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Full Length Research Paper

Antibacterial activity of crude extracts from Mexican plants against methicillin-resistant *Staphylococcus*

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The aim of this study was to evaluate the antimicrobial activity of 36 extracts from 18 vegetal species used as soap, insecticides, insect repellent and for the treatment of several diseases likely associated to microorganisms. The vegetal species were collected in Oaxaca, Puebla and Veracruz States, México. The extracts were evaluated against isolates of nosocomial infections of *Staphylococcus aureus* and *Staphylococcus* coagulase negative resistant to Methicillin by a modificated agar diffusion method. The results demonstrate an important antibacterial effect *in vitro*, against all of the strains of *Staphylococcus* tested mainly with those from *Vernonanthura oaxacana, Trixis silvatica,* and with those of *Perezia hebeclada.* The minimum inhibitory concentration for *V. oaxacana* and *P. hebeclada* was 250 µg/disc and for *T. silvatica* it was 15 µg/disc. These extracts showed an important potential that would contribute to the development of new agents against infections by *Staphylococcus.*

Key words: Crude extracts, antimicrobial activity, intrahospitalary infections, methicillin-resistant *Staphylococcus* coagulase negative.

INTRODUCTION

Nosocomial infections, also known as intrahospitalary infections, are defined as those infections that occur in hospitalized patients in whom the infections were not present, nor in incubation period, at the moment of admission to the hospita l (Edmond, 1999; Horan et al., 1992). Nosocomial infections represent an important problem of public health, due to the social, medical and economic impact. They are causes of a prolongation of hospital stays and also of a high mortality, due to the difficulty of treatment of these infections caused by infectious agents, which are transmitted from one to another patient (Aqüald-Öhman et al., 2004), generating an proper environment for the grow of multiresistant microorganisms to conventional treatments and that are capable of producing serious diseases (Fauci, 1998). Among the bacteria that causes nosocomial diseases, the grampositive *coccus* such as methicillin resistant *Staphylococcus aureus* (MRSA) and coagulase negative *Staphylococcus* (CoNS) (Pinner et al., 1996), cause infections considered emerging diseases due to their

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Abbreviations: **BUAP**, Benemérita Universidad Autónoma De Puebla, México; **CoNS**, coagulase negative *Staphylococcus;* **MIC**, minimum inhibitory concentration; **MRSA**, methicillin resistant *Staphylococcus aureus.*

multiresistance (resistance to more than four antibiotics) (Tristan et al., 2007).

The strains of MRSA are generally also resistant to other β-lactamic antibiotics and other antibacterial agents such as the macrolids (Stefani and Varaldo, 2003), added to the inherent risk that accompanies their use; like vancomycin, which is ototoxic, nefrotoxic and could cause neutropenia, anafilactic shock or thrombophlebitis. The Methicillin resistant strains of CoNS which cause nosocomial infections have undergone an increase in their prevalence (Tantracheewathorn and Vititpatarapak, 2007), making very expensive control of this type of infections, and they represent an increasingly more aggressive exposure of the patient to the chosen antibiotics, thus in the near future *Staphylococcus* could be considered a pathogen capable of provoking untreatable diseases. Therefore, the development of new strategies for the control of these pathogens is indispensible, along with the study of new natural agents as candidates to be used in the treatment of these infections (Tadeg et al., 2005; Chin et al., 2006).

Plants have shown a considerable activity against gram positive bacteria and yeasts (Tegos et al., 2002), as well as against gram negative bacteria (Pérez and Anesini, 1994); this activity is due to secondary metabolites which are found in several plant tissues (Bruneton, 1995). These secondary metabolites could be phenols, saponins, quinolones, flavones, flavonoids, flavonols, tanins, terpenoids, essential oils, alkaloids, polypeptides and other compounds (Cowan, 1999), and in spite that at least 12,000 secondary metabolites have been isolated, it is believed that they represent less than 10% of the total of the metabolites that really exist (Lincoln and Zeiger, 2006). It is considered that these compounds are more frequent and/or reactive in the growth buds of the plants, the young leaves, reproductive organs and in general in the parts of annual growth, than in the old tissues. These substances could be responsible for the defence mechanisms of the plants against damages caused by microorganisms, insects and other animals (Cowan, 1999), therefore it is considered that plants are a source of a wide variety of bioactive molecules that can be used for the development of new medicines with a wider spectrum and with less adverse effects than those produced by drugs currently used. The principal benefit of using antibacterial substances derived from plants is that they are safer and cheaper than the synthetic alternatives that are available (Recio et al., 1989; Rios and Recio, 2005).

In this work, the evaluation of antibacterial activity of 37 extracts from 18 plant species was made. Almost all the extracts evaluated have ethnobotanic antecedents and they are used in the traditional medicine or as insecticide in several communities of Puebla, Mexico State, Veracruz and Oaxaca, Mexico. The extracts were tested against nosocomial isolates of MRSA and resistant CoNS to more than five antibiotics (including Methicillin) commonly used in clinical therapy.

MATERIALS AND METHODS

Ethnobotanical data

The information of the use of plants is based on interviews with the people of the place of collection and of bibliographic sources. Table 1 shows the evaluated plant species that were selected because of their ethnobotanical antecedent, chemical and biological studies, with the exception of *Physodium oaxacanum*, which has not been studied, and has no ethnobotanical history that suggest an antimicrobial activity. Of the 18 species, ten are used in traditional medicine to treat diseases that may be related to microorganisms, these species are *Acalypha cuspidata, Baccharis conferta, Cordia dentata, Echeveria gigantean, Lantana camara, P. hebeclada, Siparuna andina, T. silvatica, Virbesina abscondita, V. oaxacana.* In addition, other plant species, which have shown antimicrobial activity were evaluated, these species were *L. camara* (Hernández et al., 2003), *Azadirachta indica* (Siddiqui et al., 1998; 1992; Thakurta et al., 2007) and *B. conferta* (Weimann et al., 2002). Another four species have references of use in crop protection (*Trichilia havanensis, Psacaliopsis purpusii, Croton ciliatoglanduliferus* and *A. indica*), which suggests the existence of bioactive substances that could have antimicrobial activity; the tetranorterpenoids isolated from *A. indica* demonstrated antibacterial activity (Siddiqui et al., 1992). *Sechium mexicanum* and *Lantana hirta* were of interest because of the antecedents of others studies of the same genus; the antialimentary activity of *Sechium pittieri* (Mancebo et al., 2001) and antimicrobial activity of *L. camara* (Hernández et al., 2003), as well as the fact that they do not have some type of study in spite that they are useful species.

