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FULL PAPER

Chemical constituents from *Macaranga hypoleuca* (Rchb.f. & Zoll.) Müll.Arg. and their radical scavenging activity

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Abstract

Euphorbiaceae is the fourth most prominent angiosperm family, with over 300 genera and 8,000 species, the largest of which is *Macaranga*. The leaves have traditionally been used to treat swelling, new cuts, ulcers, bruises, and boils. This genus is also vital for honeybee propolis production in Okinawa, Japan, and Taiwan. *Macaranga* contains a high concentration of flavonoids and stilbenoids, both of which exhibit a broad range of bioactivities. Due to that, *Macaranga hypoleuca* was selected for phytochemical and biological research. This study aimed to isolate chemical compounds from the stems and leaves of the species using various chromatographic methods and determine their isolated compounds' structure using several spectroscopic techniques. The powdered leaves of *M. hypoleuca* (2.5 kg) were macerated in methanol at room temperature for 24 hrs and repeated thrice. The crude extract of leaves obtained (700 g) was partitioned with *n*-hexane and ethyl acetate. Vacuum liquid chromatography (VLC) was used to fractionate the crude ethyl acetate (370 g) into eight fractions (HL1-8). Fraction HL5 (2.3 g) was subjected to VLC, column chromatography (CC) and preparative thin layer chromatography (p-TLC) to give a pure compound **(1)** (5.8 mg). Fraction HL6 (10.6 g) was fractionated using VLC twice, followed by purification using CC, high-performance liquid chromatography (HPLC) and p-TLC to afford pure compounds **(2)** (26.4 mg), **(3)** (6.0 mg) and **(4)** (2.0 mg). Another pure compound **(5)** (6.0 mg) was obtained from the crude acetone extract of the stem bark through fractionation, isolation and purification processes. The pure compounds were characterised based on the NMR, MS, UV-Vis and IR analyses as well as a comparison to published data. Three flavonoids known as tomentosanol D (**1**), quercetin (**2**) and kaempferol (**3**) as well as a phenolic acid namely 3,4-dihydroxybenzoic acid (**4**) were isolated from the leaves whilst a coumarin, scopoletin (**5**) was obtained from the stem bark of *M. hypoleuca*. Compounds **2** – **3** exhibited good activity on DPPH radical scavenging with the percent inhibition values of 93.85% and 93.81%, respectively.

1. Introduction

Macaranga is well-known as the largest genus within the Euphorbiaceae family and the sole genus under the Macaranginae subtribe, with over 300 species. This genus is widely distributed in tropical Africa, Madagascar, Southeast Asia, Australia and the Pacific region (Lim *et al*., 2009). This genus is typically found in rural areas, wastelands, swampy forests, and areas with abundant sunlight in secondary or disturbed forests in Malaysia (Zakaria *et al*., 2008; Aminah *et al*., 2014). Several members of this genus were traditionally used to treat a variety of ailments by the old folks. *Macaranga tanarius* root decoction was used as an antitussive to suppress coughing and also as a fever reliever (Lim *et al*., 2009); decoction of the stems and leaves was used to wash wounds and drunk by women after giving birth to prevent infections and to remove toxins from the body (Sutthivaiyakit *et al*., 2002). Besides, their tender branches have been utilised to cure fungal infections (Mazlan *et al*., 2013). In Nigeria and Congo, the leaves

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and bark of *M. barteri* were as beneficial as vermifuge and febrifuge (Asante-Kwatia *et al*., 2019). Apart from that, this genus is also vital in propolis production. The glandular trichome of *M. tanarius* fruits, which contains several prenylflavonoids, was used as propolis by the honeybees in Okinawa, Japan (Kumazawa *et al*. 2014) and Taiwan (Shahinozzaman *et al*., 2020). Previous phytochemical studies on *Macaranga* species resulted in the isolation of many phenolic compounds, particularly prenylated and geranylated flavonoids (Marliana *et al*., 2018; Segun *et al*., 2019), stilbenes (Tanjung *et al*., 2017; Kamarozaman, Ahmat, Isa *et al*., 2019) as well as terpenes (Salleh *et al*., 2017). Most of the isolated compounds exhibited a wide range of biological activities including anticancer (Pailee *et al*., 2015), antimicrobial (Ilmiawati *et al*., 2015; Kamarozaman *et al*., 2018), anti-inflammatory (Magadula, 2014), and antioxidant (Kamarozaman, Ahmat, Isa *et al*., 2019).

