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Leishmanicidal activity of different parts from *Arrabidaea brachypoda (DC.)* Bureau (Bignoniaceae)

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Abstract: Infections by protozoans of the genus *Leishmania* are the major worldwide health problem, with high endemicity in developing countries. The drugs of choice for the treatment of leishmaniasis are the pentavalent antimonials, which exert renal and cardiac toxicity. Thus, there is a strong need for safer and more effective treatments against leishmaniasis. The present study was designated to evaluate, by a bioguided assay, the leishmanicidal activity of hidro-ethanolic extracts from different parts (leves, caule and root) of one same plant the *Arrabidaea brachypoda* (DC.) Bureau. Its has been used to relieve general pain, painful joints and kidney stones in Brazilian folk medicine. Nevertheless, scientific information regarding this species is scarce; there are no reports related to its possible leishmanicidal activity. The leves extract presented the best activity on promastigotes forms of *Leishmania (L.) amazonensis*, when compared to the other extracts. It showed significant activity on *Leishmania (L.) amazonensis*. Our results are promising, showing that these compounds are biologically active on *Leishmania (L.) amazonensis*.

Key-words: *Arrabidaea brachypoda. Leishmania.* Promastigotes

1. Introduction

The Cerrado is reported as the most diverse savanna in the world, where more than 12,000 species of vascular plants have been recorded (Mendonça et al., 1998), with a high proportion of endemic species, estimated at 44% (Klink and Machado, 2005).

Many species are exclusive to this ecological region, and thus the richness and specificity of habitats reflect the high proportion of rare plants found in this biome (Ratter et al., 2003).

Leishmaniasis are a poverty related, emerging disease caused by protozoan parasites of genus *Leishmania*. Due to limited treatment options for the infection, the high costs of effective treatment, the sparsity of competent treatment facilities in many endemic countries, and the lack of a vaccine against the infection, leishmaniases areconsidered a neglected tropical disease (WHO, 2010). Effective

drugs for treatment of leishmaniases are for the most part associated with risks and/or severe side effects, limiting either usability or availability for the afflicted human populations. This is mostly due to the low specificity of the current chemotherapeutic drugs which are also harmful to mammalian cells (Chappuis et al., 2007).

First-line chemotherapy is still based on pentavalent antimonials, Amphotericin B and Pentamidine, all those drugs requiring parenteral administration, being toxic, expensive and having restricted therapeutic spectrum for all clinical forms of leishmaniasis (Davis et al., 2004).
Miltefosine has been recently shown to be active by oral route in the treatment of mucosal leishmaniasis (Soto et al., 2007). However, initial laboratory data suggest that miltefosine may not be particularly active against all forms (Yardley et al., 2005).

In this context, the discovery of new active and promising compounds with antileishmanial potential remains essential for control and prevention of leishmaniasis. A screening for new leads on the grounds of ethnomedicine and ethnopharmacology is justified, mainly because leishmaniasis is generally properly identified by the natives living in endemic area (Dedet et al., 1989), and because this approach has already been proved fruitful in the past, providing new leishmanicidal agents (Muzitano et al., 2006a,b).

Therefore, more specific therapeutic targets must be identified for new classes of anti-parasitic drugs.

The *Leishmania* live an essentially biphasic life cycle with elongated, flagellated promastigote stages thriving in the gut of the sandfly host and rounded, aflagellated amastigotes proliferating within the phagosomes of mammalian macrophages (Chappuiset al., 2007).

The genus *Arrabidaea* belongs to bignoniaceae family which includes 120 genera and about 800 species of plants distributed especially in the tropical and subtropical regions. *Arrabidaea brachypoda* is used in Brazil traditional medicine for the treatment of stones. Previous phytochemical studies of this genus plant led to the isolation of triterpenoid and flavonoids (Rocha et al, 2010).

This genus occurs in tropical America from Mexico to Argentina, including Brazilian Cerrado. Species from the genus *Arrabidaea* have been used in traditional medicine for astringent, antioxidant, anti-inflammatory, antimicrobial, antitumor and healing purposes (Zorn et al., 2001;

Bolzani et al., 2003; Leite et al., 2006; Martin et al., 2008). The roots of *Arrabidaea brachypoda* (DC.) Bureau, known as "cipó-una", is widely used in traditional medicine in Southeastern and Northeastern Brazil for kidney stones and painful joints (Alcerito et al., 2002; Rodrigues et al., 2006). Nevertheless, there are few studies related to this species in the chemical and pharmacological literature.

Based on ethnopharmacological information of the *Arrabidaea brachypoda*, the aim of this work was to evaluate the leishmanicidal effects of *Arrabidaea brachypoda* leves, caule and roots hydro-ethanolic extracts against promastigotes of *Leishmania (Lishmania) amazonensis*.

2. Materials and methods

2.1. Plant material

Arrabidaea brachypoda (DC.) Bureau (Bignoniaceae) leves, caule and roots were collected in April 2008 in Sant'Ana da Serra-João Pinheiro, Minas Gerais, Brazil. Dr[a]. Ana Maria Cristina Teixeira Braga, Department of Botanic of Federal University of Ouro Preto, identified the plant, and a voucher specimen was deposited at the Herbarium of the Federal University of Ouro Preto (voucher number 17935).

2.2. Preparation of the plant extracts and reference drug

Dried *Arrabidaea brachypoda* leves, caule and roots were triturated in a blender until a finely granulated powder was obtained. The extracts where obtained from this powder by adding hidro-ethanolic solution (70% of ethanol) under constant shaking for 10 h, followed by filtration. The residues were used for determining bioactivity.

