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Antimicrobial activity of plants used as medicinals on an indigenous reserve in Rio das Cobras, Paraná, Brazil

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ABSTRACT

Ethnopharmacological relevance: A considerable percentage of global biodiversity is located in Brazil, a country that also has rich cultural and ethnic diversity. In the community of Rio das Cobras, Paraná, plants are still widely used in the health care not only by indigenous people but also by the non-indigenous population that inhabits the region. The investigation of the efficacy and safety of these plants in the treatment of infectious diseases provides insights for future studies of these species allowing the appropriated use by the indigenous people, since few or none study has been conducted so far.

Aim of the study: Evaluate the antimicrobial activity and cytotoxicity of some plants used as medicinal on an indigenous reserve in Rio das Cobras, Paraná, Brazil.

Materials and methods: The aqueous extracts were obtained by decoction and the 50% and 70% hydroalcoholic extracts by turbo extraction. The extracts were tested against strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Leishmania amazonensis*, Poliovirus and HSV-1. Cytotoxicity assay using VERO cells were also performed.

Results: None of the extracts had a selectivity index (SI) > 1 for any of the tested bacteria. Only *Campomanesia eugenioides* and *Schinus terebinthifolius* had an SI > 1.0 for all of the tested *Candida* species. The best anti-Leishmania activity was obtained with *Zanthoxylum rhoifolium* and *Schinus terebinthifolius*. Extracts of *Cordia americana* were the most effective against herpes simplex virus type 1. *Zanthoxylum rhoifolium* was the most effective against Poliovirus, and *Ocimum gratissimum* was effective against both Poliovirus and Herpes Simplex virus. Among the plants investigated in the present study, *Zanthoxylum rhoifolium* had the fewest cytotoxic effect.

Conclusions: The plants investigated in the present study exhibited potential for future pharmacological uses, but additional studies, especially with regard to in vivo toxicity, must be conducted. The results of this preliminary survey are important for the Rio das Cobras Reserve community for the safe and effective use of plants in the treatment of some infectious diseases.

1. Introduction

The use of medicinal plants is a very important therapeutic resource for the treatment of diseases, especially among developing countries (Freitas, 1999). Interest in healing through the use of plants has existed since ancient times. Historical records from antiquity show that man had already demonstrated his awareness of several medicinal properties of plants, the knowledge of which was transmitted from generation to generation, creating a medical system known as "traditional" (Simões et al., 2007). Reports have shown healing through the use of plants many years before Christ in ancient civilizations, such as the Assyrians, Greeks, Egyptians, Chinese, Hindu, and Aztec. For centuries, plants have represented the only medicinal resource for the treatment of ailments that afflict humans. Even with the advent of pharmaceutical chemistry, they are still involved in the development of new drugs (Hostettmann et al., 2003). Nowadays, according to the World Health Organization

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(2001), approximately 65% of the world's population uses medicinal plants for primary healthcare. Most users of traditional medicines are people in developing countries, mainly among native populations motivated by the maintenance of their cultural and religious traditions or lack of access to modern medicine.

Approximately 6% of the world's plants have been screened for biological activity, and only 15% have been phytochemically evaluated (Verpoorte, 2000). Approximately 15–20% of the globe's biodiversity is located in Brazil. When only superior plants are considered, this percentage increases to 24%. In addition to this genetic collection, Brazil has rich cultural and ethnic diversity that results in considerable knowledge of traditional technologies that have been passed down from generation to generation, especially knowledge of the management and use of medicinal plants (Brasil, 2006).

In Brazil, the use of medicinal plants dates back to before the country's discovery, when indigenous people used the forest as their pharmacy. After the discovery of Brazil, records indicate the use of plants by slaves and indigenous people for healing purposes and religious rituals. These practices merged with the knowledge brought by European settlers to contribute to popular medicine (Martins et al., 1998). Later, because of biodiversity, many people continue to use these resources, although more information is needed about their efficacy and safety (Guerra and Nodari, 2001).

