Journal of Applied Pharmaceutical Science Vol. 5 (02), pp. 006-011, February, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50202
ISSN 2231-3354

Cytotoxic compounds from the leaves of *Garcinia cowa* Roxb.

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ARTICLE INFO

Article history:
Received on: 25/12/2014
Revised on: 10/01/2015
Accepted on: 22/01/2015
Available online: 27/02/2015

Key words:
Structure elucidation, NMR spectroscopy, xanthone, benzoate, *Garcinia cowa* Roxb., cytotoxic activity, MCF-7, H-460.

ABSTRACT

The aims of this study was to isolate compounds from the leaves of methanol extract of *Garcinia cowa* and to evaluated their cytotoxic activity against breast (MCF-7) and lung (H-460) cell lines. The *dichloromethane fraction* was separated by successive silica gel column chromatography to give three compounds. Based on spectroscopic comparison with those of the literature these compounds were elucidated as methyl 2,4,6-trihydroxy-3-(3-methylbut-2-enyl)benzoate **(1)**, garcinisidone-A **(2)** and methyl 4,6dihydroxy-2-(4-methoxy-5-(3-methylbut-2-enyl)-3,6-dioxocylohexa-1,4-dienyloxy)-3-(3-methylbut-2-enyl)benzoate **(3).** Compound 1, 2 and 3 had IC_{50} value of 21.0 ± 10.2 µM, 21.2 ±8.4 µM and 17.2 ± 6.2µM against MCF-7, while only compound **(2)** was found to be in active against H-460 with IC_{50} value of 18.1 ± 6.7 µM. Conclusion: The results indicate that *G. cowa* leaves could be important sources of natural cytotoxic compounds and only compound **(2)** had activity against H-460 cell lines.

INTRODUCTION

The Genus *Garcinia*, belonging to the Family Clusiaceae have been widely investigated in terms of their bioactive ingredients. The plants are small to medium sized trees, which grow up to 30 m in height and are widely distributed in the tropical regions of the world (Kijjoa and Vieira, 2009). This genus has various biological activities such as antioxidant (Muharni *et al.*, 2009 and Dachriyanus *et al.*, 2003), cytotoxic (Wahyuni *et al.*, 2009) and antimicrobial activities (Dachriyanus *et al.*, 2004). *Garcinia cowa* Roxb known as asam kandis in West Sumatera It is widely distributed throughout Indonesia and the Malay peninsula. The fruits are edible with a sour taste and used as spices in Indonesia especially in Minang tribes. (Dachriyanus *et al.*, 2003). Many parts of *G. cowa* have been used in traditional folk medicine. The bark, latex and root have been used as an antipyretic agent (Mahabusarakam, et al, 2005 and Pathong et al, 2009) while the fruit and leaves have been used for indigestion and improvement of blood circulation, and as an expectorant (Pathong *et al.*, 2009). Some pharmacological properties such as antitumor-promoting (Mukarami *et al.*, 1995), inhibition of human low-density lipoprotein peroxidation and anti-platelet activities have been reported on the crude extract of leaves (Jantan *et al.*, 2011). The chemical composition and biological activities of various parts of *G. cowa* have been investigated. Previous investigation on the fresh leaves, fruits and dried rinds of *G. cowa* has been investigated and found that (-)-hydroxycitric acid and its lactone constitute the major constituents (Jena *et al.*, 2002). Previously, we reported the isolation of [2*E*,6*E*,10*E*]-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-one (**1**), 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4- (1,1-dimethyl-2-propenyl)-9*H*- xanthen-9-one (**2**) and rubraxanthone (**3**) from the stem bark of this plant. (Wahyuni *et al.*, 2004) In continuation of our study on *Garcinia cowa* (Wahyuni *et al.*, 2004), cytotoxic properties of isolated compounds from the leaves of *Garcinia cowa* against cancer cell-lines are reported.

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MATERIALS AND METHODS

General

Vacuum liquid chromatography (VLC) was conducted on silica gel (Merck 9385) and column chromatography (CC) was conducted on either silica gel (Merck 7734). The eluates were monitored by analytical thin layer chromatography (TLC) utilizing precoated silica gel (Merck 5554). The spots were visualized under ultraviolet lights at 254. Fractions containing similar profile were combined. Radial chromatography (RC) was carried out using Harrison Research Chromatotron model 7924T on plates coated with silica gel 60 PF_{254} (Merck 7749) containing gypsum, at either 1 or 2 or 4 mm thickness.

