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Structure elucidation

Xanthones from *Garcinia cowa* Roxb. latex

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Abstract

Five xanthones named cowagarcinone A–E and six previously reported xanthones were isolated from the latex of *Garcinia cowa* Roxb. Their structures were determined on the basis of spectroscopic analysis. The crude latex and the isolated compounds were investigated for their radical scavenging activities.

Keywords: *Garcinia cowa*; Guttiferae; Xanthones; Radical scavenging

1. Introduction

Garcinia cowa Roxb. (Guttiferae) is a tree species widely distributed in Thailand where it is known as Cha-muang. It has been used in the folk medicine for various purposes. The bark has been used as an antipyretic and antimicrobial agent. The latex has been used as antifever agent (Na Pattalung et al., 1994). Some pharmacological properties of the crude extracts of leaves, e.g., antitumor-promoting activity (Murakami et al., 1995) and inflammation induction (Ilham et al., 1995) have been reported. Previous investigations of the latex and stem bark of *G. cowa* revealed the presence of prenylated xanthones, namely cowaxanthone, cowanin, cowanol, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone, β-mangostin, 7-methylgarcinone E and norcowanin. Some of these compounds have been tested for antimalarial activity and antimicrobial activity (Krahn, 1968; Lee and Chan, 1977; Na Pattalung et al., 1994; Likhitwitayawuid et al., 1997, 1998). As a continuation of our phytochemical work and our search for antioxidative natural products, we evaluated the radical scavenging activity of the crude latex of *G. cowa* and investigated the latex for its chemical constituents. As a result, six previously isolated xanthones, (**1**–**6**) were obtained together with five new xanthones (**7**–**11**). T he structures of the new compounds are reported herein.

2. Results and discussion

Acetone-soluble material from the fresh latex of *G. cowa* was separated by means of chromatography over silica gel. Cowaxanthone (**1**), cowanin (**2**), cowanol (**3**) and 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone (**4**), the previously isolated xanthones were obtained. Extensive chromatography of the minor fractions led to the isolation of mangostinone (**5**) (Asai et al., 1995), fucaxanthone A (**6**) (Ito et al., 2003) and five new compounds (**7**–**11**).

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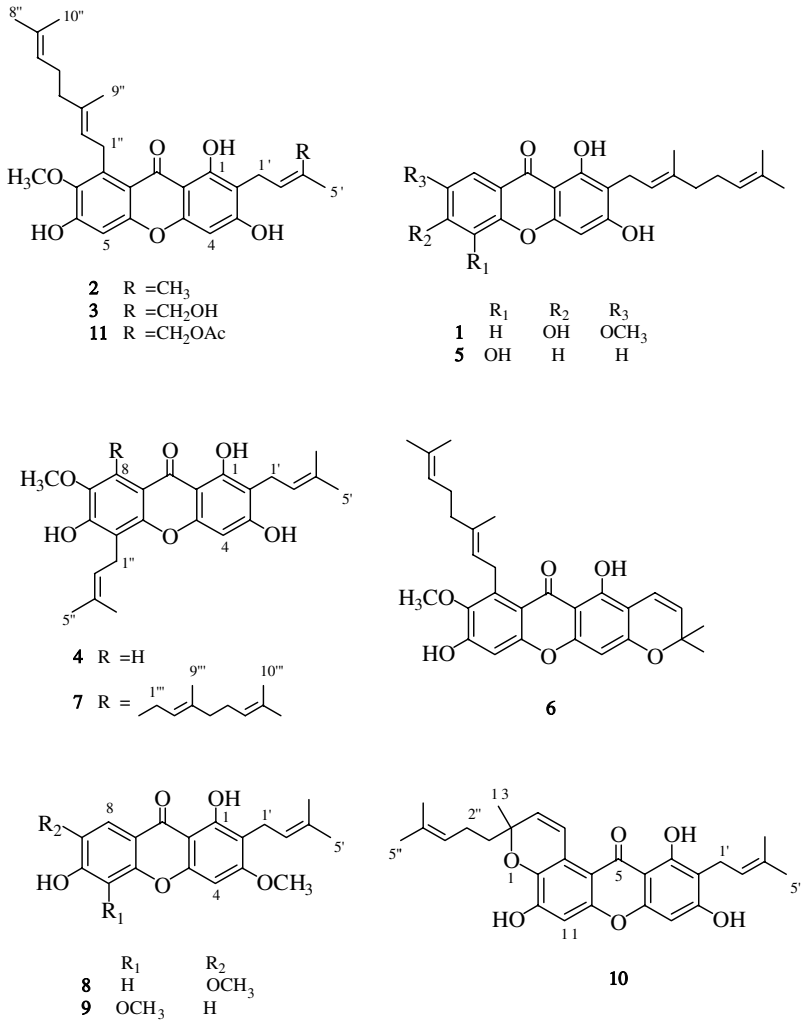

