BIOACTIVE CONSTITUENTS FROM THE LEAVES OF *MELASTOMA MALABATHRICUM* L.

Deny Susanti[1*], Hasnah M. Sirat[2], Farediah Ahmad[3], Rasadah Mat Ali[3]

[1]Department of Biomedical Science, Faculty of Science, International Islamic University Malaysia, 25200 Kuantan, Malaysia.
[2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia
[3]Medicinal Plant Division, Forest Research Institute Malaysia, 52109 Kepong, Kuala Lumpur, Malaysia

*e-mail: deny@iiu.edu.my

ABSTRACT

Phytochemical and bioactivity studies of the leaves of *Melastoma malabathricum* L. (Melastomataceae) have been investigated. The *n*-hexane extract yielded α-amyrin, patriscabatrine and auranamide, ethyl acetate extract gave quercetin and quercitrin, and methanol extract gave quercitrin and kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)glucoside. The crude extracts and isolated compounds were screened for their antioxidant and cytotoxic activities. The antioxidant assay was carried out by FTC and DPPH radical scavenging method. Kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)-glucoside, quercetin and quercitrin showed strong activities with inhibition more than 90% in the FTC method. Quercetin was found to be the most active as radical scavenger in DPPH method with IC_{50} of 0.69 μM. α-Amyrin and kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)glucoside demonstrated the strongest activities in the anti-inflammatory assay of TPA mouse ear oedema with IC_{50} of 0.11 and 0.34 mM/ear, respectively.

Keywords: Antiinflammatory, Antioxidant, Flavonoids, Melastomataceae, Melastoma malabathricum, Terpenoid

INTRODUCTION

M. malabathricum L. (senduduk) is a very common herb or shrub found throughout the tropic in the moist part mostly from India, Thailand and Malaysia, where it grow as small trees 12-13 ft. high, occasionally even up to 20 ft. (Burkill, 1966). This species has at least three varieties, i.e. large, medium and small size flower with dark purple-magenta petals, light pink-magenta petals and the rare variety white petals as reported by Corner, 1951.

The chemistry of Melastomataceae is poorly known. Previous studies of this family have characterized hydrolysable tannin. Several hydrolysable tannins have been isolated from the dry leaves of *M. malabathricum* with light pink-magenta petals. The main tannin was oligomers named nobotanin B, dimmers named malabathrins B, malabathrins C and malabathrins D, monomers named 1,4,6-tri-*O*-galloyl-□-D-glucoside, 1,2,4,6-tetra-*O*-galloyl-β-D-glucoside, strictinin, casuarictin, pedunculagin, nobotanin D, pterocarinin and oligomers nobotanin G, nobotanin H, nobotanin J (Yoshida et al., 1992), β-sitosterol, α–amyrin, uvaol, sitosterol-3-*O*-β-D-glucopiranoside, quercetin, quercitrin and rutin (Nuresti et al., 2003). To our knowledge this is the only reported chemical composition of *Melastoma* with light pink-magenta petals reported so far.

METHODS

Plant Material. *Melastoma malabathricum* was collected from Johor, Malaysia in 2002 and identified by Dr. Rusdi Tamin of Universitas Andalas, Padang, Indonesia. A voucher specimen (YANTI-2061) was deposited the Herbarium of Universitas Andalas (ANDA) Padang, Indonesia.

General methods. Mps. (uncorr.) were determined using Leica Gallen III apparatus. UV was recorded on Shimadzu UV-160 spectrophotometer in methanol. IR spectra were recorded on Perkin Elmer 1650 FTIR spectrophotometer. NMR spectra were recorded on JEOL JNH A500 Spectrometer measured at 500 MHz and 125 MHz, respectively. Vacuum liquid chromatography (VLC) and column chromatography (CC) were carried out using silica gel 230-400 mesh, Merck 9385 and 70-230 mesh, Merck 7734. Sephadex LH-20 was purchased from Pharmacia (Uppsala, Sweden). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was from Fluka (Switzerland), vitamin E and vitamin C were purchase from Merck (Darmstadt, Germany), 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was from Sigma Aldrich (St. Louis, MO, USA).

