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Triterpenoids from The Bark of *Garcinia porecta* and their Cytotoxic Activity against MCF7 Breast Cancer Lines

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ABSTRACT

The *Garcinia* genus is a well known tropical plant in the Indo-Malesiana region and mainly distributed in tropical countries including Indonesia, Thailand, and Malaysia. Previous phytochemical studies on *Garcinia* species have led to the identification and isolation of mainly prenylated xanthones. This research describes the isolation and structure elucidation of isolated triterpenoids compounds from the bark of *Garcinia porecta*. Dried powder bark of *G. porecta* was extracted with methanol and then partitioned with *n*-hexane, ethyl acetate, and *n*-butanol. The *n*-hexane extract then was separated and purified with chromatography techniques to obtain isolated compounds **1** and **2**. The chemical structure of isolated compounds were elucidated by spectroscopic methods including one and two-dimensional NMR as well as high-resolution mass spectrometric analysis and identified as lanosterol (**1**) dan arabidiol (**2**), respectively. These triterpenoids were isolated from this plant for the first time. Compound **1** and **2** showed weak cytotoxic activity against MCF-7 breast cancer cells with IC_{50} values of 60.09 dan 46.17 µM, respectively.

Keywords: arabidiol, *Garcinia porecta*, lanosterol, MCF7 breast cancer cells, triterpenoid.

INTRODUCTION

Garcinia is one genus in the family Guttiferae with number of species and is distributed in lowland forests of tropical Asia, Africa, Polynesia and New Caledonia (Merza et al., 2004; Heyne, 1982). In Indonesia about 91 species spread across the island of Sumatra, Java, Sulawesi, and Maluku (Panthong et al., 2006) and known for prenylated xanthone content (Ampofo and Waterman, 1986; Bennet and Lee, 1989; Adegboye, 2008). The previous investigation on Garcinia species had reported a wide range of biological activities such as cytotoxic, antimicrobial, antimalarial, and anti-HIV-1 protease inhibitory (Kosela et

al., 2000; Bennet and Lee, 1989). In our ongoing research to get new compounds from Garcinia species, we selected Garcinia porecta. G.porecta commonly known as "manggisutan" in Indonesia and widely distributed in tropical countries especially in Indonesia and Malaysia (Martin, 1980). In this report, we discuss the isolation and structural identification of two known triterpenoids, lanosterol (**1**) and arabidiol (**2**) from the methanol extract of the bark of G. porecta. Cytotoxicity against MCF7 breast cancer lines was evaluated for all isolated compounds.

METHODS

General Procedure

The melting points were measured on an electro thermal melting point equipment and uncorrected. The IR spectra were obtained on 1760X Perkin-Elmer using KBr discs. The HRTOFMS were obtained using a Waters Xevo QTOF MS mass spectrometer. NMR spectra were recorded on JEOL JNM A-500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) and TMS as an internal standard. Column chromatography was performed with silica gel 60 (70-230 and 200-400 mesh). Vacuum Liquid Chromatography on silica gel (SiO_2, Kieselgel 60, Merck, Darmstadt, Germany), Column chromatography on silica gel (SiO_2, Kieselgel 60, Merck, Darmstadt, Germany), TLC analysis on Kieselgel 60 F_{254} (Merck) plates; visualization was performed with UV lamp and plates were sprayed with 10% H_2SO_4 solution in ethanol and heated.

Plant Material

The bark of *G. porecta* was obtained from Bogor Botanical Garden, Bogor, Indonesia in June 2017. The plant was provided by Bogoriense Herbarium, and a voucher specimen was deposited at the herbarium.

Extraction and Isolation

The powder dried bark (2.0 Kg) of *G. porecta* was extracted with aqueous methanol (12 L) at room temperature. Evaporation of the methanolic extract to produce the brown residue (150.4 g). The residue was first suspended in H_2O and then partitioned successively with *n*-hexane, EtOAc, and *n*-butanol. The *n*-hexane extract (20.0 g) was separated by vacuum liquid chromatography on silica gel G60 using *n*-hexane-EtOAc as gradient solvent to give seven fractions (A–G). Fraction D (3.25 g) was separated on a column chromatography of silica gel, with an-hexane–acetone as solvent (10:0–1:1), to yield six subfractions (C01–C06). Subfraction C04 (185.5 mg) was chromatographed on a column of silica gel with $CHCl_3$: MeOH (9.75:0.25) as a solvent to give five subfractions (C04A-

C04E). Subfraction C04B (86.5 mg) was separated on preparative TLC of silica gel GF_{254}, with *n*-hexane–EtOAc (9:1) as a solvent, to produce **1** (18.5 mg). Fraction E (2.10 g) was separated on a column chromatography of silica gel with a gradient of *n*-hexane–acetone (10:1–1:10) as a solvent, to yield five subfractions (D01–D05). Subfraction D03(78.5 mg) was recrystallized in MeOH, to give **2** (12.6 mg).

