

ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS FROM THE HUAUTLA SIERRA BIOSPHERE RESERVE IN MORELOS (MÉXICO)

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ABSTRACT

Twenty-two extracts from nine Mexican medicinal plants of eight different families used for people neighbor to Huautla Sierra Biosphere Reserve (REBIOSH) in different infectious diseases were assayed in vitro to determine their antibacterial activity against *Staphylococcus aureus*, *Streptococcus faecalis*; *Escherichia coli*; *Proteus mirabilis*; *Salmonella typhi* and the yeast *Candida albicans*. Most plants showed antibacterial activity, while two plants showed activity against range 0.25 a 4 mg/mL almost one microorganism used. The extracts showed minimum inhibitory concentration (MIC) against *S. aureus* and *S. faecalis*, represents microorganisms Gram-positive were hexanic and acetic extracts of *Bursera copallifera* and hexanic extract of *B. grandifolia*. The ethanol extract of *Lippia graveolens* showed a MIC value of 1 mg/mL against yeast *Candida albicans*.

Key words: antimicrobial activity, minimum inhibitory concentration (MIC),

medicinal plants, Huautla Sierra Biosphere Reserve, Morelos.

RESUMEN

Se determinó la actividad antimicrobiana de veintidós extractos orgánicos de nueve especies vegetales medicinales de ocho diferentes familias, que son comúnmente empleadas por los habitantes de las comunidades vecinas a la reserva de la biosfera Sierra de Huautla (REBIOSH), Morelos, para tratar diferentes padecimientos infecciosos. Los microorganismos empleados fueron: *Staphylococcus aureus*; *Streptococcus faecalis*; *Escherichia coli*; *Proteus mirabilis*; *Salmonella typhi* y la levadura *Candida albicans*. La mayoría de los extractos de las plantas probadas inhibieron el crecimiento microbiano en un rango de 0.25 a 4 mg/mL de al menos uno de los microorganismos utilizados. Los extractos que mostraron las menores concentraciones mínimas inhibitorias (CMI) frente a los microorganismos *S. aureus* y *S. faecalis*, representativos de Gram-positivos fueron

el extracto hexánico y acetónico de *Bursera copallifera* y el hexánico de *B. grandifolia*. El extracto etanólico de *Lippia graveolens* logró inhibir a *C. albicans* con una CMI de 1 mg/mL.

Palabras clave: Actividad antimicrobiana, concentración mínima inhibitoria (CMI), plantas medicinales, Reserva de la Biosfera Sierra de Huautla, Morelos.

INTRODUCTION

According to the World Health Organization, the population most susceptible to infectious diseases are children. In the whole world, a little over two million children under five years old died from these diseases from 2000-2003 (Lerberghe, 2005). In Mexico, the mortality statistics rank acute infectious respiratory diseases among the ten principal causes of death; seventh place with for women with 6 381 deaths, and in eighth place for men with 7 463 deaths (Estadísticas de Mortalidad en México, 2005). In the state of Morelos these diseases have high frequency rates, and in many cases are initially cared for by an herbalist (Taddei-Bringas *et al.*, 1999, Monroy and Castillo, 2000; Romero *et al.*, 2004; Maldonado *et al.*, 2004).

In the Huautla Sierra Biosphere Reserve, Morelos-México (REBIOSH) (Figure 1), 1,025 species of vascular plants were documented to occur, and around 401 medicinal plant species were reported. Among them, are those used to control infectious diseases (Dorado *et al.*, 2005). In the REBIOSH, the vegetation type is characterized as tropical deciduous forest (TDF), but in some areas temperate forest can be found mainly dominated by *Quercus* spp., and in some humid

gullies the vegetation is similar to semi-deciduous forest. The TDF in the REBIOSH is found in general altitudes from 800 to 1500 m.a.s.l. Annual average temperatures are between 20° and 29°C, which is a determining factor in the distribution of the TDF (Rzedowski, 1978). A very important aspect is the fact that in this hilly region of south and southeast Morelos, more than 20 000 people live in about 29 communities inside the Reserve. Some of the species of wild medicinal flora of the REBIOSH are used by the people of the region to treat various sicknesses (Maldonado, 1997; Maldonado *et al.*, 2004; Dorado *et al.*, 2005).

