Evaluation of in vitro Schistosomicidal and Trypanocidal Activity of Leaf Extracts of Hancornia speciosa

Avaliação da Atividade Esquistossomicida e Tripanocida in vitro dos Extratos Foliares de Hancornia speciosa

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ABSTRACT

Medicinal plants are viable for treating various diseases, as the metabolites they contain demonstrate a great diversity of biological activities. In this study, we conducted a phytochemical analysis and evaluated the in vitro antiparasitic activity of extracts from the leaves of Hancornia speciosa, popularly known as mangabeira, against *Trypanosoma cruzi* and *Schistosoma mansoni* parasites. We collected the leaves of this species in the municipality of Urutaí - GO, and obtained an ethanolic extract, in which classes of organic acid compounds, coumarins, reducing sugars, catechin tannins, foaming saponins and flavonoids were identified by phytochemical analysis. The antiparasitic evaluation of the fractions obtained showed that *H. speciosa* was ineffective against *S. mansoni*. However, the hexane fraction of the extract of mangaba is moderately effective against *T. cruzi*, demonstrating IC₅₀ values of 31.71 μg mL⁻¹. This is the first time the antiparasitic activity of *H. speciosa* has been described in the literature.

Keywords: Brazilian Cerrado; Mangabeira; *T. cruzi*; *S. mansoni*; Phytochemical analysis.

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RESUMO

As plantas medicinais são alternativas viáveis para o tratamento de diversas doenças, pois os metabólitos presentes apresentam uma grande diversidade de atividades biológicas. Neste estudo, realizamos a análise fitoquímica e avaliamos a atividade antiparasitária *in vitro* de extratos das folhas de *Hancornia speciosa*, popularmente conhecida como mangabeira, contra os parasitas *Trypanosoma cruzi* e *Schistosoma mansoni*. Coletamos as folhas desta espécie no município de Urutaí - GO, e obtivemos um extrato etanólico, no qual foram identificadas por análise fitoquímica as classes de compostos de ácidos orgânicos, cumarinas, açúcares redutores, taninos catequinas, saponinas espumantes e flavonóides. A avaliação antiparasitária das frações obtidas revelou que *H. speciosa* foi ineficaz contra *S. mansoni*. Entretanto, a fração hexânica do extrato da mangaba é moderadamente eficaz contra *T. cruzi*, com valores de IC₅₀ de 31,71 μg mL⁻¹. Esta é a primeira vez que é descrita na literatura a atividade antiparasitária de *H. speciosa*.

Palavras-chave: Brazilian Cerrado; Mangabeira; *T. cruzi*; *S. mansoni*; Análise Fitoquímica.
INTRODUCTION

The biome of Brazilian Cerrado has an interesting diversity in both fauna and flora, showing a large number of known and unknown native species (Ribeiro Neto et al., 2020). It is the second largest biome in Brazil, occupying approximately 21% of the country’s area in the plateau of the South American continent (Borlaug, 1997; Klink e Machado, 2005). The states of the Federal District, Goiás, Tocantins, Maranhão, Mato Grosso do Sul, and Minas Gerais are all occupied by the Cerrado biome. Furthermore, the extensive predatory use of the Cerrado’s areas without appropriate mitigation of environmental problems, such as actions by the government to reduce the devastation of the native area has been impacting the maintenance of native species (Brasil, 2018; Ribeiro Neto et al., 2020).

In the Brazilian Cerrado, it is common to find a wide variety of plants that are used in folk medicine and ethnopharmacologically by the communities living in the regions where the biome is found (Castro Oliveira e Viveiro, 2013). The use of medicinal plants to treat diseases is one of the oldest ways that humans have found to treat illnesses (Bruning, Mosegui e Vianna, 2012). The reason for the important applications of plants or parts of them in the treatment of diverse sicknesses is due to special metabolites, natural compounds produced in plants as a by-product of primary metabolism (Kim et al., 2021). Although they do not have a vital function for the plant, they guarantee its survival, the perpetuation of the species, attract pollinators and seed dispersers, act against herbivores, and more (Böttger et al., 2018). Due to the important biological responses of the secondary metabolites, they are applied for pharmacological purposes. Recent studies estimated that in the last four years around 23.5% of drugs approved worldwide are natural products or derivatives of them (Newman e Cragg, 2020).

