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Chemical profile, cytotoxic and antiparasitic activity of *Operculina hamiltonii*

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ABSTRACT

The aim of this study was carry out a phytochemical characterization of *Operculina hamiltonii* and to assess its antiparasitic potential against *Trypanosoma cruzi*, *Leishmania brasiliensis* and *Leishmania infantum* and also its cytotoxic activity. The infusion of this plant was selected on the basis of its popular use. A lyophilized infusion was analyzed by HPLC-DAD methodology. The anti-epimastigote and antipromastigote activity and cytotoxic activity were determined in vitro assay using a concentration range of 62.5–1000 μg/mL. The HPLC results showed the presence of quercetin and chlorogenic acid as the major compounds. The infusion exhibited significant leishmanicidal activity with IC_{50} of 236.93 μg/mL for *Leishmania braziliensis* and 342.90 μg/mL for *L. infantum*. This study showed trypanocidal activity with IC_{50} 10.61 μg/mL, however, demonstrated significant cytotoxicity at concentrations equal to or greater than 250 μg/mL with IC_{50} 47.62 μg/mL. Our results suggest that, due to the cytotoxic activity of this natural product, new assays should be performed to investigate this effect, mainly to evaluate *O. hamiltonii* for possible anticancer activity.

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1. Introduction

Infectious and parasitic diseases are still neglected in developing countries, showing worrisome epidemiological data of morbidity and mortality. Diseases such as trypanosomiasis and leishmaniosis, among other prevalent diseases; even though these concerns are clear, investment in drug research is insufficient, and measures aimed at their control are still precarious in many countries (Lemos and Lima, 2002). There is no doubt about the social impact of these diseases, because their occurrence is also related to poor housing, food and hygiene conditions to which people are subjected (Paes and Silva, 1999).

Trypanosoma cruzi is the etiologic agent of Chagas disease and affects approximately eight million people in the Americas. The etiological agents of leishmaniosis are the protozoa of the genus, while American cutaneous leishmaniosis is caused by the species *Leishmania brasiliensis* and American visceral leishmaniasis by *Leishmania infantum* (Leça Júnior et al., 2015; León et al., 2015).

In geographical regions where these diseases are prevalent, local communities usually know a vast repertoire of plants that can be used therapeutically, encouraging bioprospecting studies to discover substances secondary plant metabolites with antiparasitic activity (Anosa et al., 2014).

Species of the genus *Operculina* (Convolvulaceae) are cited in ethnobotanical studies as therapeutic resources in the treatment of parasitic diseases. *Operculina hamiltonii* (G. Don) DF Austin & Staples is an ornamental vine, whose common names vary according to the region: *batata de purga*, *jalapa-brasileira*, *jalapa*, *raiz-do-jeticucu* and *mecoacã* (Austin and Staples, 1983). Its ethnomedicinal use is associated with antiparasitic and blood purifying properties, as recognized by local populations. Some studies have determined the antihelmintic activity of *O. hamiltonii* (Gomes et al., 2010; Sobral et al., 2010), but we did not find any works that examined its activity against other types of parasites such as of the genera *Leishmania* and *Trypanosoma*. Another important aspect is that the secondary metabolites found in this species, such as tannin polyphenols and flavonoids, have demonstrated antiparasitic activity (Kolodziej and Kiderlen, 2005; Tasdemir et al., 2006). Accordingly, we hypothesized that these secondary metabolites may also be effective in the treatment of other parasitic diseases such as trypanosomiasis and leishmaniasis. These parasitic diseases are very prevalent in the tropics,

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and according to Braz et al. (2014), immunocompromised patients are more affected by these diseases and require special attention regarding their diagnosis and treatment, which should be done early to avoid more severe complications (Braz et al., 2015).

This study carried out a phytochemical characterization of an extract of *O. hamiltonii* and to assess its antiparasitic potential against *T. cruzi*, *L. brasiliensis* and *L. infantum* and also its cytotoxic activity.

2. Material and methods

2.1. Plant material

Tubers of *O. hamiltonii* were collected in July in the city of Patos, Paraíba State, Brazil. They were identified by Dr. Maria Arlene Pessoa da Silva, taxonomist and curator of Herbarium Caririense Dárdano de Andrade Lima of the University of the Region of Cariri – URCA, and a voucher specimen was deposited with the identification number 4022.

2.2. Preparation of lyophilized powder

Tubers of *O. hamiltonii* were crushed to prepare an infusion, following the protocol of the Farmacopeia Brasileira (2010), as to the proportion of plant material, quantity of water and time of steeping. Subsequently, the infusion was frozen and lyophilized using Christ Alpha 1–4 apparatus.

