Research Article

Biflavonoids from Root Barks of *Garcinia cowa* with Radical Scavenging Activity

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ABSTRACT

Plants of the Clusiaceae family have received considerable attention due to their availability of interesting secondary metabolites. We have previously collected a number of phytochemicals from the *Garcinia* species. In continuation of the search for new bioactive substances from Thai natural resources, we have found that the MeOH extract obtained from the root barks of *Garcinia cowa* Roxb., exhibited a significant antioxidative activity. Subsequently isolation, four compounds of the 3,8" linked biflavonoids were yielded and identified as (+) volkensiflavone (**1**), (+) morelloflavone (**2**), (+) spicataside (**3**), and (+) fukugiside (**4**). Their chemical structures were mainly elucidated by NMR data analysis and by comparison with the reported values. The antioxidant capacity of the isolates was tested using DPPH scavenging assay and compounds **2** and **4** exhibited strong activity with IC_{50} values of 8.85 and 19.65 μg/mL, respectively. The highest activity of compound **2** which was comparable to that of the positive control, revealed the importance of both phenolic hydroxyls at C-3 and C-4 on ring E of the biflavonoid framework.

Keywords: *Garcinia cowa*, biflavonoids, antioxidation

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Introduction

Garcinia is a genus of the family Clusiaceae found worldwide such as in tropical Asia, Africa, New Caledonia, Polynesia, and Brazil [1], of which Thailand exhibits a diversity of 29 species [2]. *Garcinia* plants are well known for their bioactive constituents. *Garcinia cowa* Roxb. or Cha-Muang in Thai has been used in food preparation and as traditional folk medicine. The barks are used as an antipyresis agent, fruits and leaves are used for the improvement of blood circulation, treatment of coughs, indigestion and as a laxative; the roots and latex are used for fever relief [2]. All plant parts: roots, stem barks, twigs, latex, leaves and fruits of *G. cowa* have been examined for their bioactive constituents, notably high content of xanthones, phloroglucinols, and flavonoids/biflavonoids [2]. For examples, phloroglucinol benzophenones and xanthones isolated from the leaves and roots inhibited nitric oxide production and α-glucosidase effects [3-4]; xanthones from the roots showed antibacterial activities against *B. cereus* and MRSA-SK1 [5] and cytotoxicity property towards KB and HeLa cells [6]; tetraoxygenated xanthones of the barks and latex were found to possess interesting antimalarial activity against *Plasmodium falciparum* [7-8] but poor radical scavenging action [9] and in addition biflavonoids of the twigs expressed good antioxidative activity [10]. To date, four biflavonoids were obtained from roots [5], twigs [10] and branches [11]. Therefore, phytochemical investigations of *G. cowa* root barks led to the isolation of four biflavonoids: volkensiflavone (**1**), morelloflavone (**2**), spicataside (**3**) and fukugiside (**4**) from the MeOH extract and compound **3** is reported for the first time from this plant species. The antioxidant property of the extracts and isolated compounds was also evaluated based on the principle of scavenging the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical.

Materials and Methods

General experimental procedures

Optical rotations were measured using a Jasco-1020 polarimeter using a 10 mm microcell in MeOH. 1D and 2D NMR experiments were recorded on Bruker NEO 500 FT-NMR spectrometer in dimethyl sulfoxide-d_6 (DMSO-d_6). Chemical shifts were reported using residual DMSO-d_6 (δ_H 2.49 and δ_C 39.52 ppm) as internal standard. IR spectra were obtained using a Perkin-Elmer UATR TWO spectrophotometer. Specific optical rotations were taken on a Jasco-1020 polarimeter. The spots were monitored using TLC sheet precoated with UV fluorescent Merck silica gel 60 F_{254} and were visualized under UV light of 254 nm, and upon staining with anisaldehyde–H_2SO_4 reagent followed by heating. Column chromatography was carried out using Silicycle silica gel 60 (< 0.063 mm) and Sephadex LH-20 (GE Health care).

Plant material

The root barks of *G. cowa* were collected from Chanthaburi Province, Thailand, in January, 2007. A voucher specimen has been deposited at the Laboratory of Natural Product Research Unit, Chemistry Department of Srinakharinwirot University.