Plant material

The botanical names, families, common names, popular use, collection site, registration number in the herbarium and described studies are shown in Table 1. The collection of the plants *V. oaxacana* (leaves and stems), *T. silvatica* (leaves and stems), *B. conferta* (leaves and stems), *P. hebeclada* (roots), *L. camara* (leaves and stems), *L. hirta* (leaves and stems), *V. abscondita* (leaves and stems), *A. cuspidata* (leaves and stems), *C. dentata* (leaves and stems), *E. gigantean* (leaves), *P. oaxacanum* (leaves and stems), *Vitis tiliifolia* (stems), and *S. andina* (leaves), was carried out by investigators of the Universidad del Mar in Oaxaca and the identification of the species was made in the Herbarium of the Preparatoria Agrícola of the Universidad Autónoma Chapingo, México. The vegetal species that were not deposited in the herbarium were only compared with authentic samples of the herbarium of the Universidad Nacional Autónoma de México. The collection of *T. havanensis* (seeds), *A. indica* (seeds), *P. purpusii* (leaves and stems), *C. ciliatoglanduliferus* (leaves and stems), and *S. mexicanum* (roots), was made in the Veracruz and Puebla states, México, by investigators of the Instituto de Ciencias of the Benemérita Universidad Autónoma de Puebla (BUAP) and the identification of the material was made in the Herbarium and Botanical Garden of the BUAP. The plant material was dried in the shade in a ventilated place at room temperature, and was pulverized in a mill using a sieve of 1.5 mm.

Obtainment of extracts

The dried and ground plant material (1 g to 200 g) was subjected to extraction with one or more solvents (50 mL to 1 L) consecutively and in an increasing order of polarity (n-hexane, acetone, ethyl acetate, methanol, ethanol, and methanol-water). The plant material was macerated at room temperature for approximately two weeks in each solvent and this was eliminated under reduced

Table 1. Plant species screened for antimicrobial activity.

Plant (Family)	Common names	Popular use	Collection site/ Collector/Herbarium/ Voucher specimen [a]	Chemical/Biological studies
Acalypha cuspidata Jacq (Euphorbiaceae)	Jabachopo, Hierba de la araña	Leafs are used in the varicella and measles treatment	Pluma Hidalgo, Oaxaca/ BHC, ECP UACH/61179	Not found
Azadirachta indica A. Juss (Meliaceae)	Nim	Ornamental and insect repellent	Plantation of the Colegio de Postgraduados, Campus Veracruz. Km. 88.5, Carretera Federal Xalapa – Veracruz, Manlio Fabio Altamirano, Veracruz, Veracruz/ JFLO/Not in Herbarium	-Methanol extracts shown activity against multi-drug-resistant *Vibrio cholerae* of serotypes O1, O139 and non-O1, non-O139 (Thakurta et al., 2007). -Tetranortriterpenoid with antibacterial activity against gram-pos. and gram-neg. organisms (Siddiqui et al., 1992). -Diterpenoids with antibacterial activity (Siddiqui et al., 1988).
Baccharis conferta Kunth (Asteraceae)	Escobilla	Leafs are used to relief cold and vomit and sickness	La Marquesa Estado de México/ EBT UACH	-Triterpenes (Bohlmann and Zdero, 1976). -Coumarins (Bohlmann and Zdero, 1976). -Flavonoids (Weimann et al., 2002). -Triterpene with activity against *M. luteus* y *E. coli* (Weimann et al., 2002). -Extract with antispasmodic activity (Weimann et al., 2002; Heinrich, 2003).
Croton ciliatoglanduliferus Ortega (Euphorbiaceae)	Súliman	Crops protection	Zapotitlán de Salinas, Puebla/ JSK-AST-EMC/HUAP/8051.	-Flavonoids one of them with phytotoxic activity (Morales-Flores et al., 2007). -Diterpenes (González-Vázquez et al., 2006).
Cordia dentata Poir. (Boraginaceae)	Sasanil, Juquilote, Guilaberi	Used in the respiratory illness treatment (Waizel and Waizel, 2005) From fruit is obtained glue	San Miguel Tenango, Oaxaca/ HSR, ECP UACH/61182	-Flavonoids (Ferrari et al., 1997).
Echeveria gigantean Rose & Purpus (Crassulaceae)	Oreja de venado	Leaf is used for the eye illness treatment	Ejutla, Oaxaca/ HSR, ECP UACH	Not found
Lantana camara L. (Verbenaceae)	Zapotillo, Quebradiza	Used to treat gastrointestinal diseases (Hernández et al., 2003) The fruit is used as food Seeds and stem are toxic	San Miguel Tenango, Oaxaca/HSR, ECP/UACH/ 61184	-Triterpenes (Begum et al., 2002; Begum et al., 2006; Sharma and Sharma, 2006; Fatope et al., 2006) with allelopatic activity (Kong et al., 2006). -Antibacterial activity of hexane extract (Hernández et al., 2003).

Table 1. Contd.