2.3. Cell lines used

For in vitro studies of *T. cruzi*, the clone CL-B5 was used (Le-Senne et al., 2002). Parasites were stably transfected with the *Escherichia coli* β-galactosidase gene (lacZ) provided by Dr. F. Buckner at Instituto Conmemorativo Gorgas (Panama). Epimastigotes were grown at 28 ± 1 °C in liver infusion tryptose broth (Difco, Detroit, MI) with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA), penicillin (Ern, S.A., Barcelona, Spain) and streptomycin (Reig Jofré S.A., Barcelona, Spain), as described by Roldos et al. (2008) and harvested during the exponential growth phase. Cultures of *L. brasiliensis* and *L. infantum* were obtained from the Instituto de Investigaciones en Ciencias de la Salud, Asunción, Paraguay - IICS. The maintenance of the strains, form of cultivation and isolation of promastigote forms followed the procedures described by Roldos et al. (2008). The inhibition assays for promastigotes were performed using the strain of *Leishmania braziliensis* or *L. infantum* (MHOM/BR/75/M2903), grown at 22 ± 1 °C in Schneider's Drosophila medium supplemented with 20% FBS. For the cytotoxicity assays, we used the fibroblast cell line NCTC929 grown in Minimal Essential Medium (Sigma). The culture medium was supplemented with heat inactivated FBS (10%), penicillin G (100 U/mL) and streptomycin (100 μg/mL). Cultures were maintained at 37 ± 1 °C in a humid atmosphere with 5% CO_2. The viability of these strains was assessed according to Roldos et al. (2008), through the use of resazurin as a colorimetric method.

2.4. Reagents

Resazurin, sodium salt, was obtained from Sigma–Aldrich (St. Louis, MO) and stored at 4 ± 0.5 °C protected from light. A resazurin solution was prepared in 1% phosphate buffer, pH 7, and filter-sterilized prior to use. Chlorophenol red-β-D-galactopyranoside (CPRG; Roche, Indianapolis, IN) was dissolved in 0.9% Triton X–100 (pH 7.4). Penicillin G, streptomycin and dimethyl sulfoxide were also used.

2.5. In vitro T. cruzi epimastigote susceptibility assay

The screening assay was performed in 96-well microplates with cultures that had not reached the stationary phase (Vandesmet et al., 2015). Briefly, epimastigotes were seeded at 1×10^5 mL^{-1} in 200 μL of liver tryptose broth medium. The plates were then incubated with the test substances (0.1–50 μg/mL) at 28 ± 1 °C for 72 h, after which, 50 μL of CPRG solution were added to give a final concentration of 200 μM. The plates were incubated at 37 ± 1 °C for an additional 6 h and were then read at 595 nm. Nifurtimox (100, 50, 10, 1, 0.5 and 0.1 μg/mL) was used as reference standard. Each experiment was performed twice and independently; each concentration was tested in triplicate in each experiment. The efficacy of the essential oil was estimated by calculating the anti-epimastigotes percentage (% ATc) as follow: $\% \text{ATc} = [(A_{exp} - A_{boil}) / (A_{cont} - A_{cult})] \times 100$, where, A_{exp} = absorbance the experimental sample; A_{boil} = absorbance of the blank sample; A_{cont} = absorbance of the control; A_{cult} = absorbance of the culture medium.

2.6. In vitro leishmanicidal assay

The assay was performed using a modification of a previously reported method (Vega et al., 2005). Cultures of promastigotes of *L. brasiliensis* or *L. infantum* were grown to a concentration of 10^6 cells/mL and then transferred to the test. The compounds were dissolved in DMSO at the concentrations to be tested and were transferred to microplates. Pentamidine (100, 50, 25, 12.5, 6.25, 3.125 μg/mL) was used as the standard drug. Each test was performed in triplicate. The activity of compounds was evaluated after 72 h by direct counting of cells after serial dilutions and compared with an untreated control. The efficacy of each compound was estimated by calculating the percentage of antipromastigote activity for *L. brasiliensis* or *L. infantum* respectively (%ALb or %ALi).

2.7. Cytotoxicity assays

NCTC929 fibroblasts were plated in 96-well microplates at a final concentration of 3×10^4 cells/well. The cells were grown at 37 ± 1 °C in an atmosphere of 5% CO_2. Afterwards, the culture medium was removed, 200 μL of the compounds added, and the cells grown for another 24 h. After this incubation, 20 μL of a 2 mM solution of resazurin were added to each well. The plates were incubated for 3 h, and the reduction of resazurin was measured using dual absorbance at wavelengths of 490 and 595 nm. The value of the control (blank) was subtracted. Nifurtimox at concentrations of 600, 400, 200, 100, 50 and 25 μg/mL was used as reference. Each concentration was tested in triplicate.

2.8. Chemical, apparatus and general procedures

All chemicals were of analytical grade. Methanol, formic acid, gallic acid, caffeic acid, ellagic acid and chlorogenic acid were purchased from Merck (Darmstadt, Germany). Quercetin, rutin and luteolin were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

2.9. Chemical profile by HPLC-DAD

Chromatographic analyses were carried out under gradient conditions using Phenomenex C18 column (4.6 mm × 250 mm) packed with 5 μm diameter particles. The samples of *O. hamiltonii* infusion (IOH) were dissolved in water at 15 mg/mL and then prior to use degassed in an ultrasonic bath and filtered through a 0.45-μm membrane filter (Millipore). The mobile phase consisted of 2% formic acid in water (A) and methanol (B), and the gradient was: 5% (B) for 2 min; 25% (B) until 10 min; and 40, 50, 60, 70 and 80% (B) every 10 min. The mobile phase was filtered through a 0.45-μm membrane

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