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Biological potential of *Stillingia oppositifolia*

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Abstract: Organic extracts from leaves and stems of *Stillingia oppositifolia* Baill. ex Müll. Arg., Euphorbiaceae, were screened for antifungal and cytotoxic properties. The extracts presented Minimum Inhibitory Concentration values around 250 μg.mL^{-1} against *Candida krusei* and *Candida tropicalis*, and around 63 μg.mL^{-1} for *Paracoccidioides brasiliensis*. They were tested on three human cell lines (UACC-62, MCF-7, and TK-10), disclosing GI50 values, (concentration able to inhibit 50% of the cell growth) ranging from 50 to 100 μg.mL^{-1}. Organic extract from stems furnished hexanic, dichloromethanic and aqueous phases after partition. Chromatographic fractionation of the hexanic soluble phase of the stems yielded aleuritolic acid 3-acetate, β-sitosterol, 3-epi-β-amyrin, β-amyrone and palmitic acid. These compounds showed antifungal and cytotoxic activities in the same range as the organic crude extract and low toxic effect against mononuclear cells obtained from human peripheral blood. This is the first report on chemical and biological potential of *S. oppositifolia*.

Introduction

Stillingia oppositifolia Baill. ex Müll. Arg., popularly known as "leiterinho" in Brazil, grows at altitudes ranging from 600 to 1000 m in Araucaria forests of Minas Gerais and Rio Grande do Sul states in Brazil (Rogers, 1951). The genus *Stillingia* belongs to the Euphorbiaceae family, one of the largest families of flowering plants, with approximately 300 genera and 8000 species (Webster, 1994; Radcliffe-Smith, 2001).

Species from this family are known for producing a large amount of diterpenes and triterpens, some of them with important pharmacological activities such as antitumor and anti-inflammatory properties (Aylward et al., 2001; Aylward & Parsons, 2002). Only few works on the chemistry of *Stillingia* species have been reported in the literature. Toxic and irritant daphnane and tigliane diterpene esters were isolated from the roots of *S. sylvatica* (Adolf & Hecker, 1980), while pimarane, kaurane, atisane and tonantzitlolone diterpenes were isolated from the roots of *S. sanguinolenta*, a plant used to prepare a poultice applied after childbirth (Draeger et al., 2007).

In an effort to expand the spectrum of active agents from natural resources, we screened extracts from stems and leaves of *Stillingia oppositifolia* for antifungal and cytotoxic properties. We report herein the first biological investigation on extracts and the isolation of five compounds from *S. oppositifolia*.

Material and Methods

General experimental procedures

Nuclear Magnetic Resonance experiments (^{1}H and ^{13}C, DEPT, HMQC and HMBC) were recorded on a Brucker DRX 400 spectrometer using standard Bruker pulse sequences and conditions. Electron impact (70 eV) low-resolution mass spectra (EI-MS) were obtained on a Shimadzu QP5050A equipped with a direct insertion probe. Centrifugal Thin-Layer Chromatography (CTLC) on 1 mm thick silica gel 60 PF_{254} layer was performed on a 7924T model Chromatotron device (Harrison Research). Medium pressure liquid chromatography (MPLC) was performed on a 250 x 20 mm Büchi glass column with the mobile phases pumped at 5 mL.min^{-1} flow rate. Column chromatography (CC) was carried out on silica gel (Merck). Thin-Layer Chromatography (TLC) was run in precoated commercial plates on aluminium foil (Merck). They were eluted in a

pre-saturated chamber using solvents mixtures in different proportions of: a) chloroform:methanol:water ($CHCl_3$:MeOH:H_2O), b) hexane:dichloromethane (Hex:DCM) or c) hexane:ethyl acetate (Hex:EtOAc). The spots were visualized under visible, UV light at 254 nm and 360 nm, and after spraying the plate with an ethanol solution of vanilin-H_2SO_4 and heating with a heated plate.

