Canales-Martinez et al., Afr J Tradit Complement Altern Med., (2017) 14 (3): 74-82
doi:10.21010/ajtcam.v14i3.8

ANTIMICROBIAL ACTIVITY OF *BURSERA MORELENSIS* RAMÍREZ ESSENTIAL OIL

Canales-Martinez, M.[1*], Rivera-Yañez, C.R.[1], Salas-Oropeza, J.[1], Lopez, H.R.[1], Jimenez-Estrada, M.[2], Rosas-Lopez, R.[3], Duran, D.A.[1], Flores, C.[4], Hernandez, L.B.[4], Rodriguez-Monroy, M.A.[5]

[1] Laboratorio de Farmacognosia, Unidad de Biología, Tecnologia y Prototipos (UBIPRO), Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Av. de los Barrios No. 1, Los Reyes Iztacala, Tlalnepantla, Edo. de México. [2]Instituto de Quémica. Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán. [3]Universidad de la Cañada. Carr. Teotitlán San Antonio Nanahuatipan Km 1.7. Teotitlán de Flores Magón, Oaxaca, México. [4]Laboratorio de Fisiología Vegetal, UBIPRO y Laboratorio Nacional en Salud, Facultad de Estudios Superiores-Iztacala UNAM, Edo. Mex, Tlalnepantla, México. [5]Laboratorio de Inmunobiología, Carrera de Medicina. Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Av. De los Barrios No. 1, Los Reyes Iztacala, Tlalnepantla, Edo. de México.

Corresponding Author's E-mail: dra.margaritacanales@gmail.com

Abstract

Background: *Bursera morelensis*, known as "Aceitillo", is an endemic tree of Mexico. Infusions made from the bark of this species have been used for the treatment of skin infections and for their wound healing properties. In this work, we present the results of a phytochemical and antimicrobial investigation of the essential oil of *B. morelensis.*
Materials and Methods: The essential oil was obtained by a steam distillation method and analyzed using GC-MS. The antibacterial and antifungal activities were evaluated.
Results: GC-MS of the essential oil demonstrated the presence of 28 compounds. The principal compound of the essential oil was α-Phellandrene (32.69%). The essential oil had antibacterial activity against Gram positive and negative strains. The most sensitive strains were *S. pneumoniae*, *V. cholerae* (cc) and *E. coli* (MIC 0.125 mg/mL, MBC 0.25 mg/mL). The essential oil was bactericidal for *V. cholera* (cc). The essential oil inhibited all the filamentous fungi. *F. monilifome* (IC_{50} = 2.27 mg/mL) was the most sensitive fungal strain.
Conclusions: This work provides evidence that confirms the antimicrobial activity of the *B. morelensis* essential oil and this is a scientific support about of traditional uses of this species.

Keywords: Essential oil; Medicinal plants; Tehuacan-Cuicatlan Valley; Burseraceae; Bursera.

Introduction

The genus *Bursera* Jacq.exL comprises approximately 100 species distributed from the South of the United States to Peru (Rzedowski and Guevara-Féfer, 1992). Approximately 70 species of *Bursera* are endemic to Mexico (Rzedowski, 1986). Several species produce an aromatic resin known as "copal", which has been commonly burnt as incense in religious activities throughout the country since ancient times (Peters et al., 2003). Many *Bursera* species are important in Mexican folk medicine. Their use is related to fragrance in addition to its medicinal properties against diarrhea, fever, gingivitis, cough and measles (Hernández-Hernández et al., 2005).

Bursera morelensis Ramírez (Burseraceae), known as "Aceitillo", is an endemic tree of Mexico and is found in Guanajuato, Guerrero, Hidalgo, Morelos, Oaxaca, Puebla, Queretaro and San Luis Potosi states (Rzedowski and Guevara-Féfer, 1992). An infusion made from the bark of this species has been used for the treatment of skin infections and for its wound healing properties by the inhabitants of San Rafael, Coxcatlan, Puebla, Mexico.

