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Isolation and evaluation of the antioxidant activity of phenolic constituents of the *Garcinia brasiliensis* epicarp

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ABSTRACT

A new glycosylated biflavonone, morelloflavone-4‴-*O*-*β*-D-glycosyl, and the known compounds 1,3,6,7-tetrahydroxyxanthone, morelloflavone (fukugetin) and morelloflavone-7″-*O*-*β*-D-glycosyl (fukugeside) were isolated from the epicarp of *Garcinia brasiliensis* collected in Brazil. The structures of these compounds were established using ^{1}H and ^{13}C NMR, COSY, *g*HMQC and *g*HMBC spectroscopy. The compounds exhibited antioxidant activity. The greatest potency was displayed by morelloflavone (**2**), with IC_{50} = 49.5 mM against DPPH and absorbance of 0.583 at 400 μg/mL for the reduction of Fe^{3+}. The weakest potency was displayed by 1,3,6,7-tetrahydroxyxanthone (**1**), with IC_{50} = 148 mM against DPPH and absorbance of 0.194 at 400 μg/mL for the reduction of Fe^{3+}.

1. Introduction

The vast biodiversity of plants found in Brazil may be an important source of new pharmaceutical agents (Basso et al., 2005). *Garcinia*, also known as *Rheedia*, is the most numerous genus of the family Guttiferae, also called Clusiaceae, a large family of medicinal plants that are common in Brazil. The Guttiferae comprise 47 genera (*Vismia*, *Garcinia*, *Clusia*, *Cratoxylum*, *Harungana*, *Mesua*, *Hypericum*, *Kielmeyera*, among others) with more than 1000 species widely distributed in tropical Asia, Africa, New Caledonia, Polynesia and Brazil (Piccinelli et al., 2005).

Several members of the Guttiferae family are used in Brazilian traditional medicine to cure various ailments. They contain a wide variety of biologically active metabolites, such as anthraquinones, flavonoids, xanthones, benzophenones and phloroglucinols (Oliveira et al., 2005). This range of chemical constituents gives rise to several interesting pharmacological properties, including antidepressant activity in *Kielmeyera coriacea* (Zagoto et al., 2006), antibacterial activity in *Rheedia brasiliensis* (Almeida et al., 2008), cytotoxic activity in *Cratoxylum formosum* (Boonsri, Karalai, Ponglimanont, Kanjana-Opas, & Chantrapromma, 2006), antimalarial and antioxidant activity in *Mammea longifolia* (Rao, Ono, Ohnishi-Kameyama, & Yoshida, 2004), anti-aflatoxigenic activity in *Garcinia cowa* and *Garcinia pedunculata* (Joseph, Jayaprakasha, Selvi, & Jena, 2005) and trypanocidal activity in *Allanblackia monticola* and *Symphonia globulifera* (Lenta et al., 2007). Several chemical studies have shown that the genus *Garcinia* is an important source of biologically-active secondary metabolites, such as polyprenylated benzophenones, flavonoids and proanthocyanins, that have proven activity against diseases, such as peptic ulcer, urinary tract infections and tumours (Derogis et al., 2008).

The species *Garcinia brasiliensis* (Mart.), also known as *R. brasiliensis* Planch and Triana, is native to the Amazon region and is cultivated throughout Brazil. In Brazil, it is popularly known as bacuri, bacupari, porocó and bacuripari, and in Bolivia, it is called

Abbreviations: EAEE, ethyl acetate epicarp extract; BHT, butylhydroxytoluene; NMR, nuclear magnetic resonance; gHMQC, gradient-assisted heteronuclear multiple quantum coherence, detection of short range (^{1}J) correlations between directly connected ^{1}H and ^{13}C nuclei; *g*HMBC, gradient-assisted heteronuclear multiple bond coherence, detection of long distance (^{2}J and ^{3}J) correlations between ^{1}H and ^{13}C nuclei connected by a series of bonds; COSY, ^{1}H–^{1}H homonuclear correlation spectroscopy; UV, ultraviolet; IR, infrared; MS, mass spectrometry; DPPH, 1-diphenyl-2-picrylhydrazyl; HSCCC, high speed countercurrent chromatography; IC_{50}, 50% inhibitory concentration; CTLC, comparative thin-layer chromatography; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; *β*, internal measurement equipment.

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guapomo. It is used by the population for anti-inflammatory (Castardo et al., 2008; Santa-Cecília et al., 2011), antinociceptive (Santa-Cecília et al., 2011), antioxidant and antitumour (Coelho et al., 2008) therapies. In Thailand, Sri Lanka, Malaysia, the Philippines and India, ripe fruits are used in traditional medicine to treat abdominal pain, diarrhoea, dysentery, wound infections, suppuration and chronic ulcer (Cui et al., 2010).

