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In vitro evaluation of the leishmanicidal potential of selected plant-derived extracts against *Leishmania (Leishmania) amazonensis*

Abstract

Background: Leishmaniasis is a potentially fatal, neglected parasitic disease caused by different species of *Leishmania* sp. Natural products, especially from plants; represent a rich source for the screening of potential antiparasitic compounds.

Purpose and study design: In this study, we evaluated the leishmanicidal activity of thirteen plant extracts against the parasite *Leishmania (Leishmania) amazonensis in vitro*, the cytotoxic and hemolytic activity. The extracts with activity against the parasite, was determined the chemical constituents.

Results: The hexane extracts of *Bidens sulphurea* and *Plectranthus neochilus* were the most effective extracts against promastigote forms at 24h and 48h. The EC_{50} (50% effective concentration) value obtained for these extracts against promastigote forms were calculated to be 84.26µg/mL and 46.32µg/mL in 24h, respectively. The EC_{50} values against intracellular amastigotes were higher than 100µg/mL after 48h of incubation for both extracts. Regarding cytotoxicity in peritoneal macrophages, extracts of *B. sulphurea* showed CC_{50} values (cytotoxicity concentration of 50% of cells) of 103.9 and 80.30µg/mL at 24 and 48h, respectively, whereas the CC_{50} values for the *P. neochilus* extract were 66.95 and 34.39µg/mL at 24 and 48h, respectively. The extracts showed no significant hemolysis at the concentrations evaluated, and the CH_{50} values were higher than 100µg/mL. The chemical constituent of the hexane extracts of *B. sulphurea* and *P. neochilus* and their activity against *L. amazonensis* has not been previously described.

Conclusion: Despite the unsatisfactory results against amastigotes forms, this study shows extracts obtained from botanical sources merit further study for their leishmanicidal properties.

Keywords: *Leishmania (Leishmania) amazonensis*, natural products, hexane plant extracts, leishmanicidal activity

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Abbreviations: VL, visceral leishmaniasis; CL, cutaneous leishmaniasis; GC, gas chromatography; MS, mass spectrometry; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; EC_{50}, 50% effective concentration; CC_{50}, 50% cytotoxic concentration; HC_{50}, 50% hemolytic concentration; SD, standard deviation; SI, selectivity index

Introduction

Leishmaniasis, one of the most important neglected tropical diseases, is endemic in 98 countries, with more than 12million cases and 350million people living in areas at risk of infection.[1,2] This disease is caused by an obligate intracellular protozoan of the genus *Leishmania*,[3] and is broadly classified into three different forms: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis.[4] In Latin America, *Leishmania (Leishmania) amazonensis* is responsible for the cutaneous diffuse form of the disease,[5] that in some cases may also result in visceral leishmaniasis.[6,7] According to the Brazilian Ministry of Health, since 2005, the presence of *L.(L.) amazonensis* has been present in almost all Brazilian regions[5], thus raising concern about this infection. The first-line drugs for leishmaniasis treatment are sodium stibogluconate (Pentostan) and meglumine antimonite (Glucantime); amphotericin B and pentamidine are second-line drugs.[2] However, the current standard-of-care is unsatisfactory due to are expensive, potentially toxic and long-term treatment requirements, resulting in patient non-compliance.[2] Also, there are significant differences in the sensitivity of these species to standard drugs.[8,9]

In the last decade, the scientific investigation of medicinal plants has received considerable attention in drug development against protozoan diseases.[10–12] In this context, the evaluation of plant-derived extracts and isolated natural compounds can result in potential leads for use against infectious diseases. Recently, it was demonstrated that the hexane extracts derived from plants of Asteraceae, Lamiaceae, Myrtaceae, and Verbenaceae families showed promising activity against cariogenic bacteria.[13] As part of our ongoing interest in the antiparasitic activity of natural products and their derivatives, we evaluate here the leishmanicidal potential of thirteen selected plant-derived hexane extracts from the leaves of herbaceous or arbustive plant species (Table 1) against the parasite *L. (L.) amazonensis*.

Material and methods

Plant material and extraction

Specimens of thirteen species (Table 1) were collected in May 2010 at ¨Sítio 13 de Maio¨ (20°26'S 47°27'W 977m), localized near the city of Franca, State of São Paulo, Brazil and identified by Prof. Dr. Milton Groppo. Voucher specimens were deposited at the Herbarium of Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil. Leaves of each species were dried carefully in a

circulating air oven (Quimis-Diadema, BR) at 40°C and ground in a knife mill (Tecnal - Piracicaba, BR).

