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Original Article

Antiparasitic potential of plants used in folk medicine in Brazil

[Potencial antiparasitario de plantas utilizadas en la medicina popular en Brasil]

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Abstract

Context: Neglected Tropical Diseases (NTDs) face growing challenges due to parasite resistance and the declining efficacy of available treatments. For diseases such as Chagas and malaria, limited private sector investment further hinders therapeutic innovation, reinforcing the urgent need for more effective interventions.

Aims: To analyze the antiprotozoal potential of known medicinal species by providing novel insights into their biological activities.

Methods: Five ethnomedicinal plant species were selected based on their limited documentation regarding antiprotozoal activity. Twenty crude extracts were obtained by maceration with solvents of increasing polarity from the branches of *Anchietea salutaris* A. St.-Hil. and *Davilla rugosa* Poir., and the aerial parts of *Banisteriopsis argyrophylla* A. Juss., *Grindelia robusta* Nutt. and *Pyrostegia venusta* (Ker Gawl.) Miers. The extracts were tested *in vitro* against *Trypanosoma cruzi*, *Leishmania amazonensis*, and *Plasmodium falciparum* and evaluated for cytotoxicity to determine selectivity indexes (SI).

Results: In the preliminary screening at 25 µg/mL, all species exhibited some antiprotozoal activity. None of the extracts were considered active against *T. cruzi*. Hexane and dichloromethane extracts from *A. salutaris*, *B. argyrophylla*, and *D. rugosa* demonstrated potent antiplasmodial effects (IC_{50} < 9.2 µg/mL; SI ≥ 22). Additionally, the dichloromethane extract of *G. robusta* showed strong activity against *L. amazonensis* (IC_{50} = 2.2 µg/mL; SI = 78).

Conclusions: These findings highlight the potential of underexplored medicinal plant species as sources of bioactive compounds for developing treatments for NTDs.

Keywords: antiprotozoal agentes; *Leishmania* spp.; medicinal plants; *Plasmodium falciparum*; traditional medicine; *Trypanosoma cruzi*.

Resumen

Contexto: Las Enfermedades Tropicales Desatendidas (ETD) enfrentan desafíos crecientes debido a la resistencia de los parásitos y la eficacia cada vez menor de los tratamientos disponibles. En enfermedades como la enfermedad de Chagas y la malaria, la limitada inversión del sector privado dificulta aún más la innovación terapéutica, lo que refuerza la urgente necesidad de intervenciones más efectivas.

Objetivos: Analizar el potencial antiprotozoario de especies medicinales conocidas, aportando nuevos conocimientos sobre sus actividades biológicas.

Métodos: Se seleccionaron cinco especies de plantas etnomedicinales debido a su limitada documentación sobre actividad antiprotozoaria. Se obtuvieron veinte extractos crudos mediante maceración con solventes de polaridad creciente de las ramas de *Anchietea salutaris* A. St.-Hil. y *Davilla rugosa* Poir., y de las partes aéreas de *Banisteriopsis argyrophylla* A. Juss., *Grindelia robusta* Nutt. y *Pyrostegia venusta* (Ker Gawl.) Miers. Los extractos se probaron *in vitro* contra *Trypanosoma cruzi*, *Leishmania amazonensis* y *Plasmodium falciparum* y se evaluó su citotoxicidad para determinar los índices de selectividad (IS).

Resultados: En la evaluación preliminar a 25 µg/mL, todas las especies mostraron actividad antiprotozoaria. Ninguno de los extractos se consideró activo contra *T. cruzi*. Los extractos de hexano y diclorometano de *A. salutaris*, *B. argyrophylla* y *D. rugosa* demostraron potentes efectos antiplasmodiales (CI_{50} < 9,2 µg/mL; IS ≥ 22). Además, el extracto de diclorometano de *G. robusta* mostró una fuerte actividad contra *L. amazonensis* (CI50 = 2,2 µg/mL; IS = 78).

Conclusiones: Estos hallazgos resaltan el potencial de especies de plantas medicinales poco exploradas como fuentes de compuestos bioactivos para el desarrollo de tratamientos para enfermedades tropicales desatendidas (ETD).

Palabras Clave: agentes antiprotozoarios; *Leishmania* spp.; plantas medicinales; *Plasmodium falciparum*; medicina tradicional; *Trypanosoma cruzi*.

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Abbreviations: Balb/c: albino laboratory-bred strain of the house mouse; BGM: monkey kidney cell line; CC_{50}: Cytotoxic concentration for 50% of the cells**;** DCM: dichloromethane; DMSO: dimethyl sulfoxide; (–)-ESI-MS/MS: electrospray ionization-tandem mass spectrometry in negative mode; Hex: hexane; L929: mouse fibroblast cells; IC_{50}: half-maximal inhibitory concentration; IL-12: interleukin 12; MTT: methyl thiazolyl tetrazolium; NTDs: Neglected Tropical Diseases; PH8: *Leishmania amazonensis* strain; PMA: phorbol 12-myristate 13-acetate; SI: selectivity index; SisGen: National System of Genetic Resource Management and Associated Traditional Knowledge for Research from Brazil; THP-1: human monocyte-derived macrophage cell line.