One of the plants used for medicinal purposes in the Brazilian Cerrado is Hancornia speciosa Gomes, commonly known as “mangabeira”. It is a fruitful species found in the state of Goiás - Brazil, known for its acidic and tasty fruits, which can be used in food products. This plant is of particular interest due to its high biological potential; several of these studies on H. speciosa extracts have shown promising biological responses as antioxidant, antimicrobial, cytotoxic, antidiabetic, and anti-inflammatory agents (Bitencourt et al., 2019; Neto et al., 2020; Santos, Campos, Torquato, H. F. v., et al., 2016). In recent studies, Panontin and coworkers tested the possible use of H. speciosa extracts in cosmetic preparations, where they observed the
low genotoxicity and antimutagenic indexes of the tested fractions (Panontin et al., 2022), which reinforced the toxicological analysis proposed by the authors in previous studies of the research group (Panontin et al., 2021).

Tropical neglected diseases caused by parasites are infectious diseases that represent important opportunities to discover new lead molecules from natural products (Ndjonka et al., 2013). Chagas disease is responsible for affecting 6 to 7 million people worldwide and causing about 10,000 deaths annually (WHO, World Health Organization, 2022b). This infectious disease is caused by the parasite Trypanosoma cruzi, found in the feces of the “barbeiro” bug (Traina et al., 2017). Schistosomiasis is a parasitic disease typical of the Americas, Asia, and Africa and is caused by the parasites of genus Schistosoma sp. (Lopes et al., 2017). Approximately six million people are infected with these parasites worldwide, and at least 251.4 million people required preventive treatment for schistosomiasis in 2021 (WHO. World Health Organization, 2022a).

In both parasites, treatment focuses on the use of drugs that kill the parasite or promote control of the symptoms caused by it, but these drugs can show limited effectiveness, do not prevent reinfection (Garcia e Fox, 2014). Considering that current chemotherapy is often inadequate to treat these diseases and the alarming cases of resistance, the search for new therapeutics is urgently needed (Cribb et al., 2019; Müller Kratz et al., 2018). In addition, according to preliminary studies present in the scientific literature, there is limited data on the chemical constitution and some biological activities of H. speciosa leaf extracts (Bitencourt et al., 2019; Jácome et al., 2022). Therefore, we propose here the phytochemical analysis and testing of the antiparasitic potential of H. speciosa Gomes leaf extracts against Schistosoma mansoni and Trypanosoma cruzi.

MATERIALS AND METHODS

1. Plant material, fractions extraction, and phytochemical analysis

Intact leaves of H. speciosa absent from herbivory attacks were collected near the IF Goiano, in the city of Urutaí, Goiás, Brazil (17°22'42"S 48°14'44"W). The collected materials were exsiccated, classified and the voucher specimens (voucher number HUEG 16.433) were deposited in the Herbarium of Universidade Federal de Goiás. A sufficient mass of freshly collected leaves (170 g) was dried in an oven, miniaturized, and placed in a 2L flask. To this, 1200 mL of ethanol (P.A., 95%, Neon, São Paulo-SP, Brazil) was added. This mixture was manually stirred for 7 days and then filtered. The extract was
evaporated at 40°C under reduced pressure to dryness in a rotary evaporator (Fisatom 801 model, Fisatom, São Paulo-SP, Brazil). Part of the extract (1.0 g) formed was suspended in water and partitioned in increasing polarity (3 x 15 mL) with hexane (P.A., Dinâmica, São Paulo-SP, Brazil), dichloromethane (P.A., Neon, São Paulo-SP, Brazil) and ethyl acetate (P.A, Burdick & Jackson, Muskegon-MI, USA) (Simões et al., 2016). All fractions obtained from organic solvents were dried with dry magnesium sulfate (Vetec, Duque de Caxias-RJ, Brazil). Finally, the remaining fraction was dried at room temperature, and, together with the extracted fractions, stored in Eppendorf-type flasks in a refrigerator (-2 °C to +8°C) until they were sent for antiparasitic activities.

