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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