The Burseraceae family has been shown to have anti-inflammatory, antibacterial and cytotoxic activities (Carrera-Martínez et al., 2014; Carretero et al., 2008; Queiroga et al., 2007; Zuñiga et al., 2005; Yasunaka et al., 2005; Jutiviboonsuk et al., 2005; Sosa et al., 2002).
The chemical profile of the genus *Bursera* includes essential oils, triterpenes, steroids, bilignans, podophyllotoxin-like lignans and flavonoids (Zúñiga et al., 2005; Culioli et al., 2003; Syamasundar and Mallavarapu, 1995). Phytochemical studies of *B. morelensis* led to the identification of two lignans with cytotoxic activity (Jolad et al., 1977) and found terpenoids in the volatile fraction from the bark (Zúñiga et al., 2005). However, there are no studies regarding the antibacterial and antifungal properties of this species. In the present study, we present the results of our phytochemical and antimicrobial investigation of the essential oil of *Bursera morelensis*.

Material and methods

Plant material

B. morelensis was obtained from San Rafael, Coxcatlan, Puebla in October 2013. Voucher specimens (IZTA 42123) were deposited at the National Herbarium of Mexico (MEXU) at the Universidad Nacional Autonoma de Mexico and the herbarium IZTA at the Facultad de Estudios Superiores Iztacala.

San Rafael is a village in the municipality of Coxcatlan, located southeast of the Tehuacan-Cuicatlan Valley at coordinates 18°12' and 18°14' north and 97°07' and 97°09' west and at 957 m above sea level. The climate is dry or arid with summer rains and a mean temperature of 22°C (Fernández, 1999).

The vegetation is a thorn scrub forest with species such as *Bursera morelensis* Ramírez, *B. aptera* Ramírez, *Pachycereus weberi* (J. Coulter) Backeb, *Opuntia puberula* Pfeiffer, *Ceiba parvifolia* Rose, and *Acacia cochliacantha* Humb. & Bonpl. ex Willd (Rzedowski, 1978; Fernández, 1999; Casas et al., 2001).

The collection of specimens from the field was carried out with permission from the Secretaria de Medio Ambiente y Recursos Naturales (SGPA/DGVS/1266).

Essential oil extraction

The essential oil was obtained using the steam distillation method (2000 g of fresh plant, young stems and pieces of bark). The distillation equipment consisted of a round-bottomed 1.5 L flask with a heating mantle (SEV-Prendo, MC301-9, Mexico) attached to a double pass condenser, which in turn was coupled to a cold-water circulator. A total of 250 g of fresh plant was placed in distilled water in the round-bottomed flask and was left to distil for approximately 30 minutes. This process was repeated, with new plant material and water each time, and the essential oil that was extracted in each distillation was collected. The essential oil was stored in amber glass vials at -18°C until used.

The essential oil was analyzed in a gas chromatograph model 6850 (Agilent Technologies, USA) equipped with a column HP-5 ms (Agilent Technologies, 30 m x 0.25 mm i.d. and 0.25 µm film thickness, China). Next, 1 µL of essential oil was injected by split. The column was programmed as follows: 70°C for 2 minutes and increased to 230°C at 20°C/min. At 230°C, a programmed linear gradient increased the temperature 8°C/min to 280°C. The injector temperature was 280 °C. Helium was used as the gas carrier at a flow rate of 1.0 mL/min. The total analysis time was 21.25 min. Peak area percentages were determined using RTE integrator software (Agilent Technologies, China). The identification of the components was carried out by gas chromatography-mass spectrometry (GC-MS) using a gas chromatograph model 6850 (Agilent Technologies, USA) coupled to a mass spectrometer (MS) model 5975C (Agilent Technologies, China). The sample was ionized by electron impact at 70 eV, and the temperature achieved by the ionization source was 230°C. The oil components were identified by comparison of their retention indices and mass spectra with the NIST/EPA/NIH Mass Spectral Library 8.0.