Bioassays for cytotoxic activity

Determination of the cytotoxic activities was conducted according to the methodology described in previous papers (Supriatno et al., 2018). The MCF-7 cells were seeded into 96-well plates at an initial cell density of 3 x 10^4 cells cm^{-3}. After 24 hours of incubation, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Then six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30 - 7.65). Control wells received only DMSO. The assay was terminated after a 48 hours incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. The incubation was continued for another 4 hours, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 hours incubation was conducted. Optical density was read by using a microplate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (μM). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis run in triplicate and averaged.

RESULT AND DISCUSSION

The methanolic extract from the bark of *C. porecta* was concentrated and extracted with *n*-hexane, ethyl acetate, and *n*-butanol. The *n*-hexane extract showed the strongest cytotoxic activity against MCF7 breast cancer cells with an IC_{50} 35 µg/mL compared with ethyl acetate and *n*-butanol extract with showed an IC_{50} of 48 and 52 µg/mL, respectively. By using cytotoxic activity assay, the *n*-hexane extract was separated by column chromatography on silica gel G60 and preparative Thin Layer Chromatography (TLC) on silica gel GF_{254} to produce two cytotoxic compounds **1** and **2 (Figure 1)**.

Lanosterol (**1**)**.** White needle crystals; m.p. 139-140 °C; IR (KBr) ν_{max}(cm^{-1}) 3360, 2980, 2860, 1580 and 1156; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; MS spectrum *m/z* 426.

Arabidiol (**2**). White needle crystals; m.p. 147-149 °C; IR (KBr) ν_{max}(cm^{-1}) 3350, 1555, 1140, and 1045; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; MS spectrum *m/z* 444.

