Antioxidant activity of flavonoid compounds from the leaves of Macaranga gigantea

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Antioxidant activity of flavonoid compounds from the leaves of *Macaranga gigantea*

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ABSTRACT

Three flavonoid compounds have been isolated from the leaves of Macaranga gigantea (Euphorbiaceae) namely as glyasperin A (1), broussoflavonol F (2), apigenin (3). Their structures were elucidated by spectroscopic methods including UV, IR, HRESIMS, 1D and 2D NMR analysis. Compounds 1–3 were evaluated for their radical scavenging against 2,2-diphenyl-1-picrylhydrazyl (DPPH), showing their IC_{50} were 125.10, 708.54, and 518.01 µM, respectively. The results indicate that as glyasperin A (1) more active than ascorbic acid (329.01 µM).

Keywords: Glyasperin *A*, Broussoflavonol F, Apigenin, Flavonoid, *Macaranga gigantea*, Antioxidant

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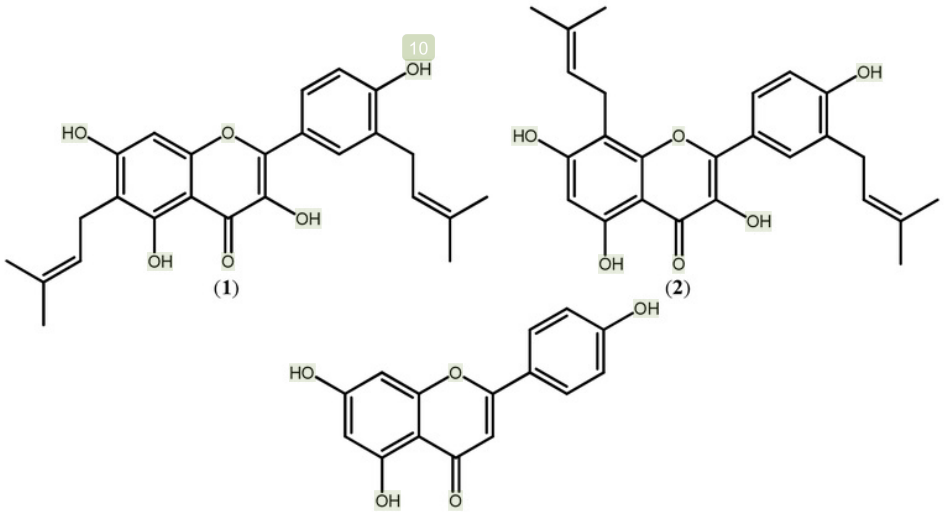
INTRODUCTION

Macaranga is one of the largest genus of the family Euphorbiaceae, comprising of about 300 species. In addition to in Indonesia, found in parts of Africa, Madagascar, Asia, the east coast of Australia, and the Pacific islands. The *Macaranga* plants are generally in the form of shrubs or trees, and grow in a place that gets a lot of sunlight in secondary forests or forests that have been damaged [1]. The phytochemical studies, this plants producing phenolic compounds, particulary flavonoid and stilbene derivatives. Structural variation of these derivatives occurs as a result of terpenoid substituents on various positions of aromatic rings. The terpenoid substituents identified include isoprenyl (C_5), geranyl (C_{10}), farnesyl (C_{15}) and geranyl-geranyl (C_{20}) [2,3,4,5]. The compound of flavonoid and stilbenoid from *Macaranga* plants exhibit various of bioactivity as antitumor, anticancer, antimalarial, antimicrobial, cyclooxygenase, and antioxidant [6,7,8,9,10,11]. In continuation of our phytochemical work of Indonesian *Macaranga* plants aiming to find new antioxidant compounds from *Macaranga gigantea*. In this paper, we report the isolation of flavonoid compounds, glyasperin A (**1**), broussoflavonol F (**2**), apigenin (**3**) from the methanol extract of the leaves of *Macaranga gigantea*. The antioxidant activity of compounds **1**– **3** against DPPH is also briefly described.

