

Apigenin, an Anticancer Isolated from *Macaranga gigantifolia* Leaves

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## ABSTRACT

Apigenin, a flavonoid compound has been isolated from the ethyl acetate fraction of methanol extract of *Macaranga gigantifolia* leaves. Isolation and purification of apigenin conducted using column and centrifugal chromatography and chemical structure characterized based on spectroscopic data. In vitro anticancer activity test against murine leukemia P-388 cell line showed potential activity as anticancer with  $IC_{50}$  14.13  $\mu$ g/mL.

**Keywords:** anticancer, apigenin, flavones, *Macaranga gigantifolia*, murine leukemia P-388 cell line

## INTRODUCTION

*Macaranga gigantifolia* Merr. (Euphorbiaceae), locally known as mahang-mahangan, is one of the *Macaranga* genus from total of 280 over than 50 species worldwide [1]. *Macaranga* species has long been used traditional medicine system such as *M. gigantea* and *M. triloba* used to treat fungal infections and leaf decoction, stomachaches [2]. Otherwise, *Macaranga hypoleuca* can be used as febrifuge, expectorant and antispasmodic and also has potential as an antioxidant, anti-bacterial [2], and anti-diabetic [3]. There are only two compounds (scopoletin and macarangin) have been isolated from *M. gigantifolia* so far [4-5]. A phytochemical review indicates the genus *Macaranga* to be a rich source of flavonoids [6]. This paper purpose is to describe the isolation and structure elucidation of apigenin compound from ethyl acetate fraction of *M. gigantifolia* leaves, and its cytotoxic activity against Murine leukemia P-388 cell lines.

## MATERIALS AND METHODS

## General

$^1H$  and  $^{13}C$ -NMR spectra were recorded with a JEOL JNM-ECA 500 spectrometer instrument. LC-MS was measured with Mariner Biospectrometry-Finnigan instrument. Column chromatography method carried out with silica gel (200-300 mesh, Kieselgel 60, Merck) for isolation and silica gel 60 F254 (Merck) for TLC

with 10%  $H_2SO_4$  in ethanol as compound detection reagent.

## Plant material

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

## Extraction and isolation

About 80 grams MeOH extract was partitioned with n-hexane and ethyl acetate (EtOAc), successively. The EtOAc fraction (17.4 g) subjected to silica gel chromatography column and eluted successively with n-hexane : EtOAc as mobile phase. 14 fractions (F1-F5) obtained and characterized with TLC. The compound spots detected using UV-Vis lamp and sprayed with 10%  $H_2SO_4$  in ethanol. Compound A obtained from purification of fraction 12 (F12) using chromatotron (centrifugal chromatography) with n-hexane : EtOAc as mobile phase.

## Cytotoxic activity

Cytotoxic activities assay conducted using MTT assay method [7-9]. Approximately  $3 \times 10^4$  of murine leukemia P-388 cells were plated in 96-well culture dishes. Incubated at 37°C for 24 h. Various concentration of sample in DMSO were added after incubation. Six desirable sample concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30-7.65), except control. After 48 h incubation, assay stopped by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium

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bromide], incubation continue for next 4h before the addition of MTT-stop solution containing sodium dodecyl sulphate (SDS). The incubation continued for next 24h. Optical density measured with microplate reader at 550 nm. IC<sub>50</sub> obtained from the plotted graph between percentage live cells compared to control against various concentration of the sample tested ( $\mu\text{M}$ ).

## RESULTS AND DISCUSSION

Dried *M. gigantifolia* leaves extracted with methanol, further 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 to obtained 14 fractions (F1-F14). Compound A obtained from further purification of fraction 12 (F12) using chromatotron (centrifugal chromatography) with n-hexane : EtOAc as mobile phase.

Compound A: yellow powder, NMR (JEOL JNM-ECA 500)  $\delta_{\text{H}}$  (500 MHz, in DMSO-d<sub>6</sub>): 6.75 (s, 1H, H-3), 6.15 (d, 1H, J= 1,95 Hz, H-5), 6.44 (d, 1H, J= 1,95 Hz, H-8), 7.91 (d, 2H, J= 9,05 Hz, H-2'/H-6'), 6.90 (d, 2H, J= 9,05 Hz, H-3'/H-5'), 12.94 (-OH). <sup>13</sup>C-NMR (DMSO, 125 MHz)  $\delta$  163.71 (C-2), 102.81 (C-3), 181.73 (C-4), 161.25 (C-5), 98.96 (C-6), 164.57 (C-7), 94.06 (C-8), 157.37 (C-9), 103.56 (C-10), 121.17 (C-1'), 128.51 (C-2'/C-6'), 116.01 (C-3'/C-5'), 161.46 (C-4'). ESI-MS (m/z) 271.2195 [M+H].

