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Research Article

PHYTOCHEMICAL ANALYSIS AND CONSTITUENTS OF HEXANE EXTRACT OF *MELASTOMA MALABATHRICUM* L.

Dil Prakash Giri[1], Meena Rajbhandari[2,*]

[1]*Department of Chemistry, Tri-Chandra Campus, Tribhuvan University*
[2]*Research Center for Applied Science and Technology, Tribhuvan University, Kathmandu*
[*]*Corresponding author: karmacharyameena@gmail.com*

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ABSTRACT

Hexane, dichloromethane, ethyl acetate, methanol and 50 % aqueous methanol extracts of leaf, flower and fruit of *Melastoma malabathricum* were screened for the presence of different classes of phytochemicals. In phytochemical screening, ethyl acetate, methanol and 50 % aqueous methanol extracts showed the presence of phenolics and flavonoids. Glycosides were found only in the 50 % methanol extract of fruit. Free radical scavenging activity of methanol extract of leaf, flower and fruit was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical. Total phenolic content was measured spectrophotometrically using Folin-Ciocalteu, total flavonoids content by using aluminum chloride and total sugar content by using anthrone reagent. Gallic acid was used as a standard for the calibration of phenolics, catechin for flavonoids and glucose for carbohydrates. The highest amount of phenolic was found in the 50 % methanol extract of leaf (242.88 ± 3.52 mg GAE/g extract), the highest amount of flavonoids was found in the ethyl acetate extract of leaf (127.41 ± 6.33 mg CE/g extract). Total sugar in 50 % methanol extract of fruit was found to be 151.12 ± 8.79 mg GE/g extract. Methanol extracts of flower showed the strongest antioxidant activity with IC_{50} value of 17.23 µg/ml. Silyl derivative of hexane extracts were prepared by using *N*-methyl-*N*-(trimetylsilyl) trifluoroacetamide for gas chromatography-mass spectroscopic (GC-MS) analysis. The GC-MS analysis of hexane extract allowed the identification of 17 phyto-constituents by comparing the fragmentation pattern of each component in mass spectrum with the standard NIST mass spectral database.

Keywords: Antioxidant, GC-MS, Total flavonoid, Total phenolic, Total sugar, Silylation

INTRODUCTION

Medicinal plants are one of our natural living treasures and their use in primary health care is a substantial benefit to all population. However, the use of medicinal plant is only based on empirical grounds. Hence, there is a need for scientific validation of such empirical knowledge. A large number of natural products, especially plant-derived drugs, continue to be discovered on the basis of traditional or empirical local medical practices. Medicinal plants are the sources of diverse chemical compounds exhibiting biological properties that were the target of several studies. It is well known that phenolics are associated with prevention of diseases assumed to be induced by oxidative stress (Halliwell & Gutteridge 1990). Natural products derived from plants have played an important role in discovery of therapeutic drugs in areas such as oncology, immune-suppression and metabolic diseases as there is still a need of lead compounds progressing into clinical trials (Farnsworth *et al.* 1985). However, information on medicinal properties of endemic plants is rather scarce, limited to certain regions and known only by local populations. Therefore, the assessment of such properties remains an interesting and necessary, particularly to discover new promising sources of drugs.

Melastoma malabathricum commonly known as Indian Rhododendron and locally known as *Angeri* or *Thulo chulesi* in Nepali, is a medicinal plant distributed in eastern and central part of Nepal at 200-1600 m above mean sea level in open or shady places. The juice of this plant is used to treat cough and cold in Nepalese traditional medicine and the fruits are used as a source of purple dye (Manandhar 2002). The whole plant is used as herbal medicine in some other Asian countries like India, Malaysia, Indonesia and Singapore and has been claimed to possess various medicinal values based on practices and traditional beliefs of different communities and tribes (Joffry *et al.* 2012).

