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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 IC_{50} 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. rizhinoides* 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-isoprenyeriodictyol from *M. pearsonii* displayed an antioxidant activity with IC_{50} 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 IC_{50} 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 GF_{254} and silica gel 60 PF_{254}. For TLC analysis, pre-coated silica gel plates (Merck

Kieselgel 60 GF_{254}, 0.25 mm thickness) were used. ^1H and ^{13}C NMR spectra were recorded with a JEOL ECS 400 spectrometer operating at 400 (^1H) and 100 (^{13}C) MHz in $CDCl_3$ using TMS as the internal standard. In addition, BioTek PowerWave XSPlate Reader and 5 % CO_2 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:$CHCl_3$ (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 Na_2CO_3 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 % CO_2 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 (IC_{50} 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 IC_{50} 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 $IC_{50} \leq 30$ 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 $CDCl_3$, 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'

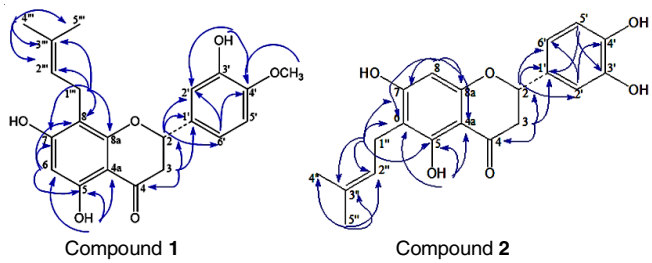

Fig. 1. HMBC correlations of compounds **1** and **2**

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-isoprenylerio-dictyol 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 $CDCl_3$, 400 MHz

No. C	δ_H (mult, *J* in Hz)	δ_C	HMBC
2	5.27 (*dd*, 12.8, 3.2)	78.5	C-2'
3	3.02 (*dd*,17.2, 12.8)$_{ax}$	43.6	C-2, C-4
	2.79 (*dd*, 17.2, 3.2)$_{eq}$		
4	-	196.8	-
4a	-	103.1	-
5	-	159.6	-
6	-	107.6	-
7	-	162.6	-
8	6.38 (*s*)	95.8	C-7
8a	-	161.6	-
1'	-	129.3	-
2'	6.98(*d*, 1.6)	113.7	C-2, C-4', C-6'
3'	-	147.7	-
4'	-	144.0	-
5'	6,89 (*d*, 8,0)	115.7	C-1', C-3'
6'	6.87 (*dd*, 8.0, 1.6)	119.2	C-2, C-4'
1"	3.30 (*d*,7.2)	21.5	C-5, C-6, C-7, C-2", C-3"
2"	5.19 (*bt*)	122.0	C-4", C-5"
3"	-	135.0	-
4"	1.81 (*s*)	18.2	C-2", C-3", C-5"
5"	1.71 (*s*)	26.2	C-2", C-3", C-4"
5-OH	12.32 (*s*)	-	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 doubletat 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 ^{13}C 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 quarternary 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-isoprenyleriodictyol from *M. conifera* [5].

Compound **2** was obtained as yellow oil. ^{1}H 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.

^{13}C 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 quartener 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 quarternary 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. ^{1}H and ^{13}C NMR spectra of the isolated compound is similar with 6-isoprenyl eriodictyol which has a molecular structure as $C_{20}H_{20}O_6$ 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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