

Chemical Composition and Anticancer Activity of *Macaranga hosei* Leaves

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The anticancer activity of methanolic extract and ethyl acetate fraction of *Macaranga hosei* leaves against HeLa cell lines were evaluated by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay. Both extracts displayed anticancer activity with IC₅₀ values of 36.18 and 7.01 μM, respectively, which can be suggested that *M. hosei* is a great potential source of anticancer agents. In addition, two isoprenylated flavanones, 4'-*O*-methyl-8-isoprenyl eriodictyol (**1**) and 6-isoprenyl eriodictyol (**2**) have been isolated from ethyl acetate fraction. The structures of both compounds have been elucidated based on their spectroscopic data, including 1D and 2D NMR spectra.

Keywords: *Macaranga hosei*, Anticancer, MTT Assay, Flavanones, Isoprenylated.

INTRODUCTION

Macaranga is one genus of the family Euphorbiaceae comprising of ± 300 species. In Indonesia, this plant known as “Mahang”. The distribution of *Macaranga* plants is relatively wide, other than Indonesia, can also be found in Africa, Madagascar, Asia, the east coast of Australia and the Pacific islands [1].

According to previous studies, phenolics such as flavonoids and stilbenoids can be isolated from this genus. The uniqueness of flavonoids and stilbenoids from this genus is the presence of terpenoids at aromatic core such as prenyl, geranyl, farnesyl and geranyl [2,3]. Prenylated flavonoids including flavanone derivatives mostly can be found in *M. triloba*, *M. trichocarpa*, *M. conivera* and *M. lowii* [3-6]. Flavonol derivatives can be obtained from *M. gigantea*, *M. recurvate*, *M. pruinosa*, *M. rizhinooides* and *M. bicolor* [2,5,7-9]. Dihydroflavone derivatives mostly can be attained in *M. conivera*, *M. alnifolia*, *M. pruinosa* and *M. lowii* [6,8,10,11].

Previous studies have revealed that the presence of isoprenoid chains plays an important role for the biological activity of prenylated aromatic compounds which made them possess better bioactivity than their mother compounds without derivatization or modification [12]. An isoprenylated flavanone compound named 4'-*O*-methyl-8-isoprenyleriodictyol from *M. pearsonii* displayed an antioxidant activity with IC₅₀ value of

536.89 μM [13]. In addition, another isoprenylated flavanones such as 4'-*O*-methyl-8-isoprenylnaringenin and lonchocarpol A from *M. hosei* leaves exhibited antioxidant activities with IC₅₀ values of 1298.0 and 1115.7 μM, respectively [14]. Prenylated flavonoids were reported to have good anticancer effects. Several of these compounds were isolated from *M. indica*, *M. kurzii* showed cytotoxic activities against cancer cell lines [15,16].

Although numbers of bioactivities have been reported in this genus, the anticancer activity from *M. hosei* leaves extract has not been investigated. In our research, the anticancer activity of methanolic extract and its ethyl acetate fraction were determined. Moreover, two compounds belong to isoprenylated flavanones named 4'-*O*-methyl-8-isoprenyl eriodictyol (**1**) and 6-isoprenyl eriodictyol (**2**) have been isolated from the methanolic extract of *M. hosei* leaves.

EXPERIMENTAL

All the reagents used were obtained from Merck Chemical, Co. without further additional purification. The isolation were monitored by thin layer chromatography (TLC) and visualized under UV 254 and 356 nm with cerium sulfate as staining agent. Vacuum liquid chromatography (VLC) and radial chromatography were carried out using silica gel 60 GF₂₅₄ and silica gel 60 PF₂₅₄. For TLC analysis, pre-coated silica gel plates (Merck

Kieselgel 60 GF₂₅₄, 0.25 mm thickness) were used. ¹H and ¹³C NMR spectra were recorded with a JEOL ECS 400 spectrometer operating at 400 (¹H) and 100 (¹³C) MHz in CDCl₃ using TMS as the internal standard. In addition, BioTek PowerWave XSPlate Reader and 5 % CO₂ incubator at 37 °C were also used in this research.

The leaves of *Macaranga hosei* were collected from Samboja, Kutai Kartanegara, East Kalimantan, Indonesia. This species was identified at the herbarium of Wanariset, Samboja, Kutai Kartanegara, East Kalimantan, Indonesia and a voucher specimen had been deposited at that herbarium.

