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PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT, AND ANTIOXIDANT ACTIVITY OF DIFFERENT PARTS OF *Melastoma malabathricum*

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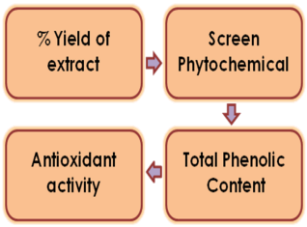
Graphical abstract



Abstract

Melastoma malabathricum Linn. commonly known as 'senduduk' in Malaysia, belongs to Melastomataceae family. The study was carried out to screen the phytochemicals present in methanol extract of leaf, flower, fruit, and stem and to evaluate the antioxidant activity, total flavonoid and total phenolic contents of these different parts. Phytochemical screening showed that all parts of this plant contained tannins, steroids, phenols and flavonoids. The flower extract was found to have highest total phenolic whereas the leaf demonstrated the highest flavonoid content followed by flower. The results of antioxidant activity from the DPPH assay showed that the flower has the highest radical scavenging activity comparable to quercetin standard while stem showed the lowest activity. The higher content of total phenolics, flavonoids and antioxidant capacity of flower suggests the possibility of its incorporation and exclusion of stem in *M. malabathricum* preparations for development of newer effective drugs.

Keywords: *Melastoma malabathricum*, DPPH, phenolics, flavonoid, quercetin

Abstrak

Melastoma malabathricum Linn. umumnya dikenali sebagai 'senduduk' di Malaysia, adalah dari famili Melastomataceae. Kajian telah dijalankan untuk menyaring kehadiran fitokimia dalam ekstrak methanol daun, bunga, buah dan batang; dan juga untuk menilai aktiviti antioksida, jumlah kandungan flavonoid dan jumlah kandungan fenolik dalam bahagian berbeza tersebut. Penyaringan fitokimia menunjukkan bahawa semua bahagian tumbuhan ini mengandungi tanin, steroid, fenol dan flavonoid. Ekstrak bunga didapati mengandungi jumlah kandungan fenolik yang tertinggi manakala daun menunjukkan jumlah kandungan flavonoid tertinggi diikuti oleh bunga. Keputusan aktiviti antioksida melalui asai DPPH menunjukkan bunga mempungai aktiviti pemerangkapan radikal tertinggi setara dengan piawai kuersetin, manakala batang menunjukkan aktiviti terendah. Jumlah kandungan fenolik, flavonoid dan keupayaan antioksida yang lebih tinggi pada bunga mencadangkan kemungkinan penglibatannya dan pengecualian batang *M. malabathricum* dalam penyediaan bagi

pembangunan ubatan baru yang efektif.

Kata kunci: Melastoma malabathricum, DPPH, fenolik, flavonoid, kuersetin

1.0 INTRODUCTION

Melastoma malabathricum Linn. belongs to the family *Melastomaceae,* and is a shrub that grows up to 18 ft height. *M. malabathricum,* is commonly found in Malaysia as well as Thailand, and is used in traditional medicine for treatment of various illnesses [1]. *M. malabathricum* contains several secondary metabolites such as flavonoid and phenolic compounds which are responsible for its therapeutic activities such as anticancer and antioxidant [2]. Majority of naturally occurring antioxidants isolated from plants are flavonoids and other phenolic compounds [3].

Reactive oxygen species is a general term which includes oxygen radicals as well as several non-radical oxidizing agents. Among these, the hydroxyl and alkoxyl free radicals are very reactive which enable them to rapidly attack molecules in cells. Reactive oxygen species may be very damaging since they can attack lipid in cell membranes, proteins in tissues or enzymes, carbohydrates and DNA. The action of reactive oxygen species on DNA may lead to DNA damage, whereas the effects on cellular membrane often involve the lipid component. The affected molecules are further degraded into a wide variety of products [4-6]. Oxidative damage associated with free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis [7]. An antioxidant is defined as any substance that when present at low concentrations compared to those of oxidizable substance, significantly delays or prevents oxidization [8]. Natural antioxidants have the ability to improve quality and stability of food, and also act as nutraceuticals, to terminate free radical chain reaction in biological systems, which in turns provide benefit to the health [9]. Free radical peroxidation of lipids in biomembranes is a major contributor to damage in cell and this often results in various pathologies. Organisms have evolved antioxidant systems to protect against free radicals and the first step includes enzymatic defenses, which involve presence of small molecule antioxidants. These are often chain breaking antioxidants and interfere in lipid peroxidation chain reaction by donating hydrogen atom for abstraction by lipid radicals. The extract of *M. malabathricum* flower has ability to scavenge free radicals [10] whereas leaf extract prevented hepatotoxic effect of reactive oxygen species [10-11] in addition to free radical scavenging effect. However, no comparison has been made between the radical scavenging ability of different parts of *M. malabathricum*.

