

A Flavonoid Glycoside Compound Isolated from *Macaranga gigantifolia* Merr Leaves

Gian Primahana¹, Akhmad Darmawan^{1,*}

¹Research Center for Chemistry, Indonesian Institute of Sciences, Kawasan PUSPIPTEK, Serpong, Banten, Indonesia.

*Corresponding author : ahmaddarmawan2013@gmail.com

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ABSTRACT

Apigenin-8-C-glycoside (1), a flavonoid glycoside compound has been isolated from the leaves of *Macaranga gigantifolia* Merr. Isolation and purification was conducted by chromatography methods and chemical structure characterization was carried out by spectroscopic methods. Cytotoxicity of apigenin-8-C-glycoside has been tested against Murine leukemia P-388 cell lines and has moderate activity with IC₅₀ values 55.4 µg/mL.

Key words: Apigenin-8-C-glycoside, *Macaranga gigantifolia* Merr., Murine leukemia P-388 cell lines.

INTRODUCTION

Macaranga gigantifolia Merr. (Euphorbiaceae), known locally as mahang-mahangan (Indonesia) is one of about 300 species member of genus *Macaranga* [1]. Some of species, including *Macaranga tanarius*, *Macaranga denticulate*, *Macaranga adenantha*, and *Macaranga alnifolia* has been extensively studied, indicated that flavonoid and terpenoid are the major compounds of the *Macaranga* genus [1-10]. Many part of this species has been used as traditional medicines. Root of *M. denticulate* has been used in Traditional Chinese Medicine for treatment icteric hepatitis [7], *M. indica* used for treatment anemia and tumor in India and Bangladesh [11], *M. peltala* used to relieve from venereal sores and also reported that active as antioxidant, antimicrobial and cytotoxic activity [12].

As part of our study about natural products drug discovery, including exploration and the potentiality bioactive plants origin from Indonesia, ethyl acetate fraction of methanol extract of *M. gigantifolia* Merr. leaves were separated using column chromatography method. Apigenin (5,7,4'-trihydroxyflavone) and its glycosides which has been reported active as anticancer, anti-inflammatory and antioxidant [13-16], is naturally abundant component from common flavonoids plants found in flowers, fruits, vegetables, beans and tea. To the best of author knowledge, there is lack of paper published about chemical content of *M. gigantifolia* plant. Previous study reported that scopoletin, macarangin, and apigenin have been isolated from *M. gigantifolia* leaves [17-19]. Here in this paper, for the first time we reported the isolation of apigenin-8-C-glycoside (1) from the methanol extract of *M. gigantifolia* Merr. leaves.

EXPERIMENT

Chemicals and instrumentation

All chemical used were obtained from Merck with pro analytical grade. Solvent used for maceration and column chromatography is technical grade and redistilled before use. 1D and 2D-NMR spectra were recorded on JEOL JNM-ECA 500 spectrometer with TMS as internal standard. LC-MS were measured with Mariner Biospectrometry-Finnigan instrument. Column chromatography method was carried out with silica gel (200-300 mesh, Kieselgel 60, Merck) using n-hexane:ethyl acetate as solvent system for isolation and silica gel 60 F₂₅₄ (Merck) for TLC with 5% H₂SO₄ in ethanol as compound detection reagent.

Plant material

The leaves of *Macaranga gigantifolia* Merr was collected from Mekongga Forest, District of Kolaka, Southeast Sulawesi, Indonesia in March 2012. The plant was determined at Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

Isolation of compound I

About 80 grams MeOH extract was partitioned with *n*-hexane and EtOAc successively. The EtOAc soluble fraction (17.4 g) was chromatographed over silica gel column, and eluted with gradient solvent system of *n*-hexane:EtOAc started from 20% ethyl acetate to 40% ethyl acetate with 5% increasement to obtain 5 fractions (F1-F5). Compound 1 (28 mg) was crystallized in the F1 bottle (eluted with 20% ethyl acetate) after evaporation and re-diluted with EtOAc and further successively purified with CHCl₃, acetone and MeOH.

Compound 1: yellow powder, ESI-MS *m/z* 431.0963 [M-H], NMR (JEOL JNM-ECA 500) δ_{H} (500 MHz, in DMSO-*d*₆): 6.78 (1H, s, H-2), 6.27 (1H, s, H-6), 8.02 (2H, d, *J*=8.9 Hz, H-2'/H-6'), 6.88 (2H, d, *J*=8.9 Hz, H-3'/H-5'), 4.68 (1H, d, *J*=9.7 Hz, H-1''), 3.37 (1H, t, H-4''), 3.24 (2H, m, H-3''/H-5''), 3.83 (1H, t, H-2''), 3.52 (1H, m, H-6''), 3.75 (1H, d, H-6''). δ_{C} (125 MHz, in DMSO-*d*₆): 163.9 (C-2), 102.4 (C-3), 182.1 (C-4), 104.0 (C-10), 162.5 (C-5), 98.1 (C-6), 160.4 (C-7), 104.6 (C-8), 155.9 (C-9), 121.6 (C-1'), 129.0 (C2'/C6'), 115.8 (C-3'/C-5'), 161.1 (C-4'), 73.4 (C-1''), 70.5 (C-4''), 78.6 (C-3''), 70.8 (C-2''), 81.8 (C-5''), 61.3 (C-6'').

