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Antiprotozoal and molluscicidal activities of five Brazilian plants

M.C.T. Truiti[1], I.C.P. Ferreira[1], M.L.M. Zamuner[1], C.V. Nakamura[2], M.H. Sarragiotto[3] and M.C. Souza[4]

[1]Departamento de Farmácia e Farmacologia, [2]Departamento de Análises Clínicas, Centro de Ciências da Saúde, [3]Departamento de Química, Centro de Ciências Exatas, [4]Departamento de Biologia, Centro de Ciências Biológicas, Universidade Estadual de Maringá, Maringá, PR, Brasil

Correspondence
M.C.T. Truiti
Departamento de Farmácia e Farmacologia
Centro de Ciências da Saúde, UEM
Av. Colombo, 5790
87020-900 Maringá, PR
Brasil
Fax: +55-3263-4596
E-mail: mcttruiti@uem.br

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Abstract

Leishmaniasis, Chagas' disease and schistosomiasis (bilharzia) are parasitic diseases with wide distribution on the American continent, affecting millions of people. In the present study, biological assays for antiprotozoal and molluscicidal activities were carried out with ethanolic extracts of plant species from the Brazilian part of the Upper Paraná River. Crude extracts were obtained by percolation with absolute ethanol from the leaves of *Cayaponia podantha* Cogn., *Nectandra falcifolia* (Nees) Castiglioni and *Paullinia elegans* Cambess., as well as from the aerial parts of *Helicteres gardneriana* St. Hil. & Naud. and *Melochia arenosa* Benth., all belonging to genera used in folk medicine. Trypanocidal activity of plants was assayed on epimastigote cultures in liver infusion tryptose. Anti-leishmanial activity was determined over cultures of promastigote forms of the parasite in Schneider's *Drosophila* medium. Microscopic countings of parasites, after their incubation in the presence of different concentrations of the crude extracts, were made in order to determine the percentage of growth inhibition. *C. podantha* and *M. arenosa*, at a concentration of 10 μg/mL, showed 90.4 ± 11.52 and 88.9 ± 2.20% growth inhibition, respectively, of epimastigote forms of *Trypanosoma cruzi*, whereas *N. falcifolia* demonstrated an LD_{50} of 138.5 μg/mL against promastigote forms of *Leishmania* (*Viannia*) *braziliensis*. Regarding molluscicidal activity, the acute toxicity of the extracts on *Biomphalaria glabrata* was evaluated by a rapid screening procedure. *M. arenosa* was 100% lethal to snails at 200 μg/mL and showed an LD_{50} of 143 μg/mL. Screening of plant extracts represents a continuous effort to find new antiparasitic drugs.

Key words
- *Cayaponia podantha*
- *Helicteres gardneriana*
- *Melochia arenosa*
- *Nectandra falcifolia*
- *Paullinia elegans*
- Antiprotozoal activity
- Molluscicidal activity

Introduction

The general floristic research on diversity in the Upper Paraná River floodplain, area of Porto Rico (PR, Brazil), carried out by Souza et al. (1), demonstrated high floristic heterogeneity characterized by forest formations. This fact is probably related both to the diversity of environments, which include dry and flooded areas like swamps, lakes and ponds located between the forest domain of Paraná State and the savanna of Mato Grosso do Sul and São Paulo States, and to anthropic disturbance. In their survey of vascular plants

the authors registered 117 families and 652 species. Many of these plants are used in folk medicine, and most of them have not been studied scientifically.

In the last few years, much research has been carried out to evaluate the effectiveness and safety of the use of plants or their metabolites for the prevention or treatment of diseases. Screening of plant extracts represents a continuous effort to find new bioactive molecules or extracts. Approximately 20% of the plants in the world or their extracts have been submitted to pharmacological or biological tests (2).