The powdered leaves were extracted with hexane, as previously reported.[13] Three extractions per species were retained, with each extraction lasting 15min. The samples were concentrated using a rotary evaporator under reduced pressure to provide the respective hexane extracts.

Gas Chromatography (GC) and gas chromatography mass spectrometry (GC-MS) analyses

Gas chromatography–mass spectrometry (GC–MS) analyses was carried out as previously reported.[13] The chemical components of the hexane extract of *Bidens sulphurea* were identified on the basis of their retention indices relative to a homologous series of *n*-alkanes (C_8–C_{40})[14] on a Rtx-5MS capillary column under the same operating conditions and computer matching with the Wiley 7, NIST 08 and FFNSC 1.2 spectral libraries of the GC-MS system.

Animals

Male BALB/c mice were maintained under controlled conditions of temperature (22±20ºC), humidity (50±10%), and light–dark cycle. All the experiments were authorized by the University of Franca´s Ethics Committee for Animal Care (Approval number: 046/15). All animals were handled using good animal practice as defined by the University of Franca in concordance with Brazilian legislation.

Parasites

L.(L.) amazonensis (MHOM/BR/PH8) was routinely in M199 medium (Gibco, New York, USA) supplemented with heat-inactivated 10% fetal bovine serum (FBS), penicillin (10000 UI/mL) and streptomycin (10mg/mL) (Cultilab, Campinas, BR) at 25°C.

Anti-promastigote assay

A preliminary screening with promastigote forms was performed in the presence of 100µg/mL previously dissolved in dimethyl sulfoxide (DMSO) (Synth, Diadema, BR). The inhibition of cell growth was determined by counting cells with a haemocytometer (Global Glass, Porto Alegre, BR) after 24 and 48h of incubation at 24°C.[15]

The plant extracts that showed greater than or equal to 50% inhibition of cell growth during 48h were further evaluated at concentrations of 0.78 to 100µg/mL. Parasites incubated with amphotericin B (Eurofarma –São Paulo, BR) was used as a positive control and M199 medium (Sigma Aldrich – St Louis, EUA) with 0.1% DMSO served as negative control. The 50% effective concentration (EC_{50}) was calculated as described below. All tests were conducted in triplicate, and three independent assays were performed.

Anti-amastigote assay

To evaluate activity against intracellular amastigote forms, peritoneal macrophages cells were seeded (2×10^5 cells/mL) into 24-wells plates containing glass coverslips (13mm). Non-adherent cells were removed, and the cells were infected with promastigote forms at a ratio 1:10 (macrophage/promastigote). Infected macrophages were incubated with the *B. sulphurea*, *P. neochilus* (6.25-100 and 3.12-50µg/mL) and amphotericin B (0.18-3µg/mL) for 48h at the same conditions described above. Parasites incubated in RPMI 1640 medium (Sigma Aldrich) with 0.1% DMSO served as the negative control. The number of amastigotes was determined by randomly counting 200 cells. The results were calculated using the negative control (0.1% DMSO) as representative of 100% cell survival. The 50% inhibitory concentration (EC_{50}) was calculated as described below. All tests were conducted in triplicate, and three independent assays were performed.

Cytotoxicity against peritoneal macrophages

The obtation of peritoneal macrophages as performed using previously reported protocol with slight modification.[16] The macrophages ($2x10^5$ cells) were incubated in the presence of a concentration range (0.78-100µg/mL) of *Bidens sulphurea*, *Plectranthus neochilus* and amphotericin B (0.002-1.56µg/mL) (Eurofarma) for 24 and 48 h. DMSO was used as a positive control (25%) and negative control was RPMI 1640 medium with 0.1% of DMSO. Cell viability was assessed by Trypan Blue exclusion (Inlab –Diadema, BR).[17] The results were expressed as the mean percentage reduction of macrophage viability compared to that in the untreated control wells, and the 50% cytotoxic concentration (CC_{50}) was calculated as described below. All tests were conducted in triplicate, and three independent assays were performed.

