IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-11, Issue-2, 2020, pp26-33
http://www.bipublication.com

Research Article

Lignans from Stem Bark of *Zanthoxylum rigidum* (Rutaceae) and their Leishmanicidal Activities

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DOI: 10.5281/zenodo.7588358

[Received: 21/06/2020; Accepted: 29/06/2020; Published: 30/06/2020]

ABSTRACT:

The genus *Zanthoxylum* (Rutaceae), comprising approximately 250 species, is well-known for its several ethnobotanical uses. The plants belonging to this genus are sources of biologically active compounds, such as alkaloids, aliphatic and aromatic amides, coumarins, lignans and cyclopeptides. Many pharmacological activities such as antiplasmodic, anti-HIV, anti-inflammatory, antihelmintic, gastroprotective and cytotoxic properties for *Zanthoxylum* species have been reported. *Zanthoxylum rigidum* is known popularly in Brazil as mamica-de-porca "pig's nipple" or mamica-de-cadela "dog's nipple" due to the particular shape of its thorn. In this research were isolated three known lignans sesamin, dimethylmatairesinol and methylpluviatolide, and other compounds as lupeol, a mixture of the steroids stigmasterol, β-sitosterol and campesterol, quercetin, and D-mannitol from stem bark of the *Zanthoxylum rigidum*. These compounds had its structures elucidated with the help of nuclear magnetic resonance spectroscopy one-dimensional (^{1}H, ^{13}C and DEPT-Q) and two-dimensional (COSY, HMQC and HMBC) and mass spectrometry (EIMS). The lignans isolated were tested for their ability to inhibit the proliferation of the promastigote forms of *Leishmania braziliensis* and *Leishmania chagasi*. The best results were obtained with sesamin and methylpluviatolide that showed IC_{50} = 22.43 and 33.60, SI = 3.87 and 2.41 and CC_{50} = 86.9 and 81.2, for *L. braziliensis* and *L. chagasi*, respectively. This study is a part of an ongoing research program to discover bioactive compounds in the Brazilian flora.

Keywords: *Zanthoxylum rigidum*, lignans, Leishmanicidal, Promastigote, *Leishmania braziliensis*, *Leishmania chagasi*.

[I] INTRODUCTION

Of the Rutaceae family, the genus *Zanthoxylum* is one of the best known, occurring practically throughout Brazil [1]. Comprising about 250 species, its main chemical constituents are

alkaloids, coumarins, lignans, amides, terpenes and cyclopeptides [2]. Ongoing studies have shown that *Zanthoxylum* genus exhibit a range of biological activities such as antichagas, tripanocidal, antiplasmodial, anti-HIV, antiinflammatory, anti-helmintic, gastropotective as well as cytotoxic [2-4].

In the Pantanal, species of *Zanthoxylum* in general, are known popularly as mamica-de-porca "pig's nipple" or mamica-de-cadela "dog's nipple" due to the particular shape of its thorn and are used in folk medicine, in the form of decoction, against different types of inflammation, rheumatism and skin stains [1]. Previous studies have described isolation of terpenes, steroids, flavonoids, alkaloids and sugars from the stem bark and cyclopeptide from the leaves of the *Z. rigidum* Humb. & Bonpl. ex Willd (Rutaceae) [1,5].

Leishmaniasis is a parasitic disease caused by a species of protozoan of the genus *Leishmania*, of which more than 20 species are known to be pathogenic to man.

The various variations of the disease are transmitted by female mosquitoes of the genus *Phlebotomus* that act as disease-transmitting agent. According to WHO, 90% of the cases of visceral leishmaniasis are registered in Bangladesh, Brazil, Nepal, India and Sudan; 90% of the cases of mucocutaneous leishmaniasis occur in Brazil, Bolivia and Peru and 90% of the cases of cutaneous leishmaniasis occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria [6,7].

In this research, we report the isolation and identification of the lignans sesamin (**1**), dimethylmatairesinol (**2**) and methylpluviatolide (**3**) from stem bark of *Z. rigidum* [**Figure 1**], which were evaluated in the inhibition of the proliferation of different forms of *Leishmania* promastigotes.