Microorganisms

The following strains of bacteria were used: *Staphylococcus aureus* ATCC 29213, *S. aureus* (clinical isolate), *S. epidermidis, Bacillus subtilis, Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* (clinical isolate donated by Hospital Angeles (Metropolitano), *Vibrio cholerae* INDRE 206 (isolated from polluted water), *Vibrio cholerae* (a clinical isolate corresponding with group 01, producing enterotoxin, serotype "Inaba", biotype "El Tor"), *Vibrio cholera* CDC V 12, *Escherichia coli* (clinical isolate), *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* (donated by the Laboratory of Microbiology of FES-Cuatitlan UNAM), *Yersinia enterocolitica* (donated by the Clinical Analysis Laboratory of University Hospital Campus Iztacala), *Proteus mirabilis* clinical isolate donated by Hospital Angeles (Metropolitano).

The fungal strains used: *Fusarium sporotrichioides* (ATCC NRLL3299), *Fusarium moniliforme* (CDBB-H-265), *Trichophyton mentagrophytes* (CDBB-H-1112), *Aspergillus niger* (CDBB-H-179), and *Rhizoctonia lilacina* (CDBB-H-306).

Antibacterial activity

Antibacterial activity was measured using the disc–diffusion method (Vanden Berghe and Vlietinck, 1991). The microorganisms were grown overnight at 37°C in 10 mL of Müeller Hinton broth (Bioxon 260-1, Estado de Mexico, Mexico). The cultures were adjusted to a turbidity comparable to McFarland no. 0.5 standard with sterile saline solution. Petri dishes containing Müeller Hinton agar (Bioxon, Edo. de Mexico, Mexico) were impregnated with these microbial suspensions. 5-mm diameter discs (Whatman no. 5) were saturated with 5 µL of essential oil. Discs of chloramphenicol (25 µg) were used as positive controls. The plates were incubated overnight at 37°C, and the

diameters of any resulting inhibition zones (mm) were measured. Each experiment was repeated at least three times. Estimation of the minimal inhibitory concentration (MIC) was carried out using the broth dilution method (Vanden Berghe and Vlietinck, 1991). Dilutions of essential oil from 4000 to 62.5 µg/mL were used. Tubes were inoculated with a microorganism suspension of 10^5 CFU/mL. MIC values were defined as the lowest essential oil concentration that prevents visible bacterial growth after 24 h of incubation at 36°C. Chloramphenicol was used a reference. Each experiment was repeated at last three times.

The bactericidal kinetic assay was performed using appropriate concentrations of essential oil (corresponding to ½MIC, MIC and MBC) (Lennette et al., 1987).

Antifungal activity

The antifungal activity assay was carried out in Petri dishes (80 mm x10 mm) containing PDA agar (30 mL) (Bioxon, Edo. de Mexico, Mexico). After each mycelial colony had developed, disks impregnated with 5 µL of essential oil were placed at a distance of 10 mm from the Petri dish's edge, and the dishes were then incubated at 23 °C for 72 h until mycelia growth had enveloped the disks containing the control and had formed crescents of inhibition around disks containing samples with antifungal activity (Ye et al., 1999). Ketoconazol (7 µg/disk) was used as positive control.

For the quantitative assays, 24-wells culture plates were used. Six dilutions of essential oil were added to PDA agar at 45°C, rapidly mixed and poured into three wells of a culture plate; after the agar solidified, a small amount (1 X 1 mm) of mycelia was inoculated in each well. Ketoconazole was used as a positive control. After incubation at 23°C for 48 h, the area of the mycelium colony was measured, and the inhibition of fungal growth and the IC_{50} was determined using the following formula:

$I (\%) = d_c - d_t / d_c \times 100$

d_c: diameter of the colony of the control culture

d_t: diameter of the colony of the treated culture

The IC_{50} values were calculated using rectangular hyperbola regression of plots, where the abscissa represented the concentration of the essential oil, and the ordinate represented the average percent of inhibition of fungal growth from three replicates.

