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# Antioxidant Activity of Garcinia cf bancana Miq

## Sri Hartati<sup>1</sup>, Triyem<sup>2</sup> and Herry Cahyana<sup>2</sup>

Research Center for Chemistry, Indonesia Institute of Sciences, Kawasan Puspiptek Serpong Tangerang. Department of Chemistry, Indonesia University. UI- Depok

## **A**bstract

Guttiferron F and essential oil were isolated from extract n-hexane of Garcinia of bancana Miq. Antioxidant activity was done by DPPH method to n-hexane, methanol, ethyl acetate, n-butanol extracts and Guttiferron F, showed activity with IC50 24.5, 22.4, 29.2, 37.6 and 25.8  $\mu$ g/mL respectively. The structure elucidation of Guttiferon F based on spectroscopy data of IR, NMR of <sup>1</sup>H and <sup>13</sup>C.

Keywords: Antioxidant, Garcinia of bancana Miq., Essential oil and Guttiferon-F.

#### INTRODUCTION

Garcinia Cf bancana Miq similarity with G. bacana Miq belonging to the Guttiferae family is distributed throughout Southern Thailand, Malaysia and Indonesia (Withmore, 1973). Some of Garcinia species used as traditional medicines as G. picroriza aand G. atroviridis used for fever (Burkill, 1935), G. mangostana used as antidhiarea and anti inflammatory (Ballasubranian et al., 1988), G. dulcis used as lymphatitis, struma and parolitis (Inuma et al, 1996). Some compounds of Garcinia species have potentially bioactivity as antioxidant (Yamaguchi et al., 2000; Terashima et al., 2002; Anne et al. 2004 and Liao et al., 2005), antibacterial and antifungal (Permana et al., 2001; Vancharin et al., 2005a, 2005b; Suksamrarn et al., 2003; Sudpodma et al., 2005 and Quan-Bin et al., 2005), cytotoxic (Odile et al., 2000; Xu et al., 2000; Chi-Kuan et al., 2002; Quan-Bin et al., 2006; Suksamrarn et al., 2006), antimalarial (Likhitwitiawuid et al., 1998a, 1998 b; Tona et al., 2004) and HIV-I inhibitory activities (Chen et al., 1996). In search to investigation antioxidant from natural product, have been done antioxidant activity assay with radical scavenger (DPPH) method of *n*-hexane, methanol, ethyl acetate and *n*butanol extracts and isolation of main active compound of bark G. cf bancana Miq.

#### **METHODS**

#### Plant material

The barks of *Garcinia cf bancana* was collected from village Kalapangan, district Sebangau Palangkaraya Central Kalimatan. Species determine was conducted by Ismail and voucher specimens deposited at the Herbarium Bogorience of Research Center for Biology–LIPI. Bogor.

#### **General** methods

Melting point was measured on Fisher Scientific Apparatus. IR spectra was obtained with a Shimadzu Perkin Elmer 16 PC-FT-IR Prestige 21 Spectrometer. 1D and 2 D NMR were performed on I NOVA Plus, Unity NMR 500 MHz spectrometer with tetra-methyl-sylane (TMS) as LC-MS standard. Mariner spectrometry, LC: Hitachi L 6200, System FSI (Electrospray Ionization), Positive ion mode, column C-18 Supelco, column length: 150 mm, ID: 2 mm, particle size: 5 um. GC-MS Shimadzu OP 5050A, detector DDSMS, temperature column 60°C; temperature detector 300°C, temperature injector 310°C, time analyze 30 min., volume injection 0.2 uL. Silica gel (65-250 mesh and 230 - 400 mesh) from E-Merck, Sephadex LH-20 from Amersham Bioscience. DPPH 1,1-diphenyl-2pycrylhidrayl was purchased from Sigma Chemical Co.

