CHEMICAL CONSTITUENTS OF *MACARANGA ANDENOCERAS* AND THEIR CYTOTOXICITY AGAINST P-388 CELLS

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ABSTRACT

Macaranga, locally known as "mahang-mahangan", is one of the plant genera belonging to Euphorbiaceae family, consisting of about 300 species. Phytochemical investigation revealed that *Macaranga* plants produce mostly prenylated flavonoid and stilbene derivatives. Structural variation of these derivatives occurs as a result of the attachment of terpenoid substituents on various positions of aromatic rings. The terpenoid substituents identified include prenyl (C_5), geranyl (C_{10}), farnesyl (C_{15}), and labdanyl (C_{20}) groups, as well as their chemical modifications, particularly on the prenyl and geranyl substituents. Some of the isolated compounds had been evaluated for their biological properties, including as antioxidants, COX inhibitors, cytotoxicities, and as plant growth regulators. In continuing our study on Indonesian *Macaranga,* isolation of secondary metabolites from *Macaranga andenoceras* collected from Manokwari, Papua, Indonesia, has been conducted. A new flavonol derivative, macaflavonol (**1**), together with four known compounds, broussonol E (**2**), glyasperin A (**3**), kaempferol (**4**), and quercetin (**5**), has been isolated from the acetone extract of the leaves of this species. These compounds were obtained using several chromatographic techniques. The structures of the isolated compounds were identified based on NMR and mass spectral data. Cytotoxic properties of compounds **1** - **5** were evaluated against murine leukemia P-388 cells, showing that compounds **1** - **3** were strongly inhibited the cells with IC_{50} 10 - 13 μM. The presence of a pyran ring in compound **1** could be important for cytotoxic activity.

Keywords : *Macaranga andenoceras*, cytotoxic, murine leukemia P-388 cells

Introduction

The genus *Macaranga* (Euphorbiaceae) comprises about 300 species, which its distribution covers the region from Africa and Madagascar in the West to tropical Asia, North Australia, and Pacific islands in the East [1]. The presence of phenolic compounds, particularly flavonoids and stilbenoids derivatives, has been well documented (for examples: [2,3]). In our previous reports, we have disclosed the presence of isoprenylated, geranylated, and farnesylated flavonoids [4-8] and phenolic derivatives containing an irregular sesquiterpenyl side chain [9,10], from Indonesian *Macaranga*. In continuation of these chemical investigation with the aim to find new bioactive compounds, we have examined a species, *M. adenoceras* Zoll., and succeeded to isolate a new isoprenylated flavonol, trivially name macaflavonol (**1**), together with four known flavonol derivatives **2** – **5** (Fig. 1). In this paper isolation and structure elucidation of these compounds will be discussed. Also, cytotoxic properties of compounds **1** - **5** against murine leukemia P-388 cells will briefly be described.

Experiment

General experimental procedures

NMR spectra were recorded with an NMR spectrometer of Agilent DD2 system operating at 500 (^{1}H) and 125 (^{13}C) MHz using residual and deuterated solvent peaks as reference standards. High resolution mass spectra were obtained with a Waters LCT Premier XE mass spectrometer using positive mode of ESI. Vacuum liquid (VLC) and planar centrifugal chromatography (PCC) were carried out using Merck silica gel 60 GF_{254} art. 7731 and 7749, respectively. For TLC analysis, precoated silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm) were used. Solvents for extraction and purification were of technical grades, which were distilled before used.

Plant material

The leaves of *M. adenoceras* were collected from Papua Island, Indonesia, in August 2012. The plant was identified by the Herbarium of State University of Papua, Indonesia, and the voucher specimen was deposited in the herbarium.