Cowagarcinone A (**7**) was a yellow gum, with a molecular formular $C_{34}H_{42}O_6$. The ^{1}H NMR spectra exhibited signals of a hydrogen bonded hydroxy proton at δ13.89 (*s*, 1-OH), a methoxy group at δ3.80 (*s*, 7-OCH_3) and an aromatic proton at δ6.34 (*s*, H-4). Two prenyl groups were present, as was evident from the following resonances: two olefinic protons at δ5.31 (*br t*, H-2′) and 5.29 (*br t*, H-2″), two pairs of methylene protons at δ3.46 (*d*, H-1′) and 3.57 (*d*, H-1″) and four allylic methyl groups at δ1.78 (*s*, H-5′), 1.85 (*s*, H-4′), 1.88 (*s*, H-4″), 1.69 (*s*, H-5″). The remaining signals appeared as the typical signals of a geranyl unit. These signals were a doublet of the methylene protons H-1‴ at δ4.08, two broad triplets of the olefinic protons H-2‴ and H-6‴ at δ5.27 and 5.03, two multiplets of the methylene protons H-4‴ and H-5‴ at δ1.96 and 2.05, respectively, and three singlets of methyl groups H-8‴, H-9‴ and H-10‴ at δ1.60, 1.83 and 1.55, respectively. The assignments of the substituents were deduced from the HMQC and HMBC spectra (Table 1). The geranyl unit was located at C-8 according to the correlations of benzylic methylene protons H-1‴ to C-7, C-8 and C-8a and the deshielding effect of the C-9 carbonyl group on H-1‴. In a NOE experiment, irradiation of the methoxy protons at δ3.80 enhanced the signal of H-1‴, thus the methoxy group should be at C-7. The methylene protons H-1′ correlated to C-2 and C-3 whereas the methylene protons H-1″ correlated to C-5, C-6 and C-10a, consequently, two prenyl units were located at C-2 and C-5, respectively. Cowagarcinone A (**7**) was then characterized as 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone.

Cowagarcinone B (**8**) was a pale yellow solid, m.p. 252–253 °C, with a molecular formula $C_{20}H_{20}O_6$. The ^{1}H NMR spectra exhibited signals of a chelated phenolic hydroxy proton at δ13.00 (*s*, 1-OH), two methoxy groups at δ3.92 (*s*, 3-OCH_3) and 4.01 (*s*, 7-OCH_3) and three aromatic protons at δ6.43 (*s*, H-4), 6.94 (*s*, H-5) and 7.61 (*s*, H-8). The aromatic proton (δ7.61) was assigned to be H-8 as it was deshielded by the C-9 car-

