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# Antileishmanial activity of some Brazilian plants, with particular reference to *Casearia sylvestris*

**LUCIANA M.R. ANTINARELLI[1], NÍCOLAS C. PINTO[2], ELITA SCIO[2] and ELAINE S. COIMBRA[1]**

[1]Departamento de Parasitologia, Microbiologia e Imunologia/ICB,
Universidade Federal de Juiz de Fora, Campus Universitário,
Bairro São Pedro, 36036-900 Juiz de Fora, MG, Brasil
[2]Laboratório de Produtos Naturais Bioativos, Departamento de Bioquímica/ICB,
Universidade Federal de Juiz de Fora, Campus Universitário,
Bairro São Pedro, 36036-900 Juiz de Fora, MG, Brasil

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**ABSTRACT**

Leishmaniasis is a complex of diseases caused by *Leishmania* protozoa which treatment is restricted to a limited number of drugs that exhibit high toxicity, collateral effects and are often costly. There are a variety of tropical plants distributed in Brazil, and for many poor people the therapy for several diseases is based mainly on the use of traditional herbal remedies. In this work, the cytotoxic activity of 17 plant methanol extracts was evaluated on several *Leishmania* species and murine macrophages. Among them, the extract of *Casearia sylvestris, Piptocarpha macropoda*, *Trembleya parviflora, Samanea tubulosa and Plectranthus neochilus* showed a promissing leishmanicidal activity, exhibiting $IC_{50}$ values below of 20 µg/mL against at least one species of *Leishmania*. *Casearia sylvestris* showed the most expressive activity against all promastigote forms of *Leishmania* species ($IC_{50}$ values of 5.4 µg/mL, 5.0 µg/mL, 8.5 µg/mL and 7.7 µg/mL for *L. amazonensis*, *L. braziliensis*, *L. chagasi* and *L. major*, respectively), being more effective than the reference drug miltefosine. In spite of the cytotoxic effect on macrophages ($CC_{50}$ value of 5.2 µg/mL), *C. sylvestris* exhibited a strong inhibition against intracellular amastigotes of *L. braziliensis* ($IC_{50}$ value of 1.3 µg/mL). Further studies, including bio-guided fractionation will be conducted to identify the active compounds.

**Key words:** Brazil, *Casearia sylvestris*, leishmanicidal activity, medicinal plants, natural products.

## INTRODUCTION

Leishmaniasis is a vector borne disease caused by protozoa parasites of the genus *Leishmania* (WHO 2010). According to the World Health Organization, leishmaniasis is considered a major Neglected Tropical Disease with expressive economic, social, and political impacts. Leishmaniasis is distributed in more than 90 countries, with an annual incidence of 1.5 to 2.0 million cases, and 350 million people under the risk of infection (WHO 2013).

Leishmaniasis comprises a complex of clinical manifestations including ulcerative skin lesions, destructive mucosal inflammation, and disseminated visceral infection in its most severe form (Murray

Correspondence to: Elaine Soares Coimbra
E-mail: elaine.coimbra@ufjf.edu.br

et al. 2005). These clinical manifestations occur due to a complex interaction between the parasite and the immune response of the mammalian host.

Current chemotherapeutic agents for the treatment of all clinical manifestations of leishmaniasis are pentavalent antimonials compounds (e.g., sodium stibogluconate and meglumine antimoniate) and amphotericin B, which unfortunately are considerably toxic (Croft and Olliaro 2011, Tempone et al. 2011, Singh and Sundar 2012). Furthermore, these drugs exhibit several limitations, including high cost and the need for daily parenteral administration (Singh and Sundar 2012). More recently the antitumor drug miltefosine was introduced as the first and still the only oral therapeutic option for the treatment of visceral leishmaniasis in India (Sundar et al. 2002, Dorlo et al. 2012).