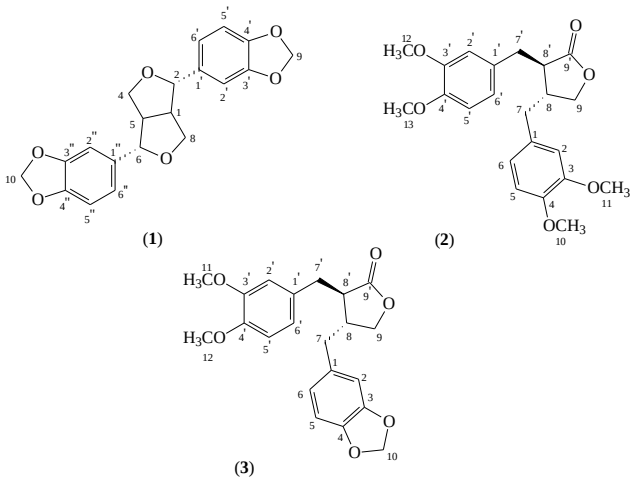

Fig 1: Lignans isolated from the stem bark of *Z. rigidum.*

[II] MATERIALS AND METHODS

2.1. General experimental procedures

Low-resolution mass spectra were performed using a gas chromatograph coupled to mass spectrometry (GC-MS), model GCMS - QP2010 Plus Shimadzu, using an ion trap and ionization by electron impact at 70 eV. IR spectrum was determined on an IRAffinity-1S Fourier Transform Infrared spectrophotometer Shimadzu Corporation. The Nuclear Magnetic Resonance spectra, ^{1}H and ^{13}C (including 2D experiments) were recorded on Bruker Magnet System AscendTM 500 (^{1}H: 500 MHz and ^{13}C: 125 MHz) spectrometers using $CDCl_3$ as solvents and TMS as an internal reference. In column chromatographic separations, silica gel 60 (70-230 *mesh*, Merck) and Sephadex LH-20 (GE Healthcare) were used; in the analytical and preparative thin layer analyzes, silica gel 60 PF_{254} (Merck) with adequate granulation and development under UV light (254 and 365 nm), acid vanillin or exposure to iodine vapors was used.

2.2. Plant material

The stem bark of *Z. rigidum* was collected in the municipality of Cuiabá in the state of Mato Grosso (Brazil), on the road to the Manso Lake,

km 20. A testimony sample is deposited at the Central Herbarium of the Federal University of Mato Grosso under number: 24985.

2.3. Extraction and isolation of the compounds

The stem bark of *Z. rigidum* (1.5 kg) was powdered and successively extracted with methanol at room temperature, providing 171.04 g of crude methanolic extract. This extract was subjected to solid-liquid partition on silica gel 60 with hexane, ethyl acetate and methanol, resulting in the fractions hexane (10.50 g), ethyl acetate (23.40 g) and methanol (91.30 g). The hexane fraction (10.50 g) was chromatographed on silica gel 60 column (70-230 *mesh*) eluted with hexane and dichloromethane, gradually increasing the polarity to obtaining of 67 fractions (150 mL). These fractions were analyzed by TLC (Thin Layer Chromatography) and reunited in 19 fractions (15 mL). In the fraction 8 (45.40 mg) a white amorphous solid precipitated and after filtration in acetone, provided the triterpene lupeol (451.80 mg). The fraction 6 (67.70 mg) provided colorless needle-shaped crystals corresponding to the mixture of the steroids β-sitosterol, campesterol and stigmasterol. The ethyl acetate fraction (23.40 g) was subjected to a new fractionation on a silica gel 60 (70-230 *mesh*) and microcrystalline cellulose (1:1) column, using hexane, ethyl acetate and methanol with increasing of polarity affording 77 fractions (15 mL). These fractions were analyzed by TLC and grouped according to similarity of retention factor (R_f) in 13 fractions (15 mL), and after this process, there was the precipitation of a crystalline solid in fraction 5, which provided sesamin (**1**, 102.0 mg). Fractions 7-9 (4.15 g) were combined by similarity and subjected to filtration on Sephadex LH-20 in methanol and 53 fractions were obtained. After analysis by TLC, in the fractions 27-31 (650.0 mg) was observed the presence of two major compounds that corresponded to dimethylmatairesinol (**2**, 22.70 mg) and methylpluviatolide (**3**, 97.80 mg), separated through a preparative TLC eluted in the $CHCl_3$:MeOH system (9:1). The methanol fraction (91.30 g) was subjected to the classic column on silica gel 60 (70-230 *mesh*) eluted with hexane, chloroform, ethyl acetate and methanol afforded 105 fractions (10 mL), combined according to their similarity in 15 fractions (15 mL). Fraction 3 yielded a yellowish amorphous precipitate which was recrystallized in acetone and provided again the mixture of steroids (420.10 mg). In fractions 5-7, precipitate a crystalline solid in the form of needles occurred, again providing lupeol (410.13 mg). In fraction 13, a yellow precipitate formed that was filtered through $CHCl_3$, providing quercetin (272.90 mg). From fraction 15 there was a spontaneous precipitation of a white crystalline solid, D-mannitol (610.40 g).

