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Flavonoids from leaves of *Mauritia flexuosa*

Djalma M. de Oliveira,[1] Ezequias P. Siqueira,[2] Yule R. F. Nunes,[3] Betania B. Cota*[,2]

[1]Departamento de Química e Exatas, Universidade Estadual do Sudoeste de Bahia, Campus Jequié, Brazil,
[2]Laboratório de Química de Produtos Naturais, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Brazil,
[3]Laboratório de Ecologia e Propagação Vegetal, Universidade Estadual de Montes Claros, Brazil.

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Abstract: The chromatographic fractionation of the *Mauritia flexuosa* L. f., Arecaceae, leaves extract, a plant known by the name of buriti palm tree, resulted in the isolation of six flavonoids: tricin-7-*O*-rutinoside, apigenin-6-*C*-arabinoside, 8-*C*-glucoside (isoschaftoside), kaempferol-3-*O*-rutinoside (nicotiflorine), quercetin-3-*O*-rutinoside (rutin), luteolin-8-*C*-glucoside (orientin) and luteolin-6-*C*-glucoside (isoorientin). The flavonoids were found out and previously reported as constituents of the Arecaceae family plants, but the occurrence of *C*-glucoside flavonoids, in the species being analyzed, is described for the first time on this study. The structural elucidations of all of the isolated compounds were performed by means of the comparison of their spectral data (^{1}H and ^{13}C NMR, UV and ESI-MS) with those ones of the literature.

Introduction

Mauritia flexuosa L. f., Arecaceae, (Miriti, Buriti) is a palm tree that provides food, as its fruit and its oil are edible. In Brazil, the oil from the fruits is also utilized, in the popular medicine, as vermifuge and analgesic. The wood and the straw are used, as raw materials, to make rafts and for building houses and artisans´ works. Plotkin & Balick (1984) reported that the sap of the trunk is utilized as a starch source for the production of wine and the sago-like pap, prepared with the stem pith, is used to cure dysenteries and diarrheas.

Previous studies have described the composition of the buriti fruits, specially the occurrence of phytosterols in the buriti oil (Costa et al., 2010). The crude buriti oil has also a high carotenoid (β-carotene, vitamin A) content and shows antioxidant activity (Aquino et al., 2012).

Williams & Harborne (1973) have suggested that the flavone tricetin 3',5'-dimethyl ether, named tricin, appears among the possible markers for the Arecaceae family. Recently, triterpenes and flavonoids were isolated from *M. flexuosa* roots found out in the Amazonia (Koolen et al., 2012).

Although some few studies about the occurrence of the natural products from *M. flexuosa* have been reported, there are not previous studies of the leaves. We describe, in this study, the chromatographic fractionation, the isolation and the structural elucidation of six flavonoids that occur in the ethanolic crude extract of the *M. flexuosa* leaves.

Materials and Methods

General procedures

The solvents were removed by using a Spress SpeedVac® vacuum centrifuge (Thermo-Savant SPD SC250 Express, Holbrook, NY, USA). Thin-layer chromatographic (TLC) analyses were conducted on pre-coated commercial silica gel G-60/F254 (0.25 mm, Merck, Darmstadt, Germany) plates. TLC plates were eluted, in a pre-saturated chamber, by using solvents mixtures in different proportions of: a) ethyl acetate: methanol: water (EtOAc:MeOH:H_2O/70:10:2.5); or b) dichloromethane: methanol (DCM:MeOH/95:5). The spots were visualized under UV light at 254 nm and 366 nm and visible, after spraying the plates with an ethanol solution of 1% diphenyl ethanolamine borate in methanol and 5% polyethylene glycol in ethanol. Gel Permeation Chromatography (GPC) was carried out by using two coupled glass columns (Büchi column n° 17980) filled with Sephadex LH-20 ™ (GE Healthcare, U.S.A.) gel and ethanol as mobile phase at flow rates of 120 mL/h. Analytical reverse phase (RP) HPLC analyses were developed using a HPLC system (Shimadzu, Kyoto, Japan), equipped with a LC10AD pump and a SPD M-20A Diode Array Detector. Analitical analyses were carried out by a Shim-pack® C18 column (5 μm, 4.6 mm