The Rio das Cobras Reserve was established in 1901 and had about 500 people at that time. Today, it is the largest reserve in the state of Paraná, with an area of 18,681.98 ha and population of approximately 2300 individuals who comprise 550 families from three ethnic groups: Guarani, Kaigang and Xetá. The population is divided into several villages, including Campo do Dia, Taquara, Pinhal, Lebre, Trevo, Papagaio, and Vila Nova (Funai, 2006; Lazzarotto et al., 2007). According to Lazzarotto et al. (2007), most inhabitants of the reserve survive from agriculture and handicrafts, but the unemployment rate is high and socioeconomic disturbances directly influence the health of the population. Although they have a health center, and treatments can be administered using allopathic medicine, the traditional methods provided by the elders of the tribes, known as Tamõi, such as teas, roots, and blessings, are still widely used, not only by indigenous people but also by the non-indigenous population that inhabits the region. In the Rio das Cobras indigenous reserve, several plants are used in traditional medicine for the treatment of different diseases according to the guidance of the elders.

Of the plants used by them, many are used to treat sores on the skin and mucous membranes or symptoms associated with flu, fever, jaundice, and diarrhea, among others. The objective of the present study was to evaluate the antimicrobial activity and in vitro cytotoxicity of the aqueous extracts (AEs) and hydroalcoholic extracts (HAEs) of some plants used as medicinal on the indigenous reserve in Rio das Cobras, Paraná, Brazil. Some plants used by this community to treat symptoms associated with infectious and others diseases are listed in Table 1.

2. Materials and methods

2.1. Ethnopharmacological survey

The survey of plants used in traditional medicine was conducted using structured interviews with key figures who are knowledgeable about the indigenous reserve in Rio das Cobras. In this survey, the names of the plants, indications, route of administration, dose (i.e., spoon, cup, glass, or other measurement), plant part used, time and season of harvest, use as a dried or fresh preparation, solvent, plant/solvent proportion, and other relevant information were asked.

2.2. Plant material

The plants were collected in the region of the indigenous reserve in Rio das Cobras, located between the cities of Nova Laranjeiras and Espigão Alto do Iguaçú, and taxonomically identified by Prof. Dr. Lívia Godinho Temponi of the Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná. The voucher specimens were deposited and cataloged in the herbarium at the Universidade Estadual de Maringá, Paraná. This research was authorized by Brazilian regulatory agencies, Conselho de Gestão do Patrimônio Genético, Fundação Nacional do Índio, and Instituto Nacional do Meio Ambiente e Recursos Renováveis.

The selected plants were *Cordia americana* L (Guajuvira), *Campomanesia eugenioides* (Cambess.) (Gabiroba), *Ocimum gratissimum* L. (Alfavaca), *Luehea paniculata* Mart. (Açoita-cavalo), *Schinus terebinthifolius* Raddi (Aroeira), and *Zanthoxylum rhoifolium* Lam. (Mamica de cadela).

2.3. Extract preparation

After collection, the pharmacogens were presorted and then dried in an oven (40 °C) with forced air circulation with temperature control. After drying, the pharmacogens were ground in a knife mill to obtain a fine powder. To prepare the AEs, the dried plants (10 g powder) were boiled in 100 ml of distilled water for 10 min to obtain specific ethnopharmacological form based on

Table 1
Summary of ethnobotanical data of the plants commonly used by the people in the Rio das Cobras Reserve in Paraná, Brazil.

Plant species (family)	Common name	Part of the plant	Ethnomedicinal use	Mode of use
Campomanesia eugenioides Cambess. (Myrtaceae)	Gabiroba or guabiroba	Leaves	Diarrhea, stomach pain, fever	Drink the tea
Luehea paniculata Mart. (Tiliaceae)	Açoita-cavalo	Bark	Wounds, stomach pain, uterine disorders, hepatitis, pneumonia, inflammation, anxiolytic, dysmenorrhea	Wash the wound with tea
Ocimum gratissimum L. (Lamiaceae)	Alfavaca	Leaves	Flu, toothache, wounds	Wash the wound with tea
Cordia americana L. (Boraginaceae)	Guajuvira	Bark and leaves	Flu, wounds	Wash the wound with tea
Schinus terebinthifolius Raddi (Anarcadiaceae)	Aroeira	Bark	Flu, toothache, sores in the mouth, sore throat, feminine diseases	Wash the wound with tea
Zanthoxylum rhoifolium Lam. (Rutaceae)	Mamica de Cadela	Bark and leaves	Headaches, anemia, jaundice, high blood pressure	Drink the tea

information transmitted from generation to generation. Fifty percent of the HAEs were prepared using turbo-extraction for 15 min at $<40\,^{\circ}C$. The plant/solvent proportion was 10 g of powder in 100 ml of hydroalcoholic solution.

