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Phenolic Constituents from the Tree Barks of *Garcinia cf cymosa* and their Antioxidant and Antibacterial Activities

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ABSTRACT

Two isoprenylated xanthones, α-mangostin (1) and β-mangostin (2) were isolated from the tree barks of *Garcinia cf cymosa*, along with the flavanol epicatechin (3). Their structures were elucidated by analysis of spectroscopic data. Compounds 1-3 exhibited moderate in vitro antibacterial activity against Staphylococcus aureus and Bacillus sp, while in the 1,2-diphenyl-picryl-hydrazyl (DPPH) antioxidant assay system only compound 3 showed moderate free radical scavenging activity, with IC50 value of 41.8 ppm.

| Antibacterial | antioxidant | flavanol | Garcinia cf cymosa | xanthones |

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1. INTRODUCTION

The genus *Garcinia* (Clusiaceae or Guttiferae) comprises about 200 species and occurs in moist tropical regions, including Southeast Asia [1,2]. Phytochemical investigations have shown that this group of plants, such as *Garcinia mangostana*, are very rich inphenolic compound, prenylated xanthones and flavonoids [3,4]. Biological studies on the constituents of the fruits of *G. mangostana* demonstrated antibacterial, antifungal, antitumor promotion, and other biological activities [3,5,6]. The present work on the tree barks of *Garcinia cf cymosa* collected from Muarobungo District, Jambi Province, Sumatra, Indonesia was investigated for the first time to evaluate the phytochemical profil of this endemic plant in comparison with that of other related species. This preliminary investigation led to the isolation of two prenylated xanthones, namely, α-mangostin (1) [7-9] and β-mangostin (2) [10,11] along with a flavanol epicatechin (3) [4]. The structural characterization, as well as antioxidant and antibacterial evaluation of these compounds against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus sp*, are discussed herein.

2. EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Fisher Jhone micromelting point apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a UV-Vis spectrophotometer 1700 Series in MeOH and infrared (IR) spectra on a Perkin Elmer 1600 Series. spectrophotometer in $CHCl_3$. The ^1H and ^{13}C NMR, COSY, HMQC, and HMBC were run on JEOL JNM-ECS 400 spectrometers. All mass spectra were taken under high resolution time of flight mass spectrum (HRTOFMS) conditions with Waters PremierXE spectrometer. Thin layer chromatography (TLC) was done on GF_{254} (Merck).

Plant Material. *Garcinia cf cymosa* was collected in Juni 2011 from the region of Muarobungo District, Jambi Province, Indonesia, and authenticated by the Herbarium Bogoriensis, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia. A voucher speciment has been deposited at the Herbarium.

Extraction and Separation. The air-dried powdered tree barks of *Garcinia cf cymosa* (5 kg) were exhaustively macerated with MeOH at room temperature, and concentrated to give a dark brown crude extract (647 g). Part of the MeOH extract (200 g) was successively fractionated by column chromatography on silica gel and eluted with a gradient of *n*-hexane and dichloromethane, and then ethyl acetate to obtain 15.2, 21.4, and 38.7 g of the respective residues after evaporating the solvents.

Part of the n-hexane extract (10 g) was chromatogtaphed on silica gel and eluted with *n*-hexane, dichloromethane, ethyl acetat, and methanol to obtain 8 major fractions. Fraction 3 (1.47 g) was purified on silica column to yield 124 vials, and vial 39 on crystalization gave pure α-mangostin (**1**) (45 mg). Part of the dichloromethane extract (6.5 g) was also submitted to silica column chromatography using *n*-hexane, dichloromethane, etil asetat, and methanol as eluents to produce 15 major fractions. Fraction 11 (0.8 g) under the same conditions yielded β-mangostin (**2**) (268 mg). Part of the ethyl asetate extract (10 g) was submitted to chromatographic separation

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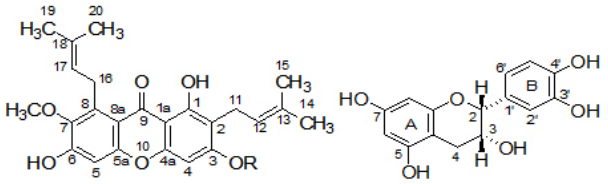
on silica column to give 21 major fractions. Fraction 3 (0.2 g) under similar conditions yielded epicatechin (**3**) (22 mg).

3. RESULTS AND DISCUSSIONS

The dried powder of tree barks of *Garcinia cf cymosa* were macerated with methanol at room temperature and the dried residue obtained was then separated with column chromatography to afford two prenylated xanthones, α-mangostin (**1**) and β-mangostin (**2**), together with a flavanol, epicatechin (**3**).

