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Phenolic composition and antiparasitic activity of plants from the Brazilian Northeast "Cerrado"

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Abstract   This work describes the antiparasitic and cytotoxic activities of three plant species from the Cerrado biome, Northeastern Brazil. Significant antiparasitic inhibition was observed against *Trypanosoma cruzi* (63.86%), *Leishmania brasiliensis* (92.20%) and *Leishmania infantum* (95.23%) when using ethanol extract from leaves of *Guazuma ulmifolia* Lam. (Malvaceae), at a concentration of 500 μg/mL. However, low levels of inhibition were observed when assessing leishmanicidal and trypanocidal (Clone CL-B5) activities of crude ethanol extracts from leaves and bast tissue of *Luehea paniculata* (Malvaceae) and leaves and bark of *Prockia crucis* (Salicaceae) at a concentration of 500 μg/mL. The extracts revealed the presence of phenolic acids such as gallic acid, chlorogenic acid, caffeic acid and rosmarinic acid, as well as flavonoids such as rutin, luteolin, apigenin and quercetin – the latter detected only in *G. ulmifolia*. *G. ulmifolia* extract displayed higher leishmanicidal activity probably due to the presence of quercetin, a potent known leishmanicidal compound. A cytotoxicity test indicated values over 50% at the highest concentration (1000 μg/mL) for all natural products,

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which were considered cytotoxic. This points out the need for further tests to enable future *in vivo* trials, including antineoplastic activity on human tumor cells.

1. Introduction

Cerrado vegetation occurs particularly in Brazil's Central Highlands, especially in the states of Mato Grosso, Goiás and Minas Gerais. Disjunct areas were later identified in the states of São Paulo and Minas Gerais (Ferri, 1955) and in Ceará, where this biome was contained almost entirely within the semiarid domain, around the southern part of the state (Figueiredo and Fernandes, 1987). Cerrado features heterogeneous vegetation, with taxonomic biodiversity that is actually higher than that of the Amazon rainforest, including over 7000 native vascular plant species (Hiruma-Lima et al., 2006). Due to this notable floristic diversification, there has been a growing interest in investigating medicinal plants from the Cerrado as sources of bioactive compounds (Guarin Neto and Morais, 2003).

Leishmaniasis is a prevalent disease in 88 countries, located in 4 continents, with an estimated 1.6 million new cases annually, of which 500.000 are visceral and 1.1 million, cutaneous or mucocutaneous (WHO, 2010). In Brazil, leishmaniasis occurs in 19 states, with over 90% of human cases occurring in the Northeast region (Maia-Elkhoury et al., 2008). Rangel and Lainson (2009) state that American cutaneous leishmaniasis in Brazil is caused by a dermotropic variety of *Leishmania*, and the transmission of the causal agents involves different species of phlebotomines (*Diptera*: *Psychodimalian*: *Phlebotominae*) in strict relationship with mammal hosts of the parasites, resulting in a number of different transmission cycles nationwide.

Several substances have been tested as anti-*Leishmania* drugs. The main ones are directed toward inhibition of the vital and specific metabolic pathways of the parasite (Figueiredo et al., 2014). To Bezerra et al. (2004), the first-line drugs to treat leishmaniasis have been pentavalent antimonials (Sb5 +). Natural products constitute an important source of new medications, because their derivatives are extremely useful as structures for synthetic modification and bioactivity optimization. Flavonoids have also been investigated in this field (Sülsen et al., 2007).

To Chai et al. (2005), the mechanism of action of pentavalent antimonials against *Leishmania* is still disputed and little understood. Liposomal amphotericin B, pentamidine, paromomycin and miltefosine are drugs of interest as they represent new therapeutic alternatives, but feature several problems such as side effects, product price and formula production (Pereira, 2011).

Chagas disease is another widespread parasitic disease occurring worldwide, caused by *Trypanosoma cruzi* and affecting about 10 million people in the Americas (WHO, 2010; Figueiredo et al., 2014). The most epidemiologically important forms of transmission are vectors – through hematophagous insects (triatomines), transfusion, congenital and oral (Coura et al., 2007). *T. cruzi* has a complex biological cycle, involving three evolutive forms (trypomastigote, epimastigote and amastigote) and several species of triatomines and mammals, wild and domestic, which act respectively as vectors and reservoirs of the parasite (Lana and Tafuri, 2005).