Despite that, the information on the phytochemical and pharmacological of *M. hypoleuca* is limited and there was no report included in a review of *Macaranga* species by Magadula in 2014. However, recent research to identify the chemical constituents of *M. hypoleuca* using pyrolysis gas chromatography-mass spectrometry (Py-GCMS QP2010) resulted in the identification of dibromo chloropropane, methoxy 2-propenal, ammonium carbamate, levoglucosan, carbinol and pentanoic acid (Gusmailina and Saputra, 2021). As part of our search for bioactive constituents from *Macaranga* species especially in Malaysia, the leaves and stem barks of *M. hypoleuca* were investigated. This study aimed to detail the isolation and elucidation of compounds and their biological activity. Through this study, three flavonoid compounds (**1** – **3**) together with a coumarin (**5**) and phenolic acid (**4**) were successfully purified and evaluated on DPPH radical scavenging assay (Figure 1).

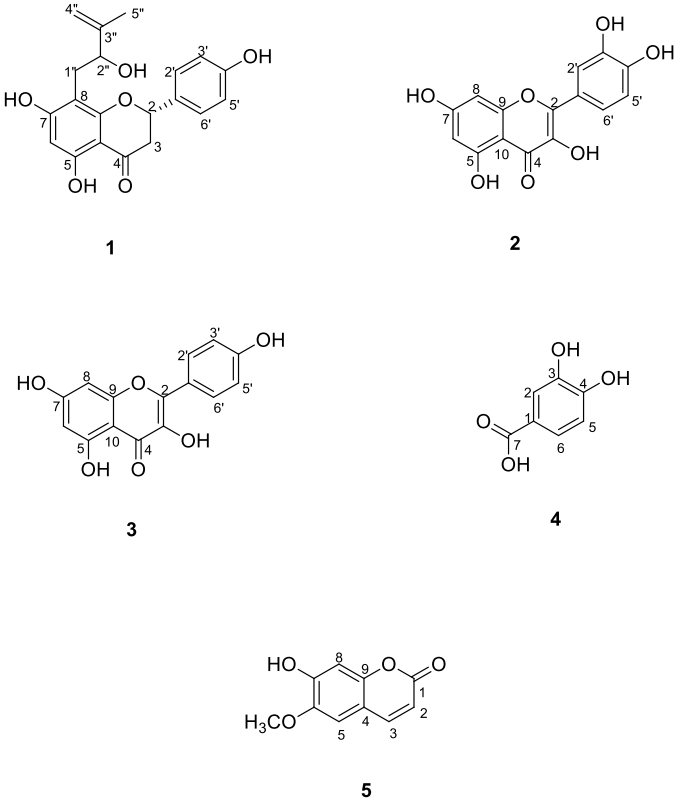

Figure 1. Structures of compounds **1**-**5**

2. Materials and methods

2.1 General experimental procedure

The 1D and 2D NMR spectra were measured using deuterated solvents on a Bruker Ultrashield spectrometer at 600 MHz (^{1}H at 600 MHz and ^{13}C-APT at 150 MHz). Chemical shift values were reported in ppm and shown as δ scale. The peak multiplicities were recorded in Hz. The UV spectra were acquired using Jasco V-700Bio Spectrophotometer while Thermo Scientific Nicolet 6700 FT-IR was used to measure the IR spectra and HR-ESI-MS was performed on Agilent 6530 Accurate-Mass Q-TOF LC/MS using positive/negative mode. Extraction and purification of the crude extract were done using vacuum liquid chromatography (VLC), column chromatography (CC) and radial chromatography (RC). Silica gel 60 F_{254} (Merck catalog number: 1.00747), G60 (230 – 400 mesh, Merck catalog number: 1.09385) and 60 PF_{254} containing gypsum (Merck catalog number: 1.07749) were used for VLC, CC and RC while silica gel 60 (0.2-0.5 mm) (Merck catalog number: 1.07733) was used for sample premix. Silica Gel 60 PF_{254} (aluminium sheets) (Merck catalog number: 1.05554) of thin-layer chromatography (TLC) was used for the analysis. The chemical spots on the TLC plates were visualized under UV light at the wavelength of 254 and 365 nm.