Detailed literature review indicated that various classes of phytochemicals have been identified in different parts of *M. malabathricum* such as fatty acids and aliphatic constituents (Dinda & Saha 1986a, Dinda & Saha 1986b, Dass & Kotoky 1988), sterols (Manzoor-I-Khuda *et al.* 1981), phenolic acids (Lowry 1968), flavonoids and their glycosides (Dinda & Saha 1988, Mohandoss & Ravindran 1993, Wong *et al.* 2012), tannins (Yoshida *et al* 1992a, 1992b), anthocyanins (Lowry 1976), amino acids (Dinda & Saha 1985) and so on. Different parts of the plant have been shown to exert diverse biological activities like antibacterial and antifungal (Grosvenor *et al.* 1995, Wiart *et al.* 2004, Johnny *et al.* 2010, Maji *et al.* 2010), cytotoxic (Susanti *et al.* 2007) and antiviral (Nazlina *et al.* 2008, Lohezic-Le Devehat *et al.* 2002), antiparasitic (Alen

et al. 2000), antioxidant (Susanti *et al.* 2007), anticoagulant (Manicam *et al.* 2010), platelet-activating factor inhibitory (Jantan *et al.* 2005), wound healing (Sunilson *et al.* 2008), anti-ulcer (Hussain *et al.* 2008), anti-diarrheal (Sunilson *et al.* 2009), anti-nociceptive (Sulaiman *et al.* 2004), and anti-pyretic (Zakaria *et al.* 2006) at different concentrations.

Although different types of polar bioactive constituents like phenolics, falvonoids, tannins and their glycosides have been reported from the different parts of the plant, their total content have not been reported elsewhere. The antioxidant activity has been reported but the method adopted was different. The chemical constituents of hexane extract have not been studied by GC-MS. Therefore, the present study was carried out to estimate the total content of phenolic, flavonoid and sugar in different extracts and their antioxidant activity by DPPH method and the determination of chemical constituents of hexane extract by GC-MS technique. This is the first attempt to investigate the constituents of hexane extract by GC-MS.

MATERIALS AND METHODS

Plant materials

The plant was collected from Nagarjun forest at an altitude between 1100 to 1400 m above mean sea level in June 2016 and was identified by comparison with the herbarium species deposited at Central Department of Botany, Tribhuvan University. Voucher specimen (MM-16-DG) was deposited at Research Centre of Applied Science & Technology (RECAST), Tribhuvan University.

Solvents and chemicals

DPPH and authentic (±)-catechin were purchased from Sigma Chemical Company, USA. Gallic acid was purchased from Merck, Darmstadt, Germany. Aluminum chloride reagent (SD fine-chemicals), Folin-Ciocalteu reagent (SD fine-chemicals) and anthrone (Thomas baker) were purchased from local vendor. All other chemicals and solvents were of analytical grade and purchased from local vendors. Absorbance was measured using Chemito UV-VIS Spectrophotometer. GC-MS analysis was carried out using Agilent Gas Chromatograph G1530 N fitted with mass selective detector G2588 A.

2.3. Extraction

Dried and powdered leaves, flowers and fruits (each 50 g) were extracted successively with hexane (200 ml, 5 times, 18 h), dichloromethane (200 ml, 5 times, 18 h), ethyl acetate (150 ml, 4 times, 18 h) and methanol (150 ml, 5 times, 18 h) by cold percolation. The residue after extraction with methanol was refluxed with 50% aqueous methanol (250 ml, 4 h). The extracts were filtered and the solvent was evaporated under reduced pressure in a Rotavapor to get respective crude extracts. The dried extracts were stored at -20 ºC for further use.

Phytochemical screening

The freshly prepared crude extracts were subjected to chemical tests to identify various classes of chemical compounds present in the extracts using standard procedures (Culie 1982).

Determination of total phenolic content

The total phenolic content in plant extracts was determined by using Folin-Ciocalteu colourimetric method based on oxidation-reduction reaction (Waterhouse 2002). Various concentrations of gallic acid solutions in methanol (10, 25, 50, 75 and 100 µg/ml) were prepared to make the standard solution. In a 20 ml test tube, 1 ml gallic acid of each concentration was added and to that 5 ml Folin-Ciocalteu reagent (10 %) and 4 ml 7 % Na_2CO_3 were added to get a final volume of 10 ml. The blue coloured mixture was shaken well and incubated for 30 minutes at 40 °C in a water bath. The absorbance was measured at 760 nm against blank. All the experiments were carried out in triplicate. The average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve. Various concentrations of the extracts (200, 100, 50 and 25 µg/ml) were prepared. Following the procedure described for standard, absorbance for each concentration of the extract was recorded. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram dry extract (mg/g).