At present, our team is investigating the field of medicinal plants with the potential to treat infectious diseases (Navarro *et al.*, 1996; Navarro and Delgado, 1999; Rojas *et al.*, 2001; Navarro *et al.*, 2006). In the last few years, several plants that are used for various diseases have been studied, and the families-genera of plants that stand out are Boraginaceae-*Cordia* (Ioset *et al.*, 1998; Ioset *et al.*, 2000), Burseraceae-*Bursera* (Camporese *et al.*, 2003; Yasunaka *et al.*, 2005), Fabaceae-*Prosopis* (Salvat *et al.*, 2004), Lamiaceae-*Ocimum* (Bozin *et al.*, 2006), Passifloraceae-*Passiflora* (Nicolls *et al.*, 1973), Rubiaceae-*Randia* (Jayasinghe *et al.*, 2002), Urticaceae-*Urtica* (Gulcin *et al.*, 2004) and Verbenaceae-*Lippia* (Pascual *et al.*, 2001). As we continued the investigation of the aforementioned families-genera, we decided to perform antimicrobial bioassays on nine medicinal species belonging to these families of medicinal plants, whose habitat is the REBIOSH.

The present research paper is focused on the scientific validation of the use of certain medicinal plants for the treatment

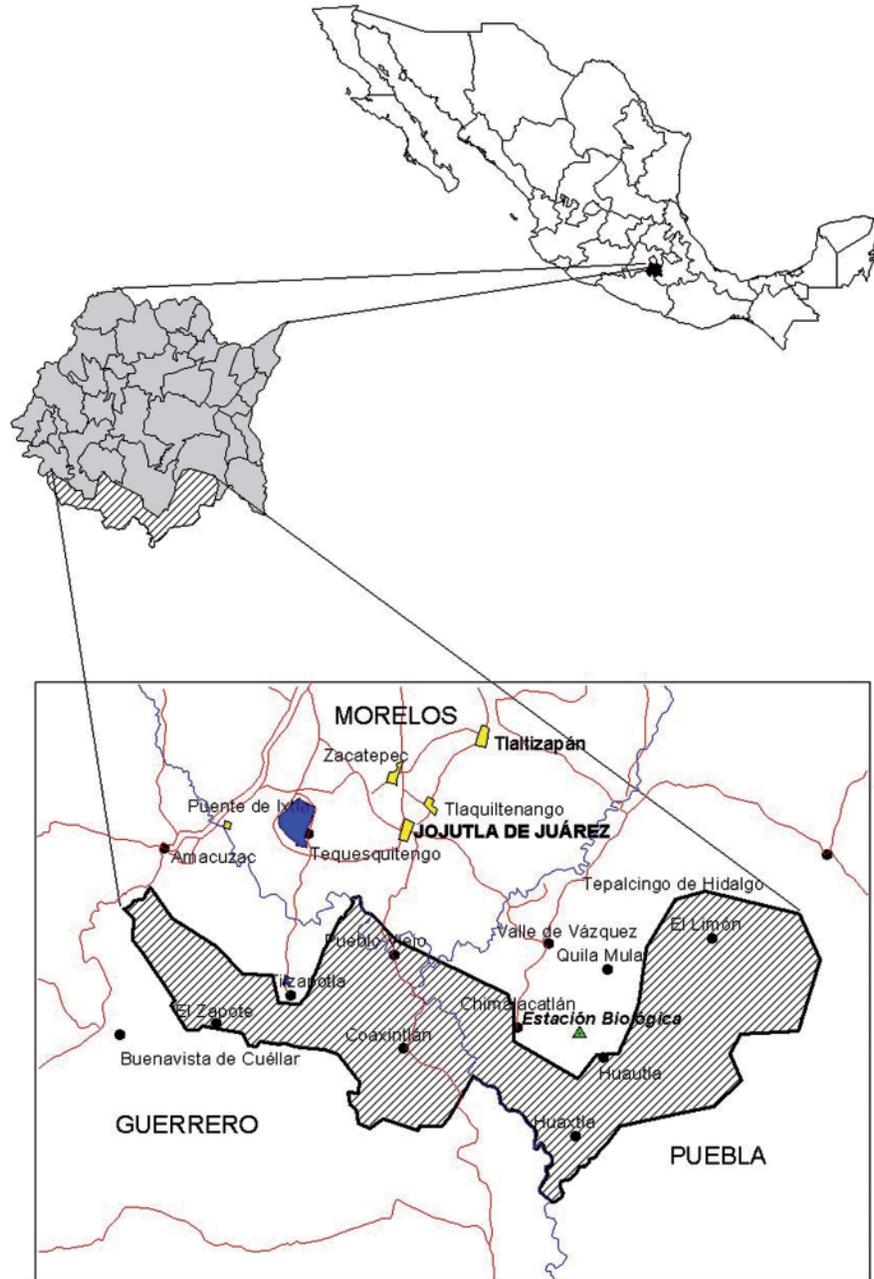


Fig. 1. Map of the Huautla Sierra Biosphere Reserve, Morelos, Mexico.

of infectious diseases by the communities next to the REBIOSH, who use this natural resource.

MATERIAL AND METHODS

Collecting the vegetable material

Plants material was collected in March and June, 2005, in the REBIOSH. The nine species were prepared for depositing in HUMO herbarium (Herbarium of the University of Morelos) of the Center of Environmental Education and Investigation of the Huautla Sierra of the Autonomous University of the State of Morelos. List the specimen accession number, as well as their common name and other ethnobotanical information (Table 1).

Preparation of extracts