The study was carried out to screen the phytochemicals present in leaf, flower, fruit or stem as well as to evaluate the antioxidant potentials, total phenolic contents and total flavonoid contents of different parts of *M. malabathricum*.

2.0 EXPERIMENTAL

2.1 Chemicals and Standards

Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, quercetin, Folin–Ciocalteu phenol reagent, sodium carbonate solution, methanol, ethanol, HCl, H_2SO_4, glacial acetic acid, iodine, zinc, ferric chloride and potassium iodide were purchased from Merck. (Germany).

2.2 Plant Materials

M. malabathricum were collected in August, 2014 from the health forest in Gong Badak, Terengganu, Malaysia. The voucher specimens (00244) was identified based on morphological characteristics [12-13] of leaves, fruits and stems using standard procedures by Norhaslinda Haron, Scientific Officer and deposited at Herbarium unit of Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (UniSZA).

2.3 Preparation of Extracts

Samples were washed with tap water and followed by distilled water and dried at 40°C for 72 hrs. The samples were ground into powder form, and each powdered sample was weighed and soaked in methanol at the ratio of 1:10 (w/v) for 72 hrs. Extracts were decanted and filtered through Whatman filter paper no. 1. The filtrate was then concentrated in a rotary evaporator at 40°C and reduced pressure. The extracts were dried at 40°C and kept in a freezer at 4°C for further use.

2.4 Determination of Percentage Yield (%)

The percentage yield of the extract was determined gravimetrically using the dry weight of extract (x) and soaked samples material (y) using this formula:

Percentage yield = (x/y) ×100

The extraction yield was calculated for each extract and results were presented as a percentage yield (%).

2.5 Preliminary Phytochemical Screening

Preliminary phytochemical screenings of extracts of different parts were carried out according to the established procedures [14-17].

2.6 Determination of Total Phenolic Content (TPC)

The total phenolic content of leaf, flower, stem and fruit of *M. malabathricum* extracts were determined according to the method as described earlier [18] with minor modifications. Extracts were diluted to 100 µg/mL concentration. The Folin-Ciocalteau reagent was also diluted at the ratio of 1:10 before use. 1.25 mL of Folin-Ciocalteau reagent was added to 0.25 mL extracts and incubated at room temperature for 2 min. I mL of sodium carbonate solution (75 g/L) was then added to the mixture and kept in a dark place for 30 min. The absorbances of the mixture were measured spectrophotometrically at 760 nm against a blank. The gallic acid aqueous solutions in the range of 2.5-160 µg/mL were used as standard.

2.7 Total Flavonoid Content

The flavonoid content of methanol extract of different parts of *M. malabathricum* was determined according to the previous method [19] with minor modifications. 0.25 mL aliquot of the diluted extract of 400 µg/mL of methanol solution of *M. malabathricum* was mixed with solution containing 50 µL of 10% aluminium chloride, 50 µL of 1 M aqueous potassium acetate and 2.15 mL of 95% ethanol. The mixtures were incubated at room temperature for 40 min; the absorbance was recorded spectrophotometrically at 415 nm. Total flavonoid was calculated using quercetin as a standard and expressed as mg/g from calibration curve.