Determination of total flavonoid content

The total flavonoid content was determined by aluminum chloride colorimetric assay (Zhishen *et al.* 1999). Various concentrations of standard catechin (10, 25, 50, 75 and 100 µg/ml) were prepared. An aliquot of 1 ml catechin of each concentration in methanol was added to a 10 ml test tube containing 4 ml double distilled water. At the zero time, 0.3 ml 5 % sodium nitrite was added, after 5 min, 0.3 ml of 10 % $AlCl_3$ was added and at 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the total volume of the mixture was made up to 10 ml by the addition of 2.4 ml double distilled water and mixed thoroughly. Absorbance of the pink coloured mixture was determined at 510 nm versus a blank containing all reagents except catechin. The average absorbance values obtained at different concentrations of catechin were used to plot the calibration curve. Various concentrations of the extracts (200, 100, 50 and 25 µg/ml) were prepared. Following the procedure described for standard, absorbance for each concentration of extract was recorded. Total flavonoid content of the extracts was expressed as mg catechin equivalents (CE) per gram of dry extract (mg/g).

Determination of total sugar content

The total sugar content in plant extracts was estimated by using anthrone reagents based colorimetric method (Hedge & Hofreiter 1962). Various concentrations of D-glucose (10, 25, 50 and 75 µg/ml) were prepared. An aliquot of 2 ml glucose of each concentration and 8 ml of freshly prepared anthrone reagent (200 mg of anthrone in 100 ml of ice-cold 95 % H_2SO_4) was mixed in 15 ml test tube. The mixture was shaken well and heated for 8 minutes at boiling water bath. Then cooled rapidly and the absorbance of green colour solution was measured at 630 nm against blank containing all reagents except sugar. All the experiments were carried out in triplicate. The absorbance values obtained are used separately to plot calibration curve.

The amount of 100 mg of each extract was dissolved in methanol followed by addition of 5 ml of 2.5 N HCl and subjected to hydrolysis by keeping it in a boiling water bath for 3 hrs. It was then cooled and neutralized with solid sodium carbonate and made the volume to 100 ml (1 mg/ml) and centrifuged. Serial dilution of supernatant was carried out to get the concentration of 100, 200, 400 and 600 µg/ml and Anthrone reagent was added to these diluted solutions, followed by heating for 8 minutes and absorbance was measured at 630 nm against blank

Calculation

The total phenolic, flavonoid and sugar contents were calculated in all the extracts separately using equation (1).

$$C = \frac{cV}{m} \qquad (1)$$

Where, C = total content of flavonoid/phenol/sugar compounds (mg/g), c = concentration of gallic acid/(±)-catechin/D-glucose established from the calibration curve in mg/ml, V = volume of extract (ml) and m = weight of plant extract.

Statistical analysis

Data were recorded as mean (±) standard deviation of three determinations of absorbance for each concentration, from which linear correlation coefficient (R^2) value was calculated using MS Office Excel 2007. The linear regression equation for a straight line is, y = mx + c where, y = absorbance of extract, m = slope of the calibration curve, x = concentration of extract, c = intercept. Using this regression equation, concentrations of extracts were calculated. From the calculated values of concentration of each extract, the total phenolics, flavonoid and sugar content were calculated.

Determination of antioxidant activities

Antioxidant activity of the methanol extracts of leaf, flower and fruit was assessed using DPPH free radical (Brand-Williams *et al.* 1995). DPPH solution (0.1 mM) was prepared by dissolving 3.9 mg of DPPH in 100 ml methanol and stirred overnight at 4 °C. Thus, prepared purple color DPPH free radical solution was stored at –20 °C for further use.

Three different concentrations (5, 10 and 15 µg/ml) of methanolic solutions of each extract were prepared by the serial dilution of the stock solution of the respective extract. To each 0.5 ml extract solution, 2.5 ml 0.1 mM methanolic DPPH solution was added. A control was prepared by mixing 0.5 ml methanol and 2.5 ml 0.1 mM methanolic DPPH solution. These samples were shaken well and kept in dark for 30 min at room temperature. The absorbance was measured at 517 nm against the blank solution consisting of MeOH. The radical scavenging activity was expressed as the radical scavenging percentage and calculated using the equation (2) where; A_s = absorbance of sample solution, A_b= absorbance of blank and A_c = absorbance of control.

$$\%\,\text{scavenging} = \frac{A_c - (A_s - A_b)}{A_c} \times 100 \qquad (2)$$

IC_{50} value is the concentration of sample required to scavenge 50 % of DPPH free radical and was calculated from the plotted graph of radical scavenging activity against the concentration of extracts.