There are a variety of tropical plants distributed in Brazil and for many poor people the therapy for several diseases is based mainly on the use of traditional herbal remedies (Lorenzi and Matos 2002). In most cases, these plants are used without any scientific base. In the last years, several works showed the leishmanicidal effect of some Brazilian plant extracts or essential oils (Santin et al. 2009, Albernaz et al. 2010, Alviano et al. 2012, Brito et al. 2013). In a previous work, we reported the antileishmanial activity of extracts of 20 plants from the Brazilian flora (Braga et al. 2007). Due to our continuous search for new alternatives for the treatment of leishmaniasis, this study aimed to investigated the leishmanicidal activity of 17 plant methanolic extracts against four *Leishmania* species and murine macrophages.

## MATERIALS AND METHODS

### Plant Material

Specimens of 17 species were collected in Juiz de Fora, Minas Gerais, Brazil. A voucher specimen was deposited at the Herbarium Leopoldo Krieger (CESJ) of the Federal University of Juiz de Fora. Table I shows the botanical name, local name, voucher specimen number and the popular uses of the plants tested.

### Preparation of Plant Extracts

The dried parts of the plants (50 g each) were powdered and macerated with methanol (3x 200 ml) for five days at room temperature. After evaporation of the solvent under reduced pressure, the respective methanolic extracts were obtained. All extracts were kept dried in tightly stoppered bottles under refrigeration (4 °C) until used for the biological tests.

### Antileishmanial Activity

#### *Parasites*

Four *Leishmania* species were used for *in vitro* screening: *L. amazonensis* (IFLA/Br/67/PH8), *L. major* (MRHO/SU/59/P), *L. braziliensis* (MHOM/Br/75/M2903) and *L. chagasi* (MHOM/Br/74/PP75). Promastigotes of *L. amazonensis* were cultured in Warren's medium [brain heart infusion- BHI (Himedia, Mumbai, Indian), plus hemin and folic acid (Sigma Chemical Co, St. Louis, MO, USA)], promastigotes of *L. major* and *L. braziliensis* were maintained in Medium BHI, and promastigotes of *L. chagasi* were maintained in Medium 199 (Cultilab, Campinas, São Paulo, Brazil), both supplemented with 10% fetal bovine serum (FBS; Cultilab, Campinas, São Paulo, Brazil) at 25 °C.

Antipromastigote assay: The antileishmanial activity was determined by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) method based on tetrazolium salt reduction by mitochondrial dehydrogenases (Mossman 1983). Briefly, promastigotes from a logarithmic phase culture were suspended to yield 2 millions of cells/mL (*L. amazonensis*) or 3 millions of cells/mL (*L. chagasi*, *L. braziliensis* and *L. major*) after Neubauer chamber counting.

The screening was performed in 96-well microtiter plates maintained at 25 °C. The analyses were made in duplicate. The extracts were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) as a stock solution. Parasites were exposed to increasing concentration of the extracts solutions (at minimum five serial dilutions) for 72h at 25 °C. Controls containing 0.5% DMSO and medium alone were also included. The viability of promastigotes was obtained by measuring the absorbance at 570 nm (Multiskan MS microplate reader, LabSystems Oy, Helsink, Finland). Amphotericin B (Cristalia, São Paulo, Brazil) and miltefosine (Cayman Chemical Company, Michigan, USA) were used as reference drugs.