Plant collection

Leaves and stems from *Stillingia oppositifolia* Baill. ex Müll. Arg., Euphorbiaceae, were collected in São Gonçalo do Rio Preto, Minas Gerais, Brazil, on October 2003. A voucher specimen (BHCB 87513) was deposited in the BHCB Herbarium at the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Extract preparation and isolation of compounds

Leaves and stems of *S. oppositifolia* were macerated in DCM:MeOH (1:1) at room temperature and the solutions were concentrated under vacuum in a rotary evaporator at temperatures below 45 °C. The residual solvent was removed in a vacuum centrifuge at 40 °C. An aliquot of organic crude extract from stems (23 g) was suspended in MeOH:H_2O (1:1) and extracted successively with Hex and DCM. This procedure afforded the phases denominated Hex (3.8 g), DCM (4.2 g) and Aqueous (11.2 g). Hex phase (3.5 g) was subjected to CC (70-230 mesh) using Hex, Hex:DCM, DCM:MeOH mixtures as eluent to yield 27 subfractions. Subfraction 13 (120 mg) was subjected to MPLC on silica gel (25-40 mesh) using a gradient system from Hex to Hex:EtOAc (80:20) and furnished compounds **1** (100 mg) and **2** (4 mg). Subfraction 17 (140 mg) was fractionated by successive CC using mixtures of Hex:DCM and $CHCl_3$:EtOAc, to afford compound **3** (15 mg). Subfractions 19-20 were grouped (150 mg) and subjected to centrifugal TLC on a Chromatotron system using Hex, DCM and MeOH mixtures and gave a material (25 mg) that after crystallization on hexane furnished compound **4** (3 mg). Subfraction 21 (840 mg) was fractionated on a Sephadex LH-20 column using ethanol as eluent to afford 10 groups of fractions. Groups 6-8 weighting 313 mg was further chromatographed on silica gel CC (70-230 mesh), eluted in Hex, Hex:DCM, and Hex:EtOAc at different proportions. This procedure resulted in the isolation of 5 mg of compound **5**.

Analysis of volatiles by Gas Chromatography-Mass Spectrometry (GC-MS)

The volatiles present in the hexanic soluble phase of *S. oppositifolia* were adsorbed on a Solid Phase Micro-Extraction (SPME) sampling device and analyzed by GC-MS. Briefly, 1 mg of the Hex phase was transferred to a 2 mL glass vial, closed with a cap sealed with a teflon coated septum (Supelco, USA) and placed in a heat block adjusted to 90 °C. A SPME fiber (PDMS/DVB TM 65 µm, Supelco, USA) was inserted through the septum and left in the headspace during 5 min. Before use, the fiber was preconditioned at 230 °C during 30 min in the GC injector port. GC-MS analysis were performed on a Shimadzu QP-5050A (SHIMADZU, JP) instrument, equipped with a PTE-5™ column (30 m, 0.25 mm, 0.25 µm, Supelco, USA). The following conditions were employed for analysis: helium at 22.3 $mL.min^{-1}$, as carrier gas; injector temperature, 230 °C; column temperature, 3 min at 80 °C, 80-300 °C at 7 $°C.min^{-1}$, 5 min at 300 °C. The split valve was closed during the first minute of injection and then opened, with a 1:10 ratio. The mass detector was set to scan from 50 to 500 atomic mass unit, at a rate of 2 $scans.sec^{-1}$. Data acquisition and handling was done via CLASS 5000 Shimadzu software. Raw data files were analyzed by Automated Mass Deconvolution and Identification System software (AMDIS), version 2.1, supplied by National Institute of Standards and Technology (NIST, USA). The compounds identification were performed by comparison of the experimental spectra with those stored in the NIST/EPA/NIH library version 2.0 using the NIST Mass Spectral Search Program.

Cytotoxicity assays with human cancer cell lines

The organic crude extracts, phases and compounds assays were performed using the tumor cell lines UACC-62 (human melanoma cancer), MCF-7 (human breast cancer) and TK-10 (human renal cancer). These cell lines were purchased from the National Cancer Institute, Maryland, USA. The cell toxicity assays were run according to the protocols established at NCI using the sulphorhodamine colorimetric assay (Monks et al., 1991). The samples were dissolved in DMSO aqueous 0.1% at concentrations of 200 $µg.mL^{-1}$.

All assays were run in triplicate wells and repeated at least once. Etoposide at 1.6 $µg.mL^{-1}$, culture medium without samples and culture medium with DMSO 0.1% were controls. Results were expressed in terms of the growth inhibition percentage (%), where the sample tested was considered cytostatic from 0-99% and cytocidal from 100-200%.