As part of a bioprospecting program seeking to identify new plant metabolites with antioxidant activity, this paper reports the identification and the evaluation of the antioxidant activities of the main phenolic constituents of the epicarp of *G. brasiliensis*. Four compounds were isolated by extraction of the epicarp with ethyl acetate: 1,3,6,7-tetrahydroxyxanthone (**1**), morelloflavone (**2**), morelloflavone-7″-*O*-*β*-D-glycoside (fukugeside) (**3**) and the novel compound morelloflavone-4‴-*O*-*β*-D-glycoside (**4**) (Fig. 1). The occurrence of xanthone (**1**), morelloflavone (**2**), and the biflavonoid fukugeside (**3**) in *G. brasiliensis* is consistent with the compounds reported in other *Garcinia* species, such as *Garcinia garderiana* (Botta, Mac-Quhae, Delle, Delle-Monache, & De Mello, 1984; Castardo et al., 2008; Luzzi et al., 1997; Rodrigues et al., 2000), *Garcinia mangostana* (Carpenter, Locksley, & Scheinmann, 1969) and *Garcinia morella* (Karanjgaokar, Radhakrishnan, & Venkatarama, 1967). Thus, the isolation of compounds **1**, **2** and **3** from the species *G. brasiliensis* indicates that these compounds may be considered as chemotaxonomic markers for the genus *Garcinia*. The structures of the isolated compounds were elucidated using IR, MS, ^{1}H and ^{13}C NMR spectroscopy and by comparison with data from the literature.

2. Materials and methods

2.1. General procedures

The extracts and fractions were concentrated using a rotary evaporator under reduced pressure at 45 °C. The ethyl acetate extract was purified by column chromatography (CC), using silica gel 60 [230–400 mesh (0.200–0.360 nm), Merck®] as the stationary phase, eluted with increasing polarity mixtures of *n*-hexane/ethyl acetate and ethyl acetate/ethanol. Comparative thin-layer chromatography (CTLC) experiments employed an aqueous suspension of silica gel PF 254 7749 (Merck®), supported on glass plates. The substances were stained with iodine vapour, vanillin-sulphuric acid (3%) reagent or 1% $FeCl_3$ in ethanol and visualised using ultra-violet radiation (λ = 254 and 366 nm).

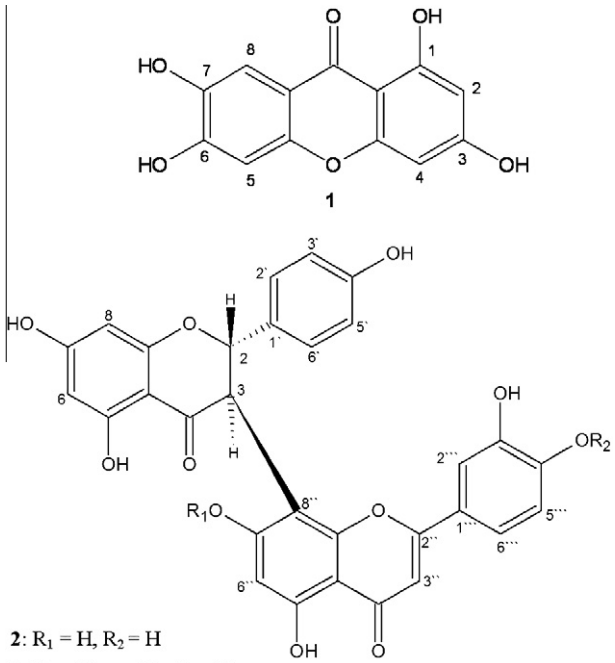

Fig. 1. Structures of 1,3,6,7-tetrahydroxyxanthone (**1**) and biflavonoids morelloflavone (**2**), morelloflavone-7″-*O*-*β*-D-glycoside (**3**) and morelloflavone-4‴-*O*-*β*-D-glycoside (**4**).

High speed countercurrent chromatography (HSCCC) was performed on a PC chromatograph equipped with a 130 × 1.6 mm internal diameter polytetrafluoroethylene (PTFE) column (PC Inc., Potomac, MD). The value of β ranged from 0.5 at the inner part of the column to 0.85 at the outside of the column. The total volume of the column was 325 mL. The column was rotated at 850 rpm. Samples were introduced using a 16-mL loop injector (PC Inc.) with the aid of a Waters (Milford, MA) pump. Melting points (in °C) were determined using a Mettler melting point apparatus (Mettler–Toledo, Leicester, UK). Absorption spectra in the ultraviolet region were collected with a Shimadzu-2550 dual beam UV–visible spectrophotometer (Shimadzu, Kyoto, Japan), as described by Mabry, Markham, and Thomas (1970), with modifications. The phenolic constituents were dissolved in ethanol (0.1%) and analysed by scanning over the range λ = 500–200 nm, both before and after the addition of $AlCl_3$ and HCl, or NaOAc and H_3BO_3. Absorption spectra in the infrared region (IR) were obtained with a Prestige-21 spectrometer (Shimadzu) using KBr pellets. The ^{1}H and ^{13}C NMR spectra were collected on a 400 MHz Bruker AVANCE DRX spectrometer (Bruker Biospin, Rheinstetten, Germany). The *g*HMQC, *g*HMBC and COSY contour maps were collected on a 500 MHz Varian (Palo Alto, CA) spectrometer equipped with a *Z*-axis gradient multinuclear probe. Tetramethylsilane (TMS) was used as an internal reference for all NMR experiments. The molecular masses of the compounds were determined using the positive ionisation mode in MALDI-TOF mass spectrometry (Microflex LT, Bruker), using alpha-cyano-4-hydroxycinnamic acid as the matrix. The *in vitro* antioxidant activity experiments were monitored by UV–visible spectrophotometry using a dual beam Shimadzu-2550 instrument. The radical-scavenging experiment was observed at λ = 517 nm, and the reducing power experiment was observed at λ = 700 nm.