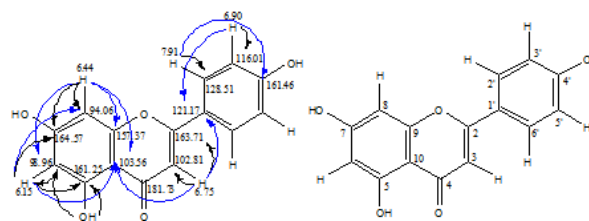


Figure 1. HMQC- and HMBC-NMR correlation of compound A and chemical structure of Apigenin

The <sup>1</sup>H-NMR spectrum of compound A (Table 1), showed six aromatic protons at  $\delta_{\text{H}}$  7.91 (2H, d, J=9.05 Hz) and 6.90 (2H, d, J=9.05 Hz) indicated the presence of AA'XX' ring system in B ring. In addition, two doublet signals at  $\delta_{\text{H}}$  6.15 (1H, d, J= 1,95 Hz) and 6.44 (1H, d, J= 1,95 Hz) showed the meta position correlation each other. This is mean that two proton signals are in the same ring system (ring A). The <sup>13</sup>C NMR spectrum of compound A showed 15 carbon resonances (Table 1) including a downfield carbonyl signal at  $\delta_{\text{C}}$  81.73 ppm (C-4). Five oxyaryl carbons can be observed at 157.37; 161.25; 161.46; 163.71 and 164.57, two quaternary sp<sup>2</sup> carbons at  $\delta_{\text{C}}$  103.56, 121.17, and seven methine sp<sup>2</sup> carbons at  $\delta_{\text{C}}$  94.06, 98.96, 128.51 (2C), 116.01 (2C).

The HMQC (<sup>1</sup>J<sub>(C-H)</sub>) and HMBC (<sup>2</sup>J<sub>(C-H)</sub> or <sup>3</sup>J<sub>(C-H)</sub>) correlations of compound A illustrated in Figure 1. The HMBC correlation between  $\delta_{\text{H}}$  6.15 with  $\delta_{\text{C}}$  161.25, 164.57, 103.56 and 94.06 together with correlations

Table 1. <sup>1</sup>H- and <sup>13</sup>C-NMR data of compound A and Apigenin

No.	Reference (Nawal & Atta, 2013)		Compound A	
	$\delta_{\text{H}}$ (mult., J/Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., J/Hz)	$\delta_{\text{C}}$
1		-		-
2		164.19		163.71
3	6.47 (s, 1H)	103.30	6.75 (s, 1H)	102.81
4		182.21		181.73
5		161.95		161.25
6	6.21 (d, 1H, J= 2.5 Hz)	99.89	6.15 (d, 1H, J= 1,95 Hz)	98.96
7		163.31		164.57
8	6.77 (d, 1H, J= 2.5 Hz)	94.55	6.44 (d, 1H, J= 1,95 Hz)	94.06
9		157.86		157.37
10		105.83		103.56
1'		121.63		121.17
2' & 6'	7.93 (d, 2H, J= 8 Hz)	128.98	7.91 (d, 2H, J= 9,05 Hz)	128.51
3' & 5'	6.92 (d, 2H, J= 8 Hz)	116.51	6.90 (d, 2H, J= 9,05 Hz)	116.01
4'		161.79		161.46

showed by proton  $\delta_{\text{H}}$  6.44 with carbons at  $\delta_{\text{C}}$  157.37, 103.56, 164.57 and 98.96 confirmed the location of  $\delta_{\text{H}}$  6.15 and 6.44 at H-8 and H-6 of the A-ring, respectively. Another set of HMBC correlations between  $\delta_{\text{H}}$  6.90 with quaternary  $\text{sp}^2$  carbon at  $\delta_{\text{C}}$  121.17 and  $\delta_{\text{H}}$  7.91 with quaternary  $\text{sp}^2$  carbon at  $\delta_{\text{C}}$  161.46 confirmed the presence of AA'XX' ring system in B ring. The olefinic proton at  $\delta_{\text{H}}$  6.75 gave  $^3\text{J}$  HMBC correlation to  $\delta_{\text{C}}$  103.56 and 121.17 indicated that compound A is flavones group. The LC-MS spectra (Figure 2) showed a molecular ion  $[\text{M}+\text{H}]^+$  271.2195 or  $[\text{M}^+]$  270.2195 indicates the molecular weight of the compound corresponds to the molecular weight of apigenin and also corresponds to molecular formula  $\text{C}_{15}\text{H}_{10}\text{O}_5$  [10].

