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Chemical Constituents from *Garcinia maingayi* and *Garcinia parvifolia* (Guttiferae) and Their Biological Activities

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ABSTRACT

Detail chemical studies on *Garcinia maingayi* have yielded one xanthone, 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone, one benzophenone, isoxanthochymol, one benzoic acid derivative 3,4-dihydroxy-methylbenzoate and two triterpenoids, stigmasterol and sitosterol. Meanwhile, investigations on *Garcinia parvifolia* have afforded one triterpenoid, α-amyrin and two xanthones, cowanin and rubraxanthone. Their structures were derived based on spectroscopic evidence, mainly 1D and 2D NMR spectroscopy. Acetylation reaction was carried out on rubraxanthone to yield triacetate rubraxanthone. It was found that the pure rubraxanthone was strongly active against the larvae of *Aedes aegypti* with LC_{50} value of 15.49 μg/ml and HL-60 cells line with an IC_{50} value of 7.5 μg/ml.

Keywords: *Garcinia maingayi*, *Garcinia parvifolia*, 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone, isoxanthochymol, 3,4-dihydroxy-methylbenzoate, cowanin, rubraxanthone, triacetate rubraxanthone

INTRODUCTION

Garcinia is best known in Malaysia as a genus of fruit trees. The genus has been the subject of phytochemical studies which revealed it to be a major source of prenylated xanthones, benzophenones and biflavanoids linked between C-3 and C-8 (Xu *et al.*, 2001; Hussain and Waterman, 1982). Some of these exhibit a wide range of biological and pharmacological activities namely cytotoxic, anti-inflammatory, antimicrobial and antifungal activity (Minami *et al.*, 1996; Minami *et al.*, 1994). A number of *Garcinia* species have been investigated but only a few have been extensively studied. *Garcinia mangostana* and *Garcinia subelliptica* are the two species that have been well studied (Minami *et al.*, 1996; Minami *et al.*, 1994; Bennett and Lee, 1989; Iinuma *et al.*, 1995; Iinuma *et al.*, 1994; Asai *et al.*, 1995; Fukuyama *et al.*, 1991; Nilar and Harrison, 2002). There is no previous record on the chemistry and bioactivity of *Garcinia maingayi*.

MATERIAL AND METHODS

Plant Material

The stem bark of *Garcinia maingayi* and *Garcinia parvifolia* were collected from Fraser's Hill in Pahang. Voucher specimens are kept in the Institute of Bioscience, University Putra Malaysia.

General

Infrared spectra were measured in a 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 with tetrametylsilane (TMS) as internal standard. Ultra violet spectra were recorded on a Shimadzu UV-160A, UV-Visible Recording Spectrometer. Chromatographic separation was carried out using silica gel Merck 9385 and Sephadex LH-20.

Extraction and Isolation of Compounds

The dried and powdered stem bark material (1.5 kg each) of *Garcinia maingayi* and *Garcinia parvifolia* were extracted successively with distilled *n*-hexane, chloroform, acetone and methanol twice for 48 hours. About 12.8 g of crude hexane extract was fractionated by column chromatography over silica gel to yield stigmasterol (**1**) and sitosterol (**2**). The other three extracts were also subjected to a silica gel column chromatography and Sephadex LH-20 column to give isoxanthochymol (**3**), 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (**4**) and 3,4-dihydroxymethylbenzoate (**5**). Fractionation of the hexane extract of *Garcinia parvifolia* (6.5 g) over a silica gel column yielded α-amyrin (**6**). Similarly, the chloroform extract was directly chromatographed on a silica gel column to give 20 fractions which when further purified in a Sephadex LH-20 column, gave cowanin (**7**). Rubraxanthone (**8**) was recrystallized as pale yellow powder in acetone after it was obtained from the column chromatography of the acetone extract.