UV (in absolute ethanol) and IR (KBr) spectra were recorded on a J ASCO V-560 spectrophotometer and a Perkin-Elmer 1650 FTIR spectrophotometer, respectively. Mass spectra were obtained on a J EOL J MS HX-110A spectrometer. ^{1}H and ^{13}C NMR spectra ($CDCl_3$) were recorded on a Varian 500 MHz NMR Spectrometer at 500 MHz (^{1}H)and 125 MHz (^{13}C), respectively and interpreted with the aid of the 1H-1H COSY, HMBC, and HMQC techniques.

Cell lines

The cell lines, NCI-H460 were purchased from the American Type Culture Collection (Manassas, VA, USA). The cancer cells were cultured in RPMI 1640 medium (Life Technologies, Paisly, UK) with 10%v/v fetal calf serum (PAA Laboratories, Linz, Austria), 100 IU/ml penicillin and 100 µg/ml streptomycin (Life Technologies, Paisly, UK) whereas the solution trypsin-EDTA were purchased from GIBCO (Auckland, New Zealand). Dimethylsulfoxide (DMSO) was purchased from BDH Laboratory (England). Culture flask (25 cm^2 and 75 cm^2) and 10 ml serological pipettes were purchased from Becton Dickson (New Jersey, USA).

Plant material

The leaves of *Garcinia cowa* Roxb were collected at Sarasah Bonta, Harau Valley, and West Sumatra at an altitude of 500 m. The voucher specimens (DR- 181) were identified by Dr Rusdi Tamin and were deposited in the herbarium of Andalas University, Padang, Indonesia. Plant materials were air dried and ground to powder before extraction.

Extraction

Ground air-dried leaves (3.2 kg) were sequently defatted with hexane (3 x 2.5 L) and extracted with dichlorometahane (dichloromethane) (10 x 2.5 L). The dichloromethane extract was evaporated to dryness under reduced pressure to give the dark mass 153 g.

Cytotoxic asssay

Breast cancer MCF-7 and Lung cancer H-460 cell lines were used in this study. Cells were cultured in RPMI-1640 medium. Cells were cultured in RPMI with 10% FBS in 25 cm2culture flasks and incubated at 37oC, with 90% humidity level and 5% CO2 level. Varying concentrations of the pure isolated compounds were prepared from the stock solutions by serial dilution (100 µg/mL, 10 µg/mL, 1 µg/mL, 0.1 µg/mL) in RPMI-1640 to give the volume of 200 µl in each well of the microtiter plate.

Each concentration was tested in quadruplicate and the culture plate was incubated for 96 hours. After incubation, 50 µL of 2 mg/mL. MTT solution was added to each well and allowed to incubate. After 4 hours, all supernatant were discarded. 100 µL DMSO was added to each well to dissolve the formazan crystals. Absorbance values at 550 nm was measured with a microplate reader. Cytotoxicity was stated as IC_{50} (Mossman, 1983).

Isolation of 3(9-methyl-8-pentaenyl) 2,4,6-trihydroxybenzoic acid methyl ester (1)

A portion of the dichloromethane extract (20 g) was subjected to column chromatography (5.0 x 80 cm) over silica gel Merck 7734 and successively eluted with hexane followed by hexane: ethyl acetate mixture in step gradient polarity manner, and finally with methanol to give 30 fractions (75 ml each). The fractions obtained were analysed on TLC and similar fractions were combined to give four fractions; A (1-2, 339 mg), B (3-11, 250 mg), C (12-21, 780 mg), D (22-30, 1.8 g).

Fraction A was further subjected to sephadex column chromatography and eluted with dichloromethane: methanol (1: 1) to gave five sub fractions (A1-A_5). Fraction A_3 (194 mg) was resubjected to sephadex column chromatography with the same eluent to yield $A3_1$ – $A3_4$. Fraction A_{33} (194 mg) was also subjected to sephadex column chromatography and eluted with dichloromethane: methanol (1: 1) to give sub fractions A33. Fraction A33-2 was radial chromatographed on a 1 mm plate thickness and eluted with n-hexane: dichloromethane (3:1) to give 15 (5 ml) fractions of which fractions 10-15 were recombined and recrystallization with ethyl acetate: hexane to give compound methyl 2,4,6-trihydroxy-3-(3-methylbut-2-enyl)benzoate **(1)** as white needles (31 mg).

Compound (1) $C_{13}H_{16}O_5$, UV (MeOH) λ_{max} (logε) nm: 226.80 (4.36); 272.00 (4.21); 314.80 (3.40). IR V_{max} (KBr) cm^{-1}: 3399, 3153, 2967, 1675, 1431, 1064 . EIMS *m/z* (rel. inten): 252 (M^+,60), 237(15), 205 (100), 220 (50).

