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In vitro antileishmanial activity of Mexican medicinal plants

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[1] Taken in part from the MSc thesis of Ronna Delgado-Altamirano.

Abstract

Aim of the study: To evaluate the anti-leishmanial activity and cytotoxicity of aqueous and organic extracts of ten plants used in Mexican traditional medicine as anti-parasitics.

Materials and methods: For the organic extracts, plant material was macerated in dichloromethane (CH_2Cl_2) and dichloromethane/methanol (CH_2Cl_2/MeOH) (1:1) during two weeks; the aqueous extracts were prepared by infusion. The extracts were tested against promastigotes and intracellular amastigotes of *Leishmania amazonensis*. The cytotoxicity was assayed in parallel on peritoneal macrophages of BALB/c mice.

Results: Four of the thirty extracts tested were active and selective against *L. amazonensis* promastigotes: *Schinus molle* (CH_2Cl_2 and CH_2Cl_2/MeOH), *Lantana camara* (CH_2Cl_2) and *Prosopis laevigata* (aqueous). These extracts had a median

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inhibitory concentration (IC_{50}) against intracellular amastigotes under 50 μg/mL and a selectivity index (SI) higher than 5, which indicates that they constitute valuable candidates to obtain secondary metabolites with leishmanicidal activity. **Conclusions:** The results derived from this study indicate that *L. camara, P. laevigata,* and *S. molle* might provide interesting new leads for the development of antileishmanial drugs.

Keywords: Infectious diseases, Pharmaceutical science

1. Introduction

The World Health Organization (WHO) considers leishmaniases as a group of neglected tropical diseases (NTD) which afflict 12–15 million people in 88 countries (World Health Organization Regional Office for Africa, 2017). They are caused by protozoan species of the genus *Leishmania* and are transmitted by sandflies of the genus *Phlebotomus* and *Lutzomyia* (Tiuman et al., 2011). *Leishmania* species undergo two main phases during their life cycle: the extracellular form, promastigote, which subsists in the sandfly midgut, and the intracellular form, the amastigote, which lives inside macrophages, monocytes, dendritic cells, and neutrophils (Kaye and Scott, 2011).

Leishmaniases can be classified into three main types, according to its clinical manifestations: cutaneous (CL), which affects only localized parts of the skin and is the most common form of the disease; mucocutaneous (MCL), which has the ability to destroy mucous tissue and is exclusively present in America; and visceral (VL) which is the less common type of leishmaniasis but causes liver and spleen distention and can be fatal if it does not receive prompt treatment (Akhoundi et al., 2016; Bifeld and Clos, 2015). Leishmaniases, as in other countries, are endemic maladies of Mexico. The prevalent species of *Leishmania* species of Mexico belong to the *L. donovani*, *L. mexicana*, and *L. braziliensis* complexes (Alvar et al., 2012).

The main chemotherapeutic treatment against leishmaniases are drugs based on pentavalent antimony (Bifeld and Clos, 2015). However, these drugs have cardiotoxic, hepatotoxic, and nephrotoxic side effects (Sundar and Chakravarty, 2010). Moreover, parasite resistance to these agents has emerged in countries such as India, where leishmaniases constitute a major public health problem (Tiuman et al., 2011). At present, there are several factors that promote expansion of leishmaniases such as wars (Alasaad, 2013; Doganay and Demiraslan, 2016), *Leishmania* habitat evolution (Okwor and Uzonna, 2016; Vélez et al., 2017), increase in world travel (Mansueto et al., 2014), and HIV co-infection (Okwor and Uzonna, 2016). These facts emphasize the importance of research to find alternative sources of bioactive molecules that could help in the treatment of this disease

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Traditionally, herbal remedies have been used to alleviate symptoms and improve human health. Even today, in many regions of the world, herbal medicine constitutes the first, and sometimes the only, option for treating leishmaniases (Abdel-Sattar et al., 2010; Musuyu Muganza et al., 2012). Nevertheless, data on the efficacy and safety of these medicinal plants are scarce. Therefore, scientific validation of traditional medicinal resources could lead to new perspectives in protozoal disease control, which is supported by the fact that medicinal plants have proven to be a valuable source of leishmanicidal compounds, which might represent novel leads for the development of drugs (Abdel-Sattar et al., 2010; Singh et al., 2014) or adjuvants in vaccine improvement (Rey-Ladino et al., 2011).