American tegumentary leishmaniasis, Chagas' disease and schistosomiasis (bilharzia) are parasitic diseases with wide distribution on the American continent, affecting millions of people, primarily those with the worst living conditions (3). Plants with antiprotozoal or molluscicidal activities can be useful tools to control these diseases.

American tegumentary leishmaniasis, whose etiological agents are flagellate protozoa of the genus *Leishmania* (family Trypanosomatidae), affects skin and mucous membranes and can cause disfiguring lesions (4). The etiological agent of Chagas' disease is *Trypanosoma cruzi*, also a flagellate protozoan belonging to the family Trypanosomatidae. This parasitic disease may cause mainly cardiomyopathy and megasyndromes (3). Clinical data demonstrate that the current drugs for the treatment of these diseases present several side effects and do not always provide the desired cure levels (3,5-7).

Schistosomiasis is an important endemic disease in Brazil. Its etiological agent, the helminth *Schistosoma mansoni* (family Schistosomatidae), requires snails of stagnant or slow-flowing fresh water to complete its developmental cycle, with the species *Biomphalaria glabrata* (family Planorbidae) being the most important vector of *S. manso*ni in Brazil. The severity of the clinical forms causes much concern and can lead to death (3). The synthetic molluscicides that have been used are very expensive, can be toxic to other organisms, and can lead to deleterious long-term effects on the environment and to the development of resistance by vector snails (8,9). Thus, other alternatives need to be evaluated.

In the present study, we evaluated the antiprotozoal and molluscicidal activities of the plant species *Cayaponia podantha* Cogn. (Cucurbitaceae) popularly known as "taiuiá", *Helicteres gardneriana* St. Hil. & Naud. (Sterculiaceae) "sacarrolha", *Melochia arenosa* Benth. (Sterculiaceae) "malva", *Nectandra falcifolia* (Nees) Castiglioni (Lauraceae) "canelinha" and *Paullinia elegans* Cambess. (Sapindaceae) "cipó-timbó".

Material and Methods

Plant material

Vegetative samples of the five species were collected from riparian vegetation on banks in different areas of the Upper Paraná River floodplain: *C. podantha* (leaves) and *H. gardneriana* (aerial parts) from the Ivinhema River, municipal district of Jatei (MS, Brazil), *M. arenosa* (aerial parts) from Garças' Lake, municipal district of Bataiporã (MS, Brazil), *N. falcifolia* (leaves) from the Baía River, municipal district of Taquaruçu (MS, Brazil), and *P. elegans* (leaves) from Figueira's Pond, municipal district of Porto Rico (PR, Brazil). Voucher specimens of each species were added to the collection of the HNUP Herbarium (Nupelia, Universidade Estadual de Maringá, PR, Brazil; Registration Numbers 1281, 2844, 1834, 1421, and 463, respectively).

Extraction

The following amounts of dried plant parts were powdered with a knife mill: 200 g *C. podantha*, 450 g *H. gardneriana*, 600 g *M. arenosa*, 580 g *N. falcifolia,* and 395 g *P.*

elegans. Crude extracts were obtained by extraction with absolute ethanol at room temperature, and the solvent was removed by rotary evaporation under reduced pressure, producing 15.8, 32.7, 71.0, 64.0, and 55.8 g of ethanolic extracts, respectively.

Culture and maintenance of the parasites

Leishmania (*Viannia*) *braziliensis* promastigotes, strain MHOM/BR1987/M11272, were grown at 25ºC in Schneider's *Drosophila* medium supplemented with 10% (v/v) heat-inactivated fetal calf serum. Cells were harvested in the late log phase (day 3 of culture), resuspended in fresh medium, counted in a Neubauer chamber and adjusted to a concentration of 4 x 10^6 promastigotes/mL. *T. cruzi* epimastigotes, strain Y, were cultivated in liver infusion tryptose medium (10) containing 10% fetal calf serum and incubated at 28ºC for 96 h. Both parasite strains were maintained through weekly transfers in the respective medium.

