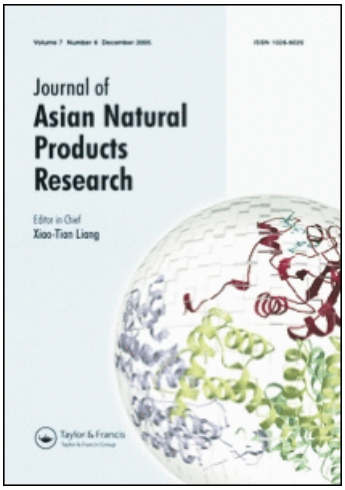

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Garcinia mangostana: a source of potential anti-cancer lead compounds against CEM-SS cell line

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Our current interest in searching for natural anti-cancer lead compounds from plants has led us to the discovery that the stem and roots of *Garcinia mangostana* can be a source of such compounds. The stem furnished 2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone (**1**), which is a new xanthone. Meanwhile, the root bark of the plant furnished six xanthones, namely α-mangostin (**2**), β-mangostin (**3**), γ-mangostin (**4**), garcinone D (**5**), mangostanol (**6**), and gartanin (**7**). The hexane and chloroform extracts of the root bark of *G. mangostana* as well as the hexane extract of the stem bark were found to be active against the CEM-SS cell line. γ-Mangostin (**4**) showed good activity with a very low IC_{50} value of 4.7 μg/ml, while α-mangostin (**2**), mangostanol (**6**), and garcinone D (**5**) showed significant activities with IC_{50} values of 5.5, 9.6, and 3.2 μg/ml, respectively. This is the first report on the cytotoxicity of the extracts of the stem and root bark of *G. mangostana* and of α-mangostin, mangostanol, and garcinone D against the CEM-SS cell line.

Keywords: *Garcinia mangostana*; xanthones; cytotoxicity; CEM-SS cell line

1. Introduction

The genus *Garcinia*, a member of the Guttiferae family is widespread in the lowland tropical rain forests of Southeast Asia and West Africa[1,2] and is rich in prenylated xanthones, triterpenes, biflavonoids, and polyprenylated benzophenone, which are biologically active.[3,4] Extensive research has shown that the *Garcinia* species exhibit a wide range of biological and pharmacological activities such as cytotoxic, antimicrobial, antimalarial, and anti-HIV-1 protease inhibitory activity.[5] *Garcinia mangostana* L. is known for its medicinal properties. The fruit hulls of this plant are reported to be used as an anti-inflammatory agent and astringent or used against diarrhea.[6] Our detailed studies on the stem and roots of this species have provided six known prenylated xanthones and a new xanthone, 2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone (**1**). We report here the structural elucidation of the new xanthone. We also report the results of cytotoxicity tests of the crude extracts, α-mangostin (**2**), mangostanol (**6**), and garcinone D (**5**), against tumor cell growth of the CEM-SS cell line.

2. Results and discussion

Compound (**1**) was obtained as yellow crystals with a melting point of 147–148°C. The $[M^+]$ at *m/z* 326 in the EI-MS spectrum corresponds to the molecular formula $C_{19}H_{18}O_5$; HR-EI-MS: *m/z* 326.1157. The UV absorption bands at 243, 262, 305, and 371 nm indicated **1** to be a hydroxylated xanthone. The IR spectrum showed strong bands at 3250 and 1648 cm^{-1}, which were

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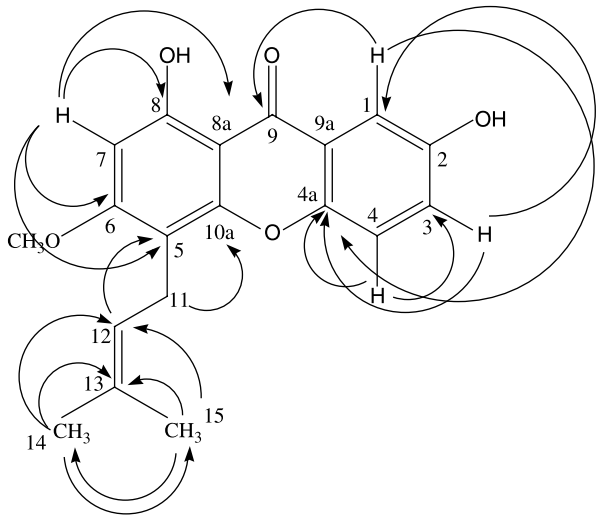

Figure 1. Some key HMBC correlations of **1**.

due to a phenolic hydroxyl and a chelated carbonyl group. The ^{1}H NMR spectrum indicated the presence of ABX-type signals at δ 7.42 (1H, d, $J = 3.0$ Hz), 7.24 (1H, d, $J = 9.1$ Hz), and 7.19 (1H, dd, $J = 9.1$, 3.0 Hz), which were assigned to H-1, H-4, and H-3, respectively. The occurrence of the doublet of doublet at δ 7.19 was due to ortho-coupling with the doublet at δ 7.24 ($J = 9.1$ Hz) and meta-coupling with δ 7.42 ($J = 3.0$ Hz). It was noted that H-1 was at a lower field as it was deshielded by the carbonyl group at C-9. The remaining one-proton singlet at δ 6.37 was clearly assigned to an isolated aromatic proton at H-7.

