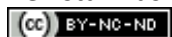


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## Original Article

## Activity of Fabaceae species extracts against fungi and *Leishmania*: vatacarpan as a novel potent anti-*Candida* agent



Dandara Braga Santana<sup>a</sup>, Raphaella Correia da Costa<sup>a</sup>, Renata Mendonça Araújo<sup>b</sup>, José Elias de Paula<sup>a,1</sup>, Edilberto Rocha Silveira<sup>c</sup>, Raimundo Braz-Filho<sup>d,e</sup>, Laila Salmen Espindola<sup>a,\*</sup>

<sup>a</sup> Laboratório de Farmacognosia, Faculdade de Ciências da Saúde, Universidade de Brasília, Campus Universitário Darcy Ribeiro, Brasília, DF, Brazil

<sup>b</sup> Instituto de Química, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil

<sup>c</sup> Departamento de Química Orgânica, Universidade Federal do Ceará, Fortaleza, CE, Brazil

<sup>d</sup> Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil

<sup>e</sup> Departamento de Química, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil

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## ABSTRACT

Leishmaniasis and fungal infection treatment efficacy is limited by toxicity and ever increasing resistance to available drugs, requiring development of alternative compounds. The richness of Cerrado plant antimicrobial secondary metabolites justifies screening of Fabaceae species extracts: *Enterolobium ellipticum* Benth., *Sclerolobium aureum* (Tul.) Baill. and *Vatairea macrocarpa* (Benth.) Ducke, against *Leishmania (Leishmania) amazonensis*, yeasts and dermatophytes. Among the 26 extracts tested, more than 50% of the total demonstrated significant antifungal activity in comparison to the drug controls (minimal inhibitory concentration 0.12 to  $\leq 31.25$   $\mu\text{g/ml}$ ). Six extracts capable of complete parasitic growth inhibition had the inhibitory concentration index for 50% values from 9.23 to 78.65  $\mu\text{g/ml}$ . The results led to the selection of the *V. macrocarpa* ethyl acetate root bark extract for chemical fractionation. This plant, traditionally referred to as *angelim-do-cerrado* or *maleiteira*, is used to treat superficial mycoses in Amazonia. A previously unreported pterocarpan vatacarpan together with the known compound musizín was isolated. Vatacarpan demonstrated a minimal inhibitory concentration value of 0.98  $\mu\text{g/ml}$  against *Candida albicans* ATCC 10231, and thus comparable or superior to fluconazole and amphotericin B. The results add to literature's information the ability of pterocarpan to act as antimicrobial agents.

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## Introduction

Natural products and their ethnopharmacological activities have been historically used as a primary source of compounds for drug discovery. However, for the last twenty years, the technical requirements for high throughput screening led the pharmaceutical industry to focus on combinatorial chemistry libraries instead. This pathway was only partly successful since it came up with structures that lacked the complex scaffold of secondary metabolites. Currently, natural products are again a source of drug discovery, providing lead compounds for clinical trials, including antimicrobial agents (Dias et al., 2012; Harvey et al., 2015).

The incidence of fungal infections has significantly increased since the late 1960s, primarily with cases of immune-compromised or hospitalized patients. Research dedicated to the development

of new therapeutic strategies has culminated in four antifungal classes currently used in clinical practice: fluoropyrimidine analogs, polyenes (such as amphotericin B, the most commonly used medication for systemic infections) azoles and equinocandins (Vandeputte et al., 2012).

Cutaneous leishmaniasis is considered a neglected tropical diseases group, with 1.5 million people worldwide manifesting skin lesions which persist for several months, or even years (Silva et al., 2013). Pentavalent antimonials ( $\text{Sb}^{+5}$ ) remain the primary treatment option, despite their toxicity and low patient tolerance, with either pentamidine or amphotericin B used as second line treatments.

The failure of the aforementioned drug strategies has been attributed to microbial resistance. The use of plants by traditional local communities and the richness of Cerrado species antimicrobial secondary metabolites have been reported (De Assis et al., 2014; Albernaz et al., 2012; Melo e Silva et al., 2009; De Mesquita et al., 2005). We screened Fabaceae extracts from the Cerrado: *Enterolobium ellipticum* Benth., *Sclerolobium aureum* (Tul.) Baill. and *Vatairea macrocarpa* (Benth.) Ducke, against *Leishmania*

\* Corresponding author.