The 50% hydroalcoholic solution was used because the extracts are prepared with Brazilian cachaça in other popular cultures, which contains approximately this alcoholic concentration (Lorenzi and Matos, 2002). After the preparation, the extracts were filtered through filter paper under reduced pressure, evaporated under a vacuum, and lyophilized. The 70% HAE was tested when 50% of the HAE showed better activity than the AE to determine the fraction with the best activity.

2.4. Antibacterial and antifungal activity assays

2.4.1. Microorganisms

Antibacterial and antifungal activity was evaluated for the following strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6623, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 28707.

2.4.2. Microorganism suspension standardization

The microbial suspensions were standardized according to the Clinical and Laboratory Standards Institute (CLSI, 2009) for bacteria and (CLSI, 2008) for yeasts. Bacteria were grown in Müller–Hinton broth (MHB; Merck) for 18–24 h and yeasts in Sabouraud dextrose broth (Merck) for 48–72 h. After growing, the microbial suspension was standardized with sterile saline to turbidity equivalent to 0.5 McFarland scale (approximately $1–2 \times 10^8$ CFU/ml for bacteria and $1–5 \times 10^6$ CFU/ml for *Candida* spp.).

2.4.3. Minimum inhibitory concentration (MIC): broth microdilution

Changes were made in the technique described by M27-A3 CLSI manual, in order to allow the serial dilution of extracts directly in the microplate. A solution of 2 mg/ml of the extracts was prepared using MHB as the solvent (test solution). One hundred microliters of MHB were added to each well of a 96-well plate. A volume of 100 μl of the test solution was added to the wells in the first row, and then a serial dilution was performed until the ninth row, resulting in concentrations that varied from 3.9 μg/ml to 1000 μg/ml. The 10th, 11th, and 12th rows were used as inoculum, drug, and medium controls, respectively. Thereafter, 5 μl of the previously standardized microorganism suspension was added to all of the wells, and the plate was incubated at 37 °C for 18–24 h. The MIC was indicated by the absence of turbidity in the medium. RPMI-1640 (Gibco Invitrogen, Grand Island, NY, USA) with 3-(*N*-Morpholino)propanesulfonic acid (MOPS buffer—Sigma-Aldrich, St. Louis, MO, USA), pH 7.0, was the medium used to cultivate the strains of *Candida* spp. Incubation was performed at $35 \pm 2\,^{\circ}C$ for 48 h. Microbial growth was observed by the presence of turbidity and/or change in color from red to yellow. The reference antibiotics used were tetracycline, vancomycin and penicillin (Sigma).

2.4.4. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

To determine the MFC and MBC, 5 μl of the suspension in which the MIC was determined was plated on Müller–Hinton Agar (MHA; Merck) for bacteria or Sabouraud Dextrose Agar for yeasts. The plates were incubated at $35 \pm 2\,^{\circ}C$ for 24 h and 48 h, respectively. The lowest concentration of the extract at which no growth of microorganisms was observed or the growth of only one colony was observed was considered the MBC. Nystatin (Sigma) was used as control.

2.5. Anti-Leishmanial activity

Promastigote forms of *Leishmania amazonensis* MHOM/BR/75/JOSEFA were cultured in Warren's medium, pH 7.0 (brain heart infusion plus folic acid and haemin), supplemented with 10% fetal calf serum (FCS; Gibco Invitrogen, Grand Island, NY, USA). The assay was performed in a 24-well plate, and promastigote forms were grown for 48 h to reach the logarithmic phase before running the assay. The extract concentrations ranged from 10 μg/ml to 1000 μg/ml, and the suspensions of *Leishmania amazonensis* promastigotes (10^6 protozoa/ml) were used. A protozoan culture without the addition of the extracts was used as a control. The growth results were assessed after 72 h of incubation at 25 °C by counting in a Neubauer haemocytometer. The results are expressed as the inhibitory concentration 50% (IC_{50}; i.e., the concentration of the extract that is able to eliminate 50% of the viable protozoa). Amphotericin B (Cristalia, São Paulo, Brazil) was used as reference drug.