x 250 mm *i.d.*), eluted with gradient of methanol/water at a flow rate of 1 mL/min. In general, semi-preparative purifications were performed by using a Shim-pack® C18 column (5 µm, 20 x 250 mm, *i.d.*), mixtures of MeOH:H_2O plus 0.1% trifluoracetic acid (TFA) or ACN:H_2O as eluents, at flow rates of 7 mL/min and detection at λ 220 and 254 nm by utilizing a Shimadzu chromatographic system (Shimadzu, Kyoto, Japan), equipped with a LC6AD pump and a UV-visible dual-wavelength detector (SPD10A). Presumptive identification of isolated compounds was based on comparison of their retention times with unknown peaks in the ethanolic extract and ethyl acetate soluble fraction of *M. flexuosa* leaves. The ethanolic extract and ethyl acetate soluble fraction were diluted with a mixture of MeOH:H_2O (1:1) to obtain a concentration of 5 mg/mL. The isolated compounds were diluted with a mixture of MeOH:H_2O (1:1) to obtain a solution of 0.5 mg/mL. All samples were centrifuged prior to injection into the chromatograph. HPLC profiles of all samples were obtained after injection of 20 µL of solutions. HPLC profiles were done by using a RP-18 column (250 mm × 4.6 mm *i.d.*; 5 µM) using elution gradient of methanol/water in 10% methanol to 100% methanol in 30 min, 100% of methanol in 5 min at a flow rate of 1 mL/min. UV spectra were recorded from 200 to 500 nm. 1D spectra were recorded on a Brucker DRX 400 spectrometer at 400 MHz, with the pulse programs provided by the manufacturer. The substances were dissolved in perdeuterated solvents containing 0.1% tetramethylsilane as internal chemical shift standard. Electrospray ionization mass spectrometry (ESI-MS) was performed by using a Bruke maXis ETD (Bruker, DE). Solutions of the compounds, at 200 µg/mL in ACN:H_2O (1:1), were infused at 3 µL/min with 0.1% formic acid in the negative and positive mode. Mass spectra were acquired over a range between *m/z* 50-1500 daltons. The instrument was operated under the following conditions: end plate offset, -500 V; capillary voltage, 4500 V; nebulizer pressure, 0.4 bar; dry gas (nitrogen) flow rate, 4.0 L/min; dry temperature, 180 °C; ISCID energy, 0-20 eV; hexapole RF, 600 Vpp; collision energy, 0-30 eV; collision RF, 600 Vpp; transfer time, 30-100 µs.

Plant material

Leaves of *Mauritia flexuosa* L. f., Arecaceae, were collected at Bonito de Minas, Minas Gerais State, Brazil, in 2010. A voucher specimen (HMC 1448) was deposited at Montes Claros Herbarium, State University of Montes Claros, Brazil.

Extraction and isolation of constituents from Mauritia flexuosa

Fresh leaves (312 g) of *M. flexuosa* were extracted at room temperature and the solution was concentrated under vacuum in a rotary evaporator at temperatures below 45 °C. The residual solvent was removed in a vacuum centrifuge at 40 °C. Crude extract (26 g) was suspended in MeOH:H_2O (8:2) and extracted with solvents of increased polarity to afford a hexane soluble layer (Hex, 3.1 g), a dichloromethane soluble layer (DCM, 0.5 g), an ethyl acetate soluble layer (EtOAc, 7.8 g) and a aqueous soluble layer (Aq, 6.3 g). An aliquot of EtOAc fraction (7.3 g) was subjected to GPC using ethanol to produce 360 fractions of 20 mL. The fractions were pooled after TLC analysis to give eighteen groups (A1 to A18). Compound **1** (7.7 mg) was obtained by semi-preparative RP-HPLC with ACN:H_2O (10:90→80:20 in 60 min) at 7 mL/min from group A7 (75 mg). Group A10 (150 mg) was submitted to semi-preparative RP-HPLC, using a mixture of ACN:H_2O (10:90→100:0 in 80 min) to yield thirty subgroups (B1 to B30). Subgroups B13 to B15 were pooled (30 mg) and purified by semi-preparative RP-HPLC using mixture of MeOH:H_2O plus 0.1% TFA (30:70→100:0 in 20 min and 100:0 in 5 min) to yield **2** (5 mg). Subgroups B16 to B17 (15 mg) and B21 to B23 (18 mg) were submitted to the semi-preparative HPLC with MeOH: H_2O plus 0.1% TFA (20:80→100:0 in 50 min) to give **3** (3.5 mg) and **4** (3.5 mg), respectively. Group A11 (100 mg) was also submitted to semi-preparative HPLC, using a mixture of MeOH: H_2O plus 0.1% TFA (20:80→100:0 in 80 min) to obtain **5** (27 mg) and **6** (34 mg).