Fungal strains

For the antifungal evaluation, the following strains from the American Type Culture Collection

(ATCC, Rochville, MD, USA) were used: *Candida albicans* ATCC 18804, *C. krusei* ATCC 20298, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 2001, *Cryptococcus neoformans* ATCC 32608 and *Paracoccidioides brasiliensis* (Pb18), (from the fungi collection of the Faculty of Medicine of the Universidade de São Paulo, São Paulo, SP, Brazil). The fungi strains were maintained on Sabouraud Dextrose Agar (SDA, Oxoid, Basingstoke, UK) and YPD (Yeast, Peptone and Dextrose).

Antifungal assays

The minimal inhibitory concentration (MIC) was determined on synthetic RPMI medium (Sigma, St Louis, MO, USA) containing L-glutamine and buffered to pH 7.0 with 0.165 mol.L^{-1}) morpholine propanesulfonic acid (MOPS; Sigma). Final inocula of 1.5 x 10^3 CFU. mL^{-1} was prepared using the spectrophotometric dilution method. Broth microdilution testing was performed in accordance with the guidelines in the CLSI M27-A_2 document (NCCLS, 2002), with modifications proposed by Johann et al. (2007). Amphotericin B (Sigma, St Louis, USA) was included as positive antifungal control being the stock solutions prepared in DMSO. RPMI medium was used without compounds or solvents as a control for growth and sterility. Solvent DMSO at the same volumes used in the assay was used as control for toxicity. After inoculation of fungal strains, the plates were incubated at 35 °C for 48 h for the *Candida* species, at 35 °C for 72 h for *C. neoformans* and 37 °C for 72 h for *P. brasiliensis*. The tests were performed in triplicate. The endpoints were determined visually by comparing the growth in the test wells with that the growth in the drug-free control wells. MIC values were expressed in µg.mL^{-1}. Extracts and phases were considered active when they exhibited MIC values less than or equal to 500 µg.mL^{-1}, while compounds were considered active when they exhibited MIC values less than or equal to 100 µg.mL^{-1}.

Cytotoxicity assays with human peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were obtained using the protocol described by Gazzinnelli et al. (1983). Briefly, peripheral venous blood from healthy adult volunteers was collected in heparinized vials and the cells separated by centrifugation on Ficoll-Hypaque gradient. Mononuclear cells were collected and washed three times in RPMI-1640 before further processing. The cell suspensions were adjusted to 2x10^6 cells.mL^{-1} (2.0x10^5 cells per well). All cultures were carried out in RPMI-1640 medium (GIBCO, Grand Island, NY), supplemented with 5% (v/v) heat-inactivated, pooled human sera type AB (Flow Laboratories, Royaune, UN) and L-glutamine (2 mM, GIBCO, Grand Island, NY). An antibiotic/antimicotic solution containing 100 U.mL^{-1} penicillin, 100 µg.mL^{-1} streptomycin and 25 µg.mL^{-1} fungisone (SIGMA, St. Louis, MO) was added. Cells were incubated with the isolated compounds for 48 h at 37 °C in a humidified atmosphere containing 5% CO_2. The cell viability was determined using the MTT (methyl thiazolyl tetrazolium) based colorimetric assay (Jiang & Xu, 2003). The results were expressed as percent inhibition of the cell viability in relation to the control without test compounds. The results were expressed in terms of percentage of cells prolipheration, where the sample tested was considered inactive from 100-70%; moderate active from 69-40% and active from 39-0%.

Results and Discussion

Antifungal and cytotoxic activities of crude extracts

Few species of *Stillingia* genus were available for biological activity. Latex obtained from stems of *S. patagonica*, Euphorbiaceae, presented proteolytic activity showing its potential source for digestive enzymes and anti inflammatory agents (Sequeiros et al., 2003). In the present study we investigated antifungal and cytotoxic activities of the crude extracts from stems and leaves of *S. oppositifolia*. The crude extract of leaves showed, in general, similar activity of that from stems. Organic extracts from stems and leaves inhibited the growth of *C. krusei, C. tropicalis* (MIC value of 250 µg.mL^{-1}) and *P. brasiliensis* (MIC value of 63 µg.mL^{-1}) (Table 1). The crude extracts presented very low cytostatic activity (around 30%) at 20 µg.mL^{-1} against UACC-62 and MCF-7 cell lines. Nevertheless, the extracts showed cytotoxic activity in a concentration-dependent manner doses (GI50 value of 50-100 µg.mL^{-1}, Table 2) on three cancer cell lines recommended by NCI. To access if these extracts are toxic to normal human cells they were tested on leukocytes in the *ex-vivo* assays. The results demonstrated that they are not toxic at 20 µg.mL^{-1} (Table 1).