INTRODUCTION

Neglected Tropical Diseases (NTDs) such as Chagas disease, leishmaniasis, and malaria continue to pose a major global health challenge due to the increasing resistance of parasites to existing drugs and the adverse effects associated with current therapies. Moreover, the few available drugs present several limitations: lack of pharmaceutical forms adapted to different age groups, the need for prolonged or hospital-based administration, and low stability under tropical conditions such as high temperature and humidity (Conceição et al., 2022). Despite their significant public health impact, especially in low-income and developing countries, there is limited interest from private enterprises in the development of new treatments (Álvarez-Bardón et al., 2020; Rao et al., 2023).

Evidence from the literature indicates that herbal extracts and plant-derived products serve as valuable sources of antiparasitic drugs (Ungogo et al., 2020). Moreover, sixty percent of the 20 approved entities between 1981 and 2019 are natural compounds or their derivatives (Newman and Cragg, 2020).

Given the global relevance of plant-derived compounds in antiparasitic drug development, it is noteworthy that Brazil, despite harboring approximately 20% of the world's plant biodiversity, has yet to translate this potential into significant innovation. Between 1995 and 2017, only 1.3% of patent applications for herbal medicines were approved, and none resulted in marketable products (França and Vasconcellos, 2019). Brazil also reports the highest incidence of malaria, leishmaniasis, and Chagas disease in Latin America and the Caribbean (Conceição et al., 2022). Although phytotherapy has been formally incorporated into the Unified Health System through the inclusion of twelve herbal medicines in the National List of Essential Medicines (Brasil, 2022), the development of innovative plant-based therapies targeting NTDs remains limited.

As part of ongoing efforts to discover new antiparasitic agents, the present study selected promising plant species based on ethnobotanical and pharmacological literature. While the selected plant species have been previously reported for various pharmacological activities, their antiprotozoal activity remains unexplored. This gap highlights the relevance of exploring these species as potential sources of new treatments for neglected tropical diseases.

Anchietea salutaris A. St.-Hil. plant is traditionally used in Brazil to treat allergies (Gambero and Gomes 1998) and herpetic skin conditions (Fenner et al., 2006). Its ethanol-soluble fraction has demonstrated acute hypotension and vasorelaxant effect on rats (Tolouei et al., 2019), while dichloromethane extracts have been associated with competitive inhibition of thromboxane A2 TP receptors (Gambero and Gomes, 1998).

Banisteriopsis argyrophylla A. Juss. is a medicinal plant traditionally recognized for its anti-inflammatory properties (Rodrigues and Carvalho, 2001). It has also demonstrated antifungal activity against *Candida* spp. (Oliveira et al., 2018) and antimicrobial effects against *Staphylococcus aureus* (Souza et al., 2024). In addition, studies have reported antioxidant and antidiabetic activities, including the inhibition of *a*-amylase, *a*-glucosidase, pancreatic lipase, and protein glycation (Oliveira et al., 2018; Quaresma et al., 2020).

Davilla rugosa Poir. is traditionally used in Brazil for its anti-inflammatory, stimulant, and antiulcer properties (Corrêa, 1984; Guaraldo et al., 2000; Sobrinho et al., 2018). The hydroalcoholic extract of its stems, along with its polar fractions, has been shown to increase motor activity in animal models, supporting its stimulant potential, while anxiolytic effects were observed exclusively with the whole extract (Guaraldo et al., 2000). Additionally, the extract provided gastroprotective effects in rats subjected to two acute ulcer models and showed no signs of toxicity in subchronic evaluations (Guaraldo et al., 2001). Beyond its use in Brazil, *D. rugosa* is also employed in the Peruvian Amazon for cystitis and stomach disorders and has demonstrated broad-spectrum antimicrobial activity, including effects against enterobacteria, non-enterobacteria Gram-negative bacteria, Gram-positive cocci, *Candida* spp., and clinical isolates of *Staphylococcus aureus* from both veterinary and human sources (Souza et al., 2024; Roumy et al., 2015).

Grindelia robusta Nutt., an exotic plant in Brazil, is traditionally used as an antitussive, expectorant, sedative, and as an anti-asthmatic agent (Kaltenbach and Schimmer, 1991). This species is one of the components of a pediatric syrup in Europe, Grintuss®, which showed efficacy and safety in a randomized

clinical trial for the treatment of cough persisting (Canciani et al., 2014). *G. robusta* also produces methylated flavonoids that contribute to its anti-inflammatory activity by inhibiting human neutrophil elastase activity (Krenn et al., 2009). Additionally, its methanolic extract has been shown to reduce the accumulation of inflammatory mediators in macrophages in J774 murine macrophage cells (La et al., 2010; Verma et al., 2010).

Pyrostegia venusta (Ker Gawl.) Miers is traditionally used to treat respiratory and skin disorders, including vitiligo (Scalon et al., 2008). Its aqueous extract has been shown to reduce bronchial hyperresponsiveness and airway inflammation in mice (Balestra et al., 2021), supporting its use in respiratory conditions. Anti-inflammatory (Veloso et al., 2012) and hyperpigmenting effects (Moreira et al., 2015) further corroborate its popular use for skin disorders such as vitiligo. Petroleum ether and methanolic stem extracts demonstrated analgesic activity in mice, aligning with their traditional application for flu and cold symptoms, and showed no acute toxicity at doses up to 5 g/kg (Usman and Chaubey, 2018). Additionally, flower extracts have exhibited antidepressant-like effects in mice (Veloso et al., 2010), antitumor activity against subcutaneous melanoma *in vivo* (Figueiredo et al., 2014), as well as wound-healing properties and broad-spectrum antimicrobial activity against Gram-positive, Gram-negative, and fungal species (Roy et al., 2012).

