CHEMICAL CONSTITUENTS OF *Sinningia mauroana* AND SCREENING OF ITS EXTRACTS FOR ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES

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Artigo

Phytochemical study of aerial parts from *Sinningia mauroana* Chautems (Gesneriaceae) yielded 17 known compounds: sitosterol, stigmasterol, betulinic acid, oleanolic acid, hederagenin, maslinic acid, ursolic acid, pomolic acid, soranjidiol, methyl 4-hydroxyphenylacetate, 6-hydroxy-7-methoxy-α-dunnione, 7-hydroxy-6-methoxy-α-dunnione, jacaranone, calceolarioside A, conandroside, luteolin, and luteolin-7-*O*-glucoside. Complete NMR data of 6-hydroxy-7-methoxy-α-dunnione and 7-hydroxy-6-methoxy-α-dunnione were obtained in two different solvents. Hexane, ethyl acetate and ethanol extracts were screened for antimicrobial, antioxidant and cytotoxic activities. The antimicrobial activity was assayed against *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *C. parapsilosis*, *C. dubliniensis*, and *C. glabrata*, using the microdillution method. All extracts were inactive (MIC > 100 μg mL^{-1}). The antioxidant activity was evaluated with the ORAC method; the ethyl acetate and ethanol extracts were active (TE relative > 800 μg g^{-1}). The cytotoxic activity was tested against PC-3 (prostate) and SKMEL-103 (melanoma) human tumor cell lines, and 3T3 fibroblast cell line, using the MTT assay. The hexane extract showed strong activity against PC-3 and SKMEL-103 human tumor cell lines (IC$_{50}$ < 0.25 and 3.07 μg mL^{-1}, respectively), and a lower activity towards 3T3 fibroblasts (IC$_{50}$ = 6.84 μg mL^{-1}). The other extracts were inactive (IC$_{50}$ > 50 μg mL^{-1}).

Keywords: *Sinningia mauroana*; Gesneriaceae; triterpenes; naphthoquinones; phenolic compounds.

INTRODUCTION

Sinningia (Gesneriaceae) is a neotropical genus of herbs and subshrubs, comprising 75 species in Brazil, distributed mainly in Southern and Southeastern regions of the country.[1]

Until the last decade, the knowledge about the chemical constitution of *Sinningia* species was scant, with only four species investigated: *S. aggregata*, *S. allagophylla*, *S. cardinalis* e *S. speciosa*.[2] Since then, new phytochemical studies were carried on *S. aggregata*[3] and *S. allagophylla*,[4] and five species were studied for the first time: *S. canescens*,[5,6] *S. hatschbachii*,[7] *S. leucotricha*,[6,8] *S. reitzii*,[9] e *S. warmingii*.[10] The chemical profile that arises from these studies shows that the genus is very chemically diverse, producing anthraquinones, naphthoquinones and derivatives, naphthochromenes, terpenoids, flavonoids, cyclohexylethanoids, and phenylethanoid glycosides.[2-10]

Some compounds isolated from *Sinningia* spp exhibit biological activity. The naphthoquinone dunniol, obtained from *S. allagophylla*, showed strong cytotoxic activity against glioma (U251), breast (MCF-7), ovarian (OVCAR-3), and ovarian resistant (NCI-ADR/RES) human tumor cell lines.[2] This plant also furnished the naphthochromene 8-methoxylapachenol, which showed anti-inflammatory and antinociceptive activities.[11] From *S. reitzii,* two naphthoquinones with biological activity were isolated: 6,7-dimethoxydunnione that inhibited strongly the grow of prostate (PC-3) and cervix (HeLa) human tumor cell lines, and 8-hydroxydehydrodunnione, which showed anti-inflammatory and antinociceptive activities.[9] Aggregatin D, a naphthoquinone derivative isolated of *S. aggregata* and *S. warmingii*, showed antinociceptive activity,[12] and cytotoxic activity against ovarian (OVCAR-03) human tumor cell line.[3] Finally, the naphthoquinone 7-hydroxy-6-methyoxy-α-dunnione, obtained of *S. canescens*, *S. leucotricha* and *S. hatschbachii*, displayed anti-inflammatory and antipyretic

activities.[13] Therefore, *Sinningia* species are a still not fully exploited source of compounds with biological activity.