Table 2. Extracts and compounds concentration (µg.mL^{-1}) required to inhibit cell growth by 50% (GI50), (means of three determinations).

Extracts/Compounds	UACC-62	MCF-7	TK-10
1	> 200	128±29	>200
3	> 200	139±33	>200
4	100±17	88±21	100±13
Crude extract (Leaves)	50±17	50±13	54±8
Crude extract (Stems)	74±9	100±34	50±5

Table 1. Antifungal and cytotoxic activities of extracts, phases and compounds of *Stillingia oppositifolia* Baill. ex Müll. Arg., Euphorbiaceae.

Extracts/phases	MIC (μg/mL)[a]							Growth inhibition (%)[b]			Proliferation (%)[c]
	C. a	*C. k*	*C. g*	*C. t*	*C. p*	*C. n*	*P. b*	*UACC-62*	*MCF-7*	*TK-10*	*PBMC*
Crude extract (Leaves)	-	250	-	250	-	500	63	34±4	34±4	-	108±5
Hex phase (Leaves)	-	-	-	-	-	500	-	-	30±10	-	89±17
DCM phase (Leaves)	-	-	-	250	-	-	-	-	26±4	-	115±18
Aqueous phase (Leaves)	-	500	-	500	-	-	-	-	-	-	110± 11
Crude extract (Stems)	-	250	-	250	-	500	63	43±5	30±10	20±3	119±14
Hex phase (Stems)	-	250	-	30	-	63	63	98±3	30±0	73±17	63±14
DCM phase (Stems)	-	-	-	-	-	250	125	71±8	-	83±25	105±18
Aqueous phase (Stems)	-	500	500	500	-	500	-	-	-	32±14	80±2
Compounds											
1	>100	nt	nt	>100	nt	>100	>100	-	-	-	102±5
3	>100	nt	nt	>100	nt	> 100	>100	58±21	69±3	-	105±11
4	>100	nt	nt	>100	nt	> 100	>100	-	83±39	-	103±9
5	>100	nt	nt	>100	nt	> 100	>100	-	-	-	116±9
Controls											
Anfotericin	1.0	0.5	1.0	0.25	0.5	1.25	0.062	nt	nt	nt	nt
Etoposide	n t	nt	nt	nt	nt	nt	nt	183±7	151±29	71±14	nt

The minus symbol (-) means inactive, nt: not tested, ± variation coefficient. (a): C.a: *Candida albicans*; C. k: *Candida krusei*; C. g: *Candida glabrata*; C. p: *Candida parapsilosis*; C. t: *Candida tropicalis*; C. n: *Cryptococcus neoformans*; P. b: *Paracoccidioides brasiliensis*. Anfotericin was tested in the range from 30 μg.mL^{-1} to 0.031 μg.mL^{-1}; (b): *UACC-62*: human melanoma cancer; *MCF-7*: human breast cancer; *TK-10*: human renal cancer. All samples tested at 20 μg.mL^{-1}. Etoposide was tested at 1.6 μg.mL^{-1}. (c): *PBMC*: peripheral blood mononuclear cells. All samples tested at 20 μg.mL^{-1}.

Antifungal and cytotoxic activities of phases

The crude extracts of *S. oppositifolia* were concentrated and subjected to solvent partitioning to furnish Hex, DCM and aqueous soluble phases. The hexanic soluble phase (Hex) from stems of *S. oppositifolia* exhibited outstanding antifungal activity against *C. neoformans* and *P. brasiliensis* among all phases (Table 1), indicating that this phase has a good potential as antifungal. Our results showed that Hex phase of stems exhibited the strongest activity against *C. tropicalis* (MIC value of 30 μg.mL^{-1}), *C. neoformans* (MIC value of 63 μg.mL^{-1}), *P. brasiliensis* (MIC 63 μg.mL^{-1}). Dichloromethanic phase (DCM) of stems presented moderated activity against *P. brasiliensis* (MIC value of 125 μg.mL^{-1}). Hex and DCM phases were cytostatic against melanoma (98 and 71%, respectively) and renal (73 and 83%, respectively) cell lines. Hex phase presented moderated *in vitro* cytotoxity effect (around 37% of inhibition) on freshly isolated PBMC at 20 μg.mL^{-1}. Aqueous phase presented better activity against *C. krusei, C. glabrata* (MIC value of 500 μg.mL^{-1}), than DCM subfraction, that did not show activity against species of *Candida* tested and minor activity against *C. neoformans* (MIC value of 500 μg.mL^{-1}).