Addressing a gap in current knowledge, this study sought to assess the antiprotozoal potential of medicinal plant species until now uninvestigated for their activity against *T. cruzi*, *P. falciparum*, and *L. amazonensis*.

MATERIAL AND METHODS

Selection of the medicinal species

An initial search was conducted in the SciFinder® database (https://www.cas.org) in 2017 using the scientific names of medicinal plants sold online in Brazil through the website "Cha & Cia Produtos Naturais" (https://www.chaecia.com.br/). Initially, the scientific names of medicinal plants were combined with the genus names of *Trypanosoma*, *Plasmodium,* or *Leishmania*. Subsequently, only the genus names were used for all medicinal species and biological targets. The selection criteria focused on plants that had no reported antiparasitic activity.

Samples

A total of five medicinal plants were commercially acquired in Brazil through the website "Cha & Cia Produtos Naturais" in Jacarei, São Paulo, in 2017 (Table 1), and their authenticity was attested by the supplying company. These included branches of *A. salutaris* and *D. rugosa*, as well as aerial parts of *B. argyrophylla*, *G. robusta* and *P. venusta*. Additionally, the plants were registered on the National System of Genetic Resource Management and Associated Traditional Knowledge for Research from Brazil (SisGen) under code A646E2B.

Extracts preparations by maceration

A portion of 100 g of each dried commercial plant was extracted three times by maceration with hexane (Hex) and sequentially with dichloromethane (DCM), ethyl acetate (EtOAc), and a mixture of methanol: water (7:3) (v/v). The extractions of each solvent were filtered and concentrated on a rotary evaporator (Buchi Waterbath B-480 model, Büchi Labortechnik AG, Flawil, Switzerland) at a temperature below 45°C, and residual solvent was removed in a centrifugal evaporator (SpeedVac®, Thermo Fisher Scientific, Rockford, Waltham, USA) at 40°C. The calculated yields are shown in Table 2. An aliquot of each extract was deposited at the plant extract collection through Bioprospecting of Natural Products, part of the Fiocruz Technological Platform Network (P09-001B).

Assays with *Trypanosoma cruzi* amastigote and trypomastigote forms

The assay was conducted using *T. cruzi* (Tulahuen strain) expressing *Escherichia coli* β-D-galactosidase as a reporter gene (Romanha et al., 2010). Approximately 4,000 mouse fibroblast (L929) cells (ATCC® cell line, NCTC clone 929, USA) were plated in RPMI-1640 medium (pH 7.2-7.4) without phenol red (Gibco, Thermo Sci, Waltham, MA, USA), supplemented with 10% (w/v) fetal bovine serum (Gibco) and 2 mM glutamine, in a 96-well microtiter plate. After an overnight incubation period, 40,000 trypomastigotes were added to the cells and incubated for 2 h. The medium containing parasites that had not penetrated the cells was then replaced with 200 µL of fresh medium, and the plate was incubated for an additional 48 h to establish the infection. After this period, the medium was replaced with solutions of extracts at 25 µg/mL in fresh medium, and the plate was incubated for 96 h at 37°C. Subsequently, 50 µL of chlorophenol red-β-D-galactopyranoside (500 µM) in 0.5% Nonidet P40 was added to each well, and the plate was incubated for 16 to 20 h at 37°C. After this incubation, absorbance was measured at 570 nm using a Spectramax M5 spectrophotometer (Molecular Devices, CA, USA). Controls with uninfected cells, infected untreated cells, and infected cells treated with benznidazole (1 µg/mL or 3.8 µM, positive control) or vehicle (1% v/v

DMSO) were performed in parallel. Samples were considered promising if they inhibited at least 50% of *T. cruzi* growth at 25 µg/mL. Results are expressed as the percentage of *T. cruzi* growth inhibition in cells treated with compounds compared to infected, untreated cells. All samples were run in quadruplicate. IC_{50} values (i.e., the concentration that inhibits 50% of parasite growth) were calculated by linear interpolation from samples tested at six different concentrations (100 to 3.13 µg/mL) using GraphPad Prism v.8.2.0 (GraphPad Software, Inc., San Diego, CA, USA). The experiments were repeated in at least two independent assays.

Cytotoxic activity against L929 cell line

For this bioassay, 4,000 L929 cells in 200 µL of RPMI-1640 medium (Gibco, Thermo Sci, Waltham, MA, USA), without phenol red (Gibco) and with 10% (w/v) fetal bovine serum (Gibco) and 2 mM glutamine, were exposed to six concentrations of extracts (100 to 3.13 µg/mL), with 1% (v/v) DMSO as a negative control. After 96 h of incubation, absorbance was measured at 570 and 600 nm in a Spectramax M5 spectrophotometer (Molecular Devices, CA, USA) using alamarBlue™ (Invitrogen) (Romanha et al., 2010). Controls with untreated cells and cells treated with 1% v/v DMSO were run in parallel. Cytotoxicity was determined using quadruplicate tests, and CC_{50} values (drug concentration cytotoxic for 50% of the L929 cells) were calculated by nonlinear regression using GraphPad Prism 8.2. The experiments were repeated in at least two independent assays. The selectivity index (SI) was calculated by the ratio of the CC_{50} value in the L929 cells to the IC_{50} value of the *T. cruzi*. Extracts with SI > 10 were considered hits (Katsuno et al., 2015).

