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SCREENING FOR ANTIBACTERIAL AND ANTIPROTOZOAL ACTIVITIES OF CRUDE EXTRACTS DERIVED FROM MEXICAN MEDICINAL PLANTS

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Abstract

Background: *Crataegus mexicana*, *Hyptis albida*, *Larrea tridentata*, *Ocimum baislicum*, *Prunus serotina*, and *Smilax* spp. are used in Mexican traditional medicine to treat respiratory and gastrointestinal diseases such as flu, cough, diarrhea, dysentery, and other parasitic or microbial infections. Therefore this study was aimed at the pharmacological prospection of these plants against eleven bacterial species and three amitochondrial protist pathogens.
Material and methods: The fruits or aerial parts of *C. mexicana*, *H. albida*, *L. tridentata*, *O. baislicum*, *P. serotina*, and *Smilax* spp. were extracted with different solvents. The antibacterial properties of organic and aqueous extracts of these plants were determined by the microdilution method and the microplate alamar blue assay against *Stenotrophomonas maltophilia*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Mycobacterium tuberculosis*, whereas anti-protozoal activities of extracts were evaluated by a vial micro-assay against strains of *Entamoeba histolytica*, *Trichomonas vaginalis*, and *Giardia lamblia*.
Results: *H. albida*, *Smilax* spp, and *C. mexicana* showed good activity against the Gram-positive strains, *S. aureus*, methicillin-resistant *S. aureus*, and *E. faecalis*. Four extracts (*C. mexicana*, *H. albida*, *O. basilicum*, and *L. tridentata*) showed good activity against *E. histolytica*, *T. vaginalis*, and *G. lamblia*.
Conclusion: The extracts of these six medicinal plants could be a source for new antibacterial and antiprotozoal drugs. For this reason they are currently under investigation to isolate and characterize their active compounds.

Keywords: antibacterial, antiprotozoal, extract, plants, traditional medicine

Introduction

Among microbial diseases, bacterial and protozoan infections have become two of the major health problems for humans over the last decades, mainly in low-income countries. On one hand, there is an increase in the number of drug-resistant bacteria and a dearth of novel classes of antibiotics (Laxminarayan et al., 2013); on the other hand, there is an increase in the number of parasitic infections in developing countries due to low-income rates and poor sanitation levels (Norhayati et al., 2003). Thus, microbial infections have motivated the public and private sectors to develop novel antibacterial and antiprotozoal agents.

Some community and hospital-acquired bacterial infections have become untreatable with available antibiotics. Particularly, penicillin-treated staphylococci have turned into virulent strains within a year. Methicilllin-resistant *Staphylococcus aureus* spurred the development of vancomycin-resistant strains, and *Mycobacterium tuberculosis* has also evolved into multidrug-resistant and extensively drug-resistant strains (Overbye and Barret, 2005). The most common resistant pathogens are penicillin-resistant *Streptococcus pneumoniae*, methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus faecium*, *Salmonella typhimurium*, *Pseudomona aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp. and *Acinetobacter baumannii* (Coates et al., 2002; Walsh, 2003). Current concern about resistance has prompted researchers to deal with a dearth of novel classes of antibacterial agents over the last two decades (Moir et al., 2012; Taylor, 2013). Natural products or their derivatives have played a critical role in the discovery and development of different classes of antibiotics, but the innovative antibiotic pipeline has decreased since the 1970s (Fernandes, 2006; Butler et al., 2013). Current clinical antibacterial agents exhibit structural similarities that make them more susceptible to bacteria resistance mechanisms. Therefore, the quest for new antibacterial agents should be directed to the discovery of structurally diverse compounds from natural sources other than microorganisms and targets other than those of commercial drugs (Taylor, 2013).