The phytochemical analysis was performed according to the literature (Marinho et al., 2022; Matos, 1988; Menezes Filho et al., 2018; Sousa Carvalho et al., 2015). The final part of the ethanolic extract was suspended in water at concentration of 150 mg mL⁻¹, with 3 mL used for each experimental determination. All phytochemical experiments were performed in triplicates, and the intensity of the reaction visualized was considered (+++) as undoubtedly positive, (++) moderately positive, (+) less positive, and (-) negative, following the procedure described by Marín and coworkers (Marin et al., 2018).

2. Antiparasitic activity

The antiparasitic activity against *S. mansoni* was tested as previously reported in the literature (Carvalho et al., 2022; Martins et al., 2017). Adult worms (49 ± 2 days) of the Luiz Evangelista (LE) *S. mansoni* strain were recovered from Balb/c mice previously infected with 200 cercariae by perfusion of their livers and mesenteric veins. The worms were transferred to a 24-well culture plate (2 pairs of worms per well) containing 2 mL of RPMI 1640 medium (Cultilab, Campinas-SP, Brazil) buffered with 20 μM HEPES, pH 7.5 and supplemented with penicillin (100 U/mL), streptomycin (100 μg mL⁻¹, Cultilab), and 10% v/v fetal bovine serum (FBS) (Cultilab). The worms were then incubated in a humidifying atmosphere at 37°C and 5% CO₂ for 24 h. After the incubation period, the samples were solubilized in dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, USA) and applied at a concentration of 50 μg mL⁻¹. Adult worms were incubated under the same conditions described above and evaluated every 24 h for 72 h by visual analysis using an inverted microscope model Primo Vert (Carl Zeiss, Oberkochen, Germany) (4 – 10x). The observed phenotypic changes were classified on a phenotypic viability scale (Ramirez et al., 2007). As controls, praziquantel (PZQ, Sigma-Aldrich, St. Louis, USA)
was previously solubilized in DMSO and added at a concentration of 1 µg.mL⁻¹ (positive control), and RPMI 1640 medium supplemented with 0.1% DMSO (negative control).

The antiparasitic activity against *T. cruzi* was also tested as previously established in the literature (Pagotti *et al.*, 2021a). The *T. cruzi* Y strain was maintained *in vivo* in male BALB/c mice and on C₂C₁₂ line cells culture (fibroblasts of *Mus musculus* mice) maintained in RPMI 1640 medium (Cultilab) supplemented with 5% FBS (Cultilab) 10,000 µg mL⁻¹ of penicillin (Cultilab), and 10,000 µg mL⁻¹ of streptomycin (Cultilab) at 37°C and 5% CO₂ with 95% humidity. After 7 days of cultivation, trypomastigote forms at a final concentration of 1 x 10⁶ parasites/well in supplemented RMPI 1640 medium (Cultilab) were added to each well of a sterile 96-well plate and the samples were solubilized in dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, USA) and applied at a concentration of 100 µg mL⁻¹. The culture was maintained at 37 °C and in 5% CO₂ atmosphere, and the number of viable parasites (mobile parasites with typical format) was determined by counting in a Neubauer chamber (Global Glass, São Paulo, Brazil) with the aid of an optical microscope (Nikon, New York, USA), after 24 h of incubation with the samples. The assay was performed at concentrations from 100 to 3.12 µg mL⁻¹ as described before. As controls, benzonidale (benznidazole - LAFEPE, Pernambuco) previously solubilized in DMSO was added at a concentration of 2.602 µg mL⁻¹ or 0.004 to 2.602 µg mL⁻¹ (positive control), and RPMI 1640 medium supplemented with 0.1% DMSO (negative control).

