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Original Article

Chemical constituents from *Bauhinia acuruana* and their cytotoxicity

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ABSTRACT

Phytochemical investigation of *Bauhinia acuruana* Moric., Fabaceae, resulted in the isolation of sixteen constituents, including two new compounds 2′-hydroxy-2,3,5-trimethoxybibenzyl (**1**), (2*R*,3*S*)-2-(3,4′-dihydroxyphenyl)-5-methoxy-6-methylchroman-3,7-diol (**2**), together with fourteen known ones (**3–16**). The structures of the compounds were established by spectroscopic analysis including HR-ESI-MS, 1D and 2D NMR data, followed by comparison with previously reported data from the literature. Compounds **1**, **2**, **6**, **7**, **8** and **9** were evaluated for their cytotoxicity, which turned out to be marginal in a panel of six human cancer cell lines.

Introduction

Bauhinia, Fabaceae, is a large genus containing about 500 species of shrubs, and small trees distributed throughout the tropical areas of Brazil, Peru, Asia, Paraguay, and Argentina (Soares and Scarminio, 2008). Many species of this genus have been widely used in folk medicine to treat diabetes, infections, pain and inflammation (Cechinel Filho, 2009).

Bauhinia acuruana Moric., is a shrub or subshrub that usually grows in mountainous areas and/or with altitudes of 600–1100 m (Vaz and Tozzi, 2003). Previous studies have shown that the essential oil from leaves of *B. acuruana* and pacharin (**6**), compound isolated from the roots of this species, showed larvicidal activity against *Aedes aegypti* (Gois et al., 2011; Góis et al., 2013). Previous investigations carried out with pacharin (**6**) and bauhiniastatin 1 (**7**) have shown that these compounds exhibited significant growth inhibition against pancreas adenocarcinoma (BXPC-3), breast adenocarcinoma (MCF-7), CNS glioblastoma (SF268), lung large cell (NCI-H460), and prostate carcinoma (DU-145) human cancer cell lines (Pettit et al., 2006).

In the search for bioactive natural compounds from *B. acuruana*, the isolation and structural elucidation of two new compounds (**1–2**), together with fourteen known compounds (**3–16**) are reported herein. In addition, the cytotoxicity of 2′-hydroxy-2,3,5-trimethoxybibenzyl (**1**), (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-5-methoxy-6-methylchroman-3,7-diol (**2**), pacharin (**6**), bauhiastatin 1 (**7**), fisetinidol (**8**), and (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-5-methoxychroman-3,7-diol (**9**) were assessed against colon carcinoma (HTC-116), glioblastoma (SF-295), ovarian carcinoma (OVCAR-8), breast adenocarcinoma (MCF-7), lung carcinoma (NCI-H292) and pro-myelocytic leukemia (HL-60) human cancer cell lines.

Materials and methods

General experimental procedures

Melting points were determined on a digital Mettler Toledo FP82HT apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR 1000 spectrometer with KBr

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pellets. Optical rotations were obtained on a Perkin-Elmer Q-200 polarimeter, at 589 nm and 25 °C. ^{1}H and ^{13}C NMR (1D and 2D) spectra were performed on Bruker Avance DPX and/or DRX-500 spectrometers, operating at 300 and 500 MHz for ^{1}H NMR, and 75 and 125 MHz for ^{13}C NMR, respectively. The chemical shifts (δ) are expressed in ppm. The high resolution mass spectra were recorded on a Shimadzu LCMS-IT-TOF spectrometer equipped with a Z-spray ESI (electrospray) source. High performance liquid chromatography (HPLC) analysis was performed on a Shimadzu chromatographer equipped with a ternary pump (Shimadzu LC-20AT) and UV detector (Shimadzu SPD-M20A), using Phenomenex RP-18 column (analytical: 250 × 4.6 μm, 5 m; semi-preparative: 250 × 10 mm, 10 μm). HPLC grade solvents were purchased from Tedia Co. (São Paulo, Brazil) and HPLC grade water was obtained by a Milli-Q purification system. Silica gel 60 (70–230 mesh, Vetec, Rio de Janeiro, Brazil) and Sephadex LH-20 (Pharmacia) were used for column chromatography. Thin layer chromatography (TLC) was performed on precoated silica gel polyester sheets (kieselgel 60 F_{254}, 0.20 mm, Silicycle, Quebec, Canada), and the spots were visualized by UV detection and/or heating after spraying with vanillin/perchloric acid/EtOH solution. The human tumor cell lines were obtained from the Banco de Células do Rio de Janeiro (RJ, Brazil) and Laboratório de Oncologia Experimental da Universidade Federal do Ceará (CE, Brazil).