Lantana hirta Graham (Verbenaceae)	Shubaroba, quebradiza	The fruit is used as food Seeds and stem are poisons	San Miguel Tenango, Oaxaca/ HSR, ECP UACH/61180	Not found
Psacaliopsis purpusii (Greenm. Ex Bradegee) H. Rob. & Brettell (Asteraceae)	Hierba del perro	Crops protection	Amozoc, Puebla/JC-AM/HUAP/6542	Not found
Perezia hebeclada (DC.) A. Gray (Asteraceae)	Hierba del Zopilote, Zazanaca	Used to fever treatment (Johnson, 1999)	Cerro del Chiquihuite, Miguel Hidalgo, Cd. de México/ EBT, BHC UACH	-Coumarins (Hernández-Carlos et al., 2003). -Terpenoids (Joseph-Nathan et al., 1972). -Quinones with response on the motility of isolated intestine of rabbits and rats (Enriquez et al., 1980).
Physodium oaxacanum Dorr & Barnett (Sterculiaceae)	Yaagguiroose (rose flowers tree)	Ornamental	San Miguel Tenango, Oaxaca/ HSR, ECP UACH/61186	Not found
Sechium mexicanum Lira & M. Nee (Cucurbitaceae)	Amole	Fruit is used as bleacher and perfume of clothes Roots are used as soap	Yaonahuac, Puebla/JFLO/HUAP/12860	Not found
Siparuna andina (Tul.) A. DC. (Monimiaceae)	Conchuda	Leaf infusion are used to dengue fever treatment	Pluma Hidalgo, Oaxaca/ ECP UACH/61178	-Activity against *Plasmodium falciparum* of hexane extract (Jenett-Siems et al., 1999) and sesquiterpene (Jenett-Siems et al., 2000).
Trichilia havanensis Jacq. (Meliaceae)	Ramatinaja, Xopiltetl, Palo de cuchara	The fruits and leafs are used for crops protection	Tuzamapan, Puebla/ MSH-JLCJ/HUAP/11024	-Limonoids (Arenas et al., 1990) with insecticide activity (Chan et al., 1973). -Triterpenes (Huerta et al., 2003) with antifeedant (Rodríguez et al., 2003) and deterrent activities (Ortego et al., 1999).
Trixis silvatica B.L. Rob. & Greenm. (Asteraceae)	Pichaga, Árbol Pericón	Leaf are used as cathartic and bark to relief pectoral muscle pain and stomach illness	Ejido Buenos Aires, Tehuantepec, Oaxaca/ HSR, ECP UACH / 61181	Not found

Table 1. Contd.

Verbesina abscondita Klatt (Asteraceae)	Tziquescui	Leafs are used in the respiratory illness treatment	Ejido Buenos Aires, Tehuantepec, Oaxaca/ HSR, ECP UACH/61183	Not found
Vernonanthura oaxacana (Schultz-Bip. ex Klatt) H. Rob (Asteraceae) (antes *Vernonia oaxacana*)	Not found	Stem and leafs are used in combination with other species in the respiratory illness treatment	Ejido Buenos Aires, Tehuantepec, Oaxaca/ HSR, ECP UACH/61185	Not found
Vitis tiliifolia Humb. & Bonpl. ex. Roem. & Schult. (Vitaceae)	Bejuco de agua	Fruit is food and stem salvia is a refresh beverage.	Pluma Hidalgo, Oaxaca/ ECP UACH/61177	Not found

[a]ECP, E. Cedillo-Portugal; HSR, H. Santiago-Romero; BHC, B. Hernández-Carlos; EBT, E. Burgueño-Tapia; JFLO, J.F. López-Olguín; JSK-AST-EMC, J. Sánchez-Kent & A. Salinas T. & E. Martínez C.; JC-AM, J. Carreto & A. Muñoz; MSH-JLCJ, M. S. Hernández H. & J. L. Contreras J. UACH: Herbarium of the División de Ciencias Forestales de la Universidad Autónoma de Chapingo, Chapingo, México. HUAP: Herbarium of the Universidad Autónoma de Puebla, Puebla, México.

pressure, and each residue was kept under refrigeration at 4 ºC, until its biological evaluation. The parts of the plant species evaluated were selected according to the ethnobotanical antecedent of each one.

The extracts were obtained with solvents of different polarity to insure a greater extraction of secondary metabolites (Cowan, 1999), and some extracts of species such as *L. camara*, *A. cuspidata, A. indica*, among others, did not show differences in their chromatographic profiles (results not shown) when they were obtained with acetate of ethyl and methanol; the reason for which only one of them was evaluated. It was confirmed that the solvents did not cause false positives in the evaluations given that the negative controls prepared with methanol, ethanol, *n*-hexane, acetone and water did not present inhibition halo in any of the assays, whereas the positive control presented an inhibition halo between 7 and 10 mm diameter.

Biological strains

The bacterial isolates were made out from samples of wound secretions, hemoculture or peritoneal dialysis liquid shown in plaques with blood agar and manitol salt agar (DIFCO) and they were obtained from patients with nosocomial infections from the University Hospital of the BUAP. Nosocomial infection is defined as the development of infection after at least 48 h or more of the hospital admission that was not present or incubating at the time of admission; and a patient does not have any nosocomial infection, if it showed no symptoms during their stay in the hospital. The samples were incubated at 37°C during 48 to 72 h and the bacterial growths were subjected to the morphological and phenotypic studies. The strains were identificated as *S. aureus* or CoNS through Gram staining, pigment production and biochemical tests such as the activity of the catalase, oxidase, coagulase, urease, DNAase, reduction of nitrates, decarboxylation of the ornithine and hemolysis in blood gelose (Murray et al., 1999; Holt et al., 1994) (Table 2).