Scientific groups in many parts of the world have investigated the anti-leishmanial potential of plants used in different traditional medicine systems (Al-Musayeib et al., 2012; Luize et al., 2005; Mans et al., 2016; Musuyu Muganza et al., 2012; Sawadogo et al., 2012; Valadeau et al., 2009). However, there are very few reports regarding the leishmanicidal evaluation of Mexican medicinal plants; only a few native plants of the Yucatan Peninsula have been studied (Getti et al., 2009; Peraza-Sánchez et al., 2007).

Mexico is acknowledged worldwide for its extensive and rich biodiversity, comprising more than 20,000 plant species, of which nearly one-third is used in traditional medicine. However, less than 2% of the Mexican flora has been examined from a phytochemical or a pharmacological perspective (Getti et al., 2009). One of most frequent uses of medicinal plants in Mexico is against parasites. Nonetheless, there are not specific records of plants used to treat leishmaniases. In this way, the aim of this study was to determine the in vitro effect of aqueous and organic extracts obtained from ten plants used in Mexican traditional medicine as anti-parasitics on *Leishmania amazonensis.*

2. Materials and methods

2.1. Plant material

2.1.1. Plant selection

An extensive bibliographic search in ethnobotanical records of Mexican traditional medicine was conducted in order to find medicinal plants widely used as anti-parasitics in Mexico. Ten plants easily accessible in *Leishmania* endemic areas (CONABIO, 2017) were selected (Table 1).

2.1.2. Plant collecting

The plants used in this study were collected in different communities of Queretaro and Guanajuato, Mexico. The specimens were identified by Heike Vibrans and deposited at the National Herbarium of Mexico (MEXU). The species, their local

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Table 1. Ten anti-parasitic Mexican plants tested on *Leishmania amazonensis.*

Plant family, species with author(s)	Local name	Collection place and date	Traditional uses	Voucher number
Anacardiaceae				
Schinus molle L.	Pirul, pirú, perú, pelon-quáhuitl	Cerro de las Campanas, Querétaro, Querétaro (20°35′29.92”N 100°24′42.3”W) April, 2014.	For malaria, mycosis, healing wounds, painful joints, rheumatism, colic, stomachache, constipation, toothache, cough, asthma, gonorrhea, varicose veins, feminine sterility, genitourinary diseases, and as a purgative (Foster and Hobbs, 2002; Márquez et al., 1999; Mendoza et al., 1997; UNAM, 2016).	1413997
Asteraceae				
Conyza filaginoides (D. C.) Hieron	Simonillo	Mercado Escobedo, Querétaro, Querétaro March, 2014.	For dysentery, digestive diseases, diarrhea, stomachache, liver pain, indigestion, and anger, colic (Márquez et al., 1999; Mendoza et al., 1997; UNAM, 2016).	1413998
Fabaceae/Leguminosae				
Acacia farnesiana (L.) Willd	Huizache, huechachín, wichachin	El Calichar, Apaseo El Grande, Guanajuato Guanajuato (20°30′35.0”N 100°31′00.8”W) April, 2014.	For diarrhea, wounds, tuberculosis, typhoid, grown spleen, pharyngitis, headache, *Herpes simplex.* As antispasmodic, astringent (González et al., 2004; Márquez et al., 1999; Mendoza et al., 1997; Milliken, 1997; UNAM, 2016).	1413999
Bauhinia variegata L.	Pata de vaca	Lomas de Casa Blanca, Querétaro, Querétaro (20°34′21.9”N 100°23′52.2”W) December, 2013.	For dysentery, diarrhea, wound healing, cough, pulmonary diseases, asthma, child-birth, antiseptic, energizer, and anti-inflammatory (UNAM, 2016).	1413996
Fabaceae/Leguminosae				
Caesalpinia pulcherrima (L.) Swartz	Flamboyán, tabachín, espuela de caballero	Primero de Mayo, Corregidora, Querétaro (20°31′34.8”N 100°27′24.8”W) March, 2014.	For fever and tonsillitis. As anti-parasitic and purgative (González et al., 2004; UNAM, 2016).	1414001
Prosopis laevigata (Willd.) M. Johnson	Mezquite	El Calichar, Apaseo El Grande, Guanajuato (20°35′25.4”N 100°24′38.3”W) April, 2014.	For dysentery, stomach diseases, ocular diseases, conjunctivitis, rash, cough, fever, toothache, pharyngitis, snoring (UNAM, 2016).	1414004