As part of our research project on Brazilian *Sinningia*, we reported here the first chemical and biological study of *S. mauroana* Chautems. This plant is a perennial subshrub (50-230 cm in height) that either lacks tubers or displays only one reduced tuber. *S. mauroana* can be considered an ornamental species, because it is a very beautiful plant that produces red-orange flowers from March to November. Its occurrence is restricted to the Coastal Atlantic Forest in the São Paulo and Paraná states (Brazil).[14] Vernacular names or uses in the folk medicine were not found for *S. mauroana*.

EXPERIMENTAL

General procedures

Optical rotations were measured in CHCl$_3$ on a JASCO PTC-203 polarimeter (λ = 589 nm, temperature = 20 ºC). Optical density was measured using a Synergy 2 (Bio-Tek) spectrophotometer. One-dimensional (^{1}H, ^{13}C) and two-dimensional (gHSQC, gHMBC) NMR spectra were recorded on Brucker spectrometers (AC 200, Avance 400, and or Avance 600) observing ^{1}H at 200, 400, or 600 MHz, and ^{13}C at 50, 100 or 150 MHz. Deuterated acetone (acetone-d_6), chloroform (CDCl$_3$) or methanol (MeOH-d_4) were used as solvents, and the chemical shifts are given in ppm (δ), with coupling constants (*J*) in Hz. TMS was used as internal reference. Geometry optimization and density functional theory (DFT) calculations on the electronic structure of the compounds employed B3LYP functional, having Los Alamos ECP as basis set as implemented in Gaussian suite program. Theoretical optical rotations were calculated after geometry optimization.[15] HPLC separations were performed in a Waters apparatus equipped with PDA detector, and a semi-preparative Nucleosil 100-5 C18 column (250 x 10 mm). Acetonitrile:water 40:60 (isocratic) was used as mobile phase, with a flow rate of 2.8 mL min^{-1},

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at room temperature, applied for 25 min. Column chromatographic separations (CC) were carried on silica gel 60 (Merck, 230-400 mesh) or sephadex LH-20 (Sigma Co), while precoated silica gel 60 GF$_{254}$ plates (Macherey-Nagel) were used for TLC and preparative TLC (PTLC). Compounds were visualized by exposure under UV$_{254/365}$ light and spraying with 5% (v/v) H$_2$SO$_4$ in ethanol solution, followed by heating on a hot plate. All solvents were analytical or spectroscopic grade, and the mixtures of solvents were prepared as v/v.

Plant material

Stems of *Sinningia mauroana* Chautems were cut off from a natural population in Antonina, Paraná State, Brazil (25º25'44'' S; 48º42'43'' W), in March/2013. The plant was collected and identified by Clarice B. Poliquesi, who deposited a voucher specimen in the Herbarium of Museu Botânico Municipal (MBM 391.575). The stems were cultivated in Curitiba, Paraná State, Brazil (25º26'34.85'' S, 49º14'22.58'' W). Aerial parts for phytochemical study were collected from these cultivated plants in March/2015. The access was registered on SISGEN under number AF5C97F.

Extraction and isolation

Dried and powdered aerial parts of *S. mauroana* (327.0 g) were extracted with hexanes (Hex, mixture of isomers), EtOAc, and EtOH, successively (three successive extraction employing 1.6 L of solvent each time), at room temperature. The solvents were removed using a rotatory evaporator, at reduced pressure, to give the respective extracts. An aliquot (20 mg) of each extract was reserved for biological studies.

The Hex extract (H, 1.38 g) was submitted to CC (silica gel) eluted with mixtures of Hex:EtOAc (95:5; 9:1; 8:2; 7:3), EtOAc and MeOH, yielding 11 fractions after TLC analysis (H$_{1-11}$). An aliquot (86.0 mg) of H$_6$ (169.1 mg; eluted with Hex:EtOAc 9:1) yielded **1** + **2** (23.2 mg) after PTLC (Hex:EtOAc 8:2). An aliquot (68.5 mg) of H$_8$ (120.7 mg; eluted with Hex:EtOAc 8:2) gave **3** (7.2 mg) after PTLC (Hex:Me$_2$CO 8:2).

