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Xanthones with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (Mangosteen)

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Abstract

Bioactivity-guided fractionation of a dichloromethane-soluble extract of *Garcinia mangostana* fruits has led to the isolation and identification of five compounds, including two xanthones, 1,2-dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2-enyl)furo[3,2-*a*]xanthen-11-one (**1**) and 6-deoxy-7-demethylmangostanin (**2**), along with three known compounds, 1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)xanthone (**3**), mangostanin (**4**), and α-mangostin (**5**). The structures of compounds **1** and **2** were determined from analysis of their spectroscopic data. All isolated compounds in the present study together with eleven other compounds previously isolated from the pericarp of mangosteen, were tested in an *in vitro* quinone reductase-induction assay using murine hepatoma cells (Hepa 1c1c7) and an *in vitro* hydroxyl radical antioxidant assay. Of these, compounds **1**–**4** induced quinone reductase (concentration to double enzyme induction, 0.68–2.2 μg/mL) in Hepa 1c1c7 cells and γ-mangostin (**6**) exhibited hydroxyl radical-scavenging activity (IC_{50}, 0.20 μg/mL).

Keywords: *Garcinia mangostana*; Clusiaceae; 1,2-Dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2-enyl)furo[3,2-*a*]xanthen-11-one; 2,3-Dihydro-4,7-dihydroxy-2-(2-hydroxypropan-2-yl)-6-(3-methylbut-2-enyl)furo[3,2-*b*]xanthen-5-one; (6-deoxy-7-demethylmangostanin); Quinone reductase induction; Hydroxyl radical-scavenging activity

1. Introduction

Cancer chemoprevention refers to intervention such as the prevention, delay or reversal of the process of carcinogenesis by ingestion of food, dietary supplements, or synthetic agents (Sporn et al., 1976). Of the various processes of carcinogenesis, blocking of tumor initiation by carcinogens is considered an important step in protecting cells through the induction of Phase II drug-metabolizing enzymes such as glutathione-*S*-transferase and quinone reductase (Talalay et al., 1995). As a part of our search for new cancer chemopreventive agents from botanical dietary supplements, the fruits of *Garcinia mangostana* L. (Clusiaceae) were chosen for further investigation since a dichloromethane-soluble extract of these fruits was found to exhibit inducing activity of quinone reductase (QR) in cultured murine hepatoma (Hepa 1c1c7) cells.

G. mangostana L. is commonly known as mangosteen, and is referred to as "the queen of fruits" in Thailand. The fruit hull of *G. mangostana* has been used as a traditional medicine in Southeast Asia for the treatment of diarrhea, inflammation, and ulcers (Farnsworth and Bunyapraphatsara, 1992; Peres et al., 2000; Suksamrarn et al., 2006). In the United States, mangosteen products are now widely available and are highly popular because of their perceived role in enhancing human health (Garrity, 2004). Mangosteen fruit juice has become a major botanical dietary supplement, and was ranked as one of the

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top-selling "botanicals" on the market in 2005 (Nutrition Business Journal, 2006). Xanthone derivatives, as the major secondary metabolites of *G. mangostana* fruits (Sen et al., 1982; Mahabusarakam et al., 1987; Ji et al., 2007), exhibit antibacterial (Suksamarn et al., 2003; Sakagami et al., 2005), antifungal (Gopalakrishnan et al., 1997), anti-inflammatory (Nakatani et al., 2004), antioxidant (Jung et al., 2006; Yu et al., 2007), antiplasmodial (Mahabusarakam et al., 2006), and cytotoxic activities (Matsumoto et al., 2005; Suksamrarn et al., 2006). The most abundant xanthone from *G. mangostana*, α-mangostin, was found to inhibit alveolar duct formation in a mouse mammary organ culture model and to suppress the carcinogen-induced formation of aberrant crypt foci in a short-term colon carcinogenesis model (Nabandith et al., 2004; Jung et al., 2006). As a result of these studies, the possible cancer chemopreventive activity of *G. mangostana* has been strongly suggested. However, there has been no report thus far on the ability of the *G. mangostana* xanthones to induce phase II drug-metabolizing enzymes and hence potentially detoxify carcinogens.