Sesamin (**1**), colorless crystal, $C_{20}H_{18}O_6$, EIMS *m/z* 354 $[M]^{+\cdot}$, IR (KBr film) ν_{max} (cm^{-1}) 2850, 1500, 1442, 1193, NMR ^1H [500 MHz, $CDCl_3$, δ (ppm), *J* (Hz)]: 6.87 (*d*, *J* = 1.50, H-2'/2"), 6.83 (*dd*, *J* = 8.2, 1,5, H-6'/6"), 6.80 (*d*, *J* = 7,90, H-5'/5"), 5.97 (*s*, H-9/10), 4.74 (*d*, *J* = 4.40, H-2/6), 3.90 (*dd*, *J* = 9.15, 3.70, H-4/8) and 4.25 (*dd*, *J* = 9.15, 6.70, H-4/8), 3.07 (*m*, H-1/5), NMR ^{13}C [125 MHz, $CDCl_3$, δ (ppm)]: 148.0 (C-3'/3"), 147.1 (C-4'/4"), 135.1 (C-1'/1"), 119.4 (C-6'/6"), 108.2 (C-5'/5"), 106.5 (C-2'/2"), 101.1 (C-9/10), 85.8 (C-2/6), 71.7 (C-4/8), 54.3 (C-1/5) [8].

Dimethylmatairesinol (**2**), green amorphous solid, $C_{22}H_{26}O_6$, EIMS *m/z* 386 $[M]^{+\cdot}$, IR (KBr film) ν_{max} (cm^{-1}) 2920, 1776, 1591, 1489, 1157, NMR ^1H [500 MHz, $CDCl_3$, δ (ppm), *J* (Hz)]: 6.79 (*d*, *J* = 8.20, H-5), 6.77 (*d*, *J* = 8.30, H-5'), 6.70 (*d*, *J* = 1.95, H-2'), 6.67 (*dd*, *J* = 8.10, 1.9, H-6'), 6.57 (*dd*, *J* = 8.10, 1.90, H-6), 6.50 (*d*, *J* = 2.0, H-2), 4.14 (*dd*, *J* = 6.40, 4.10, H-9a) and 3.91 (*dd*, *J* = 7.20, 5.10, H-9b), 3.88 (*s*, H-10, $OC\underline{H}_3$), 3.87 (*s*, H-13, $OC\underline{H}_3$), 3.85 (*s*, H-11, $OC\underline{H}_3$), 3.84 (*s*, H-12, $OC\underline{H}_3$), 2.98 (*dd*, *J* = 14.10, 5.40, H-7'a) and 2.94 (*dd*, *J* = 14.10, 6.60, H-7'b), 2.70 (*m*, H-7),

2.65 (*m*, H-8'), 2.55 (*m*, H-8), NMR ^{13}C [125 MHz, $CDCl_3$, δ (ppm)]: 178.7 (C-9', C=O), 149.0 (C-4/4'), 147.9 (C-3/3'), 130.4 (C-1), 130.2 (C-1'), 120.5 (C-6), 121.4 (C-6'), 112.3 (C-2'), 111.7 (C-2), 111.3 (C-5'), 111.0 (C-5), 71.3 (C-9), 60.0 (C-10, O<u>C</u>H$_3$), 56.0 (C-11, O<u>C</u>H$_3$), 55.9 (C-12, O<u>C</u>H$_3$), 55.8 (C-13, O<u>C</u>H$_3$) 46.6 (C-8'), 41.1 (C-8), 38.2 (C-7), 34.5 (C-7') [9].