Given the importance and the need to seek new antiparasitic drugs to combat leishmaniasis and Chagas disease, the aim of this study was to evaluate the leishmanicidal and trypanocidal potential as well as the cytotoxicity of crude ethanol extracts from three species of plants with few or almost no reports in the literature about antiparasitic activities – *Luehea paniculata* Mart. and Zucc. (Malvaceae), *Prockia crucis* P. Browne ex. L. (Salicaceae) and *Guazuma ulmifolia* Lam. (Malvaceae), medicinal trees of multiple effects – found in a Cerrado fragment located in the Northeast of Brazil – an area of interest relevant to the understanding of the biological potential of its flora.

2. Materials and methods

2.1. Place of collection of the plant material and obtaining of extracts

Leaves and bast tissue of *L. paniculata*, leaves and bark of *P. crucis* and leaves of *G. ulmifolia* were collected on November 19, 2012 (dry season), around 17:30 h, from the Cerrado located in the center of Caatinga area, Lavras da Mangabeira city, central mesoregion in South of Ceará, Northeastern Brazil. The area is characterized as a vegetation relic (Figueiredo and Fernandes, 1987), presenting itself in deep soil on a tabular relief on long dissected surfaces with penetration of the savannah flora. The patch of savannah in question is located in hills of 280–600 m in height, at the following coordinates: (06° 72′ 2432′ S and 38° 97′ 7396′ W). Botanical material was collected and exsiccates were obtained, which were deposited at the Prisco Bezerra Herbarium, Federal University of Ceará (UFC), Fortaleza, Ceará, Brazil, as per the numbering described in Table 1. The plant material was dried at 60 °C, weighed (Table 2) and subjected to maceration with ethanol PA for seven days. After this period, filtration and concentration of the extract were made distilling all the solvent on a rotary evaporator under reduced pressure (40 rpm at 60 °C) and then in a water bath at 60 °C, obtaining the crude extracts.

Table 1 Plant species and parts used in the antiparasitic and cytotoxicity tests.

Species	Family	Plant part	Exsiccate
Luehea paniculata Mart. and Zucc.	Malvaceae	Leaves and bast tissue	54.641
Prockia crucis P. Browne ex. L.	Salicaceae	Leaves and bark	54.741
Guazuma ulmifolia Lam.	Malvaceae	Leaves	54.743

Table 2 Dry weight and yield of the crude ethanol extracts.

Natural product	Solvent	Dry matter (g)	Crude extract (g)	Extract yields (%)
EELPL	Ethanol	595	61.649	10.36
EELPB	Ethanol	195	36.621	18.78
EEPCL	Ethanol	481	49.786	10.35
EEPC	Ethanol	247	23.604	9.55
EEGUL	Ethanol	558	54.457	9.76

EELPL = Ethanolic extract of *L. paniculata leaves*; EELPB = Ethanolic extract of *L. paniculata* bast; EEPCL = Ethanolic extract of *P. crucis* leaves; EEPC = Ethanolic extract of *P.crucis* bark; EEGUL = Ethanolic extract of *G. ulmifolia* leaves; AP(Li) = Antipromastigote activity of *L. infantum*; AP(Lb) = Antipromastigote activity of *L. brasiliensis*; AE = Antiepimastigote activity (*T. cruzi*); DS = Standard deviation.

2.2. Chemical, apparatus and general procedures

All chemical were of analytical grade. Methanol, formic acid, gallic acid, chlorogenic acid, caffeic acid and rosmarinic acid were purchased from Merck (Darmstadt, Germany). Catechin, quercetin, quercitrin, coumarin, vitexin, luteolin, rutin, apigenin and kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC–DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software. The quantification of compounds by HPLC–DAD way performed by reverse phase chromatographic analyses was carried out under gradient conditions using C_{18} column (4.6× 150 mm) packed with 5 μm diameter particles; the mobile phase was water containing 1% formic acid (A) and methanol (B), and the composition gradient was: 12% of B until 10 min and changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by Pereira et al. (2014) to *L. paniculata* extracts, with slight modifications and Klimaczewski et al. (2014) with slight modifications, to *P. crucis* and *G. ulmifolia* extracts. All chromatography operations were carried out at ambient temperature and in triplicate.