The presence of 3-methyl-but-2-enyl substituent was indicated by the ^{1}H NMR signals at δ 5.15 (1H, t, $J = 6.4$ Hz, H-12), 3.22 (2H, d, $J = 6.4$ Hz, H-11), 1.73 (3H, s, H-14), and 1.61 (3H, s, H-15). In the ^{1}H–^{1}H COSY experiment, the nature of the allylic and homoallylic coupling systems within the prenyl moiety was clearly demonstrated. It showed correlations between the olefinic proton at C-12 and the benzylic proton at C-11 and the geminal dimethyl group. The ^{13}C NMR analysis gave a total of 19 carbon resonances due to one methoxy, two methyls, one methylene, five methines, nine quaternary, and one carbonyl carbons. From the HMBC spectrum, the methoxy group of **1** was assigned to be at C-6 (see Figure 1). A correlation between the methylene signal at δ 3.28 (C-11, δ 20.9) with C-6 at δ 156.4 suggested that the 3,3-dimethylallyl moiety is attached to C-5. The isolated aromatic proton [δ 6.37 (1H, s, H-7)] showed four crosspeaks with the aromatic carbon signals C-5, C-6, C-8, and C-8a. Meanwhile, the proton signals at δ 7.24 (1H, d, H-4) and δ 7.19 (1H, dd, H-3) gave correlation peaks with C-2 (δ 153.4) and C-4a (δ 149.9), respectively. The remaining proton signal at δ 7.42 (1H, d, H-1) gave crosspeaks to C-4a (δ 149.9), confirming that it belongs to H-1. Hence, compound (**1**) was assigned as 2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone.This is the new xanthone.

The hexane extract of the stem bark and the hexane and chloroform extracts of the root bark of *G. mangostana* were found to be active toward the CEM-SS cell line with IC_{50} values less than 30 μg/ml. The hexane extract gave a very low IC_{50} value of 0.3 μg/ml, while the chloroform extract gave a slightly higher IC_{50} value of 14 μg/ml. Meanwhile, the hexane extract of the stem bark gave a low IC_{50} value of 17 μg/ml. α-Mangostin gave a very low IC_{50} value of 5.5 μg/ml, while

Table 1. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), assignments for **1** and their connectivities obtained from HMBC experiment.

Position	δ_H	δ_C	HMBC
1	7.42 (d, $J = 3.0$ Hz, 1H)	108.2	149.9 (C-4a, ^{3}J),
2		153.4	
3	7.19 (dd, $J = 9.1$ Hz, 3.0 Hz, 1H)	118.7	108.2 (C-1, ^{3}J), 149.9 (C-4a, ^{3}J) 111.4 (C-9a, ^{4}J)
4	7.24 (d, $J = 9.1$, 1H)	124.4	153.4 (C-2, ^{3}J), 149.9 (C-4a, ^{2}J)
4a		149.9	
5		103.4	
6		156.4	
7	6.37 (s, 1H)	89.6	103.4 (C-5, ^{3}J) 156.4 (C-6, ^{2}J) 164.4 (C-8, ^{2}J), 111.4 (C-8a, ^{3}J)
8		164.4	
8a		111.4	
9		180.8	
9a		120.8	
10a		158.8	
11	3.28 (d, $J = 6.4$ Hz, 2H)	20.9	156.4 (C-6, ^{3}J), 121.9 (C-12, ^{2}J), 131.9 (C-13, ^{3}J), 111.4 (C-8a, ^{4}J), 158.8 (C-10a ^{3}J)
12	5.15 (br t, $J = 6.4$ Hz, 1H)	121.9	
13		131.9	
14	1.73 (s, 3H)	17.6	121.9 (C-12, ^{3}J), 131.9 (C-13, ^{2}J) 25.6 (C-15, ^{4}J)
15	1.61 (s, 3H)	25.6	121.9 (C-12, ^{3}J), 131.9 (C-13, ^{2}J), 17.6 (C-14, ^{4}J)
OMe	3.86 (s, 3H)	55.8	156.4 (C-6, ^{3}J)

garcinone D gave an IC_{50} value of 3.2 μg/ml and mangostanol gave an IC_{50} value of 9.6 μg/ml, indicating these three compounds to be potential lead compounds for anti-cancer activity against the CEM-SS cell line. However, the new xanthone, 2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone, was found to be non-cytotoxic against the CEM-SS cell line. The hexane extracts of both the stem and root bark as well as the chloroform extract of the root bark can also be considered to have potential anti-cancer activity against tumor cell growth of the CEM-SS cell line.