In the present study, bioactivity-guided investigation of a dichloromethane-soluble extract of *G. mangostana* fruits, using the QR-inducing activity in Hepa 1c1c7 cells as an *in vitro* screening assay, has led to the isolation and identification of five compounds, including two new xanthones (**1** and **2**) and three known compounds (**3**–**5**). Compounds **1**–**5** along with eleven known xanthones that were previously isolated from the pericarp of *G. mangostana*, were evaluated individually in this QR induction assay. Additionally, these same compounds were tested in an *in vitro* hydroxyl radical antioxidant assay system.

2. Results and discussion

The structures of the known compounds **3**–**5** were identified by physical and spectroscopic data measurement ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, DEPT, 2D NMR, and MS) and by comparing the data obtained with published values, as 1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)xanthone (**3**) (Mahabusarakam et al., 1987), mangostanin (**4**) (Nilar and Harrison, 2002), and α-mangostin (**5**) (Sen et al., 1982).

Compound **1** was obtained as yellow powder, and the elemental composition of **1** was inferred from a sodiated ion peak at *m/z* 435.1425 (calcd for $C_{23}H_{24}O_7Na$, 435.1420) in the HRESI-TOF-MS. The ^{1}H NMR spectrum of **1** exhibited *ortho*-coupled resonances at δ_H 7.36 (1H, *d*, $J = 8.9$ Hz, H-6) and 7.50 (1H, *d*, $J = 8.9$ Hz, H-5), a singlet signal at δ_H 6.45 (1H, *s*, H-4), and two aromatic hydroxyl peaks at δ_H 13.08 (OH-1) and 11.10 (OH-3), assignable to a xanthone moiety (Hano et al., 1990). A 3-methylbut-2-enyl group was also observed from signals at δ_H 1.72 (3H, *s*, H-5′), 1.62 (3H, *s*, H-4′), 3.23 (1H, *d*, $J = 6.0$ Hz, H-1′), and 5.18 (1H, *brt*, $J = 6.0$ Hz, H-2′), as well as a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran-3-ol moiety from resonances at δ_H 5.80 (1H, *brt*, $J = 3.7$ Hz, H-1″), 5.14 (1H, *d*, $J = 4.0$ Hz, OH-1″), 4.70 (1H, *s*, OH-3″), 4.32 (1H, *d*, $J = 3.6$ Hz, H-2″), 1.18 (3H, *s*, H-4″), and 1.09 (3H, *s*, H-5″). The HMBC correlations of the signal at δ_H 3.23 (H-1′) to δ_C 159.5 (C-1), 109.9 (C-2), and 163.6 (C-3), as well as those at δ_H 6.45 (H-4) to δ_C 163.6 (C-3), 102.4 (C-1a), and 155.2 (C-4a), were suggestive of the connectivity of a 3-methylbut-2-enyl side chain at C-2. A 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran-3-ol group was positioned between C-7 and C-8 from the observed two or three-bond correlations between signals at δ_H 7.36 (H-6) to δ_C 156.6 (C-7), 126.4 (C-8), and 150.2 (C-10a), δ_H 7.50 (H-5) to 156.6 (C-7), 117.2 (C-9a), and 150.2 (C-10a), and δ_H 4.32 (H-2″) to δ_C 156.6 (C-7), and 71.6 (C-1″). Thus, the structure of compound **1** was elucidated as 1,2-dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2-enyl)furo[3,2-*a*]xanthen-11-one, with the configurations of C-1″ and C-2″ unresolved.