Plant material

The leaves and stalks of *Bauhinia acuruana* Moric., Fabaceae, were collected in May 2008, while the roots were collected in June 2011 at Tianguá County, State of Ceará, Brazil. The plant material was identified by Edson Pereira Nunes from the Herbário Prisco Bezerra (EAC), Departamento de Biologia, Universidade Federal do Ceará, Brazil where voucher specimens (#42405 and #49268) have been deposited.

Extraction and isolation

Air-dried and finely powdered roots (1.1 kg) were exhaustively extracted with EtOH (4 × 8 l) at room temperature for three weeks, and evaporated under reduced pressure to yield the crude EtOH extract (50.3 g), which was subjected to silica gel column chromatography eluted with hexane, CH_2Cl_2, CH_2Cl_2/EtOAc (1:1), EtOAc and MeOH to give hexane (0.85 g), CH_2Cl_2 (1.59 g), CH_2Cl_2/EtOAc (1:1) (3.89 g), EtOAc (8.71 g) and MeOH (21.15 g) fractions. The CH_2Cl_2 fraction was submitted to silica gel column chromatography, using a hexane/CH_2Cl_2 gradient from 4:1 to 0:1, to afford six fractions (F1–F6). Fraction F6 (254.8 mg; CH_2Cl_2) and fraction F5 (136.5 mg; hexane/CH_2Cl_2, 1:4) were individually purified by silica gel column chromatography eluted with CH_2Cl_2 to obtain compounds **1** (19.4 mg) and **3** (22.5 mg), respectively. The CH_2Cl_2/EtOAc (1:1) fraction was chromatographed on a silica gel column eluted with a gradient of hexane/EtOAc (4:1 to 0:1) to provide seven fractions (F1–F7). Separation of fraction F3 (390.5 mg; hexane/EtOAc, 4:1) by silica gel column chromatography, using hexane/CH_2Cl_2 (1:1), hexane/CH_2Cl_2 (1:3) and CH_2Cl_2 as eluent, yielded the mixture a mixture of sitosterol (**4**) and stigmasterol (**5**) (28.8 mg; hexane/CH_2Cl_2, 1:1) and pacharin (**6**; 26.0 mg; CH_2Cl_2). A part of EtOAc fraction (2.4 g) was subjected to silica gel column chromatography, eluted with a gradient of hexane/EtOAc (6:4 to 0:1) to produce six fractions (F1-F6). Fraction F2 (144.3 mg; hexane/EtOAc, 6:4) was further chromatographed on a silica gel column, using hexane/EtOAc (17:3) as eluent, to afford bauhiniastatin 1 (**7**; 26.2 mg), while fraction F6 (64.2 mg, EtOAc) was submitted to semi-preparative RP-18 HPLC analysis, using an isocratic mixture MeOH/H_2O (9:11) to yield compounds **8** (29.6 mg; t_R 4.6 min), **9** (4.2 mg; t_R 5.3 min) and **2** (21.3 mg; t_R 7.2 min).

Air-dried leaves (0.9 kg) were successively extracted at room temperature with hexane (4 × 5 l) for three weeks, EtOAc (4 × 5 l) for three weeks, and then with EtOH (4 × 5 l) for the same period. After filtration, the solvents were evaporated under reduced pressure to give: hexane (21.0 g), EtOAc (29.0 g) and EtOH (108.0 g) extracts. A part of hexane extract (15.7 g) was fractioned over silica gel by elution with hexane, CH_2Cl_2, EtOAc, and MeOH to give four fractions: hexane (9.3 g), CH_2Cl_2 (4.3 g), EtOAc (0.95 g) and MeOH (0.13 g). The hexane fraction was subjected silica gel column chromatography eluting with the mixture of hexane/CH_2Cl_2 and CH_2Cl_2/EtOAc in increasing order of polarity. Fractions eluted with hexane/CH_2Cl_2 (1:1) were combined and chromatographed on a silica gel column, using a gradient of hexane/EtOAc (19:1 to 0:1) to give compounds **10** (13.6 mg; hexane/EtOAc, 9:1), and **11** (7.7 mg; hexane/EtOAc, 17:3). The EtOAc extract (29.0 g) was submitted to silica gel column chromatography eluted with hexane/CH_2Cl_2, CH_2Cl_2, CH_2Cl_2/MeOH, and MeOH to provide twenty four fractions (F1-F24). Fraction F20 (554 mg; CH_2Cl_2/MeOH, 4:1) was subjected to repeated Sephadex LH-20 column eluted with $CHCl_3$/MeOH (1:1) to obtain quercetin 3-*O*-rhamnoside (**12**; 7.1 mg) and daucosterol (**13**; 9.5 mg).