^{1}H NMR (500 MHz, *d*-Chloroform) δ_H 5.98 (1H, *s*, H-5), 5.23 (1H, *t*, *J*= 7.0 Hz, H-2'), 4.03 (3H, *s*, 1-COOMe), 3.33 (2H, *d*, *J*= 7.0 Hz, H-1'), 1.8 (3H, *s*, H-4'), 1.74 (3H, *s*, H-5').

^{3}C NMR (125 MHz, *d*- Chloroform) δ_{C} 170.2 (1-OCO), 162.2 (C-2, C-4, C-6), 134.6 (C-3'), 122.2 (C-2'), 106.6 (C-3), 96.1 (C-5), 93.9 (C-1), 52.7 (1-OMe), 26.0 (C-5'), 21.90 (C-1'), 18.04 (C-4').

Isolation of garcinisidone-A (2)

Fraction B of dichloromethane was subjected to column chromatography over silica gel Merck 7734, eluting it with hexane: ethyl acetate (9:1) to yield three sub-fractions B1, B2 and B_3. Fraction B_3 (275 mg) was further purified on sephadex column, eluting with mixture of dichloromethane and methanol (1:1) to

give four sub fractions ($B3_1$ – $B3_4$). Fraction $B3_4$ (95 mg) was subjected to column chromatography over sephadex and eluted with mixture of dichloromethane and methanol (1:1) to give 2 sub fractions $B34_1$ and $B34_2$. Fraction $B34_2$ was subjected to recrystallization with chloroform: hexane to give compound (2) as white needles (65 mg).

Compound **(2),** $C_{24}H_{26}O_7$. UV (MeOH) λ_{max} (logε) nm: 229.60 (4.48) 281.00 (4.14): IR V_{max} (KBr disc) cm^{-1}: 3367, 2927, 1650, 1481, 1069. EIMS *m/z* (rel. inten.) 426 (M^+, 100), 370 (70), 339 (38), 315 (74).

^{1}H NMR (500 MHz, d_3- Methanol) δ_H 6.57 (1H, *s*, H-6), 6.23 (1H, *s*, H-4), 5.13 (2H, *t*, *J*= 7.5, H-2', H-2"), 3.72 (3H, *s*, 8-OMe), 3.43 (2H, *d*, *J*= 7.5 Hz, H-1"), 3.23 (2H, *d*, *J*= 7.5, H-1'), 1.78 (3H, s, H-5"), 1.72 (3H, s, H-5'), 1.64 (3H, s, H-4"), 1.61 (3H, *s*, H-4').

^{13}C NMR (125 MHz, d_3- Methanol) $\delta_{C□}$ 168.6 (C-11), 162.9 (C-1), 162.4 (C-3), 159.8 (C-4a), 148.2 (C-5a), 146.5 (C-7), 143.3 (C-8), 135.2 (C-9a), 132.0 (C-3"), 130.8 (C-3'), 128.2 (C-9), 121.9 (C-2'), 121.8 (C-2"), 112.5 (C-2), 105.3 (C-6), 98.9 (C-4), 97.0 (C-11a), 59.9 (8-OMe), 24.6 (C-4'), 24.5 (C-4"), 23.4 (C-1"), 21.3 (C-1'), 16.8 (C-5"), 16.5 (C-5').

Isolation of methyl 4,6dihydroxy-2-(4-methoxy-5-(3-methylbut-2-enyl)-3,6-dioxocylohexa-1,4- dienyloxy)-3-(3-methylbut-2-enyl)benzoate (3)

The methanol fraction (20 g) was subjected to silica gel (cat no. 7734) column chromatography (5.0 x 80 cm) and successively eluted with hexane followed by hexane: dichloromethane mixture in step gradient polarity manner, and finally with methanol to give 71 fractions (50 ml each). The fractions obtained were analyzed on TLC and the similar fractions were combined to give five fractions; fraction A (1-8), B (9-21, 2.263 g), C (22-26, 1.25 g), D (27-39, 0.342 g), and E (40-71, 2.25 g)

Fraction B of methanol fraction was subjected to column chromatography (3 x 50 cm) over silica gel Merck 7734 and successively eluted with hexane followed by mixture of hexane and dichloromethane in step gradient polarity manner and finally with methanol to give fifty three fractions (20 ml each). The combined fraction 28-42 (B_2, 389 mg) was further purified on sephadex column, eluting with dichloromethane: methanol with the same ratio to give three sub fractions $B2_a$, $B2_b$ and $B2_c$. Fraction B_{2b} (6-11, 102 mg) was purified with radial chromatography by using 1 mm plate thickness and eluted with hexane: dichloromethane (3:1) to give two sub fractions $B2b_1$ and $B2b_2$. Both of them were recrystallised with ethyl acetate and hexane to yield compound **(3)** as red needles (32 mg).