Antiamastigote assay: Inflammatory macrophages were obtained from the peritoneal cavity of BALB/c mice previously inoculated with 3% thioglycollate medium (Sigma Chemical Co; St. Louis, MO, USA). After 72h, the peritoneal exudate was collected by washing with cold Hank´s Balanced Sal Solution (Sigma Chemical Co; St. Louis, MO, USA) (Silva et al. 2012). Briefly, peritoneal macrophages added at $2x10^6$ cells/mL to coverslips (13 mm diameter) previously arranged in a 24-well plate in RPMI 1640 medium (Cultilab, Campinas, São Paulo, Brazil) supplemented with 10% inactivated FBS, and allowed to adhere at 37 °C in 5% $CO_2$. Adherent macrophages were infected with *L. braziliensis* (MRHO/BR/75/M2903) or *L. amazonensis* (IFLA/Br/67/PH8*)* promastigotes in the stationary growth phase using a 1:10 ratio at 33 °C for 4h. After this time, the no phagocytosed promastigotes were removed washing twice in sterile Phosphate Buffered Saline (PBS) and the test samples were added at nontoxic concentrations to the macrophages (5.0, 1.0 and 0.1 µg/mL) and maintained at 33 ºC 5% $CO_2$ for 72h. Cells were washed, fixed with absolute ethanol, and stained with Giemsa. Cells were then dehydrated in acetone followed by a gradient acetone-xylol (9:1; 1:1; 1:9) and finally xylol. The slides were mounted with Canada balsam for parasite counting at a optical microscopy (1000x magnification). At least 100 infected cells were counted and the results were expressed as Index infection, obtained multiplying the percentage of infected cells by mean number of amastigotes per cell. Miltefosine was used as reference drug. All procedures were performed in agreement with the Ethical Principles in Animal Research and according to protocols approved by the "Pró-Reitoria de Pesquisa/UFJF - Ethical Committee for Animal Research" (#016/2012-CEEA).

### Cytotoxicity on Mammalian Cells

Murine macrophages were obtained and cultured as described before. Briefly, the inflammatory peritoneal macrophages were used for cytotoxicity assay in a concentration of $2x10^6$ cells/mL in 96-well culture plates in RPMI 1640 medium supplemented with 10% inactivated FBS, at 37 °C and 5% $CO_2$ atmosphere. After 24h, the adherent macrophages were incubated with the extracts in a serial dilution, in duplicate at each concentration for 72h at 37 °C and 5% $CO_2$ atmosphere. The viability of the macrophages was determined with the MTT assay using a multiwall scanning spectrophotometer (Multiskan EX microplate reader), as described above, and was confirmed by comparing the morphology with the control (macrophages incubated in RPMI 1640 medium supplemented with 10% inactivated FBS), via light microscopy. Dose response curves were plotted (values expressed as percentage of control optical density) and the values were expressed as $CC_{50}$ values (50% cytotoxicity concentration). All procedures were performed in agreement with the Ethical Principles in Animal Research and according to protocols approved by the "Pró-Reitoria de Pesquisa/UFJF - Ethical Committee for Animal Research" (#015/2012-CEEA).

### Statistical Analysis

For *Leishmania* and murine macrophages assays, the $IC_{50}$ or $CC_{50}$ values, respectively, were carried out and the 95% confidence intervals were included,

calculated by Litchtfiet and Wilcoxon method using the Probit analysis, and the graphs were plotted by the program GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA). One-way ANOVA followed by Dunnett post test were used. Differences were regarded as significant when $p < 0.0001$ (***) and $p < 0.001$ (**).

## RESULTS AND DISCUSSION

In the present work, 17 methanolic plant extracts were evaluated for antileishmanial activity against promastigote forms of *Leishmania* and citotoxicity against murine macrophages. In addition the efficacy of the most promising plant extract against intracellular amastigotes of *Leishmania* sp was also evaluated. Table I shows the botanical name, local name, voucher specimen number and popular uses of the tested plants.