Methylpluviatolide (**3**), green amorphous solid, $C_{20}H_{20}O_6$, EIMS *m/z* 370 $[M]^{+\cdot}$, IR (KBr film) ν_{max} (cm^{-1}) 2920, 1774, 1598, 1510, 1160, [500 MHz, $CDCl_3$, δ (ppm), *J* (Hz)]: 6.79 (*d*, *J* = 8.10, H-5'), 6.74 (*d*, *J* = 7.65, H-5), 6.69 (*d*, *J* = 1.80, H-2'), 6.62 (*dd*, *J* = 7.35, 1.40, H-6/6'), 6.45 (*d*, *J* = 1.45, H-2), 5.96 (*d*, *J* = 1.45, H-10), 3.92 (*m*, H-9), 3.88 (*s*, H-11, OC<u>H</u>$_3$), 3.86 (*s*, H-12, OC<u>H</u>$_3$), 2.98 (*dd*, *J* = 14.05, 5.2, H-7'), 2.88 (*dd*, *J* = 14.05, 7.15, H-7), 2.61 (*m*, H-8'), 2.53 (*m*, H-8), NMR ^{13}C [125 MHz, $CDCl_3$, δ (ppm)]: 178.5 (C-9', C=O), 149.0 (C-3'), 147.9 (C-4), 146.5 (C-3), 146.3 (C-4'), 131.5 (C-1), 130.4 (C-1'), 120.6 (C-6'), 122.3 (C-6), 111.6 (C-2'), 111.3 (C-5'), 109.5 (C-2), 108.3 (C-5), 101.1 (C-10), 71.2 (C-9), 55.9 (C-11, O<u>C</u>H$_3$), 55.8 (C-12, O<u>C</u>H$_3$), 46.5 (C-8'), 41.1 (C-8), 38.3 (C-7), 34.6 (C-7') [9].

2.4. Evaluation of leishmanicidal activity

Cells

2.4.1. Cells

Murine macrophages of the J774 A.1 strain (ATCC CR-107), macrophage/monocyte cell type, derived from an adult female BALB/c sarcoma were used and cultured in RPMI-1640 cell culture medium, supplemented with streptomycin antibiotics (10 mg/mL), penicillin (6 mg/mL) and kanamycin (2 mg/mL), and 10% fetal bovine serum (FBS), kept at 37 ºC and 5% CO_2.

2.4.2. Parasites

Stationary phase promastigote forms of *L. (V.) braziliensis* and *L. (L.) chagasi* were used and maintained in Schneider culture media supplemented with streptomycin antibiotics (10 mg/mL) and penicillin (6 mg/mL), and 20% fetal bovine serum (FBS), kept at 26 ºC in a BOD incubator. The parasites used were obtained from cell culture from 6 to 7 days of growth when the promastigote forms are in the stationary phase.

2.4.3. Determination of the concentration that inhibits 50% of the growth of the parasite (IC_{50})

The *in vitro* cytotoxicity assay was performed to determine the inhibitory concentration of 50% (IC_{50}) using $1x10^5$ of promastigote forms of *L. braziliensis* and *L. chagasi*, maintained in Schneider medium, supplemented with fetal bovine serum (FBS) at 26 ºC incubated in 96-well plates with the standard drug amphotericin B (10 µg/mL) and the compounds **1**, **2** and **3**. These compounds were tested in concentrations between 0.5 to 250 µg/mL dissolved in Schneider culture medium and 0.1% DMSO. As a negative control, the parasites were grown only in Schneider and Schneider medium containing the diluent of the compounds (DMSO 0.1%). The viability of the promastigote forms was evaluated based on the MTT metabolism, being the same proportional to the absorbance value generated in a spectrophotometer. After 72h, incubation at 26 ºC, 50µl of MTT solution (2 mg/mL) were added to each well and then incubated for 4h at 24 ºC. After this period, the plate was centrifuged at 5,000 rpm for 7 minutes and the supernatants from each well were removed and 100 µl DMSO was added. The formazan crystals were dissolved by shaking and the absorbance was determined by an ELISA reader at 540 nm. The data were plotted on a linear regression curve and the results expressed in IC_{50} as described by Sereno and Lemestre (1997) [10]. This experiment was carried out in duplicate.