Results

The essential oil obtained of *B. morelensis* young stems and pieces of bark was translucent and colorless with a density of 0.86 g/mL and a yield of 4.1804 g (0.21%).

Chromatographic analysis by GC-MS of the essential oil demonstrated the presence of 28 compounds. The five principal compounds were α-Fellandrene (32.69%), β-Fellandrene (14.79%), *o*-Cymene (8.71%), Isocaryophyllene (7.48%) and α-Pinene (5.82%) (Figure 1, Table 1).

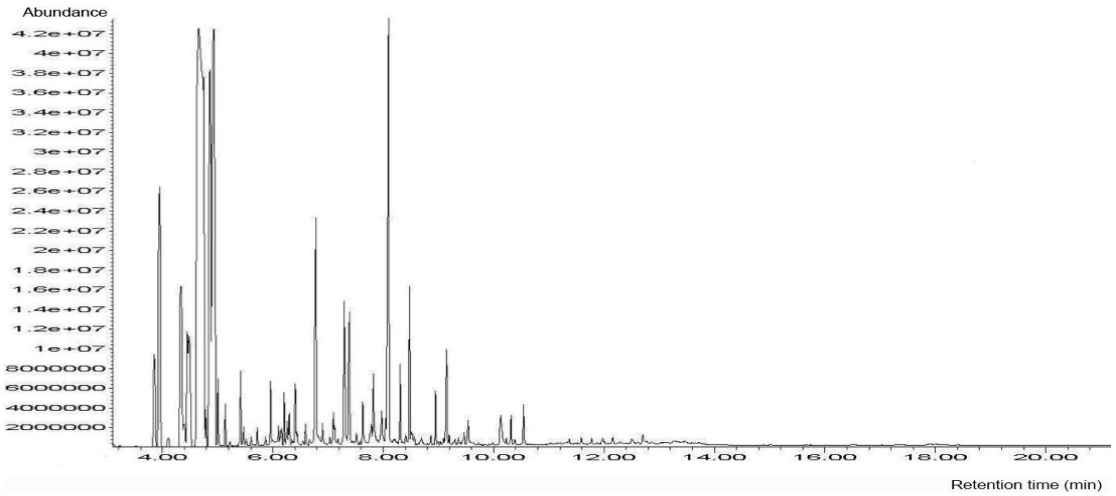

Figure 1: Chromatogram plot of *B. morelensis* essential oil.

doi:10.21010/ajtcam.v14i3.8

Table 1: Chemical composition of *Bursera morelensis* essential oil.

Compound	Retention time (min)	Abundance (%)
β-Thujene	3.856	1.99%
α-Pinene	3.945	5.82%
Sabinene	4.329	2.65%
β-Pinene	4.450	2.56%
β-Myrcene	4.490	2.15%
α-Phellandrene	4.658	32.69%
o-Cymene	4.866	8.71%
β-Phellandrene	4.938	14.79%
β-Ocimene	5..011	0.65%
γ-Terpinene	5.139	0.42%
α-Terpinolene	5.411	0.88%
4,5-Epoxycarene	5.956	0.69%
Terpinen-4-ol	6.205	0.58%
α-Terpineol	6.301	0.47%
Neoisothujol	6.405	0.71%
Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-	6.774	3.62%
Camphenol	7.095	0.46%
Pinanediol	7.295	2.96%
5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	7.383	1.80%
Bornane-2,3-diol	7.784	0.42%
Decahydronaphthalene-1-ol-3-ketone	7.976	0.60%
Isocaryophyllene	8.096	7.48%
α-Caryophyllene	8.305	0.85%
β-Cubebene	8.481	1.99%
Denderalasin	8.946	0.61%
Caryophyllene oxide	9.146	1.17%
β-Eudesmol	9.539	0.35%
Bicyclo[3.1.1.]hept-3-ene,2-formylmethyl-4,6,6-trimethyl	10.541	0.50%
Total		98.22%

