Trypanocidal Activity of Traditional Antiparasitic Medicinal Plants from the Amazon

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ABSTRACT

Background: Neglected Infectious Diseases control is an urgent need in endemic areas, but it’s not a priority in the commercial interests of the pharmaceutical industry. Chagas disease (caused by Trypanosoma cruzi) is the parasitic disease of greatest impact in Latin America. Medicinal plants continue to be an affordable front-line option in endemic areas. Thus, we aimed to advance in the pharmacological evaluation of four medicinal plants traditionally used in the Amazon against parasitic infections. Methods: The plants were collected in the Amazon region of Colombia. The dry plant material was submitted to water percolation extraction. Extracts were tested in vitro against Trypanosoma cruzi (epimastigotes), and cytotoxicity was assessed against HepG2 and MRC5 cells. Results: In vitro, against T. cruzi, extracts of Ambelania duckei and Curarea toxicofera shows concentration-dependent inhibition (IC₅₀ of 221±29 and 50±5 µg/mL respectively), comparable with benznidazole (IC₅₀: 0.7 µg/mL); while Abuta grandifolia and Aspidosperma excelsum exhibited IC₅₀'s > 500 µg/mL. All extracts showed no cytotoxicity against HepG2 and MRC5 cells. Yields of extraction were between 3.2 and 9.5% and preliminary phytochemical profile showed flavonoids and steroids in all extracts. Conclusion: Promising plants, traditionally used to treat other protozoa or those used by indigenous people to treat other parasitic diseases, could be assessed against T. cruzi. C. toxicofera exhibits good activity against epimastigotes of T. cruzi, being a species that can reasonably be considered for bioassay-guided antitrypanosomal fractionation. Key words: Medicinal plants, Trypanocidal agents, Neglected Infectious Diseases, Trypanosoma cruzi, Chagas disease, Cytotoxicity.

Key Messages: Since knowledge, uses and attitudes towards Chagas disease are generally absent, it is not easy to find useful plants to treat this disease, directly in traditional knowledge. Plants studied against other protozoa or those used by indigenous people to treat other protozoa infections, could be assessed against T. cruzi.

INTRODUCTION

Control and elimination of Neglected Infectious Diseases (NID) is fundamental to the achievement of several of the Sustainable Development Goals (SDG), proposed by the United Nations Development Program (UNDP); aimed at eradicating poverty, protecting the planet and promoting peace and prosperity for all people,¹ by 2013, half of low- and middle-income countries received less than US$ 0.35 per person per year for drug and vaccine care and research for NIDs and other emerging diseases, so it is not surprising that of the 1559 drugs approved between 1975 and 2004, only 21 were for NID treatment.² In recent years, the fight against parasitic diseases has focused on strategies to optimize existing therapies, the use of combination therapies, the reuse of drugs in other indications, and/ or the use of natural products.³⁴⁻⁷ Chagas disease (caused by Trypanosoma cruzi) is the parasitic disease of greatest impact in Latin America, nearly 90 million people in 19 endemic countries are at risk of contracting the disease and 16 million people are already infected although, in recent years, there has been an increase in the number of infected persons in Canada, the United States, and Europe.⁸⁻¹⁰ There are only two drugs available for treatment (benznidazole and nifurtimox) and, their use is limited by difficulties in administering the treatment, side effects, genotoxicity, and the impossibility of using it in patients with psychiatric, neurological, renal or hepatic disorders.¹¹⁻¹³ Since initial or acute symptoms of Chagas disease are evident only in 10% of infected people, and generally confused with other pathologies, knowledge, use and attitudes towards the disease are generally absent. Consequently, it is not easy to find useful plants to treat this disease, directly in traditional knowledge. Medicinal plants are first-line therapy in endemic areas (used by 25-75% of people).¹¹ The use of bark of Chichona (Cinchona officinalis L.) by the aborigines of South America was profited by Europeans for the discovery of quinine, in the 19th century, which derivatives such as chloroquine, amodiaquine, primaquine, and melloquine have