(*Continued*)

Table 1. (*Continued*)

Plant family, species with author(s)	Local name	Collection place and date	Traditional uses	Voucher number
Myrtaceae				
Psidium guajava L.	Guayaba, guayabo, guayabilla	Cerro de las Campanas, Querétaro, Querétaro (20°35′29.92”N 100°24′42.3”W) April, 2014.	For diarrhea, dysentery, intestinal worms, amoebic infection, vomiting, weakness, indigestion, acne, rash, scarlet fever, and scabies (Berlin et al., 1990; González et al., 2013; Leonti, 2002; UNAM, 2016).	1414002
Portulacaceae				
Portulaca oleracea L.	Verdolaga, seda, faginera	Huimilpan, Querétaro (20°22′02.5”N, 100°17′00.7”W) March–April, 2014.	For intestinal infections, intestinal parasites, intestinal worms, stomachache, constipation, diabetes, stomach, and intestinal inflammation (González et al., 2004; Maldonado, 2003; Milliken, 1997; Reid, 1996; UNAM, 2016).	1414003
Rubiaceae				
Bouvardia ternifolia (Cay.) Schltdl.	Trompetilla, cerillito, lengua de víbora	San Ildefonso, Amealco, Querétaro. (20°11′23.4”N 100°08′28.1”W) November, 2013.	For intestinal parasites, snake bite, scorpion and insect sting, erysipelas, pain, fatigue, fever, hematoma. As anti-inflammatory (Mendoza et al., 1997; UNAM, 2016).	1414000
Verbenaceae				
Lantana camara L.	Alfombrilla, gobernadora, ororuz	Cerro de las Campanas, Querétaro, Querétaro. (20°35′25.4”N 100°24′38.3”W) December, 2013. March–April, 2014.	For amebic infection, dysentery, diarrhea, vomit, stomachache, liver pain, toothache, rheumatism, earache, deafness, epilepsy, cramp, skin diseases, ulcers, tumors, diuretic, scorpion and insect sting, and snake bite (Berlin et al., 1990; González et al., 2013; Maldonado, 2003; Márquez et al., 1999; Mendoza et al., 1997; Milliken, 1997; UNAM, 2016).	1414005

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names, collection sites, voucher number (herbarium identification number), and traditional uses are summarized in Table 1.

2.2. Extracts preparation

After drying in the shade at 25 °C for three weeks, 450 g of the collected parts of each plant (Table 2) were crushed in an electric mill (IKA MF 10, mesh: pore diameter 0.5 mm). The powder obtained was divided into 3 parts 150 g each for extraction in 750 mL of each solvent: dichloromethane (CH_2Cl_2), dichloromethane/methanol (CH_2Cl_2/MeOH) (1:1), and water. Preparation of organic extracts was carried out by macerating the plant material at 25 °C with each solvent during one week, and this process was repeated once with fresh solvent. Thereafter, the plant material was filtered and the solvent was removed using a rotatory evaporator (BÜCHI R-114, St. Gallen, Switzerland). The aqueous extracts were obtained by infusion in distilled water (pH = 7, 95 °C). After cooling, the extracts were filtered, frozen and lyophilized. The dried material was then dissolved in dimethylsulphoxide (DMSO) at 20 mg/mL and stored in sealed glass vials at 4 °C for further analysis.

2.3. Animals

Female BALB/c mice (20–22 g, body weight), were obtained from The National Centre of Laboratory Animals Production (CENPALAB, Cuba) and maintained according to the "Guide for the Care and Use of Laboratory Animals". The animal use protocol was approved by the Ethics Committee of the Institute of Tropical Medicine "Pedro Kouri", Havana, Cuba (CEI-IPK 13–10). Peritoneal macrophages for cytotoxic and anti-amastigotes assays were collected as follows: female BALB/c mice were euthanized by cervical dislocation and macrophages were obtained by injection of 5 mL of RPMI-1640 medium into the peritoneal cavity, followed by needle aspiration of the cells.

