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

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**A B S T R A C T**

The aim of this study was to carry out a phytochemical characterization of *Operculina hamiltonii* and to assess its antiparasitic potential against *Trypanosoma cruzi, Leishmania braziliensis* 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 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 IC₅₀ of 236.93 μg/mL for *Leishmania braziliensis* and 342.90 μg/mL for *L. infantum*. This study showed trypanocidal activity with IC₅₀ 10.61 μg/mL, however, demonstrated significant cytotoxicity at concentrations equal to or greater than 250 μg/mL with IC₅₀ 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 leishmaniasis, 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 leishmaniasis are the protozoa of the genus, while American cutaneous leishmaniasis is caused by the species *Leishmania braziliensis* 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* (Convulvulaceae) are cited in ethnomedical use is associated with antiparasitic and blood purifying properties, as recognized by local populations. Some studies have determined the antihelmimtic 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 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,
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 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 MI) with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA), grown at 28 ± 1 °C in liver infusion tryptose broth (Difco, Detroit, MI) with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA), 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* were grown to a concentration of 10⁶ 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 (%ALD or %ALi).

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 × 10⁵ 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 efficiency of the essential oil was estimated by calculating the anti-epimastigotes percentage (% ATc) as follow: % ATc = [(Aexp − Aboil) / (Acont − Aboil)] × 100, where, Aexp = absorbance of the experimental sample; Aboil = absorbance of the blank sample; Acont = absorbance of the control; % ATc = 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 10⁶ 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 (%ALD or %ALi).

2.7. Cytotoxicity assays

NCTC929 fibroblasts were plated in 96-well microplates at a final concentration of 3 × 10⁴ cells/well. The cells were grown at 37 ± 1 °C in an atmosphere of 5% CO₂. 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, elagic 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 x 250 mm) packed with 5 µm diameter particles. The samples of *O. hamiltonii* infusion (10H) 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
filter (Millipore) and then degassed by ultrasonic bath prior to use. The flow rate was 0.7 mL/min and the injection volume was 40 μL. Stock solutions of reference standards were prepared in the HPLC mobile phase at a concentration range of 0.030–0.500 mg/mL for gallic acid, caffeic acid, ellagic acid and chlorogenic acid and 0.025–0.300 mg/mL for quercetin, rutin and luteolin. All solutions of reference standards were filtered through a 0.45-μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Quantifications were carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid and ellagic acid, 327 nm for chlorogenic and caffeic acids, and 366 nm for quercetin, rutin and luteolin. The chromatographic peaks were confirmed by comparing retention time with that of reference standards and by DAD spectra (200 to 600 nm). All chromatography operations were carried out at controlled temperature and in triplicate.

2.10. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated on the basis of the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.11. Statistical analysis

Differences between concentrations of secondary metabolites class present in HPLC were assessed by ANOVA one way and Tukey’s post-hoc test. The level of significance for the analyses was set at p < 0.05. The IC50 of antiparasitic activity was determined by linear regression. These analyses were performed by using the free software R version 3.1.1.

3. Results and discussion

The HPLC profile was determined for an infusion of O. hamiltonii (IOH) and is shown in Fig. 1 and Table 1. In addition to other minor ones, the following compounds were observed: gallic acid (retention time tR = 10.15 min, peak 1), chlorogenic acid (tR = 18.63 min, peak 2), caffeic acid (tR = 22.07 min, peak 3), ellagic acid (tR = 30.26 min, peak 4), rutin (tR = 38.11 min, peak 5), quercetin (tR = 44.76 min, peak 6) and luteolin (tR = 47.19 min, peak 7). A calibration curve was used for determination of LOD and LOQ using linear equations: gallic acid: Y = 13,179x + 1263.8 (r = 0.9998); chlorogenic acid: Y = 12,594x + 1267.9 (r = 0.9995); caffeic acid: Y = 11,983x + 1327.6 (r = 0.9999); ellagic acid: Y = 13,547x + 1308.1 (r = 0.9997); quercetin: Y = 12,564x + 1194.7 (r = 0.9996); rutin: Y = 11,839x + 1263.2 (r = 0.9999); and luteolin: Y = 13,287x + 1285.9 (r = 0.9998).

