XANTHONES AND OTHER COMPOUNDS FROM THE LATEX OF GARCINIA COWA

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Abstract. From our ongoing study on the latex of \textit{Garcinia cowa} Roxb. ex Choisy collected in Quy Chau, Nghe An province, seven compounds were isolated including four tetraoxygenated xanthones: norcowanin (1), kaennacowanol A (2), garcinone D (3), fuscaxanthone I (4); one tocochromanol: parvifoliol F (5); one sterol: stigmasterol (6) and one triterpenoid: lupeol (7). The structures of the isolated compounds were elucidated by physico-chemical spectroscopic analysis and by comparison with reported data. To the best of our knowledge, garcinone D (3), fuscaxanthone I (4) and parvifoliol F (5) were first reported as components of \textit{Garcinia cowa}. Four isolated xanthones were investigated for antioxidant activities through the extent of their abilities to scavenge the ABTS
\textsuperscript{•+} radical cation. The result showed that compounds 1 and 2 exhibited potent antioxidant activities with IC\textsubscript{50} values of 74.45 ± 8.89 µM and 64.56 ± 4.51 µM, respectively.

Keywords: \textit{Garcinia cowa}, norcowanin, kaennacowanol A, garcinone D, fuscaxanthone I.

Classification numbers: 1.1.1, 1.1.6, 1.2.1.

1. INTRODUCTION

\textit{Garcinia cowa} Roxb. ex Choisy (\textit{G. cowa}), an evergreen 8-12 metres tall tree belonging to the family of Clusiaceae, is found in the tropical forest of Viet Nam, Thailand, Malaysia and Burma. The fruits and young leaves of \textit{G. cowa} are edible while the roots and barks have been used in antipyretic drugs [1] or as antiseptic agent [2]. Prior phytochemical investigations of \textit{G. cowa} revealed that xanthones accounted for more than 50 % the amount of substances isolated from this species, making xanthones the chemotaxonomic markers for \textit{Garcinia} genus [3]. Many xanthones among them are known for their significant interesting bioactivities such as anti-inflammatory [4, 5], antimalarial [6], antibacterial [1, 7, 8] and cytotoxic activities [9 - 13].
Our previous phytochemical research of *G. cowa* latex led to the isolation of seven tetraoxygenated xanthones [14]. As a continuation of our study, seven compounds were isolated and elucidated from the latex of this species.

### 2. MATERIALS AND METHODS

#### 2.1. General

Column chromatography (CC) were carried out on silica gel 60 (Merck, 5 - 40 μm), silica gel 100 (Merck, 63 - 200 μm), Sephadex LH-20 (GE Healthcare) and C18-reversed-phase silica gel (RP-18, Merck, 15 - 25 μm). TLC plates was visualized using UV light (254 and 365 nm) and staining with vanillin-H2SO4 10 % solution. NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 and 125 MHz for 1H and 13C, respectively, at Institute of Chemistry - Vietnam Academy of Science and Technology. Chemical shifts are shown in δ (ppm) with tetramethylsilane (TMS) as an internal reference. HR-ESI-MS data were measured with an Agilent 6530 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Santa Clara, United States). Melting points were obtained from a Buchi melting point B-545 apparatus (without correction).

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and L-ascorbic acid (99 % purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium persulphate and acetate buffer were purchased from Scharlau (Australia).

#### 2.2. Plant materials

The latex of *Garcinia cowa* Roxb. ex Choisy was collected in Quy Chau, Nghe An province, in December 2015. The plant materials were identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature. The voucher specimen No. GH2015130 is deposited at Institute of Natural Products Chemistry - Vietnam Academy of Science and Technology.

#### 2.3. Extraction and isolation

The latex of *G. cowa* (3.0 kg) as a brown solid was crushed into small pieces and dried in an oven at 45 °C for three days. The dried latex (2.8 kg) was extracted with methanol (MeOH) (3 L × 3) at room temperature using conventional ultrasound-assisted technique. The solvent was then removed under reduced pressure to give a dark brown residue (500.0 g). The residue was extracted with dichloromethane (DCM) (500 mL × 3) and the solution was filtered using filter funnel. The filtrate was collected and evaporated under reduced pressure to yield DCM extract (96.7 g).

The crude DCM extract was loaded to a silica gel column chromatography (CC) eluting with a gradient of DCM-MeOH (100:0 to 0:100, v/v) to afford five fractions (Frs. G1–G5). Fraction G1 (22.4 g) was separated by silica gel CC using a gradient of n-hexane-ethyl acetate (EtOAc) (100:0 to 100, v/v) to give ten subfractions G1.1–G1.10. Purification of subfraction G1.3 by CC over silica gel using n-hexane-EtOAc (80:1, v/v) provided compound 5 (160 mg). Compounds 6 (51 mg) and 7 (48 mg) were obtained as white needles from subfractions G1.4 and G1.5, respectively, by repeated chromatography over silica gel column eluted with n-hexane-EtOAc (80:1, v/v) followed by recrystallization in n-hexane.

