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Xanthone derivatives from Cratoxylum cochinchinense roots

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Abstract

Two xanthones and two caged-prenylated xanthones, named cochinchinones A–D, respectively, and a synthetically known caged-prenylated xanthone, together with seven known compounds were isolated from the roots of *Cratoxylum cochinchinense* (Lour.) Blume. Their structures were assigned on the basis of analyses of spectroscopic data. Some of the compounds exhibited effective antioxidative properties.

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1. Introduction

Cratoxylum cochinchinense (Lour.) Blume is called "tuegliang" locally in Thailand. In traditional medicine, it has been used to treat fevers, coughs, diarrhoea, itches, ulcers and abdominal complaints (Vo. 1997). The bark of this plant was previously shown to contain triterpenoids, tocotrienols and xanthones (Bennett et al., 1993; Sia et al., 1995; Nguyen and Harrison, 1998). The use of this plant as a traditional medicine, and the results from a preliminary screening of the biological activity of crude extracts from its roots, led us to examine them further for substances that act as radical scavengers. Two new xanthones (1-2), two new caged-prenylated xanthones (3-4), a synthetically known caged-prenylated xanthone (5) and seven known compounds (6-12) were isolated. Their structures were elucidated from analyses of 1D and 2D NMR spectroscopic data, including ¹H, ¹³C NMR, NOE, COSY, HMOC and HMBC. Radical scavenging activity of the compounds was investigated.

2. Results and discussion

Separation of a dichloromethane extract of the roots of *C. cochinchinense* produced cochinchinones A–D (1–4), the caged-prenylated xanthone (5) (Thoison et al., 2000), β-mangostin (6) (Mahabusarakam et al., 1987), 1,3,7-trihydroxy-2,4-bis (3-methyl-2-butenyl)xanthone (7) (Iinuma et al., 1996), mangostin (8) (Mahabusarakam et al., 1987), macluraxanthone (9) (Iinuma et al., 1994), garcinone B (10) (Sen et al., 1982), celebixanthone (11) (Stout et al., 1962), and garcinone D (12) (Bennett et al., 1993).

Cochinchinone A, 1,3,7-trihydroxy-2-(3-methyl-2-butenyl)-4-(3,7-dimethyl-2,6-octadienyl)xanthone (1), was a yellow solid, m.p. 119–120 °C. Its molecular ion of m/z 448.2299 was in agreement with the molecular formula $C_{28}H_{32}O_5$. The IR spectrum showed the presence of O–H (3413 cm⁻¹) and C=O (1641 cm⁻¹) groups. The ¹H NMR spectrum (Table 1) exhibited signals of a hydrogen-bonded hydroxy proton at δ 12.95 (s, 1-OH) and three aromatic protons which coupled as an ABX system at δ 7.59 (d, J = 3.0 Hz, H-8), 7.36 (d, J = 9.0 Hz, H-5) and 7.24 (dd, J = 9.0, 3.0 Hz, H-6). The lower field resonance at δ 7.59 was assigned for H-8 according to the anisotropic effect from C=O. Since H-8 showed only m-coupling in the ¹H NMR spectrum, a hydroxyl group was placed at C-7.

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The characteristic signals of protons in a prenyl unit were displayed at $\delta 3.47$ (H-1', d), 5.29 (H-2', br t), 1.84 (H-4', s) and 1.76 (H-5', s). In addition, the presence of a geranyl side chain was indicated from the resonances at $\delta 3.57$ (H-1", d), 5.27 (H-2", br t), 2.04 (H-4", m), 2.09 (H-5", m),

5.05 (H-6", br t), 1.57 (H-8", s), 1.88 (H-9", s) and 1.64 (H-10", s). The correlation of H-1' to C-1, C-2 and C-3 in the HMBC spectrum (Table 1) established that the location of the prenyl unit was at C-2, whereas the correlation of H-1" to C-3, C-4 and C-4a indicated that the geranyl