In addition to the problem of bacterial resistance, parasitic infections have also drawn the attention of researchers, particularly amoebiasis, trichomoniasis, and giardiasis, which are caused by the amitochondrial protist pathogens *Entamoeba histolytica*, *Trichomonas vaginalis*, and *Giardia lamblia*, respectively (Schwebke and Burgess, 2004; Stanley, 2003; Lujan, 2006). The enteric pathogens *G. lamblia* and *E. histolytica* cause persistent diarrhea, dysentery, stomach pain, cramps, bloating, and tenderness. According to the World Health Organization, amoebiasis is the third leading cause of death due to parasitic infections, surpassed only by malaria and schistosomiasis (WHO, 1997). Giardiasis

mainly affects children under 5 years of age in developing countries, contributing to 2.5 million deaths annually from diarrheal disease (Ricci et al., 2006). On the other hand, trichomoniasis, a sexually transmitted disease associated with vaginitis, cervicitis, urethritis, prostatitis, epididymitis, cervical cancer, and infertility, accounts for 180 million infections acquired annually worldwide (Ali and Nosaki, 2007; Nanda et al., 2006).

E. histolytica, *T. vaginalis*, and *G. lamblia* infections are mainly treated with imidazole type drugs, but it has been reported that these three protist pathogens have developed drug resistance (Upcroft and Upcroft, 2001; Pal et al., 2009). This has produced an urgent need for more efficient and safer antiprotozoal agents. The development of new antiparasitic drugs has not been a priority for the pharmaceutical industry because many parasitic diseases occur in developing countries where the population cannot afford to pay for the drugs. Thus an investment in drug development against parasitosis is a risky affair (Wink, 2012).

In the continuous search for novel classes of antibacterial and antiprotozoal agents from plants (Favela-Hernández et al., 2012; Rojas et al., 1992; Bocanegra-García et al., 2009; González-Garza et al., 1989; Mata-Cárdenas et al.,, 2008), we would like to describe the antibacterial and antiprotozoal evaluation of extracts obtained from six plants that are used in Mexican traditional medicine to treat respiratory and gastrointestinal diseases such as flu, cough, diarrhea, dysentery, and other parasitic or microbial infections (Biblioteca Digital de la Medicina Tradicional Mexicana, 2014).

Crataegus mexicana Moc.Sessé, (Rosaceae) *ex C. pubescens* Kunth Steud., locally known as tejocote, manzanilla, tejocotera, manzanillo, and karash, among other names, is a Mexican medicinal plant traditionally used as an antihypertensive and cardiotonic drug. The infusions of its leaves and bark are also used to treat diarrhea, "stomach pain", and to fight against parasitic worms as well as amoebas that cause dysentery. It has also been reported that it is traditionally used for the treatment of several respiratory diseases such as flu, cough, and asthma (Biblioteca Digital de la Medicina Tradicional Mexicana, 2014). The tracheal relaxant effect of the leaves of *C. mexicana* has been investigated using isolated tracheal rings of guinea-pig as an experimental model (Arrieta et al., 2010). At the clinical level, standardized extracts of pure *Crataegus* species, such as *C. pinnatifida* and *C. oxyacantha*, and other highly hybridized species, are currently in use for the treatment of congestive heart failure and hypertension (Koch and Malek, 2011). According to literature, no chemical studies have been reported for *C. mexicana* (Edwards et al., 2012).

Hyptis albida Kunth (Lamiaceae), generally known as "salvia blanca", has been used traditionally to treat influenza and rheumatic pain as well as for the healing of wounds and as an anthelmintic (Biblioteca Digital de la Medicina Tradicional Mexicana, 2014; Rojas et al., 1992; Pereda-Miranda et al., 1990a). The leaves are also reputed to act as a potent insect repellent (Altschul, 1973). Three triterpene lactones, four triterpenes, and five flavonoids have been isolated from the acetone extract of *H. albida* (Pereda-Miranda, 1990a). The anti-inflammatory effects of the chloroform extract of this plant on lipopolysaccharide-stimulated peritoneal macrophages have been described (Sánchez-Miranda et al., 2013).