Table II shows the extracts effect on promastigote forms of different *Leishmania* species: three from the New World (*L. braziliensis, L. chagasi* and *L. amazonensis*) and one species from the Old World (*L. major*) (Santos et al. 2008). Due to this, a different sensitivity of theses parasites to the tested extracts was expected. Previous *in vitro* studies have also shown differences in sensitivity of *Leishmania* species to different reference drugs, including pentavalent antimonials, amphotericin B (Minodier and Parola 2007), miltefosine (De Morais-Teixeira et al. 2011) and crude or purified plant extracts (Braga et al. 2007, Fabri et al. 2012a, b). Among the 17 methanolic extracts tested, 11 showed leishmanicidal activity against at least one promastigote forms of *Leishmania* species with $IC_{50}$ values ranging from 5.0 to 88.3 µg/mL. Interestingly, in a general biological evaluation, the tested extracts were more active against *L. braziliensis,* responsible for cutaneous and mucocutaneous leishmaniasis; and against *L. chagasi* promastigote forms, the causative agent of fatal visceral leishmaniasis in the American continent (Cruz et al. 2009). Promastigotes of *L. amazonensis* were less sensitive to the extracts assayed.

Phytochemical screening of most of the extracts assayed in this study was previously reported and a variety of secondary metabolites was related to these extracts such as alkaloids, triterpens, sterols, tannins, saponins and flavonoids (Scio et al. 2012). In this work, among the extracts assayed, *Casearia sylvestris, Piptocarpha macropoda*, *Trembleya parviflora, Samanea tubulosa and Plectranthus neochilus* showed strong leishmanicidal activity, exhibiting $IC_{50}$ values below of 20 µg/mL, for at least one species of *Leishmania*. Biological activity of *C. sylvestris, P. macropoda* and *S. tubulosa* could be due to the presence of secondary metabolites such as alkaloids, sterols, tannins and flavonoids (Scio et al. 2012). *C. sylvestris* showed remarkable activity against promastigotes of all *Leishmania* species, with $IC_{50}$ below 10 µg/mL, being more effective than the reference drug miltefosine (Table II), which is currently the only oral drug available for the treatment of visceral leishmaniasis (De Morais-Teixeira et al. 2011). So, this plant extract was selected for further analysis against intracellular amastigote forms, since this form of parasite is found in the mammalian host and is important for human disease. Amastigotes of *L. braziliensis and L. amazonensis* were used as model in order to determine the antileishmanial property of *C. sylvestris* against intracellular forms of parasite (Fig. 1 and Table III). The effectiveness of *C. sylvestris extract* on the infection rate of the infected macrophages and the intracellular replication of the amastigote forms was determined by the infection index. Figure 1 shows the dose-dependent effect of this extract on amastigotes of *L. amazonensis* and *L. braziliensis*. Amastigotes of *L. braziliensis* were most sensitive to the treatment with *C. sylvestris* extract and the inhibition of infection index was 62.1%; 64.0% and 65.0% (0.5 µg/mL; 1.0 µg/mL and 5.0 µg/mL, respectively). Furthermore, $IC_{50}$ value for this extract against *L. braziliensis* amastigotes was very low (1.3 µg/mL), and highlighted the activity of extract against intracellular form of the

