RESORCINOL COMPOUNDS ISOLATED FROM THE BARK OF *MYRISTICA FATUA* HOUTT

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ABSTRACT

Myristica fatua Houtt. is one of the endemic plants growth in Wallace region. Isolation of the secondary metabolite compounds from the ethyl acetate fraction of the bark of *Myristica fatua* Houtt. has been done using some chromatography techniques afforded two resorcinol compounds, malabaricone C (**1**), and malabaricone B (**2**). The structures of **1-2** were determined using spectroscopic techniques, including mass spectrometry, 1D-NMR and 2D-NMR. Both compounds showed in vitro cytotoxic activity against the breast carcinoma cancer cell lines MCF-7 using alamar blue assay method, with IC₅₀ of 2.38 and 0.71 µg/mL, respectively.

Key words: *Myristica fatua* Houtt., resorcinol, malabaricone B, malabaricone C, cytotoxic

INTRODUCTION

Myristica (Myristicaceae) widely distributed from India and South East Asia to North Australia and the Pacific Islands. *Myristica* comprising of 72 species, and one of them is *Myristica fatua* Houtt (Zachariah, 2008). *M. fatua* is endemic to the Kalimantan, Sulawesi, Moluccas in Indonesia, beside the other region such as India, Philippines, New Guinea, Solomon Island, Pacific Islands, and New Caledonia. Mekongga forest is a conservation forest in Kolaka District, Northeast Sulawesi, Indonesia, which have so many uniques and endemic bioresources (Lim, 2012).

Lignans and acylphenols are the secondary metabolites major compound produced by this family (Nemethy, 1986; Woo, 1987; Cooray, 1987; Kumar, 1988; Hada, 1988; Nakatani, 1988; Herath, 1996; Herath, 1997; Pham, 2000& 2002; Talukdar, 2000; Calliste, 2010; Cuong, 2011; Chong, 2011; Manjunatha, 2011; Ragasa, 2013; Cao, 2013; Cao, 2015; Acuna, 2016; Wahab, 2016; Pandey, 2016; Sivasothy, 2016). In addition to their activities of antioxidant, antibacterial, antimicrobial, nematocide, and cytotoxic (Pham, 2000; Talukdar, 2000; Pham, 2002), antiinflammatory (Cuong, 2011; Cao, 2013; Cao, 2015), anxiogenic (Sonavane, 2002), antihelmintic (Lopez, 2015), cholinesterase inhibitor (Wahab, 2016), therapeutic (Jangid, 2014), acylphenols

also been known to have alpha-glucosidase inhibitor activity (Sivasothy, 2016).

There is no reported study on *M. fatua*, except a study for antioxidant and antibacterial activities (Viveka, 2016) and its potency as α -glucosidase inhibitor (Sivasothy, 2016). It is encourages us to do a phytochemical study about secondary metabolite compounds contained in *Myristica fatua* Houtt. In this paper we will describe the isolation of the acylphenols from *M. fatua*, the structure of malabaricone B (**1**) and C (**2**), and their cytotoxic activity against breast carcinoma MCF-7 cell lines.

MATERIALS AND METHODS

General.

The 1D- and 2D-NMR spectra were recorded by JEOL JNM-ECA 500 spectrometer with CD₃OD as a solvent and TMS as an internal standard. LC-ESI-MS were measured by the Mariner Biospectrometry-Finnigan instrument with methanol p.a (Merck) as a solvent. Column chromatography was performed using silica gel (200-300 mesh, Kieselgel 60, E. Merck) as stationary phase.

Plant material.

The bark of *Myristica fatua* were collected from the Mekongga Forest, District of Kolaka, Southeast Sulawesi, Indonesia. The plant was determined and deposited as a specimen in Herbarium Bogoriense, Research Centre for

Biology, Indonesian Institute of Sciences, Cibinong-Bogor, Indonesia.

Extraction and isolation

Dried and powdered bark of *M. fatua* 1.5k g were macerated in methanol, evaporated using rotary evaporator to get 41.9g methanol crude extract. Methanol soluble fraction (20g) was column chromatographed over silica gel and eluted with a gradient solvent system of *n*-hexane:EtOAc to obtain 10 fractions (F1- F10). Fraction 5(F5) further sephadex chromatographed using methanol: dichloromethane (1:1) as system solvent to give 7 sub-fraction (SF1-SF7). Compound **1** (20mg) and **2** (30mg) was crystallized from sub-fraction 5.4 (SF-5.4) and sub-fraction 5.5 (SF-5.5), respectively. Chemical structure identification of compound **1** and **2** performed with liquid chromatography-mass spectroscopy (LC-MS) and 1D- and 2D-nuclear magnetic resonance (NMR).