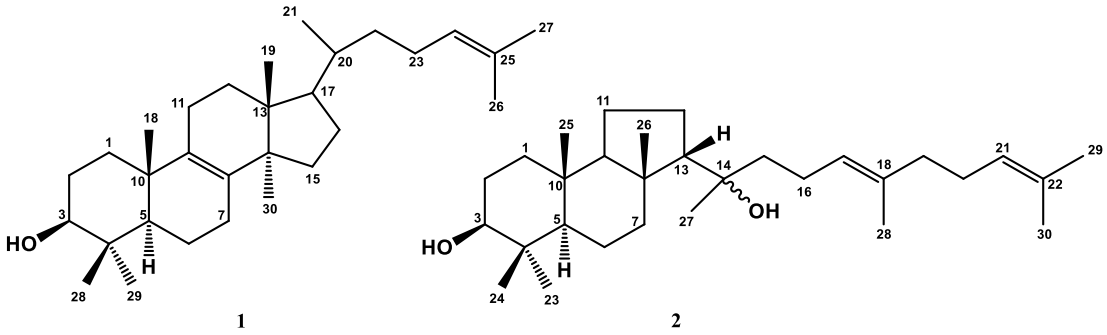

Figure 1. Chemical Structures of **1** and **2.**

Compound **1** was isolated as a white needle crystal. The molecular formula was established to be $C_{30}H_{50}O$ based on MS spectrum *m/z* 426 and NMR data (Table 1), indicating unsaturation number of six. The IR spectra revealed peaks at 3360, 2980, 2860, 1580 and 1156 cm^{-1} due to of hydroxyl, aliphatic, carbon-carbon double bond and ether groups. The ^{1}H-NMR spectrum of the compound **1** revealed the presence of seven tertiary methyl resonances at δ_H 0.89 (3×), 1.01 (2 ×), 1.70 and 1.82, one secondary methyl at δ_H 0.88 (3H, d, *J*=5.6 Hz), one elefinic methine proton at δ_H 5.20 (1H, dd, *J*=3.4, 7.2 Hz), one oxygenated proton at δ_H 3.34 (1H, dd, *J*=4.8, 6.7 Hz) and aliphatic protons resonance at δ_H 1.00-2.00.The ^{13}C-NMR spectrum revealed 30 carbon signals, which were determined d by their chemical shifts and the DEPT spectra as seven tertiary methyls, one secondary methyl, one sp^2 methine, three sp^2 quaternary carbons, one sp^3 oxygenated methine, ten sp^3 methylenes, three sp^3methines, and four sp^3 quaternary carbons. These unsaturations accounted for two out of the total six degrees of unsaturation. The remaining four degrees of unsaturation were consistent with the lanostane-type triterpenoid (Susanti et al., 2013; Connolly and Hill, 2003). To determine the connectivity of the functional group in compound **1**, ^{1}H-^{1}H COSY and HMBC experiments were conducted, and the results are shown in Figure 2. The ^{1}H–^{1}H COSY spectrum of **1** showed connectivity in H_1-H_2-H_3, H_5-H_6-H_7, H_{11}-H_{12}, H_{15}-H_{16}-H_{17} and H_{20}-H_{22}-H_{23}-H_{24}, indicating the presence of lanostane-type (Connolly and Hill, 2003). In the HMBC spectrum, the connectivity arising from the tertiary methyl protons to their attached carbons enabled the determine of the five primary methyls at C-4, C-10, C-14, C-15, and C-25, respectively. Then, a connectivity from an olefinic proton at H-24 (δ_H 5.20) to C-25 (δ_C 131.5) and C-24 (δ_C 124.7) and connectivity from the methyl proton at CH_3-26 (δ_H 1.70) and CH_3-27 (δ_H 1.82) to C-25 (δ_C 131.5) were used to

determine a double bond at C-24/C-25. This observation was feature for a lanostane-type triterpenoid (Susanti et al., 2013; Connolly and Hill, 2003). The other olefinic moiety located at C-8/C-9 was supported by connectivity from CH_3-18 (δ_H 1.01) to C-9 (δ_C 134.5) and methylene proton at C-7 (δ_H 1.99) to C-8 (δ_C 135.2). The hydroxy group at C-3 was determined based on connectivity from CH_3-28 (δ_H 0.89) and H-2 (δ_H 1.72) to C-3 (δ_C 78.7).

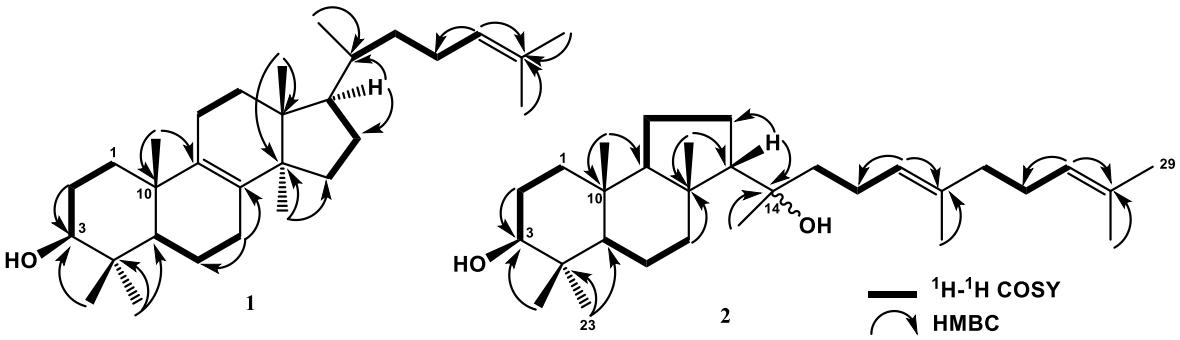

Figure 2. ^{1}H-^{1}H and HMBC Correlation of **1** and **2**

The configuration of **1** was identified by comparison those coupling constant in ^{1}H NMR spectra (^{1,2}J and ^{2,3}J) and chemical shift of ^{13}C NMR spectra with previous literature as well as from biogenetic point of view for occurrence of lanostane-type triterpenoid in Garcinia genus (Susanti et al., 2013; Connolly and Hill, 2003). A detailed comparison of the NMR data of **1** with those of lanosterol previously reported (Susanti et al., 2013) revealed very high similarity. Therefore, compound **1** was identified as lanosterol.