<sup>\*</sup>Corresponding author e-mail: hartatis2003@yahoo.com



#### **Extraction and Isolation**

The ground dried stem barks of G. cf bancana Mig (4.58 Kg) was percolated with nhexane (3x5 L) at room temperature, the solvent was evaporated in vacuum to afford a hexane extract (190 g). The residue continued to percolated with methanol (4x5 L) at room temperature, the solvent (MeOH) was evaporated in vacuum to afford MeOH extract (420 g). The MeOH extract (100 g) then suspended in H<sub>2</sub>O (1 L) and portioned with ethyl acetate (3x1 L) the separated fraction ethyl acetate from H<sub>2</sub>O and evaporated in vacuum to afford ethyl acetate extract (10 g). To the water fraction added nbutanol (3x1 L) to portioned and the separated fraction n- butanol from H<sub>2</sub>O and evaporated in vacuum to afford n- butanol extract (40 g). The nhexane extract (37 g) was subjected to silica gel flash column chromatography (CC:  $\Phi$  5 cm; < 45 um; 50 g) using gradient mixture of n-hexaneethyl acetate (0-100 %) as mobile phases, affording four fractions (FA-FD). FA fraction is colorless essential mixture oil (1.64 g) was analyzed by GC-MS. FD fraction was subjected to sephadex LH-20 column using mixture of CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:1) as mobile phase, resulting fractions  $(FD_1 - FD_3)$ , the second fraction (200 mg) was further purified by preparative thin layer

chromatography (TLC) yielded compound **5** (10.2 mg).

Each extracts and compound **5** was tested for antioxidant properties base on scavenging activity study using stable 1,1- diphenyl-2-pycrylhidrazyl (DPPH) free radical.

#### **Scavenging Activity of DPPH radicals**

The free radical-scavenging activity (Molyneux, 2004) method was tested as bleaching of stable 1,1- diphenyl-2-pycrylhidrazyl radical. In its radical from DPPH<sup>o</sup> has an absorption band at 520 nm (dark purple), which disappear upon reduction by antiradical compound. The principle reaction as bellow: (**Figure. 1**).

The reaction mixture containing test sample 25, 125, 250 and 500  $\mu$ L respectively (1000 ppm in MeOH) and 500  $\mu$ L of DPPH (0.5 mM in the MeOH) are added to each samples and dilute in MeOH until 2.5 mL and incubated at 37°C for 30 min. The absorbance was measured at 515 nm. Percent radical scavenging activity was measured by comparison with MeOH containing control. IC<sub>50</sub> values represent of compound (samples) to scavenge 50% of DPPH radical. Cuercetine was used as positive control.

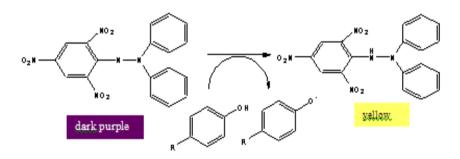


Figure 1. The principle reaction of DDPH

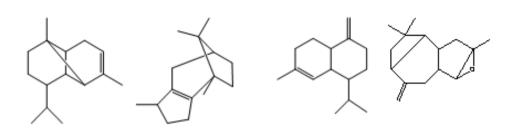
#### **RESULTS**

From the analysis of FA (essential oil) with GC-MS resulted mixture of compounds:

- a. Copaene 1 with retention time 10.42 min, with Mr = 204, molecule formula  $C_{15}$   $H_{24}$  and with percent SI (similarity) is 96%.
- b. 4,7-methanoazulene **2** have retention time 10.88 min, with Mr = 204, molecule formula  $C_{15}H_{24}$  and percent SI is 91%.
- c.  $\gamma$ -cadienene **3** with retention time 12.58 min with Mr = 204 and molecule formula  $C_{15}$   $H_{24}$  have percent SI is 95%.
- d. Cariofilen oxide **4** have retention time 13.99 min with Mr = 220 and molecule formula  $C_{15}H_{24}O$ , with percent SI is 97%. The structures **1**, **2**, **3** and **4** are (Figure. **2**):