Plant materials (stem, leaves and flowers) were shade-dried at room temperature, powdered, and individually extracted sequentially with n-hexane, acetone, ethanol and methanol at room temperature (Table 2). Each solvent was replaced three-times with fresh solvent, remaining in contact with the plant material for 48 h on each occasion. After filtration, the extracts were concentrated under low pressure at 40°C. The resulting crude extracts were stored at -20 °C until they were tested.

Microorganisms and media

The microorganisms used for the antimicrobial evaluation were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The following Gram-positive bacteria were used: *Staphylococcus aureus* (ATCC 6358), and

Streptococcus faecalis (ATCC 10231). The following Gram-negative strains were used: *Escherichia coli* (ATCC 8937), *Proteus mirabilis* (ATCC 43071), *Salmonella typhi* (ATCC 06539) and the yeast *Candida albicans* (ATCC 10231). All bacteria were maintained on tryptic soybean agar (TSA; Merck, Darmstadt, Germany) and assayed on Mueller-Hinton agar (MH; Merck). Sabouraud 4% glucose agar (SGA; Merck) was used to maintain the yeast and as the assay medium.

Antimicrobial activity assays

The antibacterial and antifungal activities of the crude extracts were determined using the agar dilution method (Jorgensen *et al.*, 1999). The hexane, acetone, ethanol and methanol extracts were individually dissolved in 2% dimethylsulfoxide (DMSO; Merck). Further two-fold dilutions of each extract were performed using sterile distilled water and were added to melted agar culture medium in 100 mm Petri dishes (Falcon Franklin Lakes, New Jersey, USA) at the following final concentrations: 0.125, 0.25, 0.50, 1, 2, 4, and 8 mg/mL. Agar culture medium and DMSO 2% were used as negative controls. The procedures followed for the antibacterial and antifungal assays are described below.