α-Mangostin (1), was isolated as a yellow powder, with mp. 193-194 ^{o}C. The molecular formula $C_{24}H_{26}O_6$ (*m/z* 410,1709) was determined by high resolution time of flight mass spectrum (HRTOFMS), the UV (λ_{max} 244, 258, 314 nm) and IR spectra were indicative of a xanthone derivative. The ^{1}H NMR spectrum showed two aromatic singlets [δ 6.29 (1H, s) and 6.83 (1H, s)], and one methoxyl group [δ 3.80 (3H. s)]. Signals due to two prenyl groups were also observed at [δ 1.77 (3H, s) and 1.83 (3H, s), 3.45 (2H, d, *J* = 6.1 Hz), 5.26 (1H, m)], and [δ 1.59 (3H, s) , 1.84 (3H, s), 4,09 (2H, d, *J* = 6.1 Hz), 5.29 (1H, m)], The ^{1}H and ^{13}C NMR spectral data of **1** closely resembled those of α-mangostin reported in the literature [7] (Table 1). The structural assignments were substantiated by 2D NMR techniques HMQC and HMBC. In the HMBC spectrum the proton resonating at [δ 3.45, H-11] showed long-range heteronuclear connectivities with C-1 (δ 160.7), C-2 (δ 111.8), and C-3 (δ 154,6). While, the proton H-16, resonating at δ 4.09, exhibited HMBC interactions with C-7 (δ 142.6), C-8 (δ 137.1) and C-8a (δ 112.3). In addition, the methoxyl signal at δ 3.80 showed a cross peak with a quaternary aromatic carbon signal at δ 142.6 (C-7). Thus, the observed NMR signals are characteristic of a 1,3,6,7-tetraoxygenated xanthone with methoxyl and prenyl moieties at C-7, C-2 and C-8, respectively. Therefore, compound **1** may be assigned as 1,3,6-trihydroxy-2,8-diprenyl-7-methoxyxanthone, known as α-mangostin.



1 : R = H
2 : R = CH_3

(3)

β-Mangostin (2), was obtained as a yellow powder, mp. 184-185 ^{o}C, and HRTOFMS spectrum at *m/z* 424.1855 established a molecular formula of $C_{25}H_{28}O_6$. Its ultraviolet (UV) spectrum, posessing characteristic absorption maxima (λ_{maks} 243, 258, 316, 319 nm) suggesting that **2** also possessed a xanthone skeleton. The ^{1}H NMR spectrum of **2** showed signals for two aromatic protons at [δ 6.48 (1H, s) and 6.84 (1H, s)], along with two methoxyl groups at [δ 3.96 (3H, s) and 3.80 (3H, s)]. The ^{1}H NMR spectrum of **2** also indicated the presence of four methyl groups on vinyl carbon [δ 1.77 (3H, s), 1.83 (3H, s), 1.59 (3H, s) and 1.84 (3H, s)], along with two benzylic methylenes [δ3.31 (2H, d) and 4.12 (2H, d)] and two olefinic methines at [δ 5,26 (1H, m) and 5,29 (1H, m)] indicative for the presence of two prenyl side chains. The ^{1}H and ^{13}C NMR spectral data of **2** were similar to those reported in the literature for β-mangostin [11,12] (Table 2). The structural assignment was confirmed by 2D NMR techniques HMQC and HMBC. The most important observations in the HMBC spectrum were the connectivities observed between the singlet proton signals of benzylic methylene signals at C-11 and C-16.The proton resonating at [δ 4.12, H-11] showed long-range heteronuclear connectivities with C-1 (δ 160.1), C-2 (δ 111.6), and C-3 (δ 164,4). While, the H-16, resonating at δ 3.31 exhibited HMBC interactions with C-7 (δ 144.4), C-8 (δ 138.0) and C-8a (δ 111.9). In addition, the methoxyl signal at δ 3.80 and 3.93 showed cross peaks with quaternary aromatic carbon signals at δ 144.4 (C-7) and 164.4 (C-3), respectively. Thus, the structure of compound **2** was therefore concluded to be 1,6-dihydroxy-3,7-dimethoxy-2,8-diprenylxanthone known as β-mangostin.