Compound **1**: Malabaricone C, ESI-MS m/z 359 [M+H]⁺; NMR (JEOL JNM-ECA 500) δ_H (500 MHz, in CD₃OD): 6.33 (2H, d, *J* 8.43 Hz, H-18/20), 7.19 (1H, t, *J* 8.43 & 7.79 Hz, H-19), 6.46 (1H, dd, *J* 2.60 & 1.95 Hz, H-15), 6.59 (1H, d, *J* 1.95, H-11), 6.64 (1H, d, *J* 7.79 Hz, H-14), 3.11 (2H, t, H-2), 1.66 (2H, p, H-3), 1.33 (8H, br.s, H-4/5/6/7), 1.55 (2H, m, H-8), 2.44 (2H, t, H-9). δ_C (125 MHz, in CD₃OD): 45.7 (C-2), 25.6 (C-3), 30.4 (C-4), 30.6 (2C, C-5/7), 30.7 (C-6), 33.1 (C-8), 36.4 (C-9), 135.8 (C-10), 116.6 (C-11), 146.1 (C-12), 144.1 (C-13), 116.3 (C-14), 120.7 (C-15), 111.5 (C-16), 163.5 (2C, C-17/21), 108.4 (2C, C-18/20), 136.9 (C-19), 209.5 (C-1, C=O).

Compound **2**: Malabaricone B, ESI-MS m/z 342 [M+H]⁺; NMR (JEOL JNM-ECA 500) δ_H (500 MHz, in CD₃OD): 6.33 (2H, d, *J* 8.43 Hz, H-18/20), 7.19 (1H, t, *J* 8.43 & 8.45 Hz, H-19), 6.96 (2H, d, *J* 8.43 Hz, H-11/15), 6.67 (2H, d, *J* 8.43 Hz, H-12/14), 3.11 (2H, t, H-2), 1.66 (2H, p, H-3), 1.33 (8H, br.s, H-4/5/6/7), 1.55 (2H, p, H-8), 2.44 (2H, t, H-9). δ_C (125 MHz, in CD₃OD): 45.7 (C-2), 25.6 (C-3), 30.4 (C-4), 30.6 (2C, C-5/7), 30.7 (C-6), 33.1 (C-8), 36.4 (C-9), 135.8 (C-10), 130.2 (2C-11/15), 116.0 (2C, C12/14), 156.3 (C-13), 111.5 (C-16), 163.5 (2C, C-17/21), 108.3 (2C, C-18/20), 136.8 (C-19), 209.8 (C-1, C=O).

Cancer cells line preparations

In this study, we used breast carcinoma (MCF-7) cells line as cancer cells. Cancer cell was cultured at 37°C of temperature with 95% moisture content and 5% CO₂ in DME medium contained 10% of FBS for 3 days (until the cell cultures undergo confluent 60-70%). Removed and replace the medium with a new medium and continued the incubation for next 24h. Wash Cultured cells with PBS 1-2 times and suspended using trypsin-EDTA solution.

Cytotoxic assay

Cytotoxic assay carried out using Alamar Blue method (Alamar Blue Assay, U.S. Patent No.5,501,959). 100μL of cells line was supplemented with 10μL of samples, incubated at temperature 37°C for 24h. The coloring process was done through the addition of Alamar blue solution for 4h. The color intensity of the cells line was measured by using ELISA plate reader at 560nm (excitation) and 590nm (emission). Percent viability is calculated using the formula as follows:

$$\frac{OD_{(cell+sample)} - OD_{(negative\ control)}}{OD_{(cells)} - OD_{(negative\ control)}} \times 100 = \% \text{ viability}$$

While IC₅₀ of the active samples was calculated using linear regression analysis between percent of survival against the concentration of the sample

RESULTS AND DISCUSSION

Compound **1** was isolated as yellowish brown crystals, its has positive ESI-MS [M+H]⁺ at m/z 359. ¹H-NMR data showed that compound **1** has 6 aromatic protons at δ 6.46, 6.64, 6.59 in 1st aromatic ring, 6.33 (2H), 7.19 in 2nd aromatic ring and 8pair of methylene proton groups at δ 3.11, 1.66, 1.33 (4-CH₂), 1.55, and 2.44. Three proton peaks at δ 6.46 (1H, dd, *J* 2.60 & 1.95 Hz), 6.59 (1H, d, *J* 1.95 Hz), 6.64 (1H, d, *J* 7.79 Hz) in 1st ring showed the AMX pattern of aromatic ring proton system, its mean that in 1st aromatic ring has 2 type of aromatic proton position: ortho position between δ 6.46 and δ 6.64, and meta position between δ 6.46 and δ 6.59 (Figure 1a). In addition, two proton peaks at δ 6.33 (2H, d, *J* 8.43 Hz) and 7.19 (1H, t, *J* 8.43 &