Spectroscopical data

Tricin-7-*O*-rutinoside (**1**), yellowish powder, $C_{29}H_{34}O_{16}$, ESI-MS (negative, *m/z*): 447.0936 [M-H-146-44]$^-$, 357.0616 [M-H-146-44-90]$^-$, 327.0510 [M-H-146-44-120]$^-$, 299.0558 [aglycone-H-30]$^-$. UV (λ_{max}, nm): 249, 269, 350. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.28 (2H, s, H-2', H-6'), 6.81 (1H, br. s, H-8), 6.75 (1H, br. s, H-3), 6.54 (1H, d, *J*=1.8 Hz, H-6), 5.09 (1H, d, *J*=7.50 Hz, H-1''), 4.48 (1H, d, *J*=1.5 Hz, H-1'''), 3.97 (6H, br.s, -OCH_3 at C-3'and C-5'), 3.42-4.03 (further glycosidic protons), 1.17 (1H, d, *J*=6.06 Hz, H-6'''). ^{13}C NMR (CD_3OD, 100 MHz) δ: 184.04 (C-4), 166.71 (C-2, C-7), 158.98 (C-5), 164.80 (C-9), 149.81 (C-3', C-5'), 140.84 (C-4´), 122.64 (C-1'), 105.71 (C-2', C-6'), 105.14 (C-3), 104.64 (C-10), 102.26 (C-1''), 101.59 (C-1'''), 101.16 (C-6), 96.55 (C-8), 78.02 (C-3''), 77.31 (C-5''), 74.90 (C-2''), 74.17 (C-4'''), 72.53 (C-3'''), 72.21 (C-2'''), 71.41 (C-4''), 69.92 (C-5'''), 67.54 (C-6''), 57.34 (3', 5'-OCH3), 18.17 (C-6''').

Apigenin-6-*C*-arabinoside-8-*C*-glucoside, isoschaftoside (**2**), yellowish powder, $C_{26}H_{28}O_{14}$, ESI-MS (negative, *m/z*): 563.1005 [M-H]$^-$, 503.0807 [M-H-60]$^-$, 473.0725 [M-H-90]$^-$, 443.0644 [M-H-90]$^-$, 383.0479 [M-H-180]$^-$, 353.0397 [M-H-210]$^-$. UV (λ_{max}, nm): 216, 271, 336. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.96 (2H, d, *J*=8.5 Hz, H-2', H-6'), 6.92 (2H, d, *J*=8.5 Hz, H-3', H-5'), 6.61 (1H, br.s, H-3), 4.88

(1H, m, H-1”), 4.76 (overlapped, 1H, m, H-1”’), 4.06 (1H, dd, *J*=12.5, 5.0 Hz, H-5b”’), 3.96 (2H, m, H-6b″, H-2”’), 3.85 (2H, m, H-6a″, H-4”’), 3.76 (2H, m, H-2”, H-4”’), 3.65 (2H, m, H-3”, H-5”), 3.54 (1H, m, H-3”’), 3.45 (2H, m, H-3”’, H-5a”’). ^{13}C-DEPT 135 NMR (100 MHz, CD_3OD) δ: 130.6 (C-2’, 6’), 116.89 (C-3’, 5’), 103.67 (C-3), 82.84 (C-5”), 76.41 (C-3”), 75.80 (C-1”’), 73.04 (C-1”, C-3”’), 71.84 (C-5”’), 72.28 (C-4”), 71.11 (C-4”’), 70.80 (C-2”’), 67.51 (C-2”), 61.77 (C-6”).

Kaempferol-3-*O*-rutinoside, nicotiflorine (**3**), yellow powder, $C_{27}H_{30}O_{15}$, ESI-MS (negative, *m/z*): 593.0866 $[M-H]^-$, 285.0096 $[M-H-146-162]^-$. UV (λ_{max}, nm): 265, 349. ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.07 (2H, d, *J*=8.5 Hz, H-2’, H-6’), 6.80 (2H, d, *J*=8.5 Hz, H-3’, H-5’), 6.42 (1H, d, *J*=2.0 Hz, H-8), 6.22 (1H, d, *J*=2.0 Hz, H-6), 5.13 (1H, d, *J*=7.8 Hz, H-1”), 4.52 (1H, d, *J*=1.8 Hz, H-1”’), 3.81 (1H, dt, *J*=10.9, 1,0 Hz, Hb-6”), 3.63 (1H, dd, *J*=3.5, 2.0 Hz, H-2”’), 3.52 (1H, dd, *J*=9.5, 3.5 Hz, H-3”’), 3.48 (1H, m, H-5”’), 3.26-3.48 (4H, m, H-2”, H-3”, H-4”, H-5”), 3.37 (1H, m, Ha-6”), 3.27 (1H, m, H-4”’), 1.12 (3H, d, *J*=6.3 Hz, C-6”’). ^{13}C NMR (CD_3OD, 100 MHz) δ: 178.63 (C-4), 166.20 (C-7), 164.74 (C-5), 161.67 (C-4’), 159.59 (C-9), 158.74 (C-2), 135.68 (C-3), 132.53 (C-2’, C-6’), 122.94 (C-1’), 116.30 (C-3’, C-5’), 105.39 (C-10), 104.72 (C-1”), 102.60 (C-1”’), 100.13 (C-6), 95.05 (C-8), 78.32 (C-5”), 77.40 (C-3”), 75.93 (C-2”), 74.06 (C-4”’), 72.46 (C-3”’), 72.46 (C-2”’), 71.62 (C-4”), 69.89 (C-5”’), 68.73 (C-6”), 18.06 (C-6”’).