Extraction and isolation

The air-dried root barks (530 g) were powdered and extracted with EtOAc (3 × 2.5 L) and then with MeOH (3 × 2.5 L) at room temperature for one week in each extraction and the filtered combined solution of each solvent extraction was evaporated to yield the EtOAc (brownish residue, 98 g) and MeOH (reddish brown sticky, 120 g) extracts, respectively. The MeOH extract exhibited stronger antioxidation activity was further chromatographed. It should be noted that an intense orange coloration upon staining with an anisaldehyde-H_2SO_4 reagent on TLC was the key biflavonoid detection during chromatographic process.

The MeOH extract (110 g) which gave TLC of many orange spots was hence chromatographed over silica gel (80 g) eluting with CH_2Cl_2, gradually increasing the polarity with MeOH to give 11 main fractions (M1–M11). Fraction M5 (1.7 g) was fractionated by column chromatography (CC) (silica gel, 80 g), employing *n*-Hexane–Acetone (92:8 to 0:100) to yield 9 sub-fractions (M5.1–M5.9). Sub-fractions of M5.4 and M5.5 (293 mg) were separated by a Sephadex LH-20 column using MeOH to afford volkensiflavone (**1**, 15 mg,) as a yellow solid. Fractions M6 (8.7 g) was subjected to CC eluting with *n*-Hexane–Acetone (60:40) to provide morelloflavone (**2**, yellow solid, 1.0 g). Fraction M8 (3.1 g) was purified by CC eluting with CH_2Cl_2–MeOH (96:5 to 0:100) to obtain 8 subfractions (M.8.1–M.8.9). Fraction M8.6 (1.1 g) was separated by CC (CH_2Cl_2-MeOH-H_2O, 8.5:1:0.5 to 6:3:1) to give 7 subfractions (M8.6.1– M8.6.7). Repeated CC of subfraction M8.6.5 (235 mg) eluting with CH_2Cl_2-MeOH-H_2O (8.5:1:0.5) afforded spicataside (**3**, 36 mg) as a pale brown solid. Fukugiside (**4**, yellow solid 0.75 g) was successfully yielded by repeated CC of fraction M10 (5 g) using a gradient of CH_2Cl_2-MeOH, 92:8 to 75:25).

Volkensiflavone (**1**): Yellow solid; $[\alpha]_D^{22}$ +165.5 (*c* 0.23, MeOH), lit [12] $[\alpha]_D^{25}$ +1.6; IR: ν_{max} 3175, 1634, 1605, 1574, 1504, 1422, 1361, 1236, 1158 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 1 and Table 2, respectively.

Morelloflavone (**2**): Pale yellow solid; $[\alpha]_D^{22}$ +234.8 (*c* 0.20, MeOH), lit [12] $[\alpha]_D^{20}$ +17; IR: ν_{max} 3199, 1638, 1600, 1578, 1509, 1361, 1258, 1160 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 1 and Table 2, respectively.

Spicataside (**3**): Pale brown solid; $[\alpha]_D^{23}$ +73.7 (*c* 0.20, MeOH), lit [13] $[\alpha]_D^{25}$ +1.0 (*c* 0.1, MeOH); IR: ν_{max} 3226, 1636, 1596, 1446, 1363, 1270, 1238, 1164, 1068 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 1 and Table 2, respectively.

Fukugiside (**4**): Yellow solid; $[\alpha]_D^{22.5}$ +96.4 (*c* 0.21, MeOH), lit [13] $[\alpha]_D^{25}$ +6.1 (*c* 0.1, MeOH); IR: ν_{max} 3264, 1641, 1598, 1518, 1448, 1368, 1258, 1167 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 1 and Table 2, respectively.

