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Article

Effects of seco-steroids purified from *Physalis angulata* L., Solanaceae, on the viability of *Leishmania* sp.

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RESUMO: "Efeitos de seco-esteróides purificados de *Physalis angulata* L., Solanaceae na viabilidade de *Leishmania* sp.". *Physalis angulata* L., Solanaceae, é uma erva anual utilizada na medicina popular em muitos países tropicais e subtropicais. Apesar dos extratos da *P. angulata* apresentarem uma grande variedade de substâncias, pouco é conhecido sobre a sua atividade farmacológica. Neste trabalho foi investigado a atividade antileishmania *in vitro* de seco-esteroides (fisalinas) purificados da *P. angulata.* O tratamento com as fisalinas B, F e G causou uma inibição concentração-dependente do crescimento de promastigotas de Leishmania amazonensis em cultura axênica, com valores de IC50 de 6,8, 1,4, e 9,2 μM respectivamente. A fisalina D foi menos ativa, com valores de IC50 de 30,5 μM. Foi também observada uma atividade leishmanicida em culturas de outras espécies de *Leishmania* (*L. major, L. braziliensis* e *L. chagasi*). Nossos resultados demonstram que as fisalinas inibem o crescimento dos promastigotas com o tratamento de espécies de *Leishmania* do Velho e do Novo Mundos e sugerem o potencial terapêutico destas moléculas na leishmaniose.

Unitermos: *Leishmania* sp., fisalinas, promastigotas, seco-esteroides.

ABSTRACT: *Physalis angulata* L., Solanaceae, is an annual herb commonly used in popular medicine in many tropical and subtropical countries. *P. angulata* extracts contain a variety of substances, but little is known about their pharmacological activities. In this work we investigated the *in vitro* antileishmanial activity of seco-steroids (physalins) purified from *P. angulata*. Addition of physalins B, F, and G caused a concentration-dependent inhibition in the growth of *L. amazonensis* promastigotes, being the IC50 values 6.8, 1.4, and 9.2 μM, respectively. Physalin D was less active and had an IC50 value of 30.5 μM. Physalins were also active in cultures of other *Leishmania* species (*L. major, L. braziliensis*, and *L. chagasi*). Our results demonstrate the potent antileishmanial activity of physalins in cultures of *Leishmania* species of the New and Old Worlds and suggest the therapeutic potential of these seco-steroids in leishmaniasis.

Keywords: *Leishmania* sp., physalins, promastigotes, seco-steroids.

INTRODUCTION

Despite of the advances in the understanding of the immunology and molecular biology of leishmaniasis, the drugs available for the treatment of this illness still present today many adverse effects, such as acute toxicity and rapid development of drug resistance (Bryceson, 2001). The drugs most used in the treatment of leishmaniasis are pentavalent antimonials, which have been used for more than 50 years (Iwu et al., 1994; Carvalho et al., 2001). Some lipid-based formulations of the amphotericin B, such as AmBisome, are now indicated in the treatment of first-line against visceral leishmaniasis in developed countries (Larabi et al., 2003). The search of new chemotherapies is therefore a focus of research in this disease. The use of natural products in the treatment of a variety of diseases has also increased due to the considerable number of medicinal plants with

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proven biological activity applicable to the treatment of some diseases.

Physalis angulata L., Solanaceae, is an annual herb distributed in many countries located in tropical and subtropical regions of the world. This plant is widely used in popular medicine as a treatment for a variety of diseases, and has antitumoral activity already demonstrated (Lin et al., 1992; Chiang et al., 1992). In addition, this plant has compounds with anti-*T. cruzi* activity (Nagafuji et al., 2004; Abe et al., 2006). Physalin H, isophysalin B and others isolated of *Physalis minima* have shown leishmanicidal activity against the promastigotes of *Leishmania major* (Choudary et al., 2005; Choudary et al., 2007). We have investigated the activities of a group of seco-steroids isolated from *P. angulata*, known as physalins, and demonstrated the immunomodulatory activity of physalins *in vitro* and in vivo (Soares et al., 2003; Soares et al., 2006). In this work we investigated the antileishmanial effects of physalins B (**1**), D (**2**), F (**3**), and G (**4**), *in vitro* against promastigotes of *Leishmania* sp. Our results suggest that these drugs could be candidate for treatment of leishmaniasis.

