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Flavonoids with antiplasmodial and cytotoxic activities of *Macaranga triloba*

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ABSTRACT

A new flavanone derivative, malaysianone A (**1**), four prenylated flavanones, 6-prenyl-3′-methoxyeriodictyol (**2**), nymphaeol B (**3**), nymphaeol C (**4**) and 6-farnesyl-3′,4′,5,7-tetrahydroxyflavanone (**5**), and two coumarins, 5,7-dihydroxycoumarin (**6**) and scopoletin (**7**), were isolated from the dichloromethane extract of the inflorescences of *Macaranga triloba*. The structures of these compounds were elucidated based on spectroscopic methods including nuclear magnetic resonance (NMR-1D and 2D), UV, IR and mass spectrometry. The cytotoxic activity of the compounds was tested against several cell lines, with **5** inhibiting very strongly the growth of HeLa and HL-60 cells (IC_{50}: 1.3 μg/ml and 3.3 μg/ml, respectively). Compound **5** also showed strong antiplasmodial activity (IC_{50}: 0.06 μM).

1. Introduction

Macaranga triloba, locally known as “Mahang merah”, is a tree endemic to Southeast Asia found at forest margins. Its water extract was used as pain relief for stomach trouble in Java [1]. Previous investigation on the chemistry and pharmacology of this genus showed that its crude extracts displayed an interesting bioactivity profile, possessing various bioactivities including antioxidant [2,3], cyclooxygenase-I and II-inhibitory [4], antibacterial [5], antitumor and antimicrobial [6], and cytotoxic effects [7–9]. The genus *Macaranga*, one of the largest genera of the Euphorbiaceae family has approximately 280 species [10]. This genus is known for a wide range of mutualistic associations with ants, ranging from facultative to strictly obligate relationships [11,12]. In Malaysia, there are approximately 40 species, growing mostly in secondary forests [13]. *Macaranga* has been reported as a plant rich in prenylated flavonoids, especially geranyl flavonoids [14–17]. This paper reports the structure elucidation of **1** and the bioactivities of the isolated flavonoids from the inflorescences of *M. triloba*.

2. Experimental

2.1. General experimental procedures

^{1}H and ^{13}C NMR were recorded in $CDCl_3$ on a Bruker 300 Ultrashield NMR spectrometer measured at 300 and 75 MHz, respectively. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) are reported in Hz. CD spectra were recorded on a JASCO J-720WI spectropolarimeter. HRESI-MS were obtained with an Agilent TOFLC/MS G6224A mass spectrometer. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum One FTIR spectrometer. Ultraviolet (UV) spectra were recorded on Shimadzu UV–vis 160i spectrophotometer. The following adsorbents were used for purification: VLC used Silica gel 60, 70–230 mesh ASTM (Merck cat no. 1.07747), radial chromatography used Si-gel 60 PF_{254} (Merck cat. no. 1.07749) and TLC analysis with Merck Kieselgel 60 F254 0.25 mm (cat. no. 1.05554). Distilled technical and analytical grade solvents were used in this study.

2.2. Plant material

The inflorescences of *M. triloba* were collected from Pasir Raja, Hulu Terengganu, Malaysia and identified by Dr. Shamsul

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Khamis, Institute of Bioscience, Universiti Putra Malaysia. A voucher specimen (UiTM17/09) was deposited at the Herbarium of Universiti Teknologi MARA, Malaysia.

2.3. Extraction and isolation

The inflorescences (1.5 kg) of *M. triloba* were air dried, ground and soaked successively with *n*-hexane, dichloromethane and methanol (3×16 L, 24 h each). The dichloromethane extract (44.41 g), a dark brown gum, was subjected to vacuum liquid chromatography (VLC) on silica gel using a gradient elution system of *n*-hexane/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10. Twenty-five fractions were obtained and spotted on TLC using the same solvent system. Similar TLC profiles were grouped to yield 6 fractions (F_1–F_6). Fraction F_3 was subjected to column chromatography (CC) using gradient elution system of *n*-hexane/$CHCl_3$ 1:9 and *n*-hexane/EtOAc 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10 to yield 47 fractions, which were pooled based on TLC profiling, to yield 15 fractions ($F1_1$–$F1_{15}$). Fraction $F1_3$ was subjected to preparative thin-layer chromatography (PTLC) to obtain **6** (5.0 mg) using solvent system CH_2Cl_2/acetone 95:5 (1 mm thickness). Fraction $F1_5$ was subjected to medium pressure liquid chromatography (MPLC) using gradient elution system of H_2O/MeCN 8:2, 7:3, 6:4, 5:5, 4:6 and 3:7 to yield 27 fractions ($F2_1$–$F2_{27}$). Fractions ($F2_1$–$F2_6$) were combined and subjected to radial chromatography (RC) to yield **2** (21.1 mg) using $CHCl_3$/acetone 9:1 (2 mm thickness). Fraction $F1_6$ was further chromatographed on reverse-phased column chromatography (RPCC) to yield 26 fractions ($F3_1$-$F3_{26}$) using H_2O/MeCN 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10. Fractions $F3_2$–$F3_{11}$ were combined and subjected to RC using $CHCl_3$/EtOAc 9.5:0.5 (2 mm thickness) yielding **3** (326.7 mg). Fractions $F3_{14}$–$F3_{19}$ were pooled and subjected to RC using $CHCl_3$/EtOAc 9:1 (1 mm thickness) to yield **4** (20.6 mg). Compound **5** (58.1 mg) was obtained from the first RPCC H_2O/MeCN 3:7 from fraction $F3_{21}$. Fraction $F3_{22}$–$F3_{23}$ was subjected to RC using $CHCl_3$/acetone 9.8:0.2 (2 mm thickness) to yield 20 fractions ($F4_1$-$F4_{20}$). Subfractions $F4_1$–$F4_6$ were combined and chromatographed on RC using *n*-hexane/ EtOAc/MeOH 8:1.9:0.1 (1 mm thickness) to yield 21 fractions ($F5_1$–$F5_{21}$). Subfractions $F5_{11}$–$F5_{15}$ were combined to obtain **1** (6.0 mg). Fraction F_4 from VLC was subjected to CC using CH_2Cl_2/acetone 10:0, 9:1, 8:2, 7:3, 6:4 and 5:5 to yield 10 fractions ($F6_1$–$F6_{10}$). Fractions $F6_1$–$F6_5$ were combined and subjected to RC to obtain **7** (6.0 mg) using CH_2Cl_2/acetone 9.8:0.2 (0.5 mm thickness).