Extraction and Isolation. The air-dried leaves of *M*. *malabathricum* (2.09 kg) of white petals was successively extracted with *n*-hexane, EtOAc and MeOH. The chromatographic separations by vacuum liquid chromatography (VLC) and column chromatography (CC) on silica gel of the *n*-hexane and ethyl acetate extracts have afforded **1** - **5**. Purification of the MeOH extract by VLC and CC has resulted in the isolation of **5** and **6**. The structures of **1** - **6** were elucidates as α-amyrin, patriscabratine, auranamide, quercetin, quercitrin and kaempferol-3-*O*-(2”,6”-di-*O*-*p*-*trans*-coumaroyl)-β-glucoside by interpretation of the spectral data (IR, FABMS, 1D and 2D NMR) as well as by comparison of the data with literature values (Mahato and Kundu, 1994; Gu, et al., 2002; Banerji and Ray, 1981; Ishiguro et al., 1991; Slowing et al., 1994; Skaltsa et al., 1994). α-Amyrin, quercitrin and quercetin have been isolated previously from *M. malabathricum* with light pink-magenta petals (Nuresti et al., 2003). Kaempferol-3-*O*-(2”,6”-di-*O*-*p*-*trans*-coumaroyl)-β-glucoside has been encountered previously in leaf hairs of *Quercus ilex* (Fagaceae) (Skaltsa *et al.,* 1994).

Antioxidant Activity, FTC (Ferric Thiocyanate) Method. The detection of lipid peroxide and preparation of solutions were carried out according to method described by Kikuzaki and Nakatani (1993). A mixture of 4.0 mg of sample in 4.0 mL of 99.5% ethanol, 4.1 mL of 2.5% linoleic acid in 99.5% ethanol, 8.0 mL of 0.02 M phosphate buffer (pH 7.0) and 3.9 mL of water contained in a screw-cap vial (38 x 75 mm) was placed in an oven at 40°C in the dark. The final concentration of sample was 0.02% w/v. To 0.1 mL of the mixture, 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% ammonium thiocyanate was added. Three minutes after the addition of 0.1 mL of 2.0×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm at every 24 hours interval until 1 day after the absorbance of the control reached its maximum value.

DPPH Method. The method established by Tagashira and Ohtake (1998) was used with slight modification. Stock solution of sample was prepared at 1 mg/mL in methanol. A test sample solution (200 μL) was added to 3.8 mL of 50 μM DPPH methanolic solution (to give a final concentration of 500, 250, 125, 62.5, 31.3 and 7.8 μg/mL). After vortexing, the mixture was incubated for 30 minutes at room temperature. The changes of DPPH in colour (from deep violet to

light yellow) were measured at 517 nm. The difference in absorbance between a test sample and a control (methanol) was expressed as % inhibition and taken as activity. The activity was shown as IC_{50} value (50% inhibitory concentration).

The percentage inhibition was calculated by the following formula:

$$\text{Percentage inhibition} = \frac{Abs._{DPPH} - Abs._{(DPPH+sample)}}{Abs._{DPPH}} \times 100\%$$

Anti-inflammatory Activity. TPA (12-*O*-tetradecanoylphorbol-13-acetate)-induced Mouse Ear Oedema method. This method was carried out according to the method described by Nik Musa'adah *et a*l. (2000). The mice were weighed, marked and arrange to six mice in one group. The crude extracts and pure compounds (20 μL) was applied topically to the inner surface of the right ear of the mice 40 minutes before TPA (25 mg/mL in Me_2CO) was applied. The left ear which acted as control was applied with the same volume of Me_2CO. The resulting of oedema was measured after six hours TPA application. The mice were killed by cervical dislocation and a 7 mm diameter section of both ears was punched out and weighed.