Cytotoxic activity

Cytotoxic activity assay was conducted using MTT assay method [20]. Approximately 3 x 10⁴ cell cm⁻³ of P-388 murine leukemia cells were plated in 96 well culture dishes and incubated at 37°C for 24 hours. Various concentrations of sample in DMSO were added after incubation. Six desirable sample concentrations were adjusted by addition of PBS (phosphoric buffer solution, pH = 7.30-7.65) solutions, except for control. After 48 h incubation, assay was stopped by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and incubation continue for next 4 h before the addition of MTT stop solution containing sodium dodecyl sulphate (SDS). After addition of MTT stop solutions, incubation was continued to the next 24 h. Optical density was measured with micro plate reader at 550 nm. IC₅₀ was obtained from the plotted graph between percentages of live cells compared to control against various concentration of the tested sample ($\mu\text{g/mL}$).

RESULT AND DISCUSSION

Dried *M. gigantifolia* leaves extracted with methanol and further were partitioned successively with *n*-hexane and EtOAc. EtOAc fraction was chromatographed over silica gel column chromatography, eluted with a gradient solvent system of *n*-hexane:EtOAc (8:2 to 6:4) and was obtained 5 fractions (F1-F5). Compound **1** was crystallized in the F1 bottle (eluted with 20% ethyl acetate) after evaporation and re-diluted with EtOAc and further successively purified with CHCl₃, acetone and MeOH.

The ¹H-NMR and ¹³C-NMR spectra of compound **1**, showed 4 aromatic protons at δ_H 6.88 (2H, d, *J*=8.9 Hz)/δ_C 115.8, and 8.02 (2H, d, *J*=8.9 Hz)/δ_C 129.0, indicated the presence of A₂B₂ ring system. In addition, singlet signals at δ_H 6.27/δ_C 98.1, and 6.78/δ_C 102.4, were belong to two proton atom which are attached to ring A and C, respectively. The position of two singlet proton which mentioned above was supported by the correlation data of HMBC (Fig. 2). Based on this chemical shift data, compound **1** was predicted as apigenin derivative. The anomeric proton (H-1'') at δ_H 4.68 (d, 1H, *J*=9.7 Hz) correlated to carbon peak at δ_C 73.4 indicated that the linkage between the sugar residue and aglycone (flavone) is *C*-glycosidic system at C-8 of the aglycon carbon atom. The *C*-glycosidic linkage was confirmed by HMBC correlations between anomeric proton at δ_H 4.68 with carbon atom at δ_C 104.6 (C-8) and 156.0 (C-8a) (Fig. 2). Mass spectroscopic analysis using LC-MS (Fig. 1) showed that compound **1** has molecular peak at *m/z* 431.0963 [M-H] correspond to molecular weight 432.0963 with molecular formula C₂₁H₂₀O₁₀. From the 1D- and 2D-NMR data, confirmed by mass spectroscopic data and compared with the reference (Table 1) [21], compound **1** was identified as Apigenin-8-*C*-glycoside (Fig. 2). This is the first report apigenin-8-*C*-glycoside isolated from *M. gigantifolia* Merr. plant.