Larrea tridentata (Sesse & Mocino ex DC.) Coville is also known as Larrea, chaparral, or creosote bush in the United States and gobernadora (governess) and hediondilla (little smelly one) in Mexico. It is a shrubby plant belonging to the family Zygophyllaceae, which dominates some areas of the southwest desert in the United States and Northern Mexico, as well as some desert areas of Argentina (Abou-Gazar et al., 2004; Arteaga et al., 2005). It is traditionally used in Mexico to treat infections, kidney problems, gallstones, diabetes, rheumatism, arthritis, wounds, skin injuries, and dissolve tumors. In the United States it is used to treat tuberculosis, venereal diseases, cancer, and wounds, as well as an expectorant and tonic (Ross, 2005). It has been reported that *L. tridentata* possesses antibacterial lignans and flavonoids (Favela-Hernández et al., 2012), cytotoxic lignans (Yokosuda et al., 2011; Lambert et al., 2005), cytotoxic triterpene glycosides (Jitsuno and Mimaki, 2010), antioxidant lignans (Abou-Gazar et al., 2004), anti-HIV lignans (Gnabre et al., 1996), furanoid lignans (Konno et al. 1990), and flavonoids (Chirikdjian, 1974; Bernhard and Thiele, 1981). The methanolic extract of *L. tridentata* has activity against several nosocomial bacteria (Bocanegra-García et al., 2009).

Ocimum basilicum is commonly known as basil or albácar. It is a culinary plant belonging to the Lamiaceae family that is extensively used as a flavoring agent in a wide variety of regions and as a popular traditional folk remedy. This plant is used as carminative, stimulant, diaphoretic, diuretic, dyspeptic, antiseptic, anesthetic, flatulence, gastritis, anti-spasmodic, anthelmintic, anti-diarrheal, analgesic and anti-tussive. Other medicinal uses of *O. basilicum* include treatment of some gastrointestinal disorders, gastrodynia, diarrhea and vomiting (Biblioteca Digital de la Medicina Tradicional Mexicana, 2014; Shirazi et al., 2014). The essential oil has antioxidant, antimicrobial, cytotoxic and larvicidal properties (Shirazi et al., 2014; Kuorwel et al., 2011; Govindarajan et al., 2013). Chemical composition of the essential oil of *O. basilicum* includes methylchavicol, neral, nerol, geranial, 1,8-cineole, linalool, citral, estragole, eugenol, methylcinnamate, camphor, ocimene, geraniol, bisabolene, caryophyllene, and methyleugenol (Shirazi et al., 2014; Kuorwel et al., 2011; Govindarajan et al., 2013; Miele et al., 2000; de Vincenzi et al., 2000). It was also reported that *O. basilicum* contains three new compounds named basilol, ocimol, and basilimoside (Siddiqui et al., 2007).

Prunus serotina (Cav. ex Spreng) McVaugh (Rosaceae) is a 60 to 90 foot-tall native North American tree, which is widely distributed in Mexico and commonly called "capulín", "taunday", "tzuúri", "chencua", American black cherry, and bird cherry (Martínez, 1991). Some people from Mexico have used the bark, leaves, inflorescences and fruits of *P. serotina* for healing cardiac, pulmonary, and intestinal ailments; particularly, the fruits are used, alone or prepared in syrups and liquors, for the treatment of cough and diarrhea (Biblioteca Digital de la Medicina Tradicional Mexicana, 2014; Argueta, 1994a). Besides their use in traditional medicine, the fruits of this plant are also part of the traditional Mexican diet and are consumed fresh, dried, as an ingredient of "tamales" or prepared in jam. *P. serotina* has also been the subject of a few chemical studies. Kaempferol, quercetin and isorhamnetin glycosides, ursolic acid derivatives and prunasin have been detected in the leaves (Biessels et al., 1974; Olszewska, 2005a, b). Cyanogenic glycosides such as amygdalin and prunasin have been isolated from the seeds (Santamour, 1998), whereas anthocyanidins have been detected in the fruit skin (Ordaz-Galindo, 1999). Previous studies carried out by our research group demonstrated that the lyophilized aqueous and methanolic extracts obtained from black cherry leaves promoted vascular smooth muscle relaxation of the rat aorta (Ibarra-Alvarado et al., 2010). A bioactivity-directed chemical study of the methanolic extract of black cherry leaves led to the isolation of three known natural products: hyperoside, prunine, and ursolic acid, which elicited a concentration-dependent relaxation of vascular muscle (Ibarra-Alvarado et al., 2009).