2.8 Determination of DPPH Radical Scavenging Activity (Antioxidant)

DPPH radical scavenging activity of the different parts of *M. malabathricum* was estimated by adapting the method of Miser-Salihoglu and co-workers [20] with minor modification. A 20 µL of extract at different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625, 0 µg/mL), was added in 96 well micro plate and 200 µL of 0.1 mM solution of DPPH was added to the mixture in wells. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was measured at 517 nm using micro plate reader. Quercetin was used as standard while dimethylsulfoxide 20 µL served as blank negative control (reaction mixture without test extract). Each sample was assayed in triplicate and then mean values and SD were calculated. The percentage inhibition of free radicals was calculated. The IC_{50} values were calculated as the concentration of a test sample required to give 50% radical scavenging activity (DPPH), the result was compared with quercetin standard.

Percentage inhibition (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

3.0 RESULTS AND DISCUSSION

3.1 Percentage Yield of Extract

The extractable matter of methanol extract of each sample was presented on Table 1; the amount of each extract from the crude powdered drug was determined and presented. The percentage yield was calculated. The highest yield was associated with fruit followed by leaf then flower, the stem yield little amount of extractable matter and has the lowest percentage yield.

Table 1 Percentage yield of methanol extract of different parts of *M. Malabathricum*

Sample	Percentage yield (%) (w/w)
Leaf	13.39 ± 0.29
Flower	8.70 ± 1.59
Fruit	17.84 ± 1.41
Stem	3.33 ± 0.46

Mean ± S.D

3.2 Phytochemical Screening

The phytochemical screening of different parts of *M. malabathricum* showed that all parts of the plant contained tannins, steroids, phenols and flavonoids; three parts (flower, fruit and leaf) contained terpenoid and fixed oil; whereas alkaloid was present only in flower and leaf extract (Table 2). All parts tested negative for the presence of glycoside and saponins.

Table 2 Preliminary phytochemical screening of different parts of *M. malabathricum*

Test	Leaf	Flower	Fruit	Stem
Saponins	-	-	-	-
Tannins	+++	+++	++	+
Alkaloids	+	+	-	-
Steroids	++	+++	+	+
Phenol	+++	+++	++	+
Terpenoid	+	+	++	-
Fix oil	+	-	+	+
Flavonoids	+ +	+ +	+	+
Glycoside	-	-	-	-

Key: - = absent, + = present in small amount, ++ = present in moderate quantity,+++ = present in large quantity.

3.3 Total Phenolic Content

The present study investigates the phenolic content of different parts of *M. malabathricum*. The results for total phenol content of different parts of plant are presented in Table 3. The result was expressed as the Gallic acid equivalents per gram of the plant extract using equation Y = 0.0089X + 0.1798, R^2 = 0.9799. The methanol extract of flower was found to have the highest total phenolic content followed by leaf then fruit, whereas stem extract was found to have the lowest phenolic content. This finding supported previous research finding [2] that leaf has high phenolic content. The mean phenolic content of different parts of *M. malabathricum* is significantly different p< 0.05.

Table 3 Results of total phenolic content of different parts of *M. malabathricum*

Sample	Total phenol content (mg of GAE /g of sample)
Leaf	199.10 ± 11.09^{b}
Flower	431.69 ± 18.24^{c}
Fruit	21.57 ± 9.20^{a}
Stem	2.47 ±2.97^{a}

Data are expressed as mean ± SD (n = 3 in each locality); means were compared by Bonferroni test (P<0.05). The mean Phenolic Content of different parts of *M. malabathricum* is significantly different P< 0.05.

Data with different lower case letters on each part of the plant are significantly different (p<0.05). The mean phenolic content with identical alphabets show no significant difference (p <0.05).

3.4 Total Flavonoid Content

All part of *M. malabathricum* used in this study contained different amount of flavonoids compounds (p<0.05; Table 4). The leaf of *M. malabathricum* demonstrated the highest flavonoid content (p<0.05) than other parts of the plant (Table 4). The high content of the flavonoid in leaf of *M. malabathricum* was associated with the high level of anti-inflammatory and antioxidant activity [21].

