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Chemical Constituents of *Garcinia parvifolia* (Guttiferae)

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ABSTRACT Detail chemical studies on *Garcinia parvifolia* have yielded two triterpenoids, stigmasterol **(1)** and β-sitosterol **(2)**, three xanthones, 6-deoxyjacareubin **(3)**, daphnifolin **(4)** and rubraxanthone **(5)**, one benzophenone, isoxanthochymol **(6)** and one alkaloid, caffeine **(7)**. These compounds were isolated using common chromatographic techniques and were identified by using spectroscopic experiments such as NMR, MS, IR and UV. This is the first report on the isolation of 6-deoxyjacareubin and daphnifolin from *Garcinia parvifolia*.

ABSTRAK Kajian kimia terperinci ke atas *Garcinia parvifolia* telah menghasilkan dua triterpinoid, stigmasterol **(1)** dan β-sitosterol **(2)**, tiga zanton, 6-deoksijacareubin **(3)**, daphnifolin **(4)** dan rubraxanton **(5)**, satu benzofenon, isoxanthochymol **(6)** dan satu alkaloid, kafein **(7)**. Struktur sebatian ini ditentukan dengan menggunakan eksperimen spekstroskopi seperti NMR, MS, IR dan UV. Ini adalah kali pertama 6-deoksijacareubin dan daphnifolin diperolehi daripada *Garcinia parvifolia*.

(*Garcinia parvifolia*, triterpenoids, xanthones, benzophenone)

INTRODUCTION

The tropical Guttiferae family is well known to be a rich source of isoprenylated xanthones and biflavonoids [1] - [6]. The family of Guttiferae is also known as Clusiaceae and this family comprises 1350 species in 47 genera [7]. The genus *Garcinia* is best known in Malaysia as a genus of fruit trees. Extracts and pure isolates of *Garcinia* species have been shown to exhibit significant antimicrobial and pharmacological activities [3],[8]. In continuation of our search for bioactive natural products from Malaysian plants, we examined the stem bark extracts of *Garcinia Parvifolia*.. We report here the isolation and structural determination of seven compounds **(1-7)**.

MATERIALS AND METHODS

Plant Material

The stem bark of *Garcinia parvifolia* was collected from Sri Aman Sarawak, Malaysia.

General

Infrared spectra were measured in KBr/NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500 MHz NMR/JEOL 400 MHz FT NMR spectrometer using tetramethylsilane (TMS) as internal standard. Ultra violet spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A, UV-Visible Recording Spectrophotometer.

Extraction and Isolation

The air-dried and powdered stem bark of *Garcinia parvifolia* (2.0 kg) was extracted successively with n-hexane, chloroform, ethyl acetate and methanol at room temperature. The extracts were evaporated to dryness under reduced pressure to yield 51.5 g of crude n-hexane extract, 12.8 g of crude chloroform extract, 32.6 g of crude ethyl acetate extract and 63.1 g of crude methanol extract. The crude n-hexane extract of *Garcinia parvifolia* (51.5 g) was chromatographed on a vacuum CC silica gel column using a stepwise gradient system (hexane/$CHCl_3$, $CHCl_3$/EtOAc and EtOAc/MeOH) to give 28 fractions (*Frs.*). *Frs. 11-12* were combined and purified in a silica gel mini column to furnish stigmasterol **(1)** (15 mg) as fine white needles. *Frs. 21-23* were combined and subjected to column chromatography (SiO_2; n-hexane/$CHCl_3$ gradients) to yield β-sitosterol **(2)** (10 mg). Fractionation of the crude chloroform extract of *Garcinia parvifolia* (12.8 g) over a silica gel column (hexane/$CHCl_3$, $CHCl_3$/EtOAc and EtOAc/MeOH gradient) provided 28 fractions. *Frs. 13* was further purified by silica gel column ($CHCl_3$/EtOAc and EtOAc/MeOH gradient) to yielded 6-deoxyjacareubin **(3)** (5 mg) after recrystallization from chloroform. *Frs. 15-16* were combined and separated over silica gel column ($CHCl_3$/EtOAc and EtOAc/MeOH gradient) to give daphnifolin **(4)** (8 mg). The crude methanol extract of *Garcinia parvifolia* (63.1 g) was fractionated by vacuum column chromatography (SiO_2; hexane/$CHCl_3$, $CHCl_3$/EtOAc and EtOAc/MeOH gradient) to yield 36 fractions. *Frs. 5-6* were combined and purified by repeated CC (SiO_2; $CHCl_3$/EtOAc and EtOAc/MeOH gradient) to give rubraxanthone **(5)** (8 g). *Fr. 13* was purified by repeated CC (SiO_2; $CHCl_3$/EtOAc and EtOAc/MeOH gradient) to give isoxanthochymol **(6)** (12 mg). The crude ethyl acetate extract of *Garcinia parvifolia* (32.6 g) was subjected to column chromatography (SiO_2; hexane/$CHCl_3$, $CHCl_3$/EtOAc and EtOAc/MeOH gradient) to give 27 fractions. *Frs. 8-9* was rechromatographed on a silica gel column ($CHCl_3$/EtOAc and EtOAc/MeOH gradient) to give caffeine **(7)** (3 mg).