Copaene





y- cadienene

Figure 2. Four essentials structure 1: 2; 3 and 4

4,7-methanoazulene

Compound 5: was isolated as yellow powder (10.2 mg), melting point 133 - 135 °C, having the  $MH^+$  at the m/z = 603. Melting point 133-135°C. The IR (in KBr) spectrum showed strong bands (3406-3257 cm<sup>-1</sup>) and sharp bands at 1726 cm<sup>-1</sup>, 1638 cm<sup>-1</sup>, 1600 cm<sup>-1</sup>. The <sup>1</sup>H-NMR data (in  $C_3D_6O$ , 500 MHz) showed chemical shift ( $\delta^1H$ , ppm) 0.99 (3H, s); 1.17 (3H, s); 1.45 (3H, s); 1.49 (3H, s); 1.59 (3H, s); 1.60 (3H, s); 1.62 (3H, s); 1.63 (3H, s); 1,68 (3H, s); 1.69 (3H, s); 1.93 (2H, dd, J=12.85; 6.1 Hz); 2.01 (m); 2.02 (m); 2.20 (d, J = 14.50 Hz; 2.69 (m); 2.70 (m); 4.50 (s); 4,51 (s); 4.93 (1H, t); 5.02 (1H, t); 5.09 (1H, b); 6.71 (1H, d, J=7.30 Hz); 7.03 (1H, d, J=7.35 Hz); 7.20 (s, 1H). The  ${}^{13}\text{C-NMR}$  (125 MHz, in  $\text{C}_3\text{D}_6\text{O}$  and DEPT) data showed chemical shift ( $\delta^{13}$ C, ppm) 17.90 (CH<sub>3</sub>); 18.12 (CH<sub>3</sub>); 18.18 (CH<sub>3</sub>); 18.38

(CH<sub>3</sub>); 23.04 (CH<sub>3</sub>); 25.74 (CH<sub>3</sub>); 25.82 (CH<sub>3</sub>); 26.16 (CH<sub>3</sub>); 26.59 (CH<sub>2</sub>); 27.21 (CH<sub>2</sub>) ; 27.33 (CH<sub>3</sub>); 32.75 (CH); 37.13 (CH<sub>2</sub>); 42.92 (CH<sub>2</sub>); 44.40 (CH); 48.88 (C); 47.60 (C); 49.50 (C); 69.00 (C); 112.05 (CH<sub>2</sub>); 114.69 (CH); 116.91 (CH); 116.91 (C); 122.07 (CH); 124.03 (CH); 125.09 (CH); 125.89 (CH); 131.38 (C); 131.46(C); 132.29 (C); 132.29 (C); 145.11 (C-OH); 149.40 (C-OH) ; 150.31 (C); 171.50 (C-OH); 196.50 (C=O); 211.00 (C=O). HMQC (Heteronuclear Multiple Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Corelation) data of compound **5** showed in Table **2**.

4. Cariofilen oxide

The Scavenging Activity of DPPH radicals result showed in **Table 1** 

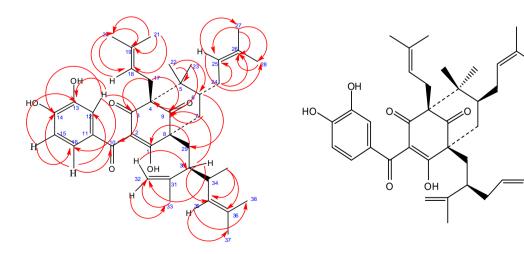


Figure 3. Correlation HMBC of compund 5

Figure 4. Guttiferon F



Table 1. The IC<sub>50</sub> antioxidant of extracts and compound 5

Sample	IC <sub>50</sub> μg/mL		
Cuercetine	10.09		
n-hexana exstract	24,50		
Methanol exstract	22,35		
Ethyl acetate exstract	29,17		
n-butanol exstract	37.56		
Compound 5 (Guttiferon F)	25.79		

#### **DISCUSSION**

#### **Essential oil**

From the analysis with GC-MS of FA (essential oil) resulted a mixture of compounds Copaene Mr = 204, molecule formula ( $C_{15}H_{24}$ ) with percent SI (similarity) is 96%. **1**; 4,7-methanoazulene, with Mr = 204, molecule formula  $C_{15}H_{24}$  and percent SI is 91%. **2**,  $\gamma$ - cadienene with Mr = 204 and molecule formula  $C_{15}$  H<sub>24</sub>, have percent SI is 95%. **3** and Cariofilen oxide with Mr = 220 and molecule formula  $C_{15}H_{24}O$ , with percent SI is 97%**4**. There compounds are sequterpen ( $C_{15}$ ), spesifik build from a head to tail isopren folowed "the isopren rule".