Table 1
The ¹H NMR and HMBC spectroscopic data of cochinchinones A–B (1–2

H-position	Cochinchinone A (1)		Cochinchinone B (2)		
	¹ H NMR	HMBC	¹ H NMR	HMBC	
4	_	_	6.40 (s, 1H)	C-2, C-3, C-4a, C-9, C-9a	
5	7.36 (d , 1H, $J = 9.0$ Hz)	C-7, C-8a, C-4b	_	_	
6	7.24 (<i>dd</i> , 1H, $J = 9.0, 3.0 \text{ Hz}$)	C-7, C-4b	_	_	
8	7.59 (d, 1H, $J = 3.0 \text{ Hz}$)	C-6, C-7, C-9, C-4b	7.48 (s, 1H)	C-6, C-7, C-9, C-4b	
1'	3.47 (d, 2H, J = 7.0 Hz)	C-1, C-2, C-3, C-2', C-3'	3.35 (d, 2H, J = 7.0 Hz)	C-1, C-2, C-3, C-2', C-3'	
2'	5.29 (br t, 1H, $J = 7.0 \text{ Hz}$)	C-4', C-5'	5.26 (br t, 1H, J = 7.0 Hz)	C-1', C-4', C-5'	
4′	1.84 (s, 3H)	C-2', C-3', C-5'	1.78 (s, 3H)	C-2', C-3', C-5'	
5'	1.76 (s, 3H)	C-2', C-3', C-4'	1.66 (s, 3H)	C-2', C-3', C-4'	
1"	3.57 (d, 2H, J = 7.0 Hz)	C-3, C-4, C-4a, C-2', C-3'	3.56 (d, 2H, J = 7.0 Hz)	C-5, C-6, C-4b, C-2", C-3"	
2"	5.27 ($br\ t$, 1H, $J = 7.0$ Hz)	C-4, C-4"	5.26 (br t, 1H, J = 7.0 Hz)	C-1", C-4"	
4"	2.04 (m, 2H)	C-2", C-5", C-6"	1.92 (m, 2H)	C-2", C-3", C-6"	
5"	2.09 (m, 2H)	C-3", C-4", C-6"	2.01 (m, 2H)	C-4", C-6", C-7"	
6"	$5.05 (br \ t, 1H, J = 7.0 Hz)$	C-5", C-10"	5.00 (br t, 1H, J = 7.0 Hz)	C-8", C-10"	
8"	1.57 (s, 3H)	C-6", C-7", C-10"	1.50 (s, 3H)	C-6", C-7", C-10"	
9"	1.88 (s, 3H)	C-2", C-3", C-4"	1.84 (s, 3H)	C-2", C-3", C-4"	
10"	1.64 (s, 3H)	C-6", C-7", C-8"	1.56 (s, 3H)	C-6", C-7", C-8"	
1-OH	12.95 (s, 1H)	C-1, C-2, C-9a	13.33 (s, 1H)	C-1, C-2, C-9a	

side chain was at C-4. Thus, the structure of cochinchinone A was deduced as 1.

Cochinchinone B, 1,3,6,7-tetrahydroxy-2-(3-methyl-2butenyl)-5-(3,7-dimethyl-2,6-octadienyl)xanthone (2), was a yellow solid, m.p. 221-222 °C. The molecular formula was determined as C₂₈H₃₂O₆ by HR-MS. The IR spectrum again showed the presence of O-H (3350 cm⁻¹) and C=O (1640 cm⁻¹) groups. The ¹H NMR spectrum (Table 1) indicated the presence of a hydrogen-bonded hydroxy group at δ 13.33 (s, 1-OH) and two isolated aromatic protons at $\delta 6.40$ (s, H-4) and 7.48 (s, H-8). The resonances at $\delta 3.35$ (d, H-1'), 5.26 (br t, H-2'), 1.78 (s, H-4') and 1.66 (s, H-5') revealed the presence of a prenyl unit. The characteristic signals of a geranyl group were observed at $\delta 3.56$ (d, H-1"), 5.26 (br t, H-2"), 1.92 (m, H-4"), 2.01 (m, H-5"), 5.00 (br t, H-6''), 1.50 (s, H-8''), 1.84 (s, H-9'') and 1.56 (s, H-10''). The prenyl group was assigned at C-2 according to the HMBC correlation (Table 1) of H-1' to C-1, C-2 and C-3 whereas the geranyl group was placed at C-5 due to the correlation of H-1" to C-5, C-6 and C-4b. The structure 2 was therefore indicated for cochinchinone B.