2.2 Plant material

The leaves and stem bark of *M. hypoleuca* were collected from Hutan Simpan UiTM Jengka, Pahang and were identified by Prof. Mohd Nazip Suratman and Dr. Shaikh Abdul Karim Yamani Zakaria, botanists from Universiti Teknologi MARA (UiTM). The voucher specimen (FSG1) was deposited at the herbarium of Forest Research Institute Malaysia (FRIM). The samples were air-dried for two weeks and kept in an air-locked plastic bag.

2.3 Extraction and isolation

The dried leaves of *M. hypoleuca* were ground into powder form. The powder of the leaves (2.5 kg) was then macerated in methanol (10 L) for 24 hrs at room temperature and repeated three times (Kamarozaman, Ahmat, Isa *et al*., 2019). The extract was filtered and evaporated using a rotary evaporator (temperature of 50°C, 120 rpm rotation) to obtain dark-green crude extract. The crude methanolic extract (700 g) was further partitioned using *n*-hexane and ethyl acetate

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successively. The ethyl acetate fraction (370 g) was semi-purified using VLC eluted with a mixture of *n*-hexane and ethyl acetate in increasing polarity to yield eight major fractions (HL1-8). Fraction HL5 (2.34 g) which showed three major spots on the TLC analysis was subjected to VLC with hex: EtOAc in increasing polarity as a solvent system to obtain seven combined sub-fractions (HL51–57). Fraction HL54 (540.7 mg) with the targeted spots was chromatographed twice using CC with the solvent system of $CHCl_3$: EtOAc and EtOAc: MeOH and hex: Acetone/ MeOH respectively, followed by purification using preparative thin-layer chromatography (p-TLC) with the eluent $CHCl_3$: EtOAc to afford **1** (5.8 mg). Fraction HL6 (10.6 g) displayed spots of interest and was re-fractionated using VLC (hex: EtOAc) to yield eight sub-fractions (HL61-68). HL65 (2.26 g) was chosen for further purification as it showed the spots of flavonoids on TLC analysis. This sub-fraction was fractionated using CC over Sephadex-LH20 gel filtration (MeOH), followed by purification using high-performance liquid chromatography (HPLC) with MeOH: H_2O as solvent system to give **2** (26.4 mg) and **3** (6 mg). Fraction HL66 (2.38 g) was re-fractionated twice using CC over Sephadex-LH20 gel filtration (MeOH) and purified using p-TLC ($CHCl_3$: MeOH) to afford **4** (2 mg).

The dried stem bark of *M. hypoleuca* (3 kg) was ground to form a powder and macerated in acetone (10 L) at room temperature for three days and repeated five times (Kamarozaman, Ahmat, Johari *et al*., 2019). The extract was filtered and evaporated using a rotary evaporator to yield crude acetone extract. The crude extract was dissolved in diethyl ether to remove the tannin. The crude acetone extract with less tannin (71.83 g) was fractionated using VLC with the mixture of *n*-hexane and EtOAc in increasing polarity to give five major fractions (HS1-5). Fraction HS3 (3.7 g) that showed one major spot was re-fractionated using VLC ($CHCl_3$: acetone) and separated *via* RC twice with the eluent of hex: EtOAc and hex: $CHCl_3$: acetone: EtOAc respectively to give **5** (6 mg).