2.4. Parasites

The *Leishmania* parasites used in this study belong to the strain *L. amazonensis* (MHOM/77BR/LTB0016), which was kindly provided by the Department of Immunology, Oswaldo Cruz Foundation (FIOCRUZ), Brazil. They were obtained from lesions on mice, isolated by aspiration through a needle and maintained as promastigotes at 26 °C in Schneider's medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS) (Sigma-Aldrich, St. Louis, MO, USA), 100 μg of streptomycin/mL, and 100 IU of penicillin/mL. The parasites were recultured in new complete Schneider's medium every 3 or 4 days but were not used after 10 *in vitro* passages.

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Table 2. Anti-leishmanial activity and cytotoxicity of the extracts prepared from medicinal plants used in Mexico as anti-parasitic.

Plant family, species with author(s)	Part used	Extract	IC_{50} ± SD (μg/mL) Promastigotes *L. amazonensis*	CC_{50} ± SD (μg/mL) Peritoneal macrophages BALB/c mice	SI	Classification
Anacardiaceae						
Schinus molle L.	Leaves and branches	CH_2Cl_2	15.4 ± 5.5	69.7 ± 0.3	5	Selective
		CH_2Cl_2/MeOH (1:1)	29.4 ± 6.0	186.8 ± 5.5	6	Selective
		Aqueous	>200	>100	–	Inactive
Asteraceae						
Conyza filaginoides (D.C.) Hieron	All parts	CH_2Cl_2	>200	62.6 ± 0.4	–	Inactive
		CH_2Cl_2MeOH (1:1)	51.1 ± 2.8	53.5 ± 3.4	1	Non-specific
		Aqueous	51.9 ± 5.4	45.3 ± 2.6	1	Non-specific
Fabaceae/Leguminosae						
Acacia farnesiana (L.) Willd	Leaves, branches and fruits	CH_2Cl_2	>200	132.8 ± 4.4	–	Inactive
		CH_2Cl_2/MeOH (1:1)	>200	>200	–	Inactive
		Aqueous	>200	>100	–	Inactive
Bauhinia variegata L.	Leaves and branches	CH_2Cl_2	>200	138.8 ± 1.8	–	Inactive
		CH_2Cl_2/MeOH (1:1)	173.1 ± 6.7	157.0 ± 6.1	1	Non-specific
		Aqueous	>200	>100	–	Inactive
Caesalpinia pulcherrima (L.) Swartz	Leaves and branches	CH_2Cl_2	173.1 ± 4.5	119.0 ± 9.5	1	Non-specific
		CH_2Cl_2/MeOH (1:1)	>200	137.9 ± 1.8	–	Inactive
		Aqueous	>200	117.8 ± 9.6	–	Inactive
Prosopis laevigata (Willd.) M. Johnson	Leaves and branches	CH_2Cl_2	195.5 ± 1.8	57.0 ± 3.5	<1	Toxic
		CH_2Cl_2/MeOH (1:1)	>200	76.5 ± 5.7	–	Inactive
		Aqueous	22.8 ± 2.9	160.7 ± 2.9	7	Selective

(*Continued*)

Table 2. (*Continued*)