Compound A was evaluated for its cytotoxicity against the P-388 murine leukemia cells with  $\text{IC}_{50}$  14.13  $\mu\text{g}/\text{mL}$ . This result suggested that phenolic group on apigenin may be an important structural feature for cytotoxic activity.

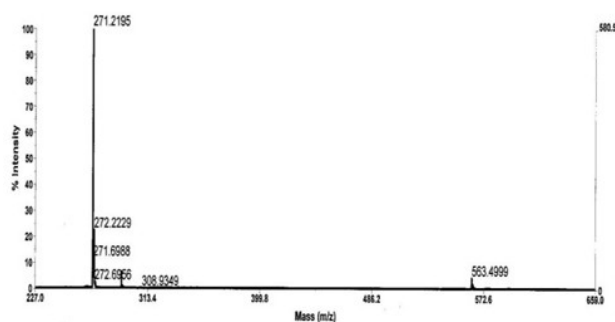


Figure 2. MS spectra of compound A

## CONCLUSIONS

Isolation and purification of ethyl acetate fraction 12 (F-12) obtained from fractionated of methanolic extract of *M. gigantifolia* leaves done using column chromatography, centrifugal chromatography, and recrystallization using different system solvent has been done. Based on spectroscopic data concluded that compound A is apigenin, which have moderate cytotoxicity activity against Murine leukemia P-388 cell lines  $\text{IC}_{50}$  14.13  $\mu\text{g}/\text{mL}$ .

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## REFERENCES

- Zakaria R, Rosely NFN, Mansor M, Zakaria MY (2008) The distribution of *Macaranga* genus (family Euphorbiaceae) in Penang Island, Peninsular Malaysia. *Journal of Bioscience*. 19(2): 91-99.
- Lim TY, Lim YY, Yule CM (2014) Bioactivity of leaves of *Macaranga* species in tropical peta swamp and non-peta swamp environments. *Journal of Tropical Forest Science*. 26(1):134-141.
- Gunawan-Puteri MDPT, Kawabata J (2010) Novel  $\alpha$  glucosidase inhibitors from *Macaranga tanarius* leaves. *Food Chemistry* 2010;123:384-389.
- Darmawan A, Kosela S, Kardono LBS, Syah YM (2012) Scopoletin, a coumarin derivatives compound isolated from *Macaranga gigantifolia* Merr. *Journal of App. Pharm. Sci.* 2(12):175-177.
- Darmawan A, Wahyudi PS, Soleh K, Kardono LBS, Sofa F (2015) Macarangin, a geranylated flavonoid and anticancer active compound isolated from ethyl acetate fraction of *Macaranga gigantifolia* leaves. *Indonesian J. Pharm.* 26(1):52-56.
- Magadula, JJ (2014) Phytochemistry and Pharmacology of the Genus *Macaranga*: a Review. *J. of Med. Plant Res.* 8(21): 489-503.
- Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbot BJ, Mayo JG, Shoemaker RH, Boyd MR (1998) Feasibility of drugs screening with panels of tumor cell lines using a microculture tetrazolium assay. *Cancer Res.*48:589-601.
- Harneti D, Tjokronegoro R, Safari A, Supratman U, Xemin L, Mukhtar MR, Mohamad K, Awang K, Hayashi, H (2012) Cytotoxic triterpenoids from the bark of *Aglaia smithii* (Meliaceae). *Phytochemistry Letters*. 5:496-499.
- Sahidin, Hakim EH, Juliawaty LD, Syah MY, Din LB, Ghisalberti EL, Latif J, Said IM, Achmad SA (2005) Cytotoxic properties of oligostilbenoids from the bark of *Hoppea dryobalanoides*. *Z. Naturforsch.* 60 (9-10):723-727.
- Nawal HM, Atta EM (2013) Cytotoxic and antioxidant activity of *Marrubium vulgare* and its flavonoid constituents. 2nd International Conference on Chemical, Environmental and Biological Sciences (ICCEBS'2013). Dubai – UEA:40-42.