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been synthesized.\[12\] The traditional Chinese herbal remedy qinghào (Artemisia annua L.), used since ancient times for the treatment of intermittent fevers, allowed to isolate artemisinin and synthesized derivatives widely used today for the treatment of malaria. Healing plants are a source of promising compounds with antiparasitic activity; in Colombia good to moderate activity was reported in twenty-one extracts of plants of the family Annonaceae, against Trypanosoma sp., Leishmania sp., Plasmodium sp., and more recently in Japan, extracts of Rhus succedanea (seeds), Cinnamomum daphnoides (fruits) and Styrax japonica (fruits) showed antiparasitic activity against T. b. rhodesiense, T. cruzi, L. donovani, and P. falciparum with $IC_{50} < 20\mu\text{g/mL}$,\[16\] the previous examples allow to recognize that extracts of medicinal plants with promising activity against Plasmodium have similar activity against other protozoan parasites. We previously studied the use of plants to treat parasitic diseases in a Uitoto (Ziora Amena) community of the Amazonas region of Colombia.\[15\] *Abuta grandifolia* (Mart.) Sandwith is jungle brush of approximately 1.5 m high, smooth leaves with three main veins, bitter taste and common name “aifo” (A+fo (uitoto)) or “abuta”; it is used as a traditional remedy in the treatment of malaria and is part of the mixture of the poison “curare”\[14,17\]. The plant is used by natives of the Amazon and Putumayo provinces of Colombia in the form of leaf infusion to treat malarial fevers, it is also used in South America claimed to have many medicinal properties.\[16,18\] Ambelantia ducket Markgr is a jungle wood tree of approximately 20 m of height, scarce, the bark of bitter flavor which, when scraping, generates sticky exudate of reddish color in little quantity; common name: “Costillo negro”, it is used like a traditional remedy in the treatment of malaria and makes part of the mixture of the poison “curare”.\[14,16\] Aspidosperma excelsum Benth, it is a woody tree of 15 m high, with a 30 cm diameter ribbed trunk, simple alternately oblong green leaves on the beam and greyish green leaf top of the common name: “Costillo blanco”, “Remoscupi” (For the use of wood to make the oars, and the quechua “caspi”, tree).\[20\] Gollaveai (uitoto),\[16,18\] the defecation of bark, drunk, is traditionally used against malaria (Colombia, Peru and Brazil)\[17,18,19\] or mixed with *C. odorata* for intestinal infections or external use against herpes.\[22\] Curarea toxicofera (Wedd.) Barneby & Krukoff is a jungle liana of approximately 10 m height and 4 cm wide, which grows around a smooth trunk tree called “castato”, its leaves are whitish by the underside, of bitter flavor; when cutting the liana, it appears abundant exudate liquid of translucent color; common name: “Bejucu de llato” (Llajo K+nai (uitoto)) or “oso perezoso” (because lazy bears climbing it). It is traditionally used as a remedy in the treatment of malaria,\[16,18\] and is part of the “curare” poison mixture.\[22\]

### MATERIALS AND METHODS

#### Plant collection and extracts preparation

A. grandifolia - Menispermaceae COI000410190 and COI000413525 (S 4° 6’ 43” W 69° 54’ 36”) leaves, A. ducket - Apocynaceae COI000413594 (N 4° 7’ 38” W 69° 55’ 11”) trunk bark, A. excelsum - Apocynaceae COI000362034 (S 4° 7’ 52” W 69° 55’ 37”) trunk bark and C. toxicofera - Menispermaceae COI000413526 (S 4° 7’ 46” W 69° 55’ 14”) liana; all Colombian origin, were collected in the municipality of Leticia-Amazon, at estimated height of 117 m a.s.l. A voucher specimen was deposited at the Colombian National Herbarium. A specialist identified all collected material and the plant name has been checked with The Plant List.\[24\] This research was carried out under the contract for access to genetic resources and derived products No. 269 of the Ministry of Environment and Sustainable Development of Colombia. Dried plants were submitted to a percolation extraction process with water as described in the USP pharmacopeia, with some modifications: 100 g of dried and ground crude material (No. 10 mesh) were moistened with a sufficient amount of extracting solvent for three hours in a well-covered container, then transferred to a percolator where it was macerated for 24 hr and then slowly percolated (1 mL/min), until 1000 mL of percolation is obtained, at which point the drug was practically depleted.\[26\] The percolation was then filtered and freeze-dried (Labconco Freezezone 4.5). All preparations were stored at 4°C, in an environment protected from daylight, until use.

