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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

^1H 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 4 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 Wiyakawaruyanti [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 (^{1}H↔^{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).

[+0.1], 330 [+0.7], 338 [+0.6], 355 [0], 360 [0.3] and 365 [−0.1] nm.

3. Results and discussion

The chloroform extract of the inflorescences of *M. triloba* was fractionated using vacuum liquid chromatography (VLC) and purified by various chromatographic techniques. Seven compounds (**1**–**7**) were isolated (Fig. 1), including a new flavanone named malaysianone A (**1**). The structural elucidation was achieved through chemical and spectroscopic analysis, including HRMS, UV, ^{1}H, ^{13}C and 2D NMR (COSY, HMQC and HMBC) and by comparison with reported data.

Compound **1** was obtained as a pale yellow amorphous solid. The UV spectrum showed maxima at 232 and 329 nm, suggestive of a flavanone moiety [21]. The IR spectrum displayed strong bands for hydroxyl (3365 cm^{-1}), conjugated carbonyl (1688 cm^{-1}), and alkene (1631 cm^{-1}) groups. The HREIMS mass spectrum revealed a $[M+H]^+$ ion at *m/z* 422.4424 corresponding to the molecular formula $C_{25}H_{26}O_6$. The ^{1}H NMR spectrum (Table 1), recorded in $CDCl_3$, displayed a set of signals at δ_H 5.52 (^{1}H, *dd*, J = 13.2, 3.0 Hz), 2.74 (^{1}H, *dd*, J = 17.1, 3.0 Hz), and 3.16 (^{1}H, *dd*, J = 17.1, 13.2 Hz), diagnostic for H-2 and H-3 of a flavanone nucleus and a one-proton signal at δ_H 12.07 characteristic of the 5-OH. A doublet at δ_H 6.01 (J_m = 2.1 Hz) was assigned to a pair of *meta*-coupled protons at H-6 and H-8 of ring A. *Ortho*-coupled protons at δ_H 6.86 and 6.93 (*d*, J_o = 8.4 Hz each) were attributed to H-6′ and H-5′ of the B ring. The presence of a pair of olefinic protons at δ_H 5.71 and 6.63 (*d*, J = 9.9 Hz each) indicated that an oxygen atom at C-3′ was cyclized with C-3″ to form a pyran ring. Comparison with tanariflavanone B (**8**) [22] confirmed this observation. The remaining signals were ascribed to the aliphatic protons of the pyran and prenyl groups [δ_H 1.44 (H-4″), δ_H 1.77 (H-5″), δ_H 2.11 (H-6″), δ_H 2.11 (H-7″), δ_H 1.60 (H-9″) and δ_H 1.69 (H-10″)]. The ^{13}C NMR spectrum of **1** showed 25 carbon resonances (Table 1) including a downfield carbonyl signal at δ_C 196.2 (C-4). Five oxyaryl carbons can be observed at δ_C 139.7, 145.1, 163.3, 164.4, and 164.9, four quaternary sp^2 carbons at δ_C 103.1, 118.9, 124.7 and 132.1, and seven methine sp^2 carbons at δ_C 95.5, 96.8, 114.6, 118.8 (2 C), 123.7 and 130.9. The flavanone nucleus was evidenced from the sp^3 methylene and methine carbons at δ_C 42.4 and 76.2, respectively. The rest of the signals were accounted for six aliphatic carbons in the pyran and prenyl groups (δ_C 17.7, 22.8, 25.7, 26.1, 40.7 and 79.1).

The HMBC correlations of **1** are illustrated in Fig. 2 and tabulated in Table 1. The correlations between δ_H 6.93 with quaternary sp^2 carbon δ_C 124.7 and oxyaryl sp^2 carbon at δ_C 139.7 together with correlations showed by proton δ_H 6.86 with another oxyaryl sp^2 carbon at δ_C 145.1 confirmed the location of δ_H 6.93 and 6.86 at H-5′ and H-6′ of the B-ring, respectively. The olefinic proton at δ_H 5.71 gave ^3J HMBC correlations to δ_C 124.7 (C-1′) and quaternary sp^3 carbon δ_C 79.1, thus supporting its position at H-1″ while its COSY correlation with δ_H 6.63 established H-2″. The ^2J and ^3J HMBC correlations observed between the methyl proton at δ_H 1.44 with methine sp^2 carbon δ_C 130.9 (C-2″), δ_C 79.1 (C-3″) and methylene sp^3 carbon δ_C 40.7 (C-5″) indicated the methyl attachment to the pyran ring. Another set of HMBC correlations between the methylene protons at δ_H 1.77 with δ_C 79.1 (C-3″) and methine sp^2 carbon δ_C 123.7 supported its connectivity to the pyran and prenyl groups. Compound **1** has a positive Cotton effect at 338 nm ($\Delta\varepsilon$ +0.6) and a negative Cotton effect at 291 nm ($\Delta\varepsilon$ −2.0), thus confirming the stereochemistry of C-2 as *S* [23]. The distinct negative Cotton effect at 273 nm ($\Delta\varepsilon$ −0.1) enabled the assignment of the absolute stereochemistry of C-3″ as *R*. The structure of **1** was thus assigned as shown in Fig. 1. Based on these assignments, this new flavanone was established as malaysianone A.