E-mail: [darvenne@unb.br](mailto:darvenne@unb.br) (L.S. Espindola).

<sup>1</sup> In memoriam.

(*Leishmania*) *amazonensis*, yeasts and dermatophytes. These antimicrobial results led to the selection of *V. macrocarpa* root bark ethyl acetate extract for further chemical studies. Here, we present the isolation, structure elucidation and antimicrobial activity of one pterocarpan. This new natural product was more active than the antifungal reference fluconazole and amphotericin B.

## Materials and methods

### Plant extracts

Plant species *Enterolobium ellipticum* Benth., *Sclerolobium aureum* (Tul.) Baill. and *Vatairea macrocarpa* (Benth.) Ducke, Fabaceae, were collected in 2010 from the Cerrado biome in the Lagoa Formosa area, Planaltina, Federal District of Brazil, south latitude 15°27'34.2"; south longitude 47°92'3.3"; at an altitude of 1071 m, and subsequently identified by botanist Prof. José Elias de Paula. Voucher numbers were kept in the University of Brasília (UB/UnB) Herbarium (Table 1). Plant organs were separated, dried, stabilized, pulverized and extracted by maceration with hexane, ethyl acetate and ethanol. The extractive solutions were concentrated in a rotary evaporator, yielding different crude extracts, which were stored at –20 °C.

### Antifungal activity method

The minimal inhibitory concentration (MIC) of each extract was determined for yeasts: *Candida albicans* ATCC 10231, *C. parapsilosis*

ATCC 22019 and *C. glabrata* LMGO 44, using CLSI M27-A3 and M27-S4 protocols (CLSI, 2008a, 2012); and for dermatophytes: *Trichophyton mentagrophytes* LMGO 09 and *Trichophyton rubrum* LMGO 06, following the CLSI M38-A2 protocol (CLSI, 2008b); with minor modifications as previously described by the research group (Da Costa et al., 2014) (see Supporting information). The LMGO (Laboratório de Micologia de Goiás) strains are clinical isolates from patients at the Federal University of Goiás Hospital, Brazil. Itraconazole (Sigma), flucytosine (Sigma) and amphotericin B (Sigma) were used as reference compounds for yeasts and dermatophytes. A fourth positive control fluconazole (Sigma) was used for yeasts only.

### In vitro antileishmanial activity

The extracts were tested against *Leishmania (Leishmania) amazonensis* (L(L)a)(MHOM/BR/PH8) promastigotes maintained in Schneider culture medium (Sigma) with 20% heat-inactivated fetal calf serum, at 22–25 °C. Extracts were dissolved in DMSO (Sigma) and diluted in Schneider's medium (Sigma). The inhibitory concentration index for 50% (IC<sub>50</sub>) of the promastigotes was determined by the MTT method. Experiments were performed as previously described (Da Costa et al., 2014) (see Supporting information) with amphotericin B (Sigma) used as the reference drug.

### General experimental procedures

Column chromatography was performed using silica gel 60 (230–400 mesh, Merck). Thin Layer Chromatography (TLC) was

**Table 1**  
Fabaceae species extract activity – determination of the minimum inhibitory concentration (MIC – µg/ml) against yeasts and dermatophytes, and IC<sub>50</sub> (µg/ml) against *Leishmania (Leishmania) amazonensis*.