The swelling induced by TPA was assessed in terms of the increase in the weight of the right ear punch biopsy over that of the left ear. The inhibitory effect (IE %) of each samples were than calculated as the ratio of weight increase of the ear sections, according to the following formula:

$$\text{Inhibitory effect (IE\%)} = \frac{L-R}{L-C} \times 100 \qquad \frac{L-R}{L-(\frac{L}{2.41})} \times 100$$

Where:

L = weight of left ear which was treated with TPA only

R = weight of right ear which was treated with TPA and the test sample

C = weight of normal ear (untreated)

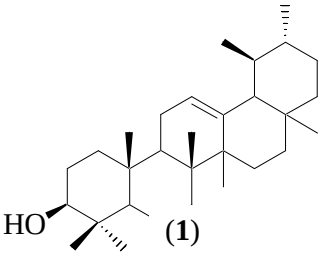
C is the calculated weight. It has been found that treating a normal ear with 0.5 μg TPA resulted in a 2.41 times increase in the weight of the ear.

Sample which has the percent of inhibition more than 50% was considered to have the anti-inflammatory activity.

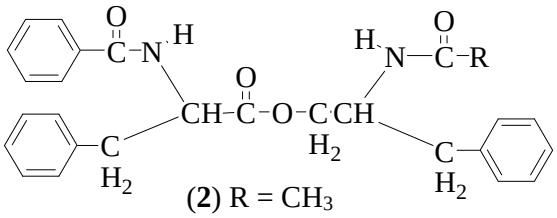
RESULTS AND DISCUSSION

Phytochemicals Investigation. The dried and powdered leaves of *M*. *malabathricum* (2.09 kg) was extracted by soxhlet extraction for 18 hours with *n*-hexane, EtOAc and MeOH, successively. The n-hexane and EtOAc extract were subjected to several chromatographic techniques (VLC and CC) to yield triterpenoid **1**, two alkaloids **2** – **3** and two flavonoids **4 - 5**, while the MeOH extract after purification afforded flavonoids **5** and **6**.

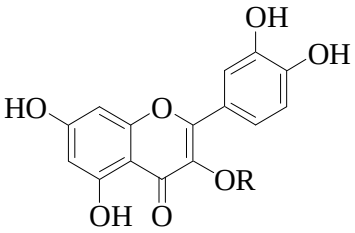
By correlating with melting points and spectral data (UV, IR, ^{1}H, ^{13}C NMR and MS) of literature values, compounds **1** - **6** were identified as α-amyrin (**1**) (Mahato, 1994) , patriscabratine (**2**) (Gu, et al., 2002), auranamide (**3**) (Banerji and Ray, 1981), quercetin (**4**) (Ishiguro, et al., 1991), quercitrin (**5**) (Slowing, et al., 1994) and kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)glucoside (**6**) (Skaltsa, *et al.,* 1994), respectively.



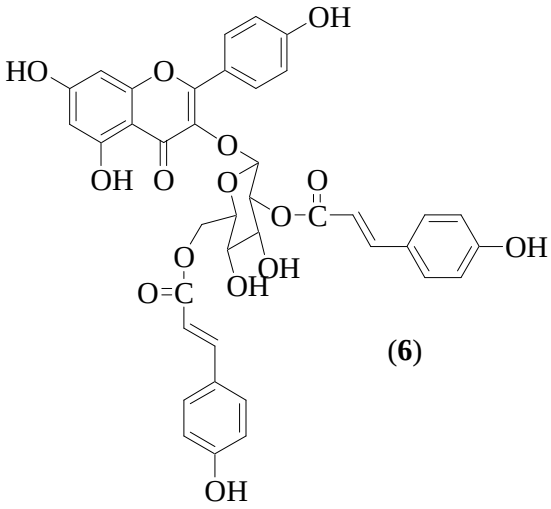
(**1**)