Plant family, species with author(s)	Part used	Extract	IC_{50} ± SD (μg/mL) Promastigotes *L. amazonensis*	CC_{50} ± SD (μg/mL) Peritoneal macrophages BALB/c mice	SI	Classification
Myrtaceae						
Psidium guajava L.	Leaves and branches	CH_2Cl_2	61.2 ± 8.1	>200	>3	Non-specific
		CH_2Cl_2/MeOH (1:1)	89.0 ± 4.1	>200	>2	Non-specific
		Aqueous	>200	96.2 ± 0.6	–	Inactive
Portulacaceae						
Portulaca oleracea L.	All parts	CH_2Cl_2	>200	170.5 ± 9.6	–	Inactive
		CH_2Cl_2/MeOH (1:1)	72.0 ± 3.2	166.8 ± 4.4	2	Non-specific
		Aqueous	83.0 ± 9.1	17.7 ± 1.4	<1	Toxic
Rubiaceae						
Bouvardia ternifolia (Cay.) Schltdl	Leaves and stems	CH_2Cl_2	71.7 ± 8.5	18.9 ± 3.0	<1	Toxic
		CH_2Cl_2/MeOH (1:1)	93.8 ± 6.8	108.5 ± 7.3	1	Non-specific
		Aqueous	>200	104.2 ± 2.0	–	Inactive
Verbenaceae						
Lantana camara L.	Leaves and stems	CH_2Cl_2	11.7 ± 4.4	>100	>9	Selective
		CH_2Cl_2/MeOH (1:1)	>200	>200	–	Inactive
		Aqueous	>200	125.9 ± 3.1	–	Inactive
Pentamidine			0.37 ± 0.01	11.7 ± 1.7	32	

– Not Determined.

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2.5. Anti-promastigote screening

Ninety-eight μL promastigotes (10^5 parasites/mL) were distributed in 96-well plates. Two microliters of the test extracts (20 mg/mL) were added to the first wells. These were diluted for final concentrations between 12.5 and 200 μg/mL in the wells. Dimethylsulphoxide (DMSO, 2 μL) was used as a negative control. The treated plates were incubated for 72 h at 26 °C, then 20 μL of a solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) at 5 mg/mL in saline solution (NaCl, 0.9%) was added to each well and the plates were incubated for 4 more hours. The medium was then removed and the precipitated formazan crystals were dissolved by adding of 100 μL of DMSO. Absorbance was determined using an EMS Reader MF, Version 2.4–0, at a wavelength of 560 nm and 630 nm as reference. The IC_{50} value was determined from the concentration-response linear curves. All evaluations were made by triplicate in independent assays. Results are expressed as mean ± standard deviation (García et al., 2012).

2.6. Cytotoxicity assay

The median cytotoxic concentration (CC_{50}) of the extracts on macrophages was determined. Peritoneal macrophages were maintained in RPMI-1640 medium (Sigma, St. Louis, Mo, USA) supplemented with antibiotics (penicillin 200 UI, streptomycin 200 μg/mL). Then, they were seeded in 96-well plates at a concentration of 30,000 cells/well and incubated for 2 h at 37 °C in 5% CO_2 to obtain a monolayer culture. To remove non-adherent cells, wells were washed with phosphate-buffered saline solution (PBS) and treated with 2 μL of the extracts or DMSO, then 98 μL medium with 10% HI-FBS and antibiotics (penicillin 200 UI, streptomycin 200 μg/mL) were added to each well. To test concentrations between 12.5 to 200 μg/mL, the plant extracts were diluted 1:2, five times. Thereafter, the treated macrophages were incubated for 72 h at 37 °C in an atmosphere of 5% CO_2. Cytotoxicity was determined as previously described, adding 15 μL of MTT solution to each well. After incubating 4 h, the formazan crystals were dissolved by addition of 100 μL of DMSO. Absorbance was measured and concentration response curves were constructed to obtain the respective IC_{50} values. Evaluations were performed by triplicate in independent assays. The results are expressed as mean ± standard deviation (García et al., 2012).

2.7. Selectivity index (SI)

The selectivity index (SI) ratio (CC_{50} for macrophages/IC_{50} for promastigotes) was used to compare the toxicity of the extracts against the murine macrophages and their activity against *Leishmania*. An extract is considered inactive when its IC_{50} for promastigotes is greater than 200 μg/mL, selective when its SI is equal to or

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greater than 5, non-specific when SI is between 1 and 5 and, toxic when its SI is less than 1. Extracts with a SI $\geq$ 5 were selected for follow-up anti-amastigote determination.

