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Bioactive Constituents of *Garcinia porrecta* and *G. parvifolia* Grown in Indonesia

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Abstract: A new xanthone, porxanthone A (1) and one porlanosterol (2) were isolated; and three known xanthones, dulxanthone E (3), dulxanthone F (4), dulxanthone G (5) from *G. porrecta*, whereas two known xanthones, parvixanthone A (6) and rubraxanthone (7), were isolated from *G. parvifolia*. The structures of these compounds were determined by spectroscopic data analysis. The results of cytotoxicity evaluation showed that (3), (4), (5), (6), (7) were inhibitory to L1210 cells, with IC_{50} values in the range of 3 to 8 μg mL^{-1}.

Key words: Porxanthone, lanosterol, cytotoxic, *Garcinia porrecta*, *G. parvifolia*

INTRODUCTION

Various Indonesian plant species have been studied for their medicinal potential for drug discovery. Plant species belong to the family of Guttiferae, Euphorbiaceae, Menispermae, Simaroubaceae, Moraceae, Meliaceae, Lauraceae and Zingiberaceae have been documented as the most frequent plant species used medicinally (Ampofo and Waterman, 1986; Kardono, 1998). Some research groups in Indonesia are studying the bioactive secondary metabolites from *Garcinia* species. These plant genera in the family of Guttiferae grown in Indonesian tropical forest were reported to be rich in natural chemical substances (Kosela *et al*., 1999). It consists of 400 species, in 35 genera, mostly grown and distributed in all over Indonesia. These plants are traditionally used for various medicinal purposes. Garcinia species, has been reported as the main sources of unique xanthones which have useful biological activities, such as anti-inflammatory, antibacterial, antifungal, antioxiant, cytotoxic and anti-HIV (Kosela *et al*., 2000). Two plant species, *G. porrecta* Laness and *G. parvifolia* (Miq.) Miq. in the family of Guttiferae, are among those documented plant species rich in xanthones, used medicinally and traditionally to treat fever. The present study report of the isolation and structure elucidation for 1 and 2.

MATERIALS AND METHODS

Location and time: Extraction, isolation and bioactivity testing were conducted in Research Centre for Chemistry-Indonesian Institute of Science, Serpong Banten Indonesia on 2001-2003. NMR analysis of isolated compounds were conducted National University of Singapore on 2003.

General experiment procedures: Melting points were measured on Electro thermal: Digital Melting Point Apparatus and uncorrected. Infra red spectra were recorded in KBr using Perkin-Elmer infrared spectrophotometer. EI and CIMS (direct) and HREIMS spectra were determined on JMS AX 500 mass spectrometer at 70 eV. ^{1}H and ^{13}C-NMR spectra were recorded at 500 and 125 MHz on a JEOL Lambda 400 instruments using $CDCl_3$. Standard JEOL LAMBDA 400 pulse sequence were used for HMQC and HMBC experiment. Chromatographic separations were carried out on silica gel 60 (63-100 μm E. Merck) and various solvents. TLC chromatographic analysis was accomplished on a pre-coated silica gel 60 GF_{254} plates (E. Merck).

Plant material: The stem bark of *G. porrecta* and the root bark of *G. parvifolia* (Guttiferae) were collected in Bogor in 1998. Plants identification were conducted by Dr. Sudarsono Riswan and voucher specimen of *G. porrecta* (#627) and *G. parvifolia* (#652) were deposited in Herbarium Bogoriense, Indonesian Institute of Sciences, Bogor, West Java, Indonesia.

Cytotoxicity evaluation: Cytotoxicity test was done against L1210 murine leukemia cell line and it is divided into three steps: isolation cell line, multiplication and

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bioassay according to method described in Sumatra (1998). The bioassay was performed in the multi-well plate tissue culture (1 mL cell/well). Three various doses of the sample were diluted in methanol and 1 mL methanol as control. The samples and control were added to cells and were incubated during 48 h in the CO_2 incubator at 37°C. The L1210 cells were obtained and derived from Institute of Physical and Chemical Research (RIKEN), Japan. Brine Shrimp Lethality Test (BSLT) was done according to method described in Meyer *et al.* (1982).