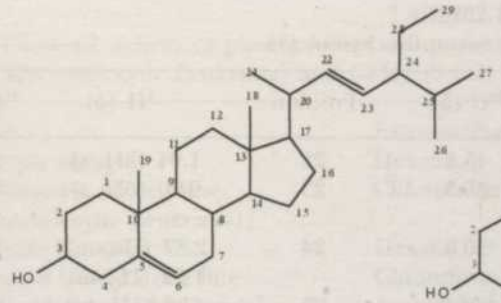
Synthesis of Triacetate Rubraxanthone (9)

Acetylation of (**8**) was carried out by dissolving (**8**) (30 mg) in pyridine (3 ml) and acetic anhydride (3 ml). The solution was left at room temperature for 24 hours. The reaction mixture was poured into iced distilled water and then extracted with ethyl acetate. The organic extract was evaporated to dryness and purified by silica gel column chromatography to yield triacetate rubraxanthone (**9**).

Isoxanthochymol (**3**): White prisms, mp 125-127 °C. UV (EtOH) λ_{max} nm (log ε): 278 (1.24), 233 (1.10). IR ν_{max} cm^{-1} (KBr): 3648, 2974, 2938, 1718, 1680, 1640, 1606, 1454, 1366, 1184. CI-MS m/z (rel. int.): 603 (M+1) (5), 469 (10), 391 (10), 279 (15), 221 (15), 149 (100), 113 (22), 74 (42). ^{1}H NMR and ^{13}C NMR see Table 1.

1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (**4**): Pale yellow needles, mp 211-213°C (Harrison *et al.*, 1993, 217-218°C). UV (EtOH) λ_{max} nm (log ε): 239.5 (1.36), 262.5 (1.28), 313.5 (0.66), 377.0 (0.25). IR ν_{max} cm^{-1} (KBr):3438 2924, 1786, 1642. EI-MS m/z (rel. int.): 312 [M^+, 48], 297 (37), 269 (82), 257 (100), 244 (16), 229 (11), 137 (12), 115 (10), 77 (12), 65 (19), 53 (19), 41 (20). ^{1}H NMR and ^{13}C NMR data agree with literature values (Harrison *et al.*, 1993).

Cowanin (**7**): Yellowish oil. ^{1}H NMR and ^{13}C NMR data are in agreement with published data (Harrison *et al.*, 1993).



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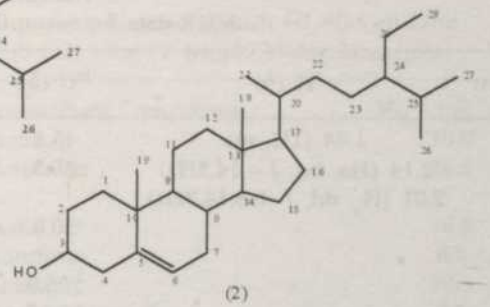

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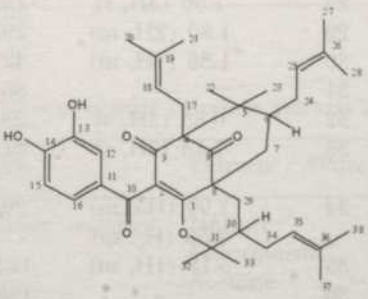

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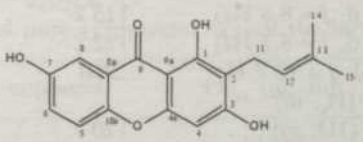

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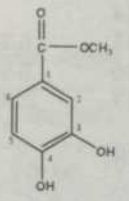

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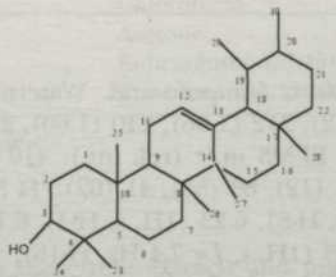

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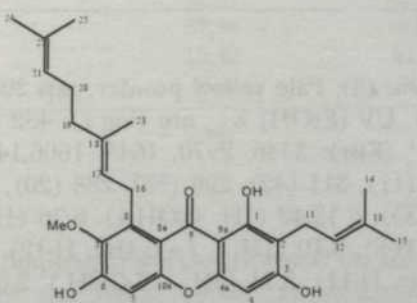

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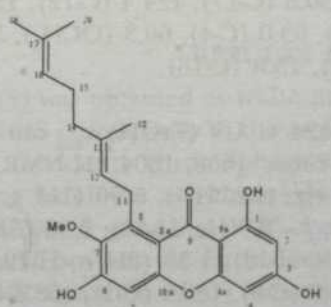