2.8. Anti-amastigote activity

The peritoneal macrophages from BALB/c mice were cultured with RPMI-1640 medium in 24-well plates at 10^6 cells/mL. Plates were incubated at 37 °C in an atmosphere of 5% CO_2 for 2 h. To remove non-adherent cells, wells were washed with PBS and L. amazonensis promastigotes were added in a ratio of 4:1 parasite/macrophage. The cultures were then further incubated for 4 h. The monolayer intracellular amastigotes were washed to remove free parasites. Subsequently, 1990 μL of the RPMI-1640 complete medium and 10 μL of the extracts or DMSO used as control were added to the wells. Four serial dilutions 1:2 resulted in concentrations between 12.5 to 100 μg/mL. Treated amastigotes were incubated for 48 h under the same conditions. Afterward, cells were fixed in absolute methanol, stained with Giemsa, and examined under light microscopy. The number of intracellular amastigotes was determined by counting the amastigotes resident in 100 macrophages per sample. Results were expressed as percentage of reduction of the infection rate (% IR) relative to those obtained with positive controls. The infection rates were obtained by multiplying the percentage of infected macrophages by the number of amastigotes per infected macrophage. The IC_{50} values were determined from the concentration-response linear curves. Evaluations were performed by triplicate in independent assays. The results are expressed as mean ± standard deviation (García et al., 2012).

2.9. Qualitative chemical profile of the active and selective extracts

A qualitative chromatographic analysis of the CH_2Cl_2 extract of *L. camara* and both organic extracts of *S. molle* was carried out using normal phase thin-layer chromatography (aluminum sheets pre-coated with silica gel 60, layer of 0.20 mm with fluorescent indicator UV_{254} Macherey-Nagel) with hexane/ethyl acetate (3:2) as the mobile phase. The aqueous extract from *P. laevigata* was analyzed with reverse phase thin-layer chromatography (pre-coated aluminum sheets RP-18W, layer 0.15 mm with fluorescent indicator UV_{254} Macherey-Nagel) with MeOH as the mobile phase. The extracts were analyzed for the presence of flavonoids, terpenoids, alkaloids, and cardiotonic glycosides. Ferric chloride 10%, *p*-anisaldehyde/sulfuric acid, Liebermann-Burchard's reagent, vanillin/sulfuric acid, Dragendorff reagent, and antimony chloride were used as chromogenic reagents. TLC plates were visualized under UV light (UV lamp 260/366 nm, San Gabriel, CA, USA), and then sprayed with the chromogenic agents.

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3. Results

3.1. Anti-promastigote screening

Table 2 shows the IC_{50} of the tested extracts against promastigotes of *L. amazonensis*, the CC_{50} on peritoneal macrophages of BALB/c mice, and their respective SI values. Four of the thirty tested extracts were active and selective against *L. amazonensis* promastigotes: *L. camara* (CH_2Cl_2, SI > 9), *S. molle* (CH_2Cl_2, SI = 5 and CH_2Cl_2/MeOH, SI = 6), and *P. laevigata* (aqueous, SI = 7). These four extracts displayed IC_{50} values less than 30 μg/mL. Although they were significantly less potent than pentamidine (IC_{50} of 0.37 ± 0.01 μg/mL), they were considerably less cytotoxic than the positive control against peritoneal macrophages. The active extracts were further evaluated against *L. amazonensis* amastigotes.

3.2. Anti-amastigote screening

The dichloromethane (IC_{50} = 25.9 ± 4.9 μg/mL) and dichloromethane/methanol (IC_{50} = 21.8 ± 4.5 μg/mL) extracts of *S. molle*, the dichloromethane extract (IC_{50} = 21.8 ± 2.4 μg/mL) of *L. camara* and the aqueous extract (IC_{50} = 35.2 ± 4.7 μg/mL) of *P. laevigata* displayed IC_{50} values against intracellular amastigotes less than 50 μg/ml, indicating their potential to fight *Leishmania* spp (Table 3).

3.3. Qualitative chemical profile of active extracts

The dichloromethane extract of *L. camara*, both organic extracts of *S. molle*, and the aqueous extract of *P. laevigata* were analyzed by thin layer chromatography to detect the major classes of secondary metabolites they contain. The results from this qualitative analysis are shown in Table 4. Terpenoids were detected in both organic extracts of *S. molle* and the dichloromethane extract of *L. camara*.

Table 3. Anti-amastigote activity and cytotoxicity of plant extracts whose SI ≥ 5.