Extraction and isolation: The dried leaves of *M. hosei* (1.0 kg) were grounded and macerated with methanolic at room temperature and filtered every 2 days. The methanolic crude extract (150 g) was obtained after evaporation by rotary evaporator. Furthermore, the crude was partitioned with *n*-hexane and ethyl acetate, respectively. The ethyl acetate fraction (35 g) was further fractionated by VLC on silica gel with *n*-hexane:EtOAc by increasing the polarity (9:1, 4:1; 7:3, 1:1 and 1:4). Further separation by VLC using *n*-hexane:EtOAc (9:1 to 3:7), followed by *n*-hexane:CHCl₃ (9:1 to 3:7) using chromatotron yielded compound **1** 12.0 mg and compound **2** 0.6 mg. Moreover, the structures of these compounds were elucidated by spectroscopic including 1D and 2D NMR.

Determination of anticancer activity

Cell culture: HeLa cervical cancer cell lines were cultured in the eagle's minimum essential medium containing 1.5 g/L of Na₂CO₃ and supplemented with 1 % of L-glutamine, 1 % of formulation of antibiotics and antimycotics, 1 % of non-essential amino acids, 1 % of sodium pyruvate and 10 % of fetal bovine serum (FBS). Furthermore, these cells were incubated with 5 % CO₂ incubator at a temperature of 37 °C.

Anticancer activity: The anticancer activities of methanolic extract and ethyl acetate fraction of *M. hosei* leaves were determined by method as described by Fahmi *et al.* [17] with modification, using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. HeLa cells were placed in 12 wells containing 25,000 cells/well. After 24 h, cells were washed with phosphate-buffered saline (PBS) and incubated with different concentrations of sample for 24 h. Furthermore, the cells were washed with PBS twice. Then 1 mL of 500 mg/mL MTT was added into each cell and incubated for 4 h. Dark blue formazan crystals formed were then dissolved in 200 mL of DMSO to measure the absorbance at a λ of 570 nm by BioTek PowerWave XSPlate Reader.

RESULTS AND DISCUSSION

Anticancer activity: The anticancer activities of methanolic extract (IC₅₀ 36.18 μg/mL) and ethyl acetate fraction (7.01 μg/mL) of *M. hosei* leaves against HeLa cells by MTT assay were found to be active as anticervical cancer. The IC₅₀ values indicated that the anticancer activities of methanolic extract belongs to moderate while ethyl acetate fraction displayed higher effect than methanolic extract. Based on National Cancer Institute, ethyl acetate fraction signified as an active anticancer due to it has IC₅₀ ≤ 30 μg/mL [18]. The anticancer activity which is showed by *M. hosei* can be assumed caused

by its bioactive compounds, one of them is prenylated flavonoids such as flavanone derivatives which known have a wide variety of biological activities.

Flavanone derivatives: Due to ethyl acetate fraction of *M. hosei* leaves was found to be more active than its methanolic extract, further separation had been conducted to isolate bioactive compounds. Two isolated flavanone derivatives, *i.e.* 4'-*O*-methyl-8-isoprenyl eriodictyol (**1**) and 6-isoprenyl eriodictyol (**2**). The position of protons and carbons of compounds **1** and **2** are presented in Tables 1 and 2, respectively. In addition, HMBC correlations of both compounds are shown in Fig. 1.

