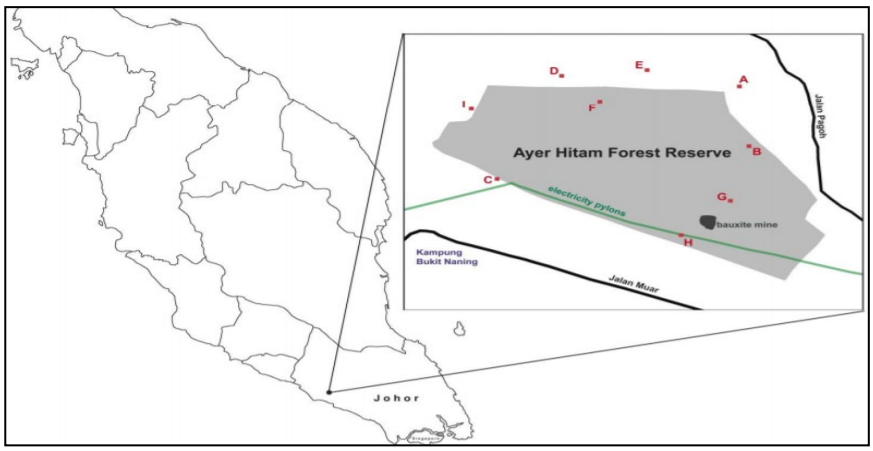

Fig. 1 *Location of Ayer Hitam Utara Forest Reserve in the state of Johor, Peninsular Malaysia*

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2.2 Preparation of Extracts

The leaves of *M. crenata* were washed under running tap water to remove debris and damaged portions, dried in the shaded area, and then dried at 50°C for 24 hours in the oven. The dried samples were ground into powder using a mechanical blender and grinder. The powdered dried leaves were then soaked in methanol in a ratio of 1:8 at room temperature in an incubator shaker for 24 hours. The extracts were then filtrated through Whatman No. 1 filter paper before evaporating the residual solvent using a rotary evaporator. The crude extracts were kept at 4°C until further analysis [9].

2.3 Qualitative Phytochemical Screening

Further, methanolic leaves extract of *M. crenata* were screened for alkaloid, flavonoid, phenol and terpenoid. The qualitative results were expressed as (+) for the presence and (-) for the absence of the respective phytochemicals.

2.3.1 Alkaloid

0.2 ml of dilute HCl and 2 ml of extract were mixed in a test tube. 1 ml of Mayer's reagent was then added. The formation of a yellowish colour indicated the presence of alkaloid [10].

2.3.2 Flavonoid

0.5 ml of diluted NaOH solution and 1 ml of stock solution were mixed in a test tube. An intense yellow colour appeared before diluted HCl was added into the mixture. The formation of a colourless solution indicated the flavonoid's presence [11].

2.3.3 Terpenoid

0.5 ml of stock solution were dissolved in 0.5 ml of chloroform. 1 ml of concentrated HCl was then added to the solution. The reddish-brown color formation indicated the presence of terpenoids [11].

2.3.4 Phenol

A small amount of the methanolic extract was mixed with 1 ml of water in a test tube, and 1-2 drops of $FeCl^3$ were added. Blue, green, red, or purple indicates a positive test [12].

2.4 Determination of Total Flavonoid Content

Flavonoid determination was carried out using the aluminium chloride method [13]. This method expressed the flavonoid contents as quercetin equivalent, and quercetin was used as a standard. The calibration curve was created using the standard form of quercetin. Using a UV-Visible spectrophotometer, the absorbance was measured at 376 nm. The following Equation 1 was used to express the total flavonoid content of the extracts in mg Quercetin equivalents (QE)/mg extract:

$$\text{DPPH radical scavenging activity (\%)} = [(\text{Absorbance of control} - \text{Absorbance of the test sample}) / (\text{Absorbance of control})] \times 100 \quad (1)$$

2.5 Statistical Analysis

Three replicates of the sample were utilised for both quantitative screening of total flavonoid content and DPPH scavenging assay. The data obtained from these replicates underwent statistical analysis and are reported as mean ± standard deviation. Statistical significance was determined using one-way ANOVA conducted in Microsoft Office Excel 2016, with differences considered significant at $p < 0.05$.