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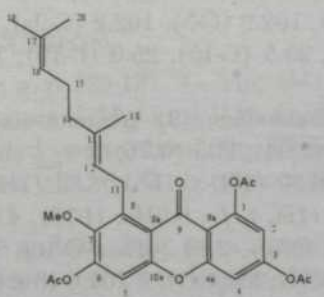

(9)

TABLE 1
NMR data for isoxanthochymol (**3**)

Position	^{1}H (δ)	^{13}C (δ)	Position	^{1}H (δ)	^{13}C (δ)
6	1.44 (1H, m)	45.3	22	1.04 (3H, s)	22.3
7	2.14 (Ha, bd, J = 14.3Hz) 2.01 (H_b, dd, J =6.8,14.3Hz)	38.3	23	0.90 (3H, s)	26.4
8	-	50.9	24	2.87 (Ha, m) 1.02 (H_b, m)	27.8
9	-	206.9	25	4.92 (1H, m)	125.3
10	-	193.7	26	-	132.4
11	-	129.0	27	1.65 (3H, s)	25.9
12	7.13 (1H, s)	115.4	28	1.50 (3H, s)	18.0
13	-	145.4	29	1.80 (2H, m)	29.2
14	-	151.2	30	1.36 (1H, m)	42.5
15	6.74 (1H, d, J = 8.3 Hz)	115.2	31	-	86.7
16	6.94 (1H, d, J = 8.3 Hz)	122.2	32	0.83 (3H, s)	28.5
17	2.48 (Ha, d, J = 13.7 Hz) 2.34 (H_b, m)	26.0	33	1.18 (3H, s)	21.2
18	4.78 (1H, m)	120.7	34	1.99 (Ha, m) 2.58 (H_b, m)	29.0
19	-	132.9	35	5.18 (1H, m)	122.2
20	1.51 (3H, s)	26.0	36	-	133.1
21	1.60 (3H, s)	18.2	37	1.73 (3H, s)	26.0
			38	1.59 (3H, s)	18.2

Rubraxanthone (**8**): Pale yellow powder, mp 208-210 °C (Ampofo and Waterman, 1986, 205-206 °C). UV (EtOH) λ_{max} nm (log ε): 432 (0.12), 312 (1.06), 240 (1.60), 214 (1.06). IR ν_{max} cm^{-1} (KBr): 3446, 2970, 1648, 1606,1466. EI-MS m/z (rel. int.): 410 (30), 341 (100), 326 (11), 311 (42), 299 (55), 288 (20), 271 (12), 69 (50), 41 (62). ^{1}H NMR (400 MHz, Me_2CO): δ 13.42 (1H, s, OH-1), 6.76 (1H, s, H-5), 6.23 (1H, s, H-4), 6.11 (1H, d, J = 1.8 Hz, H-2), 5.19 (1H, t, J = 6.4Hz, H-12), 4.95 (1H, t, J = 7.4 Hz, H-16), 4.04 (2H, d, J = 6.4 Hz, H-11), 3.71 (3H, s, 7-OCH_3), 2.09 (2H, t, J = 7.4 Hz, H-15), 1.90 (2H, t, J = 7.4 Hz, H-14), 1.83 (3H, s, H-18), 1.47 (3H, s, H-19), 1.43 (3H, s, H-20). ^{13}C NMR (100 MHz, Me_2CO): δ 181.9 (C-9), 164.6 (C-1), 164.1 (C-3), 157.2 (C-4a), 156.8 (C-6), 155.5 (C-10a), 143.8 (C-7), 137.4 (C-8), 134.3 (C-13), 130.8 (C-17), 124.4 (C-12), 124.3 (C-16), 111.2 (C-8a), 102.9 (C-5), 102.2 (C-9a), 97.9 (C-2), 93.0 (C-4), 60.3 (OCH_3), 39.6 (C-14), 26.5 (C-11), 25.5 (C-15), 25.0 (C-19), 16.9 (C-20), 15.8 (C-18).