The essential oil had antibacterial activity against all strains (Gram positive and negative). ANOVA analysis showed that there were significant differences between the strains ($P<0.0001$), but not between the bacterial groups (Gram positive and negative) ($P<0.0001$). The most sensitive strains to the essential oil were *S. pneumoniae, V. cholerae* (cc) and *E. coli* (MIC 0.125 mg/mL, MBC 0.25 mg/mL) (Table 2).

doi:10.21010/ajtcam.v14i3.8

Table 2: Antibacterial activity of *Bursera morelensis* essential oil.

Bacteria	Positive control Chloramphenicol (mm)	Inhibition zones (mm)	MIC (mg/mL)	MBC (mg/mL)
S. aureus (cc)	11.0 ± 1.00	7.50 ± 0.50	1.0	3.0
S. aureus 29213	10.0 ± 1.00	7.16 ± 0.57	1.0	3.0
S. epidermidis	30.0 ± 0.57	7.50 ± 0.50	2.0	>4.0
B. subtilis	24.0 ± 1.00	11.33 ± 0.28	3.0	4.0
S. pneumonia	30.0 ± 0.00	9.50 ± 0.50	0.125	0.25
E. faeacalis	24.0 ± 0.57	7.16 ± 0.28	3.0	>4.0
V. cholerae (INDRE 206)	8.33 ± 0.58	11.33 ± 0.57	2.0	4.0
V. cholerae (cc)	23.3 ± 1.00	20.30 ± 1.52	0.125	0.25
V. cholerae (El Tor)	7.33 ± 0.58	6.16 ± 0.28	3.0	>4.0
E. aerogenes	22.0 ± 0.50	6.00 ± 0.00	3.0	>4.0
Y. enterocolitica (CUSI)	26.50 ± 0.50	7.00 ± 0.00	3.0	>4.0
P. aeruginosa	20.0 ± 0.00	6.83 ± 0.28	3.0	>4.0
P mirabilis	17.3 ± 0.60	9.33 ± 0.57	3.0	>4.0
E. coli (cc)	21.7 ± 0.58	6.00 ± 0.00	0.125	0.25

Figures 2 and 3 show the effect of the essential oil on the survival curve for Gram negative (*V. cholerae* cc) and Gram positive bacterial (*S. aureus* clinical isolate). *V. cholerae* (cc) was inhibited at three concentrations (1/2 MIC, MIC and MBC); the essential oil was bactericidal from the first hour of interaction with the bacterium. Nevertheless, the essential oil only (MIC and MBC concentration) decreased the bacterial growth and showed a bacteriostatic activity for the Gram-positive strain *S. aureus*.