Cochinchinone C (3) was a yellow solid, m.p. 147–148 °C and $[\alpha]_D^{29} + 50^\circ$ (c 0.089, CHCl₃). A pseudomolecular ion $[M-CO]^+$ at m/z 382.1745 was consistent for the molecular formula of $C_{24}H_{26}O_6$ with the loss of 28 amu (CO). The IR spectrum also showed absorption bands of O–H stretching at 3467 cm⁻¹, conjugated C=O stretching at 1642 cm⁻¹ and unconjugated C=O stretching at 1749 cm⁻¹. The ¹³C NMR spectroscopic data (Table 3) exhibited carbon resonances at δ 180.73 (C-9) and 201.16 (C-6), confirming the presence of conjugated- and unconjugated carbonyl groups. The ¹H NMR spectrum (Table 2) revealed resonances of a hydrogen-bonded hydroxy proton at δ 12.00 (s, 1-OH) and three aromatic protons which coupled as an ABM system at δ 6.55 (dd, J = 8.4, 0.9 Hz, H-2), 7.41 (t, J = 8.4 Hz, H-3), and 6.52 (dd, J = 8.4, 0.9 Hz, H-4). The spectrum further displayed the presence of an olefinic

proton (δ 7.51, s, H-8), a methoxy group (δ 3.65, s, 7-OCH₃), a pair of non-equivalent methylene protons (δ 1.59, dd, H_b-10 and 2.39, d, H_a-10), a methine proton (δ 2.54, d, H-11) and a prenyl unit [δ 4.39 (br t, H-16), 2.64 (d, H-15), 1.37 (s, H-18) and 1.01 (s, H-19)]. The prenyl unit was placed at C-5 according to the correlation of H-15 to C-4b and C-5. The HMBC correlations (Table 2) of H_a-10 to C-4b, C-8, C-11; H_b-10 to C-7, C-8, C-11 and H-11 to C-4b, C-5, C-10 suggested that CH₂-10 and CH-11 were in between C-7 and C-4b. The signals of the methine proton H-11 and the gem-dimethyl groups (H-13, δ 1.69, s and H-14, δ 1.33, s) revealed a 2,2-dimethyltetrahydrofuran ring. It was suggested as being fused at C-5, C-4b and C-11 from the correlations of H-13 to C-11 and of H-11 to C-4b and C-5.

Cochinchinone D (4) was shown to be a methoxy derivative of the synthetically known compound (5) (Thoison et al., 2000). It was a yellow solid, m.p. 218-219 °C. and $\left[\alpha\right]_{\rm D}^{29}-58^{\circ}$ (c 6.90 × 10⁻² in CHCl₃). The molecular ion $\left[{\rm M-CO}\right]^{+}$ at m/z 398.1713 was in agreement to the molecular formula C₂₄H₂₆O₇ with the loss of 28 amu (CO). The IR spectrum showed absorption bands of O-H stretching at 3467 cm⁻¹ and of C=O stretching at 1646 (conjugated C=O) and 1738 (unconjugated C=O) cm $^{-1}$. In the 13 C NMR spectrum (Table 3), the resonances of the carbonyl carbons were at $\delta 178.00$ (C-9) and 201.00 (C-6). The 1 H NMR spectrum (Table 2), showed a singlet for 1-OH at δ 12.39 and a broad singlet for 3-OH at δ 8.05, whereas the aromatic part was tetrasubstituted as evidence from signals at $\delta 6.05$ (d, H-4) and 6.03 (d, H-2) with meta coupling (J = 2.1 Hz). The data from the ¹H NMR and HMBC spectra (Table 2) and ¹³C NMR spectra (Table 3) indicated that the non-aromatic part contained a methoxy group $(\delta 3.62, 7\text{-OCH}_3)$, an olefinic proton H-8 $(\delta 7.44, s)$, a prenyl unit (δ 4.43, H-16; 2.63, H-15; 1.40, H-18 and 1.13, H-19), a pair of methylene protons H_a -10 ($\delta 2.35, d$), H_b -10 ($\delta 1.59$, dd), a methine proton H-11 ($\delta 2.50$, d) and gem-dimethyl protons H-13 (δ 1.66) and H-14 (δ 1.31).