![Fig. 1. Representative high performance liquid chromatography profile of Operculina hamiltonii infusion. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), quercetin (peak 6) and luteolin (peak 7).](image)

A wide variety of metabolites are produced by species of the family Convolvulaceae, particularly phenolic compounds and glycosins, but also other low-molecular weight secondary metabolites with nitrogenous groups such as alkaloids, compounds that are potentially active in biological and pharmacological models (Lôbo et al., 2010). Phenolic acids have been described as antioxidant, antimicrobial and antimitogenic agents (Kim et al., 2003; Proestos et al., 2006), while flavonoids such as quercetin are recognized as antioxidant and anticarcinogenic agents and protective compounds for such organs as the kidney, liver and the cardiovascular system (Behling et al., 2008).

Studies have tested the antiparasitic activity of phenolic compounds against parasite, as Chagas’ disease (Izumi et al., 2011). The caffeic acid demonstrating poor antiparasitic activity against Giardia lambia, however, rutin present significant antiparasitic the in vitro assay (Alday-Provencio et al., 2015). Caffeic acid, chlorogenic acid and quercetin were shown by Montreux et al., 2014 to be potential agents against Leishmania amazonenses. Another study demonstrated the activity of caffeic acid against trypanostigotes of T. cruzi (Grecco et al., 2014). The plant-derived flavonoids, as luteolin, was showed great promise for acting as a lead compound in the chemotherapy of leishmaniasis (Iqbal et al., 2017).

The antiparasitic activity of IOH is presented in Table 2, showing clinically relevant results against T. cruzi, L. braziliensis and L. infantum. However, the standard clinical compound nifurtimox used as a positive control against the epimastigotes of T. cruzi exhibited 93% killing of epimastigotes at 50 μg/mL. The IC50 values for killing epimastigotes of T. cruzi were 3.02 and 10.6 μg/mL for nifurtimox and IOH respectively. For leishmanicidal activity, the standard clinical compound pentamidine was used as positive control. At the same concentration (i.e., 100 μg/mL) the standard drug used (pentamidine) killed 93.9% of the promastigotes of L. braziliensis. However, on the basis of IC50, pentamidine had more effective microbicidal action than did IOH against L. braziliensis and L. infantum (IC50 of 5.69 vs. 236.93 or 342.90 μg/mL respectively).

There are no other studies in the pertinent literature that assess the trypanocidal and leishmanicidal activity of this species. On the basis of ethnopharmacological use of this plant species, Lôbo et al. (2010) studied the antibacterial activity of O. hamiltonii and found unsatisfactory results against bacterial strains of Pseudomonas aeruginosa, Staphylococcus aureus and E. coli, and they speculated that this finding could have been due to low levels of tannins in the tuber of O. hamiltonii. Other studies point to a significant anthelmintic potential for O. hamiltonii, which appears to be effective in controlling gastrointestinal nematodes in goats in the Brazilian semi-arid region, where it is widely used in alternative programs for parasite control (Silva et al., 2010).

Comparing antiparasitic activity with the cytotoxicity displayed, the best results were seen with the natural product against T. cruzi at a concentration of 62.5 μg/mL with good antiparasitic activity (75.84%) and moderate cytotoxicity (54.73%). This result is important because of the high incidence of trypanosomiasis in the Americas as well as the existence of T. cruzi strains naturally resistant to conventional

| Components | IOH | LOD  | LOQ  |
|------------|-----|------|------|
| Gallic acid | 5.19 ± 0.01 a | 0.013 | 0.043 |
| Chlorogenic acid | 8.07 ± 0.01 b | 0.027 | 0.091 |
| Caffeic acid | 2.83 ± 0.03 c | 0.011 | 0.036 |
| Ellagic acid | 3.16 ± 0.02 d | 0.009 | 0.030 |
| Rutin | 2.79 ± 0.01 e | 0.024 | 0.079 |
| Quercetin | 6.45 ± 0.04 e | 0.018 | 0.060 |
| Luteolin | 0.91 ± 0.01 f | 0.015 | 0.048 |

Results are expressed as means ± standard deviations (SD) of three determinations. Means followed by different letters differ by the Tukey test at p < 0.05. IOH-Operculina hamiltonii infusion; LOD-limit of detection; LOQ-limit of quantification.
drugs, which explains the low percentage of cures of the disease among treated patients (Murta et al., 2008).