The molecular formula of compound **2** was assigned as $C_{23}H_{24}O_6$, from the observed sodiated ion at *m/z* 419.1475 (calcd for $C_{23}H_{24}O_6Na$, 419.1471) in the HRESI-TOF-MS. The ^{1}H NMR spectroscopic data of **2** showed the presence of a penta-substituted xanthone moiety [δ_H 7.30 (2H, *s*, H-5 and H-6), and 6.40 (1H, *s*, H-4)], a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring [δ_H 4.75 (1H, *t-like*, $J = 8.5$ Hz, H-2′), 3.06 (2H, *d-like*, $J = 8.5$ Hz, H-1′), 1.32 (3H, *s*, H-4′) and 1.15 (3H, *s*, H-5′)], and a 3-methylbut-2-enyl group [δ_H 5.18 (1H, *brt*, $J = 6.0$ Hz, H-2″, 4.02 (2H, *d*, $J = 6.0$ Hz, H-1″), 1.76 (3H, *s*, H-5″), and 1.60 (3H, *s*, H-4″)], which were similar to those of mangostanin (**4**) except for the absence of a hydroxyl group at C-6 and a methoxy group at C-7 in compound **2**. Furthermore, the HMBC correlations summarized in Fig. 1 were used to confirm the structure of **2**. Thus, the long-range connections of δ_H 4.02 (H-1″) to δ_C 127.0 (C-8), 123.4 (C-2″), 151.5 (C-7), 117.9 (C-9a), and 130.3 (C-3″), and δ_H 7.30 to δ_C 127.0 (C-8) and 117.9 (C-9a) suggested that a 3-methylbut-2-enyl group is located at C-8. The presence of a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran unit was proposed by the proton to carbon connectivities of δ_H 3.06 (H-1′) to δ_C 157.0 (C-1), 107.7 (C-2), and 167.2 (C-3), δ_H 4.75 (H-2′) to δ_C 167.2 (C-3), and δ_H 4.72 (OH-3′) to δ_C 91.7 (C-2′) and 70.0 (C-3′), as well as δ_H 6.40 (H-4) to 167.2 (C-3), 107.7 (C-2), 156.8 (C-4a), and 103.4 (C-1a). Therefore, the structure of **2** was determined as 2,3-dihydro-4,7-dihydroxy-2-(2-hydroxypropan-2-yl)-6-(3-

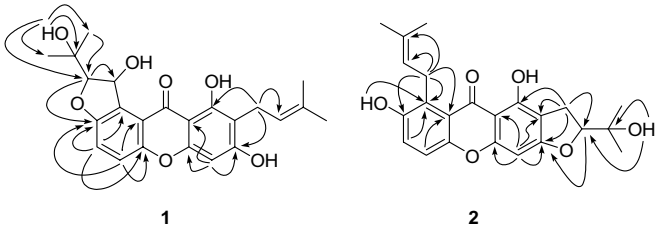


Fig. 1. Selected HMBC correlations of 1 and 2.

methylbut-2-enyl)furo[3,2-*b*]xanthen-5-one (6-deoxy-7-demethylmangostanin).

All isolated compounds obtained in the present study (**1**–**5**), together with cudraxanthone G, 8-deoxygartanin, garcinone D, garcinone E, gartanin, 8-hydroxycudraxanthone G, 1-isomangostin, γ-mangostin (**6**), mangostinone, tovophyllin A, and smeathxanthone A, previously isolated from the pericarp of *G. mangostana* (Jung et al., 2006), were tested in an *in vitro* screening assay using murine hepatoma cells (Hepa 1c1c7) for the induction of quinone reductase (QR). Of all tested compounds, only compounds **1**–**4** were found to induce QR activity as shown in Table 1. The CD (concentration required to double QR induction activity) values of compounds **1**–**4** (1.3, 2.2, 0.68, and 0.95 μg/mL, respectively) were comparable to that of isoliquiritigenin (1.1 μg/mL), used as a positive control. Moreover, compound **1** exhibited a greater chemoprevention index ($CI = IC_{50}/CD$) than isoliquiritigenin, which has been previously tested for cancer chemopreventive effects in *in vivo* models (Baba et al., 2002; Chin et al., 2007). It seems that compound **1** may be a good candidate for further evaluation as a cancer chemopreventive agent.

Additionally, the antioxidant capacity of these xanthones was evaluated in a hydroxyl radical-scavenging assay. Only γ-mangostin (**6**) from the library of 16 xanthones available was found to be active (IC_{50}, 0.20 μg/mL) whereas all other compounds were inactive ($IC_{50} > 10$ μg/mL), including the QR-inducing agents **1**–**4**. The antioxidant potency of **6** in the hydroxyl radical- scavenging assay used is comparable to those of the positive controls used, gallic acid (IC_{50}, 1.0 μg/mL), quercetin (IC_{50}, 0.38 μg/mL), and vitamin C (IC_{50}, 0.40 μg/mL). Similar data were obtained in a recently published study on this same xanthone (Yu et al., 2007).