The commercial root product of Cocolmeca is used in Mexican traditional medicine to treat obesity, syphilis, parasites, dysentery, and diabetes (Biblioteca Digital de la Medicina Tradicional Mexicana, 2014; Argueta et al., 1994b). Cocolmeca is referenced in the literature as *Smilax* spp. (Smilacaceae), but it is also named *Smilax cordifolia*, *S. acutifolia*, *S. invenusta*, and *S. schiedeana* (Martínez, 1979). Species of *Smilax* are widely distributed from Asia to America and several species have been used as traditional remedies to ameliorate infectious diseases (Challinor et al., 2012; Kuo et al., 2005; Seo et al., 2012; Hooda et al., 2011; Xu et al., 2013). *S. glauca*, *S. rotundifolia*, and *S. china* have been

traditionally used in China to treat syphilis, leprosy, and psoriasis (Challinor et al., 2012; Kuo et al., 2005; Seo et al., 2012). In Ayurveda, *S. zeylanica* is reported for the treatment of syphilis, gonorrhea, and skin diseases (Hooda et al., 2011). Pharmacological prospection of several species of *Smilax* have reported that *S. glabra* possesses anti-inflammatory, immunomodulatory, antitumor, and antimicrobial effects (Xu et al., 2013). The chemical constituents of several *Smilax* species have been reported to be triterpenoid and steroidal saponins (sarsaparrilloside, parillin), steroidal sapogenins (smilagenin, sarsapogenin), stilbenes (resveratrol), flavonoids [astilbin, (2S,3S)-5-O-β-D-glucopyranosiloxy-6-methyl-3'-methoxy-3,7,4'-trihydroxyflavone, (2S,3S)-5-O-β-D-glucopyranosiloxy-6-methyl-4'-methoxy-3,7,3'-trihydroxyflavone)], triterpenoids, phenylpropanoid glycosides (smilasides D, E, and F), epicatechins, etc. (Challinor et al., 2012; Kuo et al., 2005; Seo et al., 2012; Hooda et al., 2011; Xu et al., 2013).

The selected species are among the most used plants in Mexican traditional medicine. These plants are used in respiratory and gastrointestinal diseases that are related to antimicrobial and antiparasitic activities (Biblioteca Digital de la Medicina Tradicional Mexicana, 2014). Therefore, we decided to evaluate antibacterial and antiprotozoal effects of their extracts, because these plants could be a source of new antibacterial and antiprotozoal drugs.

Materials and Methods

Sample collection

Leaves and fruits of *C. mexicana* were collected on October 31, 2010, in Laguna de Servín, Amealco, Querétaro. A voucher specimen (A. Cabrera 2560) has been deposited at the herbarium *Jerzy Rzedowski* (HJR), Faculty of Natural Sciences, Universidad Autónoma de Querétaro. The fruits of *P. serotina* were obtained from orchards in Huejotzingo, Puebla (México) in May, 2011, and were transported to Querétaro and stored at -70°C. A voucher specimen (825) was identified by Dr. M. Martínez and Dr. L. Hernández-Sandoval and deposited in the HJR. The aerial parts of *H. albida* were collected in Guadalcazar, in the state of San Luis Potosí, México, in July 2009. This plant was identified by the taxonomist José García Pérez and a voucher specimen (SPLM20419) has been deposited in the Isidro Palacios herbarium of the Universidad Autónoma de San Luis Potosí. The leaves of *L. tridentata* were collected in Nuevo León, México in July, 2009. A voucher specimen (024772) was identified by the biologist Mauricio González Ferrara and deposited in the herbarium of the Facultad de Ciencias Biológicas (FCB), Universidad Autónoma de Nuevo León, Mexico. The aerial parts of *O. basilicum* were collected in San Nicolás de los Garza, Nuevo León, Mexico. In November, 2012 and a voucher sample (010293) was placed in the herbarium of the FCB. Finally, Dr. Carmelita García Padrón provided the roots of *Smilax* spp. in January, 2011. Dr. Carmelita is the owner of Nature Select Company located at Carpinteros de Paracho 416-A, Colonia Vasco de Quiroga, Morelia, Michoacán, Mexico.