2.2. Plant material

G. brasiliensis Mart. fruits were collected from the campus of the Federal University of Viçosa-MG, Brazil, in February (summer) of 2010. The botanical identification of the samples was confirmed by Dr. João Augusto Alves Meira Neto of the Horto Botânico of the Federal University of Viçosa. A voucher specimen (number VIC2604) was deposited at the Herbarium of the Federal University of Viçosa.

2.3. Preparation of extracts

Epicarps from *G. brasiliensis* fruit were air-dried at 40 °C for 8 days with continuous moisture monitoring. After the material was completely dry, it was pulverised in a knife grinder, producing 1052 g of ground sample. The dried, ground epicarps were subjected to exhaustive extraction in a Soxhlet apparatus using an increasing polarity solvent system, with *n*-hexane and ethyl acetate as solvents for 24 h each. The extracts were then concentrated at reduced pressure, yielding 60.2 g of hexane epicarp extract (EHE) and 102.2 g of ethyl acetate epicarp extract (EAEE).

2.4. Purification and isolation of chemical constituents

The chemical analysis of the EHE fraction, which contains the polyprenylated benzophenones 7-epiclusianone and garciniaphenone, has been previously reported (Derogis, 2008).

This paper reports the chemical analysis of the EAEE fraction. A 34.9-g portion of EAEE was separated by silica column chromatography, eluted with gradient polarity mixtures of hexane/ethyl acetate/acetic acid. A total of 143 fractions (250 mL each) were collected and sorted into five groups [G1 (4.68 g), G2 (5.58 g), G3 (7.25 g), G4 (6.43 g) and G5 (7.24 g)] by CTLC analysis. Each group was further purified by high-speed countercurrent chromatography (HSCCC), using methods described by Conway and Theory (1989). The best separation, with good distribution and a distribution constant near 1, was obtained using a hexane/ethyl acetate/methanol/water (1:1:1:1 v/v/v/v) solvent system. The solvents were mixed in a separating funnel and left to stand for twelve hours before being saturated. The lower phase was used as the stationary phase, and the upper phase was used as the mobile phase. The mobile phase was pumped in the tail → head direction of the column, using a flow rate of 1 mL/min and a column rotation of 850 rpm. The initial volume of stationary phase was 60 mL, and 81.54% of the stationary phase remained within the column after the initial loading. Next, 600 mg of each sample group was dissolved in 16 mL of a 1:1 mixture of the upper and lower phases of the solvent system. The mixtures were filtered through cotton and injected into the loop of the apparatus with a syringe. Fractions were collected from 3 mL of each group. The content of the HSCCC fractions was analysed by CTLC. Fractions 32–33 from group 2 were completely pure, and analysis of IR, ^{1}H NMR, COSY, *g*HMQC and *g*HMBC spectra led to the identification of 1,3,6,7-tetrahydroxyxanthone (**1**). This compound was a yellow crystalline powder (76 mg, 47.5% yield by mass from the initial injection). Analysis of the IR, ^{1}H NMR, COSY, *g*HMQC and *g*HMBC spectra of fractions 39–126 from group 3, fractions 106–127 from group 4 and fractions 110–128 from group 5 and comparison with previous literature reports (Elfita et al., 2009) led to the identification of the biflavonoid morelloflavone (**2**, yellow crystalline solid, 137 mg, 85.7% yield by mass from the initial injection) and the glycosylated biflavonoids morelloflavone-7″-*O*-*β*-D-glycoside (**3**, yellow solid, 92 mg, 57.5% yield by mass from the initial injection) and morelloflavone-4‴-*O*-*β*-D-glycoside (**4**, yellow crystalline solid, 30 mg, 18.75% yield by mass from the initial injection), **4** being isolated for the first time in *G. brasiliensis*.