2.6. Antiviral activity

To evaluate antiviral activity, herpes simplex virus type 1 (HSV-1) and Poliovirus were used as models of DNA and RNA viruses, respectively. VERO cells at a concentration of 2.5×10^5 cells/ml were grown in 96-well plates using Dulbecco's Modified Eagle Medium (DMEM) plus 10% FCS and 50 μg/ml gentamicin. After the formation of a confluent monolayer, the medium was removed, and the cells were washed with DMEM and infected with 25 μl of viral suspension TCID 80 for 1 h at 37 °C for adsorption. Afterward, 75 μl of the medium supplemented with FCS and gentamicin plus 100 μl of various concentrations of the extracts was added, and the cells were incubated for another 48 h (Poliovirus) or 72 h (HSV-1) in a humid atmosphere with 5% CO_2 at 37 °C. Cell controls (i.e., without viral particles or extracts) and virus controls (i.e., without the addition of extracts) were also performed. The viable cells were detected using the sulforhodamine B colorimetric method (Skehan et al., 1990). Absorbance was measured with an enzyme-linked immunosorbent assay reader (Bio-Tek Power WaveXS Microplate Fluorescence Reader) at an optical density (OD) of 530 nm. These data were used to determine the percentage of cytopathic inhibition exerted by the compounds (EC_{50}; Semple et al., 2001).

2.7. Cytotoxicity assay

VERO cells grown in DMEM plus 10% FCS and 50 μg/m gentamicin were distributed in a 96-well microplate at a 2.5×10^5 cells/well concentration and incubated in a humid atmosphere with 5% CO_2 at 37 °C until a confluent monolayer be formed. The medium was then removed, and 100 μl of fresh DMEM was added. Different extract concentrations were added to the wells in triplicate. A control that used cells without the addition of any extract was also included. The plate was incubated in a humid chamber at 37 °C with 5% CO_2 for 72 h. Viable cells were detected using the sulforhodamine B colorimetric method (Skehan et al., 1990). These data were used to calculate the CC_{50} (i.e., the extract concentration that is able to destroy 50% of the cells).

3. Results and discussion

We conducted this work to investigate the antimicrobial properties of these plants and their potentially safe uses. The extraction yields (w/w) for AE and 50% HAE extracts were: leaves of *Zanthoxylum rhoifolium* (24.8% and 20.9%); leaves of

Campomanesia eugenioides (19.7% and 18.8%); bark of *Schinus terebinthifolius* (15.8% and 15.2%); leaves (15.1% and 13.3%) and bark (15.0% and 10.9%) of *Cordia americana*; bark of *Luehea paniculata* (12.9% and 19.4%); bark of *Zanthoxylum rhoifolium* (8.3% and 8.0%). For 50% HAE extract of *Ocimum gratissimum* the yield was 17.4%.

Tests against various types of microorganisms were performed. In the investigation of antibacterial activity, the most relevant results are shown in Table 2, considering a cut-off of 1000 μg/ml. None of the extracts exhibited activity against *Escherichia coli*. Additionally, the 50% HAE of *Ocimum gratissimum* and *Cordia americana* (bark) and none of the extracts of *Zanthoxylum rhoifolium* (bark and leaves) had activity against any of the tested bacteria. The AE of the leaves of *Campomanesia eugenioides* and bark of *Cordia americana* and both aqueous and organic extracts of the leaves of *Cordia americana* had activity against only *Staphylococcus aureus*. Some activity was observed against *Bacillus subtilis* with all of the extracts of the bark of *Luehea paniculata* and *Schinus terebinthifolius*. Only *Schinus terebinthifolius* had activity against *Pseudomonas aeruginosa*. None of the extracts had a selectivity index (SI) > 1 for any of the tested bacteria. The SI is an index that shows whether an extract is active against a microorganism and is not harmful to the host cell at the concentration used. It is calculated using the following formula: $IS = CC_{50}/MIC$.

Table 3 shows the MICs and SI of the extracts tested against three *Candida* species, among which the leaves of *Campomanesia eugenioides*, bark of *Luehea paniculata*, and bark of *Schinus terebinthifolius* showed the best results against *Candida albicans*, with SI values that ranged from 7.7 to 304. The AE of *Zanthoxylum rhoifolium* (bark and leaves) and 50% HAE of the bark demonstrated activity only against *Candida albicans. Ocimum gratissimum* and *Cordia americana* (bark and leaves) did not have activity against any of the yeasts tested (data not shown). The AE of *Zanthoxylum rhoifolium* (bark and leaves) and 50% HAE of the bark demonstrated activity against only *Candida albicans. Ocimum gratissimum* and *Cordia americana* (bark and leaves) did not have activity against any of the yeasts tested.