(**2**) R = CH_3
(**3**) R = Phe



(**4**) R = H
(**5**) R = Rha



(**6**)

This is the first report on isolation of α-amyrin (**1**), patriscabratine (**2**), auranamide (**3**), quercetin (**4**), quercitrin (**5**) and kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)-β-glucoside (**6**) from the leaves of *M*. *malabathricum* with white petals. Kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)-β-glucoside (**6**) in the leaves of *M*. *malabathricum* of white petals was found for the first time in *Melastoma* species. The presence of α-amyrin (**1**), quercetin (**4**), quercitrin (**5**) and kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)-β-glucoside (**6**) in the leaves of *M*. *malabathricum* with white petals is the chemotaxonomic significant of this plants.

Antioxidant Activity. FTC (Ferric Thiocyanate) Method. FTC method measures the amount of peroxide produced during the initial stages of oxidation which are the primary products of oxidation. In this method, the reaction mixture of linoleic acid, ethanol, phosphate buffer and antioxidant (sample and standard) is incubated at 40°C and the peroxide value is determined by measuring the absorbance at 500 nm after reaction between $FeCl_3$ and thiocyanate.

Figure 1 shows the results of the antioxidant activity of the flavonoids, isolated from the leaves of *M. malabathricum*. These flavonoids showed stronger antioxidative activities than vitamin E. The percentage inhibition of these flavonoids was between 92.2-96.1% (table 1).

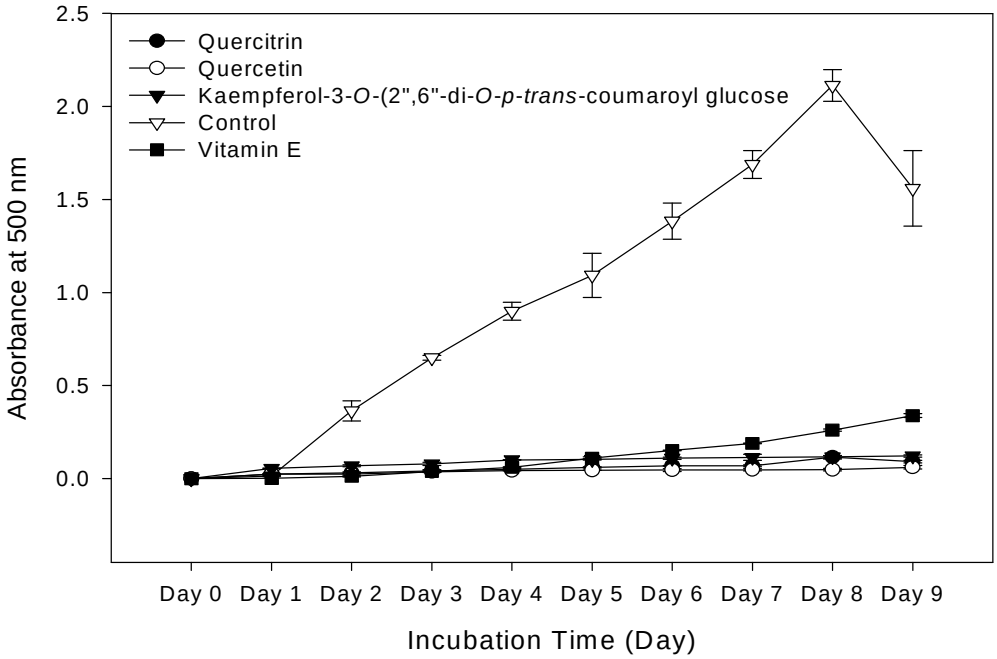


Figure 1. Antioxidant activity of flavonoids from the leaves of *M. Malabathricum* as measured by the FTC method (final sample concentration, 0.02% w/v)

DPPH Method. The DPPH method is a rapid and cheap method to measure antioxidant capacity. The radical scavenging activity was determined from the reduction in the optical absorbance at 517 nm due to scavenging of stable free radical of DPPH. Positive DPPH test suggests that the extracts and compounds are free radical scavengers.