2.5. Characterisation

2.5.1. Spectroscopic data

Morelloflavone-4‴-*O*-*β*-D-glycoside (**4**). Yellow crystalline solid. UV (ETOH, 0.1%) $\lambda_{máx/nm}$: 375, 265. IR (KBr) $\nu_{máx/cm^{-1}}$: 3405 (O–H); 1601 and 1518 (C=C); 1261 (C–O); 1725 and 1643 (C=O); 836 (C–H). MALDI-TOF/MS: *m/z* 761.2 [**4** + Ca + 2H]. ^{1}H NMR (DMSO-d_6, 400 MHz, *δ*-ppm): 5.89 (d, 1H, *J* = 12.61 Hz, H-2); 4.93 (d, 1H, *J* = 12.61 Hz, H-3); 13.08 (s, 1OH, OH-5); 5.94 (d, 1H, *J* = 4.6 Hz, H-6); 6.53 (s, 1H, *J* = 5 Hz, H-8); 6.63 (dd, 2H, *J* = 7.6 Hz, H-2′/6′); 7.15 (dd, 2H, *J* = 7.97 Hz, H-3′/5′); 6.76 (s, 1H, H-3″); 12.12 (s, 1OH, OH-5″); 6.59 (s, 1H, H-6″); 7.32 (s, 1H, H-2‴); 6.97 (d, 1H, *J* = 8.1 Hz, H-5‴); 7.49 (d, 1H, *J* = 8.1 Hz, H-6‴); 4.85 (d, 1H, *J* = 7.13 Hz, H-1⁗); 3.41 (m, 1H, H-2⁗); 4.37 (m, 1H, H-3⁗); 4.1 (m, 1H, H-4⁗); 3.75 (m, 1H, H-5⁗); 3.28 (m, 1H, H-5⁗); 12.01 (m, OH-7⁗). ^{13}C NMR (DMSO-d_6, 100 MHz, *δ*-ppm): 82.6 (CH, C-2); 49.1 (CH, C-3); 198.0 (C=O, C-4); 162.0 (C–OH, C-5); 98.1 (CH, C-6); 164.1 (C–OH, C-7); 97.6 (CH, C-8); 166.0 (C, C-9); 102.8 (C, C-10); 129.8 (C, C-1′); 128.6(CH, C-2′/C-6′); 115.7 (CH, C-3′/C-5′); 158.7 (C-OH, C-4′); 170.6 (C, C-2″); 104.1 (CH, -3″); 183.5 (C=O, C-4″); 158.3 (C–OH, C-5″); 98.2 (CH, C-6″); 161.1 (C–OH, C-7″); 83.5 (C, C-8″); 153.3 (C, C-9″); 104.6 (C, C-10″); 119.2 (C, C-1‴); 114.6 (CH, C-2‴); 145.4 (C–OH, C-3‴); 148.7 (C–O, C 4‴); 117.2 (CH, C-5‴); 121.0 (CH, C-6‴); 100.1 (CH, C-1⁗); 73.0 (CH, C-2⁗); 77.0 (CH, C-3⁗); 62.1 (CH, C-4⁗); 77.1 (CH, C-5⁗); 71.0 (CH, C-6⁗).

2.6. Evaluation of antioxidant activity

2.6.1. DPPH radical-scavenging activity

The ability of compounds **1**–**4** to scavenge DPPH free radicals was evaluated according to the method of Hatano, Kagawa, Yasuhara, and Okuda (1998). A concentration series (25, 50, 100, 200 and 400 μg/mL in ethanol) of each compound was prepared. A 4-mL aliquot of sample solution was mixed with 1 mL of DPPH (0.5 mM in ethanol). This mixture was vigorously shaken at room temperature for 30 min. The absorbance of the mixture was then measured at 517 nm. A low absorbance value indicates effective free radical scavenging. Each solution was analysed in triplicate, and the average values were plotted to obtain the IC_{50} against DPPH by linear regression. The activity of ascorbic acid, a recognised antioxidant, was used as a standard over the same range of concentrations. The radical-scavenging activity was evaluated as the percentage of inhibition according to the following equation:% inhibition = [(absorbance of control − absorbance of sample)/absorbance of control)] × 100.

2.6.2. Evaluation of reducing power

The reducing power of compounds **1–4** was evaluated according to the method of Yen and Chen (1995), with modifications. A concentration series (25, 50, 100, 200 and 400 μg mL^{-1} in ethanol) of each compound was prepared. A 25-mL test tube was loaded with 1.0 mL of sample solution, 2.5 mL of phosphate buffer (2 M, pH 6.6) and 2.5 mL of 1% (m/v) $K_3[Fe(CN)_6]$. The mixture was incubated at 45 °C for 20 min. Next, 2.5 mL of trichloroacetic acid (10% m/v) were added, and the solution was centrifuged at 4000 rpm for 15 min. A 2.5-mL aliquot of the supernatant was mixed with 2.5 mL of ultra-pure water and 0.5 mL of ferric chloride (0.1%). The absorbance of this mixture was measured at 700 nm. A greater absorbance value indicates greater reducing power. Each solution was analysed in triplicate, and the average values were plotted to obtain the IC_{50} of Fe^{3+} reduction by linear regression. The activities of solutions of ascorbic acid and BHT were used as normalisation standards.