Anti-leishmanial activity

The assay was conducted as described by Araujo et al. (5) and Ferreira et al. (11). Growth inhibition of *L.* (*V.*) *braziliensis* promastigotes was evaluated with the ethanolic extract of the plants at concentrations ranging from 0.125 to 320 µg/mL. The extracts were dissolved in DMSO (the highest concentration used was 1.6%, v/v) added to the promastigote cultures (day 3 of culture) at 4 x 10^6 promastigotes/mL, and incubated at 25ºC. After 24 h, the surviving parasites were counted in a Neubauer chamber and their number was compared with that of controls grown in the presence of DMSO only. All tests were done in triplicate and pentamidine isethionate (0.7 µg/mL; May & Baker Lab., London, UK) was used as reference drug. The LD_{50}/24 h values were determined by linear regression analysis using statistical error limits up to 10%.

Anti-*Trypanosoma cruzi* activity

The growth inhibition of *T. cruzi* epimastigotes was evaluated with the ethanolic extract of the plants (10, 100, or 1000 µg/mL). The extracts were dissolved in DMSO, added to the culture medium and the parasites (1 x 10^6 epimastigotes/mL) were cultivated for 96 h at 28ºC. Growth was evaluated by counting in a Neubauer chamber and compared to that of controls grown in medium containing only DMSO (1.0%). All tests were done in duplicate and benznidazole (10 µg/mL; N-benzyl-2-nitro-1-imidazolacetamide; Roche Pharmaceuticals, Rio de Janeiro, RJ, Brazil) was used as reference drug.

Maintenance of the snails

The snails, *B. glabrata*, SUCEN strain, were maintained in aquaria with potable water, air circulation, aquatic plants, and *Poecilia reticulata* fishes, at room temperature. Mature snails, relatively uniform in age and size (shell diameter: 10-12 mm), were used for the tests.

Molluscicidal activity

The acute toxicity on *B. glabrata* was evaluated by a rapid screening procedure (9,12). The ethanolic extracts were dissolved in 100 µl DMSO at the concentration of 400, 300, or 200 µg/mL and then added to glass beakers containing 100 mL of water from the aquaria. Two snails were placed in each container and maintained in a well-aerated place at room temperature. After 24 h the snails were placed on a Petri dish and their heartbeats were checked using a stereomicroscope. For all extracts that were 100% lethal at the concentration of 200 µg/mL, the concentrations of 150, 100, and 50 µg/mL were also tested. For these assays, ten snails were used, with a 50-mL volume of aquarium water per snail. To confirm mortality the snails were transferred to vessels containing

only distilled water and their condition was re-evaluated 24 h later. A control was carried out with DMSO. All tests were performed in duplicate and niclosamide (5 µg/mL; Bayluscide®, Bayer AG, Leverkusen, Germany) was used as reference drug.

Table 1. Effect of ethanolic extracts of *Cayaponia podantha* (Cp), *Helicteres gardneriana* (Hg), *Melochia arenosa* (Ma), *Nectandra falcifolia* (Nf), and *Paullinia elegans* (Pe) on the growth of *Leishmania* (*Viannia*) *braziliensis* promastigotes.

Plant	% Growth inhibition			
	40 µg/mL	80 µg/mL	160 µg/mL	320 µg/mL
Cp	NI	NI	38.6 ± 8.2	55.3 ± 5.2
Hg	NI	NI	44.0 ± 9.8	56.0 ± 9.0
Ma	NI	NI	NI	45.2 ± 3.3
Nf	NI	25.0 ± 9.4	59.4 ± 5.4	65.6 ± 5.4
Pe	NI	5.5 ± 0.0	11.2 ± 1.9	12.3 ± 3.4

NI = not inhibited.

Table 2. Effect of ethanolic extrats of *Cayaponia podantha* (Cp), *Helicteres gardneriana* (Hg), *Melochia arenosa* (Ma), *Nectandra falcifolia* (Nf), and *Paullinia elegans* (Pe) on the growth of *Trypanosoma cruzi* epimastigotes.