Antibacterial evaluation: Antibacterial evaluation was done according to method described in Jewetz *et al.* (1995) against *E. coli*, *S. aureus* and *B. subtilis*, with Ampicilin used as positive control of antibacterial compound.

α-Glucosidase inhibition Evaluation of α-glucosidase inhibition was done according to Prashanth *et al.* (2001). Quercetin was used as positive control of α-glucosidase inhibitor compound.

Extraction and isolation: The air-dried stem bark of *G. porrecta* (1 kg) were percolated in *n*-hexane (2.5 L) for three times, resulted to 25 g of the *n*-hexane extract. The extract then was subjected to a column chromatography on silica gel (500 g) with a mixture of hexane-ethyl acetate of increasing polarity (10, 15, 20, 25, 35, 40, 50, 60, 75 and 100% ethyl acetate) as the solvent system. The fractions with TLC similar patterns were collected and combined into three fractions. First fraction (2.5 g) was subjected to a column chromatography on silica gel (100 g) using *n*-hexane:ethyl acetate (8:2) as the solvent system resulted to the isolation of compound 1 (9 mg). Second fraction (3 g) was further subjected to a column chromatography on silica gel (150 subjected to a column chromatography on silica gel (100 g) using *n*-hexane:ethyl acetate (85:5) as the solvent system resulted to the isolation of compound 5 (300 mg). Second fraction (3 g) was further subjected to a column g) using $CHCl_3$ as the solvent system led to the isolation of compounds 2 (25 mg), 3 (12.3 mg) and 4 (20 mg). Third fraction (10.5 g) was subjected to a column chromatography on silica gel CC (200 g) using *n*-hexane: ethyl acetate (95:5) as the solvent system resulted to the isolation of compound 7 (2 g).

The air-dried root bark of *G. parvifolia* (1.5 kg) were percolated in dichloromethane (3 L) for three times, resulted to 90 g of the dichloromethane extract. About half of the extract (44 g) then was subjected to a column chromatography on silica gel CC (500 g) with a mixture of hexane-ethyl acetate of increasing polarity (10, 15, 20, 25, 35, 40, 50, 60, 75 and 100% ethyl acetate) as the solvent system. The fractions with similar TLC patterns were collected and evaporated to three fractions. First fraction (3 g) was chromatography on silica gel column chromatography (150 g) using *n*-hexane: acetone (8:2) as the solvent system led to the isolation of compounds 6 (250 mg).

RESULTS AND DISCUSSION

The methanol extract of the stem bark of *G. porrecta* and the root bark of *G. parvifolia* showed significant antioxidant activities using DPPH free radical scavenger (IC_{50}<50 μg mL^{-1}) as our preliminary activity evaluation. Garcinia species are known as the sources of xanthones either prenylated or geranylated, some of which are cytotoxic.

Compound 1 yellow powder showed a moderate cytotoxic activity in our assay (IC_{50} value = 16 μg mL^{-1}). It has high-resolution MS, $[M]^+$ = 368.1248 (cald. 368.1254) is for molecular formula $C_{21}H_{20}O_6$. It ^{1}H-NMR spectra showed typical xanthone signal at δ 8.0 (1 H, d, *J* = 8.8 Hz, H-7), 6.93 (1H, d, *J* = 8.8 Hz, H-8) and two methoxy group in the A ring at δ 3.97 (3H, s, 9-OC<u>H</u>$_3$) and 4.01 (3H, s, 10-OC<u>H</u>$_3$). Signals of _δ 6.52 (s) and methoxy signals of δ 3.93 (3H, s, 12-OC<u>H</u>$_3$) were assigned for ring C. For D ring there were two methyl singlets at δ 1.53 (6H, s), two cis-olefinic protons at δ 6.62 (1H, d, *J* = 10 Hz, H-3) and δ 5.56 (1H, d, *J* = 10 Hz, H-4) indicating a dimethyl-chromene ring. The ^{13}C signals δ 174.8 (C = O), in addition to oxygenated olefinic quarternary carbons δ 156.6, 135.8, 158.6, 155.3, 159.4 and 149.4 were assigned for carbons in B ring. These carbon and proton assignments were supported by two-dimensional NMR experiments, such as, ^{1}H-^{1}H COSY, HMQC and HMBC. The HMBC correlations showed for δ 3.97 (OC<u>H</u>$_3$) to δ 156.6 (C-9), δ 4.01 (OC<u>H</u>$_3$) to δ 135.8 (C-10) and δ 3.9 (OC<u>H</u>$_3$) to δ 158.6 (C-12). The evidence of a cross peaks in HMBC (Fig. 1) of δ 8.00 (d, H-7) to δ 174.8 (C = O) and 149.4 (C-11a); δ 6.93 to δ 156.6 (C-9), 135.8 (C-10), 117.4 (C-6a), δ 6.52 (s, H-5) to δ 107.1 (C-4a), 174.8 (C-6) and 135.8 (C-10) and no NOE NMR correlation between OMe and H-4 confirming the structure, as a new non prenylated four-membered ring xanthone, porxanthone A.