Fraction G2 (37.5 g) was fractionated by CC with a gradient of n-hexane-acetone (v/v, 60:1 to 0:100) to yield eleven subfractions G2.1-G2.11. Subfraction G2.4 (1.76 g) was...
chromatographed over silica gel with eluent of n-hexane-acetone (20:1, v/v), followed by 
purifying on RP-18 silica gel eluted with MeOH-H₂O (6:1, v/v), to give compound I (820 mg).
Subfraction G2.10 was isolated by CC over silica gel with eluent of a gradient of n-hexane-
acetone (20:1 to 10:1, v/v) to give fifteen subfractions. Compound 2 (186 mg) was obtained
from subfraction G2.10.14 (520 mg) by repeated employing CC over RP-18 silica gel with
MeOH-H₂O (6:1, v/v) as the mobile phase. Subfraction G2.8 (4.3 g) was separated by
employing CC over silica gel using n-hexane-acetone (15:1, v/v) to afford five subfractions
(G2.8.1-G2.8.5). Compound 3 (267 mg) was derived from subfraction G2.8.4 by repeated
purification on sephadex LH-20 chromatography with eluent of 5% DCM-MeOH. Subfraction
G2.10.11 was repeated chromatographed on RP-18 column eluting with MeOH-H₂O (5:1, v/v)
and on a Sephadex LH-20 column using 5% DCM-MeOH as the eluent. As a result, compound
4 (12.1 mg) was obtained as a pale yellow solid.

Norcowanin (1): Yellow needles. mp 161 - 163 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm):
13.77 (1H, s, OH-1), 6.82 (1H, br s, H-5), 6.29 (1H, br s, H-4), 5.31 (1H, m, H-2), 5.30 (1H, m,
H-3), 5.04 (1H, t, J = 7.0 Hz, H-6”), 4.37 (2H, d, J = 4.0 Hz, H-1”), 3.45 (2H, d, J = 6.5 Hz,
H-1), 2.13 (2H, m, H-5”), 2.13 (2H, m, H-4”), 1.87 (3H, s, H-4”), 1.84 (3H, s, H-10”), 1.77 (3H, s,
H-5), 1.67 (3H, s, H-9”), 1.59 (3H, s, H-8”). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 182.7 (C-9),
161.6 (C-3), 160.6 (C-1), 153.7 (C-5a), 153.7 (C-4a), 144.2 (C-7), 139.7 (C-8), 135.7 (C-3’),
139.5 (C-3”), 132.3 (C-7”), 123.7 (C-6”), 121.4 (C-2’), 121.5 (C-2”), 101.3 (C-5), 111.4 (C-8a),
108.4 (C-2), 103.7 (C-9a), 93.2 (C-4), 39.7 (C-5”), 26.3 (C-4”), 26.0 (C-1”), 25.8 (C-5”), 25.7 (C-
9”), 21.5 (C-1”), 17.9 (C-4”), 17.7 (C-8”), 16.3 (C-10”). HR-ESI-MS m/z 465.2275 [M + H]⁺
(calcd. for C₂₈H₃₃O₆, 465.2277).

Kaemnacowanol A (2): Yellow oil. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 6.64 (1H, s, H-
5), 6.20 (1H, s, H-4), 5.41 (1H, t, J = 7.5 Hz, H-2’), 5.21 (1H, d, J = 6.0 Hz, H-2’), 4.32 (2H, s,
H-4”), 4.03 (2H, d, J = 6.5 Hz, H-1”), 3.78 (3H, s, 7-OCH₃), 3.36 (2H, d, J = 7.5 Hz, H-1”), 1.97
(2H, t, J = 7.0 Hz, H-4”), 1.81 (3H, s, H-10”), 1.78 (3H, s, H-5”), 1.45 (2H, m, H-5”), 1.35 (2H,
m, H-6’), 1.11 (6H, br s, H-8”, H-9”). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 182.9 (C-9), 163.3
(C-3), 161.4 (C-1), 157.8 (C-5a), 156.6 (C-6), 156.2 (C-4a), 144.8 (C-7), 138.5 (C-8), 135.5 (C-
3”), 135.0 (C-3”), 126.8 (C-2’), 125.3 (C-2”), 112.2 (C-8a), 110.5 (C-2), 103.8 (C-9a), 102.8 (C-
5), 93.4 (C-4”), 71.5 (C-7”), 61.9 (C-4”), 61.4 (7-OCH₃), 44.1 (C-6”), 41.1 (C-4”), 29.2 (C-8”, C-
9’), 27.0 (C-1”), 23.5 (C-5”), 21.9 (C-1”), 21.7 (C-5”), 16.5 (C-10”). HR-ESI-MS m/z 513.2484
[M + H]⁺ (calcd. for C₂₉H₃₀O₆, 513.2488).

