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Research Article

Essential Oil of *Pilocarpus Microphyllus* Stapf. Against Promastigotes Forms of *Leishmania infantum*

Michel Muálem de Moraes Alves[1,2*], Lucas Pereira Lima da Cruz[1,2], Rebecca Ingryd Coelho de Freitas[1], Ana Maria Sousa Costa[1], Juliana Evelyn Oliveira Lima[1], Roberta Lillyan Rodrigues Reis[3], Leiz Maria Costa Véras[3] and Fernando Aecio de Amorim Carvalho[1,2]

[1]Medicinal Plants Research Center Federal University of Piauí, UFPI, Teresina, PI, Brazil
[2]Antileishmanial Activity Laboratoty; Federal University of Piauí, UFPI, Teresina, PI, Brazil
[3]Biodiversity and Biotechnology Research Center, Biotec, Federal University of Piauí, UFPI, Parnaíba, PI, Brazil

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Correspondence should be adressed to
Michel Muálem de Moraes Alves,
E-mail: mualemmichel@ufpi.edu.br

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Abstract

Leishmaniasis makes up a group of infectious diseases caused by different species of the genus Leishmania. The visceral form as reponsible for the hight number of deaths. The treatment is still quite limited by some factors such as long duration, adverse effects, toxicity, high costs, which reinforces the importance of the discovery of new drugs for the disease. *Pilocarpus microphyllus* Stapf. popularly known as Jaborandi, is used for various medicinal purposes. Its leaves contain important imidazole alkaloids; some with functions already well known. The objective of this work was to evaluate the antileishmania activity of the essential oil of *Pilocarpus microphyllus* Stapf. against promastigotes forms of *Leishmania infantum*. Promastigotes forms *L. infantum* in log phase of growth were plated in the amount of $1x10^6$ per well, in a 96-well plate containing essential oil obtained from the leaves of *Pilocarpus microphyllus* in eight serial dilutions, at concentrations of 800 to 6.25 µg/mL. The plate was then incubated in (BOD) oven at 26°C for 48 hours, remaining 6 hours 20 µL Resazurin 10 mol/L per well was added. At the end, the plate reading was performed on plate reader at 550 nm. The minimum concentration (IC50) was obtained by calculating probit regression. The essential oil of *P. microphyllus* Stapf. demonstrated activity on promastigote forms of *L. infantum*, with activity dependent on concentration, presented an IC50 of 6.343 µg/mL. In the literature some activities of the constituents of *P. microphyllus* are already reported. The essential oil of *P. Microphyllus* Stapf. was shown to be very promising against promastigote forms of *L. infantum*. Future studies will be conducted to investigate their cytotoxicity on host cells, elucidate their mechanism of action, and evaluate their *in vivo* activity in the treatment of disease in experimental models.

Keywords

Antileishmania Activity; Cytotoxicity; Jaborandi; Leishmaniasis; Visceral Leishmaniasis

Introduction

Leishmaniasis comprises a group of infectious diseases caused by different species of the genus Leishmania. They currently affect around 12 million people all around the world. Infections caused by these parasites promote many clinical manifestations, from subclinical forms, to skin lesions, and may even cause disseminated cutaneous, mucosal, or visceral infections.

Transmission occurs during blood repotting of female insects belonging to the genus Lutzomyia. Despite all these numbers, these protozoa are still neglected diseases [1-3].

According to the World Health Organization (WHO) approximately 0.2 to 0.4 million cases per year worldwide corresponds to visceral leishmaniasis, of which more than 90% of cases occur in developing countries [4,5].

In Brazil, visceral leishmaniasis is caused by the protozoan *Leishmania infantum*, the dog is considered the main reservoir in the urban environment, being a source of infection. It is a chronic and generalized infectious disease clinically characterized by the following manifestations irregular fever, splenomegaly and anemia, can be fatal, the lethality rate can reach about 10% when not properly treated [5-7].

The current treatments of leishmaniasis have limitations, besides adverse effects, high cost, difficulty of administration and they present a high toxicity, which demonstrates a demand for the discovery of drugs, for a treatment of low cost, and more effective. For this, natural products show themselves a remarkable alternative to possible antileishmania drugs. The plants have shown a rich source of biologically active extracts, essential oils and isolated substances, where they present several activities, thus enabling the possible discovery of new therapeutic alternatives for the treatment of several diseases, among them leishmaniasis [4,3,8,9].

Popularly known in Brazil as "jaborandi" the genus Pilocarpus, it is considered one of the most important of the Brazilian flora, presenting native species in the north and northeast of the country. *Pilocarpus Microphyllus* Stapf (Rutaceae) is the best known representative of the jaborandi group, one of the Brazilian medicinal species of great prominence present in pharmacopoeias around the world, due to the use of pilocarpine, one of its main active constituents, is an imidazole alkaloid, which is used to treat glaucoma, among others. In addition to pilocarpine *P. Microphyllus*, they present other imidazole alkaloids such as isopilosine, epiisopilosine and epiisopiloturin which are isolated, as well as other substances [10-12].

The present work had as objective to evaluate the antileishmania activity of the essential oil of *Pilocarpus microphyllus* Stapf. against promastigotes forms of *Leishmania infantum*.