IOH also showed significant activity against T. cruzi and more efficacy against L. brasiliensis, but at concentrations equal to or greater than 250 μg/mL, there was high cytotoxicity.

The cytotoxic potential of IOH against NCTC929 fibroblasts is shown in Table 2. As can be seen, concentrations greater than 1000 μg/mL did completely kill the fibroblasts, while significant cytotoxic effect was observed for nifurtimox (the reference drug) at 100 to 200 μg/mL. The order of effectiveness of killing fibroblasts was nifurtimox (IC50 = 82.39 μg/mL) < IOH (IC50 = 47.62 μg/mL). Other plants from the Convolvulaceae family have demonstrated a significant cytotoxic effect in previous studies. Ipomoea cairica and Ipomoea squamosa exhibited a cytotoxic effect against LNCaP and A549 (Lin et al., 2008) and A2780 (human ovarian cancer cell line) cell lines (Cao et al., 2007).

4. Conclusion

In conclusion, this first time was showed the fingerprint of metabolites class present in infusion of the O. hamiltonii. The results of the present investigation clearly indicate that antiparasitic activity against T. cruzi and with moderate cytotoxicity. However, too revealed that antileishmanial activity is more significant against L. brasiliensis than L. infantum. Due this fact, this infusion is a potential source of compounds to be used in the treatment of parasitic infections and safety to be used, demonstrating a moderate toxicity. In this respect, further studies are needed to investigate the possible use of IOH due to its cytotoxic potential, such as in anticancer studies. It is recommended that other studies can be necessary to identify and characterize the active components present in infusion be undertaken with a view to determining the molecular bases for the explanations of antiparasitic activity.

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Table 2

| Concentration (μg/mL) | Antileishmanial activity | Antipryramosal activity | Cytotoxicity |
|----------------------|--------------------------|-------------------------|-------------|
|                      | IOH  %Ali | IOH  %Ali | Pentamidine  %Ali | IOH  %Ali | Nifurtimox  %Ali | IOH  %SC | Nifurtimox  %SC |
| 1000                 | 100.00 | 86.75 | – | 86.86 | – | 94.92 | – |
| 600                  | – | – | – | – | – | – | – |
| 500                  | 91.34 | 82.46 | – | 85.32 | – | 94.84 | – |
| 400                  | – | – | – | – | – | – | – |
| 250                  | 70.98 | 22.99 | – | 79.07 | – | 93.80 | – |
| 200                  | – | – | – | – | – | – | – |
| 125                  | 0.00 | 0.00 | – | 77.23 | – | 100 | – |
| 100                  | – | – | – | – | – | 55.66 | – |
| 62.5                 | 0.00 | 0.00 | – | 75.84 | – | 54.73 | – |
| 50                   | – | – | – | 93.9 | – | 93 | – |
| 25                   | – | – | 89.2 | – | – | – | – |
| 12.5                 | – | – | 80.6 | – | – | – | – |
| 10                   | – | – | 84 | – | – | – | – |
| 6.25                 | – | – | 54.2 | – | – | – | – |
| 3.125                | – | – | 15.3 | – | – | – | – |
| 1                    | – | – | 43 | – | – | – | – |
| 0.5                  | – | – | 13 | – | – | – | – |
| 0.1                  | – | – | 0 | – | – | – | – |
| IC50                 | 236.93 | 342.90 | 5.69 | 10.61 | 3.02 | 47.62 | 82.39 |

%ALb: percent killing of fibroblasts; %ATc: percent killing of T. cruzi; %Ali: percent killing of L. braziliensis; %Alb: percent killing of L. infantum. A Data calculated by extrapolation using a logarithmic equation.