3. Cytotoxic activity

Fibroblasts of the C₂C₁₂ cell line (2x10⁵ cells/well) were seeded in a 96-well and cultured in DMEM medium (Dulbecco's Modified Eagle Medium, Invitrogen) supplemented with 2 mM L⁻¹ of glutamine, 10 mM NaHCO₃, 5% FBS (Cultilab) and 10,000 µg mL⁻¹ of penicillin, 10,000 µg mL⁻¹ of streptomycin (Cultilab) at 37°C in a modified atmosphere oven at 5% CO₂ and 95% humidity for 24 h. After the period, promising fractions were dissolved in DMSO and added at concentrations ranging from 3.12 to 300 µg mL⁻¹, and the cells were cultivated in the same previously described conditions for 48 h. After, the cells were washed and the viability was determined by the MTT colorimetric method (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) (Sigma Aldrich, St. Louis, USA) (Ferrari, Fornasier e Isetta, 1990). MTT was dissolved at 10 mg/mL in phosphate-buffered saline (PBS) and 20 µL of this solution was added to each well. The plates were incubated at 37°C for 4 h and the formazan precipitate was
solubilized with 100 µL of isopropyl alcohol (Synth, Diadema, Brazil). Absorbance was determined using a spectrophotometer (Libra S12–Biochrom, Holliston, USA) at a wavelength of 570 nm. DMEM medium containing 0.1% DMSO (Synth, Diadema, Brazil) was used as a negative control and 25% DMSO was used as a positive control.

4. Statistical analysis

Data were expressed as mean ± standard deviation (SD) and the experiments were performed in triplicate and repeated twice. The IC\textsubscript{50} values (concentration that inhibited trypomastigote viability by 50%) and the CC\textsubscript{50} values (concentration that was cytotoxic to 50% of the cells) were calculated by using a nonlinear regression dose–response inhibition curve. The selective index (SI), which indicates the parasite toxicity compared to the host, was calculated as the ratio between CC\textsubscript{50} and IC\textsubscript{50} (Katsuno \textit{et al.}, 2015). The analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, California, USA).

RESULTS AND DISCUSSION

1. Extraction and phytochemical analysis

The obtained extract of \textit{H. speciosa} leaves has a clear, homogenous, aspect and dark green color, yielding an extraction efficiency of 2.70% w/w. Part of the extracted mass was used on phytochemical prospecting of the main secondary metabolite groups of the ethanolic extract of \textit{H. speciosa}, as summarized in Table I. The ethanolic extract analyzed was positive for organic acids, coumarins, reducing sugars, tannins, catechins, saponins, and flavonoids. Organic acids were identified as expected from our phytochemical analysis, and as present in several species of \textit{Hancornia} described in the literature. Bastos and coworkers described the presence of organic acids in alcoholic extracts of \textit{H. speciosa} using UHPLC-MS (Bastos \textit{et al.}, 2017). In this work, the authors identified the presence of quinic acid, protocatechuic acid, caffeic acid, 3-\textit{O}-(E)-p-coumaroylquinic acid, 3-\textit{O}-(Z)-3-p-coumaroylquinic acid, among others. These organic acids, such as caffeic acid have several biological activities that have been assayed in the literature, including antioxidant, antithrombosis, antihypertensive, and antifibrotic activities (Paracatu \textit{et al.}, 2014).

In the studies carried out by Neto and coworkers, the phytochemical profile of the leaves and inner bark, as well as the phytoprospecting of the latex of \textit{H. speciosa} were
studied, with no detection of coumarins (Neto et al., 2015). However, in our studies the yellowing of the filter paper and its interaction with UV light in our experiments indicated the presence of this class, which had not been detected in other studies in the literature. Such differences may be due to different weather and cultivation conditions of the plant specimen, leading to the production of other special metabolites (Dantas et al., 2021). According to the literature, negative results do not necessarily imply the total absence of the secondary metabolite species; it is possible that the minimum detection level is below that of qualitative tests evaluated (Brum et al., 2012). Coumarins are an important class of special metabolites, and their biological responses depend on the substitutions of the cyclic moieties. Nonetheless, coumarins are known for their antiviral, antitumor, anti-inflammatory, and other biological activities, as described in the literature (Li et al., 2022; Liu et al., 2019; Weinmann, 1997; Xu et al., 2021).