Materials and Methods

Extraction of essential oil

The essential oil of *P. microphyllus* was kindly provided by Prof. Dr. Leiz Véras. A sample of the plant (TEPB voucher 27.152) was deposited at the Graziella Barroso Herbarium (Teresina, Piauí, Brazil). The plant material was air-dried for 7 days, cut into small pieces, and subjected to hydro-distillation using a Clevenger-type apparatus (300 g, 3 h) to obtain essential oil. The essential oil was then stored in a dark flask and refrigerated (at + 5 ° C) until use.

Evaluation of antileishmania activity

Log-phase growth promastigotes were plated in the amount of $1x10^6$ per well in a 96-well plate containing essential oil obtained from *Pilocarpus microphyllus* Stapf. leaves in eight serial dilutions at concentrations of 800 to 6.25 µg/mL containing Schneider's supplemented medium. After incubation in a Biological Oxygen Demand Oven (BOD) at 26°C for 48 h, remaining 6 h at the end of this period, 20 µL Resazurina® was added to each well at the concentration of 1×10^{-3} mol/L to evaluate cell viability. Plaque reading was performed on a Biotek absorbance plate reader (model ELx800) at wavelength 550 nm, and the results were expressed in terms of inhibition of growth (%).

The positive control was performed with 2 µg/mL amphotericin B (Anf B) diluted in Schneider's medium containing 1×10^6 promastigotes per well. The negative control was the Schneider's medium containing 1×10^6 promastigotes per well and, in this case, the viability was 100% for the parasite. The blank reading, for each concentration and for the controls, was necessary to disregard the absorbance resulting from the medium itself with interference or not of the compounds studied.

The assay was performed in triplicate, where the minimum Inhibitory Concentration (IC50), with 95% confidence limit, was calculated using probit regression. Analyzes of variance followed by the Bonferroni test were used, taking $p < 0.05$ as the maximum level of statistical significance.

Results and Discussion

The essential oil of *P. microphyllus* Stapf. showed a high potential against the *L. infantum* promastigotes, and its concentration-dependent activity was able to inhibit up to 100% parasite growth at concentrations of 400 and 800 µg/mL (Figure 1). The minimum inhibitory concentration (IC50) value was 6.343 µg/mL. Because it is an essential oil, it is probable that the chemical constituents present act in synergism thus promoting the observed effect.

The demand for new therapeutic alternatives such as natural products and their analogues have been very relevant for the possible discovery of new antileishmania drugs. *P. microphyllus*Stapf. has been shown to be a major research object

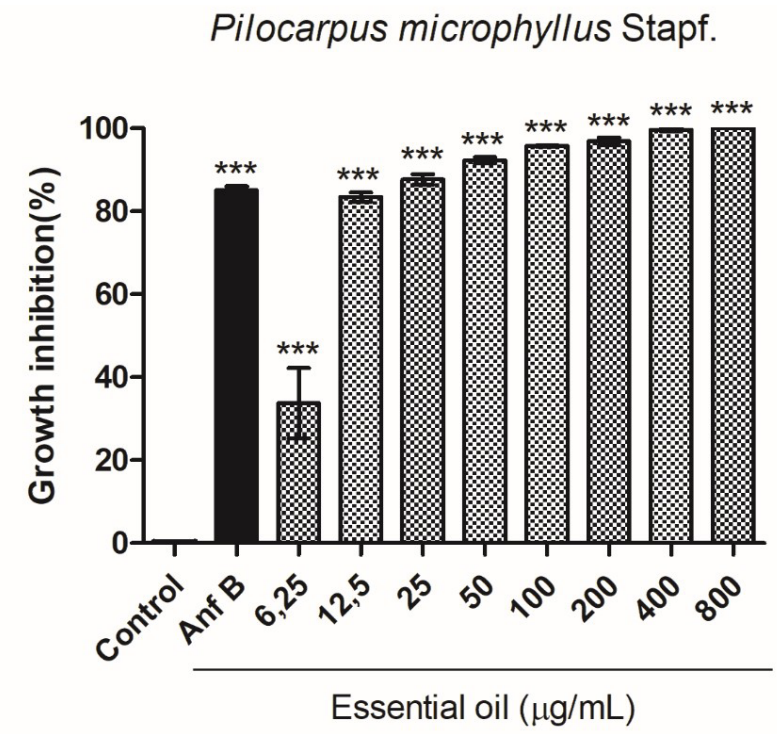

Figure 1: Antileishmania activity of *P. microphyllus* Stapf. essential oil on promastigotes forms of *L. infantum*. Promastigote forms were incubated in the presence of serial concentrations of the essential oil, which had potential action against the parasites depending on the concentration.

due to the potential that its components have shown in pharmacological and biotechnological applications, the imidazole alkaloids found in this species, such as epiisopiloturin and epiisopilosine in the treatment of neglected diseases, as well as anti- inflammatory and antinociceptive capacity, and gastroprotective effects, and schistosomicidal properties [4,10-13]. Studies also show that extracts of jaborandi have activity on *Rhipicephalus (Boophilus) microplus*, presented a carrapaticid action [14].

Conclusion

The essential oil of *Pilocarpus microphyllus* Stapf. demonstrated significant potential against promastigotes of *Leishmania infantum*. Future studies will be conducted to investigate their phytochemical constituent, cytotoxicity on host cells, elucidate their mechanism of action, and evaluate their *in vivo* activity in the treatment of disease in experimental models. In addition, the subsequent isolation and evaluation of the individual action of the essential oil components of jaborandi against the protozoan can be of great contribution for the treatment of visceral leishmaniasis in Brazil and in the world.

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