Garcinione D (3): Yellow solid, mp 202 - 203 °C. ¹H NMR (500 MHz, DMSO-D₆) δ
(ppm): 13.84 (1H, s, OH-1), 6.76 (1H, s, H-5), 6.33 (1H, s, H-4), 5.18 (1H, t, J = 7.0 Hz, H-2’),
4.15 (1H, s, OH-3”), 3.75 (3H, s, 7-OCH₃), 3.34 (1H, s, OH-6), 3.30 (2H, m, H-1”), 3.21 (1H, d,
J = 7.0 Hz, H-1’), 1.72 (1H, s, H-4”), 1.62 (1H, s, H-5”), 1.57 (2H, m, H-2”), 1.21 (6H, s, H-4”, H-
5”). ¹³C NMR (125 MHz, DMSO-D₆) δ (ppm): 181.2 (C-9), 162.2 (C-3), 159.9 (C-1), 156.9 (C-
5a), 154.6 (C-6), 154.1 (C-4a), 143.3 (C-7), 138.5 (C-8), 130.3 (C-3’), 122.5 (C-2’), 110.0 (C-2),
109.5 (C-8a), 101.8 (C-9a), 101.5 (C-5), 92.2 (C-4), 69.2 (C-3”), 60.4 (7-OCH₃), 44.8 (C-2”),
29.0 (C-4”), 25.4 (C-5”), 22.2 (C-1”), 20.9 (C-1’), 17.6 (C-4’). HR-ESI-MS m/z 429.1918
[M + H]⁺ (calcd. for C₂₉H₂₂O₂, 429.1913).

Fuscasaxanthone I (4): Pale yellow solid, mp 104 - 105 °C. ¹H NMR (500 MHz,CD₂OD) δ
(ppm): 6.72 (1H, s, H-5), 6.27 (1H, s, H-4’), 5.42 (1H, t, J = 7.5 Hz, H-2’), 5.19 (1H, t, J = 7.0
Hz, H-6”), 4.33 (2H, s, H-4”), 3.78 (1H, s, 7-OMe), 3.39 (2H, d, J = 8.0 Hz, H-1’), 3.37 (2H, d, J
= 6.5 Hz, H-1”), 2.19 (2H, m, H-5”), 1.80 (2H, overlapped, H-2”), 1.79 (3H, s, H-5”), 1.72 (3H, s,
H-8’), 1.69 (3H, s, C-9”), 1.60 (2H, t, J = 7.0 Hz, H-4”), 1.34 (3H, s, H-10”). ¹³C NMR (125
MHz, CD₂OD) δ (ppm): 183.1 (C-9), 163.5 (C-3), 161.5 (C-1), 156.7 (C-5a), 157.8 (C-6), 156.3
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(C-4a), 144.8 (C-7), 139.8 (C-8), 139.1 (C-7"), 135.2 (C-3), 126.7 (C-2), 126.1 (C-6"), 112.2 (C-8a), 110.6 (C-2), 103.8 (C-9a), 102.8 (C-5), 93.3 (C-4), 73.8 (C-3"), 61.8 (C-4'), 61.5 (7-OHm), 43.2 (C-4"), 42.3 (C-2"), 27.1 (C-8"), 25.9 (C-9"), 23.6 (C-5"), 23.2 (C-5'), 21.9 (C-1"), 21.7 (C-1'), 17.5 (C-10'). HR-ESI-MS m/z 513.2482 [M + H]⁺ (calcd. for C₉₀H₁₇O₈S, 513.2488).

**Parvifoliol F (5):** Colorless liquid. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 6.49 (1H, d, J = 3.0 Hz, H-7), 6.39 (1H, d, J = 3.0 Hz, H-5), 5.15 (1H, dt, J = 7.0, 2.0 Hz, H-11), 5.12 (1H, m, H-5), 5.12 (1H, m, H-19), 2.70 (2H, dt, J = 7.0, 2.0 Hz, H-4), 2.14 (3H, s, H-26), 2.12 (2H, m, H-10), 2.09 (2H, m, H-13), 2.08 (2H, m, H-17), 2.00 (2H, m, H-14), 1.99 (2H, m, H-18), 1.77 (2H, m, H-3), 1.69 (3H, d, J = 1.0 Hz, H-21), 1.66 (1H, m, H-9), 1.61 (6H, s, H-22, H-23), 1.60 (3H, s, H-24), 1.56 (1H, m, H-9), 1.27 (3H, s, H-25). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 147.8 (C-8), 146.0 (C-8a), 135.2 (C-16), 135.0 (C-6), 131.3 (C-20), 127.4 (C-12), 124.4 (C-19), 124.3 (C-15), 124.2 (C-11), 121.3 (C-4a), 115.7 (C-7), 112.7 (C-5), 75.4 (C-2), 39.7 (C-9, C-14, C-17), 31.4 (C-3), 26.8 (C-13), 26.6 (C-18), 25.7 (C-21), 24.0 (C-25), 22.5 (C-4), 22.2 (C-10), 17.7 (C-22), 16.1 (C-23, C-26), 16.0 (C-24). HR-ESI-MS m/z 397.3111 [M + H]⁺ (calcd. for C₂₂H₄₁O₇, 397.3107).