Air-dried and finely powdered stalks (1.2 kg) were exhaustively extracted with EtOH (4 × 5 l) at room temperature for three weeks, and evaporated under reduced pressure to yield the crude EtOH extract (70 g), which was submitted to silica gel column chromatography eluted with hexane, CH_2Cl_2, EtOAc and MeOH to give hexane (694 mg), CH_2Cl_2 (1.17 g), EtOAc (4.11 g) and MeOH (36.27 g) fractions. The hexane fraction was chromatographed on a silica gel column eluted with a gradient of hexane/EtOAc (9:1 to 0:1) to afford lupeol (**14**; 22.5 mg), and physcion (**15**; 14.7 mg). The EtOAc fraction was subjected to silica gel column chromatography using a CH_2Cl_2/MeOH gradient from 19:1 to 1:1, to obtain eleven fractions (F1–F11). Fraction F9 (339.1 mg; CH_2Cl_2/MeOH, 4:1) was further submitted to silica gel column chromatography, eluted with CH_2Cl_2/MeOH (17:3) to yield astilbin (**16**; 112.0 mg).

Spectral data

2′-Hydroxy-2,3,5-trimethoxybibenzyl = 2-[2-(2,3,5-trimethoxyphenyl)ethyl]phenol (**1**): Light brown oil; IR (KBr) $\sqrt{}_{max}$: 3419, 2960, 1600, 1493, 1458, 1202 cm^{-1}; NMR data ($CDCl_3$, 300 and 75 MHz) see Table 1; HRESIMS *m/z*: 311.1254 $[M+Na]^+$ (calcd for $C_{17}H_{20}NaO_4^+$: 311.1259).

(2*R*,3*S*)-2-(3′,4′-Dihydroxyphenyl)-5-methoxy-6-methylchroman-3,7-diol (**2**): Yellow solid; $[\alpha]_D^{20} = -2.6$ (*c* = 0.1, MeOH); IR (KBr) $\sqrt{}_{max}$: 3394, 1618 cm^{-1}; NMR data (CD_3OD, 300 and 75 MHz) see Table 1; HRESIMS *m/z*: 353.0819 $[M+Cl]^-$ calcd for $C_{17}H_{18}ClO_6^-$: 353.0792.

Cytotoxicity assay

The human tumor cell lines used in this work were MCF-7 (breast adenocarcinoma), NCI-H292 (lung carcinoma) and HL-60 (pro-myelocytic leukemia), which were obtained from the Banco de Células do Rio de Janeiro (RJ, Brazil), and HTC-116 (colon carcinoma), SF-295 (glioblastoma), OVCAR-8 (ovarian carcinoma) obtained from the Laboratório de Oncologia Experimental da Universidade Federal do Ceará (Ceará, Brazil). Cancer cells were maintained in RPMI 1640 medium or DMEN supplemented with 10% fetal bovine serum, 2 mm/l glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin at 37 °C with 5% CO_2. The cytotoxic activities of compounds **1**, **2**, **6**, **7**, **8** and **9** were tested against six human tumor cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT; Sigma Aldrich Co., St. Louis, MO, USA) reduction assay (Mosmann, 1983). For all experiments, tumor cells were plated in 96-well plates (10^5 cells/ml for

Table 1
^{1}H (300 MHz) and ^{13}C NMR (75 MHz) data of **1** (in $CDCl_3$) and **2** (in CD_3OD), including data obtained by HSQC and HMBC correlations. δ in ppm, J in Hz.