Susceptibility to antimicrobials

The susceptibility test was done on 110 strains of *Staphylococcus* isolates of nosocomial infections to 14 antibiotics routinely used in the clinic and reported by the Clinical and Laboratory Standards Institute (CLSI, 2008; CDC, 1999), using the method of diffusion in agar (Kirby-Baüer) of Müeller Hinton added with 4% NaCl (ideal conditions for carrying out the assays of antimicrobial activity for *Staphylococcus*) (McDougal and Thornsberry, 1984; Holt et al., 1994) and using discs impregnated with oxacillin (1 µg) (antibiotic normally used to test resistance to Methicillin), vancomycin (30 µg), erythromycin (15 µg), ampicillin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), cefotaxime (30 µg), cefuroxime (30 µg), pefloxacin (5 µg), dicloxacillin (1 µg), penicillin (10 µg) and gentamicin (10 µg) (Sanofi Diagnostics Pasteur, México, D.F.). Later, five strains of *S. aureus* and five of CoNS multiresistant (resistant to more than five antibiotics, including Methicillin) were selected, and that in addition were positive to the cefinase test (Becton-Dickinson) which indicated the production of the enzyme β-lactamase who awards the resistance to the β-lactamic antibiotics which are of first choice. These strains are shown in Table 2. The strains of *S. aureus* ATCC 29213 and ATCC 25923, which were resistant to Methicillin, were included as controls.

Table 2. Strains of *Staphylococcus* intrahospitalary.

Strain	Origin
ERN *S. aureus*	Hemoculture
000735 *S. aureus*	Secretion of wound
010718 *S. aureus*	Secretion of wound
020802 *S. aureus*	Liquid of dialysis peritoneal
030303 *S. aureus*	Liquid of dialysis peritoneal
MH SCN	Hemoculture
020816 SCN	Secretion of wound
EL-1 SCN	Hemoculture
030233 SCN	Liquid of dialysis peritoneal
030527 SCN	Secretion of wound

Detection of *mecA* gene

Detection of the *mecA* gene in all strains was performed by PCR amplication. Total genomic DNA was obtained from the bacteria by the phenol chloroform extraction method, as it was described by Tsen and Chen (1992). Bacteria collected from 5 mL of the all night culture in Mueller-Hinton broth were used for DNA extraction after treatment with lysostaphin and RNase (Sigma, St Louis, MO, USA). The PCR assay was performed in a DNA thermal cycler, GeneAmp PCR system 9700 (PE Applied Biosystems, Mississauga, Ontario, Canada), by using a Gene Taq amplifying kit (Wako Pure Chemicals Industries Ltd, Japan), according to the manufacturer's recommendations. Synthetic oligonucleotides used as primers were 5′-ATGAGATTAGGCATCGTTCC-3′ and 5′- TGGATGACAGTACCT GAGCC-3′ (Ryffel et al., 1990).

Antibacterial activity of the plant extracts

In a first stage, a screening of 37 plant extract (5%) was carried out by the method of diffusion in paper filter disc, based on the described method by Ali et al. (2001). Each extract was prepared in its own solvent (indicated in Table 1), except for the extracts obtained with ethyl acetate which were dissolved with acetone. Plant extract (50 mg) was placed in a microtube and was diluted to 1 ml, the solution was homogenized, and then 20 µl of the each solution was placed on discs of filter paper (Whatman No. 3) of 6 mm diameter which had been previously sterilized, so each disc containing 1 mg of plant extract. The discs were placed in sterile glass Petri dishes and the solvent were eliminated at room temperature in a biosecurity bell (class II type A/B), NUAIRE Biological Safety Cabinets) to prevent contamination. The negative control were prepared with filter paper discs impregnated with 20 µl of dissolvent (methanol, ethanol, hexane, ethyl acetate, acetone or water) which were prepared as described above (Rios et al., 1988). The positive control was prepared with discs impregnated with streptomycin (10 µg/ml) (Becton Dickinson).

The strains of *S. aureus* and CoNS were made to grow in trypticase soy agar at 37°C for 24 h. For each strain in a test tube which contained 5 ml of sterile saline solution (0.85% of NaCl), an inoculums of 2 to 5 bacterial colonies was deposited until a density comparable to tube No. 0.5 of the standards of Mac Farland which correspond to 1.5×10^8 UFC/ml was achieved. From this bacterial suspension, 100 µl were taken to inoculate Müeller Hinton agar plaques with 4% NaCl; conditions that are ideal for carrying out the tests of susceptibility to antimicrobials of strains of *Staphylococcus* (CLSI, 2008; McDougal and Thornsberry, 1984; Holt et al., 1994). The medium was inoculated in four directions over the totality of the surface of the agar to obtain uniform inoculums, making a last scanning of the swab over the edge of the Petri dish. Once the inoculums had dried, the discs impregnated with the plant extracts were placed with the treated side over the surface of the agar, and in each Petri dish the positive and negative controls were placed. The discs were lightly pressed to insure contact with the surface of the agar. After 15 min of having placed the discs, the Petri dishes were incubated at 37°C for 16 to 18 h. Each treatment was made for triplicate. The measurement of the inhibition halos was made with a Vernier through the bottom of the dish, with the aid of the illumination of the light reflected from a lamp. The plant extract that did not present inhibitory halo around the disc was considered to have no effect, if it presented an inhibition halo less than 2 mm, it was considered to have slight activity and if the inhibition halo was equal to or greater than 2 mm, it was considered to have an inhibitory effect on the growth of the bacteria.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined in the plant extracts that presented antibacterial activity against MRSA and CoNS (inhibition halo greater than 6 mm), using a modification of the method of diffusion in agar described previously (Ali et al., 2001). Extracts were prepared using the following concentrations (treatments): 5 (50 mg/ml), 2.5 (25 mg/ml), 1.25 (12.5 mg/ml), 0.625 (6.25 mg/ml) and 0.312% (3.12 mg/ml). The treatments were evaluated using 20 µl of each dilution of the extract, which were applied to the filter paper discs as described previously; thus each disc contained a concentration of 1 mg, 500, 250, 125 and 62 µg of plant extract. The discs previously impregnated with the respective treatment and the positive and negative controls were placed in the Petri dish which contained the inoculated culture medium. Each treatment was made in triplicate, and the cultures were incubated at 37°C for 18 h. The effect of the treatments on the growth of the bacteria was evaluated by measuring the inhibition halo in mm. The MIC was defined as the lowest concentration of extracts at which no visible growth was observed.