In vitro screening assays with *Plasmodium falciparum* infected erythrocytes

Activity against *P. falciparum* was tested using the chloroquine-resistant W2 clone (Oduola et al., 1988). Parasite cultures were maintained in human erythrocytes in RPMI 1640 medium (Gibco). Assays used synchronized ring-stage parasites using sorbitol incubated in 96-well plates with test samples for 48 h. Chloroquine was used as a positive control. The SYBR assay was used for evaluation (Smilkestein et al., 2004) with minor modifications (Siqueira et al., 2018), and fluorescence was read with excitation at 485 nm and emission of 535 nm using a Synergy H4 Hybrid Reader spectrophotometer (Biotek Instruments, Winooski, USA). Samples were considered promising if they inhibited 60% of *P. falciparum* at 25 µg/mL. IC_{50} values were determined for active samples in seven concentrations (50 to 0.78 µg/mL). In all tests, the compounds' activities were expressed by the 50% inhibitory concentration of the parasite growth (IC_{50}) when compared to the drug-free controls and estimated using the curve-fitting software Origin 8.0 (OriginLab Corporation, Northampton, MA, USA). The activities were considered high (≤10 µg/mL), moderate (10-20 µg/mL), or inactive (>20 µg/mL) (Chierrito et al., 2014). Each assay was performed in triplicate on the same plate and repeated in at least two independent experiments.

Cell culture and cytotoxicity test against BGM cells

The monkey kidney cell line (BGM) (ATCC®, Manassas, VA, USA), obtained from Dr. Miriam Chaves Schultz (Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil), was cultured in RPMI 1640 medium with 10% (w/v) fetal bovine serum (Gibco) and gentamicin. For cytotoxicity tests, a confluent monolayer of cells was treated with test samples at concentrations ranging from 200 to 3.13 µg/mL in DMSO, with a maximum concentration of 1% (v/v) for 24 h as a negative control. Lysosome integrity was assessed using neutral red dye uptake. The cytotoxic concentration (CC_{50}) was determined by measuring absorbance at 540 nm using a Synergy H4 Hybrid Reader spectrophotometer (BioTek Instruments, Winooski, USA) after 3 h of incubation at 37°C using at least duplicate tests. Following incubation, the medium was removed, cells were treated with a 1% (w/v) formaldehyde-1% (w/v) calcium chloride solution, the supernatant was discarded, and the dye was dissolved using a 1% (v/v) acetic acid-50% v/v ethanol solution (Borenfreund et al., 1988). The cytotoxicity concentration was determined in at least two independent assays, each performed in duplicates on the same plate, to calculate the dose that was CC_{50}, as previously described (do Céu de Madureira et al., 2002) using the Origin 8.0 software. The SI was used to assess the difference between the toxic and therapeutic dose determined from the ratio between the cytotoxicity and activity values (Bézivin et al. 2003), where an SI ≤ 10 was indicative of toxicity.

In vitro screening assays with *Leishmania amazonensis* amastigote-like forms

Amastigote forms of *L. amazonensis* (IFLA/BR/1967/PH8), isolated from skin lesions in experimentally infected hamsters, were transformed from stationary-phase promastigotes cultivated in Schneider's insect medium, supplemented with 10% (w/v) heat-inactivated fetal calf serum (Gibco) and 1% of penicillin (50 U/mL)-streptomycin (50 µg/mL) solution (Sigma-Aldrich), at pH 7.2 and 26°C (Callahan et al., 1997). After seven days under these conditions, promastigotes were induced to differentiate into 90%

amastigote-*like* forms by incubation at 32°C in Schneider's medium at pH 6.0. Samples were tested at 25 µg/mL with a parasite density of 1 × 10^6 cells/mL. Amphotericin B (0.2 µg/mL) (Fungison® Bristol-Myers Squibb B, Brazil) was used as a positive control, and DMSO (0.01% v/v) as a negative control. Parasite relative growth was assessed using methyl thiazolyl tetrazolium (MTT, 0.5 mg/mL) (Sigma-Aldrich, USA), and absorbance was measured at 570 nm in a SpectraMax M5 spectrophotometer (Molecular Devices, CA, USA) after 72 h of incubation at 32°C (Teixeira et al., 2002). Each sample was tested in triplicate, and the experiments were repeated in at least two independent experiments to ensure accuracy. Results were calculated from the measured absorbances using the formula [1-(Abs exp/Abs contr) × 100], which expresses the percentage of parasite death in relation to the controls without drug. Samples exhibiting at least 70% growth inhibition were considered active.

In vitro anti-amastigote assays with *L. amazonensis*

Human monocyte-derived macrophages (THP-1 cell line, ATCC® TIB-202) were plated at a density of 8 × 10^5 cells/mL and differentiated into macrophages by incubation with 50 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma Aldrich, USA) for 72 h at 37°C in a humidified atmosphere containing 5% CO_2. Differentiated macrophages were then infected with *L. amazonensis* amastigote forms (IFLA/BR/1967/PH8) expressing luciferase as a reporter gene (Roy et al., 2000; Tunes Guimarães et al., 2019), using a parasite-to-cell ratio of 10:1 for 3 h. Infected cells were treated with amphotericin B (Bristol-Myers Squibb) at concentrations ranging from 5.0 to 0.02 µg/mL, or with eight concentrations of *G. robusta* DCM, ranging from 0.39 to 50.0 µg/mL, in order to determine IC_{50} values. After 72 h of treatment, luciferase activity was measured by adding the One-Glo™ Luciferase Assay System substrate, and luminescence was recorded using a SpectraMax M5 spectrophotometer (Molecular Devices, CA, USA). All samples were tested in duplicates, and each experiment was independently repeated at least twice. Non-infected THP-1 macrophages were used to determine background luminescence, while untreated infected cells served as growth controls. IC_{50} values were classified as high (≤10 µg/mL), moderate (10–50 µg/mL), or weak (50–100 µg/mL), as proposed by Hassan et al. (2022). Raw data were normalized by subtracting background values and dividing by controls. Results were expressed as mean ± standard deviation (SD) values and were determined from concentration-response curves using GraphPad Prism 8.0 software.