Stigmasterol (**1**): White needle, mp 169 ^{O}C ([9] Lit.170 ^{O}C). All spectral data agree with literature values [9].

β-Sitosterol (**2**): White crystals, mp 134-136 ^{O}C ([9] Lit.136-137 ^{O}C). All spectral data agree with literature values [9].

6-Deoxyjacareubin **(3):** Yellow amorphous powder, mp 203-209 ^{O}C ([10] Lit. 211-213 ^{O}C.). UV (EtOH) λ_{max} nm (log ε): 412.5 (2.5). IR ν_{max} cm^{-1} (KBr): 3792 (broad OH), 2920 (C-H stretching), 1648 (C=O). EI-MS m/z (rel.int.): 310 (14), 295 (100), 281 (1), 147 (16), 107 (1), 77 (1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (1H, dd, *J* = 8.3, 6.4 Hz, H-8), 7.14 (1H, dd, *J* = 8.3, 6.4 Hz, H-6), 7.05 (1H, t, *J* = 8.3, H-7), 6.88 (1H, d, *J* = 10.0 Hz, H-11), 6.13 (1H, s, H-4), 5.49 (1H, d, *J* = 10.0 Hz, H-12), 1.35 (6H, s, H-14, H-15). ^{13}C NMR (100MHz, $CDCl_3$): δ 181.1 (C-9), 162.4 (C-3), 160.7 (C-1), 151.5 (C-4a), 145.7 (C-5), 145.1 (C-10a), 126.9 (C-12), 123.8 (C-7), 121.2 (C-8a), 120.4 (C-6), 114.9 (C-11), 115.6 (C-8), 103.3 (C-9a), 101.4 (C-2), 98.9 (C-4), 78.2 (C-13), 27.9 (C-14), 27.9 (C-15).

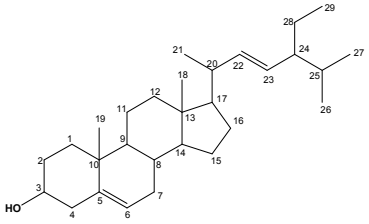
Daphnifolin **(4)**: Yellow crystals, mp 239 ^{O}C ([11] Lit. 240-242 ^{o}C). UV (EtOH) λ_{max} nm (log ε): 392.5 (2.5). IR ν_{max} cm^{-1} (KBr): 3550 (broad OH), 2946 (C-H stretching), 1656 (C=O), 1498 (C=C aromatic). EI-MS m/z (rel.int.): 274 (69), 259 (11), 245 (7), 231 (100), 202 (8), 147 (7), 136 (15), 121 (1), 101 (10), 93 (2), 65 (6), 51 (8). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, dd, *J* = 1.8, 8.2 Hz, H-8), 7.26 (1H, t, *J* = 8.2 Hz, H-7), 7.21 (1H, dd, *J* = 1.8, 8.2 Hz, H-6), 6.60 (1H, s, H-2), 3.94 (3H, s, 4-OCH_3). ^{13}C NMR (100MHz, $CDCl_3$): δ 181.2 (C-9), 158.2 (C-1), 153.7 (C-4a), 152.9 (C-3), 145.4 (C-5), 145.1 (C-10a), 130.3 (C-4), 123.3 (C-7), 120.6 (C-8a), 119.8 (C-6), 115.0 (C-8), 102.9 (C-9a), 93.9 (C-2), 60.1 (4-OCH_3).