Red blood cell lysis assay

The toxicity to red blood cells was determined as previously described with some modifications.[18] Briefly, erythrocytes were incubated with *B. sulphurea*, *P. neochilus* and amphotericin B at room temperature for 30 min and hemolysis was determined by the hemoglobin release, quantitated by the absorbance of the supernatants at 415nm. The percentage of lysis was calculated in relation to total lysis. The negative control was erythrocytes with NaCl solution 0.9%, while the positive control used erythrocytes with water. The 50% hemolytic concentration (HC_{50}) was calculated as described below. All tests were conducted in triplicate, and three independent assays were performed.

Statistical analysis

Data represent the mean number (±SD) of three independent experiments performed in triplicate. The results were compared by analysis of variance, one-way ANOVA, followed by Dunnett's test to determine significance between the negative control group and treated groups. The EC_{50}, CC_{50} and HC_{50} were calculated using dose–response curves using GraphPad Prism 5 (GraphPad Software, San Diego, California, USA). The SI was calculated using the ratio of CC_{50}/EC_{50}.[19]

Results and discussion

Several extracts and compounds isolated from plants have been investigated for their biological properties, including their leishmanicidal activit.[20,21] In the present study, thirteen hexane extracts from leaves of cultivable herbaceous or arbustive plant species (Table 1) were evaluated against *L. (L.) amazonensis*. The hexane extracts of these species were selected on the basis of previous reports in the literature or on their use as antimicrobial and antiparasitic activities in folk medicine.[13]

A preliminary screening of hexane extracts was performed at 100µg/mL against promastigote forms of *L. (L.) amazonensis* to select the most active extracts at higher concentrations. Five hexane extracts (*Artemisia camphorata, Arrabidaea chica, Eclipta alba, Foeniculum vulgare, Lippia alba)* showed no activity after 24h and activity lower than 25% in 48 h of treatment. Six extracts (*Alternanthera brasiliana,*

Coreopsis lanceolata, Pelargonium graveolens, Stachytarpheta cayennensis, Senna occidentalis and *Tagetes erecta*) showed a percentage of inhibition of cell growth of less than 50% at 24 and 48h (Table 2). On the other hand, the hexane extracts of *Bidens sulphurea* and *Plectranthus neochilus* were the most effective extracts against promastigote forms of *L. (L.) amazonensis* at 24h and 48h; they showed a percentage of inhibition of cell growth higher than 90% after 48h (Table 2).

Table 1 Classification and characteristics of the plant species selected for this study and their respective voucher number

Family	Botanical name	Voucher number	Biological activities	References
Amaranthaceae	*Alternanthera brasiliana (*L.) Kuntze	10018	Anti-inflammatory; analgesic	Moraes et al., 1994; Souza et al., 1998
Apiaceae	*Foeniculum vulgare* Mill.	12024	Diuretic; analgesic; antipyretic; antioxidant	Forster et al., 1980; Tanira et al., 1996; Oktay et al., 2003
Asteraceae	*Artemisia camphorata* Vill.	10006	Antibacterial; Anti-fungal	Itako et al., 2008; Franzener et al., 2003.
	Bidens sulphurea(Cav.) Sch. Bip.	12020	NR	Botsaris 2007
	Coreopsis lanceolata L.	10007	Antioxidant; eliminating free radicals	Crotti et al., 2013; Tanimoto et al., 2009
	Eclipta alba (L.) Hassk	10008	Anti-fungal; antiepileptic; antimicrobial	Shaikh et al., 2013; Karthikumar et al., 2007
	Tagetes erecta L.	10009	Antioxidant; Analgesic	Lorenzi & Souza, 2001; Bashir & Gilani, 2008.
Bignoniaceae	*Arrabidaea chica*(Humb.& Bonpl.) B. Verl.	10013	Collagen production; antimicrobial; leishmanicidal	Aro et al., 2013; Mafioleti et al., 2013; Rodrigues et al., 2014
Fabaceae	*Senna occidentalis* (L.) Link	10012	Toxicity	Barbosa-Ferreira et al., 2011; Barros et al., 1999
Geraniaceae	*Pelargonium graveolens* L' Hér.	12023	Antioxidant; anti-fungal.	Cávar et al., 2012; Singh et al., 2008
Lamiaceae	*Plectranthus neochilus Schltr.*	12323	schistosomicidal; Antioxidant	Caixeta et al., 2011; Viana et al., 2011
Verbenaceae	*Lippia alba (*Mill.) N.E.Br	12022	Antioxidant; Antimicrobial	Stashenko et al., 2004; Aguiar et al., 2008
	Stachytarpheta cayennensis(Rich.) Vahl.	10005	Leishmanicidal; Antimicrobial; Antispasmodic	Moreira et al., 2007; Okoye et al., 2010