References

Alday-Provencio, S., Díaz, G., Rascon, L., Quintero, J., Alday, E., Robles-Zepeda, R., Garibay-Escobar, A., Antiaarana, H., Hernandez, J., Velazquez, C., 2015. Sonoran propolis and some of its chemical constituents inhibit in vitro growth of Gaudiia lambia Trypanozoon, Planta Medica 81, 742–747.

Anosa, G.N., Udegbunam, R.I., Okoro, J.O., Okoroafor, O.N., 2014. In vivo antimalarial activities of Entando polycarpa stem bark against Plasmoidium berghei berghei in mice. Journal of Ethnopharmacology 153, 531–534.

Austen, D.F., Staples, G.W., 1983. Operculina hamiltonii (G. Don) D.F. Austin & Staples. Journal of the Arnold Arboretum 46, 487–488.

Behling, E.V., Sendão, M.C., Franscatico, H.D.C., Antunes, L.M.G., Bianchi, M.L.P., 2008. Flavonoid quercetina: aspectos gerais e ações biológicas. Alimentos e Nutrição Araquara 15, 285–292.

Braz, A.S., Andrade, C.A.F., Mota, L.M.H., Lima, C.M.R.L., 2015. Recomendações da Sociedade Brasileira de Reumatologia sobre diagnóstico e tratamento das parasitoses intestinais em pacientes com doenças reumáticas autoimunes. Revista Brasileira de Reumatologia 55, 368–380.

Cao, S., Norris, A., Wisse, J.H., Miller, J.S., Evans, R., Kingston, D.G.L., 2007. Ipomoea squamosa is a new cytotoxic macrocytic glycoflour from the leaves of Ipomoea squamosa from the Suriname rainforest. Natural Product Research 21, 872–876.

Farmacopeia Brasileira. 2010. 5 ed. Agência Nacional de Vigilância Sanitária, Brasília.

Gomes, R., Araújo, M.M., Gomes, E.N., Vieira, V.L.R., Athayde, A.C.R., 2010. Antiparasitic action in vitro of ethanolic extracts of Operculina hamiltonii (“Batata de purga”) and Momordica charantia (“Melão de São Caetano”) on eggs and larvae of gastrointestinal nematodes of goats from the semiarid of Paraiba, Brazil. Acta Veterinaria Brasilea 4, 92–99.

Grecco, S.S., Félix, M.J., Lago, J.H., Pinto, E.G., Tempone, A.G., Romoff, P., Ferreira, M.J., Sartorelli, P., 2014. Anti-trypansomal phenolic derivatives from Baccharis ucinellii. Natural Product Communications 9, 171–173.

Iqbal, K., Jamali, K., Iqbal, J., Afreen, M.S., 2017. Luteolin as a potent anti-leishmanial agent against intracellular Leishmania tropica parasite. Tropical Journal of Pharmaceutical Research 16, 337–342.

Izumi, E., Ueda-Nakamura, T., Dias Filho, B.P., Júnior, V.F.V., Nakamura, C.V., 2011. Natural products and Chagás disease: a review of plant compounds studied for activity against Trypanosoma cruzi. Natural Product Reports 28, 809–823.

Kim, D.O., Jeong, S.W., Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry 81, 321–326.

Kolodziej, H., Kiderlen, A.F., 2005. Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasites RAW 264.7 cells. Phytochemistry 66, 2056–2071.

Leçã Junior, N.F., Guedes, P.E.B., Santana, L.N., Almeida, V.A., Carvalho, F.S., Albuquerque, G.R., Wenceslau, A.A., Muniz, A.D., Silva, F.L., 2015. Epidemiology of canine leishmaniasis in southern Bahia, Brazil. Acta Tropica 148, 115–119.

Lemos, J.C., Lima, S.C., 2002. A genotypes in Colombia. Memórias do Instituto Oswaldo Cruz 97, 393.

León, C.M., Hernández, C., Montilla, M., Ramírez, J.D., 2015. Retrospective distribution of Trypanosoma cruzi I genotypes in Colombia. Memórias do Instituto Oswaldo Cruz 110, 387–393.