Compounds isolation

Due to interesting biological activity displayed by the hexanic soluble phase from stems, it was chromatographed on a silica gel open column to afford 27 groups. All groups were screened on cytotoxic assays and exhibited the best cytostatic effect against melanoma cell line (UACC-62) at 20 μg.mL^{-1} (data not shown). The groups were chosen to be purified based on the results of cytotoxic assays and their fingerprint on TLC. To our knowledge, there is not any report about phytochemical of *S. oppositifolia*.

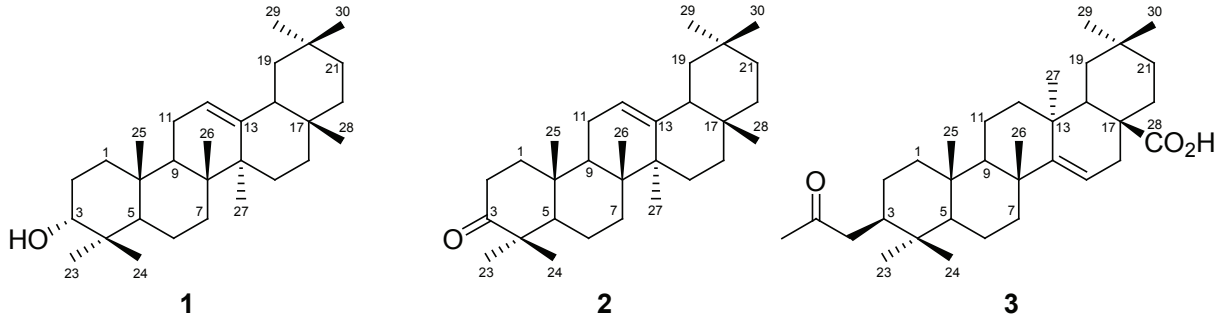
Subfractions of Hex phase from stems were purified by Si gel, Sephadex LH-20, CC or MPLC and by crystallization to afford three triterpenes: 3-*epi*-β-amyrin (**1**), β-amyrone (**2**), aleuritolic acid 3-acetate (**3**), one steroid: β-sitosterol (**4**), and a fatty acid: palmitic acid (**5**). The spectral properties, including ^{1}H NMR and ^{13}C NMR data, were identical to those previously reported in the literature (Martin et al., 1984; Mahato & Kundu, 1994; De-Eknamkul & Potduang, 2003; Lima et al., 2004). Compounds **1** and **2** are oleanane triterpenes and compound **3** is friedooleanane triterpene.

All isolated compounds were inactive against *C. albicans, C. tropicalis* and *P. brasiliensis* at concentration of 100 μg.mL^{-1} and showed cytotoxic activity in the same range than crude extracts against tumoral lines (GI50 values ≥88 μg.mL^{-1}, Table 2). The compounds demonstrated no toxicity against human leukocytes after 48 h of incubation at concentration of 20 μg.mL^{-1}.

Epi-β-amyrin (**1**) was previously isolated from leaves of *Sebastiania adenophora*, Euphorbiaceae (Macias-Rubalcava et al., 2007) and from bark of *Gelonium multiflorum* (Row & Rao, 1969). This compound was able to inhibit *Mycobacterium tuberculosis* growth (MIC value of 12.2 μg.mL^{-1}) and showed cytotoxicity against Vero cells (IC50 127.2 μg.ml^{-1}) (Woldemichael et al., 2004). Compound **2** (β-amyrenone) was isolated from light petrol extracts of the stems of *Macaranga tanarius,* Euphorbiaceae (Hui et al., 1975) and epicuticular wax of *Euphorbia cyparissias* L., Euphorbiaceae (Hemmers & Gülz, 1989). Crude extract of stems of *S. oppositifolia* showed be a good source of *epi*-β-amyrin while β-amyrenone, a related isomeric compound possessing the cetone group at C-3, was isolated as minor compound. *Epi*-β-amyrin could be a chemical marker of extracts of *S. oppositifolia* since it is not is frequently isolated on Euphorbiaceae family.