Six other known compounds were identified as 6-prenyl-3′-methoxyeriodictyol (**2**) [24], nymphaeol B (**3**) [25], nymphaeol C (**4**) [26,27], 6-farnesyl-3′,4′,5,7-tetrahydroxyflavanone (**5**) [28], 5,7-dihydroxycoumarin (**6**) [29] and scopoletin (**7**) [30]. Compounds **2**, **5**, and **6** were found for the first time in the genus *Macaranga*.

Prenylated and geranylated flavanones have been reported to possess several biological activities. In this study, four major flavonoids (**2**–**5**) were subjected to antiplasmodial and cytotoxicity activities. Biological activity of **1** cannot be tested as it was obtained in small amounts. The dichloromethane crude extract of *M. triloba* was tested for its in vitro antiplasmodial activity against a chloroquine sensitive strain of *P. falciparum*

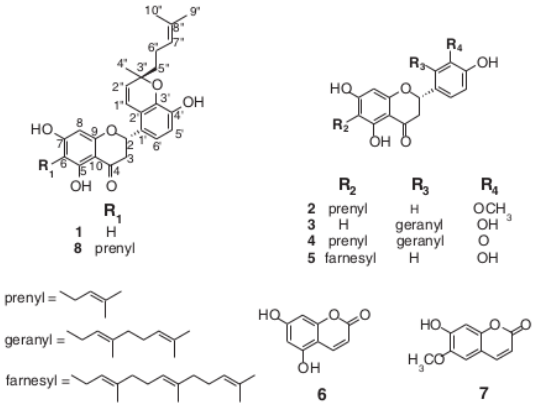

Fig. 1. Chemical constituents from the inflorescences of *Macaranga triloba*.

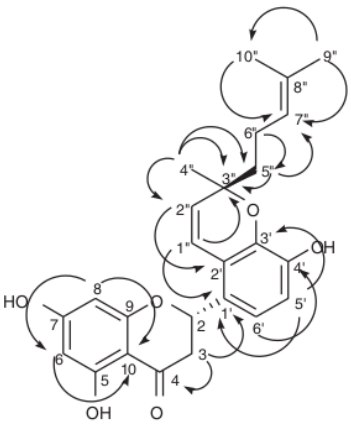

Fig. 2. Representative HMBC correlations for **1**.

Table 2
Antimalarial activity of flavonoids of *M. triloba*.

Compounds	IC_{50} (μM)
2	n.a.
3	4.02 ± 0.9
4	2.04 ± 0.6
5	0.06 ± 0.8
Chloroquine diphosphate	0.0063 ± 0.5

Each sample was tested in triplicates; the IC_{50} values were obtained from the average values of percent inhibition within a series of concentration; n.a. — no activity.

(3D7). The IC_{50} value of the crude extract (IC_{50} = 2.01 μg/ml) showed a concentration-dependent growth inhibition of the parasite *P. falciparum*, indicating the crude extract as a good potential as an antiplasmodial agent. Subsequently, the flavonoids isolated from the inflorescences of *M. triloba* were screened for their antiplasmodial activity (Table 2). Compound **5** displayed strong antiplasmodial activity with an IC_{50} value of 0.06 μM, followed by a moderate activity of **4** and **3** with IC_{50} 2.04 and 4.02 μM, respectively. Compound **2** however, showed no activity towards the *P. falciparum* strain. The presence of adjacent methoxyl and hydroxyl groups [31] or the absence of adjacent hydroxyl groups might contribute to the inactivity of compound **2**. The substituents on the flavanone skeleton clearly influenced its antiplasmodial activity which warrants further investigation.