Triacetate rubraxanthone (**9**): White crystal, mp 122-124 °C. UV (EtOH) λ_{max} nm (log ε): 341 (0.16), 271 (2.04), 203 (0.76)IR ν_{max} cm^{-1} (KBr): 2968, 1658, 1604. ^{1}H NMR (400 MHz, Me_2CO): δ 8.50 (1H, s, H-5), 7.82 (1H, d, J = 2 Hz, H-2, H-4), 5.90 (1H, t, J = 6.0 Hz, H-12), 4.85 (1H, t, J = 6.0Hz, H-16), 4.69 (2H, d, J = 6.0 Hz, H-11), 3.98 (3H, s, OMe), 2.80 (3H, s, OAc), 2.85 (6H, s OAc), 1.37 (3H, s, H-18), 1.36 (3H, s, H-19, H-20), ^{13}C NMR (100 MHz, Me_2CO): δ 201.0 (3 x C=O), 176.0 (C-9), 168.9 (C-1), 168.4 (C-3), 168.2 (C-6), 157.1 (C-4a), 155.3 (C-10a), 147.7 (C-7), 138.7 (C-8), 135.3 (C-13), 131.1 (C-17), 124.7 (C-16), 123.5 (C-12), 118.8 (C-8a), 113.7 (C-9a), 113.4 (C-5), 111.2 (C-2), 108.7 (C-4), 61.5 (OMe), 40.0 (C-14), 26.8 (C-15), 26.0 (C-11), 25.3 (C-19), 20.7 (OAc), 20.6(OAc), 20.3 (OAc), 17.2 (C-20), 16.2 (C-18).

TABLE 2
Cytotoxic activity of plant extracts and pure compound against HL-60 Cell Line (Promyelocytic Leukemia) and CEM- SS Cell Line (T lymphoblastic Leukemia)

Plant	Extracts/Pure Compounds	IC_{50} (μg/ml)
Garcinia maingayi	Hexane	10.0
[Against HL-60 cell line	Chloroform	26.5
(Promyelocytic Leukemia)]		
Garcinia parvifolia	Hexane	16.5
[Against CEM-SS cell line	Chloroform	6.5
(T-lymphoblastic Leukemia)	Acetone	19.5
	Rubraxanthone (**8**)	7.5
	Triacetate rubraxanthone (**9**)	10.3

TABLE 3
Larvicidal activity of crude extracts and pure compound against the larvae of *Aedes aegypti*

Plants	Extracts / *Pure compound	LC_{50} (μg/ml)	LC_{90} (μg/ml)
Garcinia maingayi	Hexane	145.95	249.60
	Chloroform	N.A	N.A
	Acetone	250.58	281.74
	Methanol	209.52	292.45
Garcinia parvifolia	Hexane	98.54	225.91
	Chloroform	92.07	223.80
	Acetone	67.98	192.12
	Rubraxanthone* (**8**)	15.49	21.30
	Triacetate rubraxanthone* (**9**)	N.A	N.A

N.A = Not Active

Bioassay

Bioassay on the crude extracts and pure compounds were performed on the larvae of *Aeds aegypti* according to the protocols of the World Health Organisation (1981). Cytotoxic assays were carried using the HL-60 and CEM-SS cells line. The cells were cultured and maintained in growth medium as described by Ali *et al.* (1996).

RESULTS AND DISCUSSION

Compound (**3**) was obtained as white prisms, with m.p 125-127 °C. The $(M+1)^+$ at m/z 603 in the CI-MS corresponds to the molecular formula $C_{38}H_{50}O_6$. UV absorptions at 277 and 233 nm revealed a chromophore with extended conjugation. The FTIR spectrum showed strong bands for a hydroxyl group at 3468 cm^{-1} and both non-conjugated and conjugated carbonyl groups at 1718 and 1680 cm^{-1}.