Table 1.The absorbance values and percentage of linoleic acid peroxidation of the flavonoids as measured by FTC antioxidant assay

Sample	Absorbance*	Percent Inhibition
Quercetin	0.061	96.1
Quercitrin	0.092	94.1
Kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)-β-glucoside	0.122	92.2
Vitamin E	0.339	78.3
Control	1.560	0

*Absorbance reading on the 9th day (one day after control reached maximum)

Table 2 shows the activity of isolated compounds from two species that have free radical scavenging properties. Quercetin (**4**) with IC_{50} 0.69 μM was found as the most active free radical scavenger compared to the positive control, vitamin E and vitamin C and the other flavonoids. The percent inhibition of quercetin (**4**) at concentration 7.8 μg/mL was 57.60%

Table 2. The IC_{50} of the isolated compounds from *M. malabathricum* by UV Spectrophotometry method

Sample	IC_{50} (mean ± SD)	Percent inhibition 7.8 μg/ml (mean ± SD)
Quercetin	0.69 μM ± 1.4	57.6 ± 0.5
Quercitrin	74.1 μM ± 0.4	15.4 ± 1.8
Kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)- β-glucoside	308.1 μM ± 1.7	7.5 ± 1.2
Vitamin E	17.1 mM ± 2.5	7.4 ± 0.4
Vitamin C	8.3 μM ± 1.2	30.8 ± 0.7

Data represent mean ± SD of three independent experiments performed in triplicate

Anti-inflammatory Activity with TPA (12-*O*-tetradecanoylphorbol-13-acetate)-induced Mouse Ear Oedema method. The topical application of TPA onto a mice ear induced a long lasting oedema formation. The majority of TPA actions appear to involve or be dependent on arachidonic acid release and metabolism. At the biochemical level, PGI2, PGE2 and PGF2 are elevated within minutes to hours after TPA application. Phospholipase inhibitors, cyclooxigenase inhibitors and lipoxygenase inhibitors as well as corticosteroids are effective at oedema suppression after topical application of high dose of TPA, confirming a role of arachidonic acid release and metabolism (Jeenapongsa, et al., 2003).

Oedema was induced by topical application of 0.5 mg per ear of TPA. The crude extracts (2 mg per ear); the isolated compounds (0.5 mg per ear) and the standard drug indomethacin (0.5 mg per ear) were applied topically, simultaneous with TPA. TPA-induced oedema formation was clearly observed at six hour after the topical application. The inhibitory effect of crude extracts, isolated compounds and positive control are given in table 3. Data are expressed as IC_{50} values.

Table 3. IC_{50} and inhibitory effect of the isolated compounds on mice ear oedema

Sample	IC_{50}	Percent inhibition
α-amyrin	0.34 mM/ear $\pm$ 1.1	79.21 $\pm$ 10.6
Kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)- β-glucoside	0.11 mM/ear $\pm$ 0.4	99.83 $\pm$ 8.1
Indomethacin	2.10 M/ear $\pm$ 0.5	76.00 $\pm$ 6.0

In this test, kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)glucoside (**6**) was found to be active. It gave higher percentage of oedema inhibition (99.83% $\pm$ SD 8.1) at a dose of 0.5 mg/mL compared to the reference drug, indomethacin, 76.00% (SD $\pm$ 5.0) at the same dose. The IC_{50} value of kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)glucoside (**6**) was 0.11 mM/ear (SD $\pm$ 0.4) and indomethacin as a positive control was 2.10 M/ear (SD $\pm$ 0.5).