Statistical analysis of the data

For each extract and bacterial strain, the mean and standard error were obtained of the inhibition halo (n = 3). Then, the multiple comparison of means was made with the Tukey test ($\alpha = 0.05$), which made it possible to separate and identify groups of means whose differences in inhibition halo were not statistically significant. The calculations and the statistical analysis of the data were made with tools of the statistical package Statgraphics Centurion XV. This statistical technique of separation of groups of means allowed the classification of the plant extracts as low, moderate and high antibacterial activity.

RESULTS AND DISCUSSION

Of the 37 extracts evaluated, from 18 plant species (10 families), antibacterial activity was observed in 15 extracts against at least one strain of MRSA and 17 against at least one strain of CoNS with values of low (halo of 6.25 to 9.0 mm) to high activity (halo of 13.25 to 15.50 mm) (Tables 3 and 4). It is interesting to point out that species that belong to the family Asteraceae showed the highest activity, which is congruent with the fact that several species of this family have been described with antimicrobial activity, such as the genus *Senecio* (Kiprono et al., 2000; El-Shazly et al., 2002; Loizzo et al.,

Table 3. Activity against *Staphylococcus aureus* and negative coagulase S*taphylococcus* Methicillin-resistant strains of the plant extracts (HI>6.0 mm).

Species	Plant part	Solvent/ w/w yield	Extract	Strain	HI (Mean ± SE mm) [a, b]
				000735 *S. aureus*	9.00 ± 0.0 abcdefg
		Hexane	E2	020816 *S. aureus*	9.50 ± 0.5 bcdefgh
				EI-1 SCN	12.00 ± 0.0 hijklm
		Acetone	E3	030233 SCN	7.00 ± 1.0 abc
				ERN *S. aureus*	7.50 ± 0.5 abcd
				000735 *S. aureus*	9.00 ± 1.0 abcdefg
				010718 *S. aureus*	8.00 ± 0.0 abcde
				020802 *S. aureus*	6.25 ± 0.0 a
P. purpusii	Leaf and stems	Ethanol	E4	030303 *S. aureus*	9.00 ± 0.0 abcdefg
				MH SCN	9.00 ± 0.0 abcdefg
				020816 SCN	7.50 ± 0.0 abcd
				EL-1 SCN	7.00 ± 0.0 abc
				030233 SCN	8.00 ± 0.0 abcde
				030527 SCN	10.00 ± 1.0 cdefghi
		Methanol-water 80:20	E6	ERN *S. aureus*	8.00 ± 0.0 abcde
				030233 SCN	7.00 ± 0.0 abc
		Solids from metanol extracts	E7	ERN *S. aureus*	8.50 ± 0.5 abcdef
				030233 SCN	6.25 ± 0.0 a
				ERN *S. aureus*	14.50 ± 0.5 lmnop
				000735 *S. aureus*	9.50 ± 0.5 bcdefghi
		Methanol 7.11 %		010718 *S. aureus*	11.50 ± 0.5 ghijk
L. camara	Leaf and stems		E12	020802 *S. aureus*	9.00 ± 0.0 abcdefg
				MH SCN	10.00 ± 1.0 cdefghi
				030233 SCN	15.00 ± 0.0 mnop
				030527 SCN	6.25 ± 0.0 a
				010718 *S. aureus*	6.25 ± 0.0 a
				020802 *S. aureus*	7.50 ± 0.0 abcd
		Methanol 8.0 %	E13	MH SCN	6.50 ± 0.0 ab
				030233 SCN	6.25 ± 0.0 a
V. abscondita	Leaf and stems			030527 SCN	6.25 ± 0.0 a
		Ethyl acetate 14.4 %	E14	EL-1 SCN	8.00 ± 0.0 abcde
				030233 SCN	10.00 ± 0.0 cdefghi
				010718 *S. aureus*	6.25 ± 0.0 a
				020802 *S. aureus*	10.50 ± 0.5 efghi
A. cuspidata	Leaf and stem	Methanol 3.4 %	E16	MH SCN	8.50 ± 0.5 abcdef
				030233 SCN	8.00 ± 0.0 abcde
				030527 SCN	6.25 ± 0.0 a
A. indica	seeds	Ethanol	E20	ERN *S. aureus*	6.25 ± 0.0 a

Table 3. Contd.