Fabaceae family species Voucher number	Extract Plant part (solvent)/% yield	MIC (µg/ml)					IC <sub>50</sub> (µg/ml) <i>L. (L.) amazonensis</i>
		<i>C. albicans</i> ATCC 10223	<i>C. parapsilosis</i> ATCC 22019	<i>C. glabrata</i> LMGO 44	<i>T. mentagrophytes</i> LMGO 09	<i>T. rubrum</i> LMGO 06	
<i>Enterolobium ellipticum</i> Benth (UB) 3739	RW (h)/0.37	250	250	125	500	250	>100
	RW (ea)/0.56	1.95	3.91	>1000	500	62.5	>100
	RW (e)/9.05	>1000	>1000	>1000	>1000	1000	70.05
	RB (ea)/1.44	500	125	>1000	500	500	>100
	SW (h)/0.32	>1000	1000	500	1000	500	78.65
	SW (ea)/0.61	0.98	31.25	>1000	250	250	>100
	SB (h)/0.55	1000	500	250	500	1000	25.87
	SB (ea)/2.18	>1000	15.62	>1000	125	500	9.23
	L (ea)/5.64	125	15.62	>1000	250	>1000	>100
<i>Sclerolobium aureum</i> (Tul.) Baill. (UB) 3818	RW (h)/0.42	>1000	>1000	>1000	1000	250	>100
	RW (ea)/0.62	0.48	0.12	31.25	31.25	31.25	>100
	RW (e)/6.06	1.95	3.91	15.62	31.25	31.25	>100
	RB (ea)/1.98	7.81	7.81	15.62	15.62	31.25	>100
	SW (h)/0.25	1000	500	500	500	31.25	>100
	SW (ea)/0.66	0.48	15.62	15.62	15.62	31.25	>100
	SW (e)/3.47	0.12	0.12	0.12	15.62	31.25	>100
	SB (ea)/2.05	7.81	15.62	7.81	15.62	31.25	>100
	L (ea)/4.74	250	7.81	1000	>1000	>1000	>100
<i>Vatairea macrocarpa</i> (Benth.) Ducke (UB) 3815	RW (h)/0.48	0.98	1.95	500	62.5	500	72.39
	RW (ea)/0.90	3.91	3.91	250	125	500	>100
	RB (ea)/9.78	0.98	0.98	31.25	62.5	125	71.47
	SW (h)/0.43	250	250	>1000	1000	125	>100
	SW (ea)/0.78	1.95	1.95	500	62.5	125	>100
	SW (e)/4.25	0.24	1.95	500	31.25	62.5	>100
	SB (ea)/4.84	1.95	1.95	250	31.25	31.25	>100
L (ea)/8.58	1.95	1.95	250	62.5	62.5	>100	
Itraconazole		0.125	0.0625	0.125	0.25	0.125	–
Flucytosine		0.25	0.0635	0.125	–	–	–
Fluconazole		1	0.5	4	4	2	–
Amphotericin B		4	4	16	4	–	0.067

Plant parts – RW: root wood, RB: root bark, SW: stem wood, SB: stem bark, L: leaves. Solvents – h: hexane, ea: ethyl acetate, e: ethanol; –: not tested. LMGO (Laboratório de Micologia de Goiás) strains were clinical isolates from patients of the Federal University of Goiás Hospital.

performed on precoated silica gel aluminum plates (TLC Silica gel 60 F<sub>254</sub>, Merck). The compounds were visualized by UV detection and/or sprayed with a solution of vanillin/sulphuric acid/EtOH. Optical rotation was measured on a Perkin Elmer 341 polarimeter. The IR spectra (KBR pellets) were recorded using a Perkin-Elmer FT-IR 1000 spectrometer. The high resolution electrospray ionization mass spectra (HRESIMS) were acquired using a LCMS-IT-TOF (225-07100-34) Shimadzu spectrometer. The mass spectra were recorded on a Shimadzu QP 5000/DI-50 spectrometer, operating with electron impact at 70 eV. The 1D and 2D NMR data were acquired on a Bruker Avance DPX-300 spectrometer, equipped with a 5 mm multinuclear inverse probe, using gradient field in the Z direction and a magnitude of 10 A. Chemical shifts, given on the  $\delta$  scale, were referenced to the residual undeuterated portion of the CDCl<sub>3</sub>, deuterated solvent. All standard pulse sequences were provided by the Bruker TOP-SPIN software. All experiments were conducted at room temperature.

### Extraction and isolation

A 115.70 g portion of *V. macrocarpa* root bark was dried, powdered and subsequently macerated in ethyl acetate at room temperature, yielding 11.83 g of extract after solvent evaporation under reduced pressure. An 8.28 g portion of the EtOAc extract was fractionated with hexane, EtOAc and MeOH as the binary mixture of increasing polarity and yielded 65 fractions which were analyzed by TLC.