2.3. Cell lines used

For *in vitro* studies of *T. cruzi*, the clone CL-B5 was used (Le-Senne et al., 2002). Parasites stably transfected with the *Escherichia coli* b-galactosidase gene (lacZ), were provided by Dr. F. Buckner through Instituto Conmemorativo Gorgas (Panama). Epimastigotes were grown at 28 °C in liver infusion tryptose broth (Difco, Detroit, MI) with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA), penicillin (Ern, S.A., Barcelona, Spain) and streptomycin (Reig Jofré S.A., Barcelona, Spain), as described previously (Roldos et al., 2008), and harvested during the exponential growth phase. Culture of *Leishmania brasiliensis* and *Leishmania infantum* was obtained from the Instituto de Investigaciones en Ciencias de la Salud, Asunción, Paraguay – IICS. The maintenance of strain, the form of cultivation and isolation shape promatigota followed

the procedures described by Roldos et al. (2008). The inhibition assays of promastigotes were performed using the strain of *L. brasiliensis* (MHOM/BR/75/M2903), grown at 22 °C in Schneider's Drosophila medium supplemented with 20% FBS. For the cytotoxic assays, was used the fibroblast cell line NCTC 929 grown in Minimal Essential Medium (Sigma). The culture medium was supplemented with heat-inactivated FBS (10%), penicillin G (100 U/ml) and streptomycin (100 μg/mL). Cultures were maintained at 37 °C in humid atmosphere with 5% CO_2. The viability of these strains was assessed according to Roldos et al. (2008), through the use of resazurin as a colorimetric method.

2.4. Reagents

Resazurin sodium salt was obtained from Sigma–Aldrich (St. Louis, MO) and stored at 4 °C protected from light. A solution of resazurin was prepared in 1% phosphate buffer, pH 7, and filter sterilized prior to use. Chlorophenol red-b-D-galactopyranoside (CPRG; Roche, Indianapolis, IN) was dissolved in 0.9% Triton X-100 (pH 7.4). Penicillin G (Ern, S.A., Barcelona, Spain), streptomycin (Reig Jofré S.A., Barcelona, Spain) and dimethylsulfate were also used.

2.5. In vitro epimastigote susceptibility assay

The screening assay was performed in 96-well microplates with cultures that had not reached the stationary phase (Vega et al., 2005). Briefly, epimastigotes were seeded at 1×10^5 mL^{-1} in 200 μL of liver tryptose broth medium. The plates were then incubated with the drugs (0.1–50 μg/mL) at 28 °C for 72 h, at which time 50 μL of CPRG solution was added to give a final concentration of 200 μM. The plates were incubated at 37 °C for an additional 6 h and were then read at 595 nm. Each experiment was performed twice and independently, each concentration was tested in triplicate in each experiment. The efficacy of each compound was estimated by calculating the anti epimastigotes percentual (AE%).

2.6. In vitro leishmanicidal assay

The assay was performed using a modification of a previous method. Cultures of promastigotes of *L. brasiliensis* and *L. infantum* were grown to a concentration of 10^6 cells/mL and then transferred to the test. The compounds were dissolved in DMSO to the concentrations to be tested and were transferred to microplates. Each test was performed in triplicate. The activity of compounds was evaluated after 72 h by direct counting of cells after serial dilutions and compared with an untreated control.

2.7. Cytotoxic assays

NCTC929 fibroblasts were plated in 96-well microplates at a final concentration of 3×10^4 cells/well. The cells were grown at 37 °C in an atmosphere of 5% CO_2. After that, the culture médium was removed and the compounds were added to 200 μL, and performed a new culture for 24 h. After this incubation, 20 μL of a 2 mM solution of resazurin was added to each well. The plates were incubated for 3 h and the reduction of resazurin was measured using dual absorbance at

wavelengths of 490 and 595 nm. The value of the control (blank) was subtracted. Each concentration was tested in triplicate.

3. Results and discussion

The extracts evaluated in this work showed the yields demonstrated in Table 2, where we observed a higher yield of *L. paniculata* extracts compared to *P. crucis* and *G. ulmifolia*.