**TABLE I**
**Ethnomedical data on medicinal plants.**

| Family | Botanical name [Voucher number] | Common name | Parts used[a] | Ethnomedical uses | References |
|---|---|---|---|---|---|
| Asteraceae | *Achillea millefolium* L. [CESJ 46087] | Novalgina, erva-de-carpinteiro, aquiléia, milefólio | L | Fever, headaches and general aches,colds, indigestion | Lorenzi and Matos 2002 |
| | *Anthemis cotula* L. [CESJ 48584] | Camomila-do-campo | L | Fever, gastrointestinal disorders, dysenteria, gouty arthritis | Corrêa and Penna 1984 |
| | *Bidens segetum* Mart. ex Colla [CESJ 47437] | Picão-do-mato | L | No use reported | |
| | *Piptocarpha macropoda* (DC.) Baker [CESJ 49448] | | L | No use reported | |
| | *Vernonanthura divaricata* (Spreng.) H. Rob. [CESJ 49450] | Cambará-açu | L | No use reported | |
| Euphorbiaceae | *Alchornea triplinervia* (Spreng.) Müll. Arg. [CESJ 49442] | Tapiá-vermelho, tapiágua-çu-branco, pau-óleo | L | Gastric disturbances | Lima et al. 2011 |
| Fabaceae | *Chamaecrista desvauxii* (Collad.) Killip [CESJ 23372] | Sene, acácia, carquejado-tabuleiro, flor-de-lilás, capim reis | L | Wounds in the uterus, worms, bowel, arthritis | Moreira and Guarim-Neto 2009 |
| | *Samanea tubulosa* (Benth.) Barneby & J.W. Grimes [CESJ 49743] | Amendoim-de-veado, árvore-da-chuva e pau-de-cangalha | L | Eye disorders | Hajdu and Hohmann 2012 |
| Flacourtiaceae | *Casearia sylvestris Sw.* [CESJ 49218] | Guaçatonga, bugre-branco, café-bravo, café-de-frade | L | Burns, cutaneous injuries, herpes, tonic, depurative, rheumatism, inflammation, analgesic, hemostatic, gastritis | Moreira and Guarim-Neto 2009 |
| Lamiaceae | *Leonurus sibiricus* L. [CESJ 46176] | Macaé | L | Intestinal problems, cough, bronchitis, gastric defect, rheumatism, fever diseases of eyes | Lorenzi and Matos 2002 |
| | *Ocimum basilicum* L. [CESJ 46161] | Manjericão, alfavaca | L | Gastrointestinal disorders, fever, digestive, bacterial infections, parasitosis | Lorenzi and Matos 2002 |
| | *Plectranthus neochilus* Schltr. [CESJ 46580] | Boldo | L | Treatment of respiratory infections or related symptoms, gastrointestinal disorders, skin infections, hepatic insufficiency | Caixeta et al. 2011 |
| Melastomataceae | *Trembleya parviflora* (D. Don) Cogn. [CESJ 49219] | Manacá | L | No use reported | |
| Myrtaceae | *Syzygium malaccense* (L.) Merr. and L.M.Perry [CESJ 46600] | Jambo | L | Inflammation | Dustan et al. 1997 |
| Poaceae | *Cymbopogon citratus* (DC) Stapf. [CESJ 46582] | Capim-cheiroso, erva-cidreira, Capim-cidreira, capim-limão | AP | Calmative, gastrointestinal disorders, infectious diseases, colic treatment, anxiety | Albuquerque 1989 |
| Tropaeolaceae | *Tropaeolum majus* L. [CESJ 46586] | Capuchinha, chaguinha, alcaparra-de- pobre, chagas, mastruço-do-peru | L | Scurvy, sepses, expectorant, urinary, gastrointestinal and dermatological disinfectant | Lorenzi and Matos 2002 |
| Verbenaceae | *Lippia rubella* (Moldenke) T.R.S. Silva & Salimena [CESJ 46178] | | AP | No use reported | |

[a]AP, aerial parts; L, leaves.