Position	1			2		
	δ_C	δ_H	HMBC (H → C)	δ_C	δ_H	HMBC (H → C)
1	135.90	–		–	–	
2	140.99	–	CH_2(7)	82.90	4.60 (*d*, *J* = 7.3)	C(9), C(1′), CH(2′), CH(6′)
3	153.42	–		68.81	3.99 (*m*)	
4	98.73	6.42 (*d*, *J* = 2.9)	C(2), C(3), C(5), CH(6)	28.83	2.63 (*dd*, *J* = 16.1, 8.0) 2.91 (*dd*, *J* = 16.1, 5.2)	CH(2), C(10)
5	156.41	–		158.80	–	
6	105.29	6.32 (*d*, *J* = 2.9)	C(2), CH(4), C(5)	111.41	–	
7	32.12	2.81 (*s*)	C(1), CH(6)	156.44	–	
8	32.66	2.81 (*s*)	C(1), C(1′), C(2′), CH(6′)	99.78	6.15 (*s*)	C(6), C(7), C(9), C(10)
9	–	–		154.33	–	
10	–	–		106.37	–	
1′	127.44	–		132.35	–	
2′	154.64	–		115.32	6.83 (*d*, *J* = 1.6)	CH(2), C(4′), CH(6′)
3′	115.97	6.90 (*d*, *J* = 6.0)	C(1′)	146.36	–	
4′	128.00	7.14 (*dt*)		146.36	–	
5′	120.55	6.80 (*dt*)	C(1′)	116.28	6.76 (*d*, *J* = 8.1)	C(1′), C(3′)
6′	130.10	7.12 (*d*, *J* = 6.0)	CH_2(8), C(2′)	120.09	6.70 (*dd*, *J* = 8.1, 1.6)	CH(2′), C(4′)
MeO-2	61.29	3.86 (*s*)	C(2)	–	–	
MeO-3	55.99	3.88 (*s*)	C(3)	–	–	
MeO-5	55.79	3.78 (*s*)	C(5)	60.46	3.67 (*s*)	C(5)
Me-6	–	–		8.83	2.04 (*s*)	C(5), C(6), C(7)

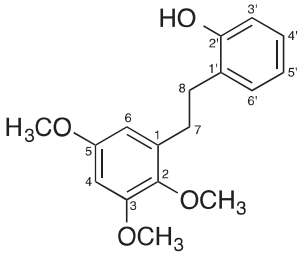
HSQC, Heteronuclear Single Quantum Coherence Spectroscopy; HMBC, Heteronuclear Multiple Bond Connectivity.

adherent cells or 3×10^5 cells/ml for leukemia). Compounds **1**, **2**, **6**, **7**, **8** and **9** dissolved in DMSO 1% were added to each well and incubated for 72 h. Control groups received the same amount of DMSO. The compound concentrations added to the cells ranged from 0.39 to 25.00 μg/ml. After 69 h of treatment, MTT (0.5 mg/ml) was added, 3 h later, the MTT formazan product was dissolved in 100 μl of DMSO, and absorbance was measured at 570 nm in plate spectrophotometer (Varioskan Flask, Thermo Scientific). Doxorubicin was used as positive control. IC_{50} values and their 95% confidence intervals for two different experiments were obtained by non linear regression using Graphpad Prism version 5.0 for Windows (GraphPad Software, San Diego, California, USA).