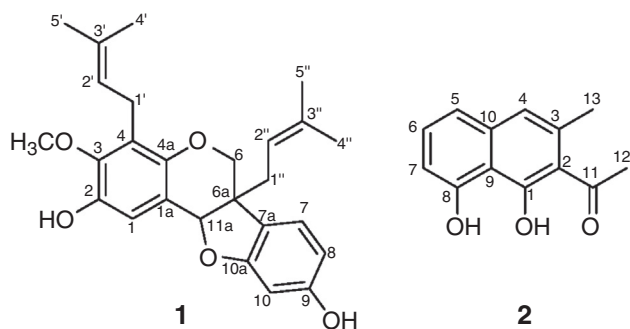
Flash chromatography of fractions 19–22 (749.9 mg), by elution with hexane, ethyl acetate and MeOH as a binary mixture of increasing polarity yielded 316 fractions analyzed by TLC.

Fraction 3 (50–61) (5.10 mg) corresponded to musizin (2).

Fraction 9 (176–179) (27.5 mg) and fraction 10 (180–183) (40.3 mg) were regrouped following TLC analysis. A new flash chromatography was conducted which yielded 141 fractions analyzed by TLC. Fraction 3 (16–21) (4.60 mg) was found to be a previously unreported pterocarpan–named herein as vatacarpan (1).

**Vatacarpan (1).** White solid; m. p. 135.2–136.4 °C; [ $\alpha$ ]<sub>D</sub> = +63° (c. 0.1, MeOH); <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz) see Table 2; HR-ESI-TOFMS (1) m/z 423.2166 [M+H]<sup>+</sup> (calcd for C<sub>26</sub>H<sub>30</sub>O<sub>5</sub>).

**Musizin (2).** Brown crystals, <sup>1</sup>H (CDCl<sub>3</sub>, 300 MHz): 6.99 (s, H-4), 7.08 (d, 7.8, H-5), 7.48 (t, 7.8, H-6), 6.84 (d, 7.8, H-7), 2.76 (s, H-12), 2.66 (s, H-13), 17.35 (s, OH-1), 10.24 (s, OH-8) and <sup>13</sup>C NMR: 205.2 (C-11), 168.8 (C-1), 158.8 (C-8), 138.4 (C-10), 133.0 (C-6), 132.4 (C-3), 122.1 (C-4), 117.7 (C-5), 113.8 (C-2), 113.2 (C-9), 111.4 (C-7), 32.4 (C-12), 25.5 (C-13).



### Results and discussion

A total of 26 extracts were prepared from the Fabaceae family: nine from *E. ellipticum*, nine from *S. aureum*, and eight from *V. macrocarpa*. Of all the extracts, 85% presented activity against a minimum of one fungal strain using the active extract criterion (MIC  $\leq$  125  $\mu$ g/ml) specified by Albernaz et al. (2010). In addition,

**Table 2**  
<sup>1</sup>H (300 MHz) and <sup>13</sup>C (75 MHz) NMR data assignments for vatacarpan (CDCl<sub>3</sub>).

Carbon number	HSQC		HMBC
	$\delta_C$	$\delta_H$	<sup>2</sup> J <sub>CH</sub> / <sup>3</sup> J <sub>CH</sub>
1	114.3	6.95 (s)	H-11a
1a	123.2	–	
2	143.7	–	
3	146.6	–	H-1/MeO-3
4	116.0	–	
4a	147.3	–	H-1/H-11a
6	70.6	4.02 (d, 10.0) 3.64 (d, 10.0)	H-11a
6a	46.7	–	
7	124.4	7.01 (d, 7.9)	
7a	123.9	–	H-8/H-10
8	107.7	6.36 (dd, 7.9, 2.2)	H-10/H-7a
9	156.8	–	H-7
10	98.6	6.34 (d, 2.2)	
10a	160.9	–	H-7
11a	83.1	5.10 (s)	H-1
1'	26.7	3.34 (d, 6.5)	
2'	122.8	5.14 (t, 6.5)	3H-4'/3H-5'
3'	131.0	–	3H-4'/3H-5'
4'	18.1	1.76 (s)	3H-5'
5'	25.9	1.66 (s)	3H-4'
1''	31.2	2.45 (d, 7.5)	H-6 $\beta$
2''	118.9	5.23 (t, 7.8)	3H-4''/3H-5''
3''	135.6	–	3H-4''/3H-5''
4''	18.2	1.54 (s)	3H-5''
5''	26.2	1.70 (s)	3H-4''
MeO-5	61.7	3.79 (s)	