#### Chemicals

Benznidazole (N-Benzyl-2-nitro-1H-imidazol-1-acetamide), resazurin and hemin were purchased from Sigma Aldrich, DMSO (dimethyl sulfoxide) and fetal bovine serum were acquired from Thermo Fisher Scientific. All chemicals provided were of reagent grade.

#### Parasites

*T. cruzi* (Y strain) was kindly donated by Tropical Parasitology Research Laboratory, University of Tolima (Colombia). The trypanmastigote forms were obtained from blood samples of mice infected with *T. cruzi* following the procedure described by Lourenço *et al.*\[27\] with some modifications. *In vivo* infection was performed in Swiss ICR mice of 23±1 g infected by intra-peritoneal with approximately 1x10⁷ parasites/mL, 15 days post-infection (at peak of parasitemia), mice were anesthetized and approximately 1 mL of blood was obtained by cardiac puncture. Blood was centrifuged at 500g for 30 min, in a biological safety box, plasma was removed and 5 mL of liver infusion tryptose culture medium, enriched with 10% fetal bovine serum and 1% hemine (LIT+10%FBS) were added, the sample was centrifuged again and medium removed; then the concentrate of red blood cells and parasites was transferred to a culture tube containing 5 mL of LIT+10%FBS medium, the tube was incubated at 28°C and shaken every 2 days until day 10 when parasitic forms and their conversion from trypanmastigote to epimastigote were checked under an optical microscope, then the culture medium was changed and incubated until day 15 when the complete conversion from trypanmastigote forms to epimastigote was achieved, parasite growth was determined by counting cells using a Neubauer hemocytometer.

#### In vitro anti-trypanosomal activity

All treatments were prepared in DMSO, final solvent concentration never exceeding 0.5%. In a 96-well plate were added, in triplicate, 100 µL of medium LIT+10%FBS containing 1x10⁶ epimastigotes/mL plus the treatments vehiculated in 100 mL of medium LIT+10%FBS (final well volume 200 µL); treatments were: 0.29-300 µg/mL of *A. grandifolia*, 62.5-500 µg/mL of *A. excelsum* and *A. ducket*; growth control was the vehicle of treatments (LIT+10%FBS + DMSO 0.5%) and active control benznidazole (BNZ) 0.25-2 µg/mL. Plates were incubated at 28°C for 6 days. Parasitic viability was determined using the resazurin colorimetric method by adding 20 µL of 0.44 µM solution in each well and incubating for 4 h at 37°C. Relative fluorescence units (RFU) were determined in LB 970 Twinkle fluorescence reader (BERTHOLD- Technologies. GmbH and Co.KG), with λ<sub>ex</sub>:540nm and λ<sub>em</sub>:590nm.

#### Cytotoxicity

Cell viability assays were developed using human cancer cell line HepG2 (liver cells) and human non-cancer cell line MRC5 (fetal lung fibroblast cells), seeded in a 96-well flat-bottomed plate (density 150000 cells/mL and 100 µL/well) and incubated at 37°C in a 5% CO₂ environment; RPMI 1640 culture medium supplemented with 10% fetal bovine serum (heat-inactivated) was used.\[28,29\] The extracts were tested at 6.25-100 µg/mL and cells were cultured for 48 hr. Viability was determined using a classical resazurin assay (0.15 mg/mL in PBS), plates were incubated for 4hr and RFU were determined as described above. Experiments
were performed in triplicate and CC₅₀ (concentration required for the reduction of cell viability by half) was determined.