Table 1 ^{1}H NMR Spectroscopic Data for Compounds **1**—**4** in DMSO-d_6

No.	**1**	**2**	**3**	**4**
	δ_H Major, Minor (multiplicity, J in Hz)			
2	5.66 (d, 12.0), 5.53 (d, 12.3)	5.69 (d, 12.0), 5.87 (d, 12.1)	5.66 (d, 12.4), 5.75 (d, 11.8)	5.78 (d, 11.9), 5.79 (d, 12.3)
3	4.98 (d, 12.0), 4.97 (d, 12.3)	4.88 (d, 12.0), 4.97 (d, 12.1)	5.38 (d, 12.4), 5.01 (d, 11.8)	5.38 (d, 11.9), 4.89 (d, 12.3)
6	5.93 (s), 5.98 (d, 1.7)	5.96 (s), 6.05 (s)	5.97(s), 5.90 (br s)	5.95 (s), 5.91(s)
8	5.93 (s), 6.02 (d, 1.7)	5.96 (s), 5.96 (s)	6.04 (s), 5.90 (br s)	6.01 (s), 5.91 (s)
2'	7.09 (d, 8.4), 7.07 (d, 8.4)	7.13 (d, 7.8), 7.07 (d, 7.6)	7.21 (d, 7.8), 7.14 (d, 7.7)	7.17 (d, 8.1), 7.17 (d, 8.1)
3'	6.34 (d, 8.4), 6.59 (d, 8.4)	6.37 (d, 7.7), 6.57 (d, 7.6)	6.53 (d, 7.8), 6.31 (d, 7.7)	6.53 (d, 8.1), 6.34 (d, 8.1)
5'	6.34 (d, 8.4), 6.59 (d, 8.4)	6.37 (d, 7.7), 6.57 (d, 7.6)	6.53 (d, 7.8), 6.31 (d, 7.7)	6.53 (d, 8.1), 6.34 (d, 8.1)
6'	7.09 (d, 8.4), 7.07 (d, 8.4)	7.13 (d, 7.8), 7.07 (d, 7.6)	7.21 (d, 7.8), 7.14 (d, 7.7)	7.17 (d, 8.1), 7.17 (d, 8.1)
3"	6.63 (s), 6.75 (s)	6.57 (s), 6.61 (s)	6.84 (s), 6.72 (s)	6.64, (s), 6.68, (s)
6"	6.22 (s), 6.04 (s)	6.21 (s), 6.05 (s)	6.48 (s), 6.72 (s)	6.48, (s), 6.71, (s)
2"'	7.91 (d, 8.7), 7.59 (d, 8.6)	7.43 (br s), 7.23 (s)	7.66 (d, 8.2), 7.96 (d, 8.3)	7.20 (d 7.8), 7.43, (d, 8.3)
3"'	6.93 (d, 8.7), 6.62 (d, 8.6)		6.66 (d, 8.2), 6.94 (d, 8.3)	
5"'	6.93 (d, 8.7), 6.62 (d, 8.6)	6.89 (d, 8.1) 6.95 (d, 8.4)	6.66 (d, 8.2), 6.94 (d, 8.3)	6.90 (d, 7.8), 6.55 (t, 8.3)
6"'	7.91 (d, 8.7), 7.59 (d, 8.6)	7.43 (d, 8.1)	7.66 (d, 8.2), 7.96 (d, 8.3)	7.02 (d, 7.8), 7.43 (d, 8.3)
1""	-	-	4.75 (d, 7.3), 5.14 (d, 7.3)	4.74 (d, 6.9) 5.13 (d, 7.6)
2""	-	-	Obscured signal, 3.05 (m)	Obscured signal, 3.08 (m)
3""	-	-	Obscured signal	Obscured signal
4""	-	-	3.17 (m), 3.07 (m)	3.16 (m), 3.08 (m)
5""	-	-	Obscured signal	Obscured signal
6""	-	-	3.66 (m), 3.71 (m)	3.67 (d, 10.9)
5-OH	12.26 (s), 12.15 (s)	12.25 (s), 12.10 (s)	12.07 (s)	12.00 (s)
5"-OH	13.04 (s), 12.93 (s)	13.07 (s), 12.97 (s)	12.88 (s), 13.11 (s)	12.90 (s), 13.10 (s)