Antibacterial activity assay

Suspensions for each strain were prepared by transferring five or six colonies, chosen after overnight growth on tryptic soybean agar, to 5 mL of Mueller-Hinton broth (MHB; Merck). Cultures were incubated at 36°C until they were visibly turbid, and the suspensions were then diluted until the turbidity matched the 0.5 McFarland

Table 1. Nine commonly used medicinal Plants of the REBIOSH.

Name scientific (family) herbarium accession number	Common Name	Popular uses (its forms of administration) (References)
<i>Bursera copallifera</i> (Sessé & Mociño) Bullock. Burseraceae (HUMO 22210)	Copal	Bronchitis, cough, cure for internal blows (oral and cutaneous) (Monroy and Castillo 2000, Argueta <i>et al.</i> , 1994)
<i>Bursera grandifolia</i> (Schldl.) Engelm. Burseraceae (HUMO 24209)	Palo mulato	Asthma, bronchitis, sinusitis, irritated throat, lung infection, influenza, tosferina (oral administration) (Monroy and Castillo 2000, Argueta <i>et al.</i> , 1994)
<i>Cordia morelosana</i> Standley Boraginaceae (HUMO 21408)	Palo prieto	Cough, bronchitis, asthma, pain of lungs, sinusitis, flu (oral administration) (Monroy and Castillo 2000, Argueta <i>et al.</i> , 1994)
<i>Lippia graveolens</i> Kunth Verbenaceae (HUMO 22725)	Orégano	Diarrhea, vomit, indigestion, dysentery (oral administration) (Monroy and Castillo 2000, Argueta <i>et al.</i> , 1994)
<i>Ocimum basilicum</i> L. Lamiaceae (HUMO 21356)	Albahaca	Diarrhea, vomit, dysentery, parasites, calculations biliaires, to calm nerves (oral administration) (Monroy and Castillo 2000, Argueta <i>et al.</i> , 1994)
<i>Passiflora mexicana</i> Juss Passifloraceae (HUMO 12766)	Itamo real	Skin diseases (Monroy and Castillo 2000, Maldonado, 1997)
<i>Prosopis laevigata</i> (Willd.) M. Johnston Fabaceae (HUMO 22363)	Mezquite	Cough, stomachache, mouth and eyes infections (Monroy and Castillo 2000, Argueta <i>et al.</i> , 1994)
<i>Randia echinocarpa</i> Mociño & Sessé ex DC. Rubiaceae (HUMO 11262)	Granjel	Kidney infection, face dots, coughs. (oral administration) (Monroy and Castillo 2000, Argueta <i>et al.</i> , 1994)
<i>Urtica dioica</i> L. Urticaceae (HUMO 14461)	Ortiga	Rheumatism and muscle pain (Monroy and Castillo 2000, Argueta <i>et al.</i> , 1994)

Table 2. Plant parts used for the preparation of the organic raw extracts used.

Species	Used part of the plant	Dry weight	Solvent
<i>Bursera copallifera</i>	Stem	875 g	2150mL H, A, M
	Leaves	300 g	1900 mL H, A, M
<i>Bursera grandifolia</i>	Stem	811 g	2100 mL H, A, E, M
	Leaves	250 g	2000 mL H, A, M
<i>Cordia morelosana</i>	Flower	100 g	1000 mL A
<i>Lippia graveolens</i>	Aerial parts	100 g	1000 mL E
<i>Ocimum basilicum</i>	Stem and leaves	100 g	1000 mL E
<i>Passiflora mexicana</i>	Aerial parts	100 g	1000 mL E
<i>Prosopis laevigata</i>	Stem and leaves	760 g	2150 mL H, A, M
<i>Randia echinocarpa</i>	Stem and Leaves	100 g	1000 mL A
<i>Urtica dioica</i>	Leaves	200 g	1500 mL E

Aerial parts: flowers, leaves and stem

Solvents: H, hexane; A, acetone; E, ethanol; M, methanol.