**TABLE II**
**Effect of plant extracts in promastigotes of *Leishmania* species and murine macrophages.**

| Plants | Parts of plants | Antileishmanial activity[a] $IC_{50}$ (µg/mL) (95% C.I.) | | | | Macrophages $CC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|
| | | *L.a*[1] | *L.m*[2] | *L.b*[3] | *L.c*[4] | |
| *Casearia cf. sylvestris* | Leaves | 5.4 (4.3-6.8) | 7.7 (6.7-8.8) | 5.0 (4.5-5.7) | 8.5 (5.8-12.4) | 5.2 (4.0-6.4) |
| *Piptocarpha cf. macropoda* | Leaves | 10.3 (9.0-11.7) | 9.2 (7.5-11.2) | 7.4 (5.7-9.5) | 26.3 (21.8-31.6) | 4.1 (3.6-4.8) |
| *Leonorus sibiricus* | Leaves | 53.2 (47.4-59.7) | 53.6 (45.5-63.1) | 46.9 (42.4-51.9) | > 108.0 | > 111.0 |
| *Cymbopogon citratus* | Aerial Parts | 33.4 (29.4-37.9) | 20.6 (17.8-23.6) | 51.1 (46.2-56.3) | 34.6 (23.2-51.8) | > 111.0 |
| *Trembleya parviflora* | Leaves | 19.7 (17.0-22.7) | > 108.0 | 17.1 (14.0-20.9) | 64.0 (54.6-75.2) | 83.8 (67.0-104.8) |
| *Vernonanthura divaricata* | Leaves | 37.1 (31.7-43.4) | > 108.0 | 28.0 (24.4-32.3) | 61.8 (49.2-77.7) | > 111.0 |
| *Alchornea triplinervia* | Leaves | > 108.0 | 36.3 (31.6-41.7) | 67.1 (59.7-75.4) | > 108.0 | 102.5 (80.5-130.6) |
| *Samanea tubulosa* | Leaves | > 108.0 | > 108.0 | > 108.0 | 19.0 (16.0-22.6) | 86.6 (70.4-106.5) |
| *Tropaeolum majus* | Leaves | > 108.0 | 61.5 (50.5-74.9) | > 108.0 | > 108.0 | > 111.0 |
| *Plectranthus neochilus* | Leaves | > 108.0 | > 108.0 | > 108.0 | 14.0 (9.5-20.7) | > 111.0 |
| Achillea millefolium | Leaves | > 108.0 | 30.8 (26.8-35.5) | > 108.0 | > 108.0 | > 111.0 |
| *Lippia rubella* | Aerial Parts | > 108.0 | > 108.0 | > 108.0 | 88.3 (70.8-110.9) | > 111.0 |
| *Ocimum basilicum* | Leaves | > 108.0 | > 108.0 | > 108.0 | > 108.0 | > 111.0 |
| Syzygium malaccense | Leaves | > 108.0 | > 108.0 | > 108.0 | > 108.0 | > 111.0 |
| *Bidens segetum* | Leaves | > 108.0 | > 108.0 | > 108.0 | > 108.0 | > 111.0 |
| *Anthemis cotula* | Leaves | > 108.0 | > 108.0 | > 108.0 | > 108.0 | > 111.0 |
| *Chamaecrista desvauxii* | Leaves | > 108.0 | > 108.0 | > 108.0 | > 108.0 | > 111.0 |
| AmB | | 0.11 (0.09-0.12) | 0.10 (0.09-0.11) | 0.12 (0.09-0.14) | 0.05 (0.05-0.06) | 122.7 (98.4-153.1) |
| Miltefosine | | 11.8 (10.0-14.0) | 8.8 (7.6-10.1) | 10.4 (9.0-12.1) | 8.3 (7.2-9.5) | 49.4 (43.8-55.7) |

[a]Data are $IC_{50}$ values in µg/mL and 95% confidence intervals are in brackets. [1]L.a.= *Leishmania amazonensis*; [2]L.m.= *L. major*; [3]L. b.= *L. braziliensis* and [4]L.c.= *L. chagasi*. These data represent the average of three independent experiments. AmB (amphotericin B) and miltefosine were used as reference drug. The highest concentration used of DMSO was 0.1% (v/v), which is not toxic to the parasites.

**TABLE III**
**Effect of *Casearia cf. sylvestris* extract on *Leishmania braziliensis*, specificity and selectivity index.**

| | Promastigotes $IC_{50}$ (µg/mL) | Amastigotes $IC_{50}$ (µg/mL) | Macrophages $CC_{50}$ (µg/mL) | [a]$SI_{PRO}$ | [b]$SI_{AMA}$ | [c]$SP_{PRO/AMA}$ |
|---|---|---|---|---|---|---|
| *Casearia sylvestris* | 5.0 | 1.3 | 5.2 | 1.0 | 4.0 | 3.8 |
| Miltefosine | 10.4 | 2.4 | 49.4 | 4.7 | 20.6 | 4.3 |