Preparation of silyl derivatives and GC-MS analysis

Hexane extracts of leaf, flowers and fruit (1 mg each) were subjected to silylation for GC by treating with 80 µl of *N*-methyl-*N*-(trimetylsilyl) trifluoroacetamide (MSTFA) at 37 °C for 30 minutes. The reaction mixture was cooled to room temperature and used for analysis. For GC measurement, 8 µl of retention time standard mixture [0.5 % (m/v) *n*-decane, *n*-dodecane, *n*-pentadecane, eicosane, *n*-octacosane, *n*-dotriacontane dissolved in hexane] was added to the samples. Analytical GC-MS of the silyl derivatives of hexane extracts of leaf, flower and fruit were carried out on Agilent Gas Chromatograph G1530 N fitted with mass selective detector G2588 A connected with DB-5 (30 m x 0.25 mm x 0.25 µm) column (J&W Scientific, Folsom, USA). Injection was performed with an Agilent G2613 A series injector. The injection port temperature was kept at 230 °C. Diluted sample (2 µl) was injected into the injection port in a split ratio 25:1. The MS Transfer Line Temperature was kept at 250 °C. Helium was used as a carrier gas with flow rate of 1.0 mL/min. The MS was operated in the electron impact mode with ionization energy of 70 eV. The oven programming was 1 min at 70°C, increasing by 1.5 °C/min to 76 °C and then by 5 °C/min to 330 °C, followed by a 10 min isothermal cool-down to 70 °C. The total run time was 65.8 minutes. Full scan mass spectra were acquired from 35 to 573 m/z with scan rate of 2.74/s. The detected compounds were identified by processing of the

raw GC/MS data with ChemStation G1701CA software and comparing with the NIST mass spectral database 2.0 d (National Institute of Standards and Technology, Gaithersburg USA) and from retention times and mass spectra of added standard compounds.

RESULTS AND DISCUSSION

Extractive values of different extracts

The plant material (each 50 g) was successively extracted with hexane, dichloromethane, ethylacetate and methanol. The residue was refluxed with 50 % aqueous methanol to get the respective extracts. The highest amount of extract was obtained with methanol and the lowest amount was obtained with ethyl acetate. The results of the yield of the different extracts are shown in Table 1.

Phytochemical screening

The phytochemical screening of hexane, dichloromethane, ethyl acetate, methanol and 50 % methanol extracts of leaf, flower and fruits indicated that phenolics and flavonoids are present in all the polar fractions. Alkaloids and reducing sugars are absent in all the extracts while quinones are present in all the extracts. Terpenoids are present only in the hexane extract of leaf and flower as well as ethyl extract of leaf and fruit, glycoside is present only in the 50 % methanol extract of fruit and saponins are present in the methanol extract of leaf and flower and 50 % methanol extract of fruit. The results are shown in Table 2.

Estimation of total phenolic content

A simple and reproducible Folin-Ciocalteu (FC) method was applied for the determination of total phenolic content using gallic acid as a standard though there was possibility of interference from other readily oxidizable compounds present in plant extracts. The absorbance values obtained at different concentrations of gallic acid was used for the construction of calibration curve. FC method is based on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybedic/phosphotungstic acid complexes to form blue colour complexes, $(PMoW^{11}O_{40})^{-4}$, which are determined spectrophotometrically at 760 nm. The total phenolic content in different extracts were calculated from the calibration curve using regression equation $Y = 0.013x$, $R^2 = 0.999$ followed by the formula $C=cV/m$ and expressed as mg gallic acid equivalent (GAE) per g of extract in dry weight (mg/g). The results indicated that the highest amount of phenolic was detected in the 50 % methanol extract of leaf (242.88±3.52 mg GAE/g extract) while the lowest amount was found in the ethyl acetate extract of leaf (145.67±5.40 mg GAE/g extract). The results are shown in Table 3.