#### **Campound 5**

Was isolated from n-hexane fraction (FD<sub>2</sub>) as yellow powder (10.2 mg), gave a molecular ion at  $[MH^+] = 603/M = 602$ , molecule formula C<sub>38</sub>H<sub>49</sub>O<sub>6</sub>. The IR spectrum characteristic showed strong bands for hydroxyl (3537 - 3257 cm<sup>-1</sup>) and both non conjugated (1725 cm<sup>-1</sup>) and conjugated (1637 cm<sup>-1</sup> and 1600 cm<sup>-1</sup>) carbonyl groups. Melting point 133-135°C. Analysis of the 1D and 2 D NMR spectra in C<sub>3</sub>D<sub>6</sub>O with homo and hetero nuclear direct and long-range correlations allowed assignment of <sup>1</sup>H and <sup>13</sup>C NMR signals for compound 5 as listed in Table. 2, revealed signals assignable six vinylic methyl groups at  $(\delta 1.49)$ ; 1.60; 1. 62; 1. 63; 1.68 and 1.69 ppm, 3 H each singlet) and three vinyl proton at ( $\delta$  4.51; 4.93; 5.02 ppm, 1H each triplet) indicated contain of three isophrenyl groups. Two saturated methyls group appeared at  $\delta$  (0.99 and 1.17 ppm, 3H each s) and were assigned methyl geminal group. (C-22 - C-23) correlated by HMBC two each other and to C-4; C-5 and C-6. It also showed two

exomethylen protons at ( $\delta$  4. 50 and 4.53 ppm each s) and one terminal methyl proton. An AMX system was evident from proton resonance at, 6.71 (1H, d, J=7.30 Hz); 7.03 (1H, d, J=7.35 Hz); 7.20 (s, 1H) and typical <sup>13</sup>C NMR signals three substituted aromatic carbons at δc 149.5; 145. 1; 131.5 and conjugated carbonyl at δc 196.5, were indicated at 3,4 - deoxygenated benzovl moietv. Resonance for six membered ring consisting non conjugated ketone at  $\delta c$  211.0. It shows the presence of three methine carbons of three substituted olefenic groups (-CH=Me x 3) doublet at 125.9; 131.8 and 132.3 and triplet at 112.1 for terminal methylene carbon  $[C(Me)=\underline{C}H_2)$ . The other assignment are at 132.9; 131.4 and 132.3 ppm (C-19; C-26 and C-36; =CMe<sub>2</sub>), 147.2 (C-36; =<u>C</u>=CH<sub>2</sub>). The compound **5** confirmed with literature (Fuller R.W. et al, 1999; Gustafson et al, 1992) and can be concluded that compound 5 is Guttiferon F. (The correlation HMBC of copound 5 list at **Table 2**. and Guttiferon F in **Fig. 2**).

#### **Antioxidant acivity**

The *n*-hexane, methanol, ethyl acetate, *n*-butanol extracts and Guttiferron F (compound **5**) were screened for DPPH Radical scavenging activity, *n*-hexane, methanol, ethyl acetate, extracts and Guttiferron F (compound **5**) are showed significant activity (IC<sub>50</sub> 24.5, 22.4, 29.2, and 25.8  $\mu$ g/mL respectively) compare to the standard cuercetin (IC<sub>50</sub> 10.09  $\mu$ g/mL) and *n*-butanol extract showed moderate activity (IC<sub>50</sub> 37,56  $\mu$ g/mL).



Table II. Chemical ship <sup>1</sup>H, <sup>13</sup>C NMR, HMQC and HMBC Data of Compound 5.