**Stigmasterol (6):** White needles, mp 174 - 176 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 5.35 (1H, m, H-6), 5.15 (1H, dd, J = 8.5, 15.0 Hz, H-23), 5.02 (1H, dd, J = 9.0, 15.5 Hz, H-22), 3.53 (1H, m, H-3), 1.02 (3H, H-18), 1.01 (3H, H-19), 0.86 (3H, H-29), 0.81 (3H, H-28), 0.79 (3H, H-26), 0.70 (3H, H-21). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 141.0 (C-5), 138.9 (C-22), 129.9 (C-23), 121.9 (C-6), 72.1 (C-3), 56.8 (C-14), 56.2 (C-17), 50.2 (C-9), 46.2 (C-24), 42.5 (C-13, C-4), 40.7 (C-20), 39.8 (C-12), 36.7 (C-10), 31.9 (C-7, C-8), 29.7 (C-27), 29.4 (C-16), 25.3 (C-25), 24.6 (C-15), 21.8 (C-21), 21.6 (C-21), 20.1 (C-28), 19.7 (C-29), 18.8 (C-19), 12.3 (C-26), 12.2 (C-18).

**Lupeol (7):** White needles, mp 215-216 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 4.69 (1H, d, J = 4.0 Hz, H-29a), 4.57 (1H, dd, J = 2.0, 2.5 Hz, H-29e), 3.19 (1H, dd, J = 5.5 Hz, H-3), 2.38 (1H, d, J = 11.0, 6.0 Hz, H-19), 1.92 (2H, m, H-21), 1.68 (3H, s, H-30), 1.66 (1H, m, H-13), 1.52 (2H, m, H-11), 1.03 (3H, s, H-28), 1.01 (2, m, H-15), 0.95 (3H, s, H-27), 0.93 (3H, s, H-26), 0.83 (3H, s, H-25), 0.78 (3H, s, H-24), 0.76 (3H, s, H-23), 0.67 (1H, br d, J = 9.5 Hz, H-5). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 150.2 (C-20), 109.3 (C-29), 79.1 (C-3), 55.4 (C-5), 50.3 (C-9), 48.3 (C-18), 47.7 (C-19), 43.1 (C-17), 42.8 (C-14), 40.0 (C-8), 38.8 (C-1, C-22), 38.1 (C-4), 37.3 (C-10), 36.3 (C-13), 35.7 (C-16), 34.5 (C-7), 29.9 (C-21), 27.8 (C-23), 25.4 (C-15), 24.1 (C-12), 22.0 (C-2), 20.9 (C-11), 19.3 (C-30), 18.4 (C-6), 18.0 (C-28), 16.2 (C-24), 16.1 (C-25), 16.0 (C-26), 14.5 (C-27).

### 2.4. Antioxidant activity: ABTS assay

The ABTS radical cation (ABTS⁺) scavenging activities of compounds 1-4 were determined using the modifications of the 96-well microtiter plate method described by Saeed N. et al. [15]. Compounds 1-4 were dissolved in dimethyl sulfoxide (DMSO) to concentration of 10000, 2000, 400, 80 μg/mL. ABTS was dissolved in deionized water to a concentration of 7 mM. ABTS⁺ was produced from the reaction between the ABTS solution and potassium acetate solution (2.45 mM) in the dark at room temperature in 16 hours. The ABTS⁺ was diluted with acetate buffer to an absorbance of 0.70 ± 0.02 at 734 nm. After that, 190 μL of ABTS⁺ solution and 10 μL of tested compounds were mixed in 96-well plate.

L-ascorbic acid was used as a positive reference and DMSO solution was used as negative control. Percentage reduction of the initial ABTS⁺ absorption in relation to the control were recorded. The ABTS⁺ radical scavenging activity was calculated using the following equation:


g = \frac{A - A_0}{A_0} \times 100\% \quad \text{where} \quad g = \text{activity percentage} \\
\text{A is the absorbance of the test solution} \\
\text{A₀ is the absorbance of the control solution}

\[ g = \frac{A - A_0}{A_0} \times 100\% \]

Where:

- **g** = activity percentage
- **A** is the absorbance of the test solution
- **A₀** is the absorbance of the control solution

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ABTS⁺⁺ scavenging effect (%) = \left[1 - \left(\frac{A_{\text{control}}}{A_{\text{sample}}}\right)\right] \times 100
\]
where \( A_{\text{control}} \) is the absorbance of the control and \( A_{\text{sample}} \) is the absorbance of the tested compounds. The IC₅₀ values were calculated from the graph plotted as inhibition percentage against the concentration.

3. RESULTS AND DISCUSSION

Compounds 1-7 were isolated from the DCM extract of the latex of \( G. \) cowa by means of repeated column chromatography over silica gel, Sephadex LH-20 and \( C_{18} \)-reversed-phase silica gel with appropriate solvent mixtures as mobile phases. Four isolated compounds 1-4 exhibited strong UV absorption band of xanthone chromophore at \( \lambda_{\text{max}} \) 254 nm. Coloured reactions of the isolated substances on the TLC plate with visualizing reagents, i.e. vanillin-H₂SO₄ 10 % solution, produced green spots which were similar to those of the polypropylated xanthones isolated before [14]. In addition, the NMR data of 1-4 revealed signals of aromatic protons and carbons, a carbonyl group, prenyl and/or geranyl groups characterized for a xanthonoid skeleton with prenyl and/or geranyl side chains. Compound 5 was determined as a tocotrienol and compounds 6, 7 were sterol and triterpenoid, respectively. The structures of the isolated compounds are shown in Figure 1.