The antimicrobial activity of *Cordia americana* has not been previously described, but other members of the Boraginaceae family demonstrated antibacterial and antifungal activity, such as *Cordia curassavica*, *Heliotropium europaeum*, and *Onosma argentatum* (Özgen et al., 2003; Hernandez et al., 2007).

With regard to *Ocimum gratissimum*, although the essential oil of the leaves demonstrated activity against bacteria, fungi, and helminthes, possibly because of the presence of eugenol (Nakamura et al., 1999, 2004; Pessoa et al., 2002; Adebolu and Oladimeji, 2005; Lemos et al., 2005), this plant did not exhibit activity against bacteria or fungi in the present study. Adebolu and Oladimeji (2005) showed that the AE of this plant did not have activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, or *Salmonella thyphimurium*. However, Iwalokun et al. (2001) found activity of the AE (decoction) against microorganisms such as *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii*, and *Escherichia coli*. Another study observed differences in the composition of the extract of *Ocimum gratissimum*, depending on the solvent used in its preparation and whether the leaves were fresh or dried, which can alter the activity (Junaid et al., 2006).

In the present study, *Zanthoxylum rhoifolium* did not have antibacterial activity. Its only antifungal activity was against *Candida albicans*, and the best results were obtained with the 50% HAE of the leaves. This plant has been reported to have antimalarial, antinociceptive, antitumoral, and gastroprotective effects. The essential oil of its leaves and alkaloids isolated from the stem bark have antibacterial activity against gram-positive and -negative bacteria and antifungal activity against *Aspergillus flavus,* but no activity against *Candida* species was found (Gonzaga et al., 2003; Jullian et al., 2006; Silva et al., 2007; Boehme et al., 2008; Peneluc et al., 2009; Pereira et al., 2010; Freitas et al., 2011). Activity against *Candida* spp. of other species of this genus, such as *Zanthoxylum leprieurii* and *Zanthoxylum xanthoxyloides*, has been reported. These extracts were made with leaves, roots, and stem bark, and the effective concentrations were > 1000 μg/ml (Ngane et al., 2000; Adeniyi et al., 2010).

Another important result obtained in the present experiments was the activity of *Campomanesia eugenioides* against *Staphylococcus aureus* and all tested *Candida* species. No antimicrobial activity had previously been reported for this plant. However, others species of the genus *Campomanesia*, such as *Campomanesia xanthocarpa*, *Campomanesia phaea*, and *Campomanesia lineatifolia*, showed mild activity against gram-negative and -positive bacteria, and *Campomanesia adamantium* exhibited activity against

Table 2
Minimum inhibitory concentration (MIC—μg/ml) of crude extracts against bacteria.

Plant	Extract	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
Campomanesia eugenioides—leaves	AE	250	> 1000	> 1000
	50% HAE	125	> 1000	1000
Luehea paniculata—bark	AE	250	> 1000	1000
	50% HAE	125	> 1000	500
Cordia americana—bark	AE	1000	> 1000	> 1000
Cordia americana—leaves	AE	1000	> 1000	> 1000
	50% HAE	1000	> 1000	> 1000
Schinus terebinthifolius—bark	AE	250	1000	1000
	50% HAE	250	1000	1000

Table 3
Minimum inhibitory concentration (μg/ml) and SI of crude extracts against some *Candida* species.

Plant	Extract	*Candida albicans*		*Candida parapsilosis*		*Candida tropicalis*	
		MIC	**SI**	**MIC**	**SI**	**MIC**	**SI**
Campomanesia eugenioides—leaves	AE	0.24	120.8	15.6	1.86	7.8	3.72
	50% HAE	0.24	304.2	62.5	1.17	31.25	2.34
Luehea paniculata—bark	AE	3.9	7.7	62.5	0.48	500	0.06
	50% HAE	0.98	73.5	62.5	1.2	125	0.6
Schinus terebinthifolius—bark	AE	0.49	104.1	3.9	13.1	15.6	3.3
	50% HAE	0.49	> 204.1	62.5	> 1.6	62.5	> 1.6
Zanthoxylum rhoifolium—bark	AE	62.5	3.0	> 1000	< 1	> 1000	< 1
	50% HAE	31.25	21.9	> 1000	< 1	> 1000	< 1
Zanthoxylum rhoifolium—leaves	AE	31.25	5.76	> 1000	< 1	> 1000	< 1

SI—Selectivity index (CC_{50}/MIC).