The presence of reducing sugars in the leaf extract was evidenced by the appearance of a dark-red precipitate in the qualitative tests. This experiment corroborates the results presented in previous studies, where the physical and chemical characterization of the fruits of H. speciosa identified the class of compounds (Souza et al., 2007). Tannins were identified by iron (III) chloride tests, evidenced by the presence of catechin tannins in the H. speciosa extract, as shown in Table I. According to previous studies, the presence of tannins in high concentrations in the ethanolic extracts of H. speciosa is attributed to their antibacterial and antifungal activities (Costa et al., 2008). Catechins have been described in previous works for the species by UHPLC-HRMS in comparison to reference standards such as (+)-catechin, (-)-catechin, and (epi)-catechin, corroborating the phytochemical analysis proposed here (Rodrigues et al., 2007).

Table I. Phytochemical profile of the main secondary metabolites on ethanolic extract of H. speciosa.

<table>
<thead>
<tr>
<th>CLASS OF COMPOUNDS</th>
<th>EXPERIMENT</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORGANIC ACIDS</td>
<td>Pascová reagent</td>
<td>+++</td>
</tr>
<tr>
<td>COUMARINS</td>
<td>UV light (254 and 365 nm)</td>
<td>+</td>
</tr>
<tr>
<td>POLYSACCHARIDES</td>
<td>Lugol test</td>
<td>-</td>
</tr>
<tr>
<td>REDUCING SUGARS</td>
<td>Fehling reagent</td>
<td>+++</td>
</tr>
<tr>
<td>PHENOLS</td>
<td>FeCl₃</td>
<td>-</td>
</tr>
<tr>
<td>TANINS</td>
<td>FeCl₃</td>
<td>Gr</td>
</tr>
<tr>
<td>ANTHRAQUINONES</td>
<td>Bornträger reagent</td>
<td>-</td>
</tr>
<tr>
<td>STEROIDS AND TRITERPENOIDS</td>
<td>liebermann-buchard’s test</td>
<td>-</td>
</tr>
</tbody>
</table>
The presence of foamy saponins was observed in ethanolic extracts of *H. speciosa*, as earlier described in previous works (Chaves *et al.*, 2020; Panontin *et al.*, 2022). Chaves and coworkers described the presence of cardiotonic glycosides for the detection of steroidal nuclei and suggested the possibility of applying the *H. speciosa* extract in cosmetic bioprospection due to its detergent properties (Chaves *et al.*, 2020). In addition, the presence of flavonoids is still evidenced in the Pew’s test (365 nm) (Table I). This class of compounds was identified and is in accordance with the literature (Neto *et al.*, 2015; Panontin *et al.*, 2021). The presence of such compounds is closely correlated to known antioxidant and anti-aging actions, further reinforcing the possibility of its use in cosmetics (Panontin *et al.*, 2021; Santos, Campos, Torquato, H. F. v., *et al.*, 2016; Zaid e Ramahi, al, 2019).

2. *Antiparasitic activity against S. mansoni, T. cruzi* and cytotoxicity

Several species of the Apocynaceae family, present in a great diversity of plants in the Brazilian Cerrado, have been described in the literature to have biological potential against parasites such as *S. mansoni*. Melo and coworkers described latexes obtained from Apocynaceae with promising effects against parasites, such as *Cryptostegia grandiflora* (Melo, Bonilla e Lucena, 2021). In the same work, many studies described *H. speciosa* latex as having antioxidant, antibacterial, anti-inflammatory, osteogenic, angiogenic, anti-biofilm, healing, and non-toxic activities. The anti-inflammatory profile of this species also draws attention, since *S. mansoni* infections can influence the development of immune responses in diseases caused by infections (Pearce e MacDonald, 2002). Therefore, compounds that present a certain anti-inflammatory potential can potentially act positively in the immune response against schistosomiasis. Previous studies in the literature determined the antifungal capacity of *H. speciosa* latex against *Candida albicans* (Silva *et al.*, 2011). Vasodilator properties were also verified in mangabeira...
leaves from the ethanolic extract. Moreover, previous reports described its effectiveness in combating and healing gastric ulcers due to its ability to stimulate the synthesis of mucus and produce an antisecretory effect (Moraes et al., 2008). However, to date, no studies of schistosomicidal and trypanocidal effects of H. speciosa leaf extracts have been presented in the literature.