Results and discussion

The EtOH extract from roots of *B. acuruana* was subjected to multiple chromatographic steps to yield two new compounds (**1–2**) and the known compounds (**3–9**). The air-dried leaves were successively extracted with hexane, EtOAc, and EtOH. Chromatographic fractionation of the hexane and EtOAc extracts of *B. acuruana* leaves yielded the compounds (**10–11**) and (**12–13**), respectively. Three known compounds (**14–16**) were isolated from the EtOH extract from stalks of *B. acuruana*. The structures of known compounds (**3–16**) were identified as 2′-hydroxy-3,5-dimethoxybibenzyl (**3**) (Takasugi et al., 1987; De Sousa et al., 2016), a mixture of sitosterol (**4**) and stigmasterol (**5**) (Da Silva et al., 2012), pacharin (**6**) (Pettit et al., 2006; Anjaneyulu et al., 1984), bauhiniastatin 1 (**7**) (Pettit et al., 2006), fisetinidol (**8**) (Imai et al., 2008), (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-5-methoxychroman-3,7-diol (**9**) (Cren-Olivé et al., 2002), 1β,6α-dihydroxy-4(14)-eudesmene (**10**) (Moujir et al., 2011), aromadendrane-4β,10α-diol (**11**) (Meira et al., 2008), quercetin 3-*O*-rhamnoside (**12**) (Slowing et al., 1994), daucosterol (**13**) (Lendl et al., 2005), lupeol (**14**) (Imam et al., 2007), physcion (**15**) (Danielsen et al., 1992), and astilbin (**16**) (Bezerra et al., 2013) by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was isolated as a brown viscous liquid and possessed a molecular formula $C_{17}H_{20}O_4$ with eight degrees of unsaturation from its high-resolution electrospray ionization spectrum (HRESIMS) at *m/z* 311.1254 $[M+Na]^+$, (calcd 311.1259). The molecular formula was further substantiated by the ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** which showed 17 carbon resonances, including three methyl, two methylene, six methine, and six quaternary carbons, including four oxygenated at δ_C 140.99, 153.42, 154.64 and 156.41 (Table 1). The IR spectrum showed absorption bands for hydroxyl group at 3419 cm^{-1} and aromatic ring at 1600 and 1458 cm^{-1}. Its ^{1}H NMR spectrum exhibited two doublets at δ_H 6.42 (*J* = 2.9 Hz, H-4) and 6.32 (*J* = 2.9 Hz, H-6) for aromatic protons *meta*-positioned, indicating the occurrence of a 1,2,3,5-tetrasubstituted benzene ring, four signals at δ_H 6.90 (*d*, *J* = 6.0 Hz, H-3′), 7.14 (*dt*, H-4′), 6.80 (*dt*, H-5′), and 7.12 (*d*, *J* = 6.0 Hz, H-6′) which allowed to postulate the presence of a 1,2-disubstituted benzene ring, and one signal at δ_H 2.81 (*s*, 4H) attributed to a $-CH_2CH_2-$ unit. In addition, three singlets at δ_H 3.78 (MeO-5), 3.86 (MeO-2), and 3.88 (MeO-3) suggested the occurrence of methoxyl groups (Table 1). These data



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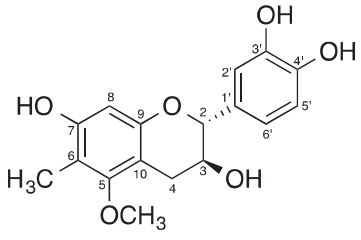

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Table 2
Cytotoxic activity of compounds **1**, **2**, **6**, **7**, **8** and **9**.

Compound	IC_{50} (μM) (95% confidence intervals)[a]					
	HCT-116 (colon carcinoma)	SF-295 (gliobastoma)	OVCAR-8 (ovarian carcinoma)	MCF-7 (breast adenocarcinoma)	NCI-H292 (lung carcinoma)	HL-60 (pro-myelocytic leukemia)
1	23.96 (19.79–28.47)	37.85 (27.08–52.08)	40.62 (25.69–64.24)	>86.80	57.63 (35.42–94.44)	19.44 (15.62–23.96)
2	>78.62	>78.62	>78.62	>78.62	>78.62	>78.62
6	19.26 (16.30–22.96)	14.44 (10.74–19.63)	23.33 (18.52–30.37)	20.00 (14.81–26.67)	11.11 (9.63–12.59)	8.15 (7.41–8.89)
7	25.70 (22.53–28.87)	21.13 (12.32–36.97)	22.89 (18.66–27.82)	21.83 (14.44–32.75)	10.91 (9.51–12.68)	10.21 (7.75–13.03)
8	>91.24	>91.24	>91.24	>91.24	>91.24	>91.24
9	>82.24	>82.24	>82.24	>82.24	>82.24	>82.24
Doxorubicin	0.18 (0.18–0.36)	0.36 (0.36–0.55)	0.55 (0.36–0.55)	0.55 (0.36–0.92)	0.36 (0.18–0.92)	0.04 (0.02–0.04)

HCT-116, colon carcinoma; SF-295, glioblastoma; OVCAR-8, ovarian carcinoma; MCF-7, breast adenocarcinoma; NCI-H292, lung carcinoma; HL-60, pro-myelocytic leukemia.
[a] The results were means of two independent experiments.