Cytotoxic activity against THP-1 line

The cytotoxicity assay was performed using human monocyte-derived macrophages from the THP-1 cell line. Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 50 U/mL penicillin, and 50 µg/mL streptomycin (all from Gibco). They were seeded at 1 × 10^6 cells per well in 96-well plates and incubated for 72 h with 50 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma Aldrich, USA) to induce differentiation into macrophages. Amphotericin B (Fungison® Bristol-Myers Squibb B, Brazil) and the dichloromethane extract of *G. robusta* were tested at seven different concentrations (100 to 1.56 µg/mL). Viability was assessed with MTT (0.5 mg/mL) (Sigma-Aldrich, USA) after 4 h of incubation. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm (Spectramax M5 spectrophotometer, Molecular Devices, CA, USA). The sample was tested in triplicate, and the experiments were repeated in at least two independent experiments. SI were calculated using CC_{50} for the THP-1 and IC_{50} against *L. amazonensis* with extracts having an SI >10 considered as hits (Katsuno et al., 2015). CC_{50} values were determined from concentration-response curves using GraphPad Prism software 8.0.

RESULTS

Selected medicinal plants

Several medicinal plant species with established traditional medicinal uses (data not showed), available for purchase on Brazilian websites, were examined for their potential antiprotozoal activities based on a thorough search of the scholarly SciFinder® database in 2017. From this exploration, the species were selected for testing (Table 1), as none of them had been previously reported for their efficacy against trypanosomiasis, leishmaniasis, or malaria. These species have various applications in Brazilian folk medicine (Table 1) and are associated with diverse pharmacological properties according to the literature. Notably, four out of the five species are known as "cipó," characterized by climbing stems and belong to distinct botanical families. It is important to note that *G. robusta* is the only species not native from Brazil.

Extract preparation

Plant species were extracted by maceration using solvents of increasing polarities, resulting in a total of 20 extracts, of which the aqueous mixtures (**4**: mixture of methanol: water 7:3 v/v) yielded high weights (Table 2). *G. robusta* produced the highest yield among all crude extracts (from 1.9 to 6.0 % w/w),

while *A. salutaris* yielded the lowest total weight (from 0.3 to 1.9 % w/w).

In vitro screening of antiprotozoal activities

The twenty crude extracts (Table 3) were submitted to preliminary screening at a concentration of 25 µg/mL against *T. cruzi*, *L. amazonensis*, and *P. falciparum*. All species were active against one or more parasites at preliminary screening, exhibiting a growth percentage greater than or equal to 50% to *T. cruzi* and 60% to *P. falciparum* and 70% for *L. amazonensis* (Table 3). Non-polar extracts from *A. salutaris, B. argyrophylla, D. rugosa,* and *P. venusta* showed some activity against *T. cruzi*, and almost all plant species were active against *P. falciparum*. In the present study, *G. robusta* was the unique species with antileishmanial activity against amastigote-like forms.

Results of antiparasitic and cytotoxic activity assays

The active samples were further investigated to determine their IC_{50}, CC_{50}, and SI values against *T. cruzi*, *L. amazonensis*, and *P. falciparum* (Table 4). None of the extracts showed activity against *T. cruzi*; all selectivity index values (SIs) remained below 10, indicating cytotoxicity. In contrast, several hexane and DCM extracts from *A. salutaris*, *B. argyrophylla*, *D. rugosa*, and *P. venusta* exhibited strong antiplasmodial activity against chloroquine-resistant *P. falciparum*, with most SI values exceeding 20 and exhibiting no cytotoxicity. Notably, the DCM extract of *G. robusta* exhibited significant activity against *L. amazonensis*, presenting the highest selectivity index among all tested extracts (SI = 78).

DISCUSSION

Native and exotic medicinal plants are commonly traded in various cities across Brazil, available at fairs, public markets, websites, and social networks. The globalization of trade has significantly influenced the commerce of these products, with local studies highlighting the contribution of both native and exotic species. For instance, a study conducted in the mesoregions of the state of Paraíba, located in the northeast of Brazil, examined the medicinal plants sold in public markets. Among the 32 species deemed highly important by medicinal plant traders, 11 were native to Brazil, while 21 were exotic (Ferreira et al., 2021). In another investigation conducted in the rural community of Canapi, situated in the state of Alagoas, Northeast Brazil, it was observed that individuals rely on both exotic and native medicinal plants for treating diseases. The study revealed a preference for exotic species, and these were being utilized in greater quantities in the community (Dantas and da Silva, 2020).

A way to identify new plants with antiprotozoal activity is to investigate those that have already shown some significant activity. This approach can reduce time, cost, and toxicity assays in comparison to conventional drug development (Surh, 2011). In the present work, the activity of the five plant species was investigated, as they had not been reported as antiprotozoal agents in the SciFinder® database in searches conducted in 2017 and 2024. In addition, these species are already used in traditional medicine, and their toxic potential is likely low.