Table 1
HMBC correlation data of **7, 10** and **11**

Position H	**7**	Position H	**10**	Position H	**11**
4	C-2, C-3, C-4a, C-9, C-9a	3	C-2, C-4, C-4a	4	C-2, C-3, C-4a, C-9, C-9a
1′	C-2, C-3, C-2′, C-3′	4	C-2, C-4b, C-12a	1′	C-1, C-2, C-2′, C-3′
2′	C-1′, C-3′, C-4′	9	C-5, C-5a, C-7, C-8, C-9a	2′	C-2, C-5′,C-4′
4′	C-2′, C-3′, C-5′	11	C-4b, C-5, C-10a, C-12, C-12a	4′	C-2′, C-3′, C-5′, C=O
5′	C-2′, C-3′ C-4′	13	C-2, C-3, C-1″	5′	C-2′, C-3′, C-4′
1″	C-5, C-6, C-10a, C-2″, C-3″	1′	C-6, C-7, C-8, C-2′, C-3′	1″	C-7, C-8, C-8a, C-2″
2″	C-4″, C-5″	2′	C-1′, C-4′, C-5′	2″	C-8, C-1″, C-4″, C-9″
4″	C-2″, C-3″, C-5″	4′	C-2′, C-3′, C-5′	4″	C-2″, C-3″
5″	C-2″, C-3″, C-4″	5′	C-2′, C-3′, C-4′	5″	C-4″
1‴	C-7, C-8, C-8a, C-2‴, C-3‴	1″	C-2, C-2″	6″	C-5″
2‴	C-8, C-4‴, C-9‴	2″	C-3″, C-4″	8″	C-6″, C-7″, C-10″
4‴	C-2‴, C-3‴, C-9‴, C-5‴	3″	C-2″, C-5″, C-6″	9″	C-2″, C-3″, C-4″
5‴	C-4‴, C-6‴, C-7‴	5″	C-3″, C-4″, C6″	10″	C-6″, C-7″, C-8″
6‴	C-8‴, C-10‴	6″	C-3″, C-4″, C-5″	1-OH	C-1, C-2, C-9a
8‴	C-6‴, C-7‴, C-10‴	6-OH	C-6, C-7, C-5a	7-OCH_3	C-7
9‴	C-1‴, C-2‴, C-3‴	12-OH	C-10a, C-11, C-12, C-12a	$OCOCH_3$	C=O
10‴	C-6‴, C-7‴, C-8‴				
1-OH	C-1, C-2, C-9a				
7-OCH_3	C-7				

bonyl group. Irradiation of the methoxy protons at δ3.92 gave a NOE enhancement of the H-4 signal and likewise irradiation of the signal at δ4.01 enhanced the H-8 signal, thus the methoxy groups should be at C-3 and C-7, respectively. A prenyl unit was also present: two methyl group singlets at δ1.68 and 1.80, a doublet from methylene protons at δ3.37 and an olefinic proton signal at δ5.24. The position of the prenyl group was deduced to be at C-2 by the HMBC correlations (Table 2) of H-1′ to C-1, C-2, and C-3. Therefore, 1,6-dihydroxy-3,7-dimethoxy-2-(3-methyl-2-butenyl)xanthone was determined for cowagarcinone B (**8**).

Cowagarcinone C (**9**) was obtained as a pale yellow solid, m.p. 152–153 °C, with the molecular formula $C_{20}H_{20}O_6$. The ^{1}H NMR spectra showed the presence of a chelated phenolic hydroxy group of 1-OH at δ13.00 and two methoxy groups at δ3.95 (3-OCH_3) and 4.02 (5-OCH_3). Two doublets corresponding to two *ortho*-coupled aromatic protons H-7 and H-8 at δ6.98 and 7.95 were observed in addition to a singlet aromatic proton H-4 at δ6.48. A prenyl moiety was also present: signals of two methyl groups δ1.68 and 1.80, two methylene protons at δ3.38 and an olefinic proton at δ5.23. The arrangement of two methoxy groups at C-3 and C-5 was indicated by the ^3J coupling of the methoxy protons to C-3 and to C-5, respectively. The 3-OCH_3 was confirmed to be adjacent to H-4 by a NOE experiment. The prenyl moiety was located at C-2 due to the correlation of H-1′ to C-1, C-2, and C-3 in the HMBC spectrum (Table 2). The positions of other protons were also confirmed. 1,6-Dihydroxy-3,5-dimethoxy-2-(3-methyl-2-butenyl) xanthone therefore was assigned for cowagarcinone C (**9**).