Table 1
Quinone reductase (QR)-inducing activity of compounds **1**–**4**

Compound	CD[a]	IC_{50}[b]	CI[c]
	μg/mL (μM)	μg/mL (μM)	
1	1.3 (3.0)	>20 (48.5)	>16
2	2.2 (5.6)	10.3 (26.0)	4.7
3	0.68 (1.8)	6.7 (17.7)	9.9
4	0.95 (2.2)	7.8 (17.6)	8.2
Isoliquiritigenin[d]	1.1 (4.2)	9.1 (35.5)	8.5

[a] Concentration required to double QR induction activity; test compounds were considered as inactive with CD > 5 μg/mL.
[b] Concentration required to inhibit cell growth by 50%.
[c] Chemoprevention index (CI) = IC_{50}/CD.
[d] Positive control.

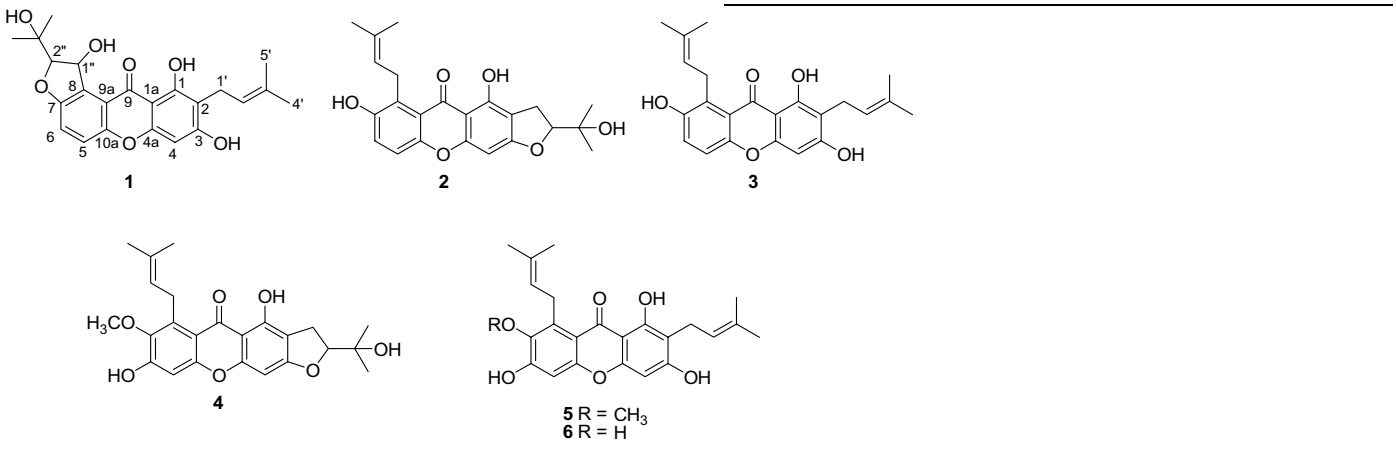
3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a Perkin–Elmer 241 automatic polarimeter. UV spectra were obtained with a Perkin–Elmer UV/Vis lambda 10 spectrophotometer. Circular dichroism (CD) spectra were recorded on JASCO J-810 spectropolarimeter. IR spectra were run on a Nicolet Protégé 460 FT-IR spectrometer. NMR spectroscopic data were recorded at room temperature on Bruker Avance DPX-300 and DRX-400 spectrometers with tetramethylsilane (TMS) as internal standard. Electrospray ionization (ESI) mass spectrometric analyses were performed with a 3-T Finnigan FTMS-2000 Fourier Transform mass spectrometer. A SunFire™ PrepC_{18}OBD™ column (5 μm, 150 × 19 mm i.d., Waters, Milford, MA) and a SunFire™ PrepC_{18} guard column (5 μm, 10 × 19 mm i.d., Waters) were used for preparative HPLC, along with two Waters 515 HPLC pumps and a Waters 2487 dual λ absorbance detector (Waters). Column chromatography was carried out with Purasil® (230–400 mesh, Whatman, Clifton, NJ) and Sephadex LH-20 (Sigma, St. Louis, MO). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm-thickness Partisil® K6F (Whatman) glass plates, while preparative thin-layer chromatography was conducted on precoated 20 × 20 cm, 500 μm-thickness Partisil® K6F (Whatman) glass plates.

3.2. Plant material

A freeze-dried powder of the fruits of *G. mangostana* (Clusiaceae), collected in China and/or southeast Asia,



used for the present study, was provided by Nature's Sunshine Products, Inc. (lot number: 0114129). A representative sample (OSUADK-CCP0007) has been deposited as a powder in the Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University.