indicated that **1** was a bibenzyl (Kittakoop et al., 2000; Boonphong et al., 2007; Apisantiyakom et al., 2004; Yang et al., 2014; Chen et al., 2014; Xu et al., 2014). Detailed analysis of its ^{1}H and ^{13}C NMR spectra showed that compound **1** was similar to compound **3** (Takasugi et al., 1987; De Sousa et al., 2016), except for the presence of the signal at δ_H 3.86 (*s*, 3H) observed in the ^{1}H NMR spectrum of **1**, which was assigned to an additional methoxyl group (MeO-2). In the HMBC spectrum, the correlations from the signal at δ_H 6.42 (*d*, *J* = 2.9 Hz, H-4) with the carbon signals at δ_C 140.99 (C-2), 153.42 (C-3), and 156.41 (C-5), and correlations from the signal at δ_H 6.32 (*d*, *J* = 2.9 Hz, H-6) with the carbon signal at δ_C 98.73 (C-4) allowed to assign the location of methoxyl groups at C-2, C-3 and C-5. Similarly, the correlations from H-6′ at δ_H 7.12 (*d*, *J* = 6.0 Hz) with C-8 (δ_C 32.66), and C-2′ (δ_C 154.64), and the correlations from H_2-7 at δ_H 2.81 (*s*) with C-1 (δ_C 135.90), and C-6′ (δ_C 130.10) allowed to assign a phenolic hydroxyl group at C-2′ and confirmed the location of the $-CH_2CH_2-$ unit, respectively (Table 1). On the basis of these spectroscopic data, compound **1** was identified as 2′-hydroxy-2,3,5-trimethoxybibenzyl.

Compound **2** was obtained as yellow solid with a specific rotation of $[\alpha]_D^{20} = -2.6$. It had the molecular formula $C_{17}H_{18}O_6$ based on its HRESIMS (observed *m/z* 353.0819 $[M+Cl]^-$, calcd 353.0792) and ^{13}C NMR data, indicating nine degrees of unsaturation. Its IR spectrum showed absorption bands for hydroxyl group at 3394 cm^{-1} and aromatic ring at 1618 cm^{-1}. The ^{1}H and ^{13}C NMR data of **2** exhibited signals of two oxygenated CH groups at δ_H/δ_C 3.99 (*m*, H-3)/68.81, and 4.60 (*d*, *J* = 7.3 Hz, H-2)/82.90, CH_2 group at δ_H/δ_C 2.63 (*dd*, *J* = 16.1 and 8.0 Hz, H-4a)/28.83, and δ_H/δ_C 2.91 (*dd*, *J* = 16.1 and 5.2 Hz, H-4b)/28.83, and two signals of CH_3 groups at δ_H/δ_C 2.04 (*s*, Me-6)/8.83, and 3.67 (*s*, MeO-5)/60.46, together with one 1,2,3,4,5-pentasubstituted (δ_H/δ_C 6.15 (*s*, H-8))/99.78, and one 1,3,4-trisubstituted (δ_H/δ_C 6.83 (*d*, *J* = 1.6 Hz, H-2′)/115.32, 6.76 (*d*, *J* = 8.1 Hz, H-5′)/116.28, and 6.70 (*dd*, *J* = 8.1 and 1.6 Hz, H-6′)/120.09) benzene rings (Table 1). A comparison of the NMR data of **2** (Table 1) with those reported for (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-5-methoxychroman-3,7-diol (**9**) (Cren-Olivé et al., 2002) showed high similarity. The difference between compounds **2** and **9** is the presence of the signal at δ_H/δ_C 2.04 (*s*, 3H)/8.83 observed in the NMR spectra of **2**, which was assigned to a methyl group of aromatic ring (Me-6). The location of the methyl group at C-6 was established according to observed correlations from the signal at δ_H 2.04 (*s*, Me-6) with the carbon signals at δ_C 111.41 (C-6), 156.44 (C-7), and 158.80 (C-5), and from the signal at δ_H 6.15 (*s*, H-8) with the carbon signals at δ_C 111.41 (C-6) in the HMBC spectrum. The relative stereochemistry of **2** was substantiated by the NOESY data, which showed NOE correlations between the *pseudoaxial* hydrogen H-2 (δ_H 4.60 (*d*, *J* = 7.3 Hz)) and H-3 (δ_H 3.99 (*m*)), and by comparison of its optical rotation with the reported data for **8** (Imai et al., 2008) and **9** (Cren-Olivé et al., 2002). In addition, were observed NOE effect between hydrogen atoms: H-2 (δ_H 4.60 (*d*, *J* = 7.3 Hz)) and H-2′ (δ_H 6.83 (*d*, *J* = 1.6 Hz)); H-2 (δ_H 4.60 (*d*, *J* = 7.3 Hz)) and H-6′ (δ_H 6.70 (*dd*, *J* = 8.1 and 1.6 Hz)); and H-3 (δ_H 3.99 (*m*)) and H-6′ (δ_H 6.70 (*dd*, *J* = 8.1 and 1.6 Hz)) (Table 1). From the above data, the structure of **2** was determined to be (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-5-methoxy-6-methylchroman-3,7-diol.