No.	<sup>13</sup> C- NMR (δ, ppm)	HMQC ¹H (δ, ppm)	groups	HMBC <sup>13</sup> C-NMR (δ, ppm)		
I	171.50	-	C-OH	-	-	-
2	116.91	-	С	-	-	-
3	211.00	-	C=O	-	-	-
4	69.00	-	С	-	-	-
5	49.50	-	С	-	-	-
6	47.60	1.45	CH	C8 (48.88)	-	-
7	42.92	2.2 (d, J=14.50 Hz)	CH₂	C9 (211.00)	-	
8	48.88	-	С	-	-	-
9	211.00	-	C=O	-	-	-
10	196.50	-	C=O	-	-	-
П	131.46	-	С	-	-	-
12	116.91	7.2 (s, 1H)	C-H	C13(145.11)	C14(149.50)	C16(125.09)
13	145.11	-	C-OH	-	-	-
14	149.40		C-OH	-	-	-
15	114.69	6.71 (d, 1H $J = 7.30 \text{ Hz}$ )	CH	C13 (145.11)		
16	125.09	7.03 (d, 1H $J = 7.35$ Hz)	CH	C10 (196.5)	CII(II6.9I)	C15(114.69)
17	26.59	2.7 (m)	CH <sub>2</sub>	C3 (211.00)	C9 (211.00)	
18	125.89	4.93 (t, 1H)	CH	C20 (25.74)	C21(17.9)	
19	132.29	-	С	-	-	-
20	25.74	1.62 (s, 3H)	CH₃	C18 (125.89)	C19 (132.29)	C21 (25.74)
21	17.90	1.49 (s, 3H)	CH₃	C18 (125.89)	C19 (132.29)	C20 (25.74)
22	23.04	1.17	CH₃	C5 (49.50)	C6 (47.60)	
23	27.33	0.99 (s, 3H)	CH₃	C4 (69.00)	C6 (47.60)	
24	27.21	2.01 (m)	CH <sub>2</sub>	C25 (124.03)	C26 (131.38)	-
25	124.03	5.02 (t, 1H)	CH	C26 (131.38)	C27 (25.82)	
26	131.38	-	С	-	-	-
27	25.82	1.63 (s, 3H)	CH₃	C21 (17.9)	C25 (124.03)	C28 (18.18)
28	18.18	1.60 (s, 3H)	CH₃	C25 (124.03)	C26 (131.38)	C27 (25.82)
29	37.13	1.93 (d d, J= 12.85; 6.1)	CH <sub>2</sub>	CI (171.50)		
30	44.40	2.69 (m, 1.55 H)	CH	C32 (112.05)	C35 (122.07)	C9 (211.00)
31	150.31	-	С	-	-	-
32	112.05	4.51 (s, 1H); 4.53(s,1H)	CH <sub>2</sub>	C30 (44.40)	C33 (18.38)	
33	18.38	1.59 (s)	CH₃	C32 (112.05)		
34	32.75	2.02 (m)	CH <sub>2</sub>	C35 (122.07)	C36 (132.29)	
35	122.07	5.09 (b, 1H)	CH	C37 (18.12)	C38 (17.9)	
36	132.29	-	С	-	-	-
37	26.16	1.69 (s, 3H)	CH₃	C35 (122.07)		
38	18.12	1.68 (s, 3H)	CH₃	C35 (122.07)	_	_



#### CONCLUSION

From the analysis with GC-MS of FA (essential oil) resulted a mixture of compounds Copaene ( $C_{15}H_{24}$ ) 1, 4,7-methanoazulene ( $C_{15}H_{24}$ ) 2,  $\gamma$ - cadienene ( $C_{15}H_{24}$ ) 3 and Cariofilen oxide ( $C_{15}H_{24}$ O) 4. There compounds are sequterpen ( $C_{15}$ ).

Compound 5. as yellow powder (10.2 mg), gave a molecular ion at  $[MH^+] = 603 / M = 602$ , melting point 133-135°C, molecule formula  $C_{38}H_{49}O_6$ . is Guttiferon F.

Antioxidant acivity of n-hexane, methanol, ethyl acetate, n-butanol extracts and Guttiferron F (compound 5) were screened for DPPH Radical scavenging activity, n-hexane, methanol, ethyl acetate, extracts and Guttiferron F (compound 5) are showed significant activity (IC<sub>50</sub> 24.5, 22.4, 29.2, and 25.8  $\mu$ g/mL respectively) compare to the standard cuercetin (IC<sub>50</sub> 10.09  $\mu$ g/mL) and n-butanol extract showed moderate activity (IC<sub>50</sub> 37,56  $\mu$ g/mL).

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