Table 1. Extractive values of different extracts of obtained from 50 g of dried plant materials

Parts of *M. malbathricum*	Yield of Different Extract (g)				
	Hexane	CH_2Cl_2	EtOAC	MeOH	50 % MeOH
Leaf	1.05	0.46	0.21	2.75	1.20
Flower	0.28	0.53	0.35	1.12	0.65
Fruit	0.20	0.513	0.17	3.32	0.41

Table 2. Results of phytochemical screening of different extracts of *M. malabathricum*

Extracts	Hexane			CH_2Cl_2			EtOAc			MeOH			50% MeOH		
	Lf	Fl	Fr	Lf	Fl	Fr	Lf	Fl	Fr	Lf	Fl	Fr	Lf	Fl	Fr
Phenolics	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Tannins	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Reducing sugars	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinones	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+

Lf-Leaf, Fl-Flower, Fr-Fruit, (+) indicates present and (–) indicates absent

Estimation of total flavonoid content

The total flavonoid content in different extracts was estimated by aluminium chloride colorimetric assay using (±)-catechin as a standard. The absorbance values obtained at different concentrations of catechin was used for the construction of calibration curve. The principle involved in aluminium chloride ($AlCl_3$) colorimetric method is that $AlCl_3$ forms acid stable complexes with either keto groups and/or group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups of the flavonoids which give

pink colour in alkaline medium the intensity of which was measured spectrophotometerically. The total flavonoid content in different extracts was calculated from the calibration curve using regression equation Y= 0.002x, R^2 = 0.995 followed by the formula C= cV/m and expressed as mg (±)-catechin equivalent (CE) per g of extract in dry weight (mg/g). The results of this investigation indicated that the highest amount of flavonoid was detected in the ethyl acetate extract of the leaf (127.41±6.33 CE/g) while the lowest amount was detected in the 50% methanol extract of the fruit (29.12±2.03 CE/g extract). The results are shown in Table 3.

Estimation of total sugar content

Anthrone reagent based colorimetric assay using D-glucose as a standard was used to determine the total sugar content in plant extracts. Plant extracts containing sugars were first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural that with anthronale, an enol form of anthrone forms a green coloured product with an absorption maximum at 630 nm which was measured against blank consisting of anthrone solution and distilled water. The absorbance values obtained at different concentrations of glucose was used for the construction of calibration curve. The total sugar content in different extracts were calculated from the calibration curve using regression equation Y = 0.013x, R^2 = 0.994 followed by the formula C = cV/m and expressed as mg glucose equivalents (GE) per g of extract in dry weight (mg/g). The total sugar was content in 50 % aqueous methanol extract was found to be (151.12 ± 8.79 GE/g). The result is shown in Table 3.

Table 3. Total phenolic, flavonoid, sugar and antioxidant activity of different extracts

Analysis	Parts	EtOAc extract	MeOH extract	50 % MeOH extract
Total phenolic content (mg GAE/g extract)	Leaf	145.67±5.40	227.04±5.06	242.88±3.52
	Flower	186.49±5.37	179.14±3.09	163.59±5.93
	Fruit	166.30±5.10	222.06±4.84	232.56±4.61
Total flavonoid content (mg CE/g extract)	Leaf	127.41±6.33	125.66±6.59	89.92±6.15
	Flower	98.14±2.32	93.28±1.91	111.11±1.76
	Fruit	115.64±5.70	102.72±5.98	29.12±2.03
Total sugar (mg GE/g extract)	Fruit	-	-	151.12±8.79
DPPH IC_{50} (µg/ml)	Leaf	-	19.19	-
	Flower	-	17.23	-
	Fruit	-	31.12	-

Determination of antioxidant activity

The DPPH assay was carried out only for the methanol extracts of leaf, flower and fruit. The absorbance values were measured at wavelength 517 nm for different concentrations of extracts and the control. These values were used to calculate the percentage inhibitions of DPPH radicals against the samples. The IC_{50} values were calculated from the plotted graph of radical scavenging activity against the concentration of extracts. The IC_{50} values in DPPH assay of methanol extracts of leaf, flower and fruit were are given in Table 3.The results indicated that all the tested extracts showed strong radical scavenging capacity. The lowest IC_{50} value was showed by the flower extract (17.23 µg/ml) followed by leaf extract (19.19 µg/ml) and fruit extract (31.12 µg/ml).