standard turbidity equivalents [10^8 colony-forming units (CFU)/mL]. Microbial suspensions were further diluted 1:10 to obtain a concentration of 10^7 CFU/mL. The diluted inoculum was applied with a loop calibrated to deliver 0.002 mL, resulting in a spot covering a circle of 5 to 8 mm diameter and containing 10^4 CFU/mL. The plates were incubated for 24 h at 36°C. Gentamicin (5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) was used as positive control. Observations were performed in duplicate, and results are expressed as the lowest concentration of plant extract that produced a complete suppression of colony growth, the minimal inhibitory concentration (MIC) (Ríos *et al.*, 1988).

Antifungal activity assay

C. albicans inoculum was prepared by diluting the yeast suspension (adjusted to McFarland 0.5 scale) in 0.85% NaCl solution to a final concentration of 10^5 cell/mL. The filamentous fungi inoculums were prepared by diluting the scraped cells mass in 0.85% NaCl to a final concentration of 10^6 spores/mL. The diluted inocula were delivered on the top of the solidified agar with a loop calibrated to deliver 0.005 mL. The plates were incubated at 29°C. *C. albicans* growth was checked both in control plates prepared without any test sample and in experimental plates, *C. albicans* was cultured 48 h. Experiments were duplicated, and results were expressed as MIC. Posi-

tive control was prepared with Nystatin (5 mg/mL; Merck).

RESULTS AND DISCUSSION

Table 3 shows the plant part used, the percentage of yield, and the MIC obtained for the corresponding evaluations of each extract. The agar dilution method used in this investigation has been employed frequently and recommended as a good method for determining the relative potency of complex extracts and for establishing their antimicrobial spectrum (Ríos and Recio, 2005). In our experience and according to previous antimicrobial studies carried out in our laboratory, crude plant extracts with MIC values between 2.5 and 8 mg/mL (Lozoya *et al.*, 1992; Navarro *et al.*, 1996) have led to the isolation of strong antimicrobial compounds (Navarro and Delgado 1999). Under this criterion, and considering that in this study only crude extracts were employed, extracts with MIC values of 8 mg/mL or below against any of the microorganisms tested were considered active. Most of the extracts used showed antimicrobial activity with the Gram-positives bacteria being the more sensitive to the extracts.

The species which showed the highest activity was *B. copallifera*, of which the acetone stems extract and the methanol leaves extract were the most active against the *S. aureus*, both of them with a MIC value of 0.25 mg/mL. In addition these same extracts showed values of 1 and 0.25 mg/mL respectively against *S. faecalis*. On the other hand, the leaves acetone extract of this species showed a wide scale of inhibition against all the microorganisms, with MIC value of 2 mg/mL against the

Gram-negatives and yeast. It is important to mention that the other species in this genus, that is to say *B. grandifolia*, also showed antimicrobial activity. The stems hexane and acetone extracts showed a MIC value of 0.5 mg/mL against the *S. aureus*. The hexane extract to *B. grandifolia* showed a MIC value of 4 mg/mL against all the microorganisms. The remainder of the plant species also showed notable antimicrobial activities. The ethanol extract of *L. graveolens* showed a MIC value of 1 mg/mL against *S. aureus* and the yeast *C. albicans*, and a MIC value of 4 mg/mL against *S. faecalis*. The ethanol extracts of *O. basilicum* and *P. mexicana* showed MIC values of 4 mg/mL against *S. aureus*.

On the other hand all the extracts of the medicinal plant *P. laevigata* showed antimicrobial activity. The hexane extract gave a MIC value of 0.5 mg/mL and the acetone extract showed a MIC value of 4 mg/mL, both against *S. aureus*. The ethanol extract of *U. dioica* showed antimicrobial activity with a MIC value of 1 mg/mL against the Gram-positive bacteria.