EXPERIMENTAL SECTION

The leaves of *Macaranga gigantea* were collected in July 2012 from Cinta Damai Village, District Banyuasin, South Sumatera, Indonesia. The plant was identified by Mr Ismail Rachman, Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia, and the voucher specimen was deposited in the herbarium. The dried and powdered leaves of *Macaranga gigantea* (1.5 kg) were macerated in methanol at room temprature three times, and the methanol extract was evaporated under reduced pressure to give a

dark brown residue (70 g). Furthermore, the methanol extract were partition with n-heksana and ethyl acetate. The ethyl acetate extract (40 g) was separated by vacuum liquid chromatography on silica gel eluted with *n* hexane-ethyl acetate mixture containing increasing amount of ethyl acetate (90:10, 80:20; 50:50 and 30:70) to give five major fraction A-E. On TLC analysis, fraction B (450 mg) showed two major spots on purification of this fraction using planar radial chromatography, and using n hexane-ethyl acetate (from 9:1 and 8:2) to yielded compound **1** (160 mg) and **2** (20 mg). The separation of fraction C (1.2 g) by flash chromatography with n hexane-ethyl acetate (from 8:1 and 7:3) to give three subfractions C_1-C_3. Further purification of subfraction C_3 (280 mg) by radial chromatography with n n hexane- chloroform 3:7, and chloroform to give compound **3** (18 mg).



Glyasperin A (**1**), pale yellow solid, UV (MeOH) $_{maks}$ nm (log ε): 205 (4.32); 230 sh (4.11); 254 (4.05); 271 (4.06); 347 (3.98); and 368 (3.98) nm; (MeOH + NaOH) 205 (4.80); 230 sh (4.18); 278 (4.05); 321 (3.91); and 413 (4.07); (MeOH + $AlCl_3$) 205 (4.30); 234 sh (4.04); 265 (4.10); 305 sh (3.68); 368 (3.75); and 434 (4.12) nm; ($AlCl_3$+ HCl) 206 (4.25); 234 sh (4.03); 264 (4.08); 306 sh (3.65); 348 (3.67); 368 (3.73); and 435 (4.11) nm; (NaOAc): 205 (4.89); 268 (4.08); 347 (3.73); and 435 (4.09) nm. IR (KBr) υ_{max} (cm^{-1}): 3321 (OH); 2964, 2912 (CH alkyl); 1645 (conj. C=O); and 1606-1448 (C=C aromatic). HRESIMS *m/z* $[M-H]^-$ 421.1647 (calcd for $C_{25}H_{25}O_6$, 421.1651). ^{1}H NMR (500 MHz, acetone-d6): see Table 1. ^{13}C NMR (125 MHz, acetone-d6): see Table 1.

Broussoflavonol F (**2**), pale yellow solid, UV (MeOH) $_{maks}$ nm (log ε): 204 (4.36); 230 sh (4.19); 256 (4.20); 340 (3.58); 273 (4.10); 346 (4.02); and 364 (3.12) nm; (MeOH + $AlCl_3$) 204 (4.80); 232 sh (4.20); 270 (4.10); and 432 (4.27) nm; ($AlCl_3$+ HCl) 204 (4.25); 232 sh (4.09); 270 (4.12); 308 sh (3.69); 350 (3.70); 368 (3.80); and 432 (4.15) nm; (NaOAc): 204 (4.90); 266 (4.10); 346 (3.75); and 435 (4.11) nm. HRESIMS *m/z* $[M-H]^-$ 421.1657 (calcd for $C_{25}H_{25}O_6$, 421.1651). ^{1}H NMR (400 MHz, acetone-d6): see Table 2. ^{13}C NMR (100 MHz, acetone-d6): see Table 2.
Apigenin (**3**), yellow solid, UV (MeOH) $_{maks}$: 205 (4.49); 228 sh (4.29); 275 (4.18); 300 (4.10); 341 (4.12), and 383 (4.00) nm, (MeOH + $AlCl_3$) 205 (4.50); 230 sh (4.25); 276 (4.17); 300 (4.08); 341 (4.11); and 382 (3,99) nm; ($AlCl_3$ + HCl) 205 (4,49); 228 sh (3,86); 276 (4,18); 300 (4,10); 343 (4,12); and 385 (4,00) nm; (NaOAc) 202 (4,76); 272 (4,18); 300 (4,05); 344 (4,01); and 356 (3,59) nm. EIMS: *m/z* (% relatif): 270 (M^+, 100); 242 (16); 153 (17); and 121 (11). ^{1}H-NMR (500 MHz, aseton *d*-6) δ_H (ppm): 13.00 (1H, *s*, 5-OH); 9.71 (1H, *s*, 7-OH); 9.28 (1H, *s*, 4'-OH); 7.92 (2H, *d*, *J* = 9.1 Hz, H-2'/H-6'); 7,01 (2H, *d*, *J* = 9.1 Hz, H-3'/H-5'); 6.62 (1H, s, H-3); 6.53 (1H, *d*, *J* = 2.5 Hz, H-8); and 6.24 (1H, *d*, *J* = 2.5 Hz, H-6).