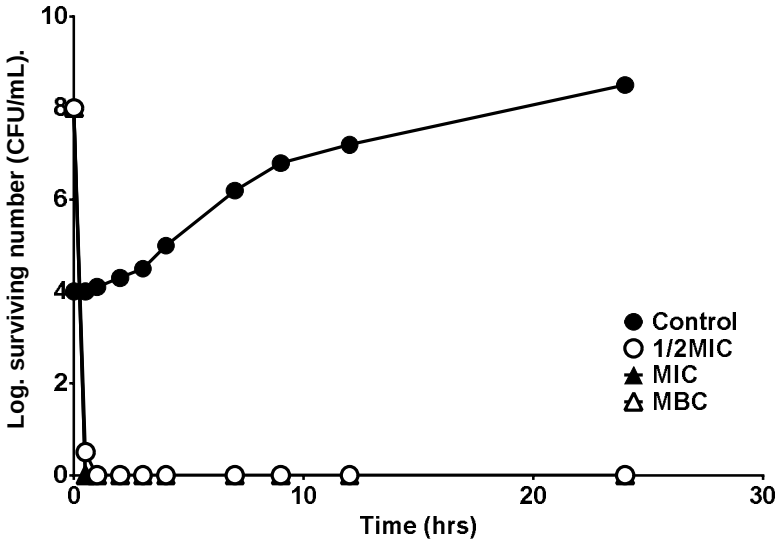

Figure 2: Survival curve of *Vibrio cholerae* (cc) exposed to essential oil of *B. morelensis*. The essential oil was added to each experimental culture at time zero. The concentrations used were as follows: 0.0625 mg/mL (1/2 MIC), 0.125 mg/mL (MIC) and 0.250 mg/mL (MBC). The control tube contained only broth.

doi:10.21010/ajtcam.v14i3.8

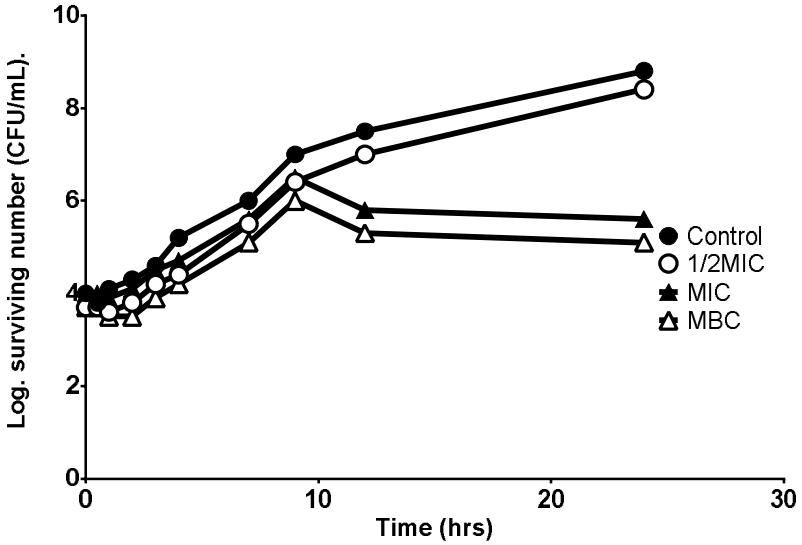

Figure 3: Survival curve of *S. aureus* (cc) exposed to the essential oil of *B. morelensis*. The essential oil was added to each experimental culture at time zero. The concentrations used were as follows: 0.5 mg/mL (1/2 MIC), 1.0 mg/mL (MIC) and 3.0 mg/mL (MBC). The control tube contained only broth.

A qualitative test demonstrated that the essential oil inhibited all the filamentous fungi strains except *F. sporotrichioides*. ANOVA analysis showed that there were significant differences between the sensitivity of the fungal strains to the essential oil ($P<0.0001$). The more sensitive strain was *F. moniliforme* (IC_{50} = 2.27 mg/mL) (Table 3).

Table 3: Antifungal activity of *Bursera morelensis* essential oil.

Filamentous fungi	Essential oil IC_{50} (mg/mL)	Ketoconazol (µg/mL)
F. moniliforme	2.27	7.55
R. lilacina	3.32	21.56
A. niger	1.60*	15.29
Aspergillus sp.	1.84*	9.75
T. mentagrophytes	1.99*	1.16

*CF_{25}: the essential oil only inhibited the 25% of the mycelial growth.

Discussion and Conclusion

Mexico has a large diversity of *Bursera* species, the majority of which are endemic to Mexico. Studies of the *Bursera* species are very limited; the relationship between the antimicrobial activity and the chemical composition of the essential oil of *B. morelensis* is reported here for the first time.