3.3. Extraction and isolation

The fruit powder of *G. mangostana* (600 g) was extracted by maceration with MeOH three times (1500 mL each) at room temperature, for up to 1 day each, with the extractions pooled and then evaporated *in vacuo*. The dried MeOH extract (110 g) was suspended with H_2O (1000 mL) and partitioned sequentially with hexanes (3 × 1000 mL), CH_2Cl_2 (3 × 1000 mL), and EtOAc (3 × 1000 mL).

The CH_2Cl_2-soluble partition (7.0 g) exhibited induction of quinone reductase (CD, 5.4 μg/mL). The CH_2Cl_2-soluble fraction (7.0 g) was subjected to HP-20 gel cc (5.5 × 35 cm, 200 g) and was fractionated into five sub-fractions (F01: H_2O–MeOH = 1:5, 1200 mL, F02: MeOH, 1200 mL, F03 and F04: MeOH-acetone = 4:1, 2400 mL, F05: acetone, 1200 mL). QR-inducing activity of these five sub-fractions was monitored using the Hepa 1c1c7 cell line, and sub-fraction F02 (CD, 3.1 μg/mL) was deemed to be active. Fraction F02 (600 mg) was subjected to Sephadex LH-20 cc (3.5 × 80 cm, 100 g) with MeOH used as solvent and afforded nine sub-fractions (F0201-F0209). The constituents of sub-fraction F0205 (162 mg) were applied to a silica gel column (2.5 × 50 cm, 50 g) with a gradient of increasing polarity (hexanes-EtOAc = 10:1–3:1). Of the sub-fractions obtained (F020501-F020503), F020503 was purified by HPLC. This separation was conducted with MeCN–H_2O (68:32, 7.0 mL/min), by isocratic elution, to afford, in order, compounds **1** (t_R 11.0 min, 3.5 mg), **2** (t_R 15.0 min, 1.2 mg), **4** (t_R 17.5 min, 2.2 mg), **3** (t_R 35.0 min, 7.8 mg), and α-mangostin (**5**, t_R 44.5 min, 14.0 mg).

3.3.1. 1,2-Dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2-enyl)furo[3,2-a]xanthen-11-one (*1*)

Yellow powder, $([\alpha]_D^{23}$ + 11.4 (acetone; *c* 0.05); UV (MeOH) λ_{max} (log ε): 238 (4.59) 268 (4.56), 319 (4.29) nm; IR ν_{max} (film): 3417, 2986, 1651, 1465, 1069 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 13.08 (1H, *s*, OH-1), 11.10 (1H, *s*, OH-3), 7.50 (1H, *d*, *J* = 8.9 Hz, H-5), 7.36 (1H, *d*, *J* = 8.9 Hz, H-6), 6.45 (1H, *s*, H-4), 5.80 (1H, *brt*, *J* = 3.7 Hz, H-1″), 5.18 (1H, *brt*, *J* = 6.0 Hz, H-2′), 5.14 (1H, *d*, *J* = 4.0 Hz, OH-1″), 4.70 (1H, *s*, OH-3″), 4.32 (1H, *d*, *J* = 3.6 Hz, H-2″), 3.23 (2H, *d*, *J* = 6.0 Hz, H-1′), 1.72 (3H, *s*, H-5′), 1.62 (3H, *s*, H-4′), 1.18 (3H, *s*, H-4″), 1.09 (3H, *s*, H-5″). ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.3 (*s*, C-9), 163.6 (*s*, C-3), 159.5 (*s*, C-1), 156.6 (*s*, C-7), 155.2 (*s*, C-4a), 150.2 (*s*, C-10a), 130.7 (*s*, C-3′), 126.4 (*s*, C-8), 122.2 (*d*, C-2′), 119.4 (*d*, C-5), 117.8 (*d*, C-6), 117.2 (*s*, C-9a), 109.9 (*s*, C-2), 102.4 (*s*, C-1a), 97.2 (*d*, C-2″), 93.0 (*d*, C-4), 71.6 (*d*, C-1″), 69.8 (*s*, C-3″), 25.9 (*q*, C-5″), 25.5 (*q*, C-4′), 25.0 (*q*, C-4″), 20.9 (*t*, C-1′), 17.7 (*q*, C-5′). HRESI-TOF-MS *m/z*: 435.1425 $[M+Na]^+$ (calcd for $C_{23}H_{24}O_7Na$, 435.1420).