NR: Not Reporte

Table 2 Screening *in vitro* of leishmanicidal activity against *L.(L.) amazonensis* promastigotes after 24 and 48h of incubation with hexane plant extracts

Species	% Inhibition of cell growth±SD	
	24 h	48 h
Alternanthera brasiliana	4.12±1.71	6.51±7.05
Artemisia camphorata	0±0	19.13±3.93
Arrabidaea chica	0±0	16.60±2.80
Bidens sulphurea	57.34±1.48	92.72±8.05
Coreopsis lanceolata	0.11±0.16	7.63 ±2.32
Eclipta alba	0±0	0.69±0.97
Foeniculum vulgare	0±0	5.66±4.38
Lippia alba	0±0	21.20±0.96
Pelargonium graveolens	25.88±4.29	41.58±2.67
Plectranthus neochilus	80.23±2.39	92.19±2.62
Stachytarpheta cayennensis	39.38±1.29	40.28±10.90
Senna occidentalis	12.64±2.04	45.12±3.30
Tagetes erecta	2.10±2.97	27.08±.04
Anfotericina B(2µg/mL)	100±0	100±0

Percentage of inhibition cell growth was calculated relative to the negative control (0.1% DMSO).
Each experiment was performed in triplicate and repeated three times.

Another study demonstrated that ethanolic extracts from *Artemisia kulbadica, Artemisia cininformes* and *Artemisia santolina* had an EC_{50} of 25.25 and 80µg/mL, respectively, against promastigotes forms of *L. (L.) major* after 24h of incubation.[22] However, no study has demonstrated the effect of *A. camphorata* extracts against *L. (L.) amazonensis*. As described previously in the literature, the hexane extract of *A. chica* showed EC_{50} values of 31.8µg/mL and 14.7µg/mL against *L.(L). amazonensis* e *L. (L.) infantum* at 120h, respectively.[23]

In another study, the aqueous and ethanolic extract of *E. alba* inhibited 100% growth of *L. (L.) donovani* promastigotes at concentration of 0.5mg/mL.[24] Besides, study demonstrated that essential oils obtained from the *L. alba* species collected at different locations in Colombia showed different leishmanicidal activities against *L.(L.) chagasi* promastigotes, which suggest that different location may show changes in the chemical composition of the plant.[25] Maquiaveli and co-workers also reported that butanol fraction of the aqueous extract of *S. cayennensis* showed EC_{50} values of 51.0 (72h) and 32.0 (24h) µg/mL against promastigote and amastigote forms, respectively.[26]

To determine the EC_{50} values of the hexane extracts of *P. neochilus* and *B. sulphurea,* promastigote forms of *L. (L.) amazonensis* were incubated with the hexane extracts for 24 and 48h. The activity of extracts has been classified as follows in the literature: highly active (EC_{50} value <10µg/mL); active, (10<EC_{50} <50µg/mL), moderately active (50 < EC_{50} < 100µg/mL) and non-active (EC_{50} >100 µg/mL).[27] Our results revealed that in 24 h the hexane extract of *B. sulphurea* showed an EC_{50} value of 84.26 µg/mL (95% Confidence Interval (95% CI) 81.23-87.56µg/mL) (moderately active), while hexane extract of *P. neochilus* showed a value of 46.32µg/mL (95% CI-38.42-57.54µg/mL), considerate as moderate activity. In 48h, both extracts were considerate active, with EC_{50} values of 40.37 (95% CI-29.64-55.64µg/mL) and 43.20µg/mL (95% CI-39.57-50.87µg/mL) for hexane extracts of *B. sulphurea* and *P. neochilus*, respectively. Amphotericin B showed an EC_{50} value of 0.011µg/mL at 24 (95% CI-0.0058-0.019µg/mL) and 0.012µg/mL 48h (95% CI-0.0063-0.022µg/mL) (Table 3).