more than 50% of the total samples demonstrated significant activity in comparison to the drug controls (MIC 0.12 to  $\leq$  31.25  $\mu$ g/ml) (Table 1). The inhibitory concentration index for 50% of the promastigotes from *L. (L.) amazonensis* (IC<sub>50</sub> determined by the MTT method) was calculated for the six extracts capable of complete parasitic growth inhibition at a concentration of 100  $\mu$ g/ml: four extracts from *E. ellipticum* and two from *V. macrocarpa* (IC<sub>50</sub> 9.23 to 78.65  $\mu$ g/ml); with no activity observed for *S. aureum* (Table 1).

Inspired by the clinical use of amphotericin B in the treatment of fungal infections and leishmaniasis, we selected the *V. macrocarpa* root bark EtOAc extract for chemical studies based on its activity, yield, quantity and polarity. Silica-gel open column chromatography of this 8.28 g extract (fungal MIC 0.98–125  $\mu$ g/ml and parasite IC<sub>50</sub> 71.47  $\mu$ g/ml) yielded 65 fractions.

Fractionation revealed an unreported compound named herein as vatacarpan (1), together with musizin (2) (Covell et al., 1961). All compound structures were established using spectroscopic techniques, including 1D and 2D NMR analysis, and comparison with previously reported data.

Compound 1, was a white solid (m.p. 135.2–136.4 °C). The infrared (IR) spectrum showed absorption bands at 3384 cm<sup>-1</sup> characteristic of hydroxyl groups and at 1620 and 1570 cm<sup>-1</sup> of the skeletal vibrations of the benzene ring. Asymmetric and symmetric C–O–C stretching of aryl-alkyl ether at 1261–1199 and 1085 cm<sup>-1</sup>, respectively, were also observed in addition to phenol absorptions at 1380 and 1165 cm<sup>-1</sup>. The HRESIMS of 1 displayed a molecular ion peak at m/z 423.2166 corresponding to the molecular formula C<sub>26</sub>H<sub>30</sub>O<sub>5</sub>.

In the <sup>1</sup>H NMR spectrum (300 MHz, CDCl<sub>3</sub>), a methoxy group at  $\delta_H$  3.79 (s) and an aromatic proton at  $\delta_H$  7.26 (H<sub>1</sub>, s) were observed, consistent with a pentasubstituted benzene moiety and three aromatic protons compatible with the presence of an ABX spin system at  $\delta_H$  6.34 (H<sub>10</sub>, d, 2.2), 6.36 (H<sub>8</sub>, dd, 7.9, 2.2) and 7.01 (H<sub>7</sub>, d, 7.9). The presence of two prenyl groups were suggested from the signals of the four methyl groups at  $\delta_H$  1.66 (H<sub>5'</sub>, s), 1.76 (H<sub>4'</sub>, s), 1.54 (H<sub>4''</sub>, s) and 1.70 (H<sub>5''</sub>, s) and the two olefinic protons at  $\delta_H$

5.14 ( $H_{2'}$ , t,  $J=6.5$  Hz) and 5.23 ( $H_{2''}$ , t,  $J=7.5$  Hz), in addition to the two methylene protons at  $\delta_H$  3.34 ( $H_{1'}$ , d,  $J=6.5$ ) and 2.45 ( $H_{1''}$ , d,  $J=7.5$ ). The correlation of these signals in the COSY spectrum confirmed the two prenyl moieties. In addition, the  $^1H$  NMR spectrum revealed signals for a diastereotopic methylene at  $\delta_H$  4.02 ( $H_{6\alpha}$ , d, 10.0) and 3.64 ( $H_{6\beta}$ , d,  $J=10.0$  Hz) and an oxymethine proton at  $\delta_H$  5.10 ( $H_{11a}$ , s) typical of the oxymethylene and oxymethine protons of the heterocyclic ring B (2H-6) and C (H-11a) of a 6a-substituted pterocarpan skeleton, respectively. The pterocarpan skeleton was supported by the correlation displayed between H-6 and H-11a in the NOESY spectrum.