Rubraxanthone **(5):** Yellow crystals, mp 206-209 ^{O}C ([4] Lit.205-206 ^{O}C). UV (EtOH) λ_{max} nm (log ε): 432 (0.12), 312 (1.06), 238 (1.60), 209 (1.06). IR ν_{max} cm^{-1} (KBr): 3430 (broad OH), 2970 (C-H stretching), 1640 (C=O), 1610 (C=C aromatic), 1466 (C=C aromatic). EI-MS m/z (rel.int.): 410 (17), 341 (100), 326 (5), 311 (20), 299 (25), 288 (13), 271 (9), 69 (32), 53 (6). ^{1}H NMR (400 MHz, MeOH): δ 6.68 (1H, s, H-5), 6.17 (1H, d, *J* = 1.8 Hz, H-4), 6.08 (1H, d, *J* = 1.8 Hz, H-2), 5.18 (1H, t, *J*=6.4 Hz, H-12), 4.97 (1H, m, H-16), 4.05 (2H, d, *J* = 6.4 Hz, H-11), 3.74 (3H, s, 7-OCH_3), 2.02 (2H, m, H-14), 1.97 (2H, m, H-15), 1.79 (3H, s, H-18), 1.53 (3H, s, H-19), 1.49 (3H, s, H-20). ^{13}C NMR (100MHz, MeOH): δ 183.1 (C-9), 165.9 (C-1), 164.8 (C-3), 158.4 (C-6), 158.1 (C-4a), 156.8 (C-10a), 144.9

(C-7), 138.7 (C-8), 135.4 (C-13), 132.0 (C-17), 125.5 (C-16), 125.2 (C-12), 112.2 (C-8a), 102.9 (C-5), 103.9 (C-9a), 98.8 (C-2), 94.0 (C-4), 61.4 (7-OCH_3), 40.8 (C-14), 27.6 (C-15), 27.0 (C-11), 25.8 (C-19), 17.7 (C-20), 16.6 (C-18).

Isoxanthochymol **(6):** Bright yellow solid, mp of 123-125 ^{O}C ([12] m.p. 125-127 ^{O}C). UV (EtOH) λ_{max} nm (log ε): 414 (2.50). IR ν_{max} cm^{-1} (KBr): 3428 (OH), 2928 (C-H stretching), 1728 (C=O), 1634 (C=C), 1444 (CH_2 bending), 1378 (CH_3 bending), 1198 (C-O). EI-MS m/z (rel.int.): 602 (1.8), 465 (38), 411 (3), 355 (6), 341 (18), 285 (5), 231 (39), 217 (6), 177 (12), 137 (5), 109 (5), 95 (15), 81 (23), 69 (100), 55 (25), 41 (3). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97 (1H, d, *J*=8.3 Hz, H-16), 6.95 (1H, s, H-12), 6.61 (1H, d, J= 8.3 Hz , H-15), 5.10 (1H, m, H-35), 4.95 (1H, m, H-25), 4.80 (1H, m, H-18), 2.74 (2H, m, H-7), 2.63 (1H, m, H-30), 2.38 (1H, m, H-6), 2.19 (1H, m, H-17a), 2.16 (1H, m, H-17b), 2.08 (2H, m, H-24), 1.86 (2H, m, H-34), 1.81 (3H, s, H-32), 1.75 (3H, s, H-33), 1.71 (3H, s, H-37), 1.70 (3H, s, H-38), 1.60 (2H, d, *J* = 6.4 Hz, H-29), 1.56 (3H, s, H-20), 1.54 (3H, s, H-27), 1.48 (3H, s, H-21), 1.45 (3H, s, H-28), 1.19 (3H, s, H-22), 1.02 (3H, s, H-23). ^{13}C NMR (100MHz, $CDCl_3$):δ 209.2 (C-9), 195.0 (C-3), 193.8 (C-10), 149.8 (C-14), 147.2 (C-1), 145.7 (C-36), 143.6 (C-13), 135.3 (C-26), 132.8 (C-19), 127.4 (C-11), 124.0 (C-16), 123.6 (C-25), 119.9 (C-35), 116.4 (C-12), 115.7 (C-15), 114.2 (C-2), 113.3 (C-18), 109.6 (C-31), 69.7 (C-4), 49.6 (C-8), 46.7 (C-5), 43.4 (C-7), 43.2 (C-6), 42.4 (C-30), 36.4 (C-32), 35.3 (C-33), 28.8 (C-17), 26.8 (C-23), 26.2 (C-29), 26.0 (C-20), 25.7 (C-27), 25.6 (C-24), 25.6 (C-34), 22.6 (C-22), 22.5 (C-37), 18.1 (C-21), 17.8 (C-28), 17.0 (C-38).