According to Tempone and co-workers, the methanol extracts of *Aristolochia cymbifera*, *Plectranthus amboinicus*, *Plectranthus barbatus* and *Lippia alba* showed EC_{50} values of 45.14; 89.17; 54.46 and 62.67 µg/mL, respectively against *L. (L.) chagasi* at 48h.[28] In addition, the methanol extract of *P. neochilus* was inactive against *Leishmania* species.[28] Another study, Antinarelli and co-workers demonstrated that the methanolic extract of *P. neochilus* showed active against *L. (L.) chagasi*, but it did not show activity against *L.(L.) amazonensis*, *L.(L.) major* and *Leishmania (Viannia) braziliensi*.[20] Despite these results, it is interesting to notice that extracts methanolic and/or hexanic from the genus *Plectranthus* has showed values of EC_{50} considered active or moderately active, because of that this genus should be better investigated about its antiparasitic activity.

Although promastigotes can be used for fast screenings of potential compounds, the clinically relevant form of the parasite is the amastigote form, which shows metabolic differences from the extracellular forms.[29,30] When the hexane extracts were evaluated against intracellular amastigotes, it was observed that after 48h of incubation with *B. sulphurea* the EC_{50} value were 371µg/mL (95% CI-254-487µg/mL) and when incubated with *P. neochilus* the EC_{50} were 141µg/mL (95% CI-90.09-192.4µg/mL), demonstrating that the hexane extracts have no activity against these parasitic forms. The EC_{50} obtained after incubation with amphotericin B were 0.095 µg/mL (95% CI-0.07-0.12µg/mL) (Table 3).

An important criterion in the research of active compounds and extracts is to determine the absence of toxic effects on the host cells. In this study, the toxicity of hexane extracts of *B. sulphurea* and *P. neochilus* was evaluated on peritoneal macrophage. The hexane extract of *B. sulphurea* showed CC_{50} value (50% cytotoxic concentration) of 103.9µg/mL and 80.30µg/mL (95% CI-99.46-128.98µg/mL and 73.15-88.15µg/mL, respectively) after 24 and 48h of incubation. Moreover, the hexane extract of *P. neochilus* showed CC_{50} value of 66.95µg/mL and 34.39µg/mL (95% CI-59.55-75.27µg/mL and 28.21-41.93) at 24 and 48h, respectively. Amphotericin B was more toxic to mammalian cell than hexane extracts, showing CC_{50} values of 4.29 and 2.98µg/mL (95% CI-3.25-6.77µg/mL and 1.41-3.87) in 24 and 48h, respectively (Table 4). However, the methanolic extract of *P. neochilus* presented a CC_{50} value of 111µg/mL when incubated with peritoneal macrophages after 72hours of incubation.[20]

According to one study, a selectivity index (SI) value greater than 10 can suggest better safety of the product for use in mammals.[31] The hexane extract of *B. sulphurea* showed a SI of 1.23 and 1.98 in 24h and 48h, respectively. In addition, the hexane extract of *P. neochilus* showed a SI of 1.44 and 0.79 in 24h and 48h, respectively, Despite the low SI, the hexane extracts showed values close to those obtained by amphotericin B, with a SI of 390 and 248.3 in 24h and 48 h, respectively (Table 4).

One of the main treatments for leishmaniasis is the use of pentavalent antimony as a first-line and amphotericin B as a second-line. One of the biggest problems associated with this regimen is the need for intravenous or intramuscular administration for both medicines.[5] Thus, there is concern about the effect of these or other proposed compounds or extracts with antileishmanial activity with respect to hemolytic activity. In determining the hemolytic activity of the hexane extracts from *B. sulphurea* and *P. neochilus*, we observed that the extracts showed no hemolytic activity at the concentrations evaluated, and the HC_{50} values were higher than 100µg/mL for *B. sulphurea* and *P. neochilus* (Table 4).

Recently, the chemical composition of the hexane extract of *P. neochilus* was determined by gas chromatography–mass spectrometry (GC-MS). A total of thirteen compounds were detected, with predominance of sesquiterpenes (88.8%). The major constituents were identified as being spathulenol (46.1%), *trans*-caryophyllene (19.0 %), caryophyllene oxide (10.7%) and germacrene D (7.8%).[13] According toAcebey and co-workers, the sesquiterpene spathulenol, isolated from an ethyl acetate extract of the bark of *Hedyosmum angustifolium,* did not show activity against *L. (L.) amazonensis* and *L. (L.) infantum in vitro*.[32] This could indicate that some other compounds may be responsible for the activity of the extract. The other major constituents were already described in extracts or essential oils, but their isolated activity was not been described.[33,34] Thus, the activity of the isolates should be better investigated against parasites from genus *Leishmania* sp.