Three vinylic protons, six vinylic methyl protons and six allylic protons were apparent in the ^1H NMR spectrum of (**3**) indicating the presence of three isoprenyl groups. The characteristic 3,4-dihydroxy benzoyl group was evident from proton resonance at δ 7.13 (1H, s), δ 6.94 (1H, d, J = 8.3 Hz) and δ 6.74 (1H, d, J = 8.3 Hz) which were assigned to H-12, H-16 and H-15 respectively. The large coupling constant indicated that H-15 and

H-16 are *ortho*-coupled aromatic protons. In addition to that, the methylene and methine protons which gave complexes of multiplets of 12H in the regions of δ 1.3 to 2.9 were observed. The ^{1}H NMR also showed 4 tertiary methyl protons which accounted for H-22, H-23, H-32 and H-33. Additional evidence for three ketone functions inferred from the FTIR spectrum was obtained from the ^{13}C NMR spectrum.

Characteristic ^{13}C NMR resonances for substituted aromatic carbons at δ 129.0 (C-11), δ 145.4 (C-13) and δ 151.2 (C-14) and a conjugated carbonyl group [δ 193.7, (C-10)] confirmed the existence of a 3,4-dihydroxy benzoyl group. Resonances consisting of a non-conjugated ketone at δ 206.9 (C-9) flanked by two quaternary carbons at δ 67.7 (C-4) and δ 50.9 (C-8) were also observed. HMBC established the substitution pattern of the isoprenyl group in **(3)**. The methylene protons of H-17 gave a cross peak to C-9 thus suggesting one of the prenyl group is located at C-4. No correlation for the other two prenyl groups was observed to identify their substitution patterns. However based on previous reports on **(3)**, (Gustasfson *et al.*, 1992) the two other prenyl moieties were placed at C-6 and C-30. The presence of isoprenyl moieties was further validated by HMBC. The methyl protons of H-37 [δ 1.73 (3H, s)], H-38 [δ 1.59 (3H, s)], H-27 [δ 1.65 (3H, s)], H-28 [δ 1.50 (3H, s)], H-20 [δ 1.51 (3H, s)] and H-21 [δ 1.60 (3H, s)] all showed linkages to the carbon atoms C-35 (δ 122.2), C-36 (δ 133.1), C-25 (δ 125.3), C-26 (δ 132.4), C-18 (δ 120.7) and C-19 (δ 132.9). Compound **(3)** was therefore identified as isoxanthochymol and the spectral data are summarized in Table 1.

Compound **(8)** was obtained as a yellow powder, mp.: 208-210 °C (Ampofo and Waterman, 1986, 205-206 °C). The [M$^+$] at m/z 410 in the EI-MS spectrum corresponds to the molecular formula $C_{24}H_{26}O_6$. The ^{1}H NMR spectra revealed signals for a H-bonded hydroxyl function (δ 13.4) at C-1 and three aromatic protons two of which are *meta*-coupled [δ 6.23 (1H, s), δ 6.11 (1H, d, J = 1.8Hz)] for H-4 and H-2] and a singlet at δ 6.76 for H-5. A single methoxy resonance occurred at δ 3.71. The remaining resonances appeared as a series of signals typical of a geranyl moiety. Also observed were an olefinic methyl proton at δ 1.83 (3H, s, H-18), a geminal-dimethyl protons at δ 1.47 (3H, s, H-19) and δ 1.43 (3H, s, H-20) in addition to a methylene proton at δ 4.04 (2H, d, J = 6.4Hz, H-11) and two vinyl methine protons at δ 5.19 (1H, t, J = 6.4Hz, H-12) and δ 4.95 (1H, t, J = 7.4Hz, H-16). Other than that, the ^{1}H NMR spectrum also exhibited two sets of methylene protons at δ 1.90 (2H, t, J = 7.4Hz) and δ 2.09 (2H, m) attributable to H-14 and H-15. The signal of the methylene proton (δ 4.04) of the chain which appeared in the low field region indicated that the geranyl group was located at C-8 which is next to the carbonyl group.

The ^{13}C NMR spectrum clearly showed 24 carbon signals. A typical conjugated carbonyl group (δ 181.9, C-9) for xanthones was observed. From the ^{13}C NMR spectrum six aromatic carbons with O-function appeared at δ 164.6, δ 164.1, δ 157.2, δ 156.8, δ 143.8 and δ 137.4. These were assigned to C-1, C-3, C-4a, C-6, C-7 and C10a respectively. The resonance at δ 60.3 was due to a methoxy group. These suggests that **(8)** is a tetraoxygenated xanthone with one methoxy and three hydroxyl group. From the DEPT spectrum five methine, three methylene, three methyl and twelve tertiary carbon signals were observed supporting the structure for rubraxanthone **(8)**, previously isolated from *Garcinia pyrifera* (Ampofo and Waterman, 1986).