Plant family, species with author(s)	Extract	SI	IC_{50} ± SD (mg/mL) Amastigotes *L. amazonensis*
Anacardiaceae			
Schinus molle L.	CH_2Cl_2	5	25.9 ± 4.9
	CH_2Cl_2/MeOH (1:1)	6	21.8 ± 4.5
Fabaceae/Leguminosae			
Prosopis laevigata (Willd.) M. Johnson	Aqueous	7	35.2 ± 4.7
Verbenaceae			
Lantana camara L.	CH_2Cl_2	> 9	21.8 ± 2.4

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Table 4. Qualitative chemical profile of active extracts.

Extract	Phenolic compounds	Terpenoids	Alkaloids	Anthraquinones	Cardiotonic glycosides
Anacardiaceae					
Schinus molle L. (CH_2Cl_2)	−	+	−	−	−
Schinus molle L. (CH_2Cl_2/MeOH)	+	+	−	−	−
Fabaceae/Leguminosae					
Prosopis laevigata (Willd.) M. Johnson (Aqueous)	+/−	−	+	+	−
Verbenaceae					
Lantana camara L. (CH_2Cl_2)	−	+	−	−	−

Alkaloids were only found in the aqueous extract of *P. laevigata*, which also contained phenolic compounds.

4. Discussion

In this study, we tested the leishmanicidal activity of thirty extracts obtained from ten plants used in Mexican traditional medicine as anti-parasitics. Firstly, as a preliminary assay of leishmanicidal activity, all the extracts were tested against *Leishmania amazonensis* promastigotes. The results derived from this analysis showed that fifteen extracts turned out to be inactive (IC_{50} greater than 200 μg/mL), eight were non-specific ($1 < SI < 5$), three resulted primarily cytotoxic against mammalian cells ($SI < 1$), and four were selective ($IS \geq 5$): both organic extracts of *S. molle*, the dichloromethane extract of *L. camara*, and the aqueous extract of *P. laevigata* (Table 2).

The extracts of *P. oleracea* (aqueous), *P. laevigata* (CH_2Cl_2) and *B. ternifolia* (CH_2Cl_2) displayed an $SI < 1$, which indicated that they were more cytotoxic against peritoneal macrophages than to promastigotes, which precludes its potential use as leishmanicidal agents. Therefore they were not considered for further analysis (Cos et al., 2006). It is important to highlight the cytotoxicity exhibited by the aqueous extract of *P. oleracea* since in Mexican traditional medicine, an infusion or a decoction prepared from this plant is drunk during nine days for the treatment of intestinal parasites (UNAM, 2016); and also it is widely consumed as a vegetable. These data show the importance of biological studies to assure the safety of herbal traditional remedies. On the other hand, the extracts which showed an SI between 1 and 5 were non-specific since their cytotoxic and inhibitory concentrations fifty against macrophages and parasites, are too close and therefore, the leishmanicidal activity cannot be attributed to a real antiparasitic effect (Cos et al., 2006).

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Regarding the four extracts that were active and selective against promastigotes, they also showed *in vitro* leishmanicidal activity on amastigotes with an IC_{50} below 40 μg/mL, which implies their true potential as natural products against *Leishmania* (Cos et al., 2006). Concerning the anti-leishmanial activity displayed by the organic extracts of *S. molle*, the most selective one was the CH_2Cl_2/MeOH extract (IC_{50} for amastigotes = 21.8 ± 4.5 μg/mL; SI = 6). These results are in agreement with those reported by Abdel-Sattar et al. (2010), who found that a methanolic extract obtained by reflux from leaves of an Arabian specimen of *S. molle*, presented an IC_{50} of 32.5 μg/mL for intracellular amastigotes of *L. infantum* and an SI of 1.2 (Abdel-Sattar et al., 2010). These findings show that anti-parasitic activity is influenced by the geographical origin of the plants, and the parasite species. Interestingly, in that same study, the authors found that the methanolic extract of *S. molle* was active against *Trypanosoma cruzi, T. brucei*, and *Plasmodium falciparum.* Moreover, Molina-Garza et al. (2014), recently showed that a methanolic extract of the aerial parts of *S. molle* was effective against *T. cruzi* epimastigotes with an IC_{50} of 16.3 ± 3.3 μg/mL (Molina-Garza et al., 2014). All of these studies demonstrate that *S. molle* produces metabolites that exhibit a broad spectrum of anti-parasitic activity. The main components present in the essential oil obtained from leaves of this plant are terpenes (dos Santos et al., 2009; Simionatto et al., 2011). Our thin-layer chromatographic analysis showed that both organic extracts of *S. molle* contain terpenoids, which might be responsible for the activity observed in this study against *L. amazonensis* promastigotes and intracellular amastigotes.