2.4. Antiplasmodial activity

The antiplasmodial activity of the dichloromethane extract and the isolated compounds was determined by methods previously described by Widyawaruyanti [18]. The samples were dissolved in DMSO and kept at −20 °C until use. The malarial parasite *Plasmodium falciparum* (3D7) clone was propagated in a 24-well culture plate in the presence of 10, 1, 0.1, 0.01 and 0.001 μg/ml range of concentrations of each compound. Chloroquine diphosphate was used as positive control. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa (Merck). The antiplasmodial activity of each compound was expressed as an IC_{50} value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control.

2.5. Cytotoxicity assay

Cytotoxicity was quantitatively estimated by non-radioactive, colorimetric assay system using tetrazolium salt, MTT (Sigma, USA) as reported by Mosmann [19]. MTT was dissolved in phosphate buffered saline at 5 mg/ml and filter sterilized to eliminate a small quantity of insoluble residue present in some batches of MTT. The MTT stock solution was added directly to all appropriate microtitre-plate well (20 per 100 μl medium) containing cells. The plate was incubated for 4 h at 37 °C to allow MTT metabolism to formazan. The supernatant was aspired and 100 μl of acid-isopropanol (0.04 M HCl in propan-2-ol) was added and mixed thoroughly to dissolve the dark blue formazan crystals. The optical density (OD) was measured on an automated spectrophotometric EL 340 multiplet/microelisa reader (Bio-Tek Instruments Inc.) using test and reference wavelength of 570 and 630 nm respectively. The cytotoxic dose that killed cell was determined from the compound's concentration that reduces the mean absorbance at 570 nm to 50% (IC_{50}) of those in the untreated control cells [20].

Malaysianone A (**1**): Pale yellow amorphous solid; m.p. 92; $[\alpha]_D^{25}$ +24.6 (*c* 0.92, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 226 (4.82), 272 (4.45) and 285 (4.46); IR ν_{max} cm^{-1} (KBr): 3365, 2945, 2830, 1688 and 1451; ^{1}HNMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 1; and HRESI-MS *m/z*: $[M+H]^+$ 422.4424 (calc. for $C_{25}H_{26}O_6$ 422.4444). CD (*c* = 0.125 mM, MeOH): 232 [Δε = +8.3], 252 [+1.3], 255 [+1.6], 273 [−0.1], 291 [−2.0], 307 [0], 313 [+0.2], 316

Table 1
^{1}H NMR spectroscopy data of **1**.

No	δ_H (*J* in Hz)	δ_C	HMBC ($^1H \leftrightarrow {}^{13}C$)
2	5.52, *dd*, (*J* = 3.0 Hz, 13.2 Hz)	76.2	C-4
3	3.16, *dd*, (*J* = 13.2, 17.1 Hz, H-3a)	42.4	C-2, C-4
	2.74, *dd*, (*J* = 3.0, 17.1 Hz, H-3b)		C-4
4	–	196.2	–
5	12.07 (−OH, *s*)	164.4	C-5, C-6, C-8
6	6.01, *d*, (J_m = 2.1 Hz)	95.5	C-10
7	–	164.9	–
8	6.01, *d*, (J_m = 2.1 Hz)	96.8	C-6, C-7
9	–	163.3	–
10	–	103.1	–
1′	–	124.7	–
2′	–	118.9	–
3′	–	139.7	–
4′	–	145.1	–
5′	6.93, *d*, (J_o = 8.4 Hz)	114.6	C-1′, C-3′
6′	6.86, *d*, (J_o = 8.4 Hz)	118.8	C-2, C-2′, C-4′
1″	5.71, *d*, (*J* = 9.9 Hz)	118.8	C-1′, C-2′, C-3′, C-3″
2″	6.63, *d*, (*J* = 9.9 Hz)	130.9	C-2′, C-3″, C-4″, C-5″
3″	–	79.1	–
4″	1.44, *d*	26.1	C-2″, C-3″, C-5″
5″	1.77, *m*	40.7	C-3″, C-7″, C-8″
6″	2.11, *m*	22.8	–
7″	5.11, *dt*, (*J* = 1.2, 5.7 Hz)	123.7	–
8″	–	132.1	–
9″	1.60, *s*	17.7	C-10″
10″	1.69, *s*	25.7	C-9″, C-8″, C-7″

Measured in $CDCl_3$ at 300 MHz (^{1}H) and 75 MHz (^{13}C).