2.4.4. Determination of cytotoxicity (CC_{50}) and selectivity index (SI) of the compounds

Macrophages J774 A.1, were grown in RPMI-1640 medium, supplemented with streptomycin

antibiotics (10 mg/mL), penicillin (6 mg/mL) and kanamycin (2 mg/mL), and 10% fetal bovine serum (FBS), maintained at 37 ºC and 5% CO_2, this culture after acquiring semi-confluence, was washed once with the Hanks buffer solution, trypsinized and the cells counted in a Neubauer chamber, adjusting the number cells to $2x10^5$ cells/mL in complete RPMI medium. From this suspension, the cells were seeded in 96-well plates and incubated at 37 ºC and 5% CO_2 for 24h. Next, different concentrations of **1**, **2** and **3** were prepared in concentrations between 0.5 to 250 µg/mL, to which the cells were added, being incubated at 37 ºC and 5% CO_2 for 24 hours. As a negative control, the cells were cultured containing the 0.1% DMSO diluent. Doxorubicin (10 mg/mL) was used as a positive control in this bioassay. The cytotoxic concentration 50% (CC_{50}) of each compound was evaluated using the alamar blue redox indicator [11]. After the incubation period, the culture medium was removed and then 20 µL of alamar blue and 180 µL of complete RPMI medium were added. After 6h of incubation, the absorbance was read at 570 nm (oxidized state) and 595 nm (reduced state) in the ELISA reader (BIORAD 640) and the visual reading of the plate was done by the redox indicator, where the blue color oxidized represents cell death and the lilac color represents viable cells. The data were analyzed using a linear regression curve and the results were expressed as CC_{50}, being considered CC_{50} cytotoxic <50 µg/mL [12]. The experiments were carried out in triplicate. The selectivity index (SI) was calculated considering the ratio between CC_{50} and IC_{50} for each compound tested (CC_{50}/IC_{50}).

[III] RESULTS AND DISCUSSION

The hexane fraction from stem bark of *Z. rigidum* resulted in the isolation of lupeol, a mixture of the steroids campesterol, stigmasterol and β-sitosterol. The ethyl acetate fraction resulted in the identification of the lignans sesamin (**1**), dimethylmatairesinol (**2**) and methylpluviatolid (**3**). From the methanol fraction, lupeol, the steroids campesterol, stigmasterol and β-sitosterol, the flavonoid quercetin and great abundance of the carbohydrate D-mannitol were obtained. The structures of lupeol, quercetin and D-mannitol were established by analysis of ^1H and ^{13}C NMR spectra, comparisons using standards by TLC analysis and literature data [13,14,15]. For confirmation of the steroids mixture, β-sitosterol, stigmasterol and campesterol, a GC-MS analysis was conducted. The analysis of the mass fragments from each component provided the peaks corresponding to the molecular ions at *m/z* 400 (campesterol), *m/z* 412 (stigmasterol) and *m/z* 414 (β-sitosterol) [16]. The structures of the lignans sesamin (**1**), dimethylmatairesinol (**2**) and methylpluviatolide (**3**), were defined based on the analysis of ^1H and ^{13}C (DEPT-Q) and HMQC and HMBC spectroscopic data, MS, IR and comparison with literature data [8,9]. The HMBC spectrum of (**1**) showed characteristic of the furofuranic ring with the correlations beetwen the hydrogens H-4 and H-8 (δ_H 3.90 and δ_H 4.25) with the carbons C-1/C-5 (δ_C 54.3) and C-2/C-6 (δ_C 85.8), as well as the correlations between the hydrogens H-1 and H-5 (δ_H 3.07) with C2/C-6 (δ_C 85.8), C-4/C-8 (δ_C 71.7) and C-1'/C-1" (δ_C 135.1) to $^3J_{C\text{-}H}$. It was also observed the coupling between the hydrogens of the methylenedioxy group at δ_H 5.97 with the quaternary carbons C-3'/C-3" (δ_C 148.0) and C-4'/C-4" (δ_C 147.1) of the aromatic system. It was possible to differentiate compounds **2** and **3** trhough the ^1H NMR spectrum. In the compound **2** the ^1H NMR spectrum showed four methoxy groups by the intense singlets at δ_H 3.88, 3.87, 3.85 and 3.84 linked to aromatic systems; in **3**, the methoxy groups were characterized by the singlets at δ_H 3.92 and 3.88. In the compound **3** also was observed a doublet at δ_H 5.96 attributed to the hydrogens of the methylenedioxy group. In the DEPT-Q spectrum, the lactonic ring was