Plant	% Growth inhibition		
	10 µg/mL	100 µg/mL	1000 µg/mL
Cp	90.4 ± 11.52	89.4 ± 12.30	95.0 ± 0.92
Hg	65.3 ± 11.53	79.7 ± 10.89	85.5 ± 16.19
Ma	88.9 ± 2.20	94.0 ± 0.49	98.7 ± 0.21
Nf	73.8 ± 7.19	79.7 ± 1.61	94.0 ± 3.58
Pe	79.6 ± 0.85	90.5 ± 2.19	96.5 ± 2.12

Table 3. Molluscicidal activity of ethanolic extracts of *Cayaponia podantha* (Cp), *Helicteres gardneriana* (Hg), *Melochia arenosa* (Ma), *Nectandra falcifolia* (Nf), and *Paullinia elegans* (Pe) against *Biomphalaria glabrata*.

Plant	% Mortality					
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL
Cp	ND	ND	ND	-	-	-
Hg	ND	ND	ND	-	-	-
Ma	-	10	60	100	100	100
Nf	-	-	-	100	100	100
Pe	ND	ND	ND	-	-	-

- = inactive; ND = not determined.

Results and Discussion

Several natural compounds have been identified for the treatment of leishmaniasis and research on plants and their metabolites can contribute to overcoming the drug resistance of *Leishmania* parasites (7). Among the plant species evaluated here, *N. falcifolia* presented the best results regarding anti-leishmanial activity, with the ethanolic leaf extract displaying an LD_{50} of 138.5 µg/mL and 65.6 ± 5.4% growth inhibition of the promastigote forms of *L.* (*V.*) *braziliensis* at the highest concentration tested, 320 µg/mL (Table 1). Extracts of *H. gardneriana* (aerial parts) and *C. podantha* (leaves), which also demonstrated reasonable potency, presented an LD_{50} of 237 and 271 µg/mL, respectively. No growth inhibition was obtained at drug concentrations lower that 40 µg/mL. The medium containing DMSO did not affect the growth of the protozoa.

Ethanolic extracts of *C. podantha* and *M. arenosa* (aerial parts) inhibited the growth of epimastigote forms of *T. cruzi* even at very low concentrations (10 µg/mL), presenting 90.4 ± 11.52 and 88.9 ± 2.20% growth inhibition of this protozoan, respectively. On the other hand, extracts of *H. gardneriana*, *N. falcifolia*, and *P. elegans* (leaves) showed similar activities only when a concentration of 1000 µg/mL was used (Table 2). The medium containing 1.0% DMSO did not affect the growth of the protozoa. Benznidazole, used as the positive control against *T. cruzi* at 10 µg/mL, showed 80% growth inhibition (data not shown).

The best results in terms of molluscicidal activity (Table 3) were obtained with the ethanolic extract of *M. arenosa*, which induced 100 and 60% snail mortality at concentrations of 200 and 150 µg/mL, respectively, with an LD_{50} of 143 µg/mL. The *N. falcifolia* extract was 100% lethal to the snails at the concentration of 200 µg/mL, but mortality was not obtained at lower concentrations. Control assays with DMSO showed

no effect on the snails. Niclosamide at 5 μg/mL was used as positive control against *B. glabrata* and showed 100% lethality (data not shown).

Although the literature indicates that ideal concentrations of plant extracts are below 100 μg/mL for molluscicidal activity (8), the results obtained for *M. arenosa*, LD_{50} of 143 μg/mL, justify the continuation of its study. This plant is native to the area and the extract was obtained from regenerating parts of the plant, factors that can be considered of importance.