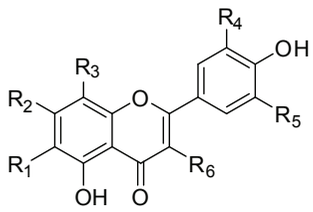
Quercetin-3-*O*-rutinoside, rutin (**4**), yellowish powder, $C_{27}H_{30}O_{16}$, ESI-MS (negative, *m/z*): 609.0820 $[M-H]^-$, 299.9964 $[M-H-(rha-O-glu)]^-$. ESI-MS (positive): *m/z* 611.1618. UV (λ_{max}, nm): 256, 270, 356. ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.67 (1H, d, *J*=2.0 Hz, H-2’), 7.63 (1H, dd, *J*=8.5, 2.0 Hz, H-6’), 6.88 (1H, d, *J*=8.5 Hz, H-5’), 6.41 (1H, d, *J*=2.0 Hz, H-8), 6.22 (1H, d, *J*=2,0 Hz, H-6), 5.11 (1H, d, *J*=7.8 Hz, H-1”), 4.52 (1H, d, *J*=1.8 Hz, H-1”’), 3.81 (1H, dt, *J*=10.9, 1.0 Hz, Hb-6”), 3.63 (1H, dd, *J*=3.5, 1.8 Hz, H-2”’), 3.54 (1H, dd, *J*=9.5, 3.5 Hz, H-3”’), 3.26-3.48 (4H, m, H-2”, H-3”, H-4”, H-5”), 3.44 (1H, m, H-5”’), 3.39 (1H, m, Ha-6”), 3.27 (1H, m, H-4”’), 1.12 (3H, d, *J*=6.1 Hz, C-6”’). ^{13}C NMR (CD_3OD, 100 MHz) δ: 179.61 (C-4), 167.19 (C-7), 162.17 (C-5), 159.51 (C-9), 158.69 (C-2), 150.45 (C-4’), 146.02 (C-3’), 136.72 (C-3), 123.70 (C-6’), 123.30 (C-1’), 117.84 (C-2’), 116.22 (C-5’), 106.06 (C-10), 104.85 (C-1”), 102.58 (C-1”’), 100.10 (C-6), 95.01 (C-8), 78.36 (C-5”), 77.41 (C-3”), 75.90 (C-2”), 74.10 (C-4”’), 72.41 (C-3”’), 72.27 (C-2”’), 71.57 (C-4”), 70.59 (C-5”’), 68.72 (C-6”), 18.03 (C-6”’).

Luteolin-8-*C*-glucoside, orientin (**5**), yellow powder, $C_{21}H_{20}O_{11}$, ESI-MS (negative, *m/z*): 447.0937 $[M-H]^-$, 357.0620 $[M-H-90]^-$, 327.0514 $[M-H-120]^-$ (base peak). UV (λ_{max}, nm): 257, 269, 350. ^{1}H NMR (400 MHz, CD_3OD) δ: 13.05 (1H, s, 5-OH), 7.57 (1H, d, *J*=8.0 Hz, H-6’), 7.47 (1H, br.s, H-2’), 6.96 (1H, d, *J*=8.0 Hz, H-5’), 6.62 (1H, s, H-3), 6.32 (1H, s, H-6), 4.94 (1H, d, *J*=10.0 Hz, H-1”), 4.07 (1H, m, H-2”), 3.97 (1H, dd, *J*=12.0, 1.5 Hz, Hb-6”), 3.83 (1H, dd, *J*=12.0, 6.0 Hz, Ha-6”), 3.66 (1H, m, H-3”), 3.51 (2H, m, H-4”, H-5”). ^{13}C NMR (100 MHz, CD_3OD) δ: 184.07 (C-4), 166.38 (C-2), 164.39 (C-7), 162.57 (C-5), 158.09 (C-9), 151.03 (C-4’), 147.24 (C-3’), 124.17 (C-1’), 121.08 (C-6’), 117.13 (C-5’), 115.33 (C-2’), 105.95 (C-8), 105.65 (C-10), 104.09 (C-3), 99.57 (C-6), 83.21(C-5”), 80.49 (C-3”), 75.39 (C-1”), 72.84 (C-2”), 72.40 (C-4”), 63.33 (C-6”).