Plant extract preparation

The freeze-dried fruits of *C. mexicana* were extracted separately by maceration (100 g) and decoction (100 g) with water and the shade-dried leaves (100 g) successively by maceration with hexane (500 ml), CH_2Cl_2 (500 ml), and CH_3OH (500 ml). Each extract was filtered separately, and the organic solvents were removed in a rotary evaporator at reduced pressure. Aqueous extracts were freeze-dried. The fruits of *P. serotina* were peeled to separate flesh and peel and both were lyophilized separately. Lyophilized peel and flesh were extracted with CH_2Cl_2/CH_3OH (1:1) by maceration and solvent was evaporated under reduced pressure. The shade-dried aerial parts of *H. albida* and *O. basilicum*, as well as the leaves of *L. tridentata*, and the roots of *Smilax* spp, were ground separately. Each powdered material (100 g) was macerated with 500 ml of CH_2Cl_2/CH_3OH (1:1). Each extract was filtered separately, and the solvents were removed in a rotary evaporator at reduced pressure; the dry extracts were then stored at 4 °C until use.

Biological assays

Sample preparation

Stock solutions for antibacterial activity were prepared by dissolving approximately 1 mg of each extract with dimethyl sulfoxide (DMSO) to achieve a concentration of 20 mg/ml. Stock solutions were kept at -20 °C until their use. Antibacterial working solutions were prepared from an aliquot of each of the stocks and diluted with Mueller-Hinton medium. The same procedure was used to prepare anti-tubercular working solutions, but using Middelbrook 7H9 medium supplemented with OADC instead. Stock solutions for antiprotozoal activity were prepared dissolving each extract and metronidazole in dimethyl sulfoxide (DMSO) to achieve a concentration of 1 mg/ml. All stock solutions were stored at -20 °C until use.

Bacterial strains and inoculum preparation

For antibacterial activity, the following strains were used: Gram-negative bacteria: *Stenotrophomonas maltophilia* ATCC 12714, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 15308, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *Enterobacter cloacae* ATCC 35030; Gram-positive bacteria: *Staphylococcus aureus* ATCC29213, methicillin-resistant *Staphylococcus aureus* MRSA ATCC BAA-44, *Listeria monocytogenes* ATCC 19111 and *Enterococcus faecalis* ATCC 29212. Strains were inoculated into plates prepared with 5% blood agar grown for 24 hours at 37 °C. To prepare inoculum for testing, three to five colonies of each culture were transferred into tubes with sterile saline, and turbidity was adjusted to 0.5 McFarland standard (1.5×10^8 CFU/ml).

Subsequently, 10 µl were transferred into 11 ml Mueller-Hinton broth to achieve 5×10^5 CFU/ml. For anti-tubercular activity, *M. tuberculosis* H37Rv American Type Culture Collection (ATCC 27294) sensitive to isoniazid, rifampicin, ethambutol and pyrizanamide was used. Mycobacteria was cultured in Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC (oleic acid albumin dextrose catalase, Becton, Dickinson and Company, Franklin Lakes, NJ) at 37 °C for 2 weeks in order to reach logarithmic phase growth. Testing inoculum was prepared diluting the bacterial suspension with culture medium to adjust turbidity to McFarland's nephelometer No. 1 standard; this bacterial suspension was further diluted to 1:20 with the same culture medium. The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of drug that inhibited the growth of bacteria.

Protozoa cultures

E. histolytica strain HM-1:IMSS, *T. vaginalis* strain GT-13, and *G. lamblia* strain 0989:IMSS were used in this study. *E. histolytica* and *T. vaginalis* were grown in PEHPS medium (Said-Fernández et al., 1988), and *G. lamblia* in TYI-S-33 supplemented with bile (Keister, 1983). All three species were subcultured three times each week. Parasites used in the assays to determine drug susceptibility were harvested when cultures had reached the middle of their respective logarithmic growth phase (Mata-Cárdenas et al., 2008).

Antibacterial activity

Antibacterial activity was determined by the microdilution method (Zgoda and Porter 2001). All tests were performed twice in different days. Levofloxacin and media with bacteria without sample were used as positive and negative controls, respectively.

Antitubercular activity

Antitubercular activity was determined by Alamar Blue assay microplate (Camacho-Corona et al., 2009). Each experiment was performed twice on different days. Isoniazid and media with bacteria without sample were used as positive and negative controls, respectively.

Antiprotozoal activity

Antiprotozoal activity was determined as previously described in the literature (Mata-Cárdenas et al., 1996). Each drug was assayed in triplicate with each protozoan species and the mean and 95% confidence limits were calculated. The Medium Inhibitory Concentration (IC_{50}) was calculated using the Probit program. Metronidazol and protozoa with medium without sample were used as positive and negative controls, respectively.