Extraction and isolation

The dried and powdered leaves of *M. adenoceras* (500 g) were macerated in acetone at room temperature (3 x 3 L) and the acetone extract was evaporated under reduced pressure to give semisolid residue (60 g). The extract was fractionated through VLC coloumn, eluted with *n*-hexane-EtOAc of increasing polarity (4:1, 3:1, 7:3, 3:2, and 1:1) to give six major fractions A-F. Refractination of the fraction A (820 mg) with sephadex LH-20 coloumn eluted with MeOH gave eight fractions A1-A8, and on purification of the fraction A6 using PCC eluted with $CHCl_3$-EtOAc (9:1) afforded macaflavonol (**1**) (3 mg). Refractination of the fraction B (1.5 g) with sephadex LH-20 coloumn eluted with MeOH gave seven fractions B1-B7, and on purification of the fraction B5 using PCC eluted with *n*-hexane- EtOAc (9:1 to 7:3) afforded glyasperin A (**3**) (240 mg). Refractination of the fraction D (1.2 g) with sephadex LH-20 coloumn eluted with MeOH give seven fraction (D1-D7) and on purification of fraction D3 using PCC eluted with *n*-

hexane-EtOAc (9:1 to 8:2) afforded broussonol E (**2**) (57 mg). Using the same [PCC, eluted with *n*-hexane-EtOAc (7:3)], purification of fraction D6 afforded kaempferol (**4**) (16 mg) and quercetin (**5**) (26 mg).

Cytotoxicity assay

The cytotoxicity assay was conducted according to the method described previously by us [11]. P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3 x 10^4 cells cm^{-3}. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequent six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30 - 7.65). Control wells received only DMSO. The assay was terminated after a 48 h incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 h incubation was conducted. Optical density was read by using a micro plate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (μM). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

Results and Discussion

Compound **1** was isolated as a yellow solid and the molecular formula $C_{25}H_{24}O_6$ was assigned to **1** from HRESIMS ($[M+H]^+$ ion at *m/z* 421.1648, calcd. for $C_{25}H_{24}O_6$ 421.1651) suggesting that **1** is a diisoprenylated flavonoid. The ^{13}C NMR spectrum of **1** (Table I) exhibited a C=O and a quarternary carbon signals at δ_C 175.1 and 135.4, respectively, that are characteristics to the flavonol. The ^{13}C NMR spectrum also showed other five =C-O- carbon signals (δ_C 161.5, 157.7, 155.0, 154.9, and 145.5), suggesting that **1** has a kaempferol structure. In the aromatic region of ^1H NMR spectrum (Table I), an ABX spin system (δ_H 7.97, 7.85, and 6.89) and a singlet signal (δ_H 6.48) were observed, indicating that either C-6 or C-8 and C-3' are substituted. A free isoprenyl group and a dimethylchromene moiety were assigned to the signals of a triple multiplet of vinil methine (δ_H 5.29), a doublet methylene (δ_H 3.47), and a pair of doblet of a *cis*-1,2-disubstituted-etene (δ_H 6.41 and 5.68), as well as four methyl singlets (δ_H 1.85, 1.78, 1.48, and 1.48). The isoprenyl group was determined to be located at C-6 from the observation of long range ^1H-^{13}C correlations in the HMBC spectrum (Table I) between a chelated –OH signal (δ_H 12.10) with three quarternary C-sp^2 carbon signals (δ_C 157.7, 109.3, and 103.5). The dimethylchromene moiety, therefore, was located at C-3'-C-4'-O- in the ring B of kaempferol structure, which also support by the long range ^1H-^{13}C correlations as shown in the Table I. Thus, structure **1** was assigned to macaflavonol, and other HMBC correlations are also consistence to the structure assignment (Table I).

The NMR data of compounds **2** – **5** (data are not shown) were consistent to the structures of broussonol E (= papyriflavonol A) [12], glyasperin A [13], kaempferol [14], and quercetin [15], respectively. Although compound **4** is widely distributed in the plant kindom, it is the first time to be isolated from *Macaranga* plants.

Table I. NMR data of compound **1** in acetone-d_6.