Acetyl aleuritolic acid was obtained from several Euphorbiaceae species as *Jatropha macrorhiza* (Torrance et al., 1977), *Croton tonkinensis* (Pham & Pham, 2002), *C. cajucara* (Maciel et al., 2006), *Discoglypremna caloneura* (Nyasse et al., 2006). This triterpene showed tumor-inhibitory properties toward the P-388 lymphocytic leukemia (Torrance et al., 1977). It was active against against *Staphylococcus aureus, Salmonella typhy, Vibrio cholera, Escherichia coli* and *Shigella dysentery* in microdilution method (MIC value of 50 μg.mL^{-1}) and was not cytotoxic to Vero cell lines *in vitro* (IC50 of 400 μg.mL^{-1}, Mathabe et al., 2008).

Many Euphorbiaceae species belonging to the genus *Acalypha* (Wang et al., 2008; Taufiq-Yap et al., 2000), *Bridelia* (Yadav & Nigam, 1975), *Croton* (Palmeira et al., 2006; Santos et al., 2008), *Euphorbia* (Kong & Min, 1996; Ekpo & Pretorius, 2007; El-Fiky et al., 2008) and *Glochidium* (Hui & Fung, 1969; Hui & Li, 1976) have furnished β-sitosterol as a chemical constituent. This compound showed to induce the macrophage tumoricidal activity, stimulate the lymphocyte blastogenesis (Park et al., 2003) and showed therapeutic angiogenic effects on damaged blood vessels (Choi et al., 2002). This compound showed hypocholesterolemic activity (Day,



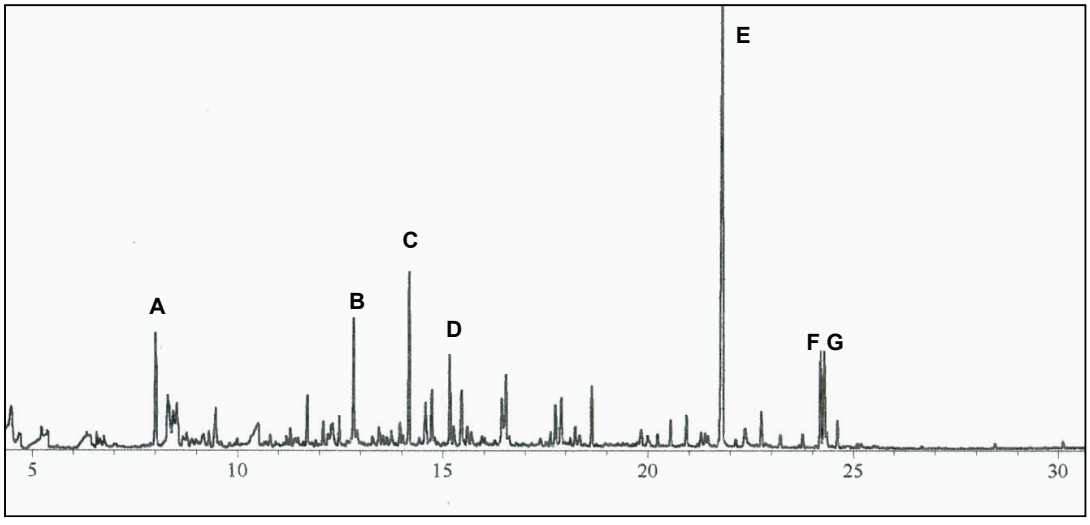


Figure 1. Total ion chromatogram of the hexanic phase from *Stillingia oppositifolia,* Euphorbiaceae. A: decahydro 2,6-dimethyl naphthalene (6.78%), B: tetradecane (5.25%), C**:** decahydro1,1,7, trimethyl,4 methylene-1H, cycloprop[e]azulene (8.74%), D: dodecanoic acid methyl ester (4.20%), E**:** hexadecanoic acid methyl ester (5, 31.93%), F: 9-dodecenoic acid methyl ester (4.53%) and G: octadecanoic acid methyl ester (4.99%).

1991), inhibitory activity on human platelet-type 12(S)-lipoxygenase [12(S)-LOX] (Schneider et al., 2004) and was active against the mutagenicity of *N*-methyl-*N*-nitrosourea and 2-aminoanthracene (Lawson et al., 1989).