Table 2
HMBC correlations data of **8** and **9**

Position H	**8**	**9**
4	C-2, C-3, C-4a, C-9, C-9a	C-2, C-3, C-4a, C-9, C-9a
5	C-6, C-7, C-8a, C-10a	–
7	–	C-5, C-6, C-8a
8	C-6, C-7, C-8a, C-9, C-10a	C-6, C-9, C-10a
1′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′, C-3′
4′	C-2′, C-3′, C-5′	C-2′, C-3′, C-5′
5′	C-2′, C-3′, C-4′	C-2′, C-3′, C-4′
1-OH	C-2	C-1, C-2, C-9a
6-OH	–	C-5, C-6, C-7
3-OCH_3	C-3	C-3
5-OCH_3	–	C-5
7-OCH_3	C-7	–

Cowagarcinone D (**10**) was a yellow solid, m.p. 92–94 °C, with the molecular formula $C_{28}H_{30}O_6$. The ^{1}H NMR spectrum exhibited a singlet signal of a chelated hydroxy group 6-OH at δ13.74. The spectrum also showed two sharp singlet signals of two isolated aromatic protons,which were considered to be H-9 and H-11 at δ6.32 and 6.83, respectively. The presence of signals of two methyl groups at δ1.78 (H-5′) and 1.85 (H-4′), methylene protons (H-1′) at δ3.46 and an olefinic methine proton (H-2′) at δ5.30 were suggestive of a prenyl moiety. Irradiation of the methylene protons (H-1′) caused a NOE enhancement of the chelated hydroxy proton 6-OH, these results suggested that the prenyl unit was at C-7. The remaining resonances were assigned to a chromene ring bearing a methyl group and C_6-sidechain: two singlets of two methyl groups at δ1.58 (H-6″) and 1.67 (H-5″), a broad triplet of an olefinic proton (H-3″) at δ5.10, a multiplet of methylene protons (H-1″) at δ1.80 and a multiplet of the methylene protons

(H-2″) at δ2.13 together with two doublets (J = 10 Hz) of vicinally coupled protons at δ8.09 (H-4), 5.80 (H-3) and a singlet of three protons at δ1.46 (H-13). The deshielding effect on the resonance of H-4 suggested that the chromene ring was attached to the xanthone nucleus in an angular fashion near the carbonyl group. Irradiation of the olefinic proton H-3″ caused the collapse of the signal of methylene protons H-2″ whereas irradiation at the signal of H-2″ affected the signal of the methylene protons H-1″. In a NOE experiment, irradiation of the olefinic proton (H-3) caused an enhancement of the methyl signal (H-13). The complete HMBC (Table 1) confirmed the structure of cowagarcinone D (**10**) as 6,8,12-trihydroxy-7-(3-methyl-2-butenyl)-2-methyl-2-(4-methyl-3-pentenyl)pyrano-(2′,3′:7,8)xanthone.

Cowagarcinone E (**11**) was a yellow gum, with the molecular formula $C_{31}H_{36}O_8$. The ^{1}H NMR spectrum showed a singlet signal of a chelated hydroxy group 1-OH at δ13.83, a singlet resonance of methoxy protons at δ3.81, and two singlet signals of two isolated aromatic protons H-4 and H-5 at δ6.35 and 6.84, respectively. Two side chains were detected: a geranyl side chain and a prenyl unit with an acetoxy group. The signals of the geranyl unit appeared as follows: two olefinic protons at δ5.27 (H-2″) and 5.03 (H-6″), three sets of methylene groups at δ4.11 (H-1″), 2.05 (H-5″) and 1.96 (H-4″) and three vinylic methyl groups at δ1.83 (H-9″), 1.60 (H-8″) and 1.55 (H-10″). Other signals were assigned to a 4-acetoxy-3-methyl-2-butenyl group, i.e.: a broad triplet at δ5.41, a doublet at δ3.59, a singlet of two protons at δ4.77, a singlet of three protons at δ2.14 and a doublet at δ1.75 arose from the olefinic proton (H-2′), benzylic methylene protons (H-1′), oxymethylene protons (H-4′), acetate methyl and vinylic methyl protons (H-5′), respectively. The enhancement of benzylic methylene protons H-1′ by irradiation at the oxymethylene protons H-4′ and the enhancement of the vinylic methyl protons H-5′ by irradiation at the olefinic proton H-2′ (δ5.41) in NOE experiments suggested the Z configuration of the C_5 unit. The HMBC correlations (Table 1) of acetyl methyl protons and oxymethylene protons H-4′ to the acetyl carbonyl group confirmed the position of the acetoxyl group. The correlation of benzylic methylene protons H-1′ to C-1 and C-2 confirmed the position of the 4-acetoxy-3-methyl-2-butenyl side chain to be C-2. The correlation of benzylic methylene protons H-1″ to C-7, C-8 and C-8a suggested that the geranyl side chain was located at C-8. Thus cowagarcinone E (**11**) was elucidated as 1,3,6-trihydroxy-7-methoxy-2-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone.