Statistical analysis

In vitro concentration-response assays were carried out to obtain 50% inhibitory and cytotoxic concentrations (IC₅₀ and CC₅₀), as a measure of anti-trypanosomal or cytotoxic activity; estimated from concentration-response curves by regression analysis using Excel. In each case, at least two independent determinations were made.

Preliminary phytochemical Analysis

The general profile of metabolites present in the extracts was evaluated or confirmed by thin-layer chromatography (TLC) on Silicagel 60G F 254, using various solvent systems comprising: Toluene/ethyl acetate/diethyleamine, Ethyl acetate/acetic acid/methanoic acid/water, Ethyl acetate/methanol/acetic acid (or water), Chloroform/acetic acid/methanol/water and visible or UV light at 254 and 365 nm, after spraying with methanol/acetic acid (or water). The general profile of metabolites present in the extracts was evaluated or confirmed by thin-layer chromatography (TLC) on Silicagel 60G F 254, using various solvent systems comprising: Toluene/ethyl acetate/diethyleamine, Ethyl acetate/acetic acid/methanoic acid/water, Ethyl acetate/methanol/acetic acid (or water), Chloroform/acetic acid/methanol/water and visible or UV light at 254 and 365 nm, after spraying with different types of reagents (Dragendorff, NP/PEG, Vanillin/H₂SO₄, Kedde, Anisaldehyde Sulfuric acid) [30].

RESULTS

The percolation extraction of plants led the extraction yields shown in Table 1.

Table 1: Yield of extracts.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Part used</th>
<th>Dry weight (mg Powder)</th>
<th>Extract</th>
<th>Yield (% w/w) of crude extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abuta grandifolia</td>
<td>Leaf</td>
<td>100</td>
<td>Water PE</td>
<td>5.8</td>
</tr>
<tr>
<td>Ambelania duckei</td>
<td>Trunk</td>
<td>100</td>
<td>Water PE</td>
<td>5.5</td>
</tr>
<tr>
<td>Aspidosperma excelsum</td>
<td>Trunk bark</td>
<td>100</td>
<td>Water PE</td>
<td>3.2</td>
</tr>
<tr>
<td>Curarea toxicofera</td>
<td>Liana</td>
<td>100</td>
<td>Water PE</td>
<td>9.5</td>
</tr>
</tbody>
</table>

PE= percolation extraction.

Figure 1: Concentration-dependent anti-trypanosomal activity of Benznidazole (dashed line) and water extracts of Ambelania duckei (dotted line) and Curarea toxicofera (plain line). Each line corresponds to an independent test.

Table 2: In vitro anti-trypanosomal activity (IC₅₀) against Trypanosoma cruzi (Y strain), cytotoxicity to HepG2 and MRC5 cells (CC₅₀) and selectivity index (SI) of active plants studied.

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC₅₀ (µg/mL±SD)</th>
<th>HepG2 CC₅₀ (µg/mL)</th>
<th>MRC5 CC₅₀ (µg/mL)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambelania duckei</td>
<td>221+/-29</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;0.45</td>
</tr>
<tr>
<td>Curarea toxicofera</td>
<td>50+/-5</td>
<td>&gt;100</td>
<td>ND</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

IC₅₀ Concentration inhibiting 50% of parasite growth. CC₅₀ Concentration killing 50% of cells. Data from at least two independent determinations.

Table 3: Qualitative phytochemical characteristics of the extracts by thin-layer chromatography.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Abuta grandifolia</th>
<th>Ambelania duckei</th>
<th>Aspidosperma excelsum</th>
<th>Curarea toxicofera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+ ) presence, (- ) absence.

In vitro anti-trypanosomal activity

Against T. cruzi, extracts of A. duckei and C. toxicofera shows concentration-dependent inhibition (Figure 1), with IC₅₀ of 221+/-29 µg/mL and 50+/-5 µg/mL respectively; while active control BNZ showed an IC₅₀ of 0.7+/-0.1 µg/mL. A. grandifolia and A. excelsum exhibited IC₅₀’s > 500 µg/mL (maximum concentration assessed).