3. Results and discussion

Compounds **1–4** were isolated from the ethyl acetate extract of *G. brasiliensis* epicarp by HSCCC. This type of chromatography is employed to separate very polar substances, which may be lost or obtained in low yield when separation by other chromatography techniques is attempted, due to irreversible adsorption onto usual stationary phases such as silica. Compound **1** was identified as 1,3,6,7-tetrahydroxyxanthone, based on the comparison of mass and NMR data with data from the literature (Holloway & Scheinmann, 1975). Compound **2** was identified as the biflavonoid morelloflavone (fukugetin) by comparing UV, IR, ^{1}H and ^{13}C NMR spectra with data from the literature (Elfita et al., 2009). Compound **3** showed ^{1}H and ^{13}C NMR signals similar to those obtained for compound **2**; however, additional signals consistent with a glucose residue were also clearly present. Detailed analysis of ^{1}H,^{1}H-COSY and ^{1}H,^{13}C *g*HMBC and *g*HMQC correlations allowed the signal at *δ* 161.1 to be assigned to the C-7″ position. This signal exhibited a long-range correlation with the signal of the anomeric proton H-1⁗ at *δ* 4.81, showing the position of the glycosidic linkage. These data are consistent with compound **3** being morelloflavone-7″-*O*-*β*-D-glycoside (fukugeside), which has been reported previously in *Garcinia xanthochymus*, *Garcinia spicata* and *Garcinia atroviridis* (Baggett et al., 2005; Konoshima & Ikeshiro, 1970; Permana et al., 2003).

The biflavonoid morelloflavone-4‴-*O*-*β*-D-glycoside (**4**) was obtained by recrystallisation from methanol as a yellow crystalline

Table 1
^{1}H and ^{13}C NMR resonances of compound (**4**) (DMSO-d_6, 400 and 100 MHz).

Position	δ_C[a,b]	δ_H, *m*, *J* [a]	*g*HMQC ($^1J_{CH}$)	*g*HMBC	
				($^2J_{CH}$)	($^3J_{CH}$)
2	82.0	5.89; *d*; (12.61)	H-2	H-3	–
3	49.9	4.93; *d*; (12.61)	H-3	H-2	–
4	195.6	–	–	H-3	H-2
5	63.0	13.08	–	H-6	–
6	97.9	5.94; *d*; (4.6)	H-6	–	H-8
7	165.8	13.94	–	H-8; H-6	–
8	96.0	6.53; *d*; (5)	H-8	–	H-6
9	162.6	–	–	H-8	H-2
10	103.3	–	–	–	H-8; H-6
1′	128.9	–	–	–	H-3′
2′	116.2	6.63; *dd*; (7.6)	H-2′/6′	H-3′	–
3′	127.0	7.15; *dd*; (7.97)	H-3′/5′	–	–
4′	158.7	10.10	–	H-3′	–
5′	127.0	7.15; *dd*; (7.97)	H-3′/5′	–	H-3′
6′	116.2	6.63; *dd*; (7.6)	H-2′/6′	–	–
2″	164.5	–	–	H-3″	–
3″	102.2	6.76; *s*	H-3″	–	–
4″	163.5	–	–	H-3″	–
5″	161.7	12.12	–	H-6″	–
6″	102.9	6.59; *s*	H-6″	–	–
7″	160.7	13.74	–	H-6″	H-3
8″	103.9	–	–	H-3	H-2; H-6″
9″	153.6	–	–	–	H-2; H-3
10″	105.7	–	–	–	H-3″; H-6″
1‴	120.5	–	–	–	H-3″; H-5‴
2‴	114.5	7.32; *s*	H-2‴	–	–
3‴	147.9	12.64	–	–	H-5‴
4‴	154.5	–	–	H-5‴	H-4‴
5‴	118.4	6.97; *d*; (8.1)	H-5‴	–	–
6‴	122.6	7.48; *d*; (8.1)	H-6‴	H-5‴	–
1⁗	100.0	4.85; *d*; (7.13)	H-1⁗	–	–
2⁗	63.0	3.41; *m*	H-2⁗	–	–
3⁗	63.0	4.37; *m*	H-3⁗	–	–
4⁗	62.0	4.1; *m*	H-4⁗	–	–
5⁗	57.0	3.75; *m*	H-5⁗	–	–
6⁗	70.0	3.28; *m*	H-6⁗	–	–
7⁗	–	12.01; *m*	–	–	–

[a] δ = values in ppm, *J* = coupling constant, expressed in Hz, *m* = multiplet; *dd* = double doublet, *d* = doublet, *m* = multiplet, *s* = singlet.
[b] The chemical shifts of carbon atoms were obtained from contour maps *g*HMQC and *g*HMBC.

solid with optical activity $[\alpha]^{25}_D$ of +127 (*c* 1.0, ethyl acetate) and a melting point of 255.4–257.8 °C. The molecular weight of compound **4** was determined by MALDI-TOF/MS to be 761.2 [**4** + Ca + 2H], consistent with a molecular formula of $C_{36}H_{32}O_{16}Ca$. The IR spectrum displayed absorption bands, ν_{max}, at 3405 (OH), 1601 and 1518 (C=C), 1261 (CO), 1725 and 1643 (C=O), and 836 cm^{-1} (CH). The UV spectrum displayed absorptions with $\lambda_{máx}$ (log ε) at 204 nm (6.39), 257 nm (5.99), 274 nm (6.02), 291 nm (6.02) and 345 nm (5.89) for the pure compound. Adding $AlCl_3$ produced absorptions at 207 nm (6.39), 221 nm (6.37), 281 nm (6.08), 358 nm (5.73) and 400 nm (5.75). Adding HCl produced absorptions at 206 nm (2.55), 220 nm (6.37), 282 nm (6.05), 297 nm (6.03), 355 nm (5.79) and 392 nm (5.74). The shifts in the UV spectra produced by adding $AlCl_3$ and HCl indicated the presence of chelatogenic hydroxyl groups. Adding NaOAc produced absorptions at 204 nm (6.47), 254 nm (5.99), 274 nm (5.99), 289 nm (5.97) and 338 nm (5.89). Adding H_3BO_3 produced absorptions at 207 nm (6.47), 265 nm (6.04) and 375 nm (5.78). The shifts in the UV spectrum produced by adding NaOAc and H_3BO_3 indicated that the hydroxyl in the *ortho*-position was absent, due the presence of a glucosyl residue linked to the oxygen atom at C-4‴.