3. Experimental

3.1 General experimental procedures

The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL FT-NMR 400 MHz spectrometer using $CDCl_3$ as a solvent and TMS as an internal standard. The EI-MS and CI-MS spectra were recorded on a Shimadzu GCMS QP5000 instrument equipped with a direct injection probe. The UV spectra were recorded in $CHCl_3$ on a Shimadzu UV-2100 spectrophotometer, while the IR spectra were recorded on a Perkin–Elmer FTIR model 1725X spectrophotometer.

Table 2. Cytotoxic activities of the plant extract and pure compounds against tumor cell growth of the CEM-SS cell line (T-lymphoblastic leukemia).

		IC_{50} (μg/ml)
G. mangostana root bark extract	Hexane extract	0.3
	Chloroform extract	14
G. mangostana stem bark extract	Hexane extract	17
Pure compounds	α-Mangostin	5.5
	Garcinone D	3.2
	Mangostanol	9.6
	2,8-Dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone	>30

3.2 Plant material

The root and stem bark of *G. mangostana* (1.8 kg) was collected from Alor Gajah, Melaka, Malaysia, in 2005. The plant materials were identified by Dr Rusea Go of the Biology Department, Universiti Putra Malaysia. A voucher specimen is deposited in the same department.

3.3 Extraction and isolation

The finely ground stem bark of *G. mangostana* (1.5 kg) was extracted with ethanol twice to yield 66 g of crude extract. The dried crude extract was purified by silica gel column chromatography using hexane, hexane–dichloromethane, dichloromethane–ethyl acetate, and dichloromethane–methanol as the eluting solvents. This provided a new xanthone, 2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone (**1**) (7 mg).

The finely ground root bark of *G. mangostana* (1.5 kg) was extracted with *n*-hexane, chloroform, and acetone twice to yield 12, 35, and 64 g of extracts, respectively. The dried extracts were purified by silica gel column chromatography using *n*-hexane, *n*-hexane–chloroform, chloroform, chloroform–ethyl acetate, ethyl acetate, and chloroform–methanol of increasing polarities as the eluting solvents. Further purifications using silica gel column chromatography and recrystallization gave the pure compounds. The hexane extract provided 4 mg of α-mangostin (**2**) and 3 mg of β-mangostin (**3**), while the chloroform extract gave 8 mg of γ-mangostin (**4**) and 5 mg of garcinone D (**5**). Meanwhile, 6 mg of mangostanol (**6**) and 2 mg of gartanin (**7**) were obtained from the acetone extract.

2,8-Dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone (**1**): UV (EtOH) λ_{max} (nm): 243, 262, 305, and 371; IR ν_{max} (cm^{-1}): 3250, 2966, 1648, and 1606; EI-MS *m/z*: 326, 311, 295, 283, 271, 258, and 241; HR-EI-MS *m/z*: 326.1157 (calcd for $C_{19}H_{18}O_5$, 326.1154). ^1H NMR and ^{13}C NMR (Table 1).

3.4 Cytotoxicity assay

The stock solution of the sample (compound) was prepared at a concentration of 5 mg/ml in dimethyl sulfoxide (DMSO). Serial dilution of the stock solution in the growth medium provided seven sample solutions at concentrations of 2.5, 5.0, 7.5, 10.0, 20.0, 30.0, and 40.0 μg/ml. The CEM-SS cell line was obtained from The National Cancer Institute in Maryland, USA. Cells were grown in a 96-well microliter plate by filling each well with 100 μl of stock culture (1×10^5 cells/ml) and incubated at 37°C for 24 h. The growth medium was removed from the wells, and each well was then treated with 100 μl of varying concentrations of sample solution. Controls were made containing only untreated cell

population in 100 μl of growth medium. The assay for each concentration of the sample was performed in triplicate and the culture plate was incubated for 3 days at 37°C, 5% CO_2, and 90% humidity. After 3 days, 10 μl of the MTT reagent (0.5 mg/ml; Roche Diagnostics, USA) was added to each well. The plate was then incubated for further 4 h at 37°C with 5% CO_2. Then, 100 μl of the solubilization solution was added to each well and the plate was allowed to stand overnight in the incubator at 37°C with 5% CO_2. Cell viability was measured using ELISA spectrophotometer (EL_x 800) at a wavelength of 550 nm. The inhibitory concentration that killed cells by 50% (IC_{50}) was determined from absorbance (OD) versus concentration curve.[7] The results are shown in Table 2.

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