The EtOAc extract (A, 1.77 g) was submitted to CC (silica gel) eluted with mixtures of Hex:EtOAc (95:5; 9:1; 8:2; 7:3; 3:2), EtOAc, and MeOH, yielding 13 fractions (A$_{1-13}$) after TLC analysis. Fraction A$_4$ (47.5 mg, eluted with Hex:EtOAc 8:2) yielded **1** + **2**. Fraction A$_5$ (24.4 mg, eluted with Hex:EtOAc 8:2) yielded **9** (1.9 mg) and **10** (1.8 mg) after PTLC (Hex:EtOAc 7:3). A$_6$ (23.8 mg, eluted with Hex:EtOAc 7:3) yielded **4** (4.4 mg); **4** + **7** (3.9 mg), and **4** + **7** + **8** (5.9 mg) after PTLC (Hex:Me$_2$CO 3:2). A$_7$ (12.4 mg, eluted with Hex:EtOAc 7:3) contained a mixture of **11** + **12** that was separated by HPLC, yielding **11** (4.8 mg, retention time = 17.03 min) and **12** (5.7 mg, retention time = 18.10 min). Compound 13 (10.8 mg) was obtained by PTLC (CH$_2$Cl$_2$:MeOH 9:1) from A$_9$ (85.1 mg, eluted with EtOAc). Fraction A$_{10}$ (39.5 mg, eluted with EtOAc) yielded **5** + **6** (5.6 mg) and **13** (1.5 mg) after PTLC (CH$_2$Cl$_2$:diethyl ether 1:1).

The ethanol extract (4.26 g) was dissolved in H$_2$O:EtOH 1:1 (120 mL) and extracted with 1-butanol (3 x 40 mL). The fraction soluble in 1-butanol (B, 2.02 g) was submitted to CC (Sephadex LH-20) eluted with MeOH to give **11** subfractions (B$_{1-11}$). B$_7$ (72.3 mg) yielded **14** (7.7 mg) and **15** (9.8 mg) after PTLC (CH$_2$Cl$_2$:MeOH 9:1). Compounds **17** (15.0 mg) and **16** (1.2 mg) were obtained from B$_{10}$ (44.8 mg), by PTLC (CH$_2$Cl$_2$:MeOH 9:1).

Biological Assays

Antimicrobial activity

The antimicrobial activity of extracts in Hex, EtOAc and EtOH

was evaluated by the microdillution method (96-well plates), as previously reported.[16] The following microorganisms were used: *Staphylococcus aureus* ATCC 14458, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 (field strain), *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C. dubliniensis* ATCC 778157, and *C. glabrata* ATCC 30070. The samples were tested at concentrations varying between 12.5-1000 μg mL^{-1}, and the minimal inhibitory concentration (MIC) was calculated in μg mL^{-1} as the lowest concentration showing complete inhibition of the tested strain. The assays were carried on in duplicate, and chloramphenicol and ketoconazole were used as positive controls for bacteria and fungi, respectively. The negative control was the solvent H$_2$O:dimethylsulfoxide (H$_2$O:DMSO, 95:5).

Antioxidant activity

The *in vitro* antioxidant capacity of the extracts was measured by the ORAC-FL assay, with fluorescein as the fluorescent probe and AAPH [2,2'-azobis(2-amidinopropane) dihydrochloride] as the free radical source. The experiments were performed in 96 wells plates as previously reported.[17] Sequential dilutions of the samples (5-500 μg mL^{-1}) were prepared in phosphate buffer:DMSO 99:1. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard in concentrations of 12.5-200 μmol L^{-1}. The reading was performed using a fluorescent filter (excitation λ = 485 nm and emission λ = 528 nm) in a microplate reader, monitoring the reaction at 37 °C every 2 min for a period of 70 min. Results were expressed as μmol of trolox equivalent (TE) per gram of dried extract (μmol TE g^{-1}). Caffeic acid, chlorogenic acid, quercetin and isoquercetin were used as positive controls, while the solvent was the negative control. All experiments were performed in triplicate.