$IC_{50}$ (Inhibitory concentration at 50% inhibition). $CC_{50}$ (Cytotoxic concentration at 50% inhibition). [a]SI: selectivity index ($CC_{50}$ of macrophages /$IC_{50}$ of promastigotes). [b]SI: selectivity index ($CC_{50}$ of macrophages /$IC_{50}$ of amastigotes). [c]SP: specificity index (ratio between promastigotes $IC_{50}$ and intracellular amastigotes $IC_{50}$). Miltefosine was used as reference drug.

parasite (Table III). In addition, Table III furnishes information about the selectivity and specificity of *C. sylvestris* extract. Regarding selectivity, when this value is greater than 1, the extract is more selective against the parasite than macrophages, otherwise the extract is more toxic for macrophages (Tempone et al. 2010). As can be observed, besides the toxic effect on macrophages, when the extract

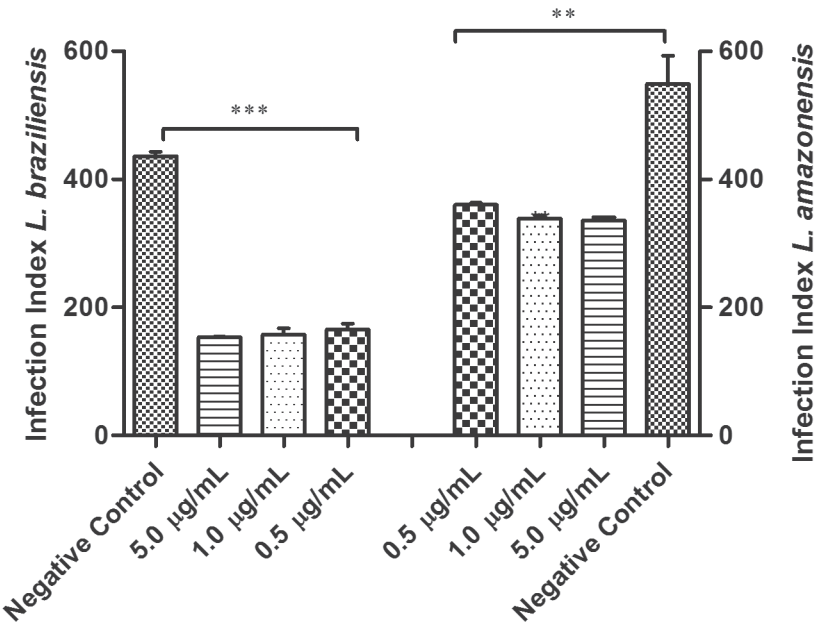


**Figure 1 -** Effect of *Casearia sylvestris* extract on *L. braziliensis* and *L. amazonensis* infected macrophages. Peritoneal macrophages previously infected with *L. braziliensis* or *L. amazonensis* promastigotes in the stationary growth phase were exposed to the compounds for 72h and the results were expressed as the infection index (percentage of infected macrophages x mean number of parasites per cells). The test considered the mean of two assays performed in duplicate. Differences were regarded as significant when $p < 0.0001$ (***) and $p < 0.001$ (**).

was assayed against amastigotes, it was much more destructive to parasite than to the host cells, being four-fold more selective against the parasite. De Muylder et al. (2011) established a cut-off regarding the specificity of compounds between these two stages of the parasite *Leishmania* sp. Specificity value > 2 was the cut-off point chosen to define a compound as being more active against the intracellular amastigote stage; while a specificity value < 0.4 indicated a more active compound against promastigotes; compounds with specificity values between 0.4 and 2 were considered as being active against both stages. It is interesting to point out that the extract was more specific for intracellular form of *L. braziliensis* (specificity index= 3.8) and this result reinforces the potential leishmanicidal activity of *C. sylvestris*.

Previously, Mesquita et al. (2005) demonstrated the antileishmanial potential of *C. sylvestris* against promastigotes forms of *L. donovani*, of which extracts displayed a significant activity with $IC_{50}$ values ranging from 0.1 to 4.9 µg/mL depending on the plant parts used.