It is widely known that members of the genus *Prosopis* produce alkaloids, which can be extracted with methanol or ethanol (Ibrahim et al., 2013; Nakano et al., 2004; Rahman et al., 2011; Samoylenko et al., 2009; Tapia et al., 2000). The 8-indolizidine alkaloids contained in these species exhibit significant *in vitro* anti-parasitic activity against *Plasmodium falciparum* and *Leishmania donovani*, comparable to the control drugs. These alkaloids also displayed *in vivo* anti-malarial effect against *P. berghei* (Samoylenko et al., 2009). Moreover, De Jesús-Gabino et al. (2010), found that a hexane extract obtained from the leaves of *P. laevigata* had antihelmintic activity in a model of gerbils infected with *Haemonchus contortus* (De Jesús-Gabino et al., 2010). These findings indicate that *Prosopis* species produce polar and non-polar compounds which possess anti-parasitic potential against protozoans and helminths. Flavonoids have been detected in polar extracts of species belonging to the *P. juliflora* complex, including *P. laevigata* (Bragg et al., 1978). In our study, we found that the aqueous extract prepared from the leaves and branches of *P. lavevigata* contains alkaloids and flavonoids. Thus, the significant anti-leishmanial activity exhibited by this extract might be attributed to these metabolites.

The dichloromethane extract prepared from the leaves and stems of *L. camara* was the most selective one (SI < 9) with an IC_{50} for amastigotes of 21.8 ± 2.4 μg/mL.

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The thin-layer chromatographic analysis showed that this extract contains mainly terpenes. Barre et al. (1997) demonstrated that a chloroform extract prepared from the leaves of *L. camara* contained this type of secondary metabolites; specifically, triterpenes of the lantadene type (Barre et al., 1997). Recently, Begum et al. (2015), isolated eight triterpenes from a methanolic extract of the aerial parts of a specimen of *L. camara* collected in Pakistan (Begum et al., 2015). They also tested the anti-leishmanial activity of these compounds against *Leishmania major* promastigotes and found that ursolic acid displayed the most potent leishmanicidal effect, with an IC_{50} of 12.4 ± 0.03 μM. Therefore, these researchers concluded that this triterpene has a great potential as an anti-leishmanial agent (Begum et al., 2015). Recently, they have also demonstrated that triterpenoids contained in the methanolic extract of *L. camara* aerial parts have nematicidal activity against *Meloidogyne incognita* (Begum et al., 2015), which reinforces the potential of this species as a source of bioactive compounds. *L. camara* is an ornamental species spread worldwide (Ghisalberti, 2000), which is resistant and easy to maintain (Begum et al., 2015). Therefore, it might represent a feasible source of molecules, specifically terpenes, with leishmanicidal activity with a high selectivity index. At present, a bio-directed phytochemical study is being carried out in order to isolate and identify the molecule(s) responsible for this activity.

5. Conclusion

Organic and aqueous extracts prepared from plants widely used in Mexican traditional medicine as anti-parasitics were tested for their leishmanicidal activity on *Leishmania amazonensis.* Four of the tested extracts were active and selective on promastigotes and intracellular amastigotes of *L. amazonensis.* The dichloromethane extract of *L. camara* was the most potent and selective one. This extract primarily contains terpenes, which very likely are responsible for the leishmanicidal activity.

Declarations

Author contribution statement

Ronna Delgado-Altamirano: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Alejandra Rojas-Molina: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abel Piñón-Tápanes: Performed the experiments.

Lianet Monzote Fidalgo, Fausto Rivero, César Ibarra-Alvarado: Analyzed and interpreted the data.

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Heike Vibrans Lindeman: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Competing interest statement

The authors declare no conflict of interest.

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Additional information

No additional information is available for this paper.

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