Cytotoxicity

None of the A. grandifolia, A. duckei, A. excelsum and C. toxicofera extracts showed cytotoxicity at concentrations ≤ 100 µg/mL on HepG2 or MRC5 cell lines. An initial estimate of the selectivity index of active species is shown in Table 2.

Preliminary phytochemical Analysis

Phytochemical screening revealed the presence of flavonoids and steroids and the absence of saponins (Table 3).

DISCUSSION

Chagas disease is among the 17 most important neglected tropical diseases for which there is no effective vaccine, so transmission control is based on prevention (i.e. vector control and drug prophylaxis), veterinary care of infected animals, reservoir control and improvements in housing, water and basic sanitation; however, despite achievements in decreasing the number of cases and screening in blood banks to reduce the risk of infections from blood transfusions, there is a need for improved diagnosis, access to treatment and research leading to the development of drugs that can treat the disease in its early and chronic stages, effectively and with reduced toxicity [31]. Natural products maintain a leading role as a source of new drugs, of 1010 new active substances approved as medicines by regulatory agencies over the last 25 years, 490 (48.5%) are of natural origin [32]. Current trypanosomiasis chemotherapy is unsatisfactory because of its unacceptable toxicity, low efficacy, undesirable course of administration and drug resistance.

Several medicinal plants show trypanocidal activity against different parasitic stages (epimastigotes, trypomastigotes and amastigotes), being...
epimastigotes form the most used since it offers as main advantages a higher susceptibility to the action of the assessed substances and easy cultivation in laboratory, resulting in a particularly useful pharmacological model for screening of plant extracts which can later be evaluated against other parasitic forms. Medicinal plant compounds have demonstrated greater chemical diversity, lower susceptibility to the development of resistance (since the activity in the plant often depends on the synergy of several different molecules), lower toxicity and innovative mechanisms of action.

Infected people are often unaware of their condition or misdiagnose it, because the symptoms are easily confused with other pathologies, thus traditional knowledge about Chagas disease is not common. Therefore, many plants that have been studied for their effects against T. cruzi are the same ones that have shown effects against other protozoa in laboratory testing or those that are used by indigenous people to treat other protozoa infections, such as leishmaniasis, sleeping sickness and malaria.

Percolation extraction in water was selected, as it is the most similar method to the traditional use and in a previous work this technique was selected according to practical, convenient and reproducible criteria; showing for C. toxicofera, a better extraction performance compared to continuous distillation and traditional preparation. The highest extraction yield was achieved with C. toxicofera, a result similar to previous work (8.9% followed by intermediate yields obtained for Anelasma gardnerianum and A. duckei and the lowest yield was obtained for the trunk bark of A. excelsum. The antiparasitic activity of extracts was classified according to the criteria shown in Table 4. A. duckei's IC50 is considerably higher than the active control Benzodiazole (three orders of magnitude) whereas C. toxicofera's IC50 is two orders of magnitude higher than active control; interesting result considering the great challenge for a primary extract to be compared with an isolated active substance. There was no evidence of selective activity (against cancer cell line), or any cytotoxic activity, in these plants.

A. grandifolia exhibits synonyms such as Abuta concolor Poepp. & Endl, Abuta guianensis Eichler, Anelasma concolor (Poepp. & Endl.) Miers, Anelasma gardnerianum Miers, Anelasma guianense Miers, Anelasma laurifolium Satog ex Benth, Anelasma laurifolium Miers, Anelasma laurifolium Satog ex Diels, Anelasma martianum Miers, Anelasma pallidum Miers, Anelasma spruceanum Miers ex Benth, Anelasma spruceanum Miers, Cocculus grandifolius Mart, Cocculus laevigatus Mart, Trichoa concolor Endl and Trichoa guianensis Klotzsch & Eichler. Previously our team showed that ethanolic extract of A. grandifolia leaves (crude alkaloid extract and traditional Huitoto remedy) has good anti-plasmodial activity against Plasmodium falciparum FCB2 (IC50 <1μg/mL and 27μg/mL respectively) and antimalarial activity in vitro against Plasmodium berghei ANKA (66 % inhibition at 250 mg/kg/day).[18,19] dichloromethane extract from leaves showed high larvicidal activity against Aedes aegypti larva[20] and ethanolic extract from the bark showed antimicrobial activity in vitro.[30] A. grandifolia do not show in vitro activity against T. cruzi epimastigotes, also, there was no evidence of cytotoxicity on HepG2 and MRC5 cells; qualitatively was detected the presence of alkaloids, flavonoids and steroids in the extract.