Table 2
The ¹H NMR and HMBC spectroscopic data of cochinchinones C–D (3–4)

H-position	Cochinchinone C (3)		Cochinchinone D (4)		
	¹ H NMR	HMBC	¹H NMR	HMBC	
2	6.55 (<i>dd</i> , 1H, <i>J</i> = 8.4, 0.9 Hz)	C-1, C-4	6.03 (d, 1H, J = 2.1 Hz)	C-1, C-4, C-9a	
3	7.41 (1H, J = 8.4 Hz)	C-1, C-4a	_	_	
4	6.52 (dd, 1H, J = 8.4, 0.9 Hz)	C-2, C-4a, C-9, C-9a	6.05 (d, 1H, J = 2.1 HZ)	C-2, C-3, C-4a, C-9, C-9a	
8	7.51 (s, 1H)	C-4b, C-7, C-8a, C-9	7.44 (s, 1H)	C-4b, C-6, C-8a, C-9	
10a	2.39 (d, 1H, J = 12.9 Hz)	C-4b, C-8, C-11	2.35 (d 1H, J = 132 Hz)	C-4b, C-7, C-8, C-12	
10b	1.59 (dd, 1H, J = 12.9, 9.9 Hz)	C-7, C-8, C-11	1.59 (dd, 1H, J = 13.2, 9.6 Hz)	C-6,C-7,C-8,C-11	
11	2.54 (d, 1H, J = 9.9 Hz)	C-4b, C-5, C-10	2.50 (d, 1H, J = 9.6 Hz)	C-4b, C-7, C-12, C-13	
13	1.69 (s, 3H)	C-11, C-12, C-14	1.66 (s, 3H)	C-11, C-12, C-14	
14	1.33 (s, 3H)	C-11, C-12, C-13	1.31 (s, 3H)	C-11, C-12, C-13	
15	2.64 (d 2H, J = 8.1 Hz)	C-4b, C-5, C-16, C-17	2.63 (d 2H, J = 7.5 Hz)	C-4b, C-5, C-6, C-16, C-17	
16	4.39 (br t, 1H, J = 8.1 Hz)	C-15, C-18, C-19	4.43 (br t, 1H, $J = 7.5$ Hz)	C-15, C-18, C-19	
18	1.37 (s, 3H)	C-16, C-17, C-19	1.40 (s, 3H)	C-16,C-17,C-19	
19	1.01 (s, 3H)	C-16, C-17, C-18	1.13 (s, 3H)	C-16,C-17,C-18	
1-OH	12.00 (s, 1H)	C-1, C-2, C-9a	12.39 (s, 1H)	C-1, C-2, C-9a	
3-OH	_	_	8.05 (<i>br s</i> , 1H)	_	
7-OCH ₃	3.65 (s, 3H)	C-7	3.62 (s, 3H)	C-7	

Table 3
The ¹³C NMR spectroscopic data of cochinchinones A–D (1–4)

C-position	1	2	C-position	3	4
1	158.27	160.00	1	162.90	160.50
2	108.90	110.02	2	109.57	95.49
3	161.13	162.28	3	138.97	167.88
4	105.00	93.34	4	107.41	96.95
4a	152.97	155.73	4a	159.44	164.50
4b	150.34	149.76	4b	88.76	88.50
5	118.87	115.32	5	84.18	83.78
6	124.12	150.23	6	201.16	201.00
7	152.43	141.60	7	84.86	84.50
8	108.89	105.95	8	135.25	133.25
8a	120.45	112.85	8a	132.14	132.25
9	180.90	180.11	9	180.73	178.00
9a	102.96	102.33	9a	106.15	100.50
1'	21.58	21.28	10	29.73	29.87
2'	121.58	122.63	11	49.43	49.27
3'	134.92	131.40	12	83.96	83.76
4'	17.94	17.79	13	30.37	30.26
5'	25.86	25.72	14	29.04	28.94
1"	21.59	22.32	15	29.21	28.98
2"	121.58	121.43	16	118.48	117.86
3"	137.94	135.41	17	135.73	135.50
4"	39.72	39.66	18	25.51	25.46
5"	26.43	26.56	19	16.69	16.88
6"	123.85	124.12	7 -OCH $_3$	54.09	53.84
7"	131.50	131.19			
8"	17.69	17.57			
9"	16.27	16.23			
10"	25.66	25.52			

In their work on bractatin and derivatives, Thoison et al. (2000) have shown that caged-prenylated xanthones can exist in pure enantiomeric form (with relatively large optical rotation) or as mixtures of enantiomers, with low optical rotation values. It is concluded therefore that cochinchinone C and D are different mixtures of the two possible enantiomers of the structures 3 and 4, respectively, in which only the relative stereochemistry is shown.