In our study, a total of fifteen compounds were detected on the *B. sulphurea* hexane extract and the major constituents were identified as being 2,4-bis(dimethylbenzyl)phenol (54.1%), (3-tert-butyl-5-hydroxymethyl-cyclohex-2-enyl)-methanol (8.1%), pulegol (7.3%) and (2-Dodecen-1-yl-succinic anhydride (7.2%) (Table 5). This is the first study describing the effects of the major constituents of *B. sulphurea* extract on protozoa.

Table 3 Effective concentration of 50% against promastigotes and amastigotes after 24 and 48h of incubation with the extracts *B. sulphurea*, *P. neochilus* and amphotericin B

Compound	EC_{50} values against promastigotes(μg/mL)(95% CI)		EC_{50} values against amastigotes(μg/mL)(95% CI)
	24 h	48h	48h
B. sulphurea	84.26(81.23-87.56)	40.37(29.64-55.64)	371.00(254.00-487.00)
P. neochilus	46.32(38.42-57.54)	43.20(36.57-50.87)	141.00(90.09-192.4)
Amphotericin B	0.011(0.0058-0.019)	0.012(0.0063-0.022)	0.095(0.07-0.012)

CI: Confidence Interval of 95%

Table 4 Cytotoxic Concentration of 50%, Hemolytic Concentration of 50% and Selectivity Index obtained after 24 and 48 h of incubation with the extracts *B. sulphurea*, *P. neochilus* and amphotericin B

Compound	CC_{50} values against murine macrophages(μg/mL)(95% CI)		HC_{50}(μg/mL) (95% CI)	Selectivity Index(SI)*	
	24 h	48h	48h	24h	48h
B. sulphurea	103.9(99.46-128.98)	80.30(73.15-88.15)	>100	1.23	1.98
P. neochilus	66.95(59.55-75.27)	34.39(28.21-41.93)	>100	1.44	0.79
Amphotericin B	4.29(3.25-6.77)	2.98(1.41-3.87)	40.42(36.69-44.15)	390	248.3

CI: Confidence Interval of 95%

*Value obtained using the EC_{50} from promastigotes assays as described by Londero and co-workers.[19]

Table 5 Chemical composition of the hexane extract of *Bidens sulphurea*

Compound	RT(min)	RI	%RA
3,3-Dimethoxy-2-butanone	3.23	826	0.8
1-Methyl-2-(3-methylpentyl)cyclopropane	5.86	914	0.7
2-ethyl-1,3-Dioxolane-4-methanol	9.45	1036	1.2
Pulegol	13.25	1141	7.6
Citronellyl propionate	25.11	1447	0.4
(3-tert-Butyl-5-hydroxymethyl-cyclohex-2-enyl)-methanol	31.16	1614	8.4
10,10-Dimethoxy-3,7-dimethyl-deca-2,6-dien-1-ol	34.58	1714	0.2
Palmitaldehyde	37.96	1818	4.9
Neophytadiene	38.31	1829	0.9
1-methyl-spiro[2.3]hexane-5-carboxylic acid menthyl ester	38.70	1842	0.2
Phytone	38.88	1847	4.5
E-phytol	41.83	1946	4.2
2-Dodecen-1-yl-succinic anhydride	42.36	1961	7.5
Oxalic acid. docecyl isohexyl ester	48.51	2276	0.8
2.4-Bis(1-methyl-1-phenylethyl)phenol	51.58	2491	56.3
Total			98.7

RT, retention time(min); RA, relative content calculated from the peak area relative to the total peak area in the GC-FID chromatogram; values are averages of three replicates; Compound identification, Comparison of the SI(Similarity Index) and retention index(RI) with those from mass spectra Wiley 7, NIST 08, and FFNSC 1.2 spectral libraries.

Conclusion

The hexane extracts were evaluated *in vitro* in relation to the protozoan *L. (L.) amazonensis* and the results demonstrate a moderate leishmanicidal activity after 24 and 48 h of incubation. Despite the unsatisfactory results against amastigotes forms, this study shows extracts obtained from botanical sources merit further study for their leishmanicidal properties.

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Conflicts of interest

The authors have declared that there are no conflicts of interest.

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