Compound 2, a norlanosterol (synonim porlanosterol) isolated as white powder. This compound did not show any cytotoxicity (IC_{50}>100 μg mL^{-1}). It has high-resolution MS, $[M]^+$ 444.3922 (cald. 444.3952) is for molecular formula $C_{30}H_{52}O_2$. Its ^{1}H-NMR spectra showed eight singlet methyl signals at δ 1.53, 1.62, 0.77, 1.19, 0.88, 1.55, 0.71 and 0.91; and ^{13}C-NMR spectra appeared at δ 17.6, 28.0, 16.0, 25.6, 16.2, 26.4, 15.2 and 28.0. From ^{13}C-NMR DEPT experiment, compound 2 has 30 C atoms (8 methyls, 10 methylenes,

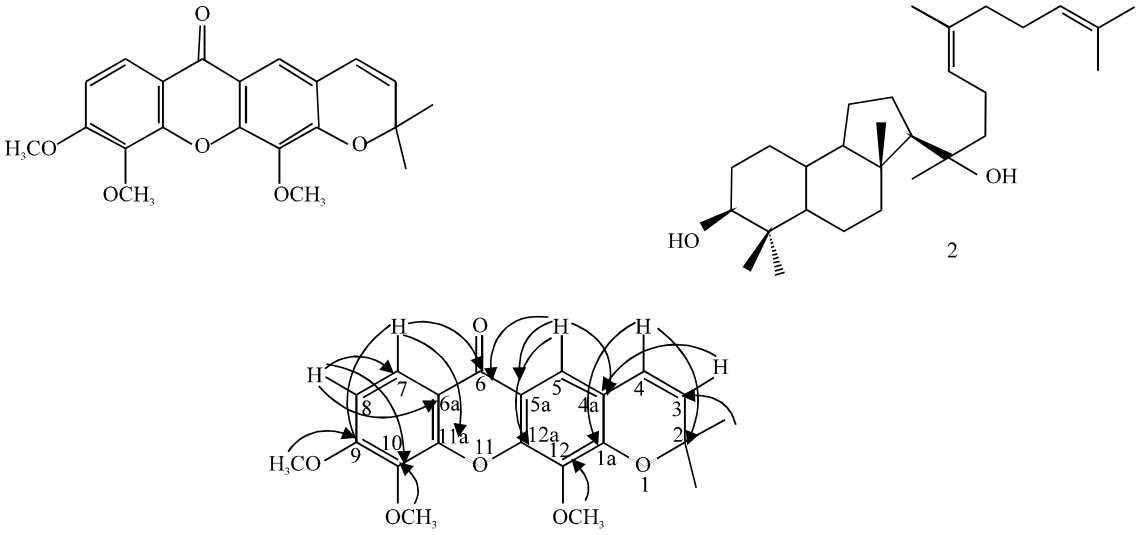

Fig. 1: Summary HMBC correlation observed for compound 1

6 methines and 6 quarternary carbons). It has two double bonds as reflected in the ^{1}H-NMR δ 5,0 (t, *J* = 5Hz, H-22), ^{13}C-NMR δ 124.2 (C-16), 134.2 (C-17), 124.2 (C-21) and 131.2 (C-22). HMQC spectra showed the correlations of δ 2.0 to δ 22.6 (C-2); δ 0.8 to δ 15.9 (C-28); δ 1.6 to δ 28.0 (C-29); δ 1.5 to δ 17.6 (C-30); δ 2.0 to δ 39.6 (C-19). The HMBC correlations were observed for δ 3.12 (H-3) to δ 37.7 (C-4) and 15.2 (C-23); δ 5.04 (H-17) to δ 39.6 (C-19) and 15.9 (C-28); δ 5.00 (H-21) to δ 26.4 (C-20) and 17.6 (C-30); δ 1.62 (H-29) to δ 131.1 (C-22) and 124.2 (C-21); δ 1.53 (H-15) to δ 24.2 (C-16) and 124.5 (C-17). Position of the other methyl angular were also supported by the HMBC experiment by the presence long range coupling between 25-Me at δ 1.55 (s) to C-10 at δ 36.76, 26-Me at δ 0.88 (s) to C-1 at δ 27.1 (C-1), 37.6 (C-7), 58.3 (C-13), 29-Me and 30-Me at δ 1.67 (s) and 0.77 (s) to C-22 at δ 131.2 and 124.2 (C-21). It is likely that this nor-lanosterol was a result of from the biocyclisation of 2,3-oxidosqualene by sterol cyclase, occurred in the plant (Alain *et al*., 1993). Based and this spectroscopic data, it was elucidated as a new norsterol or porlanosterol.

Compound 3-7 were determined as known xanthones, dulxanthone E (3) (Ampofo and Waterman, 1986), F (4) (Kardono, 1998), G (5) (Kosela *et al*., 1999), parvixanthone A (6) (Kosela *et al*., 2000) and rubraxanthone (7) (Xu *et al*., 2001), based on comparison of their physical and spectral data with the published values. The isolation of dulxanthone E (3), dulxanthone F (4), dulxanthone G (5), from *G. porrecta*, was reported for the first time from this species. Parvixanthone A (6) was previously isolated from the leaves of *G. parvifolia*. Meanwhile, rubraxanthone (7) was isolated for the first time from *G. parvifolia*. These known xanthones (3-7) showed *in vitro* cytotoxic activity to L1210 murine leukemia cell line in our assay. Their cytotoxic activity were represented by their IC_{50} values of 4.23, 3.67, 3.51, 6.04 and 7.42 μg mL^{-1}, respectively. It is likely that the prenylated side chain give somewhat enhancement to the cytotoxicity. Xanthones 5 and 6 also showed toxicity to *Artemia salina* in brine shrimp lethality test (BSLT) with LC_{50} 79.34 and 93.54 μg mL^{-1}, respectively, whereas xanthones 2 – 4 did not show toxicity (LC50>1000 μg mL^{-1}). Xanthones 3-5 show antibacterial activity against *Staphylococcus aureus* similar to ampicilin which used as a positive control, however unlike ampicilin these xanthones did not show antibacterial activity against *Escherichia coli* and *Bacillus subtilis*. Xanthone 6 shows α-glucosidase inhibitory activity with IC_{50} 52.66 μg mL^{-1} whereas quercetin that used as positive control has IC_{50} 25.73 μg mL^{-1}. Xanthones 3-6 were not consider α-glucosidase inhibitor because their IC_{50}>100 μg mL^{-1}.

Porxanthone A (1): yellowish white crystals; mp 212-214 °C; UV ($CHCl_3$) λ_{max} (log ϵ) 264 (4.21), 310 (3.75) nm; IR (Kbr) ν_{max} 1652, 1586, 1138, 1062 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.56 (1H, d, J = 10.0 Hz, H-3), 6.62 (1H, d, *J* = 10.0 Hz, H-4), 6.52 (1H- s, H-5), 8.00 (1H, d, *J* = 8.8 Hz, H-7), (1H, d, *J* = 8.8 Hz, H-8), 1.53 (6H, s, 2-Me), 3.97 (3H, s, OMe-9), 4.01 (3H, s, OMe-10), 3.93 (3H, s, OMe-12), 1.53 (3H, s), 3.97 (3H, s, OMe-9), 4.01 (3H, s, OMe-10), 3.93 (3H, s, OMe-12), ^{13}C-NMR (125 MHz, $CDCl_3$), δ: 77.0 (C-2), 127.7 (C-3), 115.88 (C-4), 107.1 (C-4a), 91.7 (C-5), 106.9 (C-5a), 174.8 (C-6), 117.9 (C-6a), 122.2 (C-7), 108.2 (C-8), 156.6 (C-9), 135.8 (C-10), 155.3 (C-1a),

149.4 (C-11a), 159.4 (C-12a), 158.6 (C-12), 28.0 (2-Me), 56.3 (9-OMe), 61.6 (10-OMe), 66.0 (12-OMe). MS m/z = $[M]^+$ 368, 338 ($[M]^+$-CH_3), 310.2 (10%, $[M]^+$-CO). HR-MS, m/z 368.1248 (calcd for $C_{21}H_{20}O_6$, 368.1254).