There were no literature reports of antimicrobial activity for most species studied, however, the chemical nature or the antimicrobial activity of some species of the same genera are described in the following research work.

The extract of *Bursera simaruba* (bark) was evaluated for its anti-bacterial properties. Activity was tested against standard strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212. The extract inhibited the growth of all of the

Table 3. Minimum inhibitory concentration (MIC, mg/mL) of the raw organic extracts. Numbers in bold indicated bioactivity.

Plant Scientific name	^a Used Part	^b Plant (yield extract %)	MIC (mg/mL)						
			^c Sa	Sf	St	Ec	Pm	Ca	
<i>Bursera copallifera</i>	S	Hexane (0.34%)	2	4	4	4	4	4	4
		Acetone (0.67%)	0.25	1	>8	>8	>8	>8	>8
		Methanol (0.29%)	>8	>8	>8	>8	>8	>8	>8
		Hexane (0.72%)	2	4	4	4	4	4	4
<i>Bursera grandifolia</i>	S	Acetone (1.60%)	0.5	0.5	2	2	2	2	2
		Methanol (19.90%)	0.25	0.25	>8	>8	>8	>8	>8
		Hexane (0.25%)	0.5	4	4	4	4	4	4
		Acetone (0.87%)	0.5	8	>8	>8	>8	>8	>8
<i>Bursera grandifolia</i>	L	Ethanol (0.40%)	2	2	>8	>8	>8	>8	>8
		Methanol (0.56%)	>8	>8	>8	>8	>8	>8	>8
		Hexane (1.86%)	>8	>8	>8	>8	>8	>8	>8
		Acetone (2.16%)	4	>8	>8	>8	>8	>8	>8
<i>Cordia morelosana</i>	F	Methanol (2.36%)	4	>8	>8	>8	>8	>8	>8
		Acetone (1.80%)	8	>8	>8	>8	>8	>8	>8
		Ethanol (2.16%)	1	4	>8	>8	>8	>8	1
		Ethanol (1.25%)	4	>8	>8	>8	>8	>8	>8
<i>Lippia graveolens</i>	S, L	Ethanol (3.95%)	4	>8	>8	>8	>8	>8	>8
		Hexane (0.33%)	0.5	8	>8	>8	>8	>8	>8
		Acetone (1.06%)	4	8	>8	>8	>8	8	>8
		Methanol (3.35%)	4	>8	>8	>8	>8	>8	>8
<i>Randia echinocarpa</i>	S, L	Acetone (1.63%)	8	8	>8	>8	>8	>8	>8
		Ethanol (10.13%)	1	1	>8	>8	>8	>8	>8
		Gentamicin	0.005	0.005	0.005	0.005	0.005	0.005	NP
		Nystatin	NP	NP	NP	NP	NP	NP	0.010

bacterial strains, except that of *Enterococcus faecalis* (Camporese *et al.*, 2003). Other studies *B. simaruba* showed, higher activity against *Staphylococcus aureus* (methicillin-sensitive and methicillin-resistant) than against *Escherichia coli* (Yasunaka *et al.*, 2005).

Two new natural products, characterized as 1-(3'-methoxypropanoyl)-2,4,5-trimethoxybenzene and a prenylated hydroquinone, 2-(2Z)-(3-hydroxy-3,7-dimethylocta-2, 6-dienyl)-1,4-benzenediol, have been isolated from the root bark of *Cordia alliodora*. Both compounds exhibited antifungal properties against the phytopathogenic mold *Cladosporium cucumerinum* (Ioset *et al.*, 2000). Also, two meroterpenoid naphthoquinones, the known cordiaquinone B and a new naphthoxirene, have been isolated from the roots of *Cordia linnaei*. The two showed activity against *Cladosporium cucumerinum* and *Candida albicans* (Ioset *et al.*, 1998).