A. duckei has no registered synonyms;[24] it shows a weak anti-trypanosomal activity and no selective action (against cancer cell line), or any cytotoxicity, at concentrations ≤ 100μg/mL. The qualitative phytochemical analysis shows the presence of flavonoids, steroids and polyphenols in the extract.

A. excelsum presents synonyms such as Aspidosperma marcgravianum Woodson and Macaglia excelsa (Benth.) Kunzke.[24] plant has been reported as active against Gram-positive cocci,[22] the hydroethanolic extract from trunk bark showed activity against chloroquine-resistant P. falciparum (W2: IC50 23.6μg/mL and FCB-2: IC50 36.0μg/mL) and low cytotoxicity (CC50 > 250μg/mL HepG2 cells), also were identified more than 20 different indole alkaloids.[18,21] This plant does not show, under our test conditions, activity against T. cruzi epimastigotes and no cytotoxicity (CC50 > 100μg/mL, on HepG2 or MRC5 cells) as previously reported for HepG2 cells. Qualitative thin-layer chromatography shows the presence of flavonoids, steroids and alkaloids (as previously reported by do Nascimento[21]).

C. toxicofera, a climbing vine native to meso and South America, exhibits synonyms such as: Abuta boliviensis Rusby, Chondrodendron bioccai Lusina, Chondrodendron polygonanthum (Diels) Diels, Cocculus toxicoferus Wedd, Hyperbaena polygonantha Diels and Chondrodendron toxicoferus (Wedd.) Krukoff & Moldenke.[15,24] The plant is used alone or in combination with prescription drugs to prevent and treat malaria in the Colombian Amazon; traditional remedy showed an IC50 of 7.3 μg/mL on Plasmodium falciparum FCR3 and on Plasmodium berghei rodent malaria the ED50 was 328.6 mg/kg/day.[19] C. Toxicofera exhibits good concentration-dependent anti-trypanosomal activity against epimastigotes of T. cruzi, being considered a species that may reasonably be considered for bioassay-guided fractionation; under our test conditions, the species does not exhibit cytotoxicity at concentrations ≤ 100 μg/mL, on HepG2 or MRC5 cells, the selectivity index is >2 making its use promising for the treatment of the disease. The phytochemical analysis shows the presence of alkaloids, flavonoids, steroids and polyphenol, coincident with previously reported for this plant.[24]

Protozoan parasites (with few exceptions) require salvage pathways for pyruvate synthesis, e.g.: adenine phosphoribosyl transferase (common for T. cruzi, B. divergens, T. brucei, T. congolense, T. cruzi, and T. vivax), and hypoxanthine-guanine phosphoribosyl transferase (common for B. divergens, T. brucei and T. cruzi).[30,61] These examples make it conceivable to consider drugs act in one or more biochemical or physiological processes, common to different protozoa, as the key to their anti-parasitic activity on different species and, also the existing differences between the pathogen and the host allow the action on the microorganism without producing toxic effects on the host.