Caffeine **(7)** - Green crystals, mp 234 ^{O}C. ([13] m.p.)UV (EtOH) λ_{max} nm (log ε): 502.5 (0.05), 495.0 (0.05), 412.5 (0.22), 389.0 (0.21), 296.0 (2.50). IR ν_{max} cm^{-1} (KBr): 3436 (N-H), 3112 (C-H stretching), 1704 (C=O), 1658 (C=C), 1548 (C=N), 1484 (C-N). EI-MS m/z (rel.int.): 194 (100), 165 (7), 136 (5), 109 (56), 94 (4), 82 (33), 67 (54), 55 (65). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (1H, s, H-4), 4.00 (3H, s, H-8), 3.59 (3H, s, H-6), 3.41 (3H, s, H-7). ^{13}C NMR (100MHz, $CDCl_3$): δ 155.4 (C-2), 151.7 (C-1), 148.7 (C-5), 141.4 (C-4), 107.6 (C-3), 33.6 (C-8), 29.7 (C-7), 27.9 (C-6).



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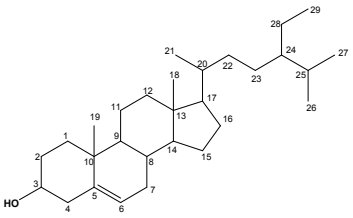

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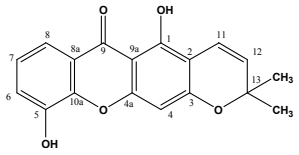

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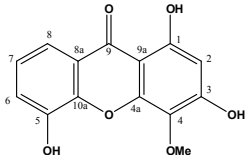

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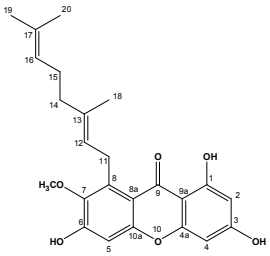

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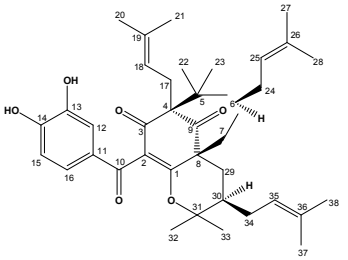

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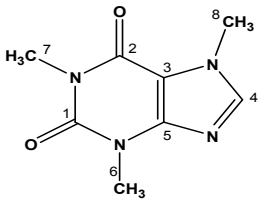

(7)

RESULTS AND DISCUSSION

6-Deoxyjacareubin **(3)** was obtained as yellow amorphous powder after recrystallisation from chloroform, m.p. 203-209 ^{O}C ([10] Lit. 211-213 ^{O}C). A positive test with alcoholic ferric chloride revealed its phenolic nature. The [M^{+}] at m/z 310 in the EI mass spectrum corresponds to the molecular formula $C_{18}H_{14}O_5$. The FTIR spectrum exhibited strong bands at 3792 cm^{-1} and 1648 cm^{-1} due to a phenolic hydroxyl and a chelated carbonyl group. The UV spectrum of **(3)** exhibited characteristic absorption bands of a xanthone at 412.5 nm.