The flavonoids were tested for cytotoxic activity against three cancer cell lines, namely HL-60 (human leukemia), MCF-7 (human breast cancer), and HeLa (human cervical cancer), using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) dye-reduction assay (Table 3). Compound **5** exhibited very strong activity against HeLa, HL-60 (IC_{50} values of 1.3, 3.3 respectively) and strong inhibition against MCF-7 cells with IC_{50} value 5.6 μg/ml. Compounds **2**, **3** and **4** demonstrated moderate activity with IC_{50} of 12.2, 17.0 and 18.2 μg/ml, respectively against HeLa cell lines and weak activity towards MCF-7 with IC_{50} 22.8, 23.5 and 23.0 μg/ml, respectively. Compounds **2** and **4** were moderately active against HL-60 cell lines with IC_{50} value of 15.1 and 11.6 μg/ml, respectively, while **3** weakly inhibited the growth of HL-60 cell line with IC_{50} 21.3 μg/ml. Based on the observation displayed on the activity of compounds **2**–**5**, we expect **1** to show similar biological activity as **3**. This is due to the similar structural property of **1** and **3**. Compound **5** which contained the farnesyl group located at C-6 was found to display strong antiplasmodial and cytotoxic activities. However, macagigantin, a farnesylated flavonol from *M. gigantea* merely showed moderate activity against P-388 cells (IC_{50} value of 11.3 μM) [32]. This compound does not possess the adjacent hydroxyl groups on ring B as compound **5**. This difference may contribute to the stronger activity displayed by compound **5**. We can conclude that the structural criteria reported in this study, showed the importance of the presence of farnesyl (on ring A) and adjacent hydroxyl groups (on ring B) on the biological activity of flavonoids.

Table 3
Cytotoxic activity of flavonoids of *M. triloba*.

Cell lines / Compounds	IC_{50} value (μg/ml)		
	HL-60	MCF-7	HeLa
2	15.1 ± 3.5	22.8 ± 0.8	12.2 ± 2.6
3	21.3 ± 3.2	23.5 ± 3.6	17.0 ± 2.8
4	11.6 ± 1.5	23.0 ± 2.4	18.2 ± 1.0
5	3.3 ± 0.6	5.6 ± 0.4	1.3 ± 0.8

IC_{50} activity (inhibition): very strong: <5 μg/ml; strong: <5–10 μg/ml; moderate: 10–20 μg/ml; weak: 20–100 μg/ml and not active >100 μg/ml.

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References

[1] Beutler JA, McCall KL, Boyd MR. A novel geranylflavone from *Macaranga schweinfurthii*. Nat Prod Lett 1999;13:29–32.
[2] Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti tyrosinase activities of four *Macaranga* species. Food Chem 2009;114:594–9.
[3] Sutthivaiyakit S, Unganont S, Sutthivaiyakit P, Suksamrarn A. Diterprenylated and prenylated flavonoids from *Macaranga denticulata*. Tetrahedron 2002;58:3619–22.
[4] Jang DS, Cuendet M, Hawthorne ME, Kardono LBS, Kawanishi K, Fong HHS, et al. Prenylated flavonoids of the leaves of *Macaranga conifera* with inhibitory activity against cyclooxygenase-2. Phytochemistry 2002;61:867–72.
[5] Schutz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from the leaves of *Macaranga pleiostemona*. Phytochemistry 1995;40:1273–7.
[6] Kumazawa S, Nakayama, Shimoi K, Goto T, Fukumoto S, Arakaki T. Flavanone compound and uses thereof. United States Patent, US 7,256,214 B2, 2007.
[7] Beutler JA, Shoemaker RH, Johnson T, Boyd MR. Cytotoxic geranyl stilbenes from *Macaranga schweinfurthii*. J Nat Prod 1998;61:1509–12.
[8] Beutler JA, Jato J, Cragg GM, Boyd MR, Schweinfurthin D. A cytotoxic stilbene from *Macaranga schweinfurthii*. Nat Prod Lett 2000;14:399–404.
[9] Li X, Xu L, Wu P, Xie H, Huang Z, Ye W, et al. Prenylflavonols from the leaves of *Macaranga sampsonii*. Chem Pharm Bull (Tokyo) 2009;57:495–8.
[10] Corner EJH. Wayside trees of Malaya, vol. 2. Kuala Lumpur: United Selangor Press; 1988.
[11] Fiala B, Maschwitz U. Studies on the South East Asian ant-plant association *Crematogaster borneensis*/*Macaranga*: adaptations of the ant partner. Insect Soc 1990;37:212–31.
[12] Fiala B, Maschwitz U, Tho YP, Helbig AJ. Studies of a South East Asian ant-plant association: protection of *Macaranga* trees by *Crematogaster borneensis*. Oecologia 1989;79:463–70.
[13] Siregar M, Sambas EN. Floristic composition of peat swamp forest in Mensemat-Sambas, West Kalimantan. Proc. Internatl. Symp. Tropic. Peatlands, 3; 2000. p. 153–64.
[14] Dinh V, Zhang HP, Duc NM, Tuu NV, Qin GW. A new geranyl flavanone from *Macaranga triloba*. J Asian Nat Prod Res 2006;8:155–8.
[15] Sultana S, Ilyas M. Chromenoflavones from *Macaranga indica*. Phytochemistry 1986;25:953–4.
[16] Yoder BJ, Cao S, Norris AJ, Miller JS, Ratovoson F, Razafitsalama J, et al. Cytotoxic prenylated stilbenes and flavonoids from *Macaranga alnifolia* from the Madagascar rainforest. J Nat Prod 2007;70:342–6.
[17] Tseng MH, Chou CH, Chen YM, Kuo YH. Alleopathic prenylflavanones from the fallen leaves of *Macaranga tanarius*. J Nat Prod 2001;64:827–8.
[18] Widyawaruyanti A, Subehan, Kalauni SK, Awale S, Nindatu M, Zaini NC, et al. New prenylated flavones from *Artocarpus champeden* and their antimalarial activity in vitro. J Nat Med 2007;61:410–3.
[19] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.
[20] Ghani NA, Ahmat N, Ismail NH, Zakaria I, Zawawi NKNA. Chemical constituents and cytotoxic activity of *Polyalthia cauliflora var. cauliflora*. Res J Med Plant 2011;6:74–82.
[21] Markham KR. Techniques of flavonoid identification. London: Academic Press Inc; 1982.
[22] Tseng MH, Kuo YH, Chen YC, Chou CH. Allelopathic potential of *Macaranga tanarius* (L.) Muell.–Arg. J Chem Ecol 2003;29:1269–86.