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\text{Figure 1. Chemical structures of compounds 1-7.}
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Norcowanin (1) was separated as yellow needles, mp 161 - 163 °C. The molecular formula of 1 was determined to be of \( C_{28}H_{32}O_{6} \) from the [M + H]⁺ protonated molecule peak at \( m/z \) 465.2275 in the HR-ESI-MS spectrum. The \(^{13}\)C-NMR spectra of 1 presented resonances of 28 carbons including a carbonyl carbon at \( \delta_C \) 182.7 (C-9). The \(^1\)H-NMR spectra showed the signals of two isolated aromatic protons resonated at \( \delta_H \) 6.82 (1H, br s, H-5), 6.29 (1H, br s, H-4); three olefinic protons at \( \delta_H \) 5.31 (2H, m, H-2'), 5.30 (2H, m, H-2''), 5.04 (1H, t, \( J = 7.0 \) Hz, H-6') and two methylene groups at \( \delta_H \) 4.37 (2H, d, \( J = 4.0 \) Hz, H-1''), 3.45 (2H, d, \( J = 6.5 \) Hz, H-1'), suggesting that 1 was a xanthone substituted with a geranyl group and a prenyl group. The shift to the downfield of methylene protons to \( \delta_H \) 4.37 ppm, due to the deshielded effects caused by
the adjacent carbonyl group, revealed that the substituent contained this methylene group was placed at C-8. In addition, the $^1$H- and $^{13}$C-NMR data of 1 were closely related to those of cowanin illustrated in our previous report [14], except for the disappearance of a methoxy group. Comparison of the HR-ESI-MS and NMR data of compound 1 with those of previously reported norcowanin [1], we concluded that 1 was norcowanin.

Kaennacowanol (2) was isolated as yellow oil. The HR-ESI-MS of 2 showed a [M + H]$^+$ protonated molecular peak at $m/z$ 513.2484, consistent with a molecular formula of C$_{25}$H$_{36}$O$_8$. The HMBC cross peak between protons of the methoxy group with carbon at $\delta$C 144.8 (C-7) revealed the location of this methoxy group was at C-7. The existence of a 4-hydroxy-3-methylbut-2-enyl group was assigned from 1D and 2D NMR data of 2 with resonances of protons appeared at $\delta$H 3.36 (2H, $d$, $J$ = 7.5 Hz, H-1$''$), 5.41 (1H, $t$, $J$ = 7.5 Hz, H-2$''$), 4.32 (2H, s, H-4$'$), 1.78 (3H, s, H-5$'$). In addition, the presence of a 7-hydroxy-3,7-dimethyloct-2-enyl group was determined from characteristic signals in the $^1$H NMR spectra, i.e. resonances of protons at $\delta$H 4.03 (2H, $d$, $J$ = 6.5 Hz, H-1$''$), 5.21 (1H, $d$, $J$ = 6.0 Hz, H-2$''$), 1.97 (2H, $t$, $J$ = 7.0 Hz, H-4$''$), 1.45 (2H, m, H-5$''$), 1.35 (2H, m, H-6$''$), 1.11 (6H, br s, H-8$'$, H-9$'$), 1.81 (3H, s, H-10$'$). Thus, the NMR data of compound 2 was quite similar to those of cowanol [14], except for the disappearance of one double bond of the geranyl group and the appearance of a hydrated tertiary saturated carbon at $\delta$C 71.5 (C-7$''$). The structure of the geranyl group was confirmed based on the HMBC correlations between H-5$''$ with C-4$''$ ($\delta$C 41.1), C-5$''$ ($\delta$C 23.5), C-7$''$ and the correlations between two equivalent methyl groups CH$_3$-8$'$,9$''$ with C-7$''$. Moreover, the HMBC correlations of protons H-1$'$ to C-1 ($\delta$C 161.4), C-2 ($\delta$C 110.5) and C-3 ($\delta$C 163.3) of the xanthone moiety indicated that the 4-hydroxy-3-methylbut-2-enyl unit was placed at C-2. The position of the 7-hydroxy-3,7-dimethyloct-2-enyl group at C-8 was assigned from the cross peaks in the HMBC spectra between H-1$'$ and C-7, C-8 ($\delta$C 138.5), C-8a ($\delta$C 112.2) (Figure 2). From the above analysis and by comparison with reported data [12], compound 2 was elucidated as kaennacowanol A.