The ^{1}H NMR spectrum of **(3)** indicated the presence of signals at δ 7.14 (1H, dd, *J* = 8.3 Hz, 6.4 Hz), δ 7.05 (1H, t, *J*=8.3 Hz) and δ 7.57 (1H, dd, *J* = 8.3, 6.4 Hz) which were assigned to H-6, H-7 and H-8 respectively. The occurrence of a doublet doublet at δ 7.14 was due to *ortho*-coupling with δ 7.05 and *meta*-coupling with δ 7.57. The H-8 signal was at a lower field as it was deshielded by the carbonyl group at C-9. In addition, the remaining one-proton singlet at δ 6.13 was attributed to an isolated aromatic proton at H-4. Other than that, signals at δ 6.88 (1H, d, *J* = 10.0 Hz, H-11), δ 5.49 (1H, d, *J* = 10.0 Hz, H-12) and δ 1.35 (6H, s, $2CH_3$) indicated the presence of a 2,2-dimethyl chromene ring substitution at the xanthone ring, with the chromene double bond *ortho* to the C-1 hydroxyl group.

The ^{13}C NMR spectrum disclosed the presence of one conjugated carbonyl group at δ181.1 (C-9) and eight substituted aromatic carbons at δ 160.7 (C-1), δ 101.4 (C-2), δ 162.4 (C-3), δ 151.5 (C-4a), δ 145.1 (C-10a), δ 145.7 (C-5), δ 121.2 (C-8a) and δ 103.3 (C-9a).

The 18 carbon signals in the ^{13}C NMR spectrum were characterized by the DEPT spectrum, which indicated that **(3)** consists of two methyl, six methine and ten quaternary carbons.

Conclusive proof for the substitution pattern of **(3)** came from analysis of the correlation peaks in the HMBC spectra. In the HMBC spectrum, the location of a hydroxyl at C-5 was supported by the observed cross peak between the hydroxyl bearing C-5 and the aromatic proton of H-6 through a ^{3}J correlation. A ^{3}J correlation was also observed between the doublet at δ6.88 (H-11) and δ78.2 (C-13) and between δ5.49 (H-12) and d 101.4 (C-2) indicating the signals at δ6.88 and δ5.49 to be for H-11 and H-12. The latter confirms the presence of the pyrano ring attached to C-2 and C-3. This assignment was further confirmed by the coupling between δ6.88 and δ5.49 as observed in the ^{1}H-^{1}H COSY experiment.

Based on these data the compound was therefore assigned 6-deoxyjacareubin **(3)**. The spectral data are summarized in Table 1.

Table 1. ^{1}H NMR (400 MHz, $CHCl_3$), ^{13}C NMR (100 MHz, $CHCl_3$) &HMBC data for 6-deoxyjacareubin (3)

Position	^{1}H (δ)	^{13}C (δ)	HMBC (C-H correlations)
1	-	160.7	103.3 (C-9a) (^{3}J), 160.7 (C-1) (^{2}J)
2	-	101.4	-
3	-	162.4	-
4	6.13 (1H, s)	98.9	103.3 (C-9a) (^{3}J), 162.4 (C-3) (^{2}J)
5	-	145.7	120.4 (C-6) (^{3}J), 145.7 (C-5) (^{2}J)
6	7.14 (1H, dd, *J* = 8.3 Hz, 6.4 Hz)	120.4	145.1 (C-10a) (^{3}J)
7	7.05 (1H, t, *J* = Hz)	123.8	145.7 (C-5) (^{3}J), 120.4 (C-6) (^{2}J)
8	7.57 (1H, dd, *J* = 8.3 Hz, 6.4 Hz)	115.6	181.1 (C-9) (^{3}J), 120.4 (C-6) (^{3}J), 145.1 (C-10a) (^{3}J)
9	-	181.1	-
11	6.88 (1H, d, *J* = 10.0 Hz)	114.9	78.2 (C-13) (^{3}J), 162.4 (C-3) (^{3}J)
12	5.49 (1H, d, *J* = 10.0 Hz)	126.9	101.4 (C-2) (^{3}J), 78.2 (C-13) (^{2}J)
13	-	78.2	-
14	1.35 (6H, s)	27.9	27.9 (C-15) (^{3}J), 126.9 (C-12) (^{3}J), 78.2 (C-13) (^{2}J)
15	1.35 (6H, s)	27.9	27.9 (C-14) (^{3}J), 126.9 (C-12) (^{3}J), 78.2 (C-13) (^{2}J)
4a	-	151.5	-
8a	-	121.2	-
9a	-	103.3	-
10a	-	145.1	-

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