UV (MeOH) λ_{max} (logε) nm: 226.40 (5.00), 266.60 (4.86); IR V_{max} (KBr) cm^{-1}: 3201, 2958, 1655, 1438, 1061; EIMS *m/z* (rel. inten.): 456 $[M-1]^+$ (100), 457 (38), 424 (65).

^{1}H NMR (500 MHz, d_6- Acetone) δ_H 6.32 (1H, *s*, H-5"'), 5.42 (1H, *s*, H-2), 5.24 (1H, *t*, *J*= 7.5 Hz, H-2"), 5.11 (1H, *t*, *J*=7.5, H-2'), 4.01 (3H, *s*, 6-OMe), 3.72 (3H, *s*, 1-'''$COOCH_3$), 3.35 (2H, *d*, *J*= 7.5 Hz, H-1"), 3.16 (2H, *d*, *J*= 7.5, H-1'), 1.76 (3H, s, 3'-Me), 1.75 (3H, s, 3'-Me), 1.67 (3H, *s*, H-5'), 1.64 (3H, s, H-5").

^{13}C NMR (125 MHz, d_6-Acetone) $\delta_{C□}$ 184.2 (C-1), 182.1 (C-4), 170.7 (C-3), 163.9 (C-2'''), 162.1 (C-4'''), 159.8 (C-6), 156.3 (C-6), 152.9 (C-6'''), 133.9 (C-3'), 131.9 (C-3"), 129.8 (C-5), 122.9 (C-2"), 121.3 (C-2'), 114.9 (C-3'''), 109.2 (C-2), 103.1 (C-5'''), 98.7 (C-1'''), 61.7 (6-OMe), 52.9 (1'''-OMe), 25.9 (C-5', C-5"), 22.9 (C-1'), 22.6 (C-1"), 18.0 (C-4'), 17.8 (C-4").

RESULTS AND DISCUSSION

Characterization of compound (1)

Compound **(1)** was isolated as white needless, which had molecular ion by EIMS at (*m/z* 252 $[M]^+$), corresponding to molecular formula $C_{18}H_{24}O_5$. The UV absorption bands at 226.8 (3.11), 272 (3.58), and 314.80 (3.25) nm indicated the presence of an aromatic moiety. The IR spectrum showed hydroxyl (3399 cm^{-1}) and conjugated ester carbonyl (1638 cm^{-1}) stretching bands. The ^{1}H NMR spectrum displayed resonances for one aromatic proton (δ 5.98, s), one prenyl unit [δ 5.23 (1H, *t*, *J*=7.0 Hz, H-2'), 3.33 (2H, *d*, *J*=7.0 Hz, H-1'), 1.8 (3H, *s*, H-4'), 1.74 (3H, *s*, H-5')] and one methoxyl group (δ 4.04, OCH_3). The carbonyl carbon resonance at δ 170.2 together with its HMBC correlation with the methoxyl group at δ 4.04 established the presence of a methyl ester group.

The position of prenyl unit and an aromatic proton were at C-2 and C-5, respectively, on the basis of HMBC correlations of the methylene protons (H-1', δ 3.33) of prenyl unit to C-2 (δ 162.2) and C-3 (δ 106.6) and the aromatic proton (H-5,δ 5.98, s) to C-1 (δ 93.91), C-3, (δ 106.6) and C-6 (δ 162.2) (Figure 4.8). The assignment of the protons and carbons of (**1**) are summarized in Table 1.

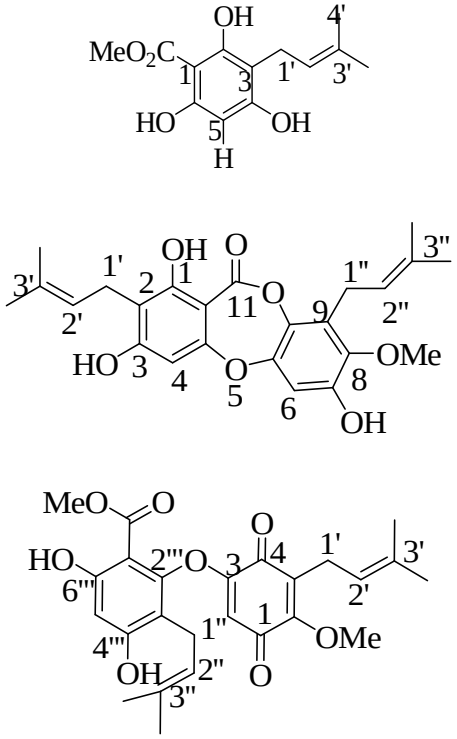

Fig. 1: Structures of the isolated compounds.