The  $^{13}C$  and DEPT  $135^\circ$  NMR spectroscopic data of **1** (Table 2) revealed 26 carbon atoms, corresponding to: four methyls ( $\delta_C$  18.1, 18.2, 25.9 and 26.2); a methoxyl ( $\delta_C$  61.7); three methylenes ( $\delta_C$  70.6, 31.2 and 26.7); one oxymethine ( $\delta_C$  83.1); one nonhydrogenated ( $\delta_C$  46.7); four olefinic carbons ( $\delta_C$  118.9, 122.8, 131.0 and 135.6); four hydrogenated aromatic carbons ( $\delta_C$  98.6, 107.7, 114.3 and 124.4); and eight quaternary aromatic carbons ( $\delta_C$  116.0, 123.2, 123.9, 143.7, 146.6, 147.3, 156.8 and 160.9). The  $^1H$ ,  $^{13}C$ -HSQC spectrum exhibited correlation between the hydrogens at  $\delta_H$  4.02 ( $H_{6\alpha}$ , d, 10.0), 3.64 ( $H_{6\beta}$ , d, 10.0 Hz) and 5.10 ( $H_{11a}$ , s) with the oxycarbons at  $\delta_C$  70.6 (C-6) and 83.1 (C-11a), respectively. These NMR signals together with the methine carbon at  $\delta_C$  46.7 (C-6a), ratified the presence of a 6a-substituted pterocarpan skeleton.

The deshielding of the methylene hydrogens 2H-1' ( $\delta_H$  3.34) when compared with the 2H-1'' ( $\delta_H$  2.45), was associated to anisotropic effect of the aromatic ring a-located at the C-1' carbon. In addition, the HMBC correlation of the 2H-1' ( $\delta_H$  3.34) with the carbons at  $\delta_C$  116.0 (C-4), and 147.3 (C-4a) established the position the one prenyl fragment at C-4 in the aromatic ring A. Other HMBC correlation of the methoxyl protons (s, 3.79) with the signal at  $\delta_C$  146.6 (C-3), and concomitant correlation of the H-1 (s, 6.95) with the signals at  $\delta_C$  123.2 (C-1a), 146.6 (C-3), and 83.1 (C-11a), indicated the position of the methoxyl and hydroxyl groups, at C-3 and C-2, respectively, confirmed the substitution pattern in aromatic ring A. The location of the hydroxyl group at the C-9 carbon of the trisubstituted aromatic ring D was based on HMBC correlation of the hydrogen at  $\delta_H$  7.01 (H-7) to the carbons at  $\delta_C$  156.8 (C-10a) and 160.9 (C-9) and concomitant correlation of the hydrogens at  $\delta_H$  6.36 (H-8) and 6.34 (H-10) with the carbon at  $\delta_C$  123.9 (C-7a), in accordance with biogenetic backgrounds (Dewick, 2002).

The relative stereochemistry of the B and D pterocarpan rings was determined by the NOESY experiment, which revealed dipolar interactions of the methylene 2H-1'' with the hydrogens H-6 and H-11a. In addition, the positive optical rotation value  $[\alpha]_D^{25} = +63^\circ$  (c. 0.1, MeOH) was decisive for the assignment of the absolute stereochemistry of the stereogenic centers C-6a and C-11a as *S* (Araújo et al., 2008). Thus, compound **1** was identified as (+)-6a*S*,11a*S*-2,9-dihydroxy-3-methoxy-4,6a-diprenyl-pterocarpan on the basis of the NMR (1D and 2D, Table 2) spectral data and HRESIMS (positive mode).

Pterocarpan, the second largest group of natural isoflavonoids, received this name from *Pterocarpus* species, the first source of this class of compounds. Since then, many structurally diverse pterocarpan have been isolated and some demonstrate interesting biological activities (Goel et al., 2013). Pterocarpan have been mainly found in a large number of Fabaceae species, and have been reported to exhibit antifungal (Jiménez-González et al., 2008) and antiprotozoal (Vieira et al., 2008) activities.