![Figure 2](image-url) **Figure 2.** Key COSY (if available) and HMBC correlations of compounds 2, 4 and 5.
Garcinone D (3) was obtained as a pale yellow solid, mp 202 - 203 °C. The molecular formula of 3 was established to be C_{24}H_{26}O_{4} by its HR-ESI-MS data (m/z 429.1918 [M + H]^+). The NMR spectra of compound 3 demonstrated signals of a xanthone with two prenyl substituents, one of them was a 3-hydroxy-3-methylbutyl group due to the appearance of a couple of equivalent methyls resonated at δ_H 1.21 (6H, s, H-4′, H-5′)/δ_C 29.0. The ^1H and ^13C NMR data of 1 indicated the existence of two isolated aromatic CH groups at δ_H 6.76 (1H, s, H-5)/δ_C 101.5, δ_H 6.33 (1H, s, H-4)/δ_C 92.2 and a methoxy group at δ_H 3.75 (3H, s, 7-OCH3)/δ_C 60.4. The unsaturated prenyl group resonated at δ_H 3.21 (1H, d, J = 7.0 Hz, H-1′)/δ_C 122.5, δ_C 130.3 (C-3′), δ_H 1.72 (1H, s, H-4′)/δ_C 17.6, δ_H 1.62 (1H, s, H-5′)/δ_C 25.4, and the 3-OH-prenyl group resonated at δ_H 3.30 (2H, m, H-1′)/δ_C 22.2, δ_H 1.57 (2H, m, H-2′)/δ_C 44.8, δ_C 69.2 (C-3′), δ_H 1.21 (6H, s, H-4″, H-5″)/δ_C 29.0. The location of the 3-OH-prenyl substituent at C-8 was evident from the shift to the downfield of the methylene group CH-1″ at δ_H 3.30/δ_C 22.2 caused by the electron attraction of the adjacent carbonyl group. By comparison of the NMR data of 3 with previously reported values [13, 16], the structure of 3 was determined as garcinone D.

Fuscaxanthone I (4) was isolated as a pale yellow solid, mp 104 - 105 °C. Its HR-ESI-MS data revealed a molecular formula of C_{23}H_{25}O_{5} through the [M + H]^+ protonated molecular peak at m/z 513.2482. Thus the molecular formula of compound 4 was the same with compound 1. In addition, the NMR spectra of 4 indicated the presence of two aromatic protons, a hydrated prenyl group and a hydrated geranyl group similar to those of 2, except for the disappearance of two equivalent methyl groups. The hydrated prenyl group was determined as a 4-hydroxy-3-methylbut-2-enyl group due to the HMBC correlations between singlet methylene protons resonating at δ_H 4.33 (2H, s, H-4′) with a methine carbon at δ_C 126.7 (C-2′), a tertiary unsaturated carbon at δ_C 135.2 (C-3′) and a methyl carbon at δ_C 23.2 (C-5′). The position of the 4-OH-prenyl was assigned at C-2 due to the long-range correlations between H-1′ (δ_H 3.39) and carbons C-1 (δ_C 161.5), C-2 (δ_C 110.6) and C-3 (δ_C 163.5) of the xanthone frame. The hydrated geranyl group was assigned as 3-hydroxy-3,7-dimethyloct-6-enyl due to the replacement of a doublet methylene group by a multiple one at higher field (δ_H 3.37/δ_C 21.9, CH-1′) and the HMBC correlations between these protons with a hydrated tertiary saturated carbon at δ_C 73.8 (C-3′). The location of the geranyl group at C-8 was confirmed by the HMBC cross peaks between H-1″ and C-8 (δ_C 139.8), C-7 (δ_C 144.8) and C-8a (δ_C 112.2) (Figure 2). Based on the analysis of the NMR and HR-ESI-MS data and comparison with reported data [17], compound 4 was assigned as fuscaxanthone I.

Compound 5 was isolated as colorless liquid. Its molecular formula, C_{23}H_{20}O_{2}, was determined by the protonated molecular peak at m/z 397.3111 [M + H]^+. The ^1H NMR of 5 revealed signals of two meta-coupled aromatic protons at δ_H 6.39 (1H, d, J = 3.0 Hz, H-5) and 6.49 (1H, d, J = 3.0 Hz, H-7). The NMR spectra of 5 also demonstrated characteristic signals of a farnesyl group, including three olefinic protons at δ_H 5.15 (1H, dt, J = 7.0, 2.0 Hz, H-11), 5.12 (1H, m, H-15) and 5.12 (1H, m, H-19), eight methylene groups at δ_H 2.70 (2H, dt, J = 7.0 Hz), 2.12 (2H, m), 2.09 (2H, m), 2.08 (2H, m), 2.00 (2H, m), 1.99 (2H, m), 1.77 (2H, m), 1.66 (1H, m) and 1.56 (1H, m); five singlet methyl groups at δ_H 2.14, 1.61, 1.61, 1.60, 1.27, and one doublet methyl group at δ_H 1.69 (3H, d, J = 1.0 Hz). The ^13C NMR of 5 exhibited signals of 12 carbons resonated at δ_C 112.7-147.8, indicated that compound 5 contained only one aromatic ring. The appearance of a hydrated tertiary saturated carbon suggested the presence of a heterocyclic ring. The signals of the farnesyl group and a –CH_{2}–CH_{2}– fragment (at δ_H 1.77 (2H, m, H-3) and 2.70 (2H, dt, J = 7.0, 2.0 Hz, H-4)) were confirmed based on correlations between protons and...
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carbons in the COSY and HMBC spectra. The location of the pyrano ring at C-4a and C-8a of the aromatic ring was assigned from the correlations between protons of one methylene group in the -CH₂-CH₂- fragment (δH 1.77) with three aromatic carbons at δC 121.3 (C-4a), 112.7 (C-5), 146.0 (C-8a). The farnesyl was determined to located at C-2 due to the correlations between methylene protons H-9 (at δH 1.66 (1H, m) and 1.56 (1H, m)) with an oxygenated carbon at δC 75.4 (C-2), a methyl carbon at δC 24.0 (C-25) and two methylene carbons at δC 31.4 (C-3), 22.2 (C-10) (Figure 2). On the basis of HR-ESI-MS, NMR data and comparison with reported values [18], compound 5 was identified as parvifoliol F.