Luteolin-6-*C*-glucoside, isoorientin (**6**), yellow powder, $C_{21}H_{20}O_{11}$, ESI-MS (negative, *m/z*): 447.0890 $[M-H]^-$, 357.0523 $[M-H-90]^-$ (base peak), 327.0477 $[M-H-120]^-$. UV (λ_{max}, nm): 257, 269, 350. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.35 (1H, dd, *J*=8.5, 1.5 Hz, H-6’), 7.34 (1H, m, H-2’), 6.89 (1H, d, *J*=8.5 Hz, H-5’), 6.52 (1H, s, H-3), 6.47 (1H, s, H-8), 4.90 (1H, d, *J*=9.0 Hz, H-1”), 4.16 (1H, t-like, *J*=9.0 Hz, H-2”), 3.89 (1H, dd, *J*=12.0, 2.0 Hz, Hb-6”), 3.75 (1H, dd, *J*=12.0, 5.0 Hz, Ha-6”), 3.49 (2H, m, H-4”, H-5”), 3.44 (1H, m, H-3”). ^{13}C NMR (100 MHz, CD_3OD) δ: 184.13 (C-4), 166.39 (C-2), 164.97 (C-7), 162.15 (C-5), 158.84 (C-9), 151.17 (C-4’), 147.16 (C-3’), 123.69 (C-1’), 120.47 (C-6’), 116.94 (C-5’), 114.31 (C-2’), 109.28 (C-6), 105.36 (C-10), 104.06 (C-3), 95.35 (C-8), 82.76 (C-5”), 80.27 (C-3”), 75.46 (C-1”), 72.77 (C-2”), 71.95 (C-4”), 63.02 (C-6”).

Results and Discussion

A EtOAc soluble fraction from ethanolic extract of *Mauritia flexuosa* L. f., Arecaceae, leaves was successively subjected to GPC and RP-HPLC to afford six flavonoids. Columns filled with Sephadex LH-20 and C-18 silica gel in semi-preparative scales were respectively utilized. The chromatographic RP-HPLC profiles of crude ethanolic extract of the *M. flexuosa* leaves as well as its soluble fraction in ethyl acetate (Figure 1) suggested the occurrence of a high content of flavonoid in this species. The flavonoids **1-6** were identified by comparison of their NMR and HRMS data with the literature. Most of the compounds, isolated in this work, such as tricin-7-*O*-rutinoside (**1**), isoschaftoside (**2**), nicotiflorine (**3**), orientin (**5**) and, isoorientin (**6**) were obtained from this plant for the first time.



1 R_1=R_3=R_6=H; R_2=*O*-rutinosyl; R_4=R_5=OCH_3
2 R_1=arabinosyl; R_2=OH; R_3=glucosyl; R_4=R_5=R_6=H
3 R_1=R_3=R_4=R_5=H; R_2=OH; R_6=*O*-rutinosyl
4 R_1=R_3=R_5=H; R_2=R_4=OH; R_6=*O*-rutinosyl
5 R_1=R_5=R_6=H; R_2=R_4=OH; R_3=glucosyl
6 R_1=glucosyl; R_2=R_4=OH; R_3=R_5=R_6=H