2.3.1 Tomentosanol D ***(1)***

Yellow amorphous (5.8 mg). UV (MeOH) λ_{max} nm: 228, 292, 336; ATR-IR νmax cm^{-1}: 3333 (OH), 2920, 2851 (CH-sp^3), 1636 (C=O), 1519 (C=C), 1091 (CO); ^{1}H-NMR (Acetone-d_6, 600 MHz) δ ppm : 12.18 (1H, *s*, OH-5), 7.43 (2H, *d*, *J* = 8.4 Hz, H-2'/H-6'), 6.92 (2H, *d*, *J* = 8.4 Hz, H-3'/H-5'), 6.00 (1H, *s*, H-6), 5.44 (1H, *dd*, *J* = 13.2, 3 Hz, H-2), 4.74, 4.89 (2H, *br s*, H2-4"), 4.34 (1H, *d*, *J* = 7.2 Hz, H-2"), 3.14 (1H, *dd*, *J* = 12.8, 16.8 Hz, H-3b), 2.76 (1H, *dd*, *J* = 16.8, 3 Hz, H-3a), 2.78, 2.94 (2H, *br s*, H2-1"), 1.70 (3H, *s*, H-5"); ^{13}CAPT NMR (Acetone-d_6, 150 MHz) δ ppm: 196.74 (C-4), 165.75 (C-7), 162.63 (C-5), 160.58 (C-9), 157.74 (C-4'), 147.33 (C-3"), 130.14 (C-1'), 127.83 (C-2'/C-6'), 115.29 (C-3'/C-5'), 109.27 (C-4"), 105.28 (C-8), 102.39 (C-10), 96.64 (C-6), 78.99 (C-2), 75.65 (C-2"), 42.63 (C-3), 29.30 (C-1"), 17.59 (C-5") (Tanaka *et al*., 1997).

2.3.2 Quercetin ***(2)***

Yellow solid (26.4 mg). UV (MeOH) λ_{max} nm: 230, 256, 270; IR ν_{max} cm^{-1}: 3318 (OH), 2945, 2833 (CH-sp^3), 1655 (C=O), 1019 (C-O); ^{1}H-NMR (Acetone-d_6, 600 MHz) δ ppm : 7.75 (1H, *d*, *J* = 2.4 Hz, H-2'), 7.65 (1H, *dd*, *J* = 1.8, 8.4 Hz, H-6'), 6.91 (1H, *d*, *J* = 9 Hz, H-5'), 6.40 (1H, *d*, *J* = 2.4 Hz, H-8), 6.20 (1H, *d*, *J* = 1.8 Hz, H-6); ^{13}C-APT NMR (Acetoned_6, 150 MHz) δ ppm: 177.34 (C-4), 165.64 (C-7), 162.49 (C-5), 158.26 (C-9), 148.78 (C-4'), 148.05 (C-2), 146.23 (C-3'), 137.21 (C-3), 124.18 (C-9), 121.73 (C-6'), 116.27 (C-5'), 116.04 (C-2'), 104.53 (C-10), 99.31 (C-6), 94.48 (C-8) (Abdullah *et al*., 2016).

2.3.3 Kaempferol ***(3)***

Yellowish-brown solid (6 mg). UV λ_{max} nm: 230, 251, 270, 370; IR ν_{max} cm^{-1}: 3331 (OH), 2942, 2831 (CH-sp^3), 1646 (C=O), 1021 (C-O); ^{1}H-NMR (Acetone-d_6, 600 MHz) δ ppm: 8.18 (1H, *d*, *J* = 8.4 Hz, H-2'/H-6'), 7.04 (1H, *d*, *J* = 8.4 Hz, H-3'/H-5'), 6.56 (1H, *d*, H-8), 6.29 (1H, *d*, H-6); ^{13}C-APT NMR (Acetone-d_6, 150 MHz) δ ppm: 176.63 (C-4), 165.10 (C-7), 162.35 (C-5), 160.22 (C-9), 157.83 (C-4'), 147.05 (C-2), 136.64 (C-3), 130.47 (C- 2'/C-6'), 123.34 (C-1'), 116.36 (C-3'/C-5'), 104.15 (C-10), 99.21 (C-6), 94.53 (C-8) (Abdullah *et al*., 2016).