Palmitic acid has been detected by GC/MS from oil seeds from various Euphorbiaceae species (Agarwal et al., 1995; Augustus et al., 2002; Mohan, 2009). In according with our observations, palmitic acid was not cytotoxic to three cell lines and human lymphocytes at 20 μg.mL^{1}. Harada et al. (2002) demonstrated that at concentrations ranging from 12.5 to 50 μg.mL^{-1}, palmitic acid shows selective cytotoxicity to human leukemic cells (Molt-4, HL-60, K-562), but no cytotoxicity to normal dermal fibroblasts cells. Carballeira (2008) suggests that the cytotoxicity of palmitic acid to cancer cell lines could be linked to the inhibition of topoisomerase I, since it inhibited this enzyme only in high concentrations.

Gas chromatography/mass spectrometry analysis

The isolated compounds from Hex phase of *S. oppositifolia* showed cytotoxic activities similar the crude extract and did not present antifungal activity at 100 g.mL^{-1}. Hex phase was analyzed by gas chromatography coupled to mass spectrometry detection (GC-MS) to access the chemical composition of volatiles. The GC-MS analysis of the Hex phase revealed the presence of decahydro 2,6-dimethyl naphthalene (A, 6.78%), tetradecane (B, 5.25%), decahydro1,1,7, trimethyl,4 methylene-1H, cycloprop[e] azulene (C, 8.74%), dodecanoic acid methyl ester (D, 4.20%), hexadecanoic acid methyl ester (E, 31.93%), 9-dodecenoic acid methyl ester (F, 4.53%) and octadecanoic acid methyl ester (G, 4.99%) as main compounds (Figure 1). Hexadecanoic acid methyl ester (E) (palmitic methyl ester), was detected in the hexanic soluble phase (Figure 1) and crude extract one (data not shown) as the most abundant component.

Most GC-MS analyses are performed to reveal the composition of essential oils obtained from hydro-distillation, a conventional extraction procedure (Bakkali et al., 2008). In this work, we explore the analysis of the volatile from hexanic phase of *S. oppositifolia* by CG-MS-SPME technique. This methodology can be successfully applied to polar and non-polar compounds in gas, liquid and solid samples and avoid that some analytes be masked by the solvent, since it is a solvent-free technique (Cuevas-Glory et al., 2007). The conditions of sample preparation (Hex phase) and the methodology used to analyze the volatiles compounds are factors that explain the absence of usual volatiles compounds that are present in essential oils, however our results has shown that several substances are closely related in this genus. Analyses of the fatty acids of the total lipids of stems of *Stillingia texuna* by gas-liquid chromatography-mass spectrometry revealed the presence of 2,4-decadienoic acid (Heimermann & Holman, 1972) and from roots of *Stillingia sylvatica* were isolated diterpene esters carrying saturated, polyunsaturated or hydroxilated fatty acids (Adolf & Hecker, 1980).

It is worthwhile to mention that, in this study, the lowest polar phase, *i.e.* hexane, was the most active, and this activity could be related to volatiles compounds. The Hex phase presented a large amount of saturated fatty acid methyl esters (45.65%), according to GC-MS analysis. Fatty acid methyl esters can disturb the lipid environment and induce an elevation in membrane fluidity (Avis & Bélanger, 2001). This fact could explain the *in vitro* activity presented by Hex phase. Regarding the biological assays results, it can be suggested that a synergic effect of constituents from the extract could be responsible for the inhibitory activity observed against fungi and cell lines.

Previous reports have shown that *S. oppositifolia* is the most important in natural regeneration in a Mixed Ombrophila Forest at São Francisco de Paula National Forest, Brazil (Narvaes et al., 2005). This is the first report about biological potential and isolation of compounds from this species, which contributes with their phytochemical knowledge.

Conclusion

At our knowledge this is the first report concerning the chemical and biological potential *S. oppositifolia* extracts. Our results demonstrated that *S. oppositifolia* extracts have antifungal activity and cytotoxic effects on breast, renal and melanoma cell lines, recommended by NCI. The Hex phase presented activity against microorganisms tested, and this activity can be associated with synergic effect between constituents of the extract. This phase presented to be a source of triterpene 3-*epi*-β-amyrin.

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