At the concentration of 50 μ M (Table 4), the twelve isolated compounds were able to scavenge the DPPH radical in the range of 1.7–81.0%. Compounds **2**, **9** and **11** exhibited the most potent radical scavenging activity with IC₅₀ values of 9.4, 19.0 and 12.3 μ M, respectively. These are

Table 4 Radical scavenging activity of compounds 1–12 (at 50 μM)

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Compounds	% Scavenging of DPPH		
1	20.7		
2	79.3		
3	1.7		
4	5.2		
5	5.2		
6	1.7		
7	20.7		
8	5.2		
9	75.9		
10	6.9		
11	79.3		
12	6.9		
BHT	51.7		

more effective than butylated hydroxytoluene (BHT) with an IC₅₀ of 28.9 μ M. The greater effectiveness of compounds **2**, **9** and **11** than the others was possibly due to the presence *ortho*-dihydroxy groups which upon donating hydrogen radicals will give higher stability to their radical forms (Shahidi and Wandasundara, 1992).

Caged-prenylated xanthones have been exclusively found in the genus *Garcinia* (Cao et al., 1998; Thoison et al., 2000; Rukachaisirikul et al., 2000). The present work is the first report of the caged-prenylated xanthones isolated from the genus *Cratoxylum*.

3. Experimental

3.1. General method

Melting points were recorded with a digital electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. Optical rotations were measured on an AUTOPOL® II automatic polarimeter. Ultraviolet spectra were measured with a UV-160A spectrophotometer (SHMADZU). Infrared spectra (IR) were obtained with a FTS165 FT-IR spectrometer. ¹H and ¹³C-Nuclear Magnetic Resonance spectra were recorded with a FT-NMR Bruker Avance 300 MHz or Varian UNITY INOVA 500 MHz spectrometer, whereas high resolution mass spectra were obtained using a MAT 95 XL. Column and quick CC were performed on silica gel 100 and silica gel 60H (Merck), respectively. Precoated TLC sheets of silica gel 60 F₂₅₄ were used. Known compounds were identified by comparison of their spectroscopic data with those in the literature.

3.2. Plant material

The roots of *C. cochinchinense* (Clusiaceae) were collected from Amphur Bannasan, Suratthani Province in the southern part of Thailand in February 2003. The voucher specimen (No. W. Nuangnaowarat 1 Suratthani: Bannasan 31/3/04) was identified by Dr. Kitichate Sridith and has been deposited in the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

3.3. Extraction and isolation

Chopped, dried roots of *C. cochinhinense* (11 kg) were sequentially extracted at room temperature with CH₂Cl₂ (42 L) and MeOH (39 L) (3 days each). Removal of solvents in vacuo produced a yellow-brown, viscous, CH₂Cl₂ extract (294 g) and a MeOH extract (292 g), respectively. An aliquot of the CH₂Cl₂ extract (66 g) was subjected to quick CC over silica gel 60H using hexane, hexane–CH₂Cl₂, CH₂Cl₂, CH₂Cl₂–Me₂CO and Me₂CO as eluents to give fractions A1–A14. Fractions A4 (8.51 g) and A6 (7.54 g) were further purified by crystallization in hexane–

CH₂Cl₂ to give the yellow solid 6 (738 mg). Fraction A7 (18.96 g) was subjected to silica gel column and eluted with hexane-CH₂Cl₂, CH₂Cl₂, CH₂Cl₂-Me₂CO to give fractions A7A (5.33 g), A7B (10.77 g) and A7C (1.94 g). Repeated chromatography of fraction A7A (2.00 g) on silica gel column using hexane–Me₂CO (9:1) as mobile phase yielded the yellow solids 7 (55 mg), 1 (20 mg), 3 (15 mg) and 8 (60 mg). Fraction A7B (1.20 g) was submitted to CC over silica gel, eluted with hexane–Me₂CO (17:3) to give the yellow solids **9** (6 mg), **5** (25 mg) and **4** (35 mg). Fraction A9 (4.06 g) was separated by CC over silica gel and eluted with hexane–Me₂CO (4:1) to give the yellow solids 10 (15 mg), 2 (67 mg) and 11 (22 mg). Fraction A11 (300 mg) was applied to a silica gel column using CH₂Cl₂-MeOH (49:1) as eluent to yield a yellow solid 12 (40 mg).