CONCLUSION

Curarea toxicofera exhibits good activity against epimastigotes of T. cruzi, being considered a species that may reasonably be considered for anti-trypanosomal bioassay-guided fractionation. Promising plants, traditionally and popularly used to treat other protozoan infections, could be assessed against T. cruzi, as traditional knowledge about the disease is not easy to obtain (because infected people are often unaware of their condition or misdiagnose it). Despite Ambelania duckei showed a weak anti-trypanosomal activity, it should be studied against other

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**Table 4: Proposed thresholds for in vitro epimastigoticide activity of anti-trypanosomal crude extracts.**

<table>
<thead>
<tr>
<th>IC50 (μg/mL)</th>
<th>Level of Activity</th>
</tr>
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<tbody>
<tr>
<td>&lt; 50</td>
<td>Very good</td>
</tr>
<tr>
<td>50 - 100</td>
<td>Good</td>
</tr>
<tr>
<td>101 - 150</td>
<td>Good to moderate</td>
</tr>
<tr>
<td>151 - 250</td>
<td>Weak</td>
</tr>
<tr>
<td>251 – 350</td>
<td>Very weak</td>
</tr>
<tr>
<td>&gt;350</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

IC50 Concentration inhibiting 50% of parasite growth.
stages of *T. cruzi*. The extracts of *A. grandifolia, A. duckei, A. excelsum* and *C. toxicofera* showed no selective activity (against cancer cell line HepG2), or any cytotoxic activity against MRC5 cells.

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**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

**ABBREVIATIONS**

*A. duckei*: Ambelania duckei; *A. excelsum*: Aspidosperma excelsum; *A. grandifolia*: Abuta grandifolia; BNZ: benznidazole; CC₉₅: 50% cytotoxic concentration; *C. toxicofera*: Curarea toxicofera; DMSO: dimethyl sulfoxide; FBS: Fetal Bovine Serum; LIT: Liver infusion tryptose medium; IC₅₀: 50% inhibitory concentration; NID: Neglected Infectious Diseases; RFU: Relative fluorescence units; SDG: Sustainable Development Goals; *T. cruzi*: Trypanosoma cruzi; TLC: thin-layer chromatography; UNDP: United Nations Development Program.

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d_organisms/resources/9789241640904/en/
Chagas disease is the parasitic disease of greatest impact in Latin America; medicinal plants continue to be an affordable front-line option in endemic areas. Since knowledge, uses and attitudes towards this disease are generally absent (because infected people are often unaware of their condition or misdiagnose it), it is not easy to find useful plants directly in traditional knowledge. We assessed, against *T. cruzi*, promising plants traditionally used to treat other protozoan infections. We aimed to advance in the pharmacological evaluation of four medicinal plants traditionally used in the Amazon against parasitic infections. This experimental pharmacology study, used *in vitro* biological models of infection and cytotoxicity and, a preliminary phytochemical approach. Plants were collected in the Amazon region of Colombia. The dry plant material was submitted to water percolation extraction. Extracts were tested *in vitro* against *Trypanosoma cruzi* (epimastigotes), and cytotoxicity was assessed against HepG2 and MRC5 cells. Finally, the general profile of metabolites present in the extracts was studied by thin-layer chromatography. *In vitro* concentration-response tests were carried out to obtain 50% inhibitory and cytotoxic concentrations as a measure of biological activity, estimated from concentration-response curves by regression analysis. Metabolite profiles were evaluated by thin-layer chromatography (TLC). *In vitro*, against *T. cruzi*, extracts of *Ambelania duckei* and *Curarea toxicofera* displayed concentration-dependent inhibition (IC$_{50}$ of 221±29 and 50±5 µg/mL respectively), comparable with benznidazole (IC$_{50}$: 0.7 µg/mL); while *Abuta grandifolia* and *Aspidosperma excliens* exhibited IC$_{50}$’s > 500 µg/mL. All extracts showed no cytotoxicity against HepG2 and MRC5 cells. Yields of extraction were between 3.2 and 9.5% and preliminary phytochemical profile showed flavonoids and steroids in all extracts. Promising plants, traditionally used to treat other protozoan infections, could be assessed against *T. cruzi*. *C. toxicofera* exhibits good activity against epimastigotes of *T. cruzi*, being a species that can reasonably be considered for bioassay-guided antitypanosomal fractionation.