Table 2 ^{13}C NMR Spectroscopic Data for Compounds **1 - 4** in DMSO-d_6

No.	**1**	**2**	**3**	**4**
	δ_C Major, Minor			
2	81.0, 82.0	80.9, 81.8	82.3, 82.9	82.3, 80.9
3	48.3, 47.5	48.2, 47.3	46.9, 48.5	46.9, 48.6
4	196.5, 196.7	196.2,	196.8, 196.3	196.7, 195.2
4a	101.7, 101.9	101.5, 101.6	101.5, 101.6	101.5, 101.6
5	163.9	163.8, 163.9	163.0, 162.9	163.0, 162.9
6	95.3, 95.6	95.3	95.4, 94.9	95.3, 95.1
7	166.6, 167.3	160.5, 160.2	167.2, 166.5	163.8, 164.4
8	96.2, 96.5	96.2, 96.3	96.4, 96.1	96.4, 96.3
8a	161.2	166.6, 167.0	163.8	166.4, 167.2
1'	128.3, 127.9	128.1	126.7	126.8, 128.3
2'	128.6, 129.1	128.5, 128.8	129.7, 128.6	129.7, 128.7
3'	114.5, 114.7	114.4, 114.6	115.0, 114.3	115.0, 114.4
4'	157.4, 157.7	157.3, 157.6	157.7, 157.2	157.3, 157.8
5'	114.5, 114.7	114.4, 114.6	115.0, 114.3	115.0, 114.4
6'	128.6, 129.1	128.5, 128.8	129.7, 128.6	129.7, 128.7
2''	163.7, 164.0	163.4	164.0	164.4, 164.1
3''	102.4, 102.2	102.2	103.3, 103.1	103.0, 102.5
4''	181.9, 181.9	181.6	181.9	181.9
4a''	103.2, 103.2	103.1	104.9, 104.7	104.7, 104.9
5''	160.6, 160.4	163.8, 163.9	160.4, 160.4	160.4
6''	98.8, 98.2	98.6	98.8	98.3
7''	163.0, 163.0	162.8, 162.8	160.8, 160.7	160.8, 160.7
8''	100.9, 100.4	100.5, 100.0	102.2, 102.5	102.1, 103.3
8a''	155.5, 154.6	155.2, 154.6	153.6	153.8, 154.6
1'''	120.9, 121.3	121.0, 121.5	121.0, 120.6	120.9, 121.3
2'''	129.0, 128.3	113.3, 113.8	128.3, 129.0	113.4, 113.9
3'''	116.1, 115.8	145.6, 146.0	115.8, 116.0	146.2, 145.8
4'''	161.3	149.7	161.3, 161.5	150.1, 150.2
5'''	116.1, 115.8	116.1, 115.1	115.8, 116.0	115.4, 116.3
6'''	129.0, 128.3	119.3, 118.0	128.3, 129.0	118.3, 119.6
1''''			99.9, 100.1	100.1, 100.1
2''''			73.1, 73.6	76.8, 76.2
3''''			76.1, 76.8	73.2, 73.7
4''''			69.6	69.7
5''''			77.1, 77.4	77.4, 77.2
6''''			60.5	60.8, 60.6

DPPH scavenging assay

The DPPH radical scavenging activity of samples was determined by reaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals in a microplate format [14-15]. DPPH solution was prepared in ethanol. The plant extract at various concentrations was diluted with DMSO to get sample solution. 5 μL of sample solution was treated with 195 μL of 100 μM DPPH solution and reacted at room temperature for 30 min in dark. The absorbance was measured at 515 nm with a control containing DPPH and ethanol. Ascorbic acid (0.78-100 μg/mL) was used as a positive control. The ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated according to the equation [16]:

$$\text{DPPH radical scavenging activity (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample.

The half-maximal inhibitory concentration (IC_{50}) of the sample against DPPH radical was calculated based on the liner regression of the percentage of remaining DPPH radical against the sample concentration. Assays were performed in triplicate and results are shown as mean ± standard deviation. The difference significance was assessed using one-way ANOVA followed by Dunnett's test for individual differences using SPSS program version 25.