MATERIALS AND METHODS

Parasites and drugs

L. amazonensis (MHOM/BR88/BA-125 Leila strain), *L. major* (MHOM/RI/-/WR-173), *L. chagas*i (MHOM/BR2000/Merivaldo2), and *L. braziliensis* (MHOM/BR88/BA-3456) promastigotes were cultivated in liver infusion tryptose (LIT) medium supplemented with 10% fetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil), 5% sterile urine and 50 µg/mL gentamycin (Sigma, St. Louis, MO, USA), pH 7.2, at 26 °C until logarithmic phase. Physalins B (**1**), D (**2**), F (**3**), and G (**4**) were obtained from plant specimens collected in Belém do Pará, Brazil, as described before (Soares et al., 2003).

Antileishmanial assay

Promastigotes of *L. amazonensis, L. major, L. chagasi,* and *L. braziliensis* were grown in axenic cultures (seeding 2x 10^6 in 5 mL) were incubated with 2, 5 or 15 µg/mL of physalins B, D, F and G. The parasites were observed and counted using a Neubauer chamber. Parasite growth inhibition was evaluated up to 5-7 days of treatment.

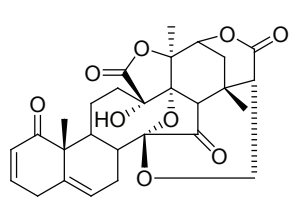
Statistical analyses

For comparing multiple groups with matched observations Friedman test was used followed by Dunns test for comparing selected groups. The inhibitory concentrations for 50% (IC50) of *Leishmania* growth, at fifth or seventh days of incubation, were calculated based in a nonlinear regression (curve fit) and the statistical analyses were made by one-way ANOVA and Newman-Keuls multiple comparison test using Prism version 4.00 for Windows, GraphPad Software (San Diego, CA). The critical level of significance was established for $p<0.05$.

RESULTS

Physalins B, G, and F, but not D, inhibit the growth of *Leishmania* spp.

To evaluate the anti-leishmania activity of physalins, promastigotes of *L. amazonensis, L. major, L. braziliensis*, and *L. chagasi* were incubated with different concentrations of the different physalins in axenic cultures. Treatment of parasites with physalin B resulted in a concentration-dependent inhibition on the proliferation of promastigotes (Figure 1). This drug reduced completely the promastigotes number since the first day of treatment when the concentration of 15 µg/mL was used. In addition, physalins F and G inhibited the



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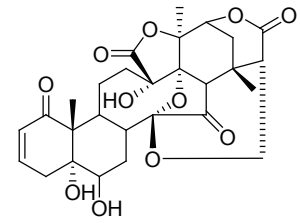

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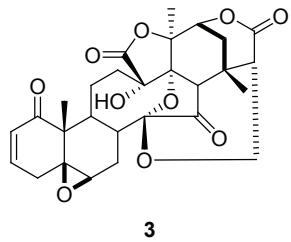

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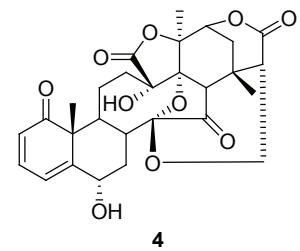

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growth of *L. amazonensis* and *L. major,* whereas addition of physalin D did not significantly alter the growth of these two parasites even at 15 µg/mL (Figure 2). Physalin F was more active against promastigotes than physalins B or G, and inhibited 100% the promastigotes when the smaller concentration was used. The estimation of IC50 values (Table 1) confirmed that physalin F was the most active (1.4 µM), having an activity comparable to the IC50 of amphotericin B (3.0 µM), a standard antileishmanial drug. Physalin D was the least active of the physalins tested, with an IC50 of 30.5 µM.