confirmed by signs for carbinolic carbon at δ_C 71.3 (**2**) and δ_C 71.2 (**3**) assigned to C-9 and two signs at δ_C 41.1 and 46.6 (**2**) and δ_C 41.1 and 46.5 (**3**) attributed to the C-8 and C-8' methyl carbons, respectively; besides the presence of a carbonyl group at δ_C 178.7 (**2**) and δ_C 178.5 (**3**) attributed to the C-9'. A detailed analysis of the HMBC spectrum corroborated with the presence of the lactonic ring by the correlations between the diastereotopic hydrogens H-9 (δ_H 3.91 and 4.14) with C-8 (δ_C 41.1), C-7 (δ_C 38.2), C-8' (δ_C 46.6) and C-9' (δ_C 178.7) for compound **2**, and, the correlation between the multiplet at δ_H 3.92 (H-9) with C-8 (δ_C 41.1), C-7 (δ_C 38.3), C-8' (δ_C 46.5) and C-9' (δ_C 178.5) indicated the lactonic ring in compound **3**. Two other signs were observed at δ_C 38.2 and 34.5 (**2**) and δ_C 38.3 and 34.6 (**3**) referring to methylenic carbons C-7 and C-7', respectively. In addition, in **3** the signal at δ_C 101.1 attributed to the methylenedioxy group was observed. The leishmanicidal potential of the compounds **1**, **2** and **3** against *L. brasiliensis* and *L. chagasi* strains was evaluated. These compounds were used in concentrations ranging from 0.5 to 250 µg/mL and analyzed at different time periods (24, 48 and 72h) in order to determine the inhibitory concentration against the tested *Leishmania* strains. The results obtained showed that among the tested compounds, only **1** and **3** with IC_{50} of 22.43 µg/mL and 33.6 µg/mL, showed the greatest efficacy against the promastigote forms of *L. braziliensis* and *L. chagasi*, respectively [**Table 1**]. The standard drug amphotericin B, used as a positive control, had an inhibitory effect on the proliferation of promastigote forms, reducing this parameter to 0.38 µg/mL and the diluting agent DMSO (0.1%) did not change the viability and proliferation of promastigote forms. Alamar blue (AB) was used as an effective tool to assess the metabolic activity and proliferation of cell lines. In this bioassay, only the reduction of resazurin to resorufin is quantified, which revealed that all compounds showed low toxicity compared to the cell line J774. A1. The results provided the values of CC_{50} (50% Cytotoxic Concentration), and was verified that the compounds **1** and **3** could be tested in concentrations below 50 µg/mL. The SI (Selectivity Index) evaluated the selectivity of the compounds against the parasite, and the highest values were observed for **1** (3.87) and **3** (2.41), indicating a greater efficacy and safety of these compounds against *L. braziliensis* and *L chagasi* compared to compound **2**. These results showed that the increase in antiproliferative activity may be related to the presence of methylenedioxy groups in the structure, according to reports found in the literature [17,18].

[Table-1] Leishmanicidal effect of lignans from the stem bark of *Z. rigidum*.

C	CC_{50} (µg/mL) J774 A.1	*L. chagasi*		*L. brasiliensis*	
		IC_{50} (µg/mL)	SI	IC_{50} (µg/mL)	SI
1	86.9 (78.01-91.05)	93.04 (82.3-107.3)	0.93	22.43 (16.81-32.4)	3.87
2	68.56 (53.25-79.14)	102.3 (97.2-112.5)	0.67	93.3 (84.1 – 98.6)	0.73
3	81.2 (75.58-89.22)	33.6 (24.27-42.78)	2.41	112.52 (96.3-124.1)	0.72

C = compound; CC_{50} = 50% cytotoxic concentration; IC_{50} = inhibitory concentration; SI = selectivity index.

[V] CONCLUSION

The phytochemical study from stem bark of *Z. rigidum* led to the isolation and structural elucidation of three lignans, already described in the literature sesamin (**1**), dimethylmatairesinol (**2**) and methylpluviatolide (**3**), besides others known compounds as lupeol, a mixture of the steroids stigmasterol, β-sitosterol and campesterol, quercetin, and D-mannitol. These lignans were tested for their leishmanicidal activity against different species of *Leishmania*, and the compounds (**1**) and (**3**) showed better results having IC_{50} values of 22.43 and 33.60 µg/mL, and SI 3.87 and 2.41, against the

promastigote forms of *L. braziliensis* and *L. chagasi*, respectively. The results for CC_{50} showed that the compounds (**1**) and (**3**) can be used in concentrations below 50 μg/mL. These results can be useful in the development of natural leishmanicidal agents for the eradication of cutaneous and visceral leishmaniasis, a neglected disease that is still very common in tropical and subtropical countries, based on the chemistry of bioactive compounds from plants in the Brazilian Pantanal.