The genus *Nectandra* is well represented in the Brazilian flora, with several species presenting many benefits to man. They have been used in popular medicine for the relief of pain, arthritis, rheumatism and diarrhea, and also as antifungals. Pharmacological studies have demonstrated the antitumoral activity of *N. rigida* Nees, the antimalarial activity of *N. cuspidata* Nees and the vascular and antimalarial activities of *N. salicifolia* Nees (13-17). In our study, *N. falcifolia* leaves presented good results regarding their antiprotozoal activity against promastigote forms of *L.* (*V.*) *braziliensis*.

Some species of the genus *Helicteres* have been used in folk medicine, such as *H. isora* L. (as an expectorant, demulcent, astringent, antigalactagogue, and for the relief of the flu, against empyema, stomach affections, and diabetes), *H. angustifolia* (analgesic, anti-inflammatory and anti-bacterial effects), *H. ovata* Lam. (depurative, emollient and antisyphilitic effects), and *H. sacarolha* Juss. (depurative and in syphilitic inflammations). Pharmacological studies have demonstrated the antidiabetic and hypolipidemic activities of *H. isora* L. (14,18-20). In our study *H. gardneriana* (aerial parts) also displayed good antiprotozoal activity against promastigote forms of *L.* (*V.*) *braziliensis*.

Among the species of the genus *Cayaponia* that have been used popularly, we may mention *C. tayuya* (Vell.) Cogn. and *C. espelina* Cogn. (anti-snake venom, tonic, diuretic, anti-asthmatic, antisyphilitic, and purgative effects, and to combat epilepsy, diarrhea and bronchitis), *C. cabocla* M. (purgative and depurative effects in cutaneous diseases and as an emmenagogue) and *C. pilosa* Cogn. (emmenagogue, antisyphilitic and purgative effects) (14,21). Our data demonstrated that *C. podantha* (leaves) presents important antiprotozoal activity against epimastigote forms of *T. cruzi* and promastigote forms of *L.* (*V.*) *braziliensis*.

Some species of the genus *Melochia* have been used in folk medicine, such as *M. corchorifolia* L. (dysentery, abdominal swellings and water-snake bites), *M. umbellata* (Houtt.) Stapf (deobstruent) and *M. pyramidata* L. (bronchitis and cough) (14,22,23). The extract obtained from the aerial parts of *M. arenosa* demonstrated molluscicidal effects and activity against *T. cruzi* epimastigotes that can be explored in the future.

Continuous research for new drugs with high activity and reduced adverse effects is very important, especially considering that in Brazil parasitic diseases constitute a serious public health problem. Screening the biodiversity of the tropical forests can reveal new phytotherapeutic drugs, but studies of this type are just beginning. The biodiversity existing in the Brazilian flora is a potential source of many new bioactive molecules (7). The biological properties of the five plant species studied here, *C. podantha*, *H. gardneriana*, *M. arenosa*, *N. falcifolia*, and *M. arenosa* had not been evaluated until the present study. The results obtained here in biological assays with these plant species that occur naturally in the studied area demonstrate that the local flora presents a great medicinal potential. The more active plant extracts were prioritized for fractionation and identification of their active components, a work that is already under way.