1, **2**, **3**, **4**, **7**, **8** and **11** are 1,3,6-trihydroxy-7-methoxyxanthones with geranyl and/or prenyl side chains whereas **5** is a trioxygenated xanthone with a geranyl side chain. **3** and **11** are oxidized forms of **2**. Pyranoxanthone **6** is a cyclized form of **2**.

Phenolic compounds are known to be antioxidants with excellent hydrogen or electron donor capacity (Shahidi et al., 1992). It was therefore of interest to test the isolated xanthones for antioxidation activity. The crude latex and pure compounds were examined for the radical scavenging activity by the DPPH assay. The crude latex was found to be able to scavenge the DPPH radical with a significant result, IC_{50} 13.20 μg/mL, whereas butylated hydroxy toluene (BHT), the standard antioxidant, exhibited activity with an IC_{50} 5.10 μg/mL. However, compounds **1**, **2**, **3**, **4**, **6**, **7** and **11** showed poor radical scavenging activity, the IC_{50} values being over 200 μM. Further search for the active component of the latex will be conducted.

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Varian UNITY INOVA 500 MHz NMR spectrometer, operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), using $CDCl_3$ solutions (a few drops of DMSO-d_6 being added wherever necessary), unless otherwise stated, with TMS as an internal standard. Inverse-detected heteronuclear correlations were measured using HMQC and HMBC pulse sequences with pulsed field gradients. UV spectra (ethanol solutions) were recorded on a SHIMADZU UV-160A spectrophotometer. IR spectra were measured with a FTS 165 FT-IR spectrometer. Low-resolution EI mass spectra and HRMS were obtained on a MS25 RFA mass spectrometer. Melting points were measured on a digital Electrothermal 9100 melting point apparatus. Analytical TLC and preparative TLC were preformed on Si gel (Merck GF_{254}). Quick CC and CC were carried out using Merck Si gel 60H and Si gel 100 (70-230 Mesh ASTM), respectively. Known compounds were identified by comparison of spectroscopic data and melting points with previously reported data.

3.2. Plant material

The latex of *Garcinia cowa* Roxb. was collected in Nakorn Sri Thammarat Province, Thailand. A specimen has been deposited in the herbarium of the Department of Biology, Prince of Songkla University, Thailand.

3.3. Isolation

The yellow viscous latex (38 g) contaminated with bark material was treated with warm acetone and the mixture was filtered to remove the bark material. Evaporation of the solvent under reduced pressure gave a yellow-brown gum (35 g). The crude material was separated by quick CC, eluted with hexane CH_2Cl_2,

$(CH_3)_2CO$, and then MeOH in a polarity gradient manner to afford fractions (A–I).