2′-Hydroxy-2,3,5-trimethoxybibenzyl (**1**), (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-5-methoxy-6-methylchroman-3,7-diol (**2**), pacharin (**6**), bauhiastatin 1 (**7**), fisetinidol (**8**), and (2*R*,3*S*)-2-(3′,4′-dihydroxyphenyl)-5-methoxychroman-3,7-diol (**9**) were evaluated for their cytotoxic activity against colon carcinoma (HTC-116), glioblastoma (SF-295), ovarian carcinoma (OVCAR-8), breast adenocarcinoma (MCF-7), lung carcinoma (NCI-H292) and pro-myelocytic leukemia (HL-60) human cell lines by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay (Mosmann, 1983), using doxorubicin as positive control. The cytotoxic activities of these compounds are summarized in Table 2. Compound **6**, which in previous studies showed significant growth inhibition against pancreas adenocarcinoma (BXPC-3), breast adenocarcinoma (MCF-7), CNS glioblastoma (SF268), lung large cell (NCI-H460), and prostate carcinoma (DU-145) human cancer cell lines (Pettit et al., 2006), exhibited cytotoxicity against pro-myelocytic leukemia (HL-60) human cell lines, with IC_{50} value of 8.15 μM, but was inactive against the other tested human cell lines. In addition compounds **1**, **2**, **7**, **8** and **9** were inactive. These results indicated that compound **6** displays cytotoxic activity, which are in accordance with other studies reporting that different oxepin derivatives (Pettit et al., 2006; Boonphong et al., 2007; Li et al., 2013) can exert cytotoxic activities on cancer cell lines.

The phytochemical investigation of *B. acuruana* led to the isolation of two new compounds, identified as 2′-hydroxy-2,3,5-trimethoxybibenzyl (**1**) and (2*R*,3*S*)-2-(3,4′-dihydroxyphenyl)-5-methoxy-6-methylchroman-3,7-diol (**2**), along with a known bibenzyl (**3**), three streoids (**4**, **5**, **13**), two oxepin derivatives (**6**, **7**), four flavonoids (**8**, **9**, **12**, **16**), two sesquitepenes (**10**, **11**), one triterpene (**14**) and one anthraquinone (**15**). This is in accordance with previous reports on the chemical constituents isolated from the *Bauhinia* genus. Notably, compound **9** was a flavonoid isolated for the first time as natural product, but reported previously as a synthetic derivative (Cren-Olivé et al., 2002).

Since previous reports described the cytotoxic properties for species of the *Bauhinia* genus, our expectative was that some of the isolated compounds could display this effect, but, unfortunately, except for pacharin (**6**) which showed cytotoxicity against pro-myelocytic leukemia (HL-60) human cell lines, the compounds **1**, **2**, **7**, **8** and **9** were inactive.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

RWSG (PhD student) did the compound isolation procedure and contributed in compound identification by NMR and literature search. LMS, HCS, FEFS and ATAP contributed in carrying out the laboratory work, and interpretation of the spectroscopic data. MASL, AMCA, TLGL and RBF contributed in the interpretation of the spectroscopic data. GCCG and PBNS contributed to biological assays. FJTG contributed to plant collection and confection of herbarium. GMPS designed the study, supervised the laboratory work and wrote the manuscript. LMS contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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