3. Results and Discussion

3.1 Qualitative Phytochemical Screening of *Miconia Crenata's* Leaves

The results show that flavonoid and phenol were present in the leaves of *M. crenata* from AHUFR, but lack of alkaloid and terpenoid. Similarly, terpenoid was also reported absent from the species according to a report by Ismail et al. [14]. The presence or absence of specific phytochemicals like flavonoids, phenolics, alkaloids, and terpenoids in plant leaves, including in *M. crenata*, can be due to several factors including the environmental conditions or habitat where it grows, plant's genetics, and the developmental stage of the plant [15].

Alkaloids and terpenoids have different biosynthetic pathways and roles in plant physiology. The absence of these compounds in the leaves of *M. crenata* might indicate that the plant does not have the necessary

biosynthetic enzymes active in the leaves for these particular compounds, or the environmental conditions do not favor their production. Occasionally, plants produce certain secondary metabolites only in response to specific stressors or during particular growth stages [15], [16]. Moreover, different parts of a plant may contain different phytochemical profiles. For instance, alkaloids might be more prevalent in the roots or seeds, while terpenoids could be more concentrated in the flowers. Table 1 present the phytochemical screening results.

Table 1 *Qualitative phytochemical screening of methanolic extract of M. crenata*

Qualitative test	Presence/Absent
Alkaloids	–
Flavonoids	+
Terpenoids	–
Phenols	+

3.1.1 Total Flavonoid Content

The total flavonoid content was expressed as quercetin equivalents. The quercetin standard solutions (0.02, 0.04, 0.06, 0.08 mg/mL) yielded a coefficient of regression (R^2) of 0.9613. The quantity of flavonoids was notably higher in the extract (508.84 ± 154.42 mg QE/mg) at 0.02 mg/mL. For comparison, the values were lower at higher concentrations: 335.81 ± 85.68 at 0.04 mg/mL, 336.14 ± 41.54 at 0.06 mg/mL and 322.684 ± 96.32 at 0.08 mg/mL. This relatively high flavonoid content in *M. crenata* was also reported by Ismail et al. (2021) which recorded a value of 507.92 mg QE/mg. This would indicate a very high and comparable flavonoid content in *M. crenata* across both studies, with only minor variation.

3.1.2 Antioxidant Activity

For antioxidant activity, a low IC50 value indicates high antioxidant potential. The IC50 value represents the concentration of a substance required to inhibit a particular biological or biochemical function by 50%. In the context of antioxidant activity, a low IC50 value indicates that a lower concentration of the substance is needed to achieve 50% inhibition of the DPPH radical. In this study, *M. crenata* collected from AHUFR recorded low IC50 value for DPPH radical scavenging at 40.56 µg/mL. This value contrasts notably with those reported by Ismail et al. [14], which were 86 µg/mL. Furthermore, other reports on another genus in Melastomataceae, *Melastoma malabathricum*, indicated IC50 values of 11 µg/mL and 280 µg/mL from the leaf methanolic extracts [17],[18].

4. Conclusion

This study has contributed to the first documentation of medicinal plants from the lowland forest areas in AHUFR. Samples of *M. crenata* tested positive for flavonoid and phenol tests but negative for alkaloid and terpenoid. The species from the study site also has a relatively low IC50 value indicates a high potent antioxidant potential. The presence of flavonoids and phenols in the species is likely responsible for the free radical scavenging effects. From the results, it is recommended to further isolate and characterise the compounds to understand their specific reactions to the plant's antioxidant activity. Besides antioxidant potential, the biological activity of the flavonoids and phenols should also be explored, including potential anti-inflammatory, anti-cancer, or cardio-protective effects in the future.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Nur Adila Jamil, Salasiah Mohamad, Alona Cuevas Linatoc;* ***data collection:*** *Nur Adila Jamil, Mohammad Ifratshim bin Muhamad Sa’ed;* ***analysis and interpretation of results*** *Nur Adila Jamil, Salasiah Mohamad, Alona Cuevas Linatoc;* ***draft manuscript preparation:*** *Nur Adila Jamil, Salasiah Mohamad, Alona Cuevas Linatoc, Mohammad Ifratshim bin Muhamad Sa’ed. All authors reviewed the results and approved the final version of the manuscript.*

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