The carbon chemical shifts of C-2, C-4 and C-6 established the attachment of hydroxyl groups at these carbons (Silverstein, 1987). Thus, the methyl ester moiety was linked at C-1. The EIMS data showed fragment ion at *m/z* 237 revealed the loss of CH_3, while the fragment ion at *m/z* 220 and *m/z* 192, supporting this compound by losing OCH_3 and CH_3-O-C=O . Based on the argument above, this compound was determined to be [3(9-metil-8-pentaenyl) 2,4,6-trihidroxy benzoic acid methyl ester]. This compound was similar with parvifoliol A (Rukachisirikul 2006), but different in substituent moiety. Thisis compound was deduced as prenylated phluoroglucinol and assigned as methyl 2,4,6-trihydroxy-3-(3-methylbut-2-enyl)benzoate **(1)** (Feld *et al*., 2004).

Table 1: The ^{1}H-NMR , ^{13}C-NMR, and HMBC data of methyl 2,4,6-trihydroxy-3-(3-methylbut-2-enyl)benzoate **(1)**

Position	δC	δH(HSQC)	HMBC
1	93.9		
2	162.2		
3	106.6		
4	162.2		
5	96.1	5.98/ H/ *s*	C-6, C-3, C-1
6	162.2		
1’	21.9	3.33/2H/*d*/ *J*= 7.0 Hz	C-3’,C-2’, -3, C-2
2’	122.2	5.23/ 1H/ *t*/ J= 7.0 Hz	C-5’, C-4’, C-1’
3’	134.6		
4’	18.0	1.8/ 3H/ *s*	C-5’, C-3’, C-2’
5’	26.0	1.74/ 3H/ *s*	C-4’, C-3’, C-2’
C=O	170.2		
OMe	52.7	4.03/ *s*/ 3H	C=O

Characterization of compound (2)

Compound **(2)** was obtained as colorless crystals, mp 178-180 ^{o}C. The EIMS spectrum gave a molecular ion peak at *m/z* 426 $[M]^+$ corresponding to the molecular formula $C_{24}H_{26}O_7$. The ultraviolet absorption and mass spectrum were very useful in structural elucidation since they were similar to depsidone and garcinisidone-A and (Ito *et al.*, 1997). The UV spectrum (MeOH) λ_{max} 281.00 (4.14) and 229.60 (4.48). The IR spectrum showed absorption bands due to hydroxyl and lactone carbonyl groups at 3367 $cm^{-1,}$ 2927 (br) and 1650 cm^{-1}, respectively. The ^{1}H and ^{13}C-NMR spectra (Table 2) coupled with the results of HSQC showed signals assignable to an *O*-methyl group [δ_H 3.72 (3H,*s*); δ_C 59.9], a lactone carbonyl group δ_C 168.6. Two aromatic proton signals at δ 6.23 (H, s) and δ 6.57 (H, s) were assigned to H-4 and H-6, respectively. Observation of two set signals at δ 3.23 (2H, *d*, *J*=7.0), δ 5.17 (H,*t*, *J*= 7.0), δ 1.61 (3H, *s*), δ 1.72 (3H, *s*) and δ 3.42 (2H, *d*, *J*=7.0), δ 5.17 (H,*t*, *J*= 7.0), δ 1.64 (3H, *s*), δ 1.78 (3H, *s*) indicated the presence prenyl side chain in the molecule.

The ^{13}C NMR spectrum showed 24 carbon atoms, including a carbonyl lactone at δ 168.6 (C-11). The deshielded carbon signals at δ 159.8, δ 146.5 and δ 135.2 were assigned to C-4a, C-7 and C-9a, respectively, were due to oxygenation of these carbons . It appeared that the ring A was substituted by hydroxyl groups at C-1 (δ_C 162.93) and C-3 (δ 162.43). It was confirmed by ^{13}C-^{1}H correlations in HMBC spectrum in which proton signal at δ 6.23 (H-4) correlated with carbon signals at δ 162.4 (C-3), δ 112.53 (C-2), and δ 98.9 (C-4). In ring B, the signals at δ 6.57 H-6) showed ^{13}C-^{1}H correlations with carbon signals at δ 148.24 (C-7), δ 143.29 (C-8) and δ 135.23 (C-9a). The locations of two prenyl groups at C-2 and C-9 and were confirmed by observation of C-H long range correlation of H-1’ (δ 3.23) with C-2 (δ112.5) and H-1” (δ 3.42) with C-9 (δ 128.2). The assignment of the protons and carbons of **76** are summarized in Table 2.