GC-MS analysis of hexane extract

The chemical composition of hexane extract of *M. malabathricum* was determined by GC-MS technique. The extracts were silylated with *N*-methyl-*N*-(trimetylsilyl) trifluoroacetamide which enhance GC performance of the samples by increasing the volatility and decreasing the surface adsorption. The silyl derivatives are formed by displacement of active hydrogen on -OH, -SH and -NH groups. Compounds containing active hydrogen atoms amendable to silylation are acids, alcohols, thiols, amines, amides, and enolizable ketones and aldehydes. Their silyl derivatives generally are more volatile, less polar, and thermally more stable. MSTFA (N-Methyl-N-trimethylsilylfluoroacetamide) is an important TMS reagent used in preparation of volatile and thermally stable derivatives for GC and MS analysis. The GC-MS analysis of the silyl derivative of hexane extract allowed the identification of 17 phyto-constituents on the basis of fragmentation pattern of each component in mass spectrum and comparing with the standard NIST mass spectral database.

A part of overlapped gas chromatogram of leaf, flower and fruit are shown in Fig. 1 with retention time ranged from 45-55 min. The overlapped gas chromatogram of all the three extracts showed somewhat similarity except the percentage of individual components. Therefore, only the composition of leaf extract was analyzed quantitatively by mass spectrometry.

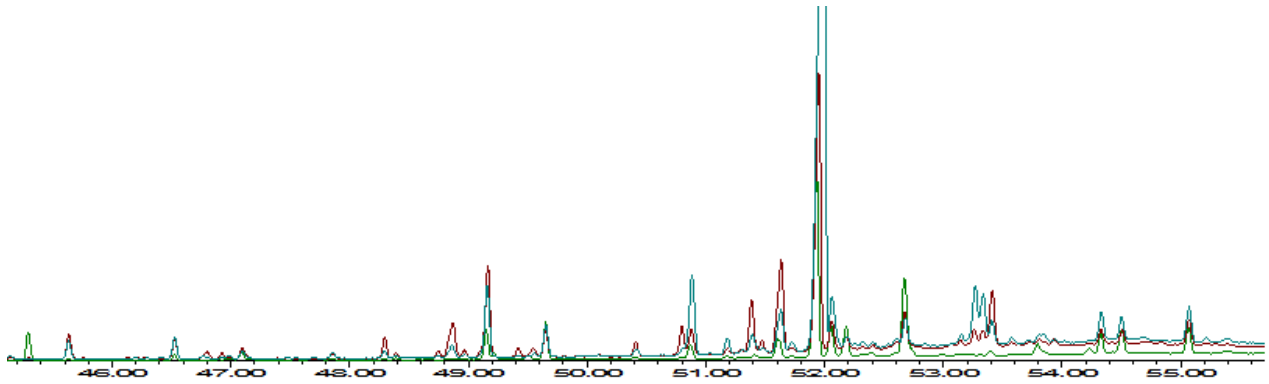

Fig. 1. Overlapped gas chromatogram of leaf (green), flower (red) and fruit (blue)

Relative amount of detected compounds were calculated on the basis of GC peak areas. The major constituents were *β*-sitosterol, *β*-amyrin, linoleic acid 2,3-bis-(O-TMS)-propyl ester, *α*-linolenic acid and palmitic acid. The identified compounds, their retention times and percentages are listed in Table 4

Table 4. Constituents of hexane extract of *M. malabathricum* leaf

Retention Time (min.)	Compounds	GC %
11.17	bis(trimethylsilyl) oxalate	1.17
15.21	Trimethylsilyl ether of glycerol	2.67
32.90	Palmitic acid, trimethylsilyl ester	5.18
35.86	Linoleic acid trimethylsilyl ester	2.97
35.97	*α*-Linolenic acid trimethylsilyl ester	5.61
36.46	Stearic acid trimethylsilyl ester	1.24
45.29	Squalene	2.89
49.15	Tetratriacontane	3.11
49.65	trimethyl(octacosyloxy)silane	3.78
50.87	Campesterol trimethylsilane	1.71
51.61	Heptacosane	2.76
51.93	*β*-Sitosterol trimethylsilyl ether	21.69
52.05	trimethyl(triacontyloxy)silane	3.81
52.68	*β*-Amyrin trimethylsilyl ether	14.47
53.79	Linoleic acid 2,3-bis-(O-TMS)-propyl ester	7.57
55.06	Betulin	3.16
56.51	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	1.44