Compounds **2** was isolated as a white needle crystal. The chemical formula was established to be $C_{30}H_{52}O_2$ based on MS spectrum m/z 444 and NMR data (Table 1), thus indicating the degree of unsaturation of five. The IR spectra revealed peaks at 3350, 2970, 2850, 1590 and 1140 cm^{-1} due to of hydroxyl, aliphatic, the double bond and ether groups. The ^{1}H-NMR spectrum revealed of eight tertiary methyl resonances at δ_H 0.89, 0.91, 0.84, 0.86, 1.20, 1.61, 1.82 and 1.70, one oxygenated methine at δ_H 3.42 (1H, dd, *J*=4.7, 6.2 Hz), two elefinic methine protons at δ_H 5.10 (1H, dd, *J*=3.4, 7.2 Hz) and δ_H 5.20 (1H, dd, *J*=3.5, 6.8 Hz) as well as aliphatic protons resonance at δ_H 1.00-2.00. The ^{13}C-NMR spectrum revealed 30 carbon signals, which were determined by their chemical shifts and the DEPT spectrum as eight tertiary methyls, two sp^2 methine, two sp^2 quaternary carbons, one sp^3 oxygenated methine, one sp^3 oxygenated carbon, ten sp^3 methylenes, three sp^3 methines, and three sp^3 quaternary carbons. These unsaturations accounted for two out of the total five degrees of unsaturation. The remaining three degrees of unsaturation were consistent with the tricylic triterpenoid (Xiang *et al*., 2006; Connolly and Hill, 2003). To determine the connectivity of the functional group in compound **2**, ^{1}H-^{1}H COSY and HMBC experiments

were conducted, and the results are shown in Figure 2. The $^{1}H–^{1}H$ COSY spectra of **2** showed connectivity in H_1-H_2-H_3, H_5-H_6-H_7, H_9-H_{11}-H_{12}-H_{13}, H_{15}-H_{16}-H_{17}, and H_{19}-H_{20}-H_{21}, indicating the presence of tricylic triterpenoid (Xiang et al., 2006; Connolly and Hill, 2003). The correlations arising from the tertiary methyl protons to their neighboring carbons enabled the determine five methyls at C-4, C-8, C-10, C-14, C-18, and C-25, respectively.

In addition, a correlation from one olefinic proton at H-21 (δ_H 5.10) to C-25 (δ_C 132.0) and C-24 (δ_C 26,4), and another olefinic proton at H-17 (δ_H 5.20) to C-18 (δ_C 135.0) and C-16 (δ_C 22.8) were used to determine a double bond at C-24/C-25 and C-17/C-18, which was characteristic for a arabidiol-type triterpenoid (Xiang et al., 2006; Connolly and Hill, 2003). The hydroxyl group at C-3 was determined based on the correlation from CH_3-24 (δ_H 0.89) and H-2 (δ_H 1.72) to C-3 (δ_C 78.7).The other hydroxy group was located at C-14 by correlations from CH_3-27 (δ_H 1.20), H-13 (δ_H 1.10) and H-15 (δ_H 1.40) to C-14 (δ_C 75.3). A comparison of the NMR data of **2** with those of arabidiol previously reported (Xiang et al., 2006), revealed the high similarity. Therefore compound **2** was identified as an arabidiol.

The cytotoxic activity of compounds **1** (60.09 mM) and **2**(46.17 μM) were evaluated against the against MCF-7 breast cancer cells according to a method described (Supriatno et al., 2018) and was used a cisplatin (IC_{50} 27.0 μM) as a positive control (Hadisaputri et al., 2012). Based on the IC_{50} value of compounds **2** showed stronger activity, suggesting that the presence of an additional hydroxyl group at C-14 and opening of D-ring of triterpenoid structure seems to increase the cytotoxic activity. These results are similar to those of previously reported that the presence of additional of the hydroxyl group and the opening of D-ring in flavonoid structure can increase the cytotoxic activity (Boussahel et al., 2015).