Le-Senne, A., Muelas-Serrano, S., Fernández-Portillo, C., Escario, J.A., Gómez-Barrio, A., 2002. Biological characterization of a beta-galactosidase expressing clone of Trypanosoma cruzi CI strain. Memórias do Instituto Oswaldo Cruz 97, 1101–1105.
Lin, R.J., Chen, C.Y., Lo, W.L., 2008. Cytotoxic activity of Ipomoea cairica. Natural Product Research 22, 747–753.

Lôbo, K.M.S., Athayde, A.C.R., Silva, W.W., Rodrigues, O.G., Vilela, V.L.R., Marinho, P.V.T., 2010. Avaliação da atividade antibacteriana e prospecção fitoquímica de Solanum paniculatum L. e Operculina hamiltonii (G. Don) DF Austin & Staples, do semi-árido paraibano. Revista Brasileira de Plantas Medicinais 12, 227–233.

Montrieux, E., Perera, W.H., García, M., Maes, L., Cos, P., Monzote, L., 2014. In vitro and in vivo activity of major constituents from Plectranthus amboinicus against Leishmania amazonensis. Parasitology Research 113, 2925–2932.

Murta, S.M.F., Nogueira, F.B., dos Santos, P.F., Campos, F.M.F., Volpe, C., Liarte, D.B., Niedé, P., Probst, C.M., Krieger, M.A., Goldenberg, S., 2008. Differential gene expression in Trypanosoma cruzi populations susceptible and resistant to benznidazole. Acta Tropica 107, 59–65.

Paes, N.A., Silva, L.A.A., 1999. Doenças infecciosas e parasitárias no Brasil: uma década de transição. Revista Panamericana de Saúde Pública 6, 99–109.

Proestos, C., Boziaris, I.S., Nychas, G.J., Komaitis, M., 2006. Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. Food Chemistry 95, 664–671.

Roldos, V., Nakayama, H., Rolón, M., Montero-Torres, A., Trucco, F., Torres, S., Vega, C., Marrero-Ponce, Y., Neguaburu, V., Yaluff, G., 2008. Activity of a hydroxybibenzyl bryophyte constituent against Leishmania spp. and Trypanosoma cruzi: in silico, in vitro and in vivo activity studies. European Journal of Medicinal Chemistry 43, 1797–1807.

Silva, C.F., Athayde, A.C.R., Silva, W.W., Rodrigues, O.G., Vilela, V.L.R., Marinho, P.V.T., 2010. Evaluation of the effectiveness of “taboa” (Typha domingensis Pers.) and “batata-de-purga” (Operculina hamiltonii (G. Don) D.F. Austin & Staples) in natura on gastrointestinal nematodes of goats, naturally infected, in the semi-arid region. Revista Brasileira de Plantas Medicinais 12, 446–471.

Sobral, F.E.S., Brandão, P.A., Freitas, F.L.S., Athayde, A.C.R., Souza, A.K.P., 2010. Operculina hamiltonii (G. DON) DF Austin & Staples (1983) e Cucurbita pepo L. no controle de ovos e larvas de helmintos gastrintestinais de Gallus domesticus. Revista Verde de Agroecologia e Desenvolvimento Sustentável 5, 131–135.

Tasdemir, D., Kaiser, M., Brun, R., Yardley, V., Schmidt, T.J., Tosun, F., Rüedi, P., 2006. Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure–activity relationship, and quantitative structure–activity relationship studies. Antimicrobial Agents and Chemotherapy 50, 1352–1364.

Vandesmet, V.C.S., Felipe, C.F.R., Kerntopf, M.R., Rolón, M., Vega, C., Corinon, C., Barbosa, A.G.R., Coutinho, H.D.M., Meneses, I.R.A., 2015. The use of herbs against neglected diseases: evaluation of in vitro leishmanicidal and trypanocidal activity of Stryphnodendron rotundifolium Mart. Saudi Journal of Biological Sciences http://dx.doi.org/10.1016/j.sjbs.2015.03.001.

Vega, C., Rolón, M., Martínez-Fernández, A.R., Escario, J.A., Gómez-Barrio, A., 2005. A new pharmacological screening assay with Trypanosoma cruzi epimastigotes expressing beta-galactosidase. Parasitology Research 95, 296–298.