Table 2: The ^{1}H-NMR , ^{13}C-NMR, and HMBC data of compound **(2).**

Position	13C NMR (ppm)	1H NMR (ppm)	HMBC
1	162.9		
2	112.5		
3	162.4		
4	98.9	6.23/*s*/H	C-3, C-2, C-4a, C-4
4a	159.8		
5a	148.2		
6	105.3	6.57/*s*/H	C-5a, C-8, C-9a
7	146.5		
8	143.3		
8-OCH_3	59.9	3.72/s/3H	C-8
9	128.2		
9a	135.2		
11	168.6		
11a	97.0		
1’	21.3	3.23/*d*/2H/ *J*= 7.0	C-1, C-2, C-2’, C-3’
2’	121.9	5.13/t/ H/ *J*= 7.0	C-4’
3’	130.8		
4’	24.6	1.61/s/3H	C-3’, C-2’, C-4’
5’	16.5	1.72/s/3H	C-3’, C-2’, C-4’
1’’	23.4	3.42/*d*/2H/ *J*=7.0	C-9, C-9a, C-8, C-2’’, C-3’’
2’’	121.8	5.13/t/ H/ *J*= 7.0	C-4”
3’’	132.0		
4”	24.5	1.64/s/3H	C-3’’, C-2’’, C-4”
5”	16.8	1.78/s/3H	C-3’’, C-2’’, C-4"

Mass fragment ions at *m/z* 370 $[M^+$-CH=C$(CH_3)_2$-H] and 315 $[M^+$ - CH=C$(CH_3)_2$-CH=C$(CH_3)_2]$ in electron impact (EI-MS) also suggested the presence of two prenyl side chains. (Ito *et al.*, 2003). The linkage of the two moieties (ring a and ring B) was interpreted as depsidone based on HMBC experiment, significant peaks in EIMS and by comparison data with spectral data of garcinisidone-A (Table 16) (Ito *et al.*, 1997). Based on these spectral assignment, compound **(2)** was deduced as garcinisidone-A.

Characterization of compound (3)

Compound **3** was obtained as red needless, mp 115-117 ^{o}C. The EIMS spectrum gave molecular ion at [*m/z* 457]$^+$ corresponding to molecular formula $C_{25}H_{28}O_8$. The UV spectrum showed an absorption band at λ_{max} 266.60 nm (4.86) and 226.40 nm (5.00). The IR spectrum exhibited absorption bands at 3201 cm^{-1} and 1655 cm^{-1} due to hydroxyl and carbonyl functionalities, respectively. The ^{13}C NMR spectrum also supported the presence three carbonyl groups by the signals at 184.16, δ182.06 and δ170.72 indicating the two *p*-benzoquinone carbonyls and an ester carbonyls, respectively (Permana, D., *et al* 2001). The ^{1}H NMR and HSQC spectra indicated the presence of two methoxyls from the signals at δ 4.01 (δ_C 61.7) and δ 3.72 (δ_C 52.9). The HMBC spectrum further suggested that the former is attached to an olefinic carbon (δ_C159.8), and the latter is located at a carbonyl carbon (δ_C 170.7) (Permana *et al*., 2001).

The ^{13}C NMR data of **(3)** indicated the presence of three hydroxylated or alkoxylated aromatic carbons at δ 163.6, δ 162.1, and δ 152.9. The ^{1}H NMR and HSQC spectra showed signals due two aromatic proton signals at δ 6.32 (H, *s*,) (δ_C 103.5) and δ 5.42 (H, *s*,) (δ_C 109.2) which were assigned to H-3''' and H-2, respectively. In the aromatic region of the HMBC spectrum, the proton signal at δ 6.32 (H-3''') further showed correlation with the carbon signals at δ 152.93 (C-4'''), 114.86 (C-5''') and 98.72 (C-1'''), while another aromatic proton signal at δ 5.42 (H-2) correlated with the carbon signals at δ 159.8 (C-3), δ 182.1 (C-4) and δ 156.3 (C-6), establishing the structure of ring A and ring B.