Compound **2** was obtained as brown crystals. The IR spectrum showed absorption bands of O–H stretching at  $3297\text{ cm}^{-1}$  and carbonyl conjugated C=O stretching at  $1628\text{ cm}^{-1}$ . Phenol absorptions were also observed at  $1378$ – $1317\text{ cm}^{-1}$  and  $1280$ – $1160\text{ cm}^{-1}$  associated to C–O stretching, and at  $1580$  and  $1440\text{ cm}^{-1}$  of the skeletal vibrations of the aromatic ring. The NMR spectra (see Supporting information) showed the presence of two methyl groups, an

chelatogetic hydroxyl group, a carbonyl group, and a naphthalene ring. Following analysis by spectroscopic means and comparison with the data from the literature, compound **2** was identified as musizin (Covell et al., 1961) otherwise named dianellidin (Dias et al., 2009) and nepodin (Li and McLaughlin, 1989).

The literature describes the activity of musizin against *C. albicans* ATCC 14053 and *Trichophyton mentagrophytes* ATCC 28185 (Dias et al., 2009), and as a potent antiprotozoal compound against malaria parasites (Lee and Rhee, 2013).

Vatacarpan (**1**) was active against *C. albicans* ATCC 10231 with a MIC value (MIC  $0.98\text{ }\mu\text{g/ml}$ ) comparable to fluconazole (MIC  $1.00\text{ }\mu\text{g/ml}$ ) and superior to amphotericin B (MIC  $4.00\text{ }\mu\text{g/ml}$ ).

The literature reports the production of pterocarpan as a response to biotic and abiotic factors, such as fungal and other pathogen challenges (Jiménez-González et al., 2008; Goel et al., 2013). Pterocarpan accumulate in infected tissue, the site of antifungal activity. For some of them high concentrations are reached rapidly, then slowly decrease as the infection ceases (Jiménez-González et al., 2008). Several studies have tried to relate the structure of the pterocarpan system with their fungicidal activity.

Investigations have shown that the roots of some plants which contain pterocarpan have a strong activity toward human pathogens (Jiménez-González et al., 2008). It is well established that lipophilic pterocarpan possess increased antifungal activity, since they cross fungal membranes more easily (Harborne, 1978). The root bark is the source of the *V. macrocarpa* extract studied. This organ is continuously challenged to soil microorganisms, humidity and oxidative stress, conditions that may enhance the production of pterocarpan.

Biological activity screening of *V. macrocarpa* extracts demonstrated the most positive results in terms of activity spectrum. The two extracts active against the protozoa were from the root (Table 1). All of the *V. macrocarpa* extracts were active against at least one fungal strain (MIC  $\leq 125\text{ }\mu\text{g/ml}$ ) (Table 1), with the majority of MIC values for *C. parapsilosis* ATCC 22019 comparable to, or better than, the drug controls. *C. parapsilosis* is regarded as reference yeast (CLSI, 2008a,b, 2012). A decade-spanning multi-centric study in Brazilian hospitals revealed an increase in the incidence of *Candida non-albicans* pathogens, primarily *C. parapsilosis* (Colombo et al., 2006). Another study in 29 Spanish hospitals reported candidemia in ICU patients caused by non-*albicans* species in 48% of cases, with *C. parapsilosis* as the most common species (Puig-Asensio et al., 2014). In dermatophytes, the MIC values ranged from  $31.25$  to  $125\text{ }\mu\text{g/ml}$  (Table 1). Literature reports the traditional use of *V. macrocarpa* root and stem bark in superficial mycoses treatment in Amazonia (Piedade and Filho, 1988). In this work, the ethyl acetate extracts from the same plant organs were active against clinical isolates of *Trichophyton mentagrophytes* LMGO 09 and *T. rubrum* LMGO 06 (MIC  $31.25$  to  $\leq 125\text{ }\mu\text{g/ml}$ ) – the dermatophytes responsible for the majority of superficial fungal infections in the Americas (Grannoum et al., 2013; Havlickova et al., 2008). *V. macrocarpa* remains a widely used treatment of diabetes mellitus symptoms (Baviloni et al., 2010; Oliveira et al., 2008), or as an antiulcer and anti-inflammatory (Jesus et al., 2009) – suggesting that it is a non-toxic species, as shown in experimental animals for heartwood methanol extract (Jesus et al., 2012).