Cytotoxic activity

The cytotoxic activity of the extracts was evaluated *in vitro* against the human tumor cell lines PC-3 (prostate) and SKMEL-103 (melanoma), and the no cancer cell line 3T3 (fibroblast), all from the American Type Culture Collection (ATCC). The assays were performed using the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide] method, as previously described.[18] Briefly, the cells were distributed in 96-well plates (100 μL cells by well), and exposed to four different concentrations of each extract (0.25, 2.5, 25, and 250 μg mL^{-1}) in DMSO (0.1%) at 37 °C, with 5% of CO$_2$, for 48 h. The final concentration of DMSO did not affect cell viability. Doxorubicin (0.025, 0.25, 2.5, and 25 μg mL^{-1}) was used as the positive control, and DMSO was the negative control. The viability of cells was quantified by the ability of living cells to reduce the yellow dye MTT to a blue formazan product. At the end of 48 h of incubation, the culture medium was replaced by fresh medium containing 0.5 μg mL^{-1} of MTT. After three hours, the formazan product was dissolved in DMSO and the optical density was measured by spectrophotometry at 570 nm. The experiments were performed in triplicate and the concentration needed to achieve 50% inhibition of cell viability (IC$_{50}$) was calculated in μmol L^{-1} by non-linear regression using the GRAPHPAD program.[19]

RESULTS AND DISCUSSION

The extracts of aerial parts of *Sinningia mauroana* yielded 17 known compounds, which were identified as sitosterol (**1**),[20] stigmasterol (**2**),[20] betulinic acid (**3**),[21] oleanolic acid (**4**),[21] 23-hydroxyoleanolic acid (hederagenin, **5**),[22] 2-α-hydroxyoleanolic acid (maslinic acid, **6**),[22] ursolic acid (**7**),[21] 19-α-hydroxyursolic acid (pomolic acid, **8**),[21] 1,6-dihydroxy-2-methylanthraquinone (soranjidiol, **9**),[23] methyl 4-hydroxyphenylacetate (**10**),[24] 6-hydroxy-7-methoxy-α-dunnione

(**11**),[25] 7-hydroxy-6-methoxy-α-dunnione (**12**),[5] jacaranone (**13**),[26] calceolarioside A (**14**),[27] conandroside (**15**),[28] luteoline (**16**),[29] and luteoline-7-*O*-glucoside (**17**).[30] Compounds **1-3** were isolated from the hexane extract, **4-13** from the ethyl acetate extract, and **14-17** from the fraction of the ethanol extract soluble in 1-butanol. The triterpenes **6-8**, the naphthoquinone **11**, and the flavonoids **16-17** are being described for the first time in *Sinningia* (Figure 1). All isolated compounds were analyzed by NMR (1D and 2D), and the data were compared with the literature.

Previously published NMR data of **11** are incomplete and disagree with our results because the spectra were recorded in different solvents,[25] but the structure was confirmed by analysis of HSQC and HMBC spectra. The NMR data of the naphthoquinones **11** and **12** are very similar when obtained in the same solvent, making it difficult to discriminate between these isomers by direct comparison with the literature data. However, the distinction is possible considering mainly the chemical shifts of the aromatic hydrogens (H-5 and H-8) and of the hydroxy group. Furthermore, the correlations observed

in the HMBC allow the unambiguous assignments of the aromatic hydrogens because C-1 is more shielded than C-4.[9]

In the ^{1}H NMR spectrum of **11**, recorded in CDCl$_3$, the signals of aromatic hydrogens are observed at δ$_H$ 7.51 (H-8) and 7.53 (H-5), while in the ^{1}H NMR spectrum of **12** these signals are at δ$_H$ 7.51 (H-5) and 7.55 (H-8). The signal of the hydroxy group is observed at δ$_H$ 6.17 and 6.02 in the ^{1}H NMR spectra of **11** and **12**, respectively (Table 1, Figure 22S). By using a more polar solvent, as deuterated acetone, the chemical shifts of the hydroxy groups are greatly deshielded, and their signals are observed now at δ$_H$ 9.00 (**11**) and 8.77 (**12**) (Table 2). On the other hand, the aromatic hydrogens became more shielded and with better resolution, with signals at δ$_H$ 7.39 (H-5) and 7.47 (H-8), for the compound **11**, and at δ$_H$ 7.37 (H-8) and 7.48 (H-5), for the compound **12** (Table 2, Figure 28S).