A total of 28 compounds were detected in the essential oil; α-Phellandrene (32.69%), β-Phellandrene (14.79%), *o*-Cymene, (8.71%), Isocaryophyllene (7.48%) and α-Pinene (5.82%) were the most abundant (Fig. 1, Table 1). Rzedowki and Ortiz (1988), detected 12 and 16 compounds (terpenoids) in the essential oil obtained from bark samples of the 8 trees at two different times (September 1982 and October 1983). The two majority of the compounds (α and β-Phellandrene) were also detected in the essential oil of *B. morelensis* collected in August and September of 2012; nevertheless, we did not detect any organic acids as reported by Carrera-Martínez (2014). These differences may be due to the essential oil extraction time, or the influence of the season. Others species of the Burseraceae family had different essential oil composition (number of terpenes detected and majoritarian compounds). Fifty compounds were detected in *Boswellia sacra*, and *E*-β-Ocimene (32.3%) and limonene (33.5%) were the most abundant (Al-Harrasi and Al-Said., 2008). Thirty-seven compounds were detected in the leaves of *Bursera graveolens*, and 27 compounds were detected in the stems. Limonene (48.2%), caryophyllene oxide (13.6%), and trans-caryophyllene (8.1%) were the most abundant in the leaves. Limonene (42.1%) and menthofuran (14.7%) were the most abundant in the stems (Leyva et al., 2007). In another study of *B. graveolens*, 11 compounds were detected in the essential oil derived from dry branches; viridiflorol (70.82%) and 3'-4'-(Methylenedioxy) (6.09%) were the most abundant in this extract (Manzano et al., 2009). This demonstrates that the type, abundance and principal compounds depend not only on the species but also on

the collection site, the part of the plant, the time of year when samples are obtained, the storage conditions and the essential oil extraction methodology (Harborne, 1988; Magiatis et al., 2002). Some of these compounds have been found in the chemical composition of essential oils from other plants with biological activity, for example, α-Phellandrene and β-Phellandrene were found in essential oils from the root and stem, respectively, of *Ligularia persica* Boiss (Mohadjerani et al., 2016). α-Pinene and β-Phellandrene were found in an essential oil from *Chamaecyparis obtuse* that showed antimicrobial activity and the inhibition of some virulence genes from *Streptococcus mutans* (Kim et al., 2016). *o*-Cymene was one of the components found in the *Echinophora platyloba* D.C methanolic extract that has antimicrobial activity (Hashemi et al., 2013).

The antibacterial activity of the *B. morelensis* essential oil depended on the bacterial strains treated ($P<0.0001$) (Table 2); nevertheless, there were no significant differences between Gram-positive and Gram-negative bacteria. The strains most sensitive to the essential oil were *S. aureus* (cc) (CMI=1 mg/mL), *S. aureus* 29213 (CMI=1 mg/mL), *S. pneumoniae* (CMI=0.125 mg/mL), *V. cholerae* (cc) (CMI=0.125 mg/mL) and *E. coli* (cc) (CMI=0.125 mg/mL) (Table 2). Essential oils act on the cellular membrane (Cichewicz and Thorpe, 1996). Our results can be better understood by taking into account the number of compounds found in an essential oil, which is why essential oils do not have cellular specific targets (Carson et al., 2002). Due to its lipophilic nature, the essential oils pass through the cytoplasmic membrane and the cell walls and disrupt their structure leading to membrane permeabilization (Bakkali et al., 2008). The permeabilization of the bacterial membrane causes ion outflow and membrane potential changes, the collapse proton pump and ATP depletion (Ultee et al., 2000, 2002; Di Pasqua et al., 2006; Turina et al., 2006). The essential oil coagulates the cytoplasm and damages the lipids and proteins (Ultee et al., 2002; Burt, 2004).

The terpenes detected in the *B. morelensis* essential oil (α and β-Phellandrene, α-Pinene, *o*-Cymene and isocaryophyllene) have antibacterial and antifungal activities (Burt, 2004; Kordali et al., 2005; Abad et al., 2007; Tangarife et al., 2011). The chemical composition of the essential oils determines their antimicrobial activity; the most abundant compounds usually determine an oil's biological and biophysical properties, but it is possible that the activity of the principal compounds is regulated by the minor compounds (Santana-Rios et al., 2001; Hoet et al., 2006). In addition, it is probable that many compounds in the essential oil can define its fragrance, density, color and the penetration of the cell wall (Cal, 2006).