V. oaxacana	Leaf and Stem	Ethyl acetate 16.54 %	E24	ERN *S. aureus*	11.50 ± 1.0	ghijk
				000735 *S. aureus*	15.25 ± 0.2	op
				010718 *S. aureus*	14.75 ± 0.2	mnop
				020802 *S. aureus*	13.00 ± 1.1	ijklmno
				030303 *S. aureus*	12.75 ± 0.7	jklmn
				MH SCN	12.75 ± 1.3	jklmn
				020816 SCN	15.50 ± 0.9	p
				EL-1 SCN	12.25 ± 0.8	hijklmn
				030233 SCN	9.50 ± 0.8	bcdefg
				030527 SCN	13.25 ± 1.0	jklmnop
T. silvatica	Leaf and Stem	Ethyl acetate 4.13 %	E26	ERN *S. aureus*	7.75 ± 0.2	abcd
				000735 *S. aureus*	11.00 ± 1.2	fghij
				010718 *S. aureus*	11.00 ± 0.4	fghij
				020802 *S. aureus*	12.50 ± 2.3	ijklmn
				030303 *S. aureus*	8.50 ± 0.9	abcdef
				MH SCN	12.25 ± 1.3	hijklmn
				020816 SCN	10.50 ± 0.9	efghi
				EL-1 SCN	10.50 ± 0.3	efghi
				030233 SCN	8.50 ± 0.9	abcdef
				030527 SCN	8.70 ± 0.6	abcdef
T. silvatica	Leaf and Stem	Methanol 4.63 %	E27	ERN *S. aureus*	7.50 ± 0.5	abcd
				000735 *S. aureus*	8.00 ± 0.0	abcde
				010718 *S. aureus*	9.00 ± 0.0	abcdefg
				020802 *S. aureus*	6.50 ± 0.5	ab
				030303 *S. aureus*	7.00 ± 0.0	abc
				MH SCN	7.00 ± 1.0	abc
				020816 SCN	6.50 ± 0.5	ab
				EL-1 SCN	6.25 ± 0.0	a
				030233 SCN	6.50 ± 0.5	ab
				030527 SCN	6.25 ± 0.0	a
B. conferta	Leaf and Stem	Ethyl acetate 3.05 %	E28	030233 SCN	6.50 ± 0.5	ab
C. dentata	Leaf and Stem	Methanol 7.5 %	E29	EL-1 SCN	13.00 ± 3.0	ijklmno
L. hirta	Leaf and Stem	Methanol 3.97 %	E33	000735 *S. Aureus*	11.00 ± 5.0	fghij
P. hebeclada	Roots	Methanol 8.0%	E34	ERN *S. aureus*	13.00 ± 1.2	jklmno
				000735 *S. aureus*	9.50 ± 0.9	bcdefg
				010718 *S. aureus*	9.50 ± 0.3	bcdefg
				020802 *S. aureus*	12.00 ± 1.1	hijkl
				030303 *S. aureus*	11.75 ± 0.2	ghijkl
				MH SCN	13.75 ± 1.9	lmnop
				020816 SCN	10.00 ± 0.0	cdefghi
				EL-1 SCN	11.50 ± 0.3	ghijk
				030233 SCN	10.50 ± 0.3	efghi
				030527 SCN	10.00 ± 0.0	cdefghi

Table 3. Contd.

P. oaxacanum	Leaf and Stem	Methanol 12.0 %	E35	ERN *S. aureus*	6.50 ± 0.5	ab
				020802 *S. aureus*	6.25 ± 0.0	a
				MH SCN	10.50 ± 0.5	efghi
				EL-1 SCN	6.25 ± 0.0	a
				030233 SCN	6.25 ± 0.0	a
				030527 SCN	6.25 ± 0.0	a
V. tiliifolia	Stem	Methanol 7.5 %	E37	ERN *S. aureus*	9.50 ± 0.5	bcdefgh
				000735 *S. aureus*	6.50 ± 0.5	ab
				010718 *S. aureus*	8.00 ± 0.0	abcde
				020802 *S. aureus*	6.25 ± 0.0	a
				MH SCN	12.00 ± 0.0	hijklm
				020816 SCN	6.50 ± 0.5	ab
Control Streptomycin				ERN *S. aureus*	8.00 ± 0.0	abcde
				000735 *S. aureus*	7.50 ± 0.5	abcd
				010718 *S. aureus*	7.00 ± 0.0	abc
				020802 *S. aureus*	7.00 ± 0.0	abc
				030303 *S. aureus*	10.00 ± 0.0	cdefghi
				MH SCN	7.00 ± 0.0	abc
				020816 SCN	10.00 ± 0.0	cdefghi
				EL-1 SCN	10.00 ± 0.0	cdefghi
				030233 SCN	10.00 ± 0.0	cdefghi
				030527 SCN	10.00 ± 0.0	cdefghi

[a]Plant extracts with antibacterial activity (HI>6.0 mm). HI: Average inhibition halo in mm. SE: Standard error. n = 3.
[b] In the HI column, means followed by the same letter are not significantly different according to Tukey's studentzed range test (honestly significant difference) (p>0.05).

Table 4. Antibacterial activity level of the active extracts with base in the separation of averages method (Tukey's test).

Group of averages (Table 3)	HI range (mm) [a](average)	Level of activity
Averages identified with the letter "a"	6.25 a 9.00	Low
Averages identified with the letters of the "b" to "o", without overlapping with "a and "p"	9.50 a 13.00	Moderated
Averages identified with the letter "p"	13.25 a 15.50	High

[a] HI, Average inhibition halo in mm.

2006; Tundis et al., 2007) (*Senesio purpusii* Greenm. Ex Brandegee is basinomia of *P. purpusii*), *Vernonia* (Freire et al*., 2002), Vernonanthura* (Portillo et al., 2005) and *Baccharis* (Salcedo et al., 2003; Feresin et al., 2003; Cobos et al., 2001) including testing with multiresistant strains of *S. aureus* such as *B. grisebachii* (Feresin et al., 2003).

The extracts with inhibitory activity of growth of the ten strains of *Staphylococcus* were those from *P. purpusii, V. oaxacana, T. silvatica* and *P. hebeclada*, and those that caused the highest levels of inhibitory activity of bacterial growth were those of ethyl acetate of *V. oaxacana* and *T. silvatica* and the methanolic extract of roots of *P. hebeclada.* These three extracts showed activity levels from moderate (9.5 to 13.0 mm) to high (13.25 to 15.50 mm) for all of the strains of MRSA and CoNS and they

showed highest activity as that observed for streptomycin, which was low (6.25 to 9.0 mm) to moderate (9.5 to 13.0 mm) (Table 4). These results justified the selection of the three previously mentioned extracts for the determination of the MIC. The extracts of ethyl acetate of *V. oaxacana* (Table 5) and of the methanolic extract of *P. hebeclada* (Table 6) showed an MIC of 12.5 mg/ml corresponding to 250 µg of plant extract per disc, producing effects on 90 and 100% of the strains of MRSA and CoNS, respectively; and for the extract of *T. silvatica*, the MIC was 6.25 mg/ml, with 125 µg of plant extract per disc, with an effect on 60% of the strains of *Staphylococcus* (Table 7).