3.3.1. Cochinchinone A (1)

Yellow solid, m.p. 119–120 °C. HREIMS m/z 448.2299 for $C_{28}H_{32}O_5$ (calcd. 448.2250). UV (EtOH) λ_{max} nm (log ε): 232 (4.44), 268 (4.42), 316 (4.04), 384 (3.70). IR (KBr) v (cm⁻¹): 3413, 1641. EIMS m/z (% rel int): 449 ([M + H]⁺, 53), 448 ([M]⁺, 15), 447 (10), 393 (18), 325 (12), 323 (30), 309 (13), 281 (10), 277 (18), 270 (23), 269 (100), 257 (13), 253 (10), 185 (75), 93 (60). For ¹H NMR and ¹³C NMR spectroscopic data, see Tables 1 and 3.

3.3.2. Cochinchinone B (2)

Yellow solid, m.p. 221–222 °C. HRMS m/z 464.2189 for $C_{28}H_{32}O_6$ (calcd. 464.2199). UV (EtOH) λ_{max} nm (log ε): 243 (4.35), 259 (4.32), 323 (4.10), 372 (3.98). IR (KBr) ν (cm⁻¹): 3350, 1640. EIMS m/z (% rel int): 465 ([M + H⁺, 18), 464 ([M]⁺, 63), 422 (10), 421(39), 409 (76), 379 (17), 353 (11), 342 (24), 339 (31), 325 (24), 297 (46), 285 (45), 257 (25), 207 (13), 178 (58), 161 (32), 121 (37), 108 (50), 91 (76), 79 (77), 69 (96), 57 (100). For ¹H NMR and ¹³C NMR spectroscopic data, see Tables 1 and 3.

3.3.3. Cochinchinone C(3)

Yellow solid, m.p. 147-148 °C. HREMS [M – CO]⁺ m/z 382.1745 for C₂₃H₂₆O₅ [M – CO]⁺ (calcd. 382.1780). Optical rotation: $[\alpha]_D^{29} + 50^\circ$ (c 8.9 × 10⁻² in CHCl₃). UV (EtOH) λ_{max} nm (log ε): 206 (4.46), 222 (4.30), 307 (3.98), 346 (3.70). IR (KBr) ν (cm⁻¹): 3467, 1749, 1642. EIMS m/z (% rel int): 382 ([M – CO]⁺, 14), 381 (52), 312 (100), 285 (35), 243 (19), 68.9 (9). For ¹H NMR and ¹³C NMR spectroscopic data, see Tables 2 and 3.

3.3.4. Cochinchinone D(4)

Yellow solid, m.p. 218–219 °C. HREIMS m/z 398.1713 for $(C_{23}H_{26})_6$ [M - CO] $^+$ (calcd. 318.1729). Optical rotation: $[\alpha]_D^{29} - 58^\circ$ (c 6.90 × 10 $^{-2}$ in CHCl₃). UV (EtOH) $\lambda_{\rm max}$ nm: 212 (4.47), 275 (4.09), 332 (4.06), 357 (4.10). IR (KBr) ν (cm $^{-1}$): 3392, 1738, 1646. EIMS m/z (% rel int): 398 ([M - CO] $^+$,10), 397 (36), 329(16), 328 (100), 300 (29), 259 (16), 68.9 (13). For 1 H NMR and 13 C NMR spectroscopic data, see Tables 2 and 3.

3.4. Radical scavenging activity

The experiments were modified from the method of Yamasaki et al., 1994. The sample solution (3.0 mM in absolute ethanol, 50 μ L) was mixed with DPPH solution (0.05 mM, 3 mL) and allowed to stand at room temperature for 30 min. The absorbance was then measured at 517 nm. The results were expressed as percentage radical scavenging, % radical scavenging = [($A_{\rm control} - A_{\rm sample}$)/ $A_{\rm control}$]×100. The DPPH solution without sample was used as control. The IC₅₀ values were obtained by linear regression analysis of the dose response curves, which were plots of % radical scavenging versus concentration. Measurements were performed in triplicate.

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