Mycobacterium tuberculosis. *Campomanesia xanthocarpa* and *Campomanesia adamantum* also had activity against *Candida albicans* (Bonilla et al., 2005; Coutinho et al., 2009; Pavan et al., 2009).

No reports of the antifungal activity of *Luehea paniculata* and others species of the same genus were found in the literature. The present study demonstrated the activity of *Luehea paniculata* against all species of *Candida* and also against *Staphylococcus aureus* and *Bacillus subtilis*. In a pharmacognostic evaluation performed by Tanaka et al. (2005), the leaves and bark of *Luehea divaricata* contained flavonoids, triterpenes, and tannins that, according to Simões et al. (2007), are compounds with antimicrobial activity.

Antioxidant, anticancer, anti-inflammatory, analgesic, antifungal, and antibacterial effects of different preparations of *Schinus terebinthifolius*, mainly its essential oil, against gram-positive and -negative bacteria have been reported (Velázquez et al., 2003; Erazo et al., 2006; Molina-Salinas et al., 2007; Gundidza et al., 2009; Santos Júnior et al., 2010). In the present study, antibacterial activity against gram-positive and -negative bacteria and an effect on *Candida* species were confirmed.

Another focus of this work was the investigation of anti-Leishmania activity. The best activity was found with the 50% HAE of the leaves of *Zanthoxylum rhoifolium*, with an IC_{50} of 143 μg/ml. However, all of the extracts of the bark of *Luehea paniculata* and leaves of *Cordia americana* and aqueous extracts of *Zanthoxylum rhoifolium* did not exhibit activity against *Leishmania amazonensis*. Because some of the 50% HAEs exhibited better activity than the AE, a 70% HAE of the plants was tested to find more effective extracts. The 70% HAE of *Cordia americana* (bark IC_{50}=275 μg/ml), *Schinus terebinthifolius* (bark IC_{50}=180 μg/ml), and *Zanthoxylum rhoifolium* (bark IC_{50}=172 μg/ml) had better activity than the AEs and their respective 50% HAEs. These results are shown in Table 4.

No studies have reported the anti-Leishmanial activity of *Luehea paniculata*, *Campomanesia eugenioides*, *Zanthoxylum rhoifolium*, and *Cordia americana*. However, some members of these families demonstrate activity against this protozoan. Myrtaceae (e.g., *Pimenta pseudocaryophyllus* and *Blepharocalyx salicifolius*), Rutaceae (e.g., *Zanthoxylum chiloperone* and *Zanthoxylum zanthoxyloides*), and the methanolic extract of the leaves of *Cordia verbenaceae* and *Cordia fragrantissima* (Boraginaceae) had activity against *Leishmania* spp. (Ferreira et al., 2002; Takahashi et al., 2004; Ahua et al., 2007; Braga et al., 2007; Corrêa, 2010; Siqueira et al., 2010). No studies have investigated the anti-Leishmanial activity of the Tiliaceae family, of which *Luehea paniculata* is a member.

Luize et al. (2005) showed that a 90% HAE of the leaves of *Ocimum gratissimum* had good activity against *Leishmania amazonensis* and some activity against *Trypanosoma cruzi*. Ueda-Nakamura et al. (2006) showed that the essential oil of this species had activity against the amastigote and promastigote forms of *Leishmania amazonensis*.

Table 4
Determination of 50% inhibitory concentration (IC_{50}) of extracts against *Leishmania amazonensis*.