The compound **1** showed a similar UV spectrum (249 nm, 270 nm and 350 nm) to the tricin-7-*O*-rutinoside characterized in barley leaves (*Hordeum vulgare* L.) (Ferreres et al., 2008). The ESI-MS (negative mode) of compound **1** showed an ion peak $[M-H-146-44]^-$ at *m/z* 447.0936 (base peak). In the ESI-MS^2 spectrum, the base peak showed losses of 90 and 120 amu, corresponding to the fragments at *m/z* 357.0616, *m/z* 327.0510, respectively. The ESI-MS^3 of the precursor fragment at *m/z* 327.0510 presented an ion peak at *m/z* 299.0558 [aglycone-H-30]$^-$. The ^{1}H NMR spectrum of **1** showed a signal of two aromatic protons at δ 7.28 (2H, s), which indicated the presence of a symmetrically tetrasubstituted phenyl group; it also showed two anomeric protons at δ 5.09 (1H, d, *J*=7.50 Hz) and δ 4.48 (1H, d, *J* = 1.5 Hz), two signals at δ 6.75 (1H, br. s) and at δ 6.54 (1H, d, *J*=1.8 Hz), corresponding to two meta-coupled protons; it showed a broad singlet at δ 3.97 (6H, br. s), referred to the protons of two equivalent methoxyl groups, and also a signal at δ 6.81 (1H, br. s). These signals were assigned to a protons system of an *O*-dimetoxylated flavonoid glycoside compound. The comparison of the ^{13}C and ^{1}H NMR data of compound **1** with the literature identified that compound as being the tricin-7-*O*-rutinoside (**1**) (Hirai et al., 1986; Zhang et al., 2012).

The ESI-MS (negative mode) of compound **2** showed a quasi-molecular ion peak at *m/z* 563.1005 corresponding to the $C_{26}H_{28}O_{14}$ formula and a similar UV spectrum (216 nm, 271 nm, 336 nm) to the apigenin-6-*C*-Glu-8-*C*-Ara (Liu et al., 2011). Furthermore, the fragmentation patterns in MS/MS exhibited losses of 60, 90, 120, 180 and 210 amu, corresponding to the ions at *m/z* 503.0807, *m/z* 473.0725 (base peak), *m/z* 443.0644, *m/z* 383.0479 and at *m/z* 353.0397 respectively, that were consistent with the characteristic fragmentations of *C*-glycosidic flavonoid (Ferreres et al., 2008). The data of the ESI-MS2 showed that the presence of fragments at *m/z* 503.0807 $[M-H-60]^-$ and at *m/z* 383.0479 $[M-H-180]^-$, and at *m/z* 443.0644 $[M-H-120]^-$ and at *m/z* 353.0397 [M-H -210]$^-$ is linked to the cleavage of *C*-pentosyl and *C*-hexosyl rings of **2**, respectively (Wu et al., 2004). Moreover, the peak as detected at *m/z* 473 (base peak) allowed to identify the compound **2** as being the isoschaftoside (apigenin-6-*C*-Ara-8-*C*-Glu) instead of its isomer, the schaftoside (apigenin-6-*C*-Glu-8-*C*-Ara). The analysis of the ^{1}H NMR spectrum of the flavonoid **2** confirmed the absence of hydrogen atoms in the A-ring spin and a 1,4-disubstituted system in the B-ring (δ 7.96, 2H, d, *J*=8.5 Hz, H-2', H-6' and δ 6.92, 2H, d, *J*=8.5 Hz, H-3', H-5'). The presence of the signals at δ 4.88 and δ 4.76, at the region of the high field, was attributed to two anomeric protons (Hooper et al., 2010). The compound **2** was identified by comparison of NMR data with the literature as being apigenin-6-*C*-arabinoside-8-*C*-glucoside (Du et al., 2011).

The ESI-MS (negative mode) of compound **3** showed a quasi-molecular ion peak at *m/z* 593.0866 (base peak), corresponding to the $C_{27}H_{30}O_{15}$ formula and of the ion at *m/z* 285.0096 $[M-H-146-162]^-$, referred to the aglycone fragment of a glucorhamnoside flavonoid. The ESI-MS (negative mode) of compound **4** showed a quasi-molecular ion peak at *m/z* 609.0820 and in the ESI-MS2 showed a loss of 309 amu corresponding to the ion fragment at *m/z* 299.9964 $[M-H-146-162]^-$ in the spectra, which suggested that the compound **4** was also a glucorhamnoside flavonoid. The ESI-MS (positive mode) of the compound 4 showed a quasi-molecular ion peak at *m/z* 611.1618 that confirmed the spectral data obtained in the negative mode.