To obtain data on antiparasitic activity against S. mansoni and T. cruzi, organic fractions of H. speciosa were obtained from the crude extract of the plant by liquid-liquid extraction with reactive solvents of increasing polarity (HS-F1 to HS-F4). We tested the action of HS-F1-F4 against S. mansoni first at a concentration of 50 µg/mL, aiming to carry out an initial screening. In these experiments, H. speciosa fractions did not show activity against adult worms of S. mansoni at a concentration of 50 µg mL⁻¹ after 24 and 72 h, as shown in Table II. On the other hand, the positive control, PZQ, caused 100% mortality at a concentration of 1.0 µg mL⁻¹.

Table 2. In vitro schistosomicidal and trypanocidal screening of H. speciosa fractions

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>SCHISTOSOMICIDAL ACTIVITY</th>
<th>TRYANOCIDAL ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% INHIBITION OF VIABILITY</td>
<td>% INHIBITION OF Flagellar MOTILITY</td>
</tr>
<tr>
<td></td>
<td>24 h 72 h</td>
<td>24 h</td>
</tr>
<tr>
<td>NEGATIVE CONTROL</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>POSITIVE CONTROL</td>
<td>100.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>HS-F1</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>HS-F2</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>HS-F3</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>HS-F4</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

a percentage of inhibition of viability of Schistosoma mansoni (LE) adult worms after incubation with fractions of H. speciosa at the concentration of 50 µg.mL⁻¹ at 24 and 72. b percentage of inhibition of flagellar motility of the trypomastigote the Trypanosoma cruzi (Y) after incubation with fractions of H. speciosa at the concentration of 100 µg.mL⁻¹ at 24 h. c RPMI 1640 medium + 0.1% DMSO. d praziquantel (Sigma-Aldrich) at concentrations of 1 µg.mL⁻¹ (schistosomicidal assay) or benzonidazole (LAPEMA) at concentration 2.602 µg.mL⁻¹ (trypanocidal assay). Data expressed ± standard deviation and the experiments were performed in triplicate and repeated twice.

The determination of antiparasitic activity against T. cruzi followed the same procedure for testing extracts at a screening concentration of 100 µg mL⁻¹. All the results of the initial screening experiments were tabulated and arranged in Table II. The data
obtained from the experiments suggest that the more non-polar fraction extracted (HS-F1) presents the highest percentage of inhibition of flagellar motility, with 62.52% ± 5.72 inhibition. Nonpolar classes of organic compounds, such as foaming saponins identified in fractions of *H. speciosa*, may be present in the hexane phase. These compounds have known hemolytic activity and may act in nonspecific lysis of cells, promoting complexation of cholesterol with cell membranes of protozoa (Ramos-Morales *et al.*, 2017).

In this sense, the IC$_{50}$, CC$_{50}$, and selectivity index (SI) values for HS-F1 were determined (Table III). The fraction HS-F1 showed moderate effectiveness in *in vitro* experiments against trypomastigote forms of *T. cruzi*, with an IC$_{50}$ value of 31.71 µg mL$^{-1}$. Furthermore, it is also proposed in the literature that slightly polar fractions permeate freely within cell membranes and end up killing parasites by affecting cytoplasmic metabolic pathways, as well as the organelles present therein. Another factor that must be considered is the fact that non-polar organic fractions cause drastic changes in the physiology of the parasite's membrane, promoting a loss of permeability and consequent death (Borges *et al.*, 2012). This hypothesis is consistent with our results, as the hexane fraction (F1) of *H. speciosa* was active against *T. cruzi*.