No.	δ_H (multiplicity, J in Hz)	δ_C	HMBC ($^1H \Leftrightarrow {}^{13}C$)
2	-	145.5	-
3	-	135.4	-
4	-	175.1	-
4a	-	103.5	-
5	-	157.7	-
6	-	109.3	-
7	-	161.5	-
8	6.48 (*s*)	94.2	C-4a, C-6, C-7, C-8a
8a	-	154.9	-
1'	-	123.2	-
2'	7.85 (*d*, 2.1)	125.9	C-2, C-4', C-6', C-9"
3'	-	121.1	-
4'	-	155.0	-
5'	6.89 (*d*, 8.6)	116.6	C-1', C-3', C-4'
6'	7.97 (*dd*, 8.6, 2.1)	128.9	C-2', C-4'
1"	3.47 (*d*, 7.0)	21.5	C-5, C-6, C-7, C-2", C-3"
2"	5.29 (*tm*, 7.0)	121.0	C-4", C-5"
3"	-	136.1	-
4"	1.85 (*s*)	17.9	C-2", C-3", C-5"
5"	1.78 (*s*)	25.9	C-2", C-3", C-4"
1"'	6.41 (*d*, 9.8)	122.0	C-2', C-3', C-4', C-3"'
2"'	5.68 (*d*, 9.8)	131.2	C-3', C-3"', C-4"', C-5"'
3"'	-	77.3	-
4"'	1.48 (*s*)	28.3	C-2"', C-3"', C-5"'
5"'	1.48 (*s*)	28.3	C-2"', C-3"', C-4"'
3-OH	6.59 (*s*)	-	C-2, C-3, C-4
5-OH	12.10 (*s*)	-	C-5, C-6, C-4a
7-OH	6.26 (*s*)	-	-

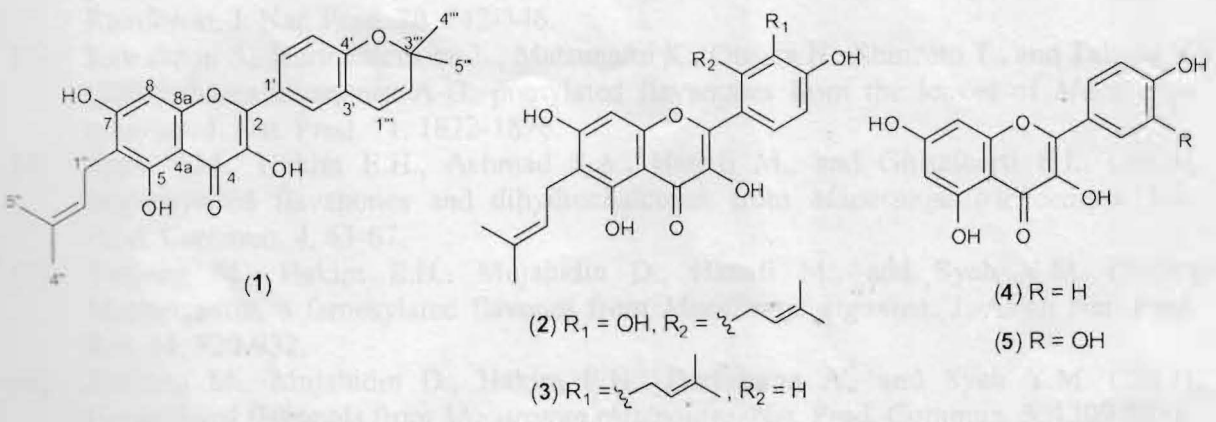

Fig. 1. The constituents isolated from *M. adenoceras*.

Cytotoxic properties of compounds **1** - **5** were evaluated against murine leukemia P-388 cells according to the method described previously [11], showing their IC_{50} were 10.52, 13.27, 12.99, 33.18, and 67.69, respectively. Compounds **1** – **3** exhibited strong cytotoxic properties while compound **4**, the unisoprenylated flavonol, was weakly active, and compound **5** was inactive. Thus, *C*-isoprenylation at the rings A and B of the flavonol greatly enhances its cytotoxic properties. The presence of a pyran ring in compound **1** could be important for cytotoxic activity.

Conclusion

Five compounds have been isolated from *Macaranga andenoceras*, one of them is new compound, namely macaflavonol Four known compounds are glyasperin A, broussonol E, Kaempferol, and Quercetin. Macaflavonol, glyasperin A, and broussonol E exhibited strong cytotoxic properties while Macaflavonol have highest activity, with IC_{50} 10,52 µM. Kaempferol is weakly active and Quercetin was not active. Presence of pyran ring in macaflavonol could be important for cytotoxic activity.

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