Fraction A and B was obtained from elution with hexane–CH_2Cl_2. Fraction A (5.31 g) was subjected to further CC using hexane–CH_2Cl_2 as an eluent to give fractions A1–A8. Fraction A4 was further purified by preparative TLC, developing with CH_2Cl_2–hexane (4:6) to give two isolated bands. Compound **6** (65.2 mg) and **7** (48 mg) were obtained from the first and second band, respectively. Fraction B (127 mg) was crystallized in hexane–CH_2Cl_2 to give 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone **4** (85 mg) as yellow needles. Fraction C (5.56 g) was crystallized from hexane–CH_2Cl_2 to give a yellow solid (2.26 g) and a filtrate (3.20 g). The solid was further subjected to CC using CH_2Cl_2 as an eluent to afford yellow needles of cowanol **3** (2.15 g). The filtrate was further purified by CC, conducted with hexane–CH_2Cl_2 in a polarity gradient manner to give fractions C1–C7. Fraction C3 (531 mg) was further subjected to CC, followed by preparative TLC using CH_2Cl_2 as a mobile phase (3 runs) to obtain a yellow solid of **8** (3.2 mg) from the first band and a yellow solid of **9** (4.1 mg) from the second band. Further chromatography of fraction C4 on CC, eluting with hexane–CH_2Cl_2, gave a yellow solid of cowanin **2** (500 mg). A solid from fraction D (630 mg) was crystallized in hexane–CH_2Cl_2 (3:2) to afford yellow needles of cowaxanthone **1** (500 mg). Fraction F (7.10 g) was a yellow-brown viscous gum. It was separated into seven fractions by CC using CH_2Cl_2–MeOH as solvent system. A solid from fraction F7 (1.15 g) was further separated into two portions by dissolving in CH_2Cl_2. The soluble portion (130 mg) was subjected to CC and eluted with CH_2Cl_2–MeOH followed by preparative TLC, developing with CH_2Cl_2, and finally recrystallized in CH_2Cl_2 to afford a yellow solid of **3** (76 mg). Fraction G (12.65 g), a yellow brown gum, was separated into fractions G1–G7 by CC eluting with CH_2Cl_2–MeOH. Further chromatography of fraction G7 (0.352 g) on CC using CH_2Cl_2–MeOH as an eluent gave four fractions. Repeat chromatography of the first and second fraction on CC and preparative TLC using CH_2Cl_2 as mobile phase (3 runs) gave **10** (7.5 mg) and **5** (8.6 mg) as a yellow solid. Preparative TLC of the third fraction, developed by $CHCl_3$ (3 runs) gave **5** and **11** (38.6 mg).

3.3.1. Cowagarcinone A (7)

Yellow gum. UV λ_{max} nm (log ε) 362 (4.09), 315 (4.62), 246 (4.85). IR (neat) ν (cm^{-1}) 3418, 1641. ^1H NMR δ13.89 (1H, *s*, 1-OH), 6.34 (1H, *s*, H-4), 5.31 (1H, *br t*, J = 6.5 Hz, H-2′), 5.29 (1H, *br t*, J = 6.5 Hz, H-2″), 5.27 (1H, *br t*, J = 6 Hz, H-2‴), 5.03 (1H, *br t*, J = 6 Hz, H-6‴), 4.08 (2H, *d*, J = 6 Hz, H-1‴), 3.80 (3H, *s*, 7-OMe), 3.57 (2H, *d*, J = 7 Hz, H-1″), 3.46 (2H, *d*, J = 7 Hz, H-1′), 2.05 (2H, *m*, H-5‴), 1.96 (2H, *m*, H-4‴), 1.88 (3H, *s*, H-4″), 1.85 (3H, *s*, H-4′), 1.83 (3H, *s*, H-9‴), 1.78 (3H, *s*, H-5′), 1.69 (3H, *s*, H-5″), 1.60 (3H, *s*, H-8‴), 1.55 (3H, *s*, H-10‴). ^{13}C NMR ($CDCl_3$) 182.38 (C-9), 161.51 (C-3), 160.56 (C-1), 155.04 (C-4a), 153.52 (C-10a), 152.28 (C-6), 142.28 (C-7), 135.67 (C-3′), 135.29 (C-3‴), 133.90 (C-8), 132.67 (C-3″), 131.26 (C-7‴), 124.32 (C-6‴), 123.59 (C-2‴), 121.54 (C-2′), 121.14 (C-2″), 113.93 (C-5), 111.96 (C-8a), 108.36 (C-2), 103.56 (C-9a), 93.23 (C-4), 62.02 (7-OCH_3), 39.72 (C-4‴), 26.57 (C-1‴), 26.34 (C-5‴), 25.86 (C-5′), 25.80 (C-5″), 25.62 (C-8‴), 22.64 (C-1″), 22.44 (C-1′), 17.97 (C-4″), 17.93 (C-4′), 17.67 (C-10‴), 16.46 (C-9‴). EIMS *m*/*z* (% rel. int.): 546 ($[M]^+$, 50), 494 (18), 477 (100), 435 (39), 421 (45), 407 (15). HRMS *m*/*z* 546.2985 (calcd. for $C_{34}H_{42}O_6$, 546.2970).

