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Antileishmanial activity and cytotoxicity of Brazilian plants

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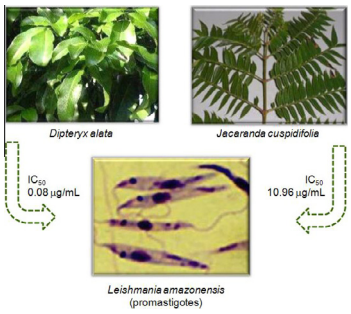
HIGHLIGHTS

- Brazilian medicinal plants were evaluated against *L. amazonensis*.
- *D. alata* and *J. cuspidifolia* leaves had high selectivity index.
- These extracts or fractions had high leishmanicidal activity and low cytotoxicity.
- The activity of *D. alata* and *J. cuspidifolia* could be due to a direct mechanism.
- The compounds that could contribute to the observed activity are discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

Leishmaniasis is a major public health problem, and the alarming spread of parasite resistance has increased the importance of discovering new therapeutic products. The present study aimed to investigate the *in vitro* leishmanicidal activity from 16 different Brazilian medicinal plants. Stationary-phase promastigotes of *Leishmania amazonensis* and murine macrophages were exposed to 44 plant extracts or fractions for 48 h at 37 °C, in order to evaluate their antileishmanial activity and cytotoxicity, respectively. The most potent extracts against *L. amazonensis* were the hexanic extract of *Dipteryx alata* (IC_{50} of 0.08 µg/mL), the hexanic extract of *Syzygium cumini* (IC_{50} of 31.64 µg/mL), the ethanolic and hexanic extracts of leaves of *Hymenaea courbaril* (IC_{50} of 44.10 µg/mL and 35.84 µg/mL, respectively), the ethanolic extract of *H. stignocarpa* (IC_{50} of 4.69 µg/mL), the ethanolic extract of *Jacaranda caroba* (IC_{50} of 13.22 µg/mL), and the ethanolic extract of *J. cuspidifolia* leaves (IC_{50} of 10.96 µg/mL). Extracts of *D. alata* and *J. cuspidifolia* presented higher selectivity index, with high leishmanicidal activity and low cytotoxicity in the mammalian cells. The capacity in treated infected macrophages using the extracts and/or fractions of *D. alata* and *J. cuspidifolia* was also analyzed, and reductions of 95.80%, 98.31%, and 97.16%, respectively, in the parasite burden, were observed. No nitric oxide (NO) production could be observed in the treated macrophages, after stimulation with the extracts and/or fractions of *D. alata* and *J. cuspidifolia*, suggesting that the biological activity could be due to mechanisms other than macrophage activation mediated by NO production. Based on phytochemistry studies, the classes of compounds

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that could contribute to the observed activities are also discussed. In conclusion, the data presented in this study indicated that traditional medicinal plant extracts present effective antileishmanial activity. Future studies could focus on the identification and purification of the antileishmanial compounds within these plants for analysis of their *in vivo* antileishmanial activity.

1. Introduction

Leishmaniasis is a disease with a wide spectrum of clinical manifestations caused by different species of protozoa belonging to the *Leishmania* genus (Desjeux, 2004). The parasites are transmitted through the bite of an infected sandfly, and more than 20 *Leishmania* species can cause this disease in humans (Grimaldi and Tesh, 1993). Ninety-eight countries and 3 territories on 5 continents have reported endemic leishmaniasis transmission, leading to the classification of leishmaniasis as one of the six most important neglected tropical diseases by the World Health Organization (Committee WHOE, 2010). Approximately 0.2–0.4 million cases of visceral leishmaniasis (VL), and about 0.7–1.2 million of cases of cutaneous leishmaniasis are registered annually, reporting the importance of this disease (Alvar et al., 2012).

Historically, chemotherapy has been used in the treatment of leishmaniasis, and it has been based on the use of pentavalent antimony compounds; however, the results obtained using these products are considered unsatisfactory, mainly due to their high toxicity and lack of efficacy in some cases of VL. Alternative treatments for leishmaniasis include amphotericin B and its lipid formulations, as well as paromomycin and miltefosine. However, the high costs, toxic side effects, and long treatment periods required limit the clinical usefulness of these drugs (Sundar et al., 2012; Kedzierski et al., 2009; Murray et al., 2005). Therefore, the development of new and cost-effective alternative therapeutic strategies for leishmaniasis has become a high priority (Frézard et al., 2009; Valadares et al., 2012a; Ribeiro et al., 2014).