Compounds 6 and 7 were isolated as white needles. Their NMR spectra and some of their physical properties, such as melting points and solubility, suggested that they were sterol and triterpenoid. The NMR data of 6 demonstrated characteristic signals of stigmasterol with an olefinic CH group at δH 5.35 (1H, m), two coupling olefinic CH groups at δH 5.15 (1H, dd, J = 8.5, 15.0 Hz, H-21)/δC 129.9 and δH 5.02 (1H, dd, J = 9.0, 15.5 Hz, H-20)/δC 138.9 and a hydrated CH group at δH 3.53 (1H, m, H-3)/δC 72.1. The NMR data of 7 revealed characteristic signals of lupeol with two inequivalent olefinic protons of the methylene group at δH 4.69 (1H, d, J = 2.0 Hz, H-29a), 4.57 (1H, d, J = 2.0 Hz, H-29e)/δC 109.3, a hydrated CH group at δH 3.19 (1H, dt, H-3)/δC 79.1, a doublet triplet proton at δH 2.38 (1H, dt, H-19) and a quaternary olefinic carbon at δC 150.2 (C-20). On the basis of the NMR data of compounds 6 and 7, and upon comparison the spectral data with those of previously reported data [19, 20], compound 6 and 7 were elucidated as stigmasterol and lupeol, respectively.

The in vitro antioxidant activities of the isolated xanthones were evaluated based on a scavenging activity study using the stable 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) free radical. Kaennacowanol A (2) and norcowanin (1) exhibited significant antioxidant activities, stronger than that of L-ascorbic acid (IC₅₀ 82.38 μM) with IC₅₀ values of 64.56±4.51 and 74.45±8.89 μM, respectively. Garcinone D (3) showed good activity with IC₅₀ value of 105.72±12.91 μM while fuscaxanthone I (4) did not show antioxidant activity in the ABTS assay. Notice that the IC₅₀ values of ascorbic acid may differ significantly in different antioxidant assay [21-23].

4. CONCLUSIONS

From our continuing phytochemical study on the latex of G. cowa collected in Quy Chau, Nghe An province, seven compounds 1-7 including four tetraoxygenated xanthones, one tocoptrienol, one sterol and one triterpenoid, were isolated by using various types of column chromatography with appropriate solvents. The xanthones were elucidated as norcowanin (1), kaennacowanol A (2), garcinone D (3) and fuscaxanthone I (4); the other compounds were assigned as a tocoptrienol: parvifoliol F (5), a sterol: stigmasterol (6) and a triterpenoid: lupeol (7) by analysis of 1D and 2D NMR spectroscopic data and by comparison with reported data. Among the isolated compounds, garcinone D (3), fuscaxanthone I (4) and parvifoliol F (5) were first isolated from G. cowa. Compound 3 showed good free radical scavenging against ABTS, while compounds 1 and 2 exhibited significant antioxidant activities with IC₅₀ values of 74.45 ± 8.89 μM and 64.56 ± 4.51 μM, respectively.

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Resources. **Tran T. Thu Thuy**: Conceptualization, Methodology, Investigation, Writing - Reviewing and Editing, Project administration.

**Declaration of competing interest.** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**REFERENCES**

1. Pattalung P., Thongtheeraparp W., Wiriyachitra P. and Taylor W. C. - Xanethones of *Garcinia cowa*, Planta Med 60 (4) (1994) 365-368.https://doi.org/10.1055/s-2006-959502.

2. Pham H. H. - Vietnamese plants, Youth Publishing House, 1999, pp. 450.

3. Ritthiwigrom T., Lapookhieo S., and Pyne S. - Chemical constituents and biological activities of *Garcinia cowa* Roxb, Maejo Int. J. Sci. Technol. 7 (2013) 212-231. https://core.ac.uk/download/pdf/26821264.pdf.

4. Fatma Sri W., Daud Ahmad Israf A., Nordin Hj L., Dachriyanus, Salau B. A., and Ashok Kumar J. - Anti-inflammatory activity of isolated compounds from the stem bark of *Garcinia cowa* Roxb, Pharmacognosy Journal 9 (1) (2017) 55-57. https://doi.org/10.5530/PJ.2017.1.10.

5. Panthong K., Hutadilok-Towatana N., and Panthong A. - Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*, Canadian Journal of Chemistry 87 (2009) 1636-1640. https://doi.org/10.1139/V09-123.

6. Likhitwitayuwuid K., Phadungcharoen T. and Krungkrai J. - Antimalarial xanthenes from *Garcinia cowa*, Planta Med. 64 (1) (1998) 70-72.https://doi.org/10.1055/s-2006-957370.

7. Auranwiwat C., Trisuwan K., Saiai A., Pyne S. G. and Ritthiwigrom T. - Antibacterial tetraoxygenated xanthenes from the immature fruits of *Garcinia cowa*, Fitoterapia 98 (2014) 179-183. https://doi.org/10.1016/j.fitote.2014.08.003.