3.3.2. Cowagarcinone B (8)

Pale yellow solid, m.p. 252–253°. UV λ_{max} nm (log ε) 360 (3.77), 319 (4.15), 300 (3.99), 260 (4.34), 242 (4.30). IR ν (cm^{-1}) 3216, 1655. ^1H NMR δ13.00 (1H, *s*, 1-OH), 7.61 (1H, *s*, H-8), 6.94 (1H, *s*, H-5), 6.43 (1H, *s*, H-4), 6.34 (1H, *s*, 6-OH), 5.24 (1H, *br t*, J = 7 Hz, H-2′), 4.01 (3H, *s*, 7-OMe), 3.92 (3H, *s*, 3-OMe), 3.37 (2H, *d*, J = 7 Hz, H-1′), 1.80 (3H, *s*, H-4′), 1.68 (3H, *s*, H-5′). ^{13}C NMR ($CDCl_3$) 179.86 (C-9), 163.85 (C-3), 159.36 (C-1), 156.24 (C-4a), 152.54 (C-10a), 152.37 (C-6), 144.32 (C-7), 131.83 (C-3′), 122.21 (C-2′), 113.63 (C-8a), 111.76 (C-2), 104.62 (C-8), 104.62 (C-9a), 102.49 (C-5), 89.58 (C-4), 56.53 (7-OMe), 55.90 (3-OMe), 25.80 (C-5′), 21.36 (C-1′), 17.80 (C-4′). EIMS *m*/*z* (% rel. int.): 356 ($[M]^+$, 27), 341 (19), 313 (72), 301 (100), 298 (14), 271 (19). HRMS *m*/*z* 356.1261 (calcd. for $C_{20}H_{20}O_6$, 356.1254).

3.3.3. Cowagarcinone C (9)

Pale yellow solid, m.p. 152–153°. UV λ_{max} nm (log ε) 345 (3.92), 315 (4.47), 281 (4.20), 246 (4.72). IR ν (cm^{-1}) 3375, 1652. ^1H NMR δ13.00 (1H, *s*, 1-OH), 7.95 (1H, *d*, J = 9.0 Hz, H-8), 6.98 (1H, *d*, J = 9.0 Hz, H-7), 6.48 (1H, *s*, H-4), 6.29 (1H, *s*, 6-OH), 5.23 (1H, *br t*, J = 7.0 Hz, H-2′), 4.02 (3H, *s*, 5-OMe), 3.95 (3H, *s*, 3-OMe), 3.38 (2H, *br d*, J = 7.0 Hz, H-1′), 1.80 (3H, *s*, H-4′), 1.68 (3H, *s*, H-5′). ^{13}C NMR ($CDCl_3$) 180.07 (C-9), 164.08 (C-3), 159.78 (C-1), 155.70 (C-4a), 154.11 (C-6), 149.53 (C-10a), 133.59 (C-5), 131.93 (C-3′), 122.03 (C-8), 122.00 (C-2′), 115.26 (C-8a), 112.34 (C-2), 112.24 (C-7), 103.24 (C-9a), 89.78 (C-4), 61.95 (5-OMe), 55.97 (3-OMe), 25.78 (C-5′), 21.59 (C-1′), 17.78 (C-4′). EIMS *m*/*z* (% rel. int.): 356 ($[M]^+$, 28), 341 (17), 326 (20), 323 (72), 301(100), 298(29), 286 (13), 271(15), 256(11). HRMS *m*/*z* 356.1263 (calcd. for $C_{20}H_{20}O_6$, 356.1254).

3.3.4. Cowagarcinone D (10)

Yellow solid, m.p. 92–94°. UV λ_{max} nm (log ε) 383 (3.70), 323 (4.32), 265 (4.43), 246 (4.40). IR ν (cm^{-1}) 3473, 1652. ^1H NMR δ13.74 (1H, *s*, 6-OH), 8.09 (1H,