Results

Antibacterial activity

Nine organic extracts and one lyophilized sample obtained from different parts of five plant species (*C. mexicana*, *H. albida*, *O. basilicum*, *P. serotina*, and *Smilax spp.*) were screened against Gram-positive, Gram-negative bacterial strains and acid-alcohol bacilli *M. tuberculosis* H37Rv, the MIC values are shown in Table 1. Of the 10 samples tested against 11 bacteria strains, only three exhibited good activity against three pathogens: *S. aureus*, methicillin-resistant *S. aureus*, and *E. faecalis*. The best activity was observed for extracts of the aerial parts of *H. albida* and the roots of *Smilax* spp., which exhibited MIC values of 25, 25 and 50 μg/ml against three Gram-positive strains: *S. aureus*, methicillin-resistant *S. aureus*, and *E. faecalis*, respectively. Furthermore, the organic extracts of *H. albida and Smilax* spp showed moderated to weak activity towards *E. coli* (MIC 100-200 μg/mL), *K. pneumoniae* (MIC 100 μg/mL), *L. monocytogenes* (MIC 200 μg/mL), and *M. tuberculosis* H37Rv (MIC 200 μg/mL).

The aforementioned three Gram-positive bacteria were also inhibited by the methanolic extract of *C. mexicana* leaves with MIC values of 50 μg/ml against *S. aureus* and *E. faecalis* and 100 μg/ml towards methicillin-resistant *S. aureus* (Table 1). In addition, the methanolic extract of *C. mexicana* leaves displayed activity against *S. maltophilia* (MIC 50 μg/ml), *A. baumannii* (MIC 200 μg/ml), *K. pneumoniae* (MIC 200 μg/ml), and *M. tuberculosis* H37Rv (MIC 100 μg/ml)

Other extracts that showed weak activity (MIC 200 μg/ml) were the aqueous extracts of *P. serotina* (*A. baumannii*), the organic extract of *P. serotina* (*S. maltophilia*, methicillin-resistant *S. aureus*, *M. tuberculosis*), cloroformic extract of *C. mexicana* leaves (*L. monocytogenes*), hexanic extract of *C. mexicana* leaves (*P. aeruginosa*, *L. monocytogenes*, *M. tuberculosis*), methanolic extract of *C. mexicana* fruits (*S. aureus*), and CH_2Cl_2 extract of *C. mexicana* fruits (*E. coli*).

Antiprotozoal activity

Ten organic extracts and one lyophilized sample obtained from different parts of six plants (*C. mexicana*, *H. albida*, *O. baislicum*, *P. serotina*, *L. tridentata*, and *Smilax spp.*) were screened against *E. histolytica*, *T. vaginalis*, and *G. lamblia*. The IC_{50} values are shown in Table 2. The organic extracts of *C. mexicana* leaves, *H. albida*, *O. basilicum*, and *L. tridentata* were active against the tested parasites. The extract of *H. albida* was the most active against *G. lamblia* and *T. vaginalis* and exhibited IC_{50} values of 16.11 and 11.42 μg/ml, respectively (Table 2).

The methanolic extract of *C. mexicana* leaves exhibited moderate activity against *G. lamblia* (IC_{50} = 153 μg/ml) and *T. vaginalis* (IC_{50} = 125 μg/ml). The extract of *O. basilicum* also showed moderate activity against *G. lamblia* (IC_{50} = 100 μg/ml). Finally, the extract of *L. tridentata* exhibited moderate inhibitory activity against *E. histolytica* (IC_{50} = 100 μg/ml), *G. lamblia* (IC_{50} = 116 μg/ml) and *T. vaginalis* (IC_{50} = 118 μg/ml).

Discussion

Antibacterial activity

Results of antibacterial activity show that three extracts have a wide range of antibacterial activity. These are obtained from *H. albida*, *Smilax* spp and *C. mexicana* (Table 1). Of particular interest is the good inhibitory activity of *Smilax* spp, against methicillin-resistant strains of *S. aureus*, suggesting that this organic extract probably contains novel antibacterial compounds useful for the treatment of resistant staphylococcal infections. This suggestion could be reinforced with previous reports that describe the potent antibacterial activity of two taxonomically related species, *S. china* and *S. glabra* (Seo et al., 2012; Xu et al., 2013). One of the aforementioned studies reported two epicatechins (smiglabrone A and cinchonain Ib), two stilbenes (smiglastilbene and *trans*-resveratrol) and two flavanones (smilachromanone and sakuranetin) as the most

bioactive molecules against *S. aureus*, methicillin-resistant *S. aureus*, and *E. faecalis*, all having MICs in the range of 0.08-1.05 mM (Xu et al., 2013).