3.3.2. 2,3-Dihydro-4,7-dihydroxy-2-(2-hydroxypropan-2-yl)-6-(3-methylbut-2-enyl)furo[3,2-b]xanthen-5-one (6-deoxy-7-demethyl-mangostanin, *2*)

Yellow powder, $([\alpha]_D^{23}$ − 40.2 (acetone; *c* 0.05); UV (MeOH) λ_{max} (log ε): 241 (4.70) 263 (4.67), 316 (4.45) nm; IR ν_{max} (film): 3384, 2976, 1666, 1614, 1484, 1165 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 13.5 (1H, *s*, OH-1), 9.76 (1H, *s*, OH-7), 7.30 (2H, *s*, H-5 and H-6), 6.40 (1H, *s*, H-4), 5.18 (1H, *brt*, *J* = 6.0 Hz, H-2″), 4.75 (1H, *t-like*, *J* = 8.5 Hz, H-2′), 4.72 (1H, *s*, OH-3), 4.02 (2H, *d*, *J* = 6.0 Hz, H-1″), 3.06 (2H, *d-like*, *J* = 8.5 Hz, H-1′), 1.76 (3H, *s*, H-5″), 1.60 (3H, *s*, H-4″), 1.32 (3H, *s*, H-4′), 1.15 (3H, *s*, H-5′). ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.8 (*s*, C-9), 167.2 (*s*, C-3), 157.0 (*s*, C-1), 156.8 (*s*, C-4a), 151.5 (*s*, C-7), 150.3 (*s*, C-10a), 130.3 (*s*, C-3″), 127.0 (*s*, C-8), 123.4 (*d*, C-2″), 123.1 (*d*, C-6), 117.9 (*s*, C-9a), 115.9 (*d*, C-5), 107.7 (*s*, C-2), 103.4 (*s*, C-1a), 91.7 (*d*, C-2′), 87.9 (*d*, C-4), 70.0 (*s*, C-3′), 25.9 (*t*, C-1″), 25.8 (*q*, C-5′), 25.6 (*q*, C-4″), 25.1 (*t*, C-1′), 24.9 (*q*, C-4′), 18.0 (*q*, C-5″). HRESI-TOF-MS *m/z*: 419.1475 $[M+Na]^+$ (calcd for $C_{23}H_{24}O_6Na$, 419.1471).

3.4. Quinone reductase (QR) induction assay

The potential QR induction activity of plant extracts, partitions, and pure compounds was assayed as described previously (Prochaska and Santamaria, 1988; Su et al., 2003). Briefly, the cells (Hepa1c1c 7; murine hepatoma; ATCC CRL-2026) were seeded onto 96-well plates at a density of 1.5×10^4 cells/mL and incubated for 24 h. The cells were then treated with test samples including isoliquritigenin as the positive control (Jang et al., 2003) and 10% DMSO as the negative control and were incubated for 48 h. Two plates are used for each test sample in order to determine both quinone reductase activity, by measuring the NADPH-dependent menadiol-mediated reduction of 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and cytotoxicity, using crystal violet staining of protein content. Both the cytotoxicity (IC_{50}) and QR activity were measured at 595 nm with an ELISA plate reader. Enzyme induction activity was expressed as a CD, the concentration required to double the specific activity of QR. IC_{50} (half-maximal inhibitory concentration of cell viability) and CI (chemoprevention index, IC_{50}/CD) values were also determined.

3.5. Hydroxyl radical-scavenging assay

Hydroxyl radical (·OH) scavenging activity was conducted according to a previous method (Halliwell and Gutteridge, 1990; Zou et al., 2002) with a minor modification using 96-well plates. The final assay volume (250 μl) contained 160 μl of freshly prepared mixed solution (1.2 mM H_2O_2 and 0.2 mM $FeSO_4$ in 50 mM phosphate buffer at

pH 7.4) with 10 μl test sample in 25% DMSO solution, which was incubated at 37 °C for 5 min. Then, 80 μl of esterase (1.0 units/mL)-treated 2′,7′-dichlorodihydrofluorescin diacetate (2 μM H_2DCF-DA) in 50 mM phosphate buffer (pH 7.4) were added and mixed well. Changes in ·OH were measured using a FL×800 fluorescence spectrophotometer (Bio-Tek) at an excitation wavelength of 485 nm and emission wavelength of 528 nm after 30 min.

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