8. Siridechakorn I., Phakhodee W., Ritthiwigrom T., Promgool T., Deachatay S., Cheenpracha S., Prawat U. and Lapookhieo S. - Antibacterial dihydrobenzopyran and xanthenoid derivatives from *Garcinia cowa* stem barks, Fitoterapia 83 (8) (2012) 1430-1434. https://doi.org/10.1016/j.fitote.2012.08.006.

9. Lapookhieo S., Cheenpracha S., Phakhodee W., Ritthiwigrom T., and Prawat U. - A new depsidone from the twigs of *Garcinia cowa*, Heterocycles 83 (2011) 1139. https://doi.org/10.3987/COM-11-12163.

10. Tian Z., Shen J., Moseman A.P., Yang Q., Yang J., Xiao P., Wu E., and Kohane I. S. - Dulxanthone A induces cell cycle arrest and apoptosis via up-regulation of p53 through mitochondrial pathway in HepG2 cells, Int J Cancer 122 (1) (2008) 31-38. https://doi.org/10.1002/ijc.23048.

11. Xu G., Kan W. L. T., Zhou Y., Song J. Z., Han Q. B., Qiao C. F., Cho C. H., Rudd J. A., Lin G., and Xu H. X. - Cytotoxic acylphloroglucinol derivatives from the twigs of *Garcinia cowa*, Journal of Natural Products 73 (2) (2010) 104-108. https://doi.org/10.1021/np9004147.
Xanthones and other compounds from the latex of Garcinia cowa

12. Kaennakam S., Siripong P., and Tip-Pyang S. - Kaennacowanols A-C, three new xanthones and their cytotoxicity from the roots of *Garcinia cowa*, Fitoterapia **102** (2015) 171-176. https://doi.org/10.1016/j.fitote.2015.03.008.

13. Xu Z., Huang L., Chen X. H., Zhu X. F., Qian X. J., Feng G. K., Lan W. J., and Li H. J. - Cytotoxic prenylated xanthones from the pericarps of *Garcinia mangostana*, Molecules **19** (2) (2014) 1820-1827. https://doi.org/10.3390/molecules19021820.

14. Nguyen T. K. A., Dinh T. H., Pham Q. L., and Tran T. T. T. - Tetraoxygenated xanthones from the latex of *Garcinia cowa* growing in Viet Nam, Vietnam Journal of Science and Technology **56** (5) (2018) 560-566. https://doi.org/10.15625/2525-2518/56/5/11826.

15. Saeed N., Khan M. R., and Shabbir M. - Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L., BMC Complement Altern Med. **12** (2012) 221. https://doi.org/10.1186/1472-6882-12-221.

16. Ragasa C., Tabin T. J., Reyes J. M. A., Carmen M., and Shen C. C. - Xanthones from *Garcinia mangostana* Linn. Pulp., Der Pharmacia Lettre **8** (20) (2016) 188-190. https://doi.org/10.1016/S0040-4020(01)98253-5.

17. Nguyen T. H. - Phytochemical and biological investigation of the bark of *Garcinia fusca* Pierre, Doctoral thesis in Faculty of Chemistry and Pharmacy, University of Regensburg, 2015. https://epub.uni-regensburg.de/32015/1/Dissertation-final-library.PDF.

18. Rukachaisirikul V., Naklue W., Phongpaichit S., Towatana N. H., and Maneenoon K. - Phloroglucinols, depsidones and xanthones from the twigs of *Garcinia parvifolia*, Tetrahedron **62** (36) (2006) 8578-8585. https://doi.org/10.1016/j.tet.2006.06.059.

19. Chaturvedula V. S. P. and Prakash I. - Isolation of stigmasterol and β-sitosterol from the dichloromethane extract of *Rubus suavissimus*, Chaturvedula and Prakash, International Current Pharmaceutical Journal **1** (9) (2012) 239-242. https://doi.org/10.3329/icpj.v1i9.11613.

20. Laghari A. H., Memon S., Nelofar A. and Khan K. M. - *Alhagi maurorum*: A convenient source of lupeol, Industrial Crops and Products **34** (2011) 1141-1145. https://doi.org/10.1016/j.indcrop.2011.03.031.

21. Ma Q., Xie H., Li S., Zhang R., Zhang M., and Wei K. M. - Flavonoids from the pericarps of *Litchi chinensis*, Journal of Agricultural and Food Chemistry **62** (2014) 1073-1078. https://doi.org/10.1021/jf405750p.

22. Vaijanathappa J., Badami S., and Bhojraj S. - *In vitro* antioxidant activity of *Enicostemma axillare*, Journal of Health Science **54** (5) (2008) 524-528. https://doi.org/10.1248/jhs.54.524.

23. Touré A. H., Xu X., Michel T., and Bangoura M. - *In vitro* antioxidant and radical scavenging of Guinean kinkeliba leaf (*Combretum micranthum* G. Don) extracts, Natural Product Research **25** (2011) 1025-1036. https://doi.org/10.1080/14786419.2010.482048.