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Short Communication

Scopoletin, a coumarin derivative compound isolated from *Macaranga gigantifolia* Merr

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ABSTRACT

Scopoletin (**1**), a coumarin derivative compound has been isolated from methanol extract of *Macaranga gigantifolia* Merr. leaves using chromatography methods. Chemical structure determination carried out based on spectroscopic data and compared with a reference. Scopoletin cytotoxicity test using MTT assay method against P-388 murine leukemia cells showed strong cytotoxic activity with IC_{50} 17.42 µg/mL.

INTRODUCTION

Macaranga gigantifolia Merr. (Euphorbiaceae) also known as mahang-mahangan (Indonesia) is one of the *Macaranga* genus member. In the course of our research for isolating the phenolic compounds from the *Macaranga* plants, methanol extract of *Macaranga gigantifolia* Merr. was explored using chromatography column method. Previous phytochemical studies of this genus showed that phenolic compounds are the major compound of *Macaranga* plants. However, there are very insufficient research project and study about *M. gigantifolia*. There are no publicated papers about *M. gigantifolia* chemical contents. Scopoletin is a common chemical compound contained in various plants (Tsukamoto *et al*., 1984; Mizuno *et al*., 1992; Bayoumi *et al*., 2008; and Simoes *et al*., 2009), but there are no publication established about chemical constituents of this plant. In this paper, we describe the structure elucidation and cytotoxic properties of phenolic compound isolated from *M. gigantifolia* Merr.

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MATERIAL AND METHODS

General

The mass spectrum was recorded with a Mariner Biospectrometry-Finnigan instrument, and ^{1}H- and ^{13}C-NMR spectra was obtained with a JEOL JNM-ECA 500 spectrometer using TMS as internal standard. Chromatographic separation process carried out using silica gel (Kieselgel 60, Merck). Purity confirmation carried out using Silica gel 60 F_{254} (Merck) with 10% H_2SO_4 in ethanol as compound detection reagent.

Plant material

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

Extraction and isolation

The dried leaves (2 kg) was extracted with 15 L methanol (MeOH) at room temperature for three times, evaporated using rotary evaporator. 80 grams MeOH extract partitioned with hexane

and ethyl acetate (EtOAc), successively. The EtOAc soluble fraction (17,4 g) was chromatographed over silica gel column, eluted successively with a gradient of *n*-hexane-EtOAc solvent (8:2-6:4) to obtained 5 fraction (F1-F5). F5 further chromatographed using column of silica gel, eluted successively with *n*-hexane-EtOAC 6:4 based on TLC result to give **1** (11 mg).

Cytotoxic activity

Cytotoxic activities assay was conducted using MTT assay method (Harneti *et al*, 2012; Sahidin *et al*, 2005; and Alley ae al, 1988). Approximately 3 x 10^4 cell cm^{-3} of P-388 murine leukemia cells were plated in 96-well culture dishes. Incubated at 37°C in humidified CO_2 incubator for 24 h. After incubation, various concentrations in DMSO solvent of the samples were added. Six desirable sample concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30-7.65), except control. After 48 h incubation, assay was stop by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and the incubation continue for next 4 h before the addition of MTT-stop solution containing sodium dodecyl sulphate (SDS), the incubation continue for next 24 h. optical density measured using microplate reader at 550 nm. IC_{50} value obtained from the plotted graph between percentage live cells compared to control (receiving only PBS and DMSO) against various concentration of the compound tested (µM).

RESULT AND DISCUSSION

Dried *M. gigantifolia* leaves extracted with methanol. The methanol extract was partitioned succesively with *n*-hexane and ethyl acetate. Ethyl acetate extract was subjected to silica gel column chromatography to give a coumarine derivate (**1**).

Table. 1: NMR data of **compound 1** (^{1}H (500 MHz) and ^{13}C in Acetone-d_6) and scopoletin.

Position	Compound 1		Reference (Zhang, 2011)	
	δ_H (mult., *J* Hz)	δ_C	δ_H (mult., *J* Hz)	δ_C
1	-	160.8		161.5
2	6.25 (d, ^{1}H, 9.75)	113.3	6.21 (d, ^{1}H, 9.3)	107.6
3	7.84 (d, ^{1}H, 9.75)	144.7	7.92 (d, ^{1}H, 9.3)	143.4
4	-	112.1		111.5
5	7.19 (s, ^{1}H)	109.9	7.21 (s, ^{1}H)	113.4
6	-	146.0		144.1
7	-	151.9		150.3
8	6.79 (s, ^{1}H)	103.7	6.78 (s, ^{1}H)	103.2
9	-	151.2		149.8
10	3.90 (s, ^{3}H)	56.7	3.87 (s, ^{3}H)	56.5

1 was obtained as colorless needle. Molecular weight data analysis result by ESI-MS (m/z 193.0101, $[M+H]^+$), combined with 1D-NMR (^{1}H- and ^{13}C-NMR (Table 1)) and 2D-NMR (HMQC and HMBC) spectral data used to characterized the chemical structure of **1**. The ^{1}H-NMR spectrum showed four aromatic protons (δ_H 6.2, 6.70, 6.82, and 7.97 ppm), and one methoxy group (δ_H 3.83 ppm), combined with ten carbons from ^{13}C-NMR, and HMQC/HMBC correlations (Figure 2) showed the chemical structure of **1** (Figure 1). Based on the spectroscopic data and reference comparative, **1** was identified as scopoletin (Zhang, 2011).

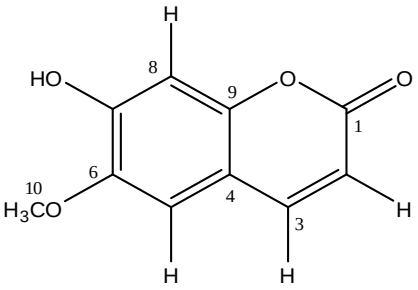

Fig. 1: Chemical structure of **compound 1.**

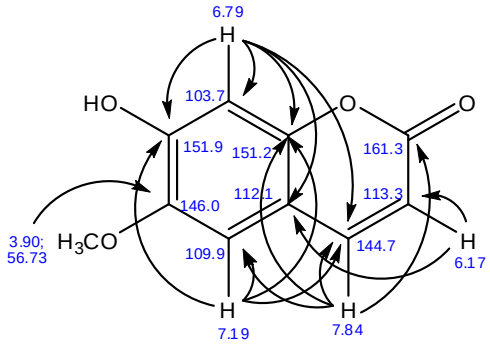

Fig. 2: HMQC and HMBC correlation for scopoletin.

Cytotoxicity activity of **1** against P-388 murine leukemia cells has been done with Artonin E (IC_{50} 0.3 µg/mL) as positive control. **1** showed strong activity with IC_{50} 17.42 µg/mL.

CONCLUSION

From this research can be concluded that one of the phenolic compounds contained in the leaves of *M. gigantifolia* plant is scopoletin, which also has strong cytotoxic activity against P-388 murine leukemia cells with IC_{50} value 17.42 µg/mL.

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