Both the compounds **3** and **4** showed a doublet at δ 1.12 (3H, d, *J*=6.3-6.1 Hz) in the ^{1}H NMR spectra as well as a signal at δ 18.06 (**3**) and at δ 18.03 (**4**) in the ^{13}C NMR spectra, that are characteristic of the rhamnosyl moieties. The signals of the anomeric protons of the rhamnose and glucose appeared at δ 5.13 (1H, d, *J*=7.8 Hz, H-1") and δ 4.52 (1H, d, *J*=1.8 Hz, H-1"') for the compound **3** and at δ 5.11 (1H, d, *J*=7.8 Hz, H-1") and δ 4.52 (1H, d, *J*=1.8 Hz, H-1"') for the compound **4**, respectively. The signals of the anomeric carbons, observed at the ^{13}C NMR spectrum, were confirmed for both the compounds (**3**, δ 104.72 and δ 102.60 and **4**, δ 104.85 and δ 102.58). The main difference among the ^{1}H NMR spectra of the compounds **3** and **4** refers to the presence of signals at δ 7.67 (1H, d, *J*=2.0 Hz, H-2'), δ 7.63 (1H, dd, *J*=8.5, 2.0 Hz, H-6') and at δ 6.88 (1H, d, *J*=8.5 Hz, H-5'), which are characteristic of the 1,3,4-tri-substituted (B ring) aromatic system derived from the quercetin, in the glycoside **4**, as well as the occurrence of signals at δ 8.07 (2H, d, *J*=8.5 Hz, H-2', H-6'), δ 6.80 (2H, d, *J*=8.5 Hz, H-3', H-5'), which are characteristic of a 1,4-disubstituted aromatic system, derived from the kaempferol in the glycoside **3**. The comparison of the spectral data with the literature confirmed the identity of these well known compounds as being kaempferol-3-*O*-rutinoside, nicotiflorine (**3**) (Mabry et al., 1976, Harborne & Mabry, 1982; Agrawal, 1989) and rutin (**4**) (Hou et al., 2005).

The ESI-MS (negative mode) of compound **5** showed a quasi-molecular ion peak at *m/z* 447.0937 $[M-H]^-$, corresponding to the $C_{21}H_{20}O_{11}$ formula. The compound 5 showed a region of the UV spectrum (257 nm, 269 nm and 350 nm) similar to that one of the luteolin (Liu et al., 2011). The ^1H and ^{13}C NMR spectra of the compound **5** exhibited characteristic signals of the aromatic systems (between δ 7.57-6.32) and of a glucopyranose moiety (between δ 4.07-3.51). In the ^1H NMR spectrum, the singlet at δ 6.32 (1H, H-6) was attributed to the A-ring aromatic proton of a 5,7,8-trisubstituted flavonoid. The doublets at δ 7.57 (*J*=8.0 Hz) and at δ 6.96 (*J*=8.0 Hz) were attributed to H-6' and H-5', respectively, of the B-ring. The anomeric proton signal at relatively low field region, at δ 4.94 (1H, d, *J*=10.0 Hz, H-1") as well as the coupling constant value was attributed to the occurrence of a *C*-glycoside flavonoid. The ^{13}C-NMR spectra and the DEPT-135 spectrum showed a quaternary carbon signal at δ 105.95, which is the characteristic of *C*-8 of the aglycon moiety. The comparison of the spectroscopic data of **5** with the literature confirmed the identity of this compound as being luteolin-8-*C*-glucoside, orientin (**5**) (Zhou et al., 2005).

The ESI-MS (negative mode) of compound **6** showed a quasi-molecular ion peak *m/z* 447.0890 $[M-H]^-$, corresponding to the $C_{21}H_{20}O_{11}$ formula, the same as the compound **5**. The fragmentation pattern in MS/MS showed losses of 90 and 120, referred to the ions at *m/z* 357.0523 and *m/z* 327.0477, respectively, demonstrating that this chemical constituent is an isomer of the compound of **5** (Ferreres et al., 2008). The singlet at δ 6.47 (1H, H-8) in the ^1H NMR spectrum, and the quaternary carbon signal at δ 109.28 were attributed to H-6/C-6 of the 5,6,7-trisubstituted A-ring system of a flavonoid. The comparison of the NMR data of **6** with the literature confirmed this compound as being the luteolin-6-*C*-glycoside, isoorientin (**6**) (Kuruüzüm-Uz et al., 2008).

The rutin, quercitrin and quercetin flavonoids were isolated from the roots of *M. flexuosa* (Koolen et al., 2012), but the occurrence of flavonoid *C*-glicosides was not yet reported in the literature. Nonetheless, flavonoid *C*-glicosides were already isolated from plants of the Arecaceae family. Flavone *C*-glycosides (84%), tricin (51%), luteolin (30%) and quercetin glycosides (24%) were found out in the leaves of the 125 species of the Palmae (Williams & Harborne, 1973). The orientin, vitexin and isoorientin flavonoids were isolated, respectively, from leaves of *Phoenix rupicola* T. and *P. loureirii* Kunth (Idaka et al., 1991). Tricin-7-*O*-rutinoside (**1**) and nicotiflorine (**3**) have the same pattern of linking of the units of sugars as the of rutin and isorhamnetin 3-*O*-β-rutinoside, both previously isolated from leaves of *Phoenix canariensis* hort. ex Chabaud (Asami et al., 1991). Tricin-7-*O*-rutinoside (**1**) was also obtained from *Phoenix humilis* Royle, hanceana Becc. var., and *Chamaerops humilis* L. (Arecaceae plants) (Hirai et al., 1986).