The molecular formula of compound **4**, $C_{36}H_{30}O_{16}$, was determined by comparative analysis of the ^{1}H NMR, COSY, *g*HMQC and *g*HMBC spectra of compounds **3** and **4**, coupled with the mass data. The ^{1}H NMR spectrum showed multiplets between δ 3.28 and 4.37 and a doublet at δ 4.85 (*J* = 7.13 Hz) that could be assigned to the anomeric hydrogen H-1⁗ of a glucosyl residue. These characteristic signals suggested the presence of a β-glucopyranosyl moiety. The chemical shift of the anomeric hydrogen indicated that the glucosyl residue adopted a *trans-diaxial* conformation, and the appearance of a long-range ($^3J_{CH}$) heteronuclear correlation with C-4‴ (δ 154.5) in the *g*HMBC spectrum confirmed that it was linked to that position of the aglycone (Table 1). The signals at δ 13.08 and 12.12 in the ^{1}H NMR spectrum indicated the presence of hydroxyl groups at C-5 and C-5″, forming a typical six-membered chelatogenic ring with the carbonylic oxygen atom. The proposed structure is also corroborated by the presence of a hydroxyl signal at δ 10.10 that showed long-range ($^3J_{CH}$) heteronuclear coupling with CH-3′ and CH-5′ (δ 127.0) in the *g*HMBC spectrum (Table 1, Fig. 2). This signal could be assigned to the hydroxyl group at C-4′. No correlation was observed between the protons at δ 13.9 (C-7-OH) and δ 13.7 (C-7″-OH), and those at C-6/C-8 (δ 97.9/96.0) and C-6″/C-8″ (δ 116.2/103.9). From the complete ^{13}C and ^{1}H assignments (Table 1), we determined that the structure of **4** was morelloflavone-4‴-*O*-β-D-glycoside.

3.1. Antioxidant activity

The reduction of DPPH· (purple) to the corresponding hydrazine (yellow) is a classic, simple and fast method for evaluating radical-scavenging activity (Gülçin, Alici, & Cesur, 2005). The reaction can

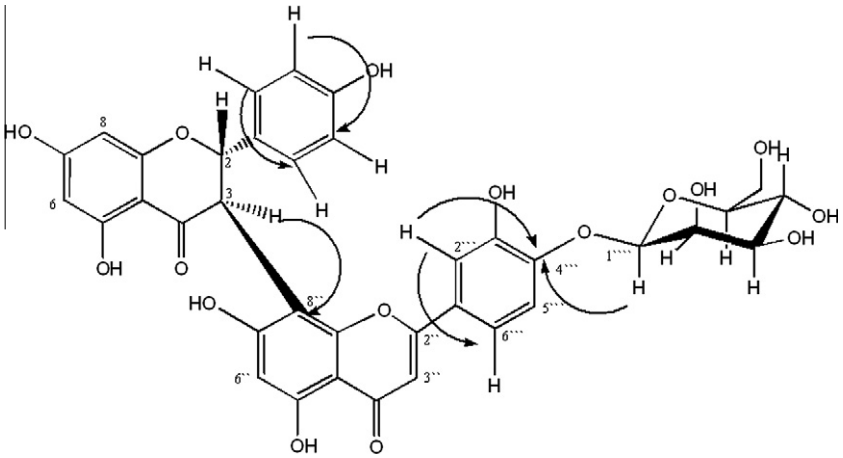

Fig. 2. Principal *g*HMBC correlations of compound **4**.

Table 2
Antioxidant activity analysis of compounds **1**–**4** by DPPH radical-scavenging and Fe^{3+} reduction experiments.

Compounds	Scavenging capacity (IC_{50} in mM)	Reducing power (absorbance at 400 μg/mL)
1	148 ± 14.2	0.194 ± 0.007
2	49.5 ± 6 60	0.583 ± 0.0104
3	52.4 ± 7.60	0.250 ± 0.043
4	62.6 ± 8.91	0.336 ± 0.151
Ascorbic acid[a]	23.5 ± 0.036	2.00 ± 0.001
BHT[a]	32.9 ± 17.5	1.99 ± 0.070

[a] Antioxidant standards.

be monitored spectrophotometrically by following the decrease in absorbance at $\lambda = 515–528$ nm. As shown in Table 2, biflavone compound (**2**) showed the greatest activity against DPPH (IC_{50}: 49.50 mM), followed by biflavones (**4**) and (**3**) and xanthone (**1**). Ascorbic acid and BHT were used as standards and produced IC_{50} values of 23.5 μg/mL and 32.9 μg/mL against DPPH.