2.3.4 3,4-Dihydrobenzoic acid ***(4)***

Brownish sticky solid (2 mg). UV (MeOH) λmax nm: 257, 295. IR ν_{max} cm^{-1}: 3288 (OH), 1666 (C=O), 1602 (C=C), 1017 (C-O); ^{1}H-NMR (Acetoned_6, 600 MHz) δ ppm: 7.51 (1H, *d*, *J* = 1.8 Hz, H-2), 7.45 (1H, *dd*, *J* = 8.3, 1.8 Hz, H-6), 6.89 (1H, *d*, *J* = 8.2 Hz, H-5); ^{13}C-APT NMR (Acetone-d_6, 150 MHz) δ ppm: 168.0 (C-7), 150.9 (C-3), 145.7 (C-4), 123.4 (C-6), 123.2 (C-1), 117.5 (C-2), 115.6 (C-5) (Abdullah *et al*., 2016).

2.3.5 Scopoletin ***(5)***

Yellowish crystal. UV (MeOH) λ_{max} nm: 231, 348, 400; IR νmax cm^{-1}: 3430 (OH), 1634 (C=O), 1012 (C-O), 1156; ^{1}H-NMR (Acetone-d_6, 600 MHz) δ ppm: 7.61 (1H, *d*, *J* = 9.5 Hz, H-3), 6.91 (1H, *s*, H-5), 6.86 (1H, *s*, H-8), 6.26 (1H, *d*, *J* = 9.5 Hz, H-2), 3.95 (3H, *s*, OCH3-6); ^{13}C-APT NMR (Acetone-d_6, 150 MHz) δ ppm: 161.3 (C-1), 150.3 (C-7), 149.9 (C- 9), 144.2 (C-6), 143.4 (C-3), 113.3 (C-2), 111.4 (C-4), 107.7 (C-5), 103.2 (C-8),

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56.4 (C-OCH_3) (Darmawan *et al*., 2012).

2.4 DPPH radical scavenging activity

The DPPH solution was prepared by dissolving 1.0 mg of DPPH stock in 1 mL of EtOH. The solution was wrapped using aluminium foil to avoid exposure to light. A stock solution of the standard and test sample was prepared at a concentration of 1 mg/mL in EtOH and diluted to 200 µg/mL. Next, the stock solution (200 µg/mL) was placed into the 96-well plate in descending volumes of 100, 80, 60, 40, 20, 10 and 5 µL. Then, the stock solution in each well was diluted with EtOH to make the total amount of 195 µL. A 5 µL of prepared DPPH solution was added to the well. Then, the plate was shaken using a shaker to ensure the mixture was mixed thoroughly. The mixtures were then incubated in the dark at room temperature for 30 minutes. After that, the absorbance was measured at the wavelength of 517 nm (Blois, 1958). The graph of inhibition (%) versus concentration (µg/mL) was plotted and the concentration of sample required for 50% inhibition was determined. The percentage inhibition towards DPPH radical was calculated using the following equation:

$$\%\ \text{DPPH Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

3. Results and discussion

Compound **1** (5.8 mg) was isolated as a brownish sticky solid. The HR-ESI-orbitrap-MS spectrum showed a molecular ion peak at *m/z* 357.0918 [M+H]+ corresponding to the molecular formula of $C_{20}H_{20}O_6$. The UV spectrum of this compound displayed maximum absorption at λ_{max} 292.2 and 336 nm corresponding to the flavanone molecule (Feng *et al*., 2017). The IR spectrum showed a broad absorbance peak at 3333 (O-H); 1636 (C=O); 2920, 2851 (alkyl); 1519 (C=C aromatic) and 1091 (C-O) cm^{-1} which support the presence of aromatic ring in the structure and this compound is a flavonoid. The ^{1}H NMR spectrum of compound **1** showed the typical characteristic of flavanone which can be observed by the presence of two methylene proton resonances at δ_H 2.76 (1H, *dd*, *J* = 7.8, 3 Hz, H-3a), 3.14 (1H, *dd*, *J* = 17.4, 13.2 Hz, H-3b) and one oxymethine proton at δ_H 5.44 (1H, *dd*, *J* = 13.2, 3 Hz, H-2). An aromatic proton at δ_H 6.00 (1H) belongs to H-6, showing that ring A is a penta-substituted ring system. In addition, the presence of a vinyl methyl at δ_H 1.70 (3H, *s*, H-5"), one benzyl methylene at δ_H 2.78-2.94 (2H, *m*, H-1"), a methine attaching to oxygenated carbon at δ_H 4.34 (1H, *m*), and a vinyl methylene at δ_H 4.74 and 4.89 (1H each, *br s*, CH2=) which assigned to a 2-hydroxy-3-methyl-3-butenyl group, also can be observed from the spectrum. An AABB spin system of a monosubstituted benzene ring was displayed by two sets of aromatic protons resonated at δ_H 7.43 (2H, *d*, *J* = 8.4 Hz, H-2'/H-6') and δ_H 6.92 (2H, *d*, *J* = 9 Hz, H-3'/H-5'). The ^{13}C-APT NMR spectrum of compound **1** displayed 18 carbon resonances which correspond to 20 carbons present in the compound. One methylene carbon appeared at δ_C 42.63 (C-3) and one oxymethine carbon at δ_C 78.99 (C-2). There are two sets of aromatic carbons at δ_C 127.83 (C-2'/C-6') and 115.29 (C-3'/C-5') and one aromatic carbon at δ_C 96.64. Four oxyaryl carbons resonated at δ_C 157.74 (C-4'), 162.63 (C-5), 165.75 (C-7) and 160.58 (C-9); two aromatic quaternary carbons at δ_C 102.39 (C-10) and 130.14 (C-1') and a carbonyl carbon (C-4) appeared at a very downfield region (δ_C 196.74). Lastly, the carbon resonances at δ_C 29.30, 75.65, 147.33, 109.27, and 17.59 are attributed to C-1", C-2", C-3", C- 4", and C-5" respectively, which belong to 2-hydroxy-3-methyl-3-butenyl group substituted at C-8 of ring A. Based on the spectral data analyses, compound **1** was identified as tomentosanol D. This compound was also reported to be present in the leaves of *M. conifera* (Jang *et al*. 2002).

Compound **2** (26.4 mg) which was purified as a yellow amorphous solid has the molecular formula of $C_{15}H_{10}O_7$ as displayed by the HR-ESI-orbitrap-MS spectrum at *m/z* 301.0323 $[M-H]^-$. The UV spectrum showed maximum absorptions at λ_{max} 230 nm, 256 nm and 370 nm indicating the occurrence of the conjugated system of the double bond in the molecule which showed the typical characteristic of flavone (Feng *et al*., 2017). The IR spectrum displayed broad absorption bands at 3318 cm^{-1} which is due to the presence of hydroxyl group, CH-sp^3 stretching bands at 2945 cm^{-1} and 2833 cm^{-1}, stretching band of C-O at 1019 cm^{-1}; while the stretching band of C=O of ketone appeared at 1665 cm^{-1} support that this compound is a flavonoid (Feng *et al*., 2017). The ^{1}H NMR spectrum of this compound showed the presence of ABX moiety as one doublet-*meta* at δ_H 7.75 (1H, *d*, *J* = 2.4 Hz, H-2'), one doublet-doublet proton at δ_H 7.65 (1H, *dd*, *J* = 2.4, 8.4 Hz, H-6') and one doublet-*ortho* resonance at δ_H 6.91 (1H, *d*, *J* = 8.4 Hz, H-5'). In addition, a pair of doublet-*meta* proton appeared at δ_H 6.40 (1H, *d*, *J* = 1.8 Hz, H-8) and δ_H 6.20 (1H, *d*, *J* = 2.4 Hz, H-6) belongs to ring A. The ^{13}C-APT NMR spectrum of **2** displayed 15 carbon resonances which correspond to the number of carbons present in the compound. The spectrum showed the presence of one carbonyl at a very downfield region at δ_C 177.3 (C-4), five oxyaryl carbons at δ_C 162.4 (C-5), 165.6 (C-7), 158.2 (C-9), 146.2 (C-3') and 148.7 (C-4'); five aromatic carbons at δ_C 94.4 (C-8), 99.3 (C-6), 116.0 (C-2'), 116.2 (C-5') and 121.7 (C-6'); and four quaternary carbons at δ_C 148.0 (C-2), 137.2 (C-3), 104.5 (C-10) and δ_C 124.1 (C-1'). Based on the comparison with the literature data by Abdullah *et al*. (2016), compound **2** was elucidated as

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quercetin.