CONCLUSION

Analyses of total phenolic, flavonoid contents and DPPH free radical scavenging activity showed that the ethyl acetate, methanol and 50 % aqueous methanol extracts of *M. malabathricum* leaf, flower and fruit are the greatest source of phenolic and flavonoid compounds with strong antioxidant activity. The 50 % aqueous methanol extract of the fruit contained carbohydrates. The finding of high amount of phenolics, falvonoids and strong antioxidant activity has supported the literature reports where various kinds of phenolics have been identified. Phenolic compounds possess a diverse range of beneficial biological activities, which contribute to their potent effects on inhibiting carcinogenesis. The presence of phytoconstituents like *β*-amyrin, and fatty acids indicated that the hexane extract could be a good resource of bioactive compounds.

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REFERENCES

Alen, Y., Nakajima, S., Nitoda, T., Baba, N., Kanzaki, H. and Kawazu, K. 2000. Antinematodal activity of some tropical rainforest plants against the pinewood nematode *Bursaphelenchus xylophilus. Zeitschrift fur Naturforschung* C **55**: 295-299.

Brand-Williams, W., Cuveiler, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Food Science and Technol*ogy **28**: 25-30.

Culie, I. 1982. Methology for analysis of vegetable drugs, Practical manuals on industrial utilization of medicinal and aromatic plant, Bucharest. *Phytochemistry* **63**: 97-104

Dass, K.K. and Kotoky, J.J. 1988. A new aliphatic constituent of *Melastoma malabathricum* Linn. *Journal of the Indian Chemical Society* **65**: 385-386

Dinda, B. and Saha, M.K. 1985. Free amino acids of *Melastomam abathricum. Journal of the Indian Chemical Society* **62**: 789-790.

Dinda, B. and Saha, M.K. 1986a. Fatty acid sterols from *Melastoma malabathricum. Journal of the Indian Chemical Society* **63**: 144-145.

Dinda, B. and Saha, M.K. 1986b. Aliphatic compounds from *Melastoma malabathricum*. *Journal of the Indian Chemical Society* **63**: 764-766.

Dinda, B. and Saha, M.K. 1988. A flavonol-diglycoside from *Melastoma malabathricum. Journal of the Indian Chemical Society* **65**: 209-211.

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D. and Guo, Z. 1985. *Bulletin of WHO* **63:** 965-981.

Grosvenor, P.W., Supriono, A. and Gray, D.O. 1995. Medicinal plants from Riau province, Sumatra, Indonesia. Part 2: antibacterial and antifungal activity. *Journal of Ethnopharmacology* **45**: 97-111.

Halliwell, B. and Gutteridge, J.M.C. 1990. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in Enzymology* **186**: 1-85.

Hedge, J.E. and Hofreiter, B.T. 1962. In: *Carbohydrate Chemistry*. Whistler R. L.; BeMiller J. N., Academic Press, New York, pp. 420.

Hussain, F., Abdulla, M.A., Noor, S.M., Ismail, S. and Ali, H.M. 2008. Gastroprotective effects of *Melastoma malabathricum* aqueous leaf extract against ethanol-induced gastric ulcer in rats. *American Journal of Biochemistry and Biotechnology* **4**: 438-441.

Jantan, I., Rafi, I.A.A. and Jalil, J. 2005. Platelet-activating factor (PAF) receptor-binding antagonist activity of Malaysian medicinal plants. *Phytomedicine* **12**: 88-92.

Joffry, S.M, Yob, N.J., Rofiee, M.S., Meor Mohd. Affandi, M.M.R., Suhaili, Z., Othman, F., Md. Akim, A., Desa, M. N.M. and Zakaria, Z.A. 2012. *Melastoma malabathricum* (L.) Smith ethnomedicinal uses, chemical constituents, and pharmacological properties: *A Review Evidence-Based Complementary and Alternative Medicine* **2012**: 258434. doi: 10.1155/2012/258434

Johnny, L., Yusuf, U.K. and Nulit, R. 2010. The effect of herbal plant extracts on the growth and sporulation of *Colletotrichum gloeosporioides*. *Journal of Applied Biosciences* **34**: 2218-2224.

Mazura, M.P., Susanti, D. and Rasadah, M.A. 2007. Anti-inflammatory action of components from *Melastoma malabathricum*. *Pharmaceutical Biology* **45**: 372-375.