Rubraxanthone triacetate **(9)** was obtained from the acetylation reaction on rubraxanthone **(8)**. Recrystalization from acetone gave the triacetate as an amorphous white solid, of m.p 122-124 °C. The [M$^+$] at m/z 536 in the EI-MS spectrum corresponds to the molecular formula $C_{30}H_{32}O_9$. The IR spectrum didn't display any hydroxyl

functional groups indicating they were successfully replaced by the acetyl moieties. From the ^{1}H NMR spectrum, the absence of the chelated hydroxyl group further supports that the acetylation chemical reaction has replaced the hydroxyl groups in **(8)**. As compared to **(8)**, chemical shifts for the aromatic, vinylic and methylene protons in triacetate rubraxanthone **(9)** have appeared more down field at δ 8.50 (1H, s, H-5), δ 7.82 (1H, d, J = 2 Hz, H-2, 1H, d, J = 2 Hz, H-4), δ 5.90 (1H, t, J = 6.0 Hz, H-12) and δ 4.69 (2H, d, J = 6.0 Hz, H-11) due to anisotropic effect. The presence of the methyl protons of the acetate group was confirmed by the ^{1}H NMR spectrum at δ 2.80 (3H, s, OAc). The two other methyl protons were observed at δ 2.85.

The ^{13}C NMR spectrum exhibited the typical conjugated carbonyl group at δ 176.0 (C-9) together with six oxygenated aromatic carbons at δ 168.9 (C-1), δ 168.4 (C-3), δ 168.2 (C-6), δ 157.1 (C-4a), δ 155.3 (C-10a) and δ 147.7 (C-7). The methine aromatic carbons resonances appeared at δ 113.4 (C-5), δ 111.2 (C-2) and 108.6 (C-4) in the lower field region due to anisotropic effect by the ketone group of acetyl moieties. The methyl carbons for the acetyl groups were observed at δ 20.7 (OAc), δ 20.6 (OAc), δ 20.3 (OAc).

The other compounds, stigmasterol **(1)**, sitosterol, **(2)**, 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone **(4)**, 3,4-dihydroxy-methylbenzoate **(5)**, α-amyrin **(6)** and cowanin **(7)** were identified by spectral data and by comparison with literature data (Harrison *et al.*, 1993; Holland *et al.*, 1978).

All the crude extracts and pure compounds obtained were bioassayed against the larvae of *Aedes aegypti*. It was found that the crude hexane, acetone and methanol extract of *Garcinia maingayi* were weakly active against the larvae with LC_{50} values of 145.9, 250.58 and 209.58 mg/ml respectively. The hexane, chloroform and acetone extract of *Garcinia parvifolia* showed moderate activities against the larvae by giving LC_{50} values of less than 100 μg/ml. Pure rubraxanthone **(8)** exhibited a strong activity against the larvae with LC_{50} value of 15.49 μg/ml. However, its derivative triacetate rubraxanthone **(9)** did not display any toxicity against the larvae. This suggests that the hydroxyl functional groups could be responsible for the toxicity against the larvae.

Biological activities of crude extracts and pure compounds were also carried out against HL-60 and CEM-SS cancer cells line. The crude hexane and chloroform extracts of *Garcinia maingayi* were considered to be active against HL-60 cell line with the IC_{50} values of less than 30 μg/ml. Meanwhile, the crude hexane and acetone extracts of *Garcinia parvifolia* were also considered to be active against CEM-SS cells line with the IC_{50} values of less than 30 μg/ml; meanwhile the crude chloroform extract showed a significant activity with an IC_{50} value of 6.5 μg/ml. Pure rubraxanthone **(8)** exhibited a strong activity against CEM-SS cell line with the IC_{50} values of 7.5 μg/ml. However the replacement of the hydroxyl groups on **(9)** didn't cause any significant change to its toxicity against the cancer cell lines

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