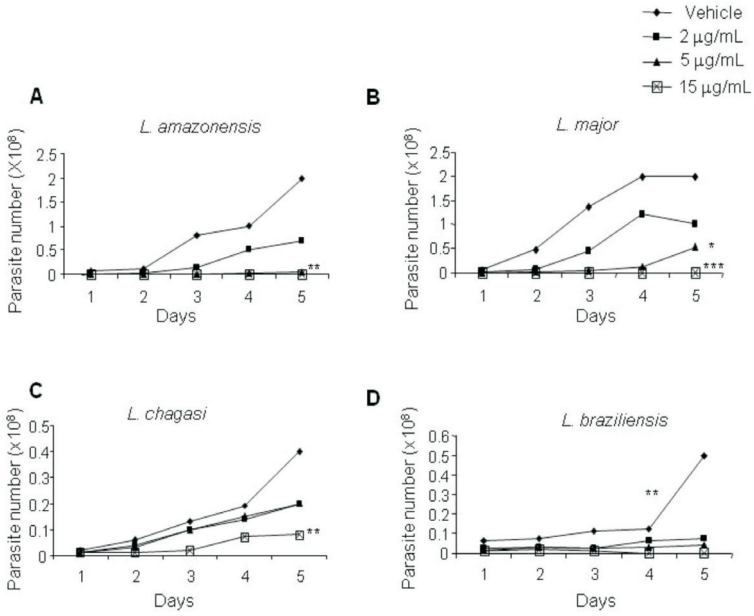

Figure 1. Effects of physalin B on promastigotes of *Leishmani*a ssp. Stationary-phase promastigotes of *L. amazonensis, L. major, L. chagasi,* and *L. braziliensis* were submitted to treatment with 2, 5 and 15 mg/mL of physalin B or vehicle in axenic cultures. The number of parasites/mL was determined by daily counting using a hemacytometer. Results shown are from one representative of three independent experiments performed. **p*<0.05, ***p*<0.01, and ****p*<0.001, compared to control group.

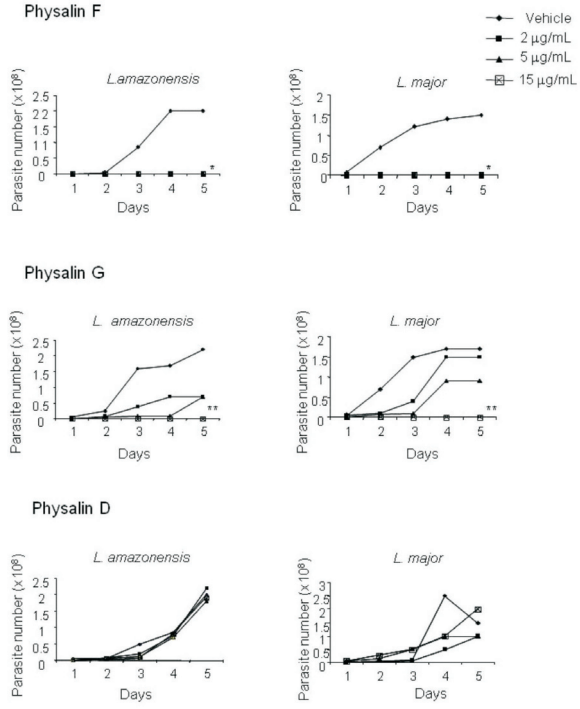

Figure 2. Effects of physalins F, G, and D on promastigotes of *Leishmania* ssp. Stationary-phase promastigotes of *L. amazonensis* and *L. major* were submitted to treatment with 2, 5, and 15 mg/mL of physalin B or vehicle in axenic cultures. The number of parasites/mL was determined by daily counting using a hemacytometer. Results shown are from one representative of two independent experiments performed. **p*<0.05 and ***p*<0.01, compared to control group.

Table 1. IC50 values for anti-*L. amazonensis* activity.

Drug	IC50 (μM)
Physalin B	6.7
Physalin D	30.5
Physalin F	1.4
Physalin G	9.2
Amphotericin B	3.0

DISCUSSION

Previous studies have shown that physalin H, isophysalin B, 5 beta, 6 beta-epoxyphysalin B and others isolated of *Physalis minima* have *in vitro* antileishmanial activitiy against promastigotes of *L. major* (Choudhary et al., 2005; Choudhary et al., 2007). In the present study we demonstrated the antileishmanial activity of physalins B, F, and G isolated from *Physalis angulata in vitro* in axenic cultures of different Leishmania species, including *L. amazonensis, L. braziliensis, L. chagasi* (New World), and *L. major* (Old World).

Whereas physalins B, F, and G had potent antileishmanial activity, this was not found with physalin D, which had a high IC50 value. The lack of immunomodulatory/anti-inflammatory activity by physalin D, but not of physalins B, F, and G, has been described before (Soares et al., 2003; Soares et al., 2006). In addition, Jacobo-Herrera (2006) also found that physalins B and F, but not D, inhibited the activation of NF-kB, an important transcription factor in inflammatory responses, and suggested that the presence of a double bond and an epoxy ring between carbons 5 and 6 in physalins B and F, respectively, which are not present in physalin D, are critical for the anti-inflammatory activity.

Although some reports have demonstrated a lack of activity of physalin D, Januario (2002) reported an antimycobacterial activity of physalin D, in addition to physalin B. Magalhães (2006) also showed that both physalins B and D have antitumoral activity, although Chiang (1992) reported lack of activity of physalin D in human leukemia cells in vitro.

In conclusion, this study demonstrated an anti-leishmania action of physalins B, F, and G against different species, with IC50 values comparable to that of amphotericin B, a drug of reference for leishmaniasis treatment. The *in vivo* activity of physalin F has been demonstrated in a model of cutaneous leishmaniasis in BALB/c mice infected with L. amazonensis. These results suggest the use of physalins as an alternative drug in treatment of the cutaneous leishmaniasis.

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