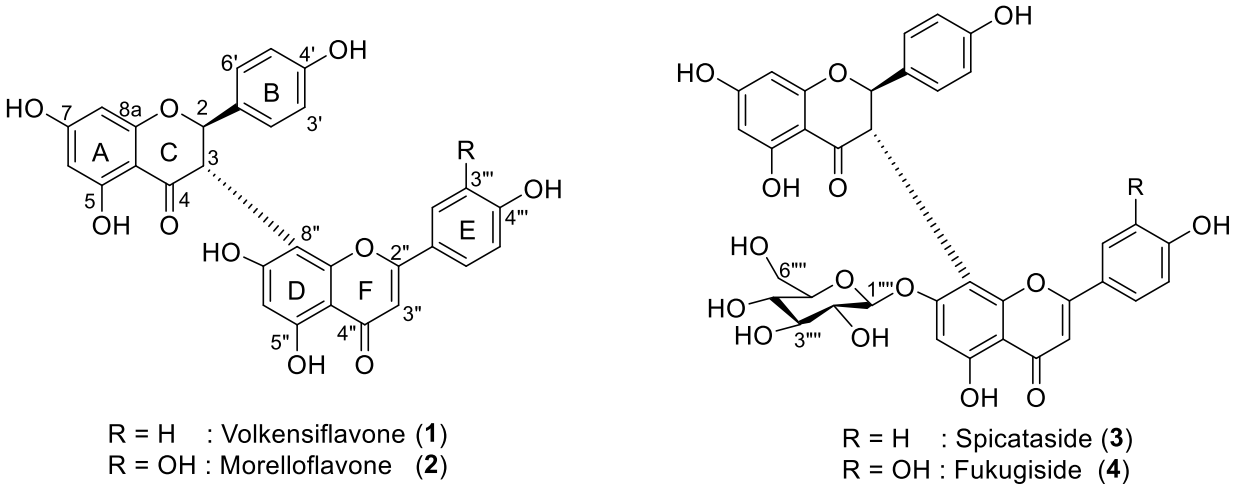

Figure 1 Chemical structures of compounds **1**–**4**.

Results and Discussion

The dried powder of the *G. cowa* root barks was thoroughly extracted with EtOAc and then with MeOH. The resulting fractions were screened for antioxidation activity using DPPH radical scavenging activity procedure and the MeOH extract, which found much interesting activity (Table 3) with IC_{50} of 47.40 μg/mL than the less polar EtOAc fraction (IC_{50} 182.01 μg/mL), was subjected to further investigation for bioactive substituents. Chromatographic separation of this MeOH soluble extract yielded four biflavonoids of the 3,8" linked type, **1**–**4** (Figure 1). Several common chemical and

spectroscopic characteristics are evident for the isolated compounds. They display an intense orange color with anisaldehyde–H_2SO_4 reagent on TLC. Their IR spectra show absorption bands for hydroxyl (3175–3264 cm^{-1}), conjugated carbonyl (1634–1641 cm^{-1}) and aromatic (1605–1574 cm^{-1}) functional groups. Their ^1H and ^{13}C NMR spectra exhibit two sets of resonances which is a characteristic of biflavonoid system and agree well with the published data for flavanone-flavone type [17].