Table 1: Antibacterial activity of plant extracts

Bacteria/Samples	**Minimum Inhibitory Concentration (MIC, µg/mL)**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Stenotrophomonas maltophilia ATCC 12714	>200	**200**	**50**	>200	>200	>200	>200	>200	>200	>200	4
Escherichia coli ATCC 25922	>200	>200	>200	>200	>200	>200	**200**	**200**	**100**	>200	0.78
Acinetobacter baumannii ATCC 15308	**200**	>200	**200**	>200	>200	>200	>200	>200	>200	>200	4
Pseudomona aeruginosa ATCC 27853	>200	>200	>200	>200	**200**	>200	>200	>200	>200	>200	4
Enterobacter cloacae ATCC 35030	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	0.78
Klebsiella pneumoniae ATCC 700603	>200	>200	**200**	>200	>200	>200	>200	**100**	**100**	>200	4
Staphylococcus aureus ATCC29213	>200	>200	**50**	>200	>200	**200**	>200	**25**	**25**	>200	6.25
Staphylococcus aureus MRSA ATCC BAA-44	>200	**200**	**100**	>200	>200	>200	>200	**25**	**25**	>200	6.25
Listeria monocytogenes ATCC 19111	>200	>200	>200	**200**	**200**	>200	>200	**200**	**200**	>200	2
Enterococcus faecalis ATCC 29212.	>200	>200	**50**	>200	>200	>200	>200	**50**	**50**	>200	0.78
Mycobacterium tuberculosis H37Rv	>200	**200**	**100**	>200	**200**	>200	>200	**200**	**200**	>200	0.02

Plant extracts were screened for antibacterial activity. Samples: **1**.- *Prunus serotina* (lyophilized flesh and peel fruits). **2**.- *P. serotina* (flesh and peel, CH_2Cl_2/CH_3OH 1:1). **3**.- *Crategus mexicana* (leaves, CH_3OH). **4**.- *C. mexicana* (leaves, CH_2Cl_2). **5**.- *C. mexicana* (leaves, C_6H_{12}). **6**.- *C. mexicana* (fruits, H_2O, maceration). **7**.- *C. mexicana* (fruits, H_2O, decoction). **8**.- *Hyptis albida* (aerial parts, CH_2Cl_2/CH_3OH 1:1). **9**.- *Smilax* spp. (roots, CH_2Cl_2:CH_3OH 1:1). **10**.- *Ocimum basilicum* (aerial parts, CH_2Cl_2/CH_3OH 1:1). **11**.- Levofloxacin (antibacterial) and isoniazid (antitubercular).

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Table 2: Antiprotozoal activity (IC_{50} in μg/ml) of plant extracts

Samples	*Entamoeba histolytica*	*Giardia lamblia*	*Trichomonas vaginalis*
1	> 300	>300	>300
2	> 300	>300	>300
3	> 300	**153**	**125**
4	> 300	> 300	> 300
5	> 300	> 300	> 300
6	> 300	> 300	> 300
7	> 300	> 300	> 300
8	> 300	**16.11**	**11.42**
9	255	> 300	> 300
10	> 300	**100.31**	> 300
11	**100**	**116**	**118**
Metronidazole	0.711	0.512	1.04

Plant extracts were screened for antiprotozoal activity. Samples: **1**.- *Prunus serotina* (lyophilized flesh and peel fruits). **2**.- *P. serotina* (flesh and peel, CH_2Cl_2/CH_3OH 1:1). **3**.- *Crategus mexicana* (leaves, CH_3OH). **4**.- *C. mexicana* (leaves, CH_2Cl_2). **5**.- *C. mexicana* (leaves, C_6H_{12}). **6**.- *C. mexicana* (fruits, maceration). **7**.- *C. mexicana* (fruits, decoction). **8**.- *Hyptis albida* (aerial parts, CH_2Cl_2/CH_3OH 1:1). **9**.- *Smilax* spp. (roots, CH_2Cl_2:CH_3OH 1:1). **10**.- *Ocimum basilicum* (aerial parts, CH_2Cl_2/CH_3OH 1:1). **11**.- *Larrea tridentata* (leaves, CH_2Cl_2/CH_3OH 1:1)