The plant extract obtained with ethyl acetate from the seeds and stems of *V. oaxacana* showed a moderate (9.5 to 13.0 mm) to high (13.25 to 15.50 mm) activities against MRSA and CoNS and an MIC of 1.25% (250 µg/disc); however, when the evaluation of the methanol extract of the same species was made, this did not show activity against any strain of *Staphylococcus*, which made evident that the compounds with the antibacterial activity are of medium polarity. It was mentioned that in this genus there are few biological studies such as the significant antimicrobial activity of *Vernonanthura tweedieana* against *Candida albicans, Cryptococcus neoformans, Microsporum gypseum, Saccharomices cerevisiae* and *Trichophyton mentagrophytes* (Portillo et al., 2005).

The species *P. hebeclada* do not have biological study and the moderate activity (9.5 to 13 mm) exhibited against MRSA and CoNS and MIC of 1.25% (250 µg/disc) of the methanol extract of roots suggested the probable activity of the cumarins (Hernández-Carlos et al., 2003) and terpenoids (Joseph-Nathan et al., 1972) described in this species (Enriquez et al., 1980).

The moderate activity of the ethyl acetate extract of *T. silvatica* leaves against MRSA and CoNS and the MIC of 0.625% (125 µg/disc) obtained in this study is novel; there are no reports of antibacterial activity in species of the genus *Trixis,* however, tripanocide activity has been described in *T. vauthieri* (Macedo et al., 1997) as well as antiulcerogenic activity in *T. divaricata* (Pereira et al., 2005), both due to flavonoids, although sesquiterpenes have also been isolated in species of *Trixis* (Kotowicz et al., 2001; Bohlmann et al., 1976; Bohlmann et al., 1981; De Riscala et al., 1988). Plant extracts obtained with ethyl acetate had higher antibacterial activity than those obtained with other solvents such as water, methanol, ethanol and *n*-hexane; these suggest that the extracts with antibacterial activity might contain flavonoids or terpenes (sesquiterpenes and triterpenes) according to our results about the genera that showed significant antimicrobial activity. This leads us to propose that the active extracts obtained with ethyl acetate must be identified in future studies.

The profile of susceptibility that was obtained in this study for the strains of *Staphylococcus* showed resistance to ampicillin, ceftazidime, gentamicin, pefloxacin, penicillin, trimethoprim-sulfamethoxazole, and oxacillin, which could be due to the fact that these antimicrobial drugs are used routinely, indiscriminately and at times empirically for the treatment of diverse human infections (Rojas et al., 2001), exerting a selective pressure, which contributes to making the bacteria increasingly more difficult to eradicate in patients suffering from nosocomial infections (Edmon, 1999). The strains that are isolated and classified in this study such as *S. aureus* and CoNS are resistant to Methicillin, and they all presented the gene *mecA*, a characteristic that implies resistance to β-lactamic antibiotics and are generally resistant to aminoglucosides, macrolides, tetracyclines and others (Chrystal, 2002; Stefani and Veraldo, 2003). It is important to mention that in similar studies with susceptible strains without the profile of resistance such as that presented by the strains used in this present study, streptomycin was used as the positive control (10 µg/ml) obtaining inhibition halos of approximately 38.7mm in strains *S. aureus* (Martínez et al*.,* 1996; Martínez et al., 1997). In this work, an inhibition diameter of 7 to 10 mm was observed, due to the fact that the activity assays were made with multiresistant strains (resistant to more than five antibiotics) to the antibiotics normally used in the clinic. Thus, the inhibition halo observed in the disc positive control of Streptomycin (7 to 10 mm), was smaller than that obtained in the disc with the most active extract, *V. oaxacana* (leaves, ethyl acetate), in which an inhibition halo of 9.5 to 15.5 mm diameter was observed (Table 3); therefore, in all of the assays, the negative control did not show inhibition halo, the same as what was reported by Ates and Erdogrul, 2003, which shows that the dissolvent is not responsible for the antibacterial activity against the microorganisms.

The results of this study show that the extracts of leaves obtained with ethyl acetate of *T. silvatica, V. oaxacana* and those obtained with methanol of roots of *P. hebeclada* possess substances with antibacterial activity against gram positive bacteria such as MRSA and CoNS. The cytoplasmic membrane of the gram positive bacteria is considerably more selective to permeability by amphipathic toxins and the bombs resistant to multidrugs provide limited protection (Lewis, 2001). The above suggests that the antibacterial activity of the plant extracts depends on the plant part and species, from which the extract is obtained, as well as the solvent and the virulence of the pathogen.

This study is the first report of the antibacterial activity of the plant extracts of *V. oaxacana, T. silvatica, P. hebeclada* and *P. purpusii* against MRSA and CoNS resistant to Methicillin. In addition, the results suggest that the extracts of these plants could be analyzed in the future to obtain the active compounds against multi-resistant grampositive bacteria which cause nosocomial infections, or as disinfectants in the contaminated areas within the hospitals, after doing an evaluation of their hepatotoxic and nephrotoxic effect.

Also, we suggest a study to look for the effects of these

Table 5. Minimum inhibitory concentration of the plant extract obtained with ethyl acetate from leaves of *V. oaxacana* against Methicillin resistant *Staphylococcus*.