Compound **3** (6 mg) was obtained as a yellowish-brown solid with the molecular formula of $C_{15}H_{10}O_6$ as showed by the HR-ESI-orbitrap-MS spectrum at *m/z* 285.0383 $[M-H]^-$. The UV and IR spectra of this compound resembled the characteristics of compound **2**. The ^{1}H NMR spectrum showed a simple skeleton that consists of an AABB spin system that appeared as doublet-*ortho* at δ_H 8.18 (1H, *d*, *J* = 8.4 Hz, H-2'/H- 6') and δ_H 7.04 (1H, *d*, *J* = 8.4 Hz, H-3'/H-5'), due to the absence of hydroxy group at C-3' at ring B. A pair of doublet-*meta* protons can be observed at δ_H 6.56 (1H, *d*, H-8) and δ_H 6.29 (1H, *d*, H-6) which belongs to ring A. The ^{13}C-APT NMR spectrum of **3** exhibited 13 carbon signals representing 15 carbons present in the compound. The spectrum showed the presence of one carbonyl at a very downfield region at δ_C 176.6 (C-4). Four oxyaryl carbons were resonated at δ_C 162.3 (C-5), 165.1 (C-7), 157.8 (C-4') and 160.2 (C-9), whereas six aromatic carbons were observed at 99.2 (C-6), 94.5 (C-8), 130.4 (C-2'/C-6'), and 116.3 (C-3'/C-5'). Lastly, four quaternary carbons resonated at δ_C 147.0, 136.6, 104.1 and 123.3 attributed to C-2, C-3, C-10 and C-1'. The data of compound **3** was consistent with the report by Abdullah *et al*. (2016) which was elucidated as kaempferol.

Compound **4** (2 mg) was obtained as a brownish sticky solid. The HR-ESI-orbitrap-MS spectrum showed a molecular ion peak at *m/z* 153.01683 $[M-H]^-$ corresponding to the molecular formula of $C_7H_6O_4$. The UV spectrum of this compound displayed maximum absorption at λ_{max} 295 nm indicating the presence of a conjugated aromatic type of compound due to the π-π* transition of benzene chromophore (Abdullah *et al*., 2016). The IR spectrum showed absorption bands at 3288 (O-H); 1666 (C=O); 1602 aromatic C=C); and 1017 (C-O) cm^{-1} which support the presence of aromatic ring in the structure (Abdullah *et al*., 2016). The ^{1}H NMR spectrum displayed a doublet-*meta* proton signal at δ_H 7.51 (1H, *d*, *J* = 1.8 Hz, H-2), doublet-doublet proton resonance at δ_H 7.45 (1H, *dd*, *J* = 8.3, 1.8 Hz, H-6), and doublet-*ortho* proton at δ_H 6.89 (1H, *d*, *J* = 8.2 Hz, H-5) which indicates the presence of ABX spin system of benzene. The ^{13}C-APT NMR spectrum displayed seven carbon signals represented by three aromatic methine carbons at δ_C 117.5 (C-2), 115.6 (C-5) and 123.4 (C-6), one carbonyl carbon at the very downfield region at δ_C 168.0 (C-7), two oxyaryl carbons at δ_C 145.7 (C-4) and 150.9 (C-3), and one quaternary carbon at δ_C 123.2 assigned to C-1 where the carboxylic acid moiety substituted. Based on the spectroscopic data analysis, compound **4** was identified as 3,4-dihydroxybenzoic acid and first time isolated from *Macaranga* species.