In a literature review of the genus *Lippia*, (Pascual *et al.*, 2001) found that *in vitro* screening with extracts of *L. alba*, and *L. dulcis* demonstrated an inhibitory activity against many of the Gram-positive bacteria most frequently responsible for infections in humans (*Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*), and thus, may provide a scientific basis for the ethnomedical use of this species against bacterial respiratory infections (Cáceres *et al.*, 1991).

Other studies report that the essential oils from genus *Lippia* have also been shown to exhibit antimicrobial activity against other microorganisms. For example *L. sidoides* shows a inhibitory activity against

E. coli, *S. aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis*, and a low activity against *Pseudomonas aeruginosa* (Lemos *et al.*, 1990).

The ethanolic extracts of *L. palmeri* and *L. formosa* exhibit activity against *S. aureus*, *S. faecalis* and *B. subtilis* (Dimayuga and Keer García, 1991). Extracts isolated from *L. dulcis*, shows antimicrobial activity against enteropathogenic *Salmonella typhi* and *Shigella flexneri* (Cáceres *et al.*, 1993).

Some species of the genus *Prosopis* have been reported to exhibit antimicrobial activity. This is the case for *P. juliflora*. One alkaloid from this plant, which has been named juliprosinene, showed antibacterial activity against *Escherichia coli* strains 11303 HER 1024 and K-125 HER 1037, *Klebsiella pneumoniae* C3HER 1111, *Pseudomonas aeruginosa* PA 01 HER 1018, *Staphylococcus aureus* B-827, and *Shigella sonnei* YER HER 1048 (Ahmad *et al.*, 1989). In another study of this plant, a mixture of alkaloids containing mainly juliprosine and isojuliprosine was tested for antifungal activity against dermatophytes, *Aspergillus* and *Candida* species in comparison with griseofulvin and econazole. Although econazole was found to be the most active one, the alkaloidal mixture was found more effective than griseofulvin (Ahmad and Khursheed, 1989).

Methanol extracts of the barks of two species of *Prosopis* were evaluated to determine their antimicrobial activity. *Prosopis kuntzei* exhibited antimicrobial activity against *S. aureus* ATCC 8095, INE 2213, and *Ps. aeruginosa* ATCC 25617, with

CMI below 1 mg/mL. On the other hand, *Prosopis ruscifolia* had activity against *S. aureus* ATCC 8095, INE 2213, *Ent. faecium* INEI 2464 and *Kl. pneumoniae* ATCC 25922, with MIC of 0.5, 0.38, 0.75 and 0.75 mg/mL respectively (Salvat *et al.*, 2004).

Recent research has discovered a new potent anti-infective and antiparasitic compound, 2,3-dihydro-1H-indolizinium chloride, from *Prosopis glandulosa* var. *glandulosa*. It shows antibacterial activity against methicillin-resistant *Staphylococcus aureus* and *Micobacterium intracellulare* with values of IC₅₀ 0.35 and 0.9 µg/mL respectively (Samoylenko *et al.*, 2009).

An investigation of *Urtica dioica* showed that a water extract exhibited antioxidant, antimicrobial, antiulcer and analgesic activities (Gulcin *et al.*, 2004).

The results obtained in the current investigation indicate that *B. copallifera*, *B. grandifolia*, *L. graveolens*, *P. leavigata* and *U. dioica* possesses antibacterial and/or antifungal properties, showing a strong correlation between the reported uses of these plant species by the communities of the Huautla Sierra in the State of Morelos and in Mexican folk medicine and the obtained experimental data. It also supports the importance of ethnopharmacology as a guide in the selection of plants for the discovery of bioactive compounds.

Phytochemical bioguided studies are currently under way in order to isolate and characterize the compounds responsible for the antimicrobial activities of these plants.

ACKNOWLEDGMENTS

The authors would like to thank Biol. Juan Carlos Juárez from the CEAMISH/UAE-Morelos for assistance in the collection and identification of plant specimens.

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Recibido: 9 enero 2009. Aceptado: 7 agosto 2009.