Compound **1** was obtained as a yellow solid with $[\alpha]_D^{22}$ +165.5 (*c* 0.23, MeOH) and its IR data showed strong absorption bands at 3175 (OH), 1634 (conjugated CO), 1605 and 1574 (aromatic) cm^{-1}. The ^1H- and ^{13}C-NMR spectra recorded in DMSO-d_6 (Tables 1 and 2) exhibited two sets of signals in a relative 1.6:1 ratio. The ^{13}C NMR, DEPT and HMQC data offered the presence of 30 carbons attributable to 14 methines and 14 quaternary carbons including two conjugated carbonyl carbons at δ_C 196.5 and 181.9 ppm, suggesting of a biflavonoid system for **1**. Two sets of methine doublet at δ_H 5.66 (J = 12.0 Hz) and 4.98 (J = 12.0 Hz) on ring C were shown in the ^1H NMR spectrum including COSY correlations observed between these protons indicating the presence of a flavanone unit. By HMQC and HMBC correlations the aromatic protons (ring A) at δ_H 5.93 (H-6/8, s, 2H) was assigned to be located at C-6 (δ_C 95.3) and C-8 (δ_C 96.2) positions. The doublets of *ortho* coupled aromatic protons (rings B and E) appearing at δ_H 7.09 (H-2'/6', d, J = 8.4 Hz, 2H) and δ_H 6.34 (H-3'/5', d, J = 8.4 Hz, 2H) of ring B, together with δ_H 7.91 (H-2'''/6''', d, J = 8.7 Hz, 2H) and δ_H 6.93 (H-3'''/5''', d, J = 8.6 Hz, 2H) of ring E were attributed to the position C-2'/6' (δ_C 128.6), C-3'/5' (δ_C 114.5), C-2'''/6''' (δ_C 129.0), and C-3''',5''' (δ_C 116.1), respectively by HMQC connectivity. Two singlets at δ_H 6.63 and 6.22 were assigned to H-3'' and H-6'' of rings F and D, respectively, together with the two singlet signals of chelated hydroxy at δ_H 13.04 (OH-5$''$) and 12.26 (OH-5) were observed.

Connections among the rings A/B/C and D/E/F, including the linkage between flavanone and flavone units were provided by analysis of its HMBC and NOESY spectra (Figure 2). The NOESY correlations of methine proton at δ_H 5.66 (H-2) and δ_H 4.98 (H-3) to aromatic protons H-2'/6' (δ_H 7.09), as well as HMBC correlations of H-2 to C-2'/6' (δ_C 128.6), and of H-3 to C-2'/6' indicating of rings B/C connection. The HMBC correlation of the chelated hydroxy OH-5 and H-6 to C-4a (δ_C 101.7) confirming rings A/C connection. Connectivity between H-3'' (δ_H 6.63) to C-2''(δ_C 163.7); H-2'''/6''' (δ_H 7.91) to C-2'' (δ_C 163.7) and C-3'' (δ_C 102.4) in HMBC spectra together with NOESY correlations of H-3'' (δ_H 6.63) to H-2'''/6''' (δ_H 7.91) suggesting rings E/F link. The HMBC spectrum, correlations displayed from H-6'' to C-5'', and from OH-5'' to C-4a'' indicating of rings D/F connection. Furthermore, HMBC interactions seen between the methine protons at H-3 and C-8'', C-8a'' and C-7'' supporting the linkage of the flavanone and flavone subunits via C-3 and C-8'' positions.

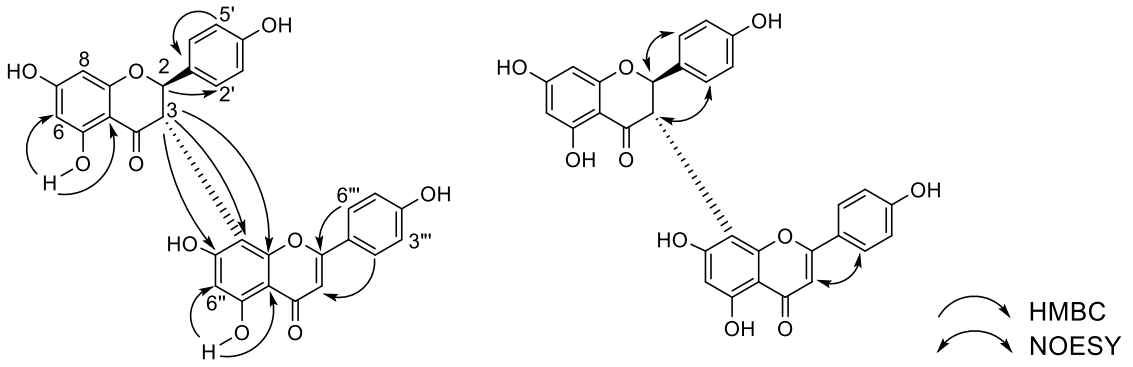

Figure 2 HMBC and NOESY correlations for volkensiflavone (**1**)