Lohezic-Le Devehat, F., Bakhtiar, A., Bezivin, C., Amoros, M. and Boustie, J. 2002. Antiviral and cytotoxic activities of some Indonesian plants. *Fitoterapia* **73**: 400-405.

Lowry, J.B. 1968. The distribution and potential taxonomic value of alkylated ellagic acids. *Phytochemistry* **7**: 1803-1813.

Lowry, J.B. 1976. Anthocyanins of the melastomataceae, myrtaceae and some allied families. *Phytochemistry* **15**: 513-516.

Manicam, J.O., Abdullah, E.R., Mohd Tohit, Z., Seman, C., Sieo, C. and Hamid, M. 2010. In vitro anticoagulant activities of *Melastoma malabathricum* Linn. aqueous leaf extract: a preliminary novel finding. *Journal of Medicinal Plants Research* **4**: 1464-1472.

Maji, S., Dandapat, P. and Ojha, D. 2010. In vitro antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated human pathogens. *Journal of Phytology* **2**: 57-64.

Mohandoss, S. and Ravindran, P. 1993. Flavonoids from *Melastoma melabathricum. Fitoterapia* **64**: 277-278.

Manaandhar, N.P. 2002. Plants and people of Nepal. Timber Press, Portland, Oregon, USA.

Manzoor-I-Khuda, M., Chowdhury, S.A., Reza, T. and Chowdhury, A.K. 1981. Chemical Investigation on *Melastoma Malabathricum*. Part 1: Isolation of melastomic acid and beta- sitosterol from the roots. *Journal of the Bangladesh Academy of Sciences* **5:** 55-59.

Nazlina,I.; Norha, S., Noor Zarina, A.W. and Ahmad, I.B. 2008. Cytotoxicity and antiviral activity of *Melastoma malabathricum* extracts. *Malaysian Journal of Applied Biology* **37**: 53-55.

Sunilson, J.A.J., Anandarajagopal, K., Kumari, A.V.A.G. and Mohan, S. 2009. Antidiarrhoeal activity of leaves of *Melastoma malabathricum* Linn. *Indian Journal of Pharmaceutical Sciences* **71**: 691-695.

Sunilson, J.A.J., James, J., Thomas, J., Jayaraj, P., Varatharajan, R. and Muthappan, M. 2008. Antibacterial and wound healing activities of *Melastoma malabathricum* Linn. *African Journal of Infectious Disease* **2**: 68-73.

Sulaiman, M.R., Somchit, M.N., Israf, D.A., Ahmad, Z. and Moin, S. 2004. Antinociceptive effect of *Melastoma malabathricum* ethanolic extract in mice. *Fitoterapia* **75**: 667-672.

Susanti, D., Sirat, H., Ahmad, M F., Ali, R.M.; Aimi, N. and Kitajima, M. 2007. Antioxidant and cytotoxic flavonoids from the flower of *Melastoma malabathricum* L. *Food Chemistry* **103**: 710-716.

Waterhouse, A. 2002. Determination of total phenolics. In: *Current Protocols in Food Analytical Chemistry* (Ed Wrolstad, R.E). Units I1.1.1-I1.1.8, John Wiley and Sons, New York.

Wiart, C., Mogana, S. and Khalifah, S. 2004. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia* **75**: 68-73.

Wong, K.C., Hag Ali, D.M. and Boey, P.L. 2012. Chemical constituents and antibacterial activity of *Melastoma malabathricum* L. *Natural Products Research* **26**: 609-618.

Yoshida, T., Nakata, F., Hosotani, K., Nitta, A. and Okuda, T. 1992a. Dimeric hydrolysable tannins from *Melastoma malabathricum.* *Phtochemistry* **31**: 2829-2833.

Yoshida, T., Nakata, F., Hosotani, K., Nitta, A. and Okuda, T. 1992b. Tannins and related polyphenols of melastomataceous plants. V. Three new complex tannins from *Melastoma mala bathricum* L. *Chemical and Pharmaceutical Bulletin* **40**(7): 1727-1732.

Zakaria, Z.A., Raden, Mohd. Nur, R.N.S. and Hanan Kumar, G. 2006. Antinociceptive, anti-inflammatory and antipyretic properties of *Melastoma malabathricum* leaves aqueous extract in experimental animals. *Canadian Journal of Physiology and Pharmacology* **84**:1291-1299.

Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* **64**: 555-559.