This work evaluated cytotoxicity using murine fibroblasts, as well as anti-parasite bioactivity against the epimastigote forms of *T. cruzi* and promastigote forms of *L. brasiliensis* and *L. infantum* of ethanol extracts from three species of plants occurring in a fragment of Cerrado in the midst of the Caatinga scrublands of northeastern Brazil.

Fig. 1 features the results for cytotoxic activity at concentrations of 1000–125 μg/mL for the natural products from the three species studied, as well as the LC_{50} values (median lethal concentration capable of killing 50% of tested cells – murine fibroblasts). The extracts from leaves (EELLP) and bast tissue (EEBTLP) of *L. paniculata*, as well as the ethanol extract from the bark of *P. crucis* (EEBPC) showed low toxicity at the concentration of 500 μg/mL, with LC_{50} values above 730 μg/mL for both. For its part, the leaf extract from *P. crucis* (EELPC) and leaf extract from *G. ulmifolia* (EELGU) displayed high cytotoxicity at the same concentration.

Fibroblasts are cells found in the conjunctive tissue of mammals. These cells are usually chosen for cytotoxicity tests because they are easy to maintain and produce results that show high correlation with biological results; also, they are present in wounds and constitute the main cell type present in regeneration (Ratner et al., 2004). Toxicity tests are devised in order to evaluate or predict toxic effects on biological systems and measure the relative toxicity of the substances (Forbes and Forbes, 1994). In that regard, the results can provide valuable information for the triage of natural products that can be regarded as probable candidates to be become medications (Morais-Braga et al., 2013).

While evaluating anti-*Leishmania* activity, the best result was observed in EELGU, tested at four concentrations, with mortality of 95.23% (500 μg/mL) and 28.27% (250 μg/mL)

for *L. infantum* cells, and 92.20% (500 μg/mL) and 44.22% (250 μg/mL) for *L. brasiliensis* cells. EC_{50} values of the tests with the respective parasites were 316.54 μg/mL and 282.63 μg/mL. The other extracts showed results of low to moderate anti-promastigote activity, with no cell mortality from EEBTLP at concentrations of 500 and 250 μg/mL. All extracts except EELGU showed EC_{50} values over 850 μg/mL in anti-*Leishmania* tests.

With regard to the evaluation of anti-*Trypanossoma* activity, the best result was also observed for EELGU, with 68.93% inhibition of the protozoan at the concentration of 500 μg/mL and EC_{50} = 454.68 μg/mL. The results of anti-parasite action from the studied extracts are shown in Table 3.

Plant extract triage is a valid strategy given that these natural products are explored in order to discover trypanocidal agents (Luize et al., 2006; Pizzolatti et al., 2008; Figueiredo et al., 2014). Several secondary metabolites with various structural patterns have proven active against *T. cruzi* (Saúde-Guimarães and Faria, 2007). For leishmaniasis, several studies have recommended the use of plants for treatment, both in ulcerated lesions from cutaneous leishmaniasis and in *in vitro* models (Paula-Junior et al., 2006; Bezerra et al., 2004; Santos et al., 2013).

Studies involving *Leishmania* spp. have focused on the extracellular form of the parasite, known as promastigote, instead of the amastigote form, due to easier *in vitro* growth and not requiring another cell culture such as macrophages (Castilhos et al., 2011).