CONCLUSIONS

Phytochemical study, which employed various chromatographic techniques, resulted in the isolation of a series of flavonoids, triterpenoids and alkaloids. These compounds were successfully isolated and characterized using modern spectroscopic techniques. The compounds are α-amyrin (**1**), patriscabratine (**2**), auranamide (**3**), quercetin (**4**), quercitrin (**5**) and kaempferol-3-*O*-(2",6"-di-*O*-*p*-*trans*-coumaroyl)-β-glucoside (**6**).

Traditionally, the Melastomataceae family used for the treatment of many ailment, such as diarrhea, puerperal infection, dysentery, leucorrhoea, wound healing, post partum treatment and haemorrhoids. Interistingly the *in vitro* and *in vivo* assay, the isolated compounds from this plant exhibited many biological activities such as anti lipid peroxidation, radical scavenger and anti-inflammatory.

ACKNOWLEDGEMENT

The authors wish to thank IRPA via project no 74051 for financial support.

REFERENCES

Burkill, I. H. (1966). *A dictionary of the economic products of the Malay Peninsula*. Kuala Lumpur, Singapore (pp. 1462-1464). Government of Malaysia and Singapore by the Ministry of Agriculture and Co-Operatives

Banerji, A. and Ray, R. (1981). Auranamide, a New Phenylalanine Derivative Isolated from *Piper aurantiacum* Wall. *Indian J. Chem.* 20B: 597-598

Corner, E.J.H. (1951). *Wayside trees of Malaya*. Malayan Nature Society: Kuala Lumpur. Vol 1. (pp 445-453)

Gu, Z. B., Yang, J. G., Liu, W. Y., Li, T. Z., Qiu, Y. and Zhang, W. D. (2002). A New Alkaloid from *Patrinia scabra*. *Chin. Chem. Lett.* 13(10): 957-958

Ishiguro, K., Nagata, S., Fukumoto, H., Yamaki, M., Takagi, S. and Isoi, K. (1991). *Phytochemistry*. 30(9): 3152-3154

Jeenapongsa, R., Yoovathaworn, K., Sriwatanakul, K. M., Pongprayoon, U. and Sriwatanakul, K. (2003). Anti-inflammatory Activity of (*E*)-1-(3,4-dimethoxyphenyl)butadiene from *Zingiber cassumunar* Roxb. *J. Ethnopharmacol*. 87(2-3): 143-148

Kikuzaki, H. and Nakatani N. (1993). Antioxidant Effect of Some Ginger Constituents. *J. Food Sci*. 58(6): 1407-1410

Mahato, S. B. and Kundu A. P. (1994). ^{13}C NMR Spectra of Pentacyclic Triterpenoids- A Compilation and some Salient Feature. *Phytochemistry*. 37(6): 1517-1575

Nik Musa'adah, M.and Rasadah, M.A. (2000). Inhibitor Effect of *Solanum torvum* on TPA-Induced Mouse Ear Oedema. *J. Trop. Forest Prod*. 6: 222-224

Nuresti, S., Baek, S. H. and Asari, A. (2003). Chemical Components of *Melastoma Malabathricum*. ACGC Chemical Research Communications. 16, 28-33

Skaltsa, H., Verykokidou, E., Harvala, C., Karabourniotis, G. & Manetas, Y. (1994). UV-B Protective Potential and Flavonoid Content of Leaf Hairs of *Quercus ilex*. *Phytochemistry*. 37(4), 987-990

Slowing, K., Sollhuber, M., Carretero, E. and Villar, A. (1994). Flavonoid Glycoside from *Eugenia jambos*. *Phytochemistry*. 37(1): 255-258

Tagashira, M. and Ohtake, Y. (1998) A New Antioxidative 1,3-Benzodioxole from *Melissa officinalis*. *Planta Med*. 64(6): 555-558

Yoshida, T., Nakata, F., Hosotani, K., Nitta, A., Okuda, T. (1992). Dimeric Hydrolysable Tannin from *Melastoma malabathricum*. *Phytochemistry*, 31, 2829-2833