The ^{13}C NMR data of **11** and **12** are very similar in both used solvents, with deuterated acetone causing small deshield effects (Tables 1 and 2, Figures 23S, 29S and 32S). For identification purpose, it is important note that hydrogenated aromatic carbons
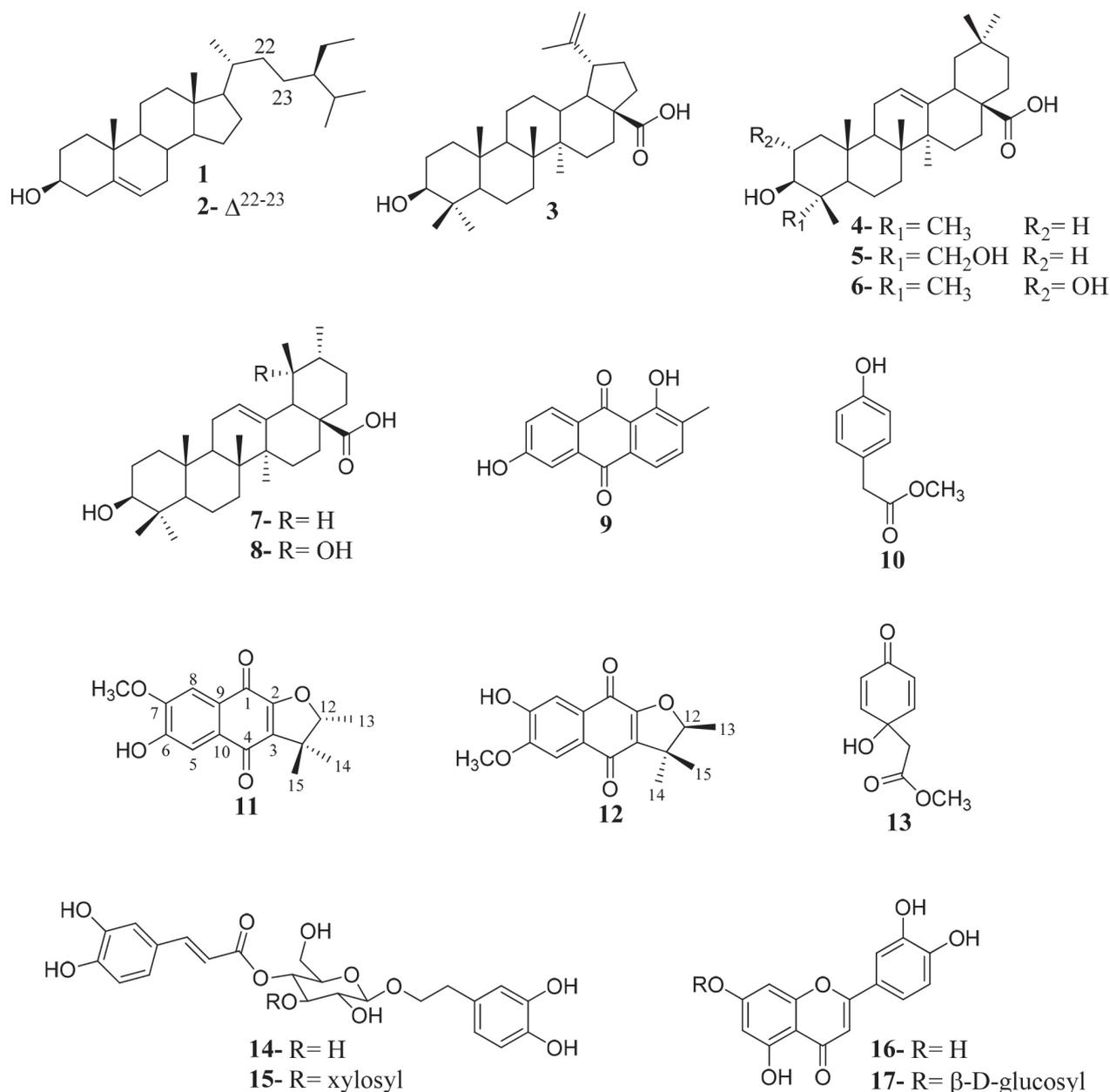