Strain	Plant extract concentration (%)/ Streptomycin	HI [a] (Average ± SE mm) [b]
ERN *S. aureus*	5.000	10.0 ± 0.0
	2.500	8.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	8.0 ± 0.0
000735 *S. aureus*	5.000	15.5 ± 0.5
	2.500	14.5 ± 0.5
	1.250	11.0 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	7.5 ± 0.5
010718 *S. aureus*	5.000	14.5 ± 0.5
	2.500	13.0 ± 1.0
	1.250	12.0 ± 1.0
	0.625	12.0 ± 1.0
	0.312	0.0 ± 0.0
	Streptomycin	7.0 ± 0.0
020802 *S. aureus*	5.000	11.0 ± 0.0
	2.500	9.0 ± 0.0
	1.250	6.5 ± 0.5
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	7.0 ± 0.0
030303 *S. aureus*	5.000	11.5 ± 0.5
	2.500	9.5 ± 0.5
	1.250	7.0 ± 1.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
MH SCN	5.000	14.0 ± 0.5
	2.500	13.0 ± 0.5
	1.250	9.0 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	7.0 ± 0.0
020816 SCN	5.000	10.5 ± 0.5
	2.500	8.5 ± 0.5
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0

Table 5. Contd.

EL-1 SCN	5.000	11.0 ± 1.0
	2.500	8.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
030233 SCN	5.000	8.0 ± 0.0
	2.500	6.2 ± 0.0
	1.250	0.0 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
030527 SCN	5.000	11.5 ± 0.0
	2.500	8.5 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0

[a] HI, Average inhibition halo in mm; [b] SE, Standard error; n = 3.

Table 6. MIC of the plant extract obtained with methanol from leaves of *P. hebeclada* against Methicillin resistant *Staphylococcus*.

Strain	Plant extract concentration (%)/Streptomycin	HI [a](Average ± SE mm) [b]
ERN *S. aureus*	5.000	11.0 ± 0.0
	2.500	8.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	8.0 ± 0.0
000735 *S. aureus*	5.000	10.0 ± 0.0
	2.500	8.5 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	7.5 ± 0.5
010718 *S. aureus*	5.000	10.0 ± 0.0
	2.500	9.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	7.0 ± 0.0
020802 *S. aureus*	5.000	14.0 ± 0.0
	2.500	9.5 ± 0.0
	1.250	7.5 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0

Table 6. Contd.

030303 *S. aureus*	5.000	11.5 ± 0.0
	2.500	8.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
MH SCN	5.000	10.5 ± 0.0
	2.500	8.5 ± 0.0
	1.250	6.5 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	7.0 ± 0.0
020816 SCN	5.000	10.0 ± 0.0
	2.500	8.5 ± 0.5
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
EL-1 SCN	5.000	11.0 ± 0.0
	2.500	8.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
030233 SCN	5.000	10.0 ± 0.0
	2.500	7.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
030527 SCN	5.000	10.0 ± 0.0
	2.500	8.5 ± 0.0
	1.250	6.5 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0

[a] HI, Average inhibition halo in mm. [b] SE: Standard error. n = 3.

Table 7. MIC of the plant extract obtained with ethyl acetate from leaves of *T. silvatica* against Methicillin resistant *Staphylococcus*.

Strain	Plant extract concentration (%) or Streptomycin	HI [a] (Average ± SE mm) [b]
ERN *S. aureus*	5.000	8.0 ± 0.0
	2.500	7.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	8.0 ± 0.0

Table 7. Contd.

000735 *S. aureus*	5.000	13.0 ± 0.0
	2.500	11.0 ± 1.0
	1.250	10.0 ± 1.0
	0.625	8.0 ± 1.0
	0.312	0.0 ± 0.0
	Streptomycin	7.5 ± 0.0
010718 *S. aureus*	5.000	11.5 ± 0.5
	2.500	10.5 ± 0.5
	1.250	8.5 ± 0.5
	0.625	6.5 ± 0.5
	0.312	0.0 ± 0.0
	Streptomycin	7.0 ± 0.0
020802 *S. aureus*	5.000	8.5 ± 0.0
	2.500	7.5 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	70 ± 0.0
030303 *S. aureus*	5.000	10.0 ± 0.0
	2.500	8.0 ± 0.0
	1.250	6.2 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
MH SCN	5.000	10.0 ± 0.0
	2.500	9.0 ± 0.0
	1.250	8.0 ± 0.0
	0.625	6.2 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	7.0 ± 0.0
020816 SCN	5.000	10.0 ± 0.0
	2.500	8.5 ± 0.0
	1.250	7.5 ± 0.5
	0.625	6.2 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
EL-1 SCN	5.000	10.0 ± 0.0
	2.500	8.0 ± 0.0
	1.250	7.0 ± 0.0
	0.625	6.2 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0

Table 7. Contd.

30233 SCN	5.000	7.0 ± 0.0
	2.500	6.2 ± 0.0
	1.250	0.0 ± 0.0
	0.625	0.0 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0
030527 SCN	5.000	9.5 ± 0.0
	2.500	8.0 ± 0.0
	1.250	7.5 ± 0.0
	0.625	6.2 ± 0.0
	0.312	0.0 ± 0.0
	Streptomycin	10.0 ± 0.0

[a] HI: Average inhibition halo in mm. [b] SE: Standard error. n = 3.

extracts as potentiators in the use of the β-lactamic antibiotics as first choice in the clinic against infections from *Staphylococcus*, as has been done in other studies with *Heliobacter pylori* (Shin et al., 2005), and thus propose the potential use of natural antimicrobial product along with other component to increase their activity.

Conclusions

The assays of antibacterial activity showed that 15 of the 37 plant extracts at 5% (50 mg/ml) of the 18 species belonging to the ten families of plants, presented inhibitory activity of growth to strains of Methicillin resistant *S. aureus* and 17 plant extracts presented inhibitory activity to the strains of multiresistant CoNS; some extracts did not inhibit all of the strains and in others only one strain was inhibited. Five extracts of four plant species of the family Asteraceae showed antibacterial activity against all of the strains of Methicillin resistant *S. aureus* and multiresistant CoNS and of these, three species (*V. oaxacana, T. silvatica* and *P. hebeclada*) were the most active species against *S. aureus* and CoNS resistant to Methicillin and those that cause nosocomial infections.

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