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References

1. Souza MC, Kita KK, Romagnolo MB et al. (2004). Riparian vegetation of the upper Paraná River floodplain, Paraná and Mato Grosso do Sul States, Brazil. In: Agostinho AA, Rodrigues L, Gomes LC et al. (Editors), *Structure and Functioning of the Paraná River and its Floodplain*. LTER-site 6 (PELD sítio6). EDUEM, Maringá, PR, Brazil.
2. Suffredini IB, Sader HS, Gonçalves AG et al. (2004). Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. *Brazilian Journal of Medical and Biological Research*, 37: 379-384.
3. Funasa (1998). *Guia de Vigilância Epidemiológica*. Ministério da Saúde, Brasília, DF, Brazil.
4. Silveira TGV, Arraes SMAA, Bertolini DA et al. (1999). Observações sobre o diagnóstico laboratorial e a epidemiologia da leishmaniose tegumentar no estado do Paraná, sul do Brasil. *Revista da Sociedade Brasileira de Medicina Tropical*, 32: 413-423.
5. Araujo CAC, Alegrio LV & Leon LL (1998). Antileishmanial activity of compounds extracted and characterized from *Centrolobium sclerophyllum*. *Phytochemistry*, 49: 751-754.
6. Rassi A, Neto VA, Siqueira AF et al. (2002). Tratamento da fase crônica da doença de Chagas com nifurtimox associado a corticóide. *Revista da Sociedade Brasileira de Medicina Tropical*, 35: 547-550.
7. Napolitano HB, Silva M, Ellena J et al. (2004). Aurapten, a coumarin with growth inhibition against *Leishmania major* promastigotes. *Brazilian Journal of Medical and Biological Research*, 37: 1847-1852.
8. Marston A & Hostettmann K (1985). Plant molluscicides. *Phytochemistry*, 24: 639-652.
9. Bilia AR, Braca A, Mendez J et al. (2000). Molluscicidal and piscicidal activities of Venezuelan Chrysobalanaceae plants. *Life Sciences*, 66: PL53-PL59.
10. Camargo EP (1964). Growth and differentiation in *Trypanosoma cruzi*. Origin of metacyclic trypanosomes in liquid media. *Revista do Instituto de Medicina Tropical de São Paulo*, 6: 93-100.
11. Ferreira ICP, Lonardoni MVC, Machado GMC et al. (2004). Antileishmanial activity of extract from *Aspidosperma ramiflorum*. *Memórias do Instituto Oswaldo Cruz*, 99: 325-327.
12. Hostettmann K, Kizu H & Tomimori T (1982). Molluscicidal properties of various saponins. *Planta Medica*, 44: 34-35.
13. Le Quesne PW, Larrahondo JE & Raffauf RF (1980). Antitumor plants. X. Constituents of *Nectandra rigida*. *Journal of Natural Products*, 43: 353-359.
14. Correa MP (1984). *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas*. Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro, RJ, Brazil.
15. Böhlke M, Guinaudeau H, Angerhofer CK et al. (1996). Costaricine, a new antiplasmodial bisbenzylisoquinoline alkaloid from *Nectandra salicifolia* trunk bark. *Journal of Natural Products*, 59: 576-580.
16. Slish DF, Ueda H, Arvigo R et al. (1999). Ethnobotany in the search for vasoactive herbal medicines. *Journal of Ethnopharmacology*, 66: 159-165.
17. Muñoz V, Sauvain M, Bourdy G et al. (2000). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. *Journal of Ethnopharmacology*, 69: 127-137.
18. Venkatesh S, Reddy GD, Reddy YSR et al. (2004). Effect of *Helicteres isora* root extracts on glucose tolerance in glucose-induced hyperglycemic rats. *Fitoterapia*, 75: 364-367.
19. Chakrabarti R, Vikramadithyan RK, Mullangi R et al. (2002). Antidiabetic and hypolipidemic activity of *Helicteres isora* in animal models. *Journal of Ethnopharmacology*, 81: 343-349.
20. Chang YS, Ku YR, Lin JH et al. (2001). Analysis of three lupine type triterpenoids in *Helicteres angustifolia* by high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 26: 849-855.
21. Lorenzi H (2002). *Plantas Medicinais no Brasil: Nativas e Exóticas Cultivadas*. Instituto Plantarum, Nova Odessa, SP, Brazil.
22. Bhakuni RS, Shukla YN & Thakur RS (1987). Chemical constituents of *Melochia corchorifolia* Linn. *Indian Journal of Chemistry*, 26B: 1161-1164.
23. Lorenzi H (1991). *Plantas Daninhas do Brasil: Terrestres, Aquáticas, Parasitas, Tóxicas e Medicinais*. Instituto Plantarum, Nova Odessa, SP, Brazil.