To evaluate the chemotherapeutic properties of extracts, it is important to determine the fraction of cytotoxic potential. The literature establishes a criterion to indicate the cytotoxic concentrations for natural products, where values lower than 100 µg mL$^{-1}$ are considered strongly cytotoxic, between 100 and 500 µg mL$^{-1}$ are considered moderately cytotoxic, and higher than 500 µg mL$^{-1}$ are considered weakly/non-cytotoxic for mammalian cells (Ríos *et al.*, 2008; Souza *et al.*, 2017). In this sense we evaluated the fraction HS-F1 as moderately cytotoxic against the cells tested, presenting CC$_{50}$/48h values of 216.8 µg mL$^{-1}$, exhibiting a considerable difference compared to the reference drug as described in past works (Pagotti *et al.* 2021a). Similar results have been reported in cytotoxic tests for fractions of *H. speciosa* (Santos, Campos, Torquato, H. F. V., *et al.*, 2016), for the extracts obtained from fruits, which respect the cytotoxic potential of the ethanolic fraction against *Artemia salina* (LD$_{50}$ = 219.2 µg mL$^{-1}$) (Assumpção *et al.*, 2014). In addition, we determined the selectivity index (SI) to parallel the trypanocidal activity of HS-F1 against *T. cruzi* and toxicity by MTT assays in selected cells. The results pointed out that the SI value for HS-F1 is 6.83, so the fraction tested is more effective against the trypomastigote forms.
Table 3. *In vitro* trypanocidal and cytotoxic activities and determination of selective index (SI).

<table>
<thead>
<tr>
<th>CONCENTRATIONS (μg mL⁻¹)</th>
<th>HS-F1</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td>69.50±3.29</td>
</tr>
<tr>
<td>50</td>
<td>58.07±2.87</td>
</tr>
<tr>
<td>25</td>
<td>52.50±6.06</td>
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<td>15.5</td>
<td>28.65±3.40</td>
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<td>6.25</td>
<td>22.11±7.12</td>
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<tr>
<td>3.12</td>
<td>4.81±3.51</td>
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<tr>
<th>IC₅₀/24h</th>
<th>CC₅₀/48h</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.71 μg mL⁻¹</td>
<td>216.8 μg mL⁻¹</td>
<td>6.83</td>
</tr>
<tr>
<td>(25.06-41.07)</td>
<td>(155.70-390.70)</td>
<td></td>
</tr>
</tbody>
</table>

*Trypanocidal activity was determined against trypomastigote forms of* Tripanosoma cruzi *after 24 h. b* Determination of 50% inhibitory concentration (IC₅₀) value after 24 h incubation. c*Determination of the Cytotoxic Concentration of 50% of the cells (CC₅₀) dSelectivity Index (SI). Parenthesis are confidence intervals.*

There are several reports in the literature where *T. cruzi* parasites are affected by low-polar fractions of plant extracts, such as the work described by Silva et al. (2017), who describe the promising antiparasitic action of the hexane fraction of the ethanolic extract of *Pfaffia glomerata* against *T. cruzi*. The authors describe that derivatives of steroids and saponins found in the low polar fraction are responsible for the antiparasitic effects observed in in vitro experiments (Silva *et al.*, 2017). Recent revisions have demonstrated the antiparasitic activity of other non-polar fractions of plants, such as *Guarea polymera* (Meliaceae), *Marila laxiflora* (Clusiaceae), *Conobea scoparioides* (Scrophulariaceae), *Otoba novogranatensis* (Myristicaceae), and *Otoba parviflora* (Myristicaceae), which also act against *T. cruzi*, reinforcing the idea that interactions between non-polar molecules of *H. speciosa* can show highly promising results at lower concentrations (García-Huertas e Cardona-Castro, 2021; Weniger *et al.*, 2001). Therefore, the work presented up to this point is still in progress since the molecules that act as possible trypanocidal agents on fraction HS-F1 must be elucidated. However, for the first time, the antiparasitic activity of *H. speciosa* fractions against *T. cruzi* was described.
CONCLUSIONS

In this work, we investigated the phytochemical classes of metabolites of *H. speciosa* and, for the first time, accessed its *in vitro* antiparasitic activity against *T. cruzi* and *S. mansoni*. Despite the antiparasitic potential against schistosomiasis of the tested fractions not showing good results, the non-polar fraction obtained from the extract showed promising results against trypanosomiasis. It is important to note that *H. speciosa* is a native species of the Brazilian Cerrado, known in traditional medicine as a natural anti-inflammatory. Additional studies are necessary to elucidate the chemical constitution of the complex mixture of mangabeira leaf extracts, and the action of the most promising fraction. In this sense, *in vivo* tests with this fraction are still necessary.

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