Parasitic diseases have imposed significant health and economic burdens. Treatments for Chagas disease, leishmaniasis, and malaria face numerous limitations, primarily due to the high toxicity of available drugs, prolonged treatment regimens, and the emergence of drug-resistant parasite strains. In the case of Chagas disease, low cure rates in the chronic stage remain a major challenge (Rao et al., 2018). In the IC_{50} determination assays, all extracts that had shown preliminary activity against *T. cruzi*, except for the dichloromethane extract of *P. venusta*, which failed to maintain its activity, displayed cytotoxicity against L929 cells (Table 4), resulting in low selectivity indexes.

In most countries, combinations of antimalarials are used to treat malaria, although plants are often used as popular medicine in poor and endemic areas for treatment and as prophylaxis of this disease (Ceravolo et al., 2024). Indeed, it is estimated that less than 10% of plant species have been studied for their pharmacological properties (Farnesworth and Soejarto, 1991). In this research, some plant species exhibited antiplasmodial activity with a good selectivity index (SI values above 10). Hexane and DCM crude extracts from *A. salutaris*, *B. argyrophylla*, and *D. rugosa*, as well as the DCM extract from *P. venusta*, were shown to be active, presenting IC_{50} values of 10 µg/mL and SI values ≥22, highlighting these species as potential candidates for *in vivo* assays.

The activity of hexane extracts from *A. salutaris, B. argyrophylla, D. rugosa*, and *G. robusta* against blood forms of *P. falciparum* could be attributed to the monoterpenes, diterpenes, sesquiterpenes, triterpenes, or aliphatic hydrocarbons. In *A. salutaris*, the triterpenes friedelin and epifriedelanol have been isolated (Matida et al., 1992), and *a*- and *β*-amyrins, as well as methyl esters of fatty acids and aliphatic hydrocarbons, have been identified in hexane leaf extracts by HR-GC/MS (Di Stasi et al., 1999).

Table 1. Information on purchased plant species with family, scientific and local names, plant part consumed, distribution, traditional use and results of research in SciFinder® Database in 2023.

Family	Scientific name	Local name[1]	Plant part consumed	Distribution[2]	Traditional use	Reference for traditional use	SciFinder® database 2024		
							T[3] n species[6] (n genus)[7]	L[4] n species[6] (n genus)[7]	P[5] n species[6] (n genus)[7]
Violaceae	*Anchietea salutaris* A. St.-Hil.	Cipó Suma, piraguaia	Roots, branches, and hut husks	Brazil and Bolivia	To treat darters and manifestations with a herpetic nature.	Fenner et al., 2006	0 (0)	0 (0)	0 (0)
Malpighiaceae	*Banisteriopsis argyrophylla* (A. Juss.) B. Gates	Cipó-prata	Aerial parts and roots	Brazil	Anti-inflammatory agent in ovarian hemorrhages, nephritis, and blennorrhagia.	Rodrigues and Carvalho, 2001	0 (1)	0 (1)	0 (0)
Dilleniaceae	*Davilla rugosa* Poir.	Cipó Caboclo, capa-homem	Branches	Meso and South America -Brazil	Purgative, tonic, anti-inflammatory, antiulcer, stimulating, aphrodisiac, and astringent agent	Corrêa, 1984	0 (0)	0 (0)	0 (0)
Asteraceae	*Grindelia robusta* Nutt.	Grindelia, girassol silvestre	Aerial parts	United States	Antitussive, expectorant, sedative, and spasmolytic	Kaltenbach and Schimmer, 1991	0 (1)	0 (1)	0 (0)
Bignoniaceae	*Pyrostegia venusta* (Ker Gawl.) Miers.	Cipó São João	Aerial parts	Meso and South America -Brazil	To treat vitiligo, diarrhea, infections, and inflammatory diseases of the respiratory system	Scalon et al., 2008	0 (0)	0 (0)	0 (0)

1: Common name in Brazil. 2: Tropicos.org. Missouri Botanical Garden. https://tropicos.org/name/Search: SciFinder® database. https://www.cas.org/cas-products/scifinder. The search terms used in the SciFinder® database 2024 were 3: T: *Trypanosoma*. 4: L: *Leishmania*. 5: *Plasmodium*. 6: Number of articles identified by plant species. 7: Number of articles identified by botanical genus.

Table 2. Extracts produced from five medicinal plants chosen to be tested in antiprotozoal assays.

Species	Crude extract (%) (w/w)			
	Hex[1]	DCM[2]	EtOAc[3]	Aq[4]
Anchietea salutaris	0.3	0.4	0.3	1.9
Banisteriopsis argyrophylla	1.6	1.1	1.5	6.7
Davilla rugosa	0.1	0.3	0.3	3.8
Grindelia robusta	5.1	5.2	1.9	6.0
Pyrostegia venusta	0.9	1.3	0.4	6.6

Yields are expressed as g/100 g of dried plant. 1: hexane extract. 2: dichloromethane extract. 3: ethyl acetate and 4: mixture of methanol: water (7:3) (v/v) extracts.

Table 3. Results on the *in vitro* antiprotozoal activities of extracts of five Brazilian plant species.