The presence of two prenyl side chains in **(3)** was indicated by the occurrence of two sets signals of prenyl unit, one are those at δ 3.35 (2H, *d*, *J*= 7.5, H-1") (δ 5.24 (H, *t*, *J*=7.5 Hz, H-2"), δ 1.76 (3H, *s*, H-4'), δ 1.64 (3H, *s*, H-5") and the other are those δ 3.16 (2H, *d*, *J*= 7.5 Hz, H-1'), δ 5.11 (1H, *t*, *J*=7.5 Hz, H-2'), δ 1.75 (3H, *s*, H-4"), δ 1.67 (3H, *s*, H-5') in its ^{1}H and ^{13}C spectra. The HMBC spectrum further showed that the proton signal at δ 3.16 (H-1') correlated with the carbonyl carbon at 182.1 (C-4), the olefinic carbon signals at δ 129.8 (C-5), 156.3 (C-6) and 121.3 (C-2'), established the attachment of this prenyl at C-5 (ring B). The proton signal at δ 3.35 (H-1") was found to correlate with hydroxyl aromatic carbon at δ 162.1 (C-6'"), the olefinic carbons at δ 131.9 (C-1") and δ 122.9 (C-2"). Thus, suggesting that this prenyl is attached to an aromatic carbon at C-3''' (ring A). The assignment of the protons and carbons of **77** are summarized in Table 3.

Table 3: The ^{1}H-NMR , ^{13}C-NMR, and HMBC Data of (**3**)

Position	^{13}C NMR (ppm)	^{1}H NMR (ppm)	HMBC
1	184.2		
2	109.2	5.42 (1H, *s*)	C-6, C-3, C-4
3	159.8		
4	182.1		
5	129.8		
6	156.3		
6-OCH_3	61.7	4.01 (3H, *s*)	C-6
1'	22.9	3.16 (2H, *d*, *J* = 7.5 Hz)	C-2', C-5, C-3', C-6, C-4
2'	121.3	5.11 (1H,*t*, *J*= 7.5 Hz)	
3'	133.9		
4'	18.0	1.76 (3H, *s*)	C-2', C3'
5'	25.9	1.67 (3H, *s*)	C-2', C3'
1''	22.6	3.35 (2H, *d*, *J* = 7.5 Hz)	C-3''', C-2'', C-3'', C-4'''
2''	122.9	5.24 (1H, *t*, *J*= 7.5 Hz)	
3''	131.9		
4"	17.8	1.75 (3H, *s*)	C-2', C-3'
5"	25.9	1.64 (3H, *s*)	C-2'', C-3''
1'''	98.7		
2'''	163.9		
3'''	103.1	6.32 (H, *s*)	C-4''', C-1'''
4'''	152.9		
5'''	114.9		
6'''	162.1		
-$OCOCH_3$	170.7		
-OCH_3	52.9	3.72 (3H, *s*)	C=O

In this manner, it was apparent that the two prenyl units are located in different ring . A correlation of the methoxyl protons resonating at δ 4.01 with the carbon signal at δ 156.3 (C-6) suggesting the position of this methoxyl group at C-6. Thus, **3** was assigned as new prenylated benzoquinone and assigned as 3-(1-methoxycarbonyl-4,6-dihydroxyphenoxy)-6-methoxy-3,5-di(3-methyl-2-butenyl)-1,4-benzoquinone.

Cytotoxic activity of isolated compounds

The IC_{50} values of less 10 µg/mL, is considered as potent, while the IC_{50} values of between 10 to 30 µM/mL is considered as having good activity and IC_{50} values of between 30 µM/mL 100 µM/mL is considered weak activity.

All isolated compounds, had been evaluated for their cytotoxic activity against MCF-7 and H-460 cell lines. The compounds **(1), (2) and (3)** showed good activities towards MF-7. Only compound (2) was active against H-460 (Table 4).

Table 4: Cytotoxic activity of isolated compounds towards cancer cell lines**.**

compounds	IC_{50} (µM)	
	MCF-7	H-460
(1)	21.0 ± 10.2	>100
(2)	21.2 ±8.4	18.1 ± 6.7
(3)	17.2 ± 6.2	>100

MCF-7 = Breast cancer cells
H-460 = Lung cancer cell cells

CONCLUSION

The potential value of the leaves of *G. cowa* from the West Sumatra region of Indonesia has been reported for the first time here. Three compounds, methyl 2,4,6-trihydroxy-3-(3-methylbut-2-enyl)benzoate **(1),** garcinisidone-A **(2)** and 3-(1-methoxycarbonyl-4,6-dihydroxyphenoxy)-6-methoxy-3, 5-di(3-methyl-2-butenyl)-1,4-benzoquinone **(3)** were isolated from this leaves. Structure elucidations of these compounds were carried out by spectroscopic methods. All isolated compounds were active against MCF-7 cell line and only garcinisidone-A **(2)** was active against H-460.