Porlanosterol (2): white powder; mp 164-166°C; IR (KBr) ν_{max} 3450, 1050 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 1.61 (m, H-1), 2.00 (m, H-2), 3.12 (t, *J*=7.4 Hz, H-3), 0.69 (b, H-5), 1.76 (m, H-6 (m), 1.79 (m, H-7), 1.63 (m, H-9), 1.55 (m, H-11), 0.91 (m, H-12), 0.88 (m, H-13), 1.53 (m, H-15), 1.20 (m, H-16), 5.03 (t, *J* = 5.5 Hz), 1.52 (s, H-19), 1.99 (m, H-28), 1.83 (m, H-19), 5.0 (t, *J* = 5 Hz, H-20), 1,62 (s, H-25), 10.77 (s, H-30), 1.19 (s, H-27), 0.71 (s, H-24), 0.91 (s, H-23), 1.55 (s-H-25), 0.88 (s, H-26). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 27.1 (C-1), 22.6 (C-2), 78.9 (C-3), 38.5 (C-4), 55.5 (C-5), 19.4 (C-6), 37.6 (C-7), 39.1 (C-8), 59.9 (C-9), 36.8 (C-10), 21.2 (C-11), 26.5 (C-12), 58.3 (C-13), 75.9 (C-14), 41.6 (C-15), 24.2 (C-16), 124.2 (C-17), 134.2 (C-18), 15.9 (C-19), 39.6 (C-20), 26.4 (C-21), 124.2 (C-22), 131.2 (C-22), 27.96 (C-29), 17.6 (C-25), 25.5 (C-26), 15.2 (C-27), 27.97 (C-23), 16.2(C-29), 26.4 (C-30). MS m/z = $[M]^+$ 426.30. HR-MS m/z 444.3922 (calcd for $C_{30}H_{52}O_2$, 444.3952).

Dulxanthone E (3): yellow powder; m.p. 192-193°C; UV ($CHCl_3$) λ_{max} nm (log ϵ) 274 (4.21), 320 (3.75); IR (KBr) ν_{max} 1652, 1586, 1138, 1062 cm^{-1}.

Dulxanthone F (4): yellow powder; m.p. 152-153°C; UV ($CHCl_3$) λ_{max} nm (log ϵ) 276 (5.05), 333 (4.77); IR (KBr) ν_{max} 1646, 1580, 1152, 1060 cm^{-1}; MS m/z = $[M]^+$ 384.3

Dulxanthone G (5): yellow powder; m.p. 183-184°C; UV ($CHCl_3$) λ_{max} nm (log ϵ) 276 (5.65), 342 (5.39); IR (KBr) ν_{max} 1647, 1569, 1159 cm^{-1} ; MS m/z = $[M]^+$ 414.12.

Parvixanthone A (6): yellow powder; m.p. 94-96°C; UV (MeOH) λ_{max} nm (log ϵ) 242 (4.40), 258 (4.37), 354 (3.86); IR (KBr) ν_{max} 3400, 1720, 1650, 1580, 1050 cm^{-1}. MS m/z = $[M]^+$ 494.10.

Rubraxanthone (7): yellow powder; m.p. 201-203°C. UV (MeOH) λ_{max} (log ϵ) 242 (4.60), 312 (4.41), 355 (4.06); IR (Kbr) ν_{max} 3450, 1720, 1655, 1580, 1050 cm^{-1}; MS m/z = $[M]^+$ 410.10.

From *G. porrecta,* a new xanthone, porxanthone A (1) and one known sterol derivative (2) and three known xanthones (3-5) were isolated. From *G. parvifolia*, whereas two known xanthones (6-7), were isolated from. All known xanthones (3-7) showed inhibitory activity to L1210 cells, with IC_{50} values in the range of 3 to 8 μg mL^{-1}.

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