The chemical structures of the other biflavonoids **2**–**4** were also determined in the same manner as for **1**. Compounds **2**–**4** were isolated as yellow amorphous and their NMR data measured in DMSO-d_6 (as shown in Table 1 and Table 2) also revealed as two sets of resonances. By comparison of their IR and NMR spectroscopic data with the literature value and with those of compound **1** together with the extensive 1D- and 2D-NMR data analysis, compounds **2**–**4** shared the same flavanone-flavone scaffold as for **1** (Figure 1). In fact, compounds **3** and **4** are glucoside analogs of volkensiflavone (**1**) and morelloflavone (**2**), respectively. The metabolites **1**, **2** and **4** have been found previously from the same plant of *G. cowa* [10], whilst spicataside (**3**) was obtained from other *Garcinia* species [17-20].

The relative configuration of the two stereogenic centers at C-2 and C-3 was suggested from the large coupling constant value of 11.9–12.4 Hz presented between H-2 and H-3 in the ^{1}H NMR spectra of the flavanone unit in compounds **1**–**4** indicated for their *trans* diaxial arrangement [19, 21]. Furthermore, the positive specific optical rotation values for **1**–**4** (**1** : $[\alpha]_D^{22}$ +165.5; **2** : $[\alpha]_D^{22}$ +234.8; **3** : $[\alpha]_D^{23}$ +73.7; and **4** : $[\alpha]_D^{22.5}$ +96.4) are in good agreement with earlier reports. All isolated metabolites are therefore characterized as (+) volkensiflavone (**1**), (+) morelloflavone (**2**), (+) spicataside (**3**), and (+) fukugiside (**4**).

The antioxidant activity of the two extracts and compounds **1**–**4** was evaluated by DPPH radical scavenging activity assay and compared with those of well-known antioxidant, ascorbic acid. Bioactive xanthones and biflavonoids are main contents in the respective less polar (EtOAc) and more polar (MeOH) soluble fractions of *Garcinia* extracts [2-12]. The xanthone constituents were reported as weak radical scavenging substances [9]. As shown in Table 3, the MeOH extract expressed approximately 4-time more potent than that of the less polar one (IC_{50} 182.01 μg/mL). Morelloflavone (**2**) displayed the highest effect with IC_{50} 8.85 μg/mL and the activity of which was comparable to that of the positive control, ascorbic acid, followed by **4** (IC_{50} 19.65 μg/mL). From the preliminary SAR observations, the absence of a phenolic hydroxyl at C-3 on ring E of these biflavonoid systems suggested for the weak activity for compounds **1** and **3**. Thus, both hydroxyls at positions 3 and 4 on ring E enhance the activity.

Table 3 The antioxidant activity of the extracts and compounds determined with DPPH radical scavenging. Results are expressed as mean ± SD (n = 2-3)

Sample	DPPH radical scavenging activity IC_{50} (μg/mL)
Ascorbic acid	6.24 ± 0.86
EtOAc extract	182.01 ± 81.49*
MeOH extract	47.40 ± 15.12
Volkensiflavone (**1**)	>100
Morolelllavone (**2**)	8.85 ± 3.00
Spicataside (**3**)	> 100
Fukugiside (**4**)	19.65 ± 1.24

*Indicates that this value is significantly different compared with the positive control (ascorbic acid) at $p < 0.05$.

Conclusions

We discovered that the MeOH extract of *G. cowa* root barks is a good source of antioxidant compounds. Four biflavonoids of the 3,8" linked type were obtained and two of them were strong antioxidant agents. Compound **2** exhibited the strongest activity which was comparable to that of the positive control, ascorbic acid.

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References

1. Santo BLSE, Santana LF, Junior WHKJ, Araújo FO, Bogo D, et al. Medicinal potential of *Garcinia* species and their compounds. Molecules. 2020;25:4513-42.
2. Ritthiwigrom T, Laphookhieo S, Pyne SG. Chemical constituents and biological activities of *Garcinia cowa* Roxb. Maejo Int J Sci Technol. 2013;7(2):212-31.