Figure 1. Proton pattern of the 1st and 2nd aromatic ring of Compound 1

Figure 2. HMQC and HMBC correlations of compound 1

7.79Hz) in 2nd ring showed the AB₂ pattern of aromatic ring proton system, indicated that 2nd aromatic ring has two symmetric proton peak at δ 6.33, which has correlation with proton peak at δ 7.19 in ortho position (Figure 1b). Two methylene proton peaks of compound 1 showed downfield chemical shift at δ 3.11 and 2.44 indicated that their position were located close to the electronegative atom or attached to aromatic ring. HMBC data (Figure 2) showed that proton peak at δ 3.11 has a correlation with carbonyl group at δ 209.5, and another proton peak (δ 2.44) has three different correlations with three different carbon atom (δ 135.8; 120.7; 116.6) from 2nd aromatic ring.

¹³C-NMR data showed 18 peak of carbon atoms including a downfield carbonyl signal at δ 209.5. Three carbon peaks at δ 30.6, 108.4, and 163.5 showed higher intensity compared to other carbon peaks, indicated that compound 1 has three symmetric carbon atoms. Five sp² methine carbon peaks can be observed at 108.4 (2C), 116.3, 116.6, 120.7, and 136.9, two quaternary sp² carbon at δ 111.5 and 135.8, and four oxyaryl carbons at δ 144.1, 146.1, and 163.5(2C).

The HMQC ($^1J_{(C-H)}$) and HMBC ($^2J_{(C-H)}$ to $^4J_{(C-H)}$) correlations of compound 1 (Figure 2). The HMQC correlation between a downfield proton atom at δ 3.11 with a downfield methylene carbon atom at δ 45.7 indicated that both atom were located near the electronegative

Figure 6. ESI-MS spectra of compound 2

Tabel I. ^{13}C - and ^1H -NMR data for malabaricone C (1) (^1H :500 MHz, ^{13}C : 125 MHz, in CD_3OD ; carbon numbering does not follow the official rule and is used here for simplicity)

1			Malabaricone C (Pham, 2000)	
	δ_{C}	δ_{H} (m, J (Hz))	δ_{C}	δ_{H} (m, J (Hz))
1	209.5		208.7	
2	45.7	3.11 (t)	44.6	3.09 (t)
3	25.6	1.66 (p)	24.3	1.62 (p)
4	30.4	1.33 (br.s)	29.2	1.30 (m)
5	30.6		29.2	1.26 (br.s)
6	30.7		29.2	
7	30.6		28.9	
8	33.1	1.55 (m)	31.4	1.49 (p)
9	36.4	2.44 (t)	35.0	2.39 (t)
10	135.8		135.3	
11	116.6	6.59 (d, 1.95)	115.2	6.62 (d, 2.0)
12	146.1		143.8	
13	144.1		141.8	
14	116.3	6.64 (d, 7.79)	114.9	6.67 (d, 8.0)
15	120.7	6.46 (dd, 2.60 & 1.95)	120.0	6.50 (dd, 2.0 & 8.0)
16	111.5		110.1	
17	163.5		161.8	
18	108.4	6.33 (d, 8.43)	107.5	6.31 (d, 8.1)
19	136.9	7.19 (t, 8.43 & 7.79)	135.7	7.12 (t, 8.1)
20	108.4	6.33 (d, 8.43)	107.5	6.31 (d, 8.1)
21	163.5		161.8	

atom, were supported by the HMBC correlation between proton atom at δ 3.11 with carbonyl group at δ 209.5, the same things happen to methylene group at δ_{H} 1.66/ δ_{C} 25.6. Other HMBC correlations between a downfield proton atom at δ 2.44 with carbon quaternary

sp^2 (1st aromatic ring) at δ 135.8 showed the influence of the aromatic ring to the chemical shift value of the carbon atom at δ 36.4. The ESI-MS spectra (Figure 3) showed a molecular ion $[\text{M}+\text{H}]^+$ 359.46 or $[\text{M}]^+$ 358.46, corresponds to molecular formula $\text{C}_{21}\text{H}_{26}\text{O}_5$.

Tabel II. ^{13}C - and ^1H -NMR data for malabariconesB (2) (^1H :500 MHz, ^{13}C : 125 MHz, in CD_3OD ; carbon numbering does not follow the official rule and is used here for simplicity)