Table 1. NMR data for compound (**1**) in comparison with literature value for α-mangostin

C	^{13}C-NMR (ppm)		H	^{1}H-NMR (ppm)	
	Compound I	α-Mangostin		Compound 1	α-Mangostin
1	160,7	161,7			
2	111,8	111,1			
3	154,6	155,7			
4	93,4	93,2	4	6,29	6,38
4a	161,7	162,9			
5a	155,9	157,3			
5	101,6	102,7	5	6.83	6,80
6	155,2	156,2			
7	142,6	144,5			
8	137,1	138,1			
8a	112,3	112,0			
9	182,1	182,8			
1a	103,7	103,6			
16	26,7	26,9	16	4,09	4,12
17	123,2	124,8	17	5,29	5,27
18	136,0	131,4			
19	18,3	18,3	19	1,84	1,82
20	25,8	25,9	20	1,69	1,64
11	21,5	22,0	11	3,45	3,34
12	121,5	124,8	12	5,26	5,27
13	132,3	131,4			
14	18,0	17,9	14	1,77	1,77
15	25,9	25,8	15	1,83	1,63
7-OCH_3	62,2	61,3	7-CH_3	3,80	3,78

Epicatechin (3), was successfully isolated as a white powder, mp. 248-249 ^{o}C, HRTOFMS spectrum at *m/z* 290.1027 established a molecular formula of $C_{15}H_{14}O_6$, and infrared (IR) spectrum, possesed characteristic absorptions for hydroxyl group (3417 cm^{-1}) and an aromatic ring (1514 cm^{-1}) for a phenolic compound. Analysis of ^{13}C NMR spectrum revealed the presence of two aromatic rings, together with two oxymethine groups (δ 66.8 and 79.3) and a methylene group (δ 29.6) indicative for the presence of –CH(O-)CH(O-)-CH_2- moiety. Therefore, compound **3** could be proposed as a flavan-3-ol. The ^{1}H NMR spectrum

of **3** showed the presence of signals for an ABX aromatics system of ring B[δ 6.78 (1H, d, *J* = 8.3 Hz), 6.83 (1H, dd, *J* = 2.0, 8.3 Hz), 7.05 (1H, d, *J* = 1.4 Hz) and two broad singlets for two meta oriented aromatic protons of ring A [δ 6,02 (1H, d, *J* = 2,2 Hz, H-6) and 5,92 (1H, d, *J* = 2,2 Hz, H-8)]. Furthermore, by COSY and HMBC correlations, the signals observed at 4.87 ppm (brs), 4.20 (brs), 2.92 ppm (dd, *J* = 5.5, 4.8 Hz) and 2.54 ppm (dd, 8.3, 8.9 Hz) were assigned as H-2, H-3, H-4a, and H-4b, respectively. Taking note on the coupling constants for H-2, H-3 and H-4, the relative stereochemistry of the phenyl group at C-2 and hydroxyl group at C-3 were both as alpha (α). Accordingly the structure of compound **3** was established as (2S,3S)-3,5,7,3',4'-pentahydroxyflavan known as epicatechin. The ^{1}H and ^{13}C NMR spectral data of **3** (Table 3) also closely resembled those of epicatechin reported in the literature[4].

Table 2. NMR data for compound **2** in comparison with literature value for β-mangostin

C	^{13}C-NMR (ppm)		H	^{1}H-NMR (ppm)	
	Compound **2**	β-mangostin		Compound **2**	β-mangostin
1	160,1	160,6			
2	111,6	111,1			
3	164,4	162,9			
4	89,7	89,9	4	6,48	6,36
4a	156,1	155,7			
5a	157,4	157,3			
5	102,5	102,7	5	6.84	6,39
6	156,2	156,2			
7	144,4	144,5			
8	138,0	138,1			
8a	111,9	112,2			
9	182,7	182,8			
1a	104,0	103,6			
16	21,8	21,9	16	3,31	3,34
17	124,6	123,5	17	5,20	5,27
18	131,4	132,4			
19	17,8	17,9	19	1,63	1.63
20	25,8	25,9	20	1,77	1,77
11	26,8	26,9	11	4,12	4,12
12	123,2	123,32	12	5,27	5,27
13	131,3	131,4			
14	18,2	18,3	14	1,65	1,69
15	25,9	25,9	15	1,83	1,82
3-OCH_3	56,4	56,5	3-OCH_3	3,96	3,93
7-OCH_3	62,2	61,3	7-OCH_3	3,80	3,83

Antibacterial Activity. The isolated compounds, α-mangostin (**1**), β-mangostin (**2**), and epicatechin (**3**) were evaluated for their antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus sp.* using disk-diffusion method. Zone of inhibition was determined after incubation at 37 ^{o}C for 48 h. Zone diameter of each compounds against *E. coli*, *S. aureus*, and *Bacillus sp*. are tabulated in Table 4. The data indicated that α-mangostin (**1**), showed high antibacterial activity against *S. aureus* and *Bacillus sp*. by producing zone of inhibitions of 3.4 and 3.2 mm, respectively, greater than that of chloramphenicol as a standard, with a zone of inhibitions of 2.6 - 2.7 mm. However, α-mangostin (**1**) showed very low activity against *E. coli* compared to standard. β-Mangostin (**2**) also showed similar tendency, but with higher antibacterial effects with 6.3 and 5.0 mm of zone inhibiition, respectively against *S. aureus* and *Bacillus sp*. On the other hand, compound **3** showed only antibacterial activity against *Bacillus sp.*