3. Raksat A, Phukhatmuen P, Yang J, Maneerat W, Charoensup, R., Andersen, R.J., et al. Phloroglucinol benzophenones and xanthones from the leaves of *Garcinia cowa* and their nitric oxide production and α-glucosidase inhibitory activities. J Nat Prod. 2020;83:164-8.
4. An NTK, Hien NV, Thuy NT, Phuong DL, Bach HG, Tra NT., et. al. Garcicowanones C-E, three new hydrated-geranylated xanthones from the roots of *Garcinia cowa* Roxb. ex Choisy, and their α-glucosidase inhibition activities. Nat Prod Res. 2022;1-9.
5. Tayana N, Suteerapataranon S, Deachathai S. Phytochemistry and bioactive compounds from *Garcinia cowa* roxb. Asia-Pac J Sci Technol. 2017;22(03):1-8.
6. Kaennakam S, Siripong P, Tip-pyang S. Kaennacowanols A–C, three new xanthones and their cytotoxicity from the roots of *Garcinia cowa*. Fitoterapia. 2015;102:171-6.
7. Likhitwitayawuid K, Phadungcharoen T, Krungkrai J. Antimalarial xanthones from *Garcinia cowa*. Planta Med. 1998;64:70-2.
8. An NTK, Ha DT, Long PQ, Thuy TTT. Tetraoxygenated xanthones from the latex of *Garcinia cowa* growing in Vietnam. Vietnam J Sci Techno. 2018;56(5):560-6.
9. Mahabusarakam W, Chairerk P, Taylor WC. Xanthones from *Garcinia cowa* Roxb. latex. Phytochemistry. 2005; 66:1148-53.
10. Panthong K, Hutadilok-Towatana N, Panthong A, Cowaxanthone F. A new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. Can J Chem. 2009;87:1636-40.
11. Shen J, Yang Y. Chemical constituents of branch of Garcinia cowa Roxb. Zhongcaoyao. 2007; 38:993-4.
12. Chen FC, Lin YM, Hung JC. Phenolic compounds from heartwood of *Garcinia multiflora*. Phytochemistry. 1975;14:300-3.
13. Brusotti G, Papetti A, Serra M, Temporini C, Marini E, Orlandini S, et al. *Allanblackia floribunda* Oliv: An aphrodisiac plant with vasorelaxant properties. J Ethnopharmacol. 2016; 192:480-5.
14. Gerhäuser C, Klimo K, Heiss E, Neumann I, Gamal-Eldeen A, Knauft J, et al. Mechanism-based in vitro screening of potential cancer chemopreventive agents. Mutat Res. 2003;523:163-72.
15. Van Amsterdam, F.T.M., Roveri, A., Maiorino, M., Ratti, E. & Ursini, F. Lacidipine: a dihydropyridine calcium antagonist with antioxidant activity. Free Radic Biol Med. 1992; 12:183-7.
16. Adebiyi OE, Olayemi FO, Ning-Hua T, Guang-Zhi Z. *In vitro* antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*. Beni-Suef Univ J Basic Appl Sci. 2017;6:10-4.
17. Jamila N, Khairuddean M, Khan S, Khan N. Complete NMR assignments of bioactive rotameric (3⟶8) biflavonoids from the bark of *Garcinia hombroniana*. Magn Reson Chem. 2014; 52:345-52.

18. Chen FC, Lin YM, Hung JC. New biflavanone glucoside from *Garcinia multiflora*. Phytochemistry. 1975;14:818-20.
19. Osorio E, Londoño J, Bastida J. Low-density lipoprotein (LDL)-antioxidant biflavonoids from *Garcinia madruno*. Molecules. 2013;18:6092-100.
20. Salleh WMNHW, Sazali, NSAN, Ahmad F, Taher M. Biflavonoids from the leaves and stem bark of *Garcinia griffithii* and their biological activities. Marmara Pharm J. 2017;21(4):889-7.
21. Mountessou BYG, Tchamgoue J, Dzoyem JP, Tchuenguem RT, Surup F, Choudhary, MI, et al. Two xanthones and two rotameric (3⟶8) biflavonoids from the cameroonian medicinal plant *Allanblackia floribunda* Oliv. (Guttiferae). Tetrahedron Lett. 2018;59:4545-50.