d, *J* = 10.0 Hz, H-4), 6.83 (1H, *s*, H-11), 6.32 (1H, *s*, H-9), 6.22 (1H, *br s*, 12-OH), 5.80 (1H, *d*, *J* = 10.0 Hz, H-3), 5.30 (1H, *br t*, *J* = 7.0 Hz, H-2′), 5.10 (1H, *br t*, *J* = 7.0 Hz, H-3″), 3.46 (2H, *d*, *J* = 7.0 Hz, H-1′), 2.13 (2H, *m*, H-2″), 1.85 (3H, *s*, H-4′), 1.80 (2H, *m*, H-1″), 1.78 (3H, *s*, H-5′), 1.67 (3H, *s*, H-5″), 1.58 (3H, *s*, H-6″), 1.46 (3H, *s*, H-13). ^{13}C NMR ($CDCl_3$) 182.55 (C-5), 161.73 (C-8), 160.47 (C-6), 155.34 (C-9a), 153.03 (C-10a), 150.77 (C-12), 136.82 (C-12a), 135.86 (C-3′), 132.16 (C-4″), 131.45 (C-3), 123.65 (C-3″), 121.44 (C-4), 121.39 (C-2′), 119.58 (C-4a), 108.55 (C-4b), 108.36 (C-7), 103.78 (C-5a), 102.32 (C-11), 93.42 (C-9), 79.39 (C-2), 40.38 (C-1″), 25.86 (C-5′), 25.67 (C-5″), 25.65 (C-13), 22.77 (C-2″), 21.45 (C-1′), 17.93 (C-4′), 17.67 (C-6″). EIMS *m*/*z* (% rel. int.): 462 ([M]$^+$, 24), 407 (18), 379 (100), 337 (17), 323 (41). HRMS *m*/*z* 462.2045 (calcd. for $C_{28}H_{30}O_6$, 462.2034).

3.3.5. Cowagarcinone E (**11**)

Yellow gum. UV λ_{max} nm (log ε) 362 (3.57), 312 (4.04), 262 (4.17), 244 (4.24). IR *v* (cm^{-1}) 3385, 1711, 1641. ^{1}H NMR δ13.83 (1H, *s*, 1-OH), 6.84 (1H, *s*, H-5), 6.35 (1H, *s*, H-4), 5.41 (1H, *br t*, *J* = 7 Hz, H-2′), 5.27 (1H, *br t*, *J* = 7 Hz, H-2″), 5.03 (1H, *br t*, *J* = 7.0 Hz, H-6″), 4.77 (2H, *s*, H-4′), 4.11 (2H, *d*, *J* = 7 Hz, H-1″), 3.81 (3H, *s*, 7-OMe), 3.59 (2H, *d*, *J* = 7 Hz, H-1′), 2.14 (3H, *s*, OCOM*e*), 2.05 (2H, *m*, H-5″), 1.96 (2H, *m*, H-4″), 1.83 (3H, *s*, H-9″), 1.75 (3H, *s*, H-5′), 1.60 (3H, *s*, H-8″), 1.55 (3H, *s*, H-10″). ^{13}C NMR ($CDCl_3$) 181.99 (C-9), 172.17 (OC═O), 161.58 (C-3), 160.86 (C-1), 155.86 (C-10a), 155.30 (C-4a), 154.55 (C-6), 142.64 (C-7), 137.15 (C-8), 135.57 (C-3″), 131.28 (C-3′), 130.45 (C-7″), 128.63 (C-2′), 124.33 (C-6″), 123.30 (C-2″), 112.27 (C-8a), 108.03 (C-2), 103.48 (C-9a), 101.60 (C-5), 93.57 (C-4), 63.92 (C-4′), 62.06 (7-OCH_3), 39.72 (C-4″), 26.59 (C-1″), 26.53 (C-5″), 25.61 (C-8″), 21.16 (C-5′), 20.99 (OCO*Me*), 20.90 (C-1′), 17.67 (C-10″), 16.50 (C-9″). EIMS *m*/*z* (% rel. int.): 536 ([M]$^+$, 5), 467 (12), 407 (100), 389 (15), 365 (18), 361 (20), 339 (17). HRMS *m*/*z* 536.2409 (calcd. for $C_{31}H_{36}O_8$, 536.2400).

3.4. Radical scavenging activity

Ethanolic solutions (50 μL) of test material at the concentrations of 2.5, 1.25, 0.75, 0.5, 0.25, 0.125, 0.06 and 0.03 mg/mL were mixed with ethanolic solutions of DPPH (0.05 mM, 3 mL). The mixed solutions were allowed to stand at 37 °C for 30 min and the absorbance measured at 517 nm. BHT was used as a positive control. The IC_{50} values were obtained by linear regression analysis of the dose response curves, which were plots of % inhibition versus concentration.

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