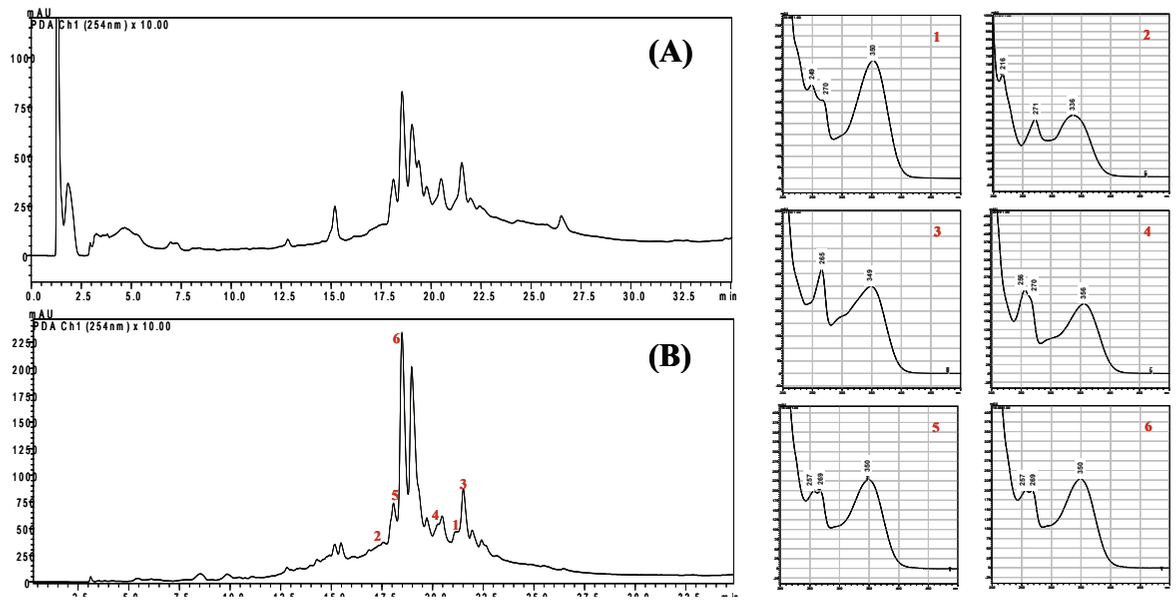


Figure 1. HPLC profiles of ethanolic extract (A) and ethyl acetate soluble fraction (B) of *Mauritia flexuosa* leaves at λ 254 nm. RP-18 column, 250 mm × 4.6 mm *i.d.*; 5 µM, mobile phase: gradient of methanol/water in 10% methanol to 100% methanol in 30 min, 100% of methanol in 5 min at a flow rate of 1 mL/min. UV spectra (200-500 nm) of tricin-7-*O*-rutinoside (**1**), isoschaftoside (**2**), nicotiflorine (**3**), rutin (**4**), orientin (**5**) and isoorientin (**6**).

Conclusions

The distribution of flavonoids in the Arecaceae family has already been well described. By means of this research, it was possible to show the presence of six glucosidic flavonoids in the *M. flexuosa* leaves, being half of *O*-glycosides and the other half of *C*-glycosides, thus contributing to the phytochemical knowledge of the Buriti, a plant mainly known by its fruits, which have been used as food and medicine by the rural populations in Brazil.

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Authors' contributions

YRFN contributed to collecting, the preparation of the herbarium and the identification of the plant. BBC designed the study, performed the isolation of the compounds, running all the laboratory work. DMO contributed to the compounds identification. EPSF performed the GPC chromatography and contributed to the critical reading of the manuscript. BBC, DMO, EPSF contributed to the critical reading of the manuscript. All the authors read the final manuscript and they approved the submission.

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***Correspondence**

Betania Barros Cota
Laboratório de Química de Produtos Naturais, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz
Av. Augusto de Lima, 1715, 30190-002 Belo Horizonte-MG, Brazil
betania@cpqrr.fiocruz.br
Tel.: 1 55 31 33497845
Fax: 1 55 31 32953115