The reducing power assay is based on the reduction of Fe^{3+} in potassium ferricyanide to Fe^{2+} to form a blue complex, which can be monitored at $\lambda = 700$ nm. The greater the reducing power of the analyte, the greater the concentration of complex formed, leading to higher absorbance values. The biflavone compounds **2**, **3** and **4** exhibited the strongest reducing activity, with compound **2** giving an absorbance of 0.583 being the most potent. Compound **1** showed the lowest activity with 0.094 of absorbance. Ascorbic acid and BHT gave absorbance values of 2.00 and 1.99 for the reduction of Fe^{3+}.

The high antioxidant activity of phenolic substances is often attributed to their −OH moieties, which are potent H· donors because electron delocalisation across the molecule efficiently stabilises the resulting phenoxy radicals, which can be observed for compound **2**. Another important feature of phenolic compounds is the planarity of the molecule, which permits conjugation and electron delocalisation, present in compound **1**. These factors are associated with an increase in radical stability. According to Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahoru, 2005, intramolecular H-bonds between neighbouring –OH and C=O groups increase aromatic-ring conjugation and raise the H· donation power of the molecule, because the resulting radicals are more delocalised. The presence of a dihydroxy group *ortho* to the C=O moiety of the biflavonoids also increases antioxidant activity, as seen in compounds **2** and **3**. All compounds (**1**–**4**) exhibit powerful antioxidant activities because they possess all of these structural features.

4. Conclusion

Fractionation by preparative HSCCC was an efficient method for the isolation of compounds **1**–**4** from the epicarp of *G. brasiliensis*. It allowed the rapid separation of xanthone (**1**) and biflavonoids (**2**–**4**). Compound **3** exhibited the best antioxidant activity, probably because of the presence of a catechol group, an α,β-unsaturated carbonyl subunit and free hydroxyl groups. We also identified a previously unreported metabolite of *G. brasiliensis*, morelloflavone-4‴-*O*-*β*-D-glycoside (**4**).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2011.10.110.

References

Almeida, L. S. B., Yatsuda, M. R., Santos, M. H., Nagem, T. J., Alencar, S. M., Koof, H., et al. (2008). Antimicrobial activity of *Rheedia brasiliensis* and 7-epiclusianone against Streptococcus mutans. *Phytomedicine, 15*, 886–891.

Baggett, S., Protiva, P., Mazzolo, E. P., Yang, H., Ressler, E. T., Basile, M. J., et al. (2005). Bioactive benzophenones from *Garcinia xanthochymus* fruits. *Journal of Natural Products, 68*. 354-36.

Basso, L. A., Silva, L. H. P., Fett-Neto, A. G., Azevedo, W. F., Junior, Moreira, I. S., Calixto, J. B., et al. (2005). The use of biodiversity as source of new chemical entities against defined molecular targets for treatment of malaria, tuberculosis, and T cell mediated diseases – A Review. *Memórias do Instituto Oswaldo Cruz, 100*, 575–606.

Boonsri, S., Karalai, C., Ponglimanont, C., Kanjana-Opas, A., & Chantrapromma, K. (2006). Antibacterial and cytotoxic xanthones from the roots of *Cratoxylum formosum*. *Phytochemistry, 67*(7), 723–727.

Botta, B., Mac-Quhae, M., Delle, M. F., Delle-Monache, G., & De Mello, J. F. (1984). Chemical investigation of the genus *Rheedia*. V. Biflavonoids and xanthochymol. *Journal of Natural Products, 47*, 1053.

Carpenter, I., Locksley, H. D., & Scheinmann, F. (1969). Xanthones in higher plants: biogenetic proposals and a chemotaxonomic survey. *Phytochemistry, 8*, 2013–2015.

Castardo, J. C., Prudente, A. S., Ferreira, J., Guimarães, C. L., Monache, F. D., Filho, V. C., et al. (2008). Anti-inflammatory effects of hydroalcoholic extract and two biflavonoids from *Garcinia gardneriana* leaves in mouse paw oedema. *Journal of Ethnopharmacology, 118*, 405–411.

Coelho, L. P., Serra, M. F., Pires, A. L. A., Cordeiro, R. S. B., Rodrigues Silva, P. M., Santos, M. H., et al. (2008). 7-Epiclusianone, a tetraprenylated benzophenone, relaxes airway smooth muscle through activation of the nitric oxide-cGMP pathway. *The Journal of Pharmacology and Experimental Therapeutics, 327*(1), 206–214.

Conway, W. D. (1989). *Countercurrent chromatography; apparatus, theory and application*. Weinheim: VCH.

Cui, J., Hu, W., Cai, Z., Liu, Y., Li, S., Tao, W., et al. (2010). New medicinal properties of mangostins: Analgesic activity and pharmacological characterization of active ingredients from the fruit hull of *Garcinia mangostana*. *Pharmacology, Biochemistry and Behavior, 95*, 166–172.