The results of this work demonstrate that the extracts of *L. paniculata* and *P. crucis* were not clinically relevant for any of the tested protozoa, as they did not show values equal to or higher than 50% inhibition at a concentration of 500 μg/mL (EC_{50} values above 850 μg/mL). To Rosas et al. (2007), this level of inhibition (50%) at a concentration of 500 μg/mL is considered to be clinically relevant. For its part, the leaf extract of *G. ulmifolia* showed considerable leishmanicidal potential, inhibiting 95.23% (*L. infantum*) and 92.20% (*L. brasiliensis*) of the growth of tested strains at a concentration of 500 μg/mL, with EC_{50} values of 316.54 and 282.63 μg/mL, respectively. A significant result was also observed when evaluating anti-*Trypanossoma* potential, as the test using this extract
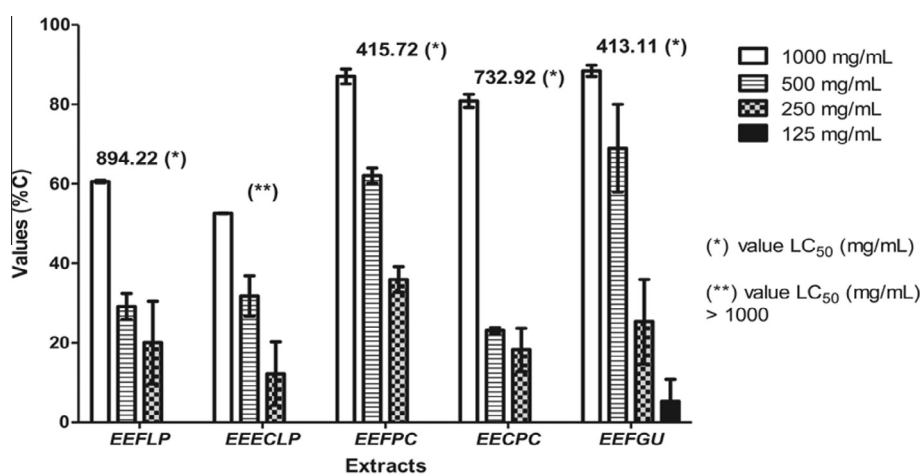

Figure 1 Graph showing the percent cytotoxicity of extracts under study on murine fibroblasts. In which: (*) LC_{50} values (median lethal concentration capable of killing 50% of tested cells); (**) LC_{50} value over concentration of 1000 μg/mL.

Table 3 Antipromastigote activities of *L. brasiliensis* and *L. infantum* and antiepimastigote activity (*T. cruzi*) of ethanol extracts from three species native to a fragment of Cerrado in Northeastern Brazil.

Natural product	Conc. (µg/mL)	%AP(Li)	± %DS	%AP(Lb)	± %DS	%AE	± %DS
EELPL	1000	35.32	1.19	39.00	0.22	28.30	1.98
	500	30.56	1.05	27.64	0.22	23.18	0.57
	250	17.13	0.61	19.18	0.59	0.58	3.39
EELPB	1000	1.15	0.39	29.39	1.58	0.00	0.42
	500	0.00	1.99	25.69	0.71	0.00	0.49
	250	0.00	1.61	19.57	1.58	0.00	3.46
EEFPC	1000	49.98	1.07	45.65	0.15	55.82	1.56
	500	25.34	0.41	29.22	0.12	25.96	1.56
	250	18.24	0.14	21.12	1.17	14.46	0.92
EECPC	1000	23.25	0.07	23.26	1.37	25.28	1.12
	500	13.59	0.75	14.42	0.23	10.68	0.26
	250	10.39	0.57	11.18	0.26	0.00	1.24
EEFGU	1000	95.45	0.72	92.36	0.17	63.86	1.20
	500	95.23	0.39	92.20	0.04	61.15	0.99
	250	28.27	0.45	42.22	0.01	24.32	1.91
	125	5.80	0.44	0.00	2.14	4.03	0.99