The *Arrabidaea brachypoda* leaves, caule and roots extracts were concentrated under reduced pressures using rotary evaporator; and then dried under vacuum. These were used for the assays in the range of 0.125 to 40.0 µg/mL. Amphotericin B was used as reference drug.

2.3 Leishmanicidal activity against promastigotes

Promastigotes of *Leishmania (L.) amazonensis* (MHOM/BR/71973/M2269) were grown on a 24-wells plate in Schneider's Drosophila medium (Sigma, USA) supplemented with 10.0% (v/v) heat-inactivated fetal bovine serum and 1.0% penicillin (10000UI/mL)/streptomycin (10.0mg/mL) (Sigma, USA). Cells were harvested in the log phase, resuspended in fresh medium, counted in Neubauer's

chamber and adjusted to a concentration of $1x10^6$ cells/mL. The crude extracts in the range of 0.125 to 40.0 µg/mL were added to promastigote cultures, at $1x10^6$ cells/mL, solubilized in dimethylsulfoxide (DMSO) (the concentration used was 0.6%, v/v in all wells) and incubated at 25°C. After 72 h of incubation, the surviving parasites were counted in a Neubauer's chamber and compared with controls, with just DMSO in concentration of 0.6% v/v, for the determination of 50.0% and 90% inhibitory growth concentration (IC_{50} and IC_{90}, respectively). All tests were performed in triplicate on three different occasions and Amphotericin B (Eurofarma) was used as the reference drug (Pereira et al., 2010).

2.4 Statistical analysis

The leishmanicidal activity was expressed as growth inhibition. Statistical analysis was performed using nonlinear regression to obtain the values of IC_{50} and IC_{90} (concentration that inhibits growth by 50.0% and 90.0% of promastigotes, respectively), these values followed by variance analyses and Tukey's test. Differences were significant when the *p* value was lower than 0.05.

3. Results and discussion

There is no vaccine available against leishmaniasis. Drug resistance, variable efficacy, toxicity, parenteral administration, and requirement for long courses of administration are the main drawbacks of current leishmanicidal drugs (Croft et al. 2006). There is an urgent need for new drugs for the treatment of these diseases, which mainly affect people in developing countries.

Plants are an important source of therapeutic agents in the search for new and selective agents for the treatment of tropical diseases caused by protozoan. Several studies have shown that natural products represent a diverse source of compounds in drug discovery and in the development of novel antiprotozoal agents (Chan-Bacab and Pena-Rodríguez, 2001).

The antileishmanial activity of plant extracts has been attributed to compounds belonging to diverse chemical groups, such as isoquinoline alkaloids, indole alkaloids, quinones, terpenes (Araújo et al., 1998) and benzophenones (Pereira et al., 2010).

The results showed that the leaves extract was more active of what the extracts from caule and roots (Table 01), in this order, as much in the determination of the IC_{50} value how

much of the IC_{90} value (Figure 01). Presenting the extract of leves a comparable efficiency the drug of reference, Amphotericin B, in the determination of the IC_{50}.

Table 01 – Values of IC_{50} and IC_{90} from positive controls and extracts hidro-etanolics from caule, leaves and roots of the *Arrabidaea brachypoda*.

	Anphotericin B	EtOH caule	EtOH leves	EtOH roots
IC_{50} (µg/mL)	3,68	9,32	4,85	15,32
IC_{90} (µg/mL)	8,82	23,52	14,89	34,36

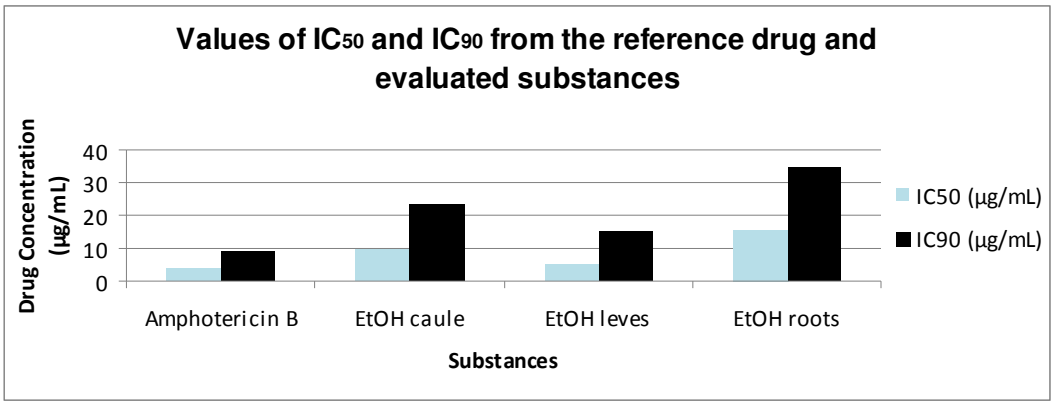

Figure 01: Comparative values of IC_{50} and IC_{90} of the drug of reference and the different evaluated parts of the plant.

4. Conclusion

More studies are needed to determine whether these substances are selective for *Leishmania* protozoan.

In conclusion, our results indicate that the hidro-ethanolic extract from *A. brachypoda* leves showed important leishmanicidal activity in vitro. Extracts from *A. brachypoda* may to supply a potential new drug for the treatment of leishmaniasis that could become available for low-income populations.

Is important to point out advantage of extract of leves to present optimum result, since these represent a renewable part of the plant, that can be gotten without killing it, also representing a part of the vegetable of easy collection and study.

This study is part of a continued search for new drugs with high activity and few side effects that can be used to treat diseases associated with protozoan parasites, such as leishmaniasis.

These important results make them, important and potential new compounds for development of new drugs against leishmaniasis, but a further detailed evaluation about their mechanism of action is still needed.

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