TABLE-1
NMR DATA OF COMPOUND 1 IN CDCl₃, 400 MHz

No. C	δ _H (mult, J in Hz)	δ _C	HMBC
2	5.36 (dd, 12.0, 3.2)	78.5	C-4, C-1', C-2', C-6'
3	3.03 (dd, 17.1, 12.0) _{ax}	42.5	C-2, C-4
	2.70 (dd, 17.1, 3.2) _{eq}		C-1'
4	-	197.1	-
4a	-	102.3	-
5	-	161.6	-
6	5.98(s)	95.8	C-4a, C-5, C-7, C-8
7	-	164.8	-
8	-	107.4	-
8a	-	160.0	-
1'	-	132.0	-
2'	6.87 (d, 2.4)	114.4	C-2, C-4', C6'
3'	-	146.9	-
4'	-	148.2	-
5'	6.89 (d, 8.4)	112.3	C-1', C-3'
6'	6.82 (dd, 8.4, 2.4)	117.8	C-2', C-4'
1''	3.04 (d, 7.0)	21.8	C-7, C-8, C-8a, C-2'', C-3''
2''	5.05 (t, 8.6)	123.2	C-3'', C-4'', C-5''
3''	-	130.8	-
4''	1.55 (s)	18.1	C-2'', C-3'', C-5''
5''	1.52 (s)	26.0	C-2'', C-3'', C-4''
5-OH	12.05 (s)	-	C-4a, C-5, C-6
7-OH	10.70 (s)	-	C-7, C-8
3'-OH	9.01 (s)	-	C-2', C-4'
4'-OCH ₃	3.73 (s)	56.1	C-4'

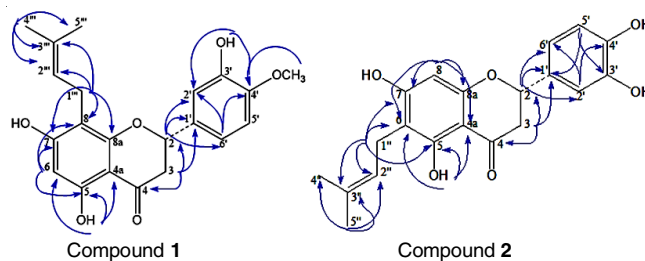


Fig. 1. HMBC correlations of compounds **1** and **2**

Compound **1** was obtained as white powder. ¹H NMR spectra analysis of compound **1** displayed the characteristic of flavanone such as three protons signals of doublet-doublet at δ_H 5.36 ppm (*J* = 12.0, 3.2 Hz, H-2), 3.03 ppm (*J* = 12.0; 17.1 Hz, H-3_{ax}) and 2.70 ppm (*J* = 17.1; 3.2 Hz, H-3_{eq}). ¹H NMR spectra analysis of compound 4'-*O*-methyl-8-isoprenyl eriodictyol exhibited three aromatic protons signals of ABX system such as doublet signal at δ_H 6.89 ppm (*J* = 8.4 Hz, H-5'), doublet signal at δ_H 6.87 ppm (*J* = 2.4 Hz, H-2') and doublet-doublet

TABLE-2
NMR DATA FOR COMPOUND 2 IN CDCl₃, 400 MHz

No. C	δ_{H} (mult, J in Hz)	δ_{C}	HMBC
2	5.27 (<i>dd</i> , 12.8, 3.2)	78.5	C-2'
3	3.02 (<i>dd</i> , 17.2, 12.8) _{ax} 2.79 (<i>dd</i> , 17.2, 3.2) _{eq}	43.6	C-2, C-4
4	-	196.8	-
4a	-	103.1	-
5	-	159.6	-
6	-	107.6	-
7	-	162.6	-
8	6.38 (<i>s</i>)	95.8	C-7
8a	-	161.6	-
1'	-	129.3	-
2'	6.98(<i>d</i> , 1.6)	113.7	C-2, C-4', C-6'
3'	-	147.7	-
4'	-	144.0	-
5'	6.89 (<i>d</i> , 8.0)	115.7	C-1', C-3'
6'	6.87 (<i>dd</i> , 8.0, 1.6)	119.2	C-2, C-4'
1''	3.30 (<i>d</i> , 7.2)	21.5	C-5, C-6, C-7, C-2'', C-3''
2''	5.19 (<i>bt</i>)	122.0	C-4'', C-5''
3''	-	135.0	-
4''	1.81 (<i>s</i>)	18.2	C-2'', C-3'', C-5''
5''	1.71 (<i>s</i>)	26.2	C-2'', C-3'', C-4''
5-OH	12.32 (<i>s</i>)	-	C-4a, C-5, C-6

signal at δ_{H} 6.82 ppm ($J = 8.4, 2.4$ Hz, H-6') [3]. This compound showed one substituent of isoprenyl (vinyl signal as triplet at δ_{H} 5.05 ppm; methylene signal as doublet at δ_{H} 3.04 ppm, two methyl signals as singlet at δ_{H} 1.55 and 1.52 ppm) and one methoxy signal as singlet (δ_{H} 3.73 ppm). The presence of one proton singlet signal at δ_{H} 5.98 ppm indicated that isoprenyl substituent bonded at C-6 or C-8.