EELPL = Ethanolic extract of *L. paniculata* leaves; EELPB = Ethanolic extract of *L. paniculata* bast; EEPCL = Ethanolic extract of *P. crucis* leaves; EEPC = Ethanolic extract of *P.crucis* bark; EEGUL = Ethanolic extract of *G. ulmifolia* leaves; AP(Li) = Antipromastigote activity of *L. infantum*; AP(Lb) = Antipromastigote activity of *L. brasiliensis*; AE = Antiepimastigote activity (*T. cruzi*); DS = Standard deviation.
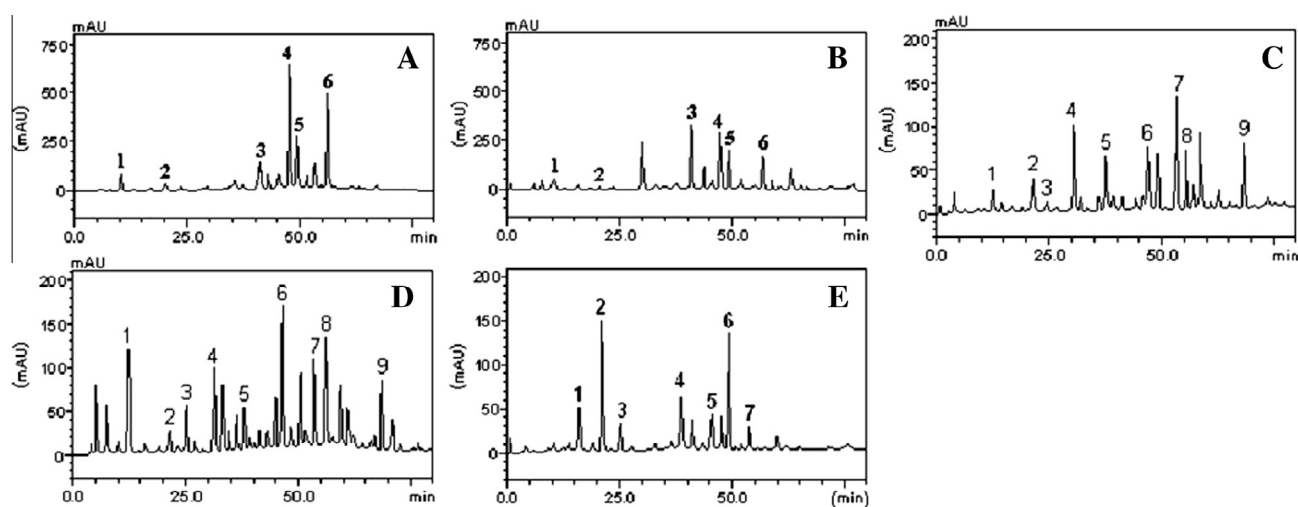

Figure 2 Representative high performance liquid chromatography profile for *L. paniculata* leaves extract (A); *L. paniculata* sapwood extract (B); *P. crucis* leaves extract (C); *P. crucis* bark extract (D); *G. ulmifolia* leaves extract (E). Detection UV was at 325 nm, where: (A and B): gallic acid (peak 1), chlorogenic acid (peak 2), vitexin (peak 3), rosmarinic acid (peak 4), rutin (peak 5) and luteolin (peak 6); (C and D): gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), coumarin (peak 4), rutin (peak 5), quercitrin (peak 6), kaempferol (peak 7), luteolin (peak 8) and apigenin (peak 9); (E): catechin (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rutin (peak 4), quercitrin (peak 5), quercetin (peak 6) and luteolin (peak 7).

showed 68.93% inhibition in the growth of the strain of *T. cruzi* at a concentration of 500 µg/mL.

One important criterion when searching for active compounds with leishmanicidal and trypanocidal activity is the evaluation of their toxicity in host cells. The cytotoxic activity of other plants has been evaluated in different human cell models, such as: peritoneal macrophages (Houghton et al., 2007), human lymphocytes (Reyes-Chilpa et al., 2008), MRC-5 cells (Cabral et al., 2010) and murine fibroblasts (Santos et al., 2013; Figueiredo et al., 2014).

The results of the cytotoxicity test in this study indicated values over 50% at the highest concentration (1000 µg/mL) for all natural products, meaning they were regarded as toxic. At concentrations in which cytotoxicity was low (extracts of *L. paniculata* and *P. crucis*), antiepimastigote and antipromastigote effects were irrelevant. Ethanol extract from the leaves of *G. ulmifolia*, which had considerable antiparasitic effect in this study, showed high cytotoxic activity at the concentrations of 1000 µg/mL (88.41%) and 500 µg/mL (68.93%), with LC_{50} value of 454.68 µg/mL. This aspect becomes irrelevant, as

the cytotoxic potential of *G. ulmifolia* – in which the reactivity of the extract in mammal cells was verified – represents an essential step in natural products research that precedes *in vivo* tests, which confirm or negate the viability and safety of their therapeutic use in humans. The cytotoxicity presented can further be useful in studies on antineoplastic activity in tumor cells.

To Simões et al. (2010), toxic substances present in the plant may be limited to a given season of the year or to certain environmental conditions, or yet to specific varieties. Block et al. (2005), emphasize that the mechanism of action of cytotoxicity is related to the ability by plants to induce cell apoptosis.