Table 4 Total flavonoid content of different parts of *M. malabathricum*

Sample	TFC (mg of QE/ g of extract)
L	60.29 ± 3.33^{d}
FL	38.17 ± 5.36^{c}
FR	18.94 ± 1.93^{b}
S	1.63 ± 0. 97^{a}

Data are expressed as mean ± SD (n = 3 in each locality); means were compared by Bonferroni test (p<0.05). The mean flavonoid content of different parts of *M. malabathricum* is significantly different p< 0.05.

Data with different lower case letters on each part of the plant are significantly different (p<0.05). The mean flavonoid content with identical alphabets show no significant difference (p <0.05).

3.5 Antioxidant activity (DPPH Method)

The ability of methanol extracts of different parts of *M. malabathricum* to donate hydrogen and act as an antioxidant was studied using the DPPH radical scavenging method. The IC_{50} value of each extract was determined and the flower extract was found to have lowest concentration (48 μg/mL) to achieve IC_{50}. This value was comparable with quercetin. Flower extract of *M. malabathricum* showed the highest antioxidant activity (IC_{50} = 48 μg/mL; Table 5). The high phenolic content in flower extract may contribute to the antioxidant activity. This was followed by fruit (IC_{50} = 152 μg/mL), leaf (IC_{50} = 280 μg/mL) and stem (IC_{50} = 856 μg/mL). Furthermore, the flower exhibited the highest percentage inhibition of free radicals (86.06%), whereas stem has the lowest antioxidant activity (60.44%) as shown in Table 6. Additionally, the mean DPPH radical scavenging activities of different parts of *M. malabathricum* are significantly different at p< 0.05. Radical scavenging activity is the ability of a compound to scavenge free radical which in turn prevents their toxic effect to cell. The phenolic compound has the ability to scavenge free radical by donating hydrogen atom [9].

Table 5 Results of DPPH scavenging activity of different parts of *M. malabathricum* and standard Quercetin

Sample	IC_{50} (μg/mL)
Leaf	280
Flower	48
Fruit	152
Stem	856
Quercetin	48

Table 6 Percentage inhibition of DPPH free radical scavenging activity of extracts of different parts of *M. malabathricum*

Sample	Percentage DPPH free radical inhibition (%)
Leaf	80.12 ± 0.63[b]
Flower	86.06 ± 0.30[b]
Fruit	86.01 ± 0. 38[b]
Stem	60.44 ± 6.04[a]
Quercetin	86.92 ± 0. 17[b]

Data are expressed as mean ± SD (n = 3 in each locality); means were compared by Dunnett's C test ($p<0.05$). The mean DPPH radical scavenging activity of different parts of *M. malabathricum* is significantly different $p< 0.05$.

Data with different lower case letters on each part of the plant are significantly different ($p<0.05$). The mean DPPH radical scavenging activity with identical alphabets show no significant difference ($p <0.05$).

Overall, the flower part of *M. malabathricum* has the highest ability to scavenge free radicals than other parts, including the leaf. However the finding of this study is contrary to finding of earlier researchers [22], which showed that both methanol and dichloromethane extract of aerial part of *M. malabathricum* has no scavenging activity against DPPH free radicals. Although various studies have reported the antioxidant activity in either leaf [2, 23-24] or flowers [10], they have not compared different parts of *M. malabathricum*. The results of this study are quite similar with the study of Fernando and coworkers [25] who studied phenolic content and antioxidant activity of *Withania somnifera* (L.) Dunal. These researchers found that leaf had the highest phenolic content followed by fruit, stem and root. It is suggested that herbal practitioners should include the flower and possibly remove the stem part to improve the efficacy of *M. malabathricum* herbal preparations.

4.0 CONCLUSION

In conclusion, the total flavonoid contents of different parts of *M. malabathricum* are significantly different from each other with leaf having the highest content. An almost similar profile was shown by total phenolics, except that flower had the highest value. As for antioxidant capacity, all parts except stem had high activity. Moreover, the flower part of *M. malabathricum* which is underutilized in traditional herbal medicine has highest DPPH free radical scavenging activity (86.06%), which can be related to its higher phenolic content. The higher content of total phenolics, flavonoids and antioxidant capacity of flower suggests the possibility of its incorporation for development of newer effective *M. malabathricum* preparations.

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