DPPH scavenging activity test: Determination of the antioxidant activity of the isolated performed using reagent DPPH (2,2-diphenyl-1-pikrihidrazil) using methods of reduction of free radicals as measured by UV spectrometer at λ 517 nm [12,13,14]. Determination of antioxidant activity done by the dissolving a compounds assay with

methanol, then added solution of 0.1 M buffer acetate (pH 5.5) and added DPPH radical solution of 5.10^{-4} M. Determination of the inhibition of isolated compounds against DPPH radical was observed using a spectrometer at λ 517 nm after incubation for 30 min at 20°C.

RESULTS AND DISCUSSION

Three flavonoids, glyasperin A (**1**), broussoflavonol F (**2**), apigenin (**3**) have been isolated from the the leaves of *Macaranga gigantea*. Their structures were elucidated by spectroscopic methods including UV, IR, HRESIMS, 1D and 2D NMR spectrum.

Table 1. NMR spectroscopic data of glyasperin A (1)

No.C	δ_H (mult, *J* Hz)	δ_C	HMBC
2	-	147.0	-
3	-	136.4	-
4	-	176.4	-
4a	-	103.8	-
5	-	158.7	-
6	-	111.6	-
7	-	162.9	-
8	6.56 (*s*)	93.7	C-4a, C-6, C-7, C-8a
8a	-	155.5	-
1'	-	128.9	-
2'	8.00 (*d*, 2.5)	130.1	C-2, C-4', C-6'
3'	-	132.9	-
4'	-	157.9	-
5'	6.97 (*d*, 8.5)	115.6	C-1', C-3', C-4'
6'	7.91 (*dd*, 8.5; 2.5)	127.8	C-2, C-2', C-4'
1''	3.30 (*d*, 7.4)	21.8	C-2, C-3, C-4', C-2''
2''	5.23 (*tm*, 7.4)	123.2	C-2, C-3, C-4', C-2''
3''	-	131.5	-
4''	1.77 (*s*)	25.8	C-3
5''	1.64 (*s*)	18.0	C-1', C-3', C-4'
1'''	3.34 (*d*, 7.3)	29.9	C-3''', C-4''', C-2''', C-3''', C-4'''
2'''	5.33 (*tm*, 7.3)	123.1	C-1''', C-4''', C-5'''
3'''	-	131.5	-
4'''	1.74 (*s*)	25.8	C-2''', C-3''', C-5'''
5'''	1.74 (*s*)	17.8	C-2''', C-3''', C-4'''
3-OH	7.85 (*br*, *s*)	-	C-3
5-OH	12.41 (*br*, s)	-	C-4a, C-5, C-6
7-OH	9.78 (*br*, s)	-	C-7
4'-OH	8.89 (*br*, s)	-	C-4'