The presence of phenolic content in the species was detected and quantified using the analytical method of high-performance liquid chromatography (HPLC). The extracts revealed the presence of phenolic acids such as gallic acid, chlorogenic acid, caffeic acid and rosmarinic acid, as well as flavonoids such as rutin, luteolin, apigenin and quercetin – the latter compound detected only in *G. ulmifolia* (Fig. 2).

Research results indicate that some of the phenolic compounds found in the extracts of the plants studied can show anti-kinetoplastidae activity (Morais-Braga et al., 2013). In *L. paniculata*, although low, the level of phenolic compounds found in the leaf extract is higher than in the bast tissue extract, with the most abundant compounds in the leaf extract being rosmarinic acid (1%) and luteolin (0.79%), whereas in the bast tissue vitexin (0.49%) and rosmarinic acid (0.41%) are most abundant. In *P. crucis*, the leaf extract contains kaempferol (1.57%) and coumarin (1.07%) at the highest rates, while quercitrin (1.89%) and gallic acid (1.5%) are most common in bark extract. In the leaf extract of *G. ulmifolia*, a natural product with significant antiparasitic results, chlorogenic acid (2.35%) and quercetin (2.15%) were found to be major compounds.

In a study on anti-*Leishmania* and anti-*Trypanosoma* activity by compounds isolated from *Croton lobatos* L., Lagnika et al. (2009) investigated, among other compounds, the action of chlorogenic acid, and found irrelevant activity. Tasdemir et al. (2006), in an *in vitro* study, indicated quercetin and its derivatives (7,8-dihydroxyflavone) as having potent and efficient antiparasitic activity against *Leishmania donovani*, with IC$_{50}$ of 1.0 μg/mL, although without significant activity for *T. cruzi*. Those authors further observed that the majority of most active compounds with trypanocidal and leishmanicidal effects have a typical flavone structure (2, 3 and C-4 of keto function) and/or a catechol substructure without replacements in the B-ring, in the benzochromone skeleton and in 7,8-dihydroxyflavone. Mittra et al. (2000) demonstrated a significant inhibitory effect *in vitro* by luteolin and quercetin on the growth of promastigote and amastigote forms of *L. donovani*. Muzitano et al. (2006), observed quercetin, isolated from *Kalanchoe pinnata*, to have leishmanicidal activity against *Leishmania amazonensis*. Matsuda et al. (2004) demonstrated that quercetin is a potent immunomodulator, capable of directly inducing death by apoptosis of *Trypanosoma brucei* gambiense, without affecting the viability of normal cells. These results highlight the use of quercetin as a potential antimicrobial and anti-inflammatory agent for the treatment of trypanosomosis.

An extract, however, is a mixture of several compounds, which in their chemical interactions can combine synergistically,

antagonistically or indifferently, and thus alter the effect that each would have by itself (Morais-Braga et al., 2013). Furthermore, the low concentrations of phenolic compounds may have been responsible for the weak bioactivity of products from *L. paniculata* and *P. crucis* against *L. brasiliensis*, *L. infantum* and *T. cruzi*.

Although cited in several works for its use by local populations to clean internal ulcers and wounds (Brandão, 1991; Ritter et al., 2002; Carvalho, 2006; Tanaka et al., 2005), no records were found of works investigating the anti-*Leishmania* potential of *L. paniculata*, or its anti-*Trypanossoma* effect. *G. ulmifolia*, another medicinal tree with multiple uses, is also hailed in natural medicine for cleaning ulcers and healing open wounds (Teske and Trentini, 1997), and this is the first report on these activities for that specie, as well as for *P. crucis*.

4. Conclusions

The ethanol leaf extract from *G. ulmifolia* was considered efficient against the tested parasite strains, emerging as a potential alternative source of natural products with activity against *T. cruzi*, *L. brasiliensis* and *L. infantum*, possibly due the presence of quercetin, a potent known leishmanicidal compound. The cytotoxicity seen in the extract from *G. ulmifolia* indicates the need for further tests, enabling future *in vivo* trials, including on antineoplastic activity in tumor cells.

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