*V. macrocarpa* and *V. guianensis* are often described in the literature as sources of lectins, a very diverse class of proteins. Lectins found in the seeds of these species, among other Fabaceae, show various biological activities, such as alternative antimicrobial agents (Vasconcelos et al., 2014), as histological markers, or as tools for cancer research (Sousa et al., 2015).

Interesting observations for *E. ellipticum* were (1) only the ethyl acetate extracts demonstrated activity against *C. parapsilosis* ATCC 22019 (MIC  $3.91$  to  $125\text{ }\mu\text{g/ml}$ ); (2) this plant species provided the highest number of anti-*Leishmania* extracts; and (3) its stem bark

ethyl acetate extract demonstrated the most leishmanicidal activity ( $IC_{50}$  9.23  $\mu\text{g/ml}$ ) (Table 1). This species is locally known as “brincos-de-saguim” because its fruit resembles “monkey’s ears” and it is used by traditional communities to treat pulmonary infections (Corrêa, 1984). Other species of the same genus are used in Brazil to treat parasitism and gonorrhoea (Mimaki et al., 2004).

*S. aureum* extracts were inactive against *L. (L.) amazonensis*, but exhibited high activity in 71% of the tests conducted against ATCC strains and clinical isolates of pathogenic fungi. *C. glabrata* LMGO 44 results (MIC 0.12 to 31.25  $\mu\text{g/ml}$ ) (Table 1) are therefore an important consideration in the search for leader compounds given the increased clinical incidence of this species (Puig-Asensio et al., 2014). It is important to note that *S. aureum* is used by the Cerrado communities for the treatment of fungal infections and as a hepatoprotector – suggesting that it is a non-toxic species. Quilombola and some indigenous Brazilian people use a decoction of the stem bark as a contraceptive (Rodrigues, 2007). The Mumbuca community use a *S. aureum* infusion as a hepatoprotector in Tocantins State, Brazil (Coelho et al., 2005). Another *Sclerolobium* species is used in the treatment of skin diseases (Muñoz et al., 2000).

Previous results from the Cerrado plant extract library, built by our research group, have demonstrated that root and stem extracts, mainly from bark are often active against fungi and protozoa (Da Costa et al., 2014). These pathogen exposed plant organs should be prioritized for target compounds isolation.

Antifungal (85%) and leishmanicidal (23%) activity were observed for 26 extracts from three species of the Fabaceae family. *E. ellipticum* was distinguished by its activity against *L. (L.) amazonensis*, especially the stem bark ethyl acetate extract ( $IC_{50}$  9.23  $\mu\text{g/ml}$ ). *S. aureum* presented no activity against the parasite, but curiously six root/stem wood and root/stem ethyl acetate or ethanol extracts exhibited differential antifungal activity (MIC 0.12 to 31.25  $\mu\text{g/ml}$ ). *V. macrocarpa* extracts were also active against both micro-organisms. Its root and stem bark are used in superficial mycoses treatment in Amazonia—traditionally referred to as *angelim-do-cerrado* or *maleiteira* (Piedade and Filho, 1988). The results found in *Trichophyton* models are consistent with this traditional use.

Fractionation of *V. macrocarpa* root bark EtOAc extract resulted in the isolation of a previously unreported vatacarpan (1) and known compound musizin (2). Vatacarpan demonstrated notable activity against *C. albicans* ATCC 10231 (MIC 0.98  $\mu\text{g/ml}$ ), an opportunistic pathogenic fungi which is the main etiological agent of candidiasis (Pierce and Lopez-Ribot, 2013).

This work documents the activity of Cerrado plant extracts against resistant clinical fungal isolates and *Leishmania*. Furthermore, it confirms the need to conserve the Cerrado biome hotspot with the view to developing lead molecules (Da Costa et al., 2014).

## Authors' contributions

LSE, RBF and ERS conceived and designed the experiments; JEDP collected and identified the plants with the research group; DBS, RCDC and RMA conducted the experiments; RBF elucidated the structures of the compounds; LSE, RBF, ERS, RMA, DBS and RCDC, contributed to data analysis; LSE and ERS contributed with reagents/materials/analysis tools and LSE, RBF, DBS, RCDC, ERS and RMA for paper writing. All the authors approved the final manuscript.

## Conflicts of interest

The authors declare no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2015.07.012.

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