Plant	Extract	$IC_{50} \pm SD$ (μg/ml)
Campomanesia eugenioides—leaves	AE	388 ± 53
	50% HAE	555 ± 64
Ocimum gratissimum—whole plant	50% HAE	500 ± 0
Cordia americana—bark	AE	850 ± 71
	50% HAE	700 ± 0
Schinus terebinthifolius—bark	AE	201 ± 25
	50% HAE	688 ± 88
Zanthoxylum rhoifolium—bark	50% HAE	850 ± 71
	70% HAE	172 ± 30
Zanthoxylum rhoifolium—leaves	50% HAE	143 ± 25
	70% HAE	167 ± 14

According to Rangel (2010), the stem wood of *Schinus terebinthifolium* had good activity against *Leishmania amazonensis* and *Leishmania chagasi*. Abdel-Sattar et al. (2010) reported the activity of the methanolic extract of the leaves of *Schinus molle* against *Leishmania infantum*, *Trypanosoma brucei*, and *Plasmodium falciparum*.

Table 5 shows the activity of the extracts tested against HSV-1 and Poliovirus. Almost all of the extracts had activity against the tested virus. Only the 70% HAE extract of the bark of *Luehea paniculata* and AE of the bark of *Cordia americana* and *Zanthoxylum rhoifolium* did not exhibit activity against HSV-1. The 70% HAE of the leaves of *Campomanesia eugenioides* and bark of *Cordia americana* and 50% and 70% HAEs of the leaves of *Cordia americana* did not exhibit activity against Poliovirus.

The literature has not reported the antiviral activity of *Campomanesia eugenioides*, but other plants from this family have shown anti-herpes virus and anti-reovirus activity (Nawawi et al., 1999; Simoni et al., 2007). The antiviral activity of *Luehea paniculata* has not yet been described.

Ayisi and Nyadedzor (2003) showed that the AE of *Ocimum gratissimum* is an effective inhibitor of HIV-1 and HIV-2 replication. In the present work, the 50% HAE extract of this species had activity against HSV-1 and Poliovirus. Activity against HSV-1 has been shown with the dichloromethane and methanol extracts of other species, such as *Ocimum sanctum*, *Ocimum basilicum*, and *Ocimum americanum* (Yucharoen et al., 2011). The anti-HSV-1 activity of *Cordia americana* and *Schinus terebinthifolius* and anti-Poliovirus activity of *Schinus terebinthifolius* have not yet been described in the literature, but studies have shown the antiviral activity of other members of the Boraginaceae and Anarcadiaceae families (Tan et al., 1991; Lim et al., 1997; Gundidza, 1993; González et al., 2004).

Finally, the methanolic extracts of other *Zanthoxylum* species, such as *Zanthoxylum chalybeum*, *Zanthoxylum coreanum*, *Zanthoxylum piperitum*, *Zanthoxylum planispinum*, and *Zanthoxylum schinifolium*, exhibited activity against measles virus (Olila and Opuda-Asibo, 2002; Song et al., 2010). To our knowledge, the present study is the first to report the antiviral activity of *Zanthoxylum rhoifolium*.

To further evaluate the pharmacological potential of these plant extracts, cytotoxicity research is needed. The in vitro results with VERO cells are shown in Table 6. The extracts that demonstrated the best results were the 50% HAE of the leaves and bark of *Zanthoxylum rhoifolium*, followed by the 50% HAE of the bark of *Ocimum gratissimum*. The greatest in vitro cytotoxic effects were observed with the aqueous and 70% HAE of *Campomanesia eugenioides*, followed by the aqueous extracts of the bark of *Luehea paniculata* and bark and leaves of *Cordia americana*.

The Selectivity Index is the ratio of biological activity and cytotoxicity, from which we can evaluate the level of selectivity. For antibacterial activity, all of the extracts had MICs higher than the CC_{50} values; therefore, the Selectivity Index was <1 for all of them. For the yeasts, *Luehea paniculata* had activity against all of the species, but the Selectivity Index was <1 for *Candida albicans* (Table 3). By comparing the Selectivity Index of each of the extracts tested, we found that the best activity against HSV-1 was exerted by the 50% HAE of *Ocimum gratissimum*, 50% and 70% HAEs of the leaves of *Cordia americana*, 70% HAE of the bark of *Cordia americana*, 70% HAE of the leaves of *Campomanesia eugenioides*, and 50% HAE of *Schinus terebinthifolius*. The best results against Poliovirus were obtained with the 50% HAE of *Ocimum gratissimum*, followed by the 50% HAE of the leaves of *Zanthoxylum rhoifolium* and 50% HAE of the bark of *Cordia americana* (Table 5). All of the extracts of *Schinus terebinthifolius* presented a strong reddish color, which could interfere with the Selectivity Index results of *Candida* spp. and the tested virus (Tables 3 and 5).