Spectra analysis of ¹³C NMR from compound 1 showed 21 carbon signals which are distinguished well. The compound consists of six carbons methine, two carbons of methylene, three carbons of methyl and ten quaternary carbons. The carbonyl signal showed at δ_{C} 197.1 ppm and one signal of oxycarbon-methine was shown at δ_{C} 78.5 ppm. Five signals of oxyaryl carbon were shown at δ_{C} : 164.8, 161.6, 160.0, 148.2 and 146.9 ppm. Those signals indicated flavanone with eriodictyol moiety.

The isoprenyl and methoxy positions of compound 1 were elucidated based on HMQC and HMBC. Long-range correlation between proton signal of 5-OH at δ_{H} 12.05 ppm with two quaternary carbon atoms (δ_{C} 161.6 ppm, C-5; 102.3 ppm, C-4a) and one aromatic methine carbon (δ_{C} 95.8 ppm, C-6) showed that isoprenyl substituent bonded at C-8. Correlation of methoxy proton signal at δ_{H} 3.73 ppm with oxyaryl carbon signal (δ_{C} 148.2 ppm) displayed that the methoxy group bonded at C-4'.

Based on NMR spectra analysis, it can be elucidated that compound 1 is 4'-*O*-methyl-8-isoprenyl eriodictyol. This compound gave NMR parameter which is suitable with 4'-*O*-methyl-8-isoprenyl eriodictyol from *M. conifera* [5].

Compound 2 was obtained as yellow oil. ¹H NMR spectra analysis of compound 2 showed the characteristic of flavanone as well, three protons signals of doublet-doublet at δ_{H} 5.27 ppm ($J = 12.8, 3.2$ Hz, H-2), 3.02 ppm ($J = 12.8, 17.2$ Hz, H-3_{ax}) and 2.79 ppm ($J = 17.2, 3.2$ Hz, H-3_{eq}) and three protons aromatic signals of ABX system at δ_{H} 6.98 ppm ($J = 1.6$ Hz, H-2'), δ_{H} 6.89 ppm ($J = 8.0$ Hz, H-5') and doublet-doublet at

δ_{H} 6.87 ppm ($J = 8.0, 1.6$ Hz, H-6'). The isolated compound displayed one substituent of isoprenyl (vinyl signal as triplet at δ_{H} 5.19 ppm, methylene signal as doublet at δ_{H} 3.30 ppm and two methyl signals as singlet at δ_{H} 1.81 and 1.71 ppm) together with one aromatic proton signal as singlet in A ring at δ_{H} 6.38 ppm exhibited that isoprenyl bounded at C-6 or C-8.

¹³C NMR spectra analysis of compound 2 indicated 20 carbon signals which are separated completely, consist of six methine carbon atoms, two methylene carbon atoms, two methyls and ten quaternary carbon atoms. This compound has also eriodictyol structure with one isoprenyl substituent.

Isoprenyl position of compound 2 was elucidated by HMQC and HMBC. Correlation of long-range between proton signal of 5-OH at δ_{H} 12.32 ppm with three quaternary carbon atoms signals (δ_{C} 159.6 ppm, C-5; 107.6 ppm, C-6; 103.1 ppm, C-4a) indicated the presence of isoprenyl substituent at C-6.

Based on data of NMR (including 1D and 2D), compound 2 was elucidated as 6-isoprenyl eriodictyol. ¹H and ¹³C NMR spectra of the isolated compound is similar with 6-isoprenyl eriodictyol which has a molecular structure as C₂₀H₂₀O₆ and positive ion mass m/z [M]⁺ 356.126 [19].

Conclusion

Two isoprenylated flavanones named 4'-*O*-methyl-8-isoprenyl eriodictyol (1) and 6-isoprenyl eriodictyol (2) have been isolated from the methanolic extract of *M. hosei* leaves. In addition, the present study revealed that methanolic extract and ethyl acetate fraction of *M. hosei* leaves exhibited significant anticancer activity against HeLa cell lines. It can be suggested that *M. hosei* is a great potential source as anticancer agents and assumed that two isolated compounds belong to isoprenylated flavanones may play important role in anticancer property.

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