Species	Growth inhibition (%)											
	T. cruzi				*L. amazonensis*				*P. falciparum*			
	Hex[1]	DCM[2]	EtOAc[3]	Aq[4]	Hex[1]	DCM[2]	EtOAc[3]	Aq[4]	Hex[1]	DCM[2]	EtOAc[3]	Aq[4]
Anchietea salutaris	94.8	92.5	-	-	-	-	-	-	85.1	78.7	-	-
Banisteriopsis argyrophylla	-	87.8	-	-	-	-	-	-	80.3	84.8	67.8	-
Davilla rugosa	78.7	-	-	-	-	-	-	-	85.9	90.9	86.5	-
Grindelia robusta	-	-	-	-	-	82.4	-	-	64.2	-	-	-
Pyrostegia venusta	-	100.0	-	-	-	-	-	-	-	79.0	60.0	-
Controls												
Amphotericin B (0.2 μg/mL)					74.5							
Benznidazole (1 μg/mL)	72.8											
Chloroquine (0.193 μg/mL)									50.0			

1: Hexane extract. 2: Dichloromethane extract. 3: Ethyl acetate and 4: Mixture of methanol: water (7:3) (v/v) extracts. (-): Inactive sample in preliminary screening at 25 μg/mL.

Table 4. Results of activity and cytotoxicity of crude extracts of five Brazilian plant species against *Trypanosoma cruzi*, *Plasmodium falciparum* and, *Leishmania amazonensis.*

Species	Extract	*T. cruzi* (IC_{50})	L929 (CC_{50})	SI	*L. ama.* (IC_{50})	THP-1 Φ (CC_{50})	SI	*P. falciparum* (IC_{50})	BGM (CC_{50})	SI
Anchietea salutaris	Hex[1]	76.7 ± 2.6	75.3 ± 9.2	1	-	n.a.	n.a.	3.5 ± 1.2	≥200	≥57
	DCM[2]	38.9 ± 2.8	59.9 ± 6.8	2		n.a.	n.a.	7.1 ± 1.0	≥200	≥28
Banisteriopsis argyrophylla	Hex[1]	-	n.a.	n.a.	-	n.a.	n.a.	8.1 ± 0.3	≥200	≥25
	DCM[2]	72.9 ± 0.6	26.9 ± 2.6	<1	-	n.a.	n.a.	5.8 ± 0.2	≥200	≥34
	EtOAc[3]	-	n.a.	n.a.	-	n.a.	n.a.	18.6 ± 0.9	≥200	≥11
Davilla rugosa	Hex[1]	45.7 ± 0.9	56.2 ± 14.4	1	-	n.a.	n.a.	9.2 ± 1.8	≥200	≥22
	DCM[2]	-	n.a.	n.a.	-	n.a.	n.a.	3.6 ± 0.9	114.5 ± 0.7	32
	EtOAc[3]	-	n.a.	n.a.	-	n.a.	n.a.	20.8 ± 3.8	IN	n.a.
Grindelia robusta	Hex[1]	-	n.a.	n.a.	-	n.a.	n.a.	18.4 ± 1.6	140.9 ± 26.7	8
	DCM[2]	-	n.a.	n.a.	2.2 ± 0.2	175.5 ± 40.4	78	-	n.a.	n.a.
Pyrostegia venusta	DCM[2]	IN	n.a.	n.a.	-	n.a.	n.a.	4.5 ± 2.0	≥200	≥44
	EtOAc[3]	-	n.a.	n.a.	-	n.a.	n.a.	22.7 ± 1.6	IN*	n.a.

1: Hexane extract; 2: Dichloromethane extract; 3: Ethyl acetate extract. *T.* cruzi: *Trypanosoma cruzi*. L929: Mouse fibroblast cell line. SI: Selectivity Index = CC_{50} /IC_{50}. *L. ama.*: *Leishmania amazonensis*. THP-1Φ: Human leukemia monocytic cell line differentiated into macrophage-*like* cells. *P. falciparum* W2: Chloroquine-resistant *Plasmodium falciparum* clone. BGM: Monkey kidney epithelial cell line. IC_{50}: Concentration of extract or positive control at μg/mL causing 50% growth inhibition. Values represent standard deviation from at least two independent experiments. CC_{50}: cytotoxic concentration for 50% of the cells. Values represent standard deviation from at least two independent experiments. (-): inactive sample in preliminary screening at 25 μg/mL. n.a.: not applicable; the value was not determined because the extract showed no activity in the preliminary assay and was therefore not tested in the dose–response assay to calculate the IC_{50}. IN: inactive sample in dose-response assay. Positive controls are expressed as IC_{50} ± standard deviation: Amphotericin B against *L. amazonensis*: 0.07 ± 0.007, Benznidazole against *T. cruzi*: 0.5 ± 0.1, Chloroquine against *P. falciparum* W2: 0.137 ± 0.014.

In *D. rugosa*, the terpenoids friedelin, sitostenone, and betulinic acid have been isolated from the stems (David et al., 2006). Regarding *G. robusta*, polyacetylenes such as matricarianol and its acetate derivatives have been isolated from the roots (Schulte et al., 1964), and monoterpenes such as bornyl acetate (Kaltenbach et al., 1993), pinene, and borneol (El-Shamy et al., 2000; Fraternale et al., 2007) have been identified as major compounds in the essential oil of this plant.

Based on previous studies, no specific class of natural products, such as more polar diterpenoids, could be associated with the activity observed in the DCM extracts of *A. salutaris*, *B. argyrophylla*, *D. rugosa*, and *P. venusta*. It is possible that moderately polar compounds also contribute to these effects, beyond known terpenoids. The antiplasmodial activity detected may also be associated with the combined presence of flavonols, flavanones, and methylated or less hydroxylated flavonoid derivatives, which are plausible candidates considering the reported presence of phenolic compounds in these species (David et al., 2006; Quaresma et al., 2020; Timmermann et al., 1994; Tolouei et al., 2019; Veloso et al., 2010; 2012).