Figure 1. *Chemical structures of isolated compounds*

(C-5 and C-8) show quite different chemical shifts, with the carbon *ortho* to the one bonded to the methoxy group being more shielded (δ_C 107.8-109.4) than the carbon *ortho* to the one attached to the hydroxy group (δ_C 112.3-113.0), independent of the solvent used. As the position of methoxy and hydroxy groups are changed in **11** and **12**, the assignments of C-5 and C-8 also are inverted. Another interesting feature is the chemical shifts of oxygenated carbons (C-6 and C-7). Both show very close chemical shifts (around δ_C 150), but C-7 is always more protected than C-6, independent of used solvent or attached substituent (Tables 1 and 2).

The NMR assignments were corroborated by HSQC and HMBC spectra of **11** and **12**. In the HMBC spectrum of **11** (CDCl₃, Table 1, Figure 25S), the hydrogen at δ_H 7.51 showed a cross-peak with a carbon at δ_C 178.2, which is typical of C-1 in the framework of the α-dunniones,[9] while the hydrogen at δ_H 7.53 showed correlation with a carbon at δ_C 181.5 that is characteristic of C-4. Therefore, the hydrogens at δ_H 7.51 and 7.53 were located at C-8 and C-5, respectively. The position of the methoxy group in C-7 was deduced from correlations among H-5 and the methoxy group (at δ_H 4.02) with an oxygenated carbon at δ_C 149.6 (C-7). Otherwise, in the HMBC spectrum of **12** (CDCl₃, Table 1, Figure 27S) the hydrogen at δ_H 7.51 showed a cross-peak with a carbon at δ_C 182.2 (C-4), while the other aromatic hydrogen (δ_H 7.55) showed a cross-peak with a carbon at δ_C 178.2 (C-1). Now, H-8 (δ_H 7.55) and the methoxy group (δ_H 4.03) showed correlations with a carbon at δ_C 150.9 (C-6). These and the remaining correlations (Table 1) confirmed **11** and **12** as isomers that differ in the position of the substituents in the aromatic ring. The same conclusion could be achieved from HSQC and HMBC spectra in deuterated acetone (Table 2, Figures 31S and 34S).

The absolute configuration of naphthoquinones of the α-dunnione type has been previously determinated by different methods. The octant rules were applied to analyze the CD spectra of (-)-6,8-dihydroxy-7-methoxy-α-dunnione, which had its absolute configuration determinated as *R*.[31] This conclusion was extended to levorotatory α-dunnione and derivatives, including compound **11**, which was previously isolated as the levorotatory isomer.[25] Oddly, both papers show the structures for the *S* isomer.[25,31] In another approach, the *R* absolute configuration was established for (+)-α-dunnione by X-ray diffraction analysis of (+)-4-bromophenyl-hydrazone-dunnione.[32] This result was used to assign the absolute configuration of several α-dunnione derivatives, including compound **12**.[5,33] Considering these contradictory reports, we decide to use computational calculations to deduce the absolute configuration of **11** and **12**. It is possible to calculate the optical rotation of a specific enantiomer employing the density functional theory (DFT)[15]; comparison between the calculated and experimental values leads to the assignment of absolute configuration. The optical rotation calculated for compound **11** was 125.1, with positive signal for the *S* isomer and negative for the *R* isomer. The experimental optical rotation measured here was -98.9 (lit. -98),[25] and accordingly, this compound had its absolute configuration assigned as *R*. For compound **12**, the calculated value was -127.4 for the *S* isomer, and the experimental optical rotation was -80.7 (lit. -86.9).[5] Therefore, its absolute configuration was assigned as *S*. It is remarkable that the position of distant substituent affects the signal of optical rotation of **11** and **12**.