Table 5
Antiviral activity of extracts and SI.

Plant	Extract	HSV-1		Poliovirus	
		$EC_{50} \pm SD$ (μg/ml)	SI	$EC_{50} \pm SD$ (μg/ml)	SI
Campomanesia eugenioides—leaves	AE	1.4 ± 0.3	21	20 ± 11	1.5
	50% HAE	5.5 ± 2.0	13	42 ± 33	1.8
	70% HAE	0.6 ± 0.2	52	> 1000	< 1
Luehea paniculata—bark	AE	3.5 ± 2.1	9.1	15 ± 5	2.1
	50% HAE	3.3 ± 1.1	22	19 ± 6.6	3.8
Ocimum gratissimum—whole plant	50% HAE	7.8 ± 5.6	62	12 ± 1.4	40.3
Cordia americana—bark	AE	> 1000	< 1	30 ± 26	1
	50% HAE	15 ± 4.2	16	20 ± 4.6	12.0
	70% HAE	4.5 ± 1.5	55	> 1000	< 1
Cordia americana—leaves	AE	5.4 ± 2.0	7.1	12 ± 2.8	2.6
	50% HAE	4.3 ± 1.3	52	> 1000	< 1
	70% HAE	3.5 ± 2.8	33	> 1000	< 1
Schinus terebinthifolius—bark	AE	2.4 ± 1.2	21.3	21 ± 22	2.5
	50% HAE	2.1 ± 0.6	> 48	27 ± 1.4	> 3.8
Zanthoxylum rhoifolium—bark	AE	> 1000	< 1	23 ± 5	8.2
	50% HAE	20 ± 12	34	175 ± 21	3.9
Zanthoxylum. rhoifolium—leaves	AE	26 ± 6.9	6.9	22 ± 2.1	8.4
	50% HAE	19 ± 8	37	47 ± 40	15.1

$SI = CC_{50}/EC_{50}$.

Table 6
In vitro cytotoxicity of the plant extracts.

Plant	Extract	CC_{50} (μg/ml) ± SD
Campomanesia eugenioides—leaves	AE	29 ± 4.0
	50% HAE	73 ± 3.5
Luehea paniculata—bark	AE	30 ± 7.6
	50% HAE	72 ± 7.6
Ocimum gratissimum—whole plant	50% HAE	483 ± 202
Cordia americana—bark	AE	30 ± 4.3
	50% HAE	237 ± 12
Cordia americana—leaves	AE	32 ± 5.8
	50% HAE	223 ± 31
Schinus terebinthifolius—bark	AE	51 ± 23
	50% HAE	> 100[1]
Zanthoxylum rhoifolium—bark	AE	185 ± 53
	50% HAE	683 ± 29
Zanthoxylum rhoifolium—leaves	AE	180 ± 19
	50% HAE	700 ± 141

[1] There was color interference of the crude extract above this concentration.

4. Conclusions

The present results showed that none of the studied plants were effective against the gram-positive or -negative bacteria assayed. Good results against *Candida* sp were found using the extracts obtained from the leaves of *Campomanesia eugenioides* and *Schinus terebinthifolius*. However, the HAE exhibited the best results, and the local population uses only the AE to treat their diseases, which presented low antimicrobial activity. The best anti-Leishmania activity was obtained with the HAEs of *Zanthoxylum rhoifolium* and aqueous extract of *Schinus terebinthifolius*. Although many of the studied plants presented antiviral activity, the best anti-HSV-1 activity results were obtained with the 50% HAE of *Ocimum gratissimum* and 50% and 70% HAEs of *Cordia americana*. Anti-Poliovirus activity was observed with the 50% HAE of *Ocimum gratissimum* and leaves of *Zanthoxylum rhoifolium*. *Campomanesia eugenioides* had the most cytotoxic effects in vitro, whereas *Zanthoxylum rhoifolium* was less cytotoxic. To our knowledge, no studies have investigated the biological activity of some of these plants. The indiscriminate use of medicinal plants without knowing their efficacy or safety presents serious risks to the population. The plants investigated in the present study may have potential for pharmacological uses, but new studies, especially with regard to in vivo toxicity, must be conducted. The results of this preliminary survey are important to inform the community of the Rio das Cobras Reserve about the safe and effective use of these plants in the treatment of some infectious diseases.

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