Glyasperin A (**1**) was obtained as pale yellow solid, showed a quasimolecular ion $[M-H]^-$ at *m/z* 421.1647 consistent to the molecular formula $C_{25}H_{25}O_6$. The UV spectrum of **1** exhibited absorption maxima for a flavonol strucrure at λ_{max} 205, 230 sh, 254, 271, 347, and 368 nm, and showed a bathocromic shift on addition of $AlCl_3$, $AlCl_3$+ HCl, and NaOAc [15]. The IR spectrum indicated absorptions for hydroxyl (3321 cm^{-1}), conyugated carbonyl (1645 cm^{-1}), and aromatic (1606-1448 cm^{-1}) groups. The ^{1}H-NMR spectrum of compound **1** showed three aromatic proton signals for ABX system at δH 8.00 (1H, *d*, *J* = 2.5 Hz); 7.91 (1H, *dd*, *J* = 8.5, 2.5 Hz); and 6.97 ppm (1H, *d*, *J* = 8.5 Hz) corresponding to the group substitutent at C-3 'and C-4' in ring B flavonols which also showed an isoprenyl group at C-3'. A singlet signal at 6:56 ppm in the aromatic region of the ^{1}H-NMR spectrum showed one isoprenyl group at A ring of flavonol strucrure. The existence of two isoprenyl chain of compound **1** showed the presence of four methyl groups (δH 1.74, 1.71, 1.70, and 1.60 ppm), two methylene groups (3:34 and 3:30 ppm), two vinyl groups (δH 5:33, and 5:23 ppm). In the ^{13}C NMR (APT experiment, Table 1) showed 22 carbon signals representing 25 carbon atoms were observed. Two of signals at δ_C 136.4 and 176.5 are characteristic for C-3 and C-4 of a flavonol structure [x]. Five carbon signals (δC 147.0; 155.5; 157.9; 158.7; and 162.9 ppm) characteristic for the region oksiaril signals which indicate that the structure is a derivative of kaempferol. The correlation of the one bond and the two/three bond ^{1}H-^{13}C compound **1** can be seen in the HMQC and HMBC spectra (Table-1). The presence of an isoprenyl group at C-6 shows in the HMBC spectrum, the long-range correlation between a proton signal chelate-OH group at δH 12.41 ppm with three quaternary atoms at δC 103.8 (C-4a), 111.6 (C-6), and 158.7 (C-5). This is supported by the correlation between a methylene proton signal at δH 3.30 ppm with three quaternary atoms at δC 111.6 (C-6), 158.7 (C-5); and162.9 (C-7). Based on data from 1D and 2D NMR of compound **1** is 6,3 '-

diisoprenylkaempferol or known as glyasperin A [16]. Other HMBC correlations consistent with the structure **1** are shown in Table 1.

Broussoflavonol F (**2**) was also obtained as pale yellow solid. The ion peak at *m/z* 421.1657 $[M-H]^-$ in the HRESIMS spectrum gave the molecular $C_{25}H_{25}O_6$, which was isomeric with **1**. Based on UV, ^1H NMR, ^{13}C NMR and HMQC spectrum, the compound **2** identical with compound **1**. The placement of isoprenyl at C-8 shown in HMBC spectrum (Table 2). The presence of long-range correlations in the HMBC spectrum of **2** between the singlet proton of a chelated –OH group at δ_H 12.09 with two quarternary at δ_C 104.1 (C-4a), 159.8 (C-5), and one methin carbon at δC 98.9 (C-6) showed isoprenyl at C-8. The presence of isopreny group at C-8, the correlation between a methylene proton signal at δ_H 3.55 with four quaternary atoms at δ_C 103.8 (C-4a), 133.3 (C-3"), 154. 9 (C-8a), and 161.9 (C-7). Based on data from 1D and 2D NMR, compound **2** is 8,3'-diisoprenylkaempferol or known as broussoflavonol F [16]. Other HMBC correlations consistent with the structure **2** are shown in Table 2.

Table 2. NMR spectroscopic data of broussoflavonol F (2)

No.C	δ_H (mult, *J* Hz)	δ_C	HMBC
2	-	147.1	-
3	-	136.4	-
4	-	176.7	-
4a	-	104.1	-
5	-	159.8	-
6	6.34 (*s*)	98.9	C-4a, C-5, C-7, C-8
7	-	161.9	-
8	-	107.1	-
8a	-	154.9	-
1'	-	123.7	-
2'	8.04 (*d*, 2.4)	129.8	C-2, C-3', C-4', C-2''
3'	-	129.1	-
4'	-	157.7	-
5'	7.01 (*d*, 7.2)	128.2	C-2, C-2', C-4'
6'	8.05 (*dd*, 7.2; 2.4)	115.7	C-1', C-2', C-4'
1''	3.55 (*d*, 7.4)	22.2	C-5, C-6, C-7, C-2'', C-3''
2''	5.39 (*d*, 1.6)	123.3	C-1'', C-4'', C-5''
3''	-	133.3	-
4''	1.65 (*s*)	18.1	C-2'', C-3'', C-5''
5''	1.80 (*s*)	25.9	C-2'', C-3'',C-4''
1'''	3.40 (*d*, 7.6)	29.0	C-2', C-3', C-4', C-2''', C-3'''
2'''	5.31 (*t*, 1.6)	123.0	C-1''', C-4''', C-5'''
3'''	-	132.0	-
4'''	1.74 (*s*)	25.9	C-2''', C-3''', C-5'''
5'''	1.74 (*s*)	17.8	C-2''', C-3''', C-4'''
3-OH	7.89 (*br,s*)	-	C-3
5-OH	12.09 (*br, s*)	-	C-4a, C-5, C-6
7-OH	6.58 (*br, s*)	-	C-7
4'-OH	8.96 (*br, s*)	-	C-4'