Compound **5** was purified as a yellowish crystal. The UV spectrum of this compound displayed maximum absorption at λ_{max} 231, 348 and 400 nm corresponding to coumarin. The IR spectrum showed a broad absorption band at 3331 cm^{-1} indicating the presence of stretching vibration of the O-H bond, and the absorbance peak of C=O stretch appear at 1668 cm^{-1}. The presence of a carbon-carbon double bond (C=C) is detected by the absorbance peak at 1448 cm^{-1} and C-O stretching at 1019 cm^{-1} representing the methoxy and hydroxy group (Firmansyah *et al*., 2020). The ^{1}H NMR spectrum of **5** displayed a pair of doublet-*ortho* protons appearing at δ_H 7.61 (1H, *d*, *J* = 9.5 Hz) and 6.26 (1H, *d*, *J* = 9.5 Hz), assigned to H-3 and H-2 respectively. A pair of one proton singlet resonances also can be observed at δ_H 6.91 (1H, *s*) and 6.86 (1H, *s*) which belongs to H-5 and H-8, and a singlet representing three protons belonging to the methoxy group can be seen at δ_H 3.95 ppm. The ^{13}C-APT NMR spectrum showed 10 carbon signals representing a total of 10 carbons in the compound. The spectrum displayed one carbonyl ester appeared at the downfield region at δ_C 206.9 (C-1), three oxyaryl carbons at δ_C 144.2 (C-6), 149.9 (C-9), and 150.3 (C-7), two methine olefinic carbons at δ_C 113.3 (C-2) and 143.4 (C-3), two aromatic carbons at δ_C 107.7 (C-5) and 103.2 (C-8), one aromatic quaternary carbon at δ_C 111.4 (C-4) and one methyl carbon for methoxy appeared at the upfield region of δ_C 56.4 (C-10). Hence, compound **5** was determined as scopoletin.

Compounds **1**, **2** and **3** were tested for DPPH radical scavenging activity (Table 1). All three compounds displayed percent inhibition greater than 50% at the concentration of 100 µg/mL. Compound **2** exhibited the highest percent inhibition of 93.85%, followed by compound **3** with the percent inhibition of 93.81%. The significant activity in compounds **2** and **3** is due to a high number of hydroxyl groups which is important in neutralizing the free radical (Pyrzynska and Pekal, 2013). Compound **2** has a slightly higher percent inhibition when compared to compound **3** as it has more hydroxyl groups in the structure. The presence of catechol moiety stabilised the structure through the delocalisation of the spare electron (Li *et al*, 2012). Meanwhile, the percent inhibition of compound **1** (50.34%) is much lower than compound **3** due to the presence of 2-hydroxyl-3-methyl-3-butenyl moiety at C-8 of ring A which reduces the activity. Besides, the number of the hydroxyl group in compound **1** is less compared to compound **3** which makes it less active.

The results were in good agreement with Abas and co-researchers (2003) who reported the radical scavenging properties of compound **2** isolated from *Cosmos caudatus* Kunth with a percent inhibition of

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81.82% at the concentration of 125 µg/ml. Meanwhile, compound **3** was isolated from *Acacia nilotica* (L.) Willd. Ex. Del. displayed the very strong scavenging activity of 92.61% at 25 µg/mL concentration and the scavenging effect became almost stable beyond this concentration i.e. 90.86% at 50 µg/mL (Singh *et al*., 2008).

Table 1. DPPH radical scavenging activity of isolated compounds from *M. hypoleuca*

Compounds	% Inhibition
Tomentosanol D	50.34
Quercetin	93.85
Kaempferol	93.81
Trolox	95.83

Trolox was used as a positive control.

4. Conclusion

Phytochemical study on the leaves and stem bark of *M. hypoleuca* resulted in the isolation of three flavonoids, a coumarin and a phenolic acid which upon further elucidation using modern spectroscopic techniques such as UV, IR NMR and MS confirmed that the flavonoids are tomentosanol D (**1**), quercetin (**2**), and kaempferol (**3**), a phenolic acid identified as 3,4-dihydrobenzoic acid (**4**) and a coumarin known as scopoletin (**5**). Quercetin (**2**), and kaempferol (**3**) exhibited good DPPH radical scavenging activity with the percent inhibition values of 93.85% and 93.81%, respectively.

Conflict of interest

The authors declare no conflict of interest.

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