Regarding the cytotoxicity effects on macrophages, *C. sylvestris* and *P. macropoda* were the most cytotoxic to mammalian cells (Table II). Cytotoxicity tests with natural products are important because they represent a potential source for the isolation of compounds for the development of new antiprotozoal agents (Brenzan et al. 2007).

*Casearia sylvestris*, which is popularly named "erva-de-lagarto", "língua-de-tiú", cafezinho-do-mato", "corta-lengua", is distributed throughout Brazil (Ferreira el al. 2011). Pharmacological studies have highlighted the promising bioactive potential of extracts and compounds isolated from *C. sylvestris*, especially its antitumor (Ferreira et al. 2010), anti-ulcer (Da Silva et al. 2008), antifungal (Alves et al. 2000), antibacterial (Chiappeta et al. 1983), antioxidant (Menezes et al. 2004) and

molluscicide (Alves et al. 2000) activities. Regarding antiprotozoal properties of *C. sylvestris*, some authors have reported activity against *Trypanosoma cruzi* (Mesquita et al. 2005). Phytochemical studies have attributed the high antitumor activity of *C. sylvestris* to the numerous secondary metabolites, especially diterpenes, which are likely based on its larger hydrophobicity and facility to penetrate across cell membrane and interact with intracellular targets (Ferreira et al. 2010). Secundary metabolites of *C. sylvestris*, such as alkaloids, sterols, saponins, tannins and flavonoids may be associated with significant activity against *Leishmania* sp. However more investigations are necessary to determine the activity of each extract component separately and in combination to ensure whether they act alone or synergistically.

## CONCLUSION

In conclusion, the methanolic extracts of *Casearia sylvestris* and *Piptocarpha macropoda* showed significant leishmanicidal activity, specially *C. sylvestris* extract, which may be a potential source of active compounds for the development of novel therapeutic agents to treat leishmaniasis.

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## RESUMO

As leishmanioses são um complexo de doenças causadas por protozoários *Leishmania*, cujo tratamento é restrito a um número limitado de fármacos que apresentam toxicidade elevada, efeitos colaterais e geralmente custos elevados. Existe uma enorme variedade de plantas tropicais distribuídas no Brasil e para muitas pessoas pobres a terapia para várias doenças baseia-se principalmente no uso de remédios tradicionais obtidos de plantas. Neste trabalho, a atividade citotóxica de 17 extratos metanólicos de plantas foi avaliada em várias espécies de *Leishmania* e em macrófagos murinos. Dentre eles, os extratos de *Casearia sylvestris*, *Piptocarpha macropoda*, *Trembleya parviflora*, *Samanea Tubulosa* e *Plectranthus neochilus* mostraram atividade leishmanicida promissora, exibindo valores de $CI_{50}$ abaixo de 20 µg/mL em pelo menos uma das espécies de *Leishmania*. *Casearia sylvestris* apresentou a atividade mais expressiva em todas as formas promastigotas de espécies de *Leishmania* (valores de $CI_{50}$ de 5,4 µg/mL, 5,0 µg/mL, 8,5 µg/mL and 7,7 µg/mL em *L. amazonensis*, *L. braziliensis*, *L. chagasi* e *L. major*, respectivamente), sendo mais eficaz que o fármaco de referência miltefosina. Apesar do efeito citotóxico em macrófagos (valor de $CC_{50}$ de 5,2 µg/mL), *C. sylvestris* exibiu uma forte inibição em formas amastigotas de *L. braziliensis* (valor de $CI_{50}$ de 1,3 µg/mL). Mais estudos, incluindo fracionamento bio-guiado, serão realizados para identificar os compostos ativos.

**Palavras-chave**: Brasil, *Casearia sylvestris*, atividade leishmanicida, plantas medicinais, produtos naturais.

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