ACKNOWLEDGEMENT

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES, finance code: 001), FAPEMAT (TANR, 042/2016), and Instituto Nacional de Áreas Úmidas (INAU) for financial support.

REFERENCES

1. Moccelini, S. K, et al., (2007), Estudo fitoquímico das cascas das raízes de *Zanthoxylum rigidum* Humb. & Bonpl. ex Willd (Rutaceae). Química Nova, Vol-32, Issue 1, pg 131-133.
2. Beirigo, P. J. S, et al., (2016), [1-8-NαC]-Zanriorb A1, a proapoptotic orbitide from leaves of *Zanthoxylum riedelianum*. Journal of Natural Products, Vol-79, Issue 5, pg 1454-1458.
3. Lee, S. W, et al., (2020), Enhanced bioactivity of *Zanthoxylum schinifolium* fermented extract: anti-inflammatory, anti-bacterial, and anti-melanogenic activity. Journal of Bioscience and Bioengineering, Vol-129, Issue 5, pg 638-645.
4. Freitas, F. F. B. P, et al., (2011), Gastroprotective activity of *Zanthoxylum rhoifolium* Lam. in animal models. Journal of Ethnopharmacology, Vol-137, Issue 1, pg 700-708.
5. Ribeiro, T. A. N, et al., (2012), A new cyclopeptide and other constituents from the leaves of *Zanthoxylum rigidum* Humb. & Bonpl. ex Willd. (Rutaceae). Helvetica Chimica Acta, Vol-95, Issue 6, pg 935-939.
6. Chappuis, F, et al., (2007), Visceral leishmaniasis: what are the needs for diagnosis, treatment and control?. Nature Reviews Microbiology, Vol- 5, pg 873–882.
7. Bahmani, M, et al., (2017), A rare case report of extensive cutaneous Leishmaniasis lesions on ear from Dehloran city, West of Iran and the treatment process. International Journal of Advanced Biotechnology and Research, Vol-8, Issue-3, pg 1747-1749.
8. Wang, et al., (2009), Preparative separation and purification of sesamin and sesamolin from sesame seeds by high-speed countercurrent chromatography. Cereal Chemistry, Vol-86, Issue 1, pg 23-25.
9. Heleno, V. C. G, et al., (2006) Detailed ^{1}H and ^{13}C NMR structural assignment of three biologically active lignan lactones. Spectrochimica Acta Part A, Vol-63, pg 234–239.
10. Sereno, D, and Lemesre, J. L, (1997), Use of an enzymatic micromethod to quantify amastigote stages of *Leishmania amazonensis in vitro*. Parasitology Research. Vol-83, Issue 4, pg 401-403.
11. Al-Nasiry, S, et al., (2007), The use of alamar blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells, Human Reproduction. Vol-22, Issue 5, pg 1304-1309.
12. Froelich, B. O, et al., (2007), Plants traditionally used against malaria: phytochemical and pharmacological investigation of *Momordica foetida*, Revista Brasileira de Farmacognosia. Vol-17, Issue 1, pg 1-7.
13. Mahato, S. B. and Kundu, A. P, (1994), ^{13}C NMR Spectra of pentacyclic triterpenoids - A compilation and some salient features. Phytochemistry, Vol-37, Issue 6, pg 1517-1575.

14. Sa, F. A. S, (2017), Phytochemical analysis and antimicrobial activity of *Myrcia tomentosa* (Aubl.) DC. leaves. Molecules, Vol-22, pg 1100-1110.
15. Sousa, E. A, et al., (2019), Composição química e atividade antileishmania de *Tocoyena hispidula*. Química Nova, Vol-42, Issue 2, pg 192-196.
16. Branco, A. and Pizzolatti, M. G, (2002), CGAR e CGAR-EM na análise dos constituintes químicos isolados do extrato hexânico de *Sebastiania argutidens* (Euphorbiaceae). Química Nova, Vol-25, Issue 15, pg 15-19.
17. Correa, J. E, et al., (2006), Minor alkaloids from *Guatteria dumetorum* with antileishmanial activity, Planta Medica. Vol-72, Issue 3, pg 270-272.
18. Rodrigues-Silva, D, et al., (2009), In vitro antileishmanial activity of hydroalcoholic extract, fractions, and compounds isolated from leaves of *Piper ovatum* Vahl against *Leishmania amazonenses*, Acta Protozoologica, Vol-48, Issue 1, pg 73-81.