	2		Malabaricone B (Pham, 2000)	
	δ_{C}	δ_{H} (m, J (Hz))	δ_{C}	δ_{H} (m, J (Hz))
1	209.8		208.6	
2	45.7	3.11 (t)	44.7	3.10 (t)
3	25.6	1.66 (p)	24.4	1.64 (p)
4	30.4	1.33 (br.s)	29.5	1.29 (br.s)
5	30.6		29.0	
6	30.7		29.0	
7	30.6		29.2	
8	33.1	1.55 (m)	31.6	1.51 (p)
9	36.4	2.44 (t)	35.0	2.46 (t)
10	135.8		134.6	
11	130.2	6.96 (d, 8.43)	129.3	6.96 (m, 8.4)
12	116.0	6.67 (d, 8.43)	115.0	6.69 (m, 8.4)
13	156.3		153.6	
14	116.0	6.67 (d, 8.43)	115.0	6.69 (m, 8.4)
15	130.2	6.96 (d, 8.43)	129.3	6.96 (m, 8.4)
16	111.5		110.1	
17	163.5		161.6	
18	108.3	6.33 (d, 8.43)	107.8	6.32 (d, 8.2)
19	136.8	7.19 (t, 8.43)	135.7	7.14 (t, 8.2)
20	108.3	6.33 (d, 8.43)	107.8	6.32 (d, 8.2)
21	163.5		161.6

Figure 7. Viability plots of compound 1 and compound 2 against breast carcinoma (MCF-7) cells line.

Based on the NMR data, supported by ESI-MS data and reference (Table I), compound 1 was identified as malabaricone C (Figure 2)(Pham, 2000).

Compound 2 was isolated as yellowish brown, its has positive ESI-MS $[\text{M}+\text{H}]^+$ at m/z 343. ^1H -NMR data showed that compound 2

has 7 aromatic proton at δ 6.33 (2H), 7.19 in 1st aromatic ring, 6.96 (2H), 6.67 (2H) in 2nd aromatic ring and 8 pair of methylene proton groups at δ 3.10, 1.66, 1.33 (4- CH_2), 1.55, and 2.49. Two proton peaks at δ 6.67 (2H, d, J 8.43 Hz) and 6.96 (2H, d, J 8.43 Hz) in 1st ring indicated the presence of AA'XX' pattern

of aromatic ring proton system, its mean that in 1st aromatic ring has four symmetric proton peaks with one type of ortho aromatic proton position at δ 6.96 and 6.67 (Figure 4a). In addition, two proton peaks at δ 6.33 (2H, d, J 8.43 Hz) and 7.19 (1H, t, J 8.43 Hz) in 2nd ring showed the AB₂ pattern of aromatic ring proton system (Figure 4b), indicated that 2nd aromatic ring has two symmetric proton peak at δ 6.33, which has correlation with proton peak at δ 7.19 in ortho position. Both AA'XX' and AB₂ patterns were supported by HMQC and HMBC correlation data (Figure 5). ¹³C-NMR data showed 16 peak of carbon atoms including a downfield carbonyl signal at δ 209.8. Five carbon peaks at δ 30.6, 108.4, 116.0, 130.3, and 163.5 showed higher intensity compared to other carbon peaks, indicated that compound **2** has five symmetric carbon atoms. Seven sp² methine carbon peaks can be observed at 108.4 (2C), 116.0 (2C), 130.3 (2C), and 136.9, two quaternary sp² carbon at δ 111.5 and 135.0, and three oxyaryl carbons at δ 156.3, and 163.5 (2C).

The HMQC (¹J_(C-H)) and HMBC (²J_(C-H) to ⁴J_(C-H)) correlations of compound **2** illustrated in Figure 5. The HMQC correlation between a downfield proton atom at δ 3.10 with a downfield methylene carbon atom at δ 45.8 indicated that both atom were located near the electronegative atom, were supported by the HMBC correlation between proton atom at δ 3.10 with carbonyl group at δ 209.8, the same things happen to methylene group at δ_H 1.66/ δ_C 25.8. Other HMBC correlation between a downfield proton atom at δ 2.49 with carbon quaternary sp² (1st aromatic ring) at δ 135.0 showed the influence of the aromatic ring to the chemical shift value of the carbon atom at δ 36.1. The ESI-MS spectra (Figure 6) showed a molecular ion [M+H]⁺ 343.45 or [M]⁺ 342.45 indicates the molecular weight of the compound corresponds to molecular formula C₂₁H₂₆O₄.

Based on the NMR data, supported by ESI-MS data and reference (Table II),

compound **2** was identified as malabaricone B (Figure 5) (Pham, 2000).

Cytotoxic assay

Compound **1-2** were examined for their cytotoxicity against the breast carcinoma (MCF-7) cells line (Figure 7) and showed that both compounds were active as anticancer compounds against breast carcinoma (MCF-7) cells line with the IC₅₀ values 2.68 and 0.71 μ g/mL, respectively.

CONCLUSION

Isolation and purification of ethyl acetate fraction 5 (F-5) of the bark of *M. fatua* using column chromatography method, and structure elucidation based on spectroscopic data analysis result have been done. Can be concluded that compound **1** and **2** are malabaricone C and malabaricone B, respectively. Both compounds have high cytotoxicity activity against breast carcinoma (MCF-7) cells line with IC₅₀ values are 2.68 and 0.71 μ g/mL, respectively.

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