The hexane, EtOAc, and EtOH extracts were evaluated for antimicrobial, antioxidant and cytotoxic activities. The antimicrobial assays were made using Gram-positive and Gram-negative bacteria, and fungi. The hexane extract was inactive against all tested microorganisms, while the EtOAc and EtOH extracts inhibited the growth only of Gram-positive bacteria (*Staphylococcus aureus* and *S. epidermidis*), with minimal inhibitory concentration (MIC) of 250 µg mL⁻¹ (Table 1S). These values of MIC indicate a very weak antimicrobial activity of *S. mauroana* extracts, considering the expected MIC ≤ 100 µg mL⁻¹ for significant activity of extracts.[34] These results are consistent with the compounds found in each extract. Previous studies demonstrated that betulinic acid (**3**), isolated here from the hexane extract, was inactive toward various microorganisms, while oleanolic (**4**) and ursolic (**7**) acids, isolated now from the EtOAc extract, showed only a weak activity against Gram-positive bacteria.[35] Furthermore, phenolic compounds, as

Table 1. NMR data for **11** (400 MHz) and **12** (600 MHz), in CDCl₃

position	11			12		
	δ_H mult. (*J* in Hz)	δ_C	HMBC	δ_H mult. (*J* in Hz)	δ_C	HMBC
1		178.2			178.2	
2		158.7			158.7	
3		130.0			130.1	
4		181.5			182.2	
5	7.53 s	112.3	4, 9	7.51 s	107.8	4, 7, 9
6		150.6			150.9	
7		149.6			149.3	
8	7.51 s	108.0	1, 6, 10	7.55 s	112.3	1, 10
9		125.7			126.5	
10		129.2			128.1	
11		45.6			45.2	
12	4.56 q (6.6)	91.6	14, 15	4.56 q (6.6)	91.5	14, 15
13	1.44 d (6.6)	14.2	11, 12	1.43 d (6.6)	14.2	11, 12
14	1.27 s	20.7	3, 11, 12, 15	1.47 s	26.0	3, 11, 12, 15
15	1.47 s	25.9	3, 11, 12, 14	1.27 s	20.6	3, 11, 12, 14
OCH₃	4.02 s	56.6	7	4.03 s	56.5	6
OH	6.17 s		5, 6	6.02 s		7

Table 2. NMR data for **11** (400 MHz) and **12** (600 MHz), in acetone-d_6

position	11			12		
	δ_H mult. (*J* in Hz)	δ_C	HMBC	δ_H mult. (*J* in Hz)	δ_C	HMBC
1		178.1			178.4	
2		159.6			159.5	
3		130.6			130.7	
4		182.5			182.6	
5	7.39 s	113.0	4, 9	7.48 s	109.0	4, 7, 9
6		151.9			153.0	
7		151.4			152.6	
8	7.47 s	109.0	1, 6, 10	7.37 s	112.8	1, 6, 10
9		125.5			127.3	
10		129.8			128.5	
11		45.8			45.8	
12	4.57 q (6.6)	91.8	14, 15	4.57 q (6.6)	91.8	14, 15
13	1.41 d (6.6)	14.5	11,12	1.40 d (6.6)	14.5	11, 12
14	1.26 s	20.8	3, 11, 12, 15	1.46 s	26.2	3, 11, 12, 15
15	1.44 s	26.2	3, 11, 12,14	1.26 s	20.8	3, 11, 12, 14
OCH$_3$	4.00 s	56.7	7	4.02 s	56.7	6
OH	9.00 s		5	8.77 s		7, 8

those present in the EtOAc and EtOH extracts, also are known to be active against Gram-positive bacteria.[34] Similar results have been found for other *Sinningia* species, as *S. hatschbachii* and *S. warmingii*.[7,10]