ACKNOLEDGMENTS

All authors thank the Malaysian Ministry of Science, Technology and the Environment for financial support under the Intensified Research in Priority Areas Research Grant (IRPA, EAR No. 09-02-04-0313), International Foundation for Science (IFS no F/3967-1), National L'Oreal Fellowship Grant, and Directorate of Higher Education, Indonesian Ministry of Education (HB XIV no: 297/SP3/PP/DP2M/II/2006).

REFERENCES

Dachriyanus, Izati M, Fahmi R, Antimicrobial compounds from the bark of *Garcinia parvifolia* Mig. Jurnal Matematika & Pengetahuan Alam, 2004; 13(1): 20-24.

Dachriyanus, Danita J, Jubahar J. Isolation of antioxidant compounds from the bark of *Garcinia cowa* Roxb. Jurnal Matematika & Pengetahuan Alam, 2003; 12(2): 67-72.

Feld H, Rycroft DS, and Zapp J. Chromenes and Prenylated Benzoic Acid Derivatives from the Liverwort *Pedinophyllum interruptum* Z. Naturforsch. 2004; 59b: 825 – 828.

Ito C, Miyamoto Y, Nakayama M, Kawai Y, Rao S, Furukawa H. Novel Depsidone. Chem Pharm Bull, 1997; 45:1403-1413.

Jantan I, Jumuddin FA, Saputri FC, Rahman K. Inhibitory effects of the extracts of Garcinia species on human low-density lipoprotein peroxidation and platelet aggregation in relation to their total phenolic contents. J Med Plants Res, 2011; 5: 2699-2709.

Jena BS, Jayaprakasha GK, Sakariah KK. Organic Acid from leaves, fruit, and rinds of *Garcinia cowa*. J Agr Food Chem, 2002; 50: 3431-3434.

Kijjoa A and Vieira L. M. M. 2009. Triterpenes from the plants of the Family Clusiaceae (Guttiferae): Chemistry and biological activities. In: Natural Products: Chemistry, Biochemistry and Pharmacology. (Ed. G. Brahmachari), Alpha Science International Ltd., Oxford, Ch. 13.

Mahabusarakam W., Chairerk P. and Taylor W.C. Xanthones from *Garcinia cowa* Roxb. Latex. *Phytochemistry*, 2005, *66*, 1148-1153.

Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods, 1983; 16: 55-63.

Muharni, Supriyatna, Bahti H, and Dachriyanus. Phenolic compounds from the stem bark of manggis hutan (*Garcinia bancana* Miq.) and their antioxidant activity, Indo J Chem, 2009; 9(2): 32 –327.

Mukarami A, Jiwajimda S, Koshimizu K, Ohigashi H. Screening for *in vitro* Antitumor promoting activities of edible plants from Thailand. Cancer Lett, 1995; 95: 137-146.

Panthong K., Hutadilok-Towatana N. and A. Panthong A., Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. Can J Chem, 2009; 87: 1636-1640.

Permana D., Lajis N.H., Mackeen M.M., Ali A.M., Aimi N., Kitajima M., Takayama H. Isolation and bioactivities of constituents of the roots of *Garcinia atriviridis*. J Nat Prod, 2001; 64: 976-979.

Rukachaisirikul, V., Naklue, W., Phongpaichit, S., Towatana, N.H., Maneenoon, K. Phloroglucinols, depsidones and xanthones from the twigs of *Garcinia parvifolia*. Tetrahedrone, 2006; 20: 1-8.

Silverstein RM, Webster FX. Spectrometric Identification of Organic Compounds, 6th ed, Wiley, Singapore, 1997.

Wahyuni FS, Lusianti M, Almahdy, Dachriyanus, Isolation of cytotoxic compound on the breast cancer from the bark of *Garcinia griffithii* T. Anders, Jurnal Farmasi Indonesia, 2009; 4(4): 177-187.

Wahyuni FS, Lindsay TB, Dachriyanus, Dianita R, Jubahar J, Lajis NH, Sargent MV. A new ring-reduced tetraprenyltoluquinone and a prenylated xanthone from *Garcinia cowa*. Aust J Chem, 2004; 57: 223-226.

How to cite this article:

Fatma Sri Wahyuni, Khozirah Shaari, Johnson Stanslas, Nordin Hj Lajis, Dachriyanus Hamidi., Cytotoxic compounds trom the leaves of Garcinia Cowa Roxb. J App Pharm Sci, 2015; 5 (02): 006-011.