Additionally, compounds commonly reported for *B. argyrophylla*, *D. rugosa*, and *P. venusta* exhibit relatively higher polarity and could predominantly account for the antiplasmodial activities found in the ethyl acetate extracts, a solvent typically employed for the extraction of phenolic compounds such as flavonoids. In *B. argyrophylla*, catechin, procyanidins, glycosylated flavonoids (notably quercetin and kaempferol derivatives), and megastigmane glucosides were identified by (–)-ESI-MS/MS (Quaresma et al., 2020). Active fractions from *B. argyrophylla* leaf extracts against *Candida* spp. were also found to contain (–)-catechin as well as quercetin and kaempferol derivatives (Oliveira et al., 2018). In *D. rugosa*, flavonoids such as naringenin, quercetin, and 4'-*O*-methyltaxifolin have been isolated from the stems (David et al., 2006). Similarly, *P. venusta* displays a broad distribution of flavonoids across flowers, roots, stem bark, and aerial parts (Dinda et al., 2002; Dubey and Misra, 1976; Ferreira et al., 2000). Furthermore, roots of *P. venusta* produce several flavonoids, have demonstrated vasorelaxant effects in isolated rat aortic rings (Reyes-Martínez, 2019).

In this work, only the DCM extract from the aerial parts of *G. robusta* showed high activity against *L. amazonensis* and high selectivity (SI > 10) for the parasite. The search in the SciFinder® database in 2024 (Table 1) revealed just one report of antileishmanial activity for acid diterpenes, specifically five compounds isolated from *G. camporum*, which showed activity against *L. infantum* with IC_{50} values ranging from 9.67 to 63.6 µg/mL (Bailen et al., 2020). Although the *B. argyrophylla* extracts have not shown any activity against *L. amazonensis*, the essential oils from another species of the same genus, *B. campestris*, have previously demonstrated activity against *L. amazonensis* with IC_{50} values exceeding 150 µg/mL and were cytotoxic, with SI values against Vero cells ranging from -0.5 to > 0.34 (Rocha et al., 2020).

Although *G. robusta* Nutt. may contain medium-polar compounds such as previously reported methylated flavonols (Krenn et al., 2009), flavones (Timmermann et al., 1994), and sapogenins (Kreutzer et al., 1990) in some concentration in the DCM extract; they are more likely to occur in the more polar extracts, such as ethyl acetate. According to previous studies, it is hypothesized that diterpene acids are the most promising antileishmanial candidates. These compounds have been isolated from species of the same genus, *G. camporum* (Hoffmann et al., 1988) and *G. squarrosa* extracts (Timmermann et al., 1985), using non-polar solvents such as DCM, and some of the diterpene acids from *G. camporum* have demonstrated antileishmanial activity (Bailen et al., 2020).

Several medicinal plants used in folk medicine have been reported to possess both antiparasitic and immunomodulatory properties, both *in vitro* and *in vivo*. These plants are known to produce mediators such as IL-12 during *Leishmania* infections (Rodrigues et al., 2015) or have demonstrated significant effects against the *Plasmodium* parasite by delaying inflammatory immune responses to malaria or by affecting the balance of pro-inflammatory and anti-inflammatory cytokines in a Balb/c mouse model (Afolayan et al., 2020). The active extracts selected in this study may act in a similar manner, as they have been reported to possess various biological activities. One hypothesis for the observed activity of these species is the presence of flavonoids, which have been reported in all species (David et al., 2006; Quaresma et al., 2020; Timmermann et al., 1994; Tolouei et al., 2019; Veloso et al., 2010; 2012).

Considering the antiparasitic properties reported for the selected medicinal plants, as evidenced throughout this study, the findings reinforce the idea that traditionally used species like *A. salutaris, B. argyrophylla, D. rugosa, P. venusta,* and *G. robusta* possess significant potential as sources of bioactive metabolites. These metabolites could be valuable in the development of antimalarial or antileishmanial drugs, thereby expanding the possibilities for treating these parasitic diseases.

CONCLUSION

The present study contributes to identifying medicinal plant species as potential antiprotozoal agents. To the best of our knowledge, this is the first report describing activity for the *A. salutaris*, *B. argyrophylla, D. rugosa, P. venusta,* and *G. robusta* plant species. All these extracts, particularly the *A. salutaris* and *P. venusta* against *P. falciparum*, and *G. robusta* against *Leishmania* spp. are promising candidates for bioassay-guided fractionation to isolate and/or identify bioactive compounds using techniques like LC-MS/MS or ESI-LC/MS. Additionally, *in vivo* assays to validate their antiprotozoal effects could also explore potential immunomodulatory mechanisms, including cytokine modulation (e.g., interleukin-12), induction of reactive oxygen and nitrogen species, and evaluation of mitochondrial function, which may help elucidate the therapeutic potential of these species.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Ceravolo IP	Moreira POL	Maia DNB	Fonseca DCS	Chaves ALS	Murta SMF	Alves TMA	Cota BB
Concepts or ideas								x
Design	x							x
Definition of intellectual content	x							x
Literature search	x							x
Experimental studies	x	x	x	x	x	x	x	x
Data acquisition	x	x	x	x	x			x
Data analysis	x	x				x	x	x
Statistical analysis	x	x						
Manuscript preparation	x							x
Manuscript editing	x							x
Manuscript review	x	x	x	x	x	x	x	x

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