Derogis, P. B., Martins, F. T., Souza, T. C., Moreira, M. E. C., Filho, J. D. S., Doriguetto, A. C., et al. (2008). Complete assignment of the ^{1}H and ^{13}C NMR spectra of garciniaphenone and keto-enol equilibrium statements for prenylated benzophenones. *Magnetic Resonance Chemistry, 46*, 278–282.

Elfita, E., Muharni, M., Latie, M., Darwati, D., Widiyantoro, A., Supriyatna, S., et al. (2009). Antiplasmodial and other constituents from four Indonesian *Garcinia spp*. *Phytochemistry, 70*, 907–912.

Gülçin, İ., Alici, H. A., & Cesur, M. (2005). Determination of in vitro antioxidant and radical scavenging activities of propofol. *Chemical and Pharmaceutical Bulletin, 53*(3281), 281–285.

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1998). *Chemistry Pharmacology Bulletin, 36*, 10190.

Holloway, D. M., & Scheinmann, F. (1975). Phenolic compounds from the heartwood of *Garcinia mangostana*. *Phytochemistry, 14*, 2517–2518.

Joseph, G. S., Jayaprakasha, A. T., Selvi, B. S., & Jena, K. K. (2005). Antiaflatoxigenic and antioxidant activities of *Garcinia* extracts. *International Journal of Food Microbiology, 101*(2), 153–160.

Karanjgaokar, C. G., Radhakrishnan, P. V., & Venkatarama, K. (1967). *Tetrahedron Letter*, 3195–3198.

Konoshima, M., & Ikeshiro, Y. (1970). Fukugiside, the first biflavonoid glycoside from *Garcinia spicata* Hook f. *Tetrahedron Letter, 11*, 1717–1720.

Lenta, B. N., Vonthronsenec, C., Weniger, B., Devkota, K. P., Ngoupayo, J., Kaiser, M., et al. (2007). Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds from *Allanblachia monticola* and *Symphonia globulifera*. *Molecules, 12*, 1548–1557.

Luzzi, R., Guimaraes, C. L., Verdi, L. G., Simionatto, E. L., Delle-Monache, F., Yunes, R. A., et al. (1997). Isolation of biflavonoids with analgesic activity from *Rheedia gardneriana* leaves. *Phytomedicine, Chicago, 4*(2), 141–144.

Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). *Systematic identification of flavonoids*. New York: Springer-Verlag (capitulo 3 e 4).

Oliveira, T. T., Nagem, T. J., Leão, M. A., Fonseca, C. C. A., Pereira, H. J. V., Santos, M. H., et al. (2005). Atividade antidiabética de *Bauhinia sacra*, corantes naturais, ácido cinâmico e 7-epiclusianona. *News Laboratory, 72*, 172–190.

Permana, D., Lajis, N. H., Shaari, K., Ali, A. M., Mackeen, M. M., Kitajima, M., et al. (2003). A new prenylated hydroquinone from the roots of *Garcinia atroviridis* Griff ex. T. Anders (Guttiferae). *Journal of Chemical Sciences, 58*, 332–335.

Piccinelli, A. L., Cuesta-Rubio, O., Chica, B. M., Mahmood, N., Pagano, B., Pavone, M., et al. (2005). Structural revision of clusianone and 7-epi-clusianone and anti-HIV activity of polyisoprenylated benzophenones. *Tetrahedron, 61*, 8206–8211.

Rao, L. J. M., Ono, H. Y., Ohnishi-Kameyama, M., & Yoshida, M. (2004). Occurrence of antioxidant and radical scavenging proanthocyanidins from the Indian minor spice nagkesar (*Mammea longifolia* Planch and Triana syn). *Bioorganic and Medicinal Chemistry, 12*(1), 31–36.

Rodrigues, C. A., Oliveira, A. E., Silva, A. F. S., Cechinel, F. V., Guimaraes, C. L., Yunes, R. A., et al. (2000). A comparative study of stationary phase for separation of biflavonoids from *Rheedia gardneriana* using column chromatography. *Zeitschrift Fuer Naturforschung Section C Journal of Biosciences, Tuebingen, 55*(7–8), 524–527.

Santa-Cecília, F. V., Vilela, F. C., Rocha, C. Q., Dias, D. F., Cavalcante, G. P., Freitas, L. A. S., et al. (2011). Anti-inflammatory and antinociceptive effects of *Garcinia brasiliensis*. *Journal of Ethnopharmacology, 133*, 467–473.

Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I., & Bahoru, T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 579*(1–2), 200–213.

Yen, G. C., & Chen, H. Y. (1995). *Journal of Agricultural and Food Chemistry, 67*, 415.

Zagoto, J. N., Bracht, A., Pagadigorria, C. L. S., Ishii-Iwamoto, E. L., Cortez, D. A. G., & Yamamoto, N. S. (2006). Effects of the *Kielmeyera coriacea* extract on energy metabolism in the rat live. *Journal of Ethnopharmacology, 105*(1–2), 47–54.