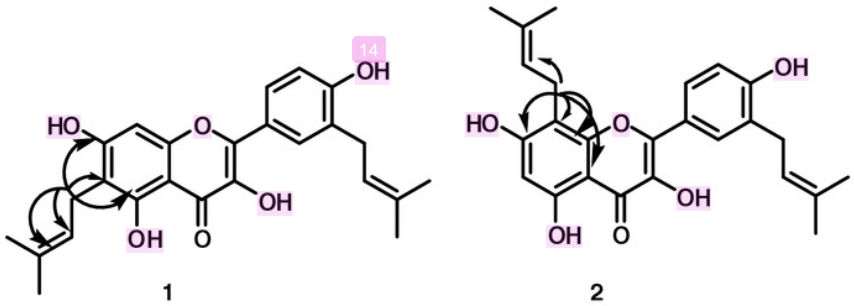


Figure 1. The difference of HMBC spectrum for compound 1 and 2

Apigenin (**3**) was obtained as yellow solid, showed ion peak at *m/z* 270 $[M]^+$ in the EIMS spectrum. The UV spectrum of **3** exhibited absorption maxima for a flavon strucrure at λ_{max} 205, 228 sh, 275, 300, 341, and 383, and showed a bathocromic shift on addition of $AlCl_3$, $AlCl_3$+ HCl, and NaOAc. The ^{1}H-NMR spectrum of compound **3** showed a pair of doublets (J = 9.1 Hz) in the aromatic region at δ_H 7.92 and 7.01 (each 2H), suggested the signal of a *p*-hydroxyphenyl at B ring. The presence of a pair of doublets (J = 2.5 Hz) at δ_H 6.53 and 6.24 in A ring corresponding to the proton at H-8 and H-6. The presence of a singlet signal at δ_H 6.62 suggested that the placement H-3 for a flavon strucrure. Based on data from UV, EIMS and ^{1}H NMR, compound **3** is 5,7,4'-trihydroxyflavone or known as apigenin [17].

The antioxidant activity of glyasperin A (**1**), broussoflavonol F (**2**), apigenin (**3**) at different concentration (1000, 500, 250, 100,and 50 µM) were evaluated against the DPPH radical scavenging. The IC_{50} values of glyasperin A (**1**), broussoflavonol F (**2**), apigenin (**3**) showed radical scavenging activity with IC_{50} values 125.10, 708.54, and 518.01 µM, respectively. Ascorbic acid as positive control have IC_{50} 329.01 µM. The results of antioxidant activity showed glyasperin A (**1**) more active than ascorbic acid. Glyasperin A (**1**), and broussoflavonol F (**2**) are isomeric structure. The placement of isoprenyl at C-6 to increasing antioxidant activity than at C-8.

CONCLUSION

Two isomeric isoprenyl flavonols, glyasperin A (**1**) and broussoflavonol F (**2**) together apigenin (**3**) have been isolated from the leaves of *Macaranga gigantea*. Apigenin is a flavone derivative and first time found in the genera of *Macaranga*. The antioxidant activity of compounds **1-3** were evaluated by measuring their ability to scanvenge the DPPH radical showed glyasperin A>apigenin>broussoflavonol F. The result indicate thet compound **1** to give very high activity than ascorbic acid as positive control. The structure-activity relationship of compounds **1–2** against DPPH radical scavenging suggested that the presence of isoprenyl group at C-6 on glyasperin A more active than the isoprenyl group at C-8 on broussoflavonol F.

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