Table 1. NMR data for Compounds **1** and **2**

Position of C	**1***		**2****	
	δ_H ($\sum$ H, mult., *J*=Hz)	δ_C (mult.)	δ_H ($\sum$ H, mult., *J*=Hz)	δ_C (mult.)
1	1.67 (1H, m) 1.42 (1H, m)	35.6 (t)	1.56 (1H, m) 1.31 (1H, m)	38.4 (t)
2	1.72 (1H, m) 1.47 (1H, m)	27.5 (t)	1.72 (1H, m) 1.46 (1H, m)	27.4 (t)
3	3.34 (1H, dd, 4.8, 6.7)	78.7 (d)	3.42 (1H, dd, 4.5, 6.4)	78.6 (d)
4	-	38.9 (s)	-	38.8 (s)
5	1.07 (1H, m)	38.7 (d)	0.94 (1H, m)	55.3 (d)
6	1.60 (1H, m) 1.36 (1H, m)	19.6 (t)	1.63 (1H, m) 1.38 (1H, m)	18.2 (t)
7	1.99 (1H, m) 1.89 (1H, m)	26.5 (t)	1.56 (1H, m) 1.31 (1H, m)	40.3 (t)
8	-	135.2 (s)	-	35.2 (s)

Table 1. NMR data for Compounds **1** and **2** (continued)

Position of C	**1***		**2****	
	δ_H (∑ H, mult., *J*=Hz)	δ_C (mult.)	δ_H (∑ H, mult., *J*=Hz)	δ_C (mult.)
9	-	134.5 (s)	0.94 (1H, m)	58.9 (d)
10	-	37.3 (s)	-	38.2 (s)
11	1.98 (1H, m) 1.89 (1H, m)	21.9 (t)	1.90 (1H, m) 1.65 (1H, m)	20.4 (t)
12	1.66 (1H, m) 1.42 (1H, m)	35.6 (t)	1.86 (1H, m) 1.60 (1H, m)	25.1 (t)
13	-	44.6 (s)	1.10 (1H, m)	57.8 (d)
14	-	49.9 (s)	-	75.3 (s)
15	1.42 (1H, m) 1.17 (1H, m)	35.1 (t)	1.40 (2H, m)	44.3 (t) 22.8 (t)
16	1.90 (1H, m) 1.65 (1H, m)	28.5 (t)	1.94 (2H, m)	
17	1.15 (1H, m)	50.5 (d)	5.10 (1H, dd, 3.4, 7.2)	124.7 (d)
18	1.01 (3H, s)	19.8 (q)	-	135.0 (s)
19	0.89 (3H, s)	16.7 (q)	1.98 (2H, t, 7.2)	39.7 (t)
20	1.32 (1H, dd, 2.6, 5.6)	30.3 (d)	2.00 (2H, t, 7.2)	26.4 (t)
21	0.88 (1H, d, 5.6)	19.5 (q)	5.20 (1H, dd, 3.5, 6.8)	123.5 (d)
22	1.53 (2H, m)	36.4 (t)	-	132.0 (s)
23	1.94 (2H, m)	24.7 (t)	0.89 (3H, s)	22.4 (q)
24	5.20 (1H, dd, 3.4, 7.2)	124.7 (d)	0.91 (3H, s)	23.4 (q)
25	-	131.5 (s)	0.84 (3H, s)	16.1 (q)
26	1.70 (3H, s)	18.6 (q)	0.86 (3H, s)	15.8 (q)
27	1.82 (3H, s)	10.6 (q)	1.20 (3H, s)	26.1 (q)
28	1.01 (3H, s)	25.0 (q)	1.61 (3H, s)	16.1 (q)
29	0.89 (3H, s)	23.5 (q)	1.82 (3H, s)	18.6 (q)
30	0.89 (3H, s)	20.4 (q)	1.70 (3H, s)	24.6 (q)

*measured in pyridine-d_5, 500 MHz for ^{1}H and 125 MHz for ^{13}C-NMR
**measured in CD_3OD, 500 MHz for ^{1}H and 125 MHz for ^{13}C-NMR

CONCLUSIONS

Two known triterpenoids, lanosterol (**1**) and arabidiol (**2**) were obtained from the bark of *Garcinia poreccta*. Compounds **1** and **2** showed weak cytotoxic activity against MCF-7 breast cancer cells with IC_{50} values of 25.6 and 20.8 µM, respectively, indicating the presence of an additional hydroxyl group at C-14; and the opening of D-ring of triterpenoid structure seems to increase the cytotoxic activity.

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