Antioxidant activity. DPPH radical scavenging assay was used to evaluate the antioxtdant activity of the isolated compoumds **1-3**, and the results were compared with that of ascorbic acid as a standard. The data obtained (see Table 5). Indicated that α-mangostin (**1**) and β-mangostin **(2)** shown no antioxidant activity with, IC_{50} 566,6 and 250,5 ppm, respectively, while epicatechin (**3**) is moderately active, with IC_{50} 41.8 ppm. These results indicated that the phenolic constituents obtained from polar fraction was responsible for the antioxidant phenomena.

Table 3. NMR data for compound 3 in comparison with literature value for epicatechin

C	^{13}C-NMR (ppm)		H	^{1}H-NMR (ppm)	
	Compound 3	Epicatechin		Compound 3	Epicatechin
2	79,3	80,0	2	4,87	4,82
3	66,8	67,6	3	4,20	4,18
4	29,6	29,4	4α	2,54	2,74
			4β	2,92	2,85
4a	99,6	100,0			
5	157,0	157,5			
6	95,6	96,0	6	6,02	5,94
7	157,4	157,8			
8	95,2	96,4	8	5,92	5,91
8a	157,5	158,1			
1`	132,1	132,4			
2`	115,1	115,4	2`	7,05	6,97
3`	145,6	146,1			
4`	145,7	145,9			
5`	115,3	116,0	5`	6,78	6,75
6`	119,2	119,5	6`	6,83	6,78

Table 4. Antibacterial activity of compounds 1-3

Bacteria strain	Concentration (ppm)					Chloramphenicol
	2	4	6	8	10	10
E coli	1,7	2,6	2,0	1,7	1,8	4,5
S aureus	1,6	1,8	2,4	2,4	3,4	2,6
Basillus sp	2,2	2,4	2,3	3,0	3,2	2,7

α-Mangostin (1): yellow powder; mp. 193-194 ^{o}C; HRTOFMS *m/z* 410.1709 (calcd for $C_{24}H_{26}O_6$ *m/z* 410.17.29); UV (EtOH) (λ_{max} 243, 258, 314, 319 nm; IR (KBr) γ_{max} 3417m 2920, 1643, 1612, 1558, 1458 cm^{-1}. ^{1}H and ^{13}C NMR data (see Table 1)

β-Mangostin (2): light yellow powder; mp. 184-185 ^{o}C; HRTOFMS *m/z* 424.1855 (calcd for $C_{25}H_{28}O_6$ *m/z* 424.1855); UV (EtOH) (λ_{max} 243, 258, 316, 319 nm; IR (KBr) γ_{max} 3398. 2993, 2924, 2858, 1647, 1600, 1558, 1427 cm^{-1}. ^{1}H and ^{13}C NMR data (see Table 2).

Epicatechin (3): white powder; mp. 248-249 ^{o}C; HRTOFMS *m/z* 290.1027 (calcd for $C_{15}H_{14}O_6$ *m/z* 290.0790); UV (EtOH) (λ_{max} 243, 258, 316, 319 nm; IR (KBr) γ_{max} 3417. 2962, 2920, 2854, 1643, 1612, 1558, 1458, 1076 cm^{-1}. ^{1}H and ^{13}C NMR data (see Table 3).

Table 5. Antioxidant activity of compounds 1-3

Compounds	Regression	R	IC_{50} (ppm)
1 (Y1)	Y = 0,0087 x + 0,701	0,937	566,6
2 (Y2)	Y = 0,195 x + 1,142	0,958	250,5
3 (Y3)	Y = 0,917 x + 11,64	0,925	41,8

Antibacterial and Antioxidant Activities. The isolated compounds, α-mangostin (**1**), β-mangostin (**2**), and epicatechin (**3**) were evaluated for their antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus sp.* using disk-diffusion method. Zone of

inhibition was determined after incubation at 37 ^{o}C for 48 hr and compared to that of chloramphenicol as the standard. For zone diameters of each isolated compounds **1**-**3** against *E. coli*, *S. aureus*, and *Bacillus sp*. (see Table 4). DPPH radical scavenging assay were used to evaluate the scavenging ability of radicals in vitro by solutions of compoumds **1**-**3** (50 µM) in MeOH (450 ml) and the final absorbance was recorded on microplate reader at 516 nm. The results were tabulated and compared with that of ascorbic acid as a standard (see Table 5).

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