Table 1. ¹H- and ¹³C-NMR Data of Compound 1 and apigenin-8-*C*-glycoside

| No. | Compound 1 | | Apigenin-8- <i>C</i> -glucoside [21] | |
|-------|---|---------------------------------------|---|---------------------------------------|
| | ¹ H-NMR (δ _H , ΣH, mult. <i>J</i> in Hz) | ¹³ C-NMR (δ _C) | ¹ H-NMR (δ _H , ΣH, mult. <i>J</i> in Hz) | ¹³ C-NMR (δ _C) |
| 2 | - | 163.9 | - | 163.85 |
| 3 | 6.78 (s) | 102.4 | 6.76 (s) | 102.39 |
| 4 | - | 182.1 | - | 182.01 |
| 5 | - | 162.5 | - | 163.85 |
| 6 | 6.27 (s) | 98.1 | 6.25 (s) | 98.31 |
| 7 | - | 160.4 | - | 160.42 |
| 8 | - | 104.6 | - | 104.66 |
| 9 | - | 156.0 | - | 156.04 |
| 10 | - | 104.0 | - | 103.77 |
| 1' | - | 121.6 | - | 121.59 |
| 2'/6' | 8.02 (2H, d, 8.9) | 129.0 | 8.01 (d) | 128.96 |
| 3'/5' | 6.88 (2H, d, 8.9) | 115.8 | 6.88 (d) | 115.86 |
| 4' | - | 161.1 | - | 161.25 |
| 1'' | 4.68 (d, 9.7) | 73.4 | 4.70 (d) | 73.43 |
| 2'' | 3.83 (t) | 70.8 | 3.84 (t) | 70.88 |
| 3'' | 3.24 (m) | 78.6 | 3.27 (m) | 78.70 |
| 4'' | 3.37 (t) | 70.5 | 3.38 (t) | 70.55 |
| 5'' | 3.24 (m) | 81.8 | 3.26 (m) | 81.84 |
| 6'' | 3.52 (m) | 61.3 | 3.52 (m) | 61.30 |
| | 3.75 (d) | | 3.77 (d) | |

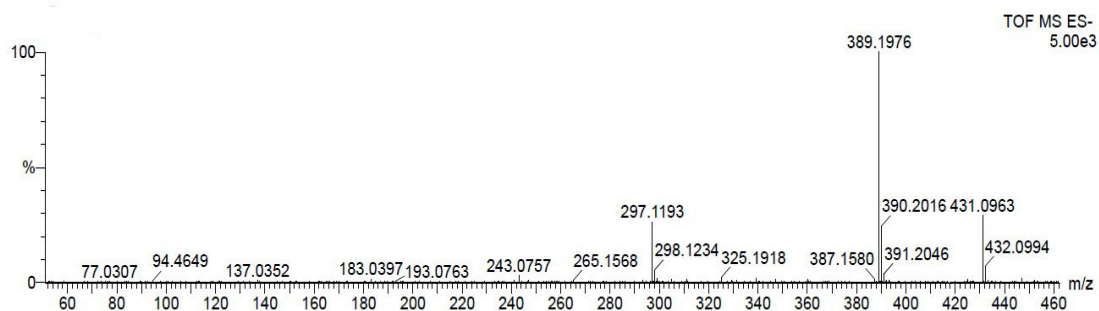


Figure 1. LC-MS spectrum of compound 1

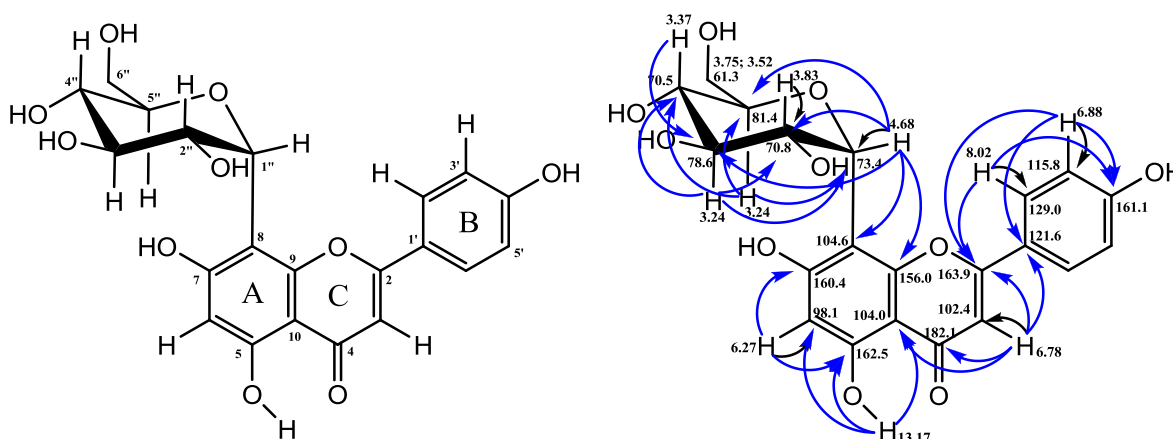


Figure 2. Chemical structure of apigenin-8-C-glycoside (1) and their HMQC-HMBC Correlation

Cytotoxicity activity of compound **1** against Murine Leukimia P-388 cancer cell lines was performed using MTT assay. Calculation of IC_{50} showed that Apigenin-8-C-glycoside has moderate activity with IC_{50} 55.4 μ g/mL.

CONCLUSION

After isolation, purification and characterization of the phenolic compound isolated from ethyl acetate fraction of the methanol extract *M. gigantifolia* leaves, it can be concluded that compound **1** is apigenin-8-C-glycoside, which have moderate cytotoxicity activity against *Murine leukemia* P-388 cell lines with IC_{50} 55.4 μ g/mL.

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