[23] Kawakami S, Harinantenaina L, Matsunami K, Otsuka H, Shinzato T, Takeda Y. Macaflavanones A–G, prenylated flavanones from the leaves of *Macaranga tanarius*. J Nat Prod 2008;71:1872–6.
[24] Wollenweber E, Roitman JN. New reports on surface flavonoids from *Chamaebatiaria* (Rosaceae), Dodonaea (Sapindaceae), *Elsholtzia* (Lamiaceae), and *Silphium* (Asteraceae). Nat Prod Commun 2007;2:385–9.
[25] Phommart S, Sutthivaiyakit P, Chimnoi N, Ruchirawat S, Sutthivaiyakit S. Constituents of the leaves of *Macaranga tanarius*. J Nat Prod 2005;68:927–30.
[26] Kumazawa S, Goto H, Hamasaka T, Fukumoto S, Fujimoto T, Nakayama T. A new prenylated flavonoid from Propolis collected in Okinawa, Japan. Biosci Biotechnol Biochem 2004;68:260–2.
[27] Yakushijin K, Shibayama K, Murata H, Furukawa H. New prenylflavanones from *Hernandia nymphaefolia* (Presl.) Kubitzki. Heterocycles 1980;14:397–402.
[28] Ahsan M, Gray AI, Waterman G, Armstrong JA. Farnesyl acetophenone and flavanone compounds from the aerial parts of *Boronia ramosa*. J Nat Prod 1994;57:673–6.
[29] Batsuren D, Batirov EKH, Malikov VM. Coumarins of *Haplophyllum dauricum*. 5,7-Dihydroxycoumarin and its C-glucoside. Chem Nat Compounds 1982;18:616–7.
[30] Farid HAR, Kunert O, Haslinger E, Seger C. Elucidation of iridoide and coumarin derivatives from *Xeromphis nilotica* (Rubiaceae). Monatsh Chem 2002;133:1453–8.
[31] Osman CP, Ismail NH, Ahmad R, Ahmat N, Awang K, Jaafar FM. Anthraquinones with antiplasmodial activity from the roots of *Rennelia elliptica* Korth. (Rubiaceae). Molecules 2010;15:7218–26.
[32] Tanjung M, Hakim EH, Mujahidin D, Hanafi M, Yana MS. Macagigantin, a farnesylated flavonol from *Macaranga gigantean*. J Asian Nat Prod Res 2009;11:929–32.

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