The antioxidant capacity, measured by ORAC method, was expressed as μmol of trolox equivalent (TE) by gram of dried extract (μmol TE g^{-1}). Active extracts should exhibit TE $\geq$ 800 μmol g^{-1}. Accordingly, the EtOAc and EtOH extracts of *S. mauroana* are active, while the hexane extract is inactive (Table 3). Again, these results are consistent with the compounds isolated from each extract. Jacaranone

Table 3. Antioxidant capacity of *S. mauroana* extracts

samples	ORAC assay (μmol TE g^{-1})
Hexane extract	215.9 ± 0.5
EtOAc extract	873.9 ± 112.6
EtOH extract	1251.2 ± 29.2
Quercetin[a]	5.60 ± 0.08[b]
Isoquercetin[a]	5.15 ± 0.09[b]
Caffeic acid[a]	2.85 ± 0.03[b]
Chlorogenic acid[a]	2.65 ± 0.03[b]

[a] positive controls; [b] data of pure compounds are expressed as relative trolox equivalent. The values are average of triplicate assays ± standard deviation.

(**13**), obtained from EtOAc extract, showed antioxidant capacity in a previous study.[36] The presence of phenolic glycosides (**14-15**) and flavonoids (**16-17**) justify the higher antioxidant activity of EtOH extract. In fact, the antioxidant capacity of phenylethanoid glycosides and flavonoids had been demonstrated by several previous studies.[37-39] Calceolarioside A (**14**) was found to be a strong antioxidant.[39] Luteolin (**16**) and luteolin-7-*O*-glucoside (**17**) also showed significant antioxidant capacity.[38]

The cytotoxic activity of the extracts was evaluated against PC-3 (prostate) and SKMEL-103 (melanoma) human tumor cell lines, besides the 3T3 (fibroblast) as cell line control. The EtOAc and EtOH extracts were considered inactive (IC$_{50}$ > 50 μg mL^{-1}) (Table 4). On the other hand, the hexane extract displayed strong cytotoxic activity (IC$_{50}$ $\leq$ 10 μg mL^{-1}) against all tested cell lines. The most susceptible cell line was PC-3 (IC$_{50}$ < 0.25 μg mL^{-1}), followed by SKMEL-03 (IC$_{50}$ 3.07 μg mL^{-1}) and 3T3 (IC$_{50}$ 6.84 μg mL^{-1}). In comparison with the control cell lines (3T3 – fibroblast), the hexane extract showed high selectivity toward the PC-3 (prostate) cell lines. In comparison with the positive control doxorubicine, the hexane extract was more active against SKMEL-103 cell lines and less active against the control cell line 3T3 (Table 4). These results can be explained by presence in the hexane extract of sitosterol (**1**) and betulinic acid (**3**). Previous studies showed that **1** induce apoptosis in PC-3 cell lines,[40] while **3** is active against several human tumor cell lines, including metastatic melanoma and prostate.[41]

Table 4. Cytotoxic activity of *S. mauroana* extracts

Cell line	IC$_{50}$ (μg mL^{-1}) ± standard deviation			
	Hexane extract	EtOAc extract	EtOH extract	Doxo
PC-3 (prostate)	< 0.25	103.3 ± 0.05	90.25± 0.08	0.20 ± 0.01
SKMEL-103 (melanoma)	3.07 ± 0.18	> 250	> 250	4.03 ± 0.08
3T3 (fibroblast)	6.84 ± 0.13	57.28 ± 0.10	93.85± 0.04	0.54 ± 0,14

Doxo: doxorubicine; IC$_{50}$: average of triplicate assays ± standard deviation.

CONCLUSION

The phytochemical study of *S. mauroana* aerial parts revealed that this plant accumulates several classes of secondary metabolites, like other *Sinningia* species previously studied. Among the isolated compounds, phenolic glycosides and flavonoids are responsible for the high antioxidant activity of ethanolic extract. On the other hand, the hexane extract shows a strong and selective activity against prostate (PC-3) human tumor cell lines, which was related with the presence of sitosterol and betulinic acid.

SUPPLEMENTARY MATERIAL

Data and spectra of NMR of isolated compounds from aerial parts of *S. mauroana* are available in http://quimicanova.sbq.org.br, as PDF file.

ACKNOWLEDGMENTS

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