Previous research reported two flavonoids from *H. albida* (cirsimaritin and isosakuranetin), both active against *S. aureus* with MIC values of 31.25 and 50 μg/ml, respectively (Rojas et al., 1992). *H. albida* has ursolic, betulinic, and oleanolic acids (Pereda-Miranda et al., 1990b), which inhibit *S. aureus* (ursolic acid, MIC = 8μg/ml; betulinic acid, MIC = 64 μg/ml; oleanolic acid, MIC = 32μg/ml) and *E. faecalis* (ursolic and oleanolic acids, MIC = 4 and 8 μg/ml, respectively) (Fontanay et al., 2008; Chung et al., 2014). Despite these reports, our current research is focused on the isolation of different molecules responsible for the inhibitory properties of *H. albida* against sensitive and methicillin-resistant *S. aureus* and *E. faecalis*.

The methanolic extract obtained from leaves of *C. mexicana* is currently under investigation in order to isolate and characterize the active antibacterial compounds and to provide the first report on the chemistry of this plant.

Antiprotozoal activity

Results of antiprotozoal activity showed that the extract of *H. albida* was the most active against *G. lamblia* and *T. vaginalis* (Table 2). It is important to point out that there are no reports on the antiprotozoal activity of this plant and further studies should be done in order to isolate its active antiprotozoal compounds.

The methanolic extract of *C. mexicana* leaves exhibited moderate activity against *G. lamblia* and *T. vaginalis* (Table 2), which could explain in part the traditional use of its leaves and bark against dysentery caused by amoebas (Biblioteca Digital de la Medicina Tradicional Mexicana, 2014). In view of the antiprotozoal activity of *C. mexicana*, further research should be conducted to characterize its bioactive molecules.

The extract of *O. basilicum* showed moderate activity against *G. lamblia* (Table 2), which supports its use in traditional medicine to treat gastrointestinal disorders (Shirazi et al., 2014). Recently, the essential oil of *O. basilicum* and two of its main components (linalool and eugenol) were reported to inhibit the growth of *G. lamblia in vitro* (Almeida et al., 2007). However, the present research gives evidence of the anti-giardial activity exhibited by the organic extract obtained from the aerial parts of *O. basilicum*, which could be attributed to its polar compounds, thus current research is aimed at the isolation of its bioactive molecules.

Finally, the extract of *L. tridentata* exhibited inhibitory activity against *E. histolytica*, *G. lamblia* and *T. vaginalis* (Table 2). A previous report on *L. tridentata* demonstrated that one of its compounds, nordihydroguairetic acid, inhibited the growth of *E. invadens* (Segura, 1978). That research allowed us to suggest that the antiprotozoal activity of *L. tridentata* in our study could be attributed to one or more of its lignans and flavonoids previously isolated by our research group (Favela-Hernández et al., 2012). Therefore, further work should be carried out to test the antiprotozoal activity of the isolates in the search for new agents against these protist pathogens.

Conclusions

Organic extracts obtained from *H. albida*, *Smilax* spp., and *C. mexicana* exhibited good activity against three Gram-positive strains, *S. aureus*, methicillin-resistant *S. aureus*, and *E. faecalis*, with MIC values of 25, 50, and 100 μg/ml. These crude extracts are currently under investigation in order to isolate, characterize, and determine the antibacterial properties of their chemical constituents. According to the

antiprotozoal properties of crude extracts, *H. albida* exhibited potent activity against *G. lamblia* and *T. vaginalis* with IC_{50} values of 16.11 and 11.42 µg/ml, while the methanolic extracts of *C. mexicana*, *O. basilicum*, and *L. tridentata* showed moderate activity against the three amitochondrial parasites with IC_{50} values over 100 µg/ml. Among these plants, *H. albida* is the most attractive source to discover new antiprotozoal agents and is now under investigation.

Conflicts of interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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