In recent years, considerable attention has been given to studying plants in an attempt to search for new antileishmanial drugs (Weniger et al., 2001; Newman et al., 2007). The medicinal value of plants lies in their active compounds, which produce a measurable physiological action in the body (Basile et al., 2000; Cowan, 1999). Identification of natural products and the wide variety of separation techniques available increase the probability of finding novel products that in turn could lead to the development of new pharmaceutical compounds (Saklani and Kutty, 2008).

The present study investigated the *in vitro* antileishmanial activity of 16 different Brazilian plant species (mainly from Cerrado), in order to evaluate their activity against *Leishmania amazonensis*. Studies were carried out to establish their minimum inhibitory concentrations (IC_{50}) in *Leishmania*, as well as their cytotoxic effects on murine macrophages (CC_{50}), to determine the selectivity index (SI) of each extract or fraction. The products that presented higher SI values were used in the treatment of macrophages infected with *L. amazonensis*. The possible mechanism that could be involved in the elimination of parasites by infected cells was also discussed.

2. Materials and methods

2.1. Plants and preparation of extracts

The extracts used in this study were obtained from a bank of extracts at the Laboratório de Fitoquímica of Faculdade de Farmácia da UFMG (Belo Horizonte, Minas Gerais, Brazil). The geographic location of the plant collection site is shown in Fig. 1. The voucher species were deposited in the UFMG Herbarium (BHCB), in the Universidade Federal de Mato Grosso do Sul Herbarium (CGMS), or in the Universidade Federal do Rio de Janeiro Herbarium (R). The voucher locations of the plants are shown in Table 1. About 1 kg of leaves, roots, and stem barks of the plants were dried at 40 °C with forced air circulation. The powdered dry materials were extracted by maceration using hexane and ethanol successively at room temperature. The solvents were evaporated under reduced pressure to provide a solid residue. Dry ethanolic extracts of *Campomanesia lineatifolia* and *Chrysobalanus icaco* were suspended in water and sequentially partitioned using four solvents with different polarities to yield hexane, dichloromethane, ethyl acetate, and butanolic fractions. *Licania tomentosa* was extracted by maceration using dichloromethane, ethanol, and ethyl acetate. The dry ethanol extract of *Jacaranda cuspidifolia* was suspended in water and sequentially partitioned with $CHCl_3/H_2O$ (1:1). Dimethyl sulfoxide (DMSO) was used as the solvent at the concentration of 0.1% in the final test solution.

2.2. Phytochemical analysis

The presence of tannins, flavonoids, coumarins, quinones, alkaloids, triterpenes, steroids and saponins were evaluated in the hexanic extract of *D. alata* and in the ethanolic extract and in the chloroformic fraction of *J. cuspidifolia* by thin layer chromatography (TLC) analysis and specific reagents (Wagner et al., 1984).

2.2.1. Determination of flavonoid content

The total flavonoid content was determined using the method adapted from Brazilian Pharmacopeia IV (Brazilian Pharmacopeia, 2010). Briefly, an aliquot of 2 mL of the ethanol solution (20 μg/mL) of extracts and fraction of *D. alata* and *J. cuspidifolia* from each sample was added to 1 mL of $AlCl_3{\cdot}6H_2O$ solution (2%, w/v). This mixture was combined with methanolic acetic acid solution (5%, v/v) and, after 30 min; absorbances were read at λ 425 nm. The blank referred to the same solution without $AlCl_3{\cdot}6H_2O$ reagent. Tests were carried out in triplicate. The total flavonoid content was calculated from the calibration curve of quercetin standard. Results were expressed in terms of percentage (%).

2.2.2. Determination of phenolic content

The quantitative phenolic content in samples was estimated using the method adapted from Brazilian Pharmacopeia IV (Brazilian Pharmacopeia, 2010). Briefly, an aliquot of 2 mL of the ethanol solution (20 μg/mL) of extracts and fraction of *D. alata* and *J. cuspidifolia* was added 2 mL of Folin–Denis reagent. This mixture was combined with Na_2CO_3 solution and, after 30 min, the absorbances were read at λ 715 nm. The blank referred to the same solution without Folin–Denis reagent. Tests were carried out in triplicate. The total phenolic content was calculated from the calibration curve of pyrogallol standard. Results were expressed in %.

2.3. Parasites and mice

L. amazonensis (IFLA/BR/1967/PH-8) was used in this study. The stationary-phase promastigotes were grown at 24 °C in Schneider's