The *B. morelensis* essential oil also has antifungal activity; five of the strains tested were inhibited. *F. moniliforme* was the most sensitive strain to the essential oil (66.67% of growth inhibition). The antifungal activity of the essential oil is due to the alteration of the cell membrane; terpenes specifically inhibit the respiration and ion transport processes (Burt, 2004; Kosalec et al., 2005; Kordali et al., 2005; Abad et al., 2007; Tangarife et al., 2011). Another essential oil fungal inhibition mechanism is the disruption ergosterol synthesis (Ricci et al., 2005; Parveen et al., 2004)

Interestingly, during the antifungal assay the mycelium grew vertically and not on the agar and produced no spores, particularly in *Aspergillus* strains. This may be evidence that the essential oil is an anti-sporulating agent. Spore formation is inhibited by some essential oils, although the growth of aerial hyphae is unaffected. Biochemical studies showed that the inhibition of spore formation may be closely related to the inhibition of filamentous fungal cell respiration (Inouye et al., 1998). Further study is necessary to confirm this observation.

Taken together, the above results demonstrate that the essential oil of *B. morelensis* has several compounds that may be responsible for antimicrobial activity, thus confirming its use in Mexican traditional medicine.

Acknowledgments

This work was supported by the PAPCA 2014-FESI-UNAM under Grant 9 and PAPIIT, UNAM under Grant IN211614.

References

1. Abad, M.J., Ansuategui, M., y Bermejo, P. (2007). Active antifungal substances from natural sources. Arkivoc. VII: 116-145.
2. Al-Harrasi, A., Al-Said S. (2008). Phytochemical Analysis of the Essential Oil from Botanically Certified Oleogum Resin of *Boswellia sacra* (Omani Luban). Molecules. 13: 2181-2189.
3. Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008). Biological effects of essential oils – A review. Food Chem Toxicol. 46: 446–475.
4. Burt, S. A. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. International J Food Microbiol. 94: 223-253.
5. Cal, K. (2006). Skin penetration of terpenes from essential oils and topical vehicles. Planta Med. 72: 311–316.

6. Carrera-Martínez, C.A., Rosas-López, R., Rodríguez-Monroy, M.A., Canales-Martínez, M., Román-Guerrero, A., Jiménez-Alvarado, R. (2014). Chemical composition and i*n vivo* anti-inflammatory activity of *Bursera morelensis* Ramírez Essential Oil. J Essent Oil Bear Pl. 17: 158-168.
7. Carretero, M.E., López-Pérez, J.L., Abad, M.J., Bermejo, P., Tillet, S., Israel, A., Noguera-P., B. (2008). Preliminary study of the anti-inflamatory activity of hexane extract and fractions from *Bursera simaruba* (Linneo) Sarg. (Burseraceae) leaves. J Ethnopharmacol. 116: 11-15.
8. Carson, C.F., Mee, B.J., Riley, T.V. (2002). Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob Agents Ch. 46: 1914–1920.
9. Casas, A., Valiente-Banuet, A., Viveros, J.L., Caballero, J., Cortés, L., Dávila, P., Lira, R., Rodríguez, I. (2001). Plant resources of the Tehuacan-Cuicatlan Valley, Mexico. Econ Bot. 55: 129–166.
10. Cichewicz, R.H., Thorpe, P.A. (1996). The antimicrobial properties of chile peppers (*Copsicum* species) & their uses in Mayan Medicine. J Ethanopharmacol. 52: 61-70.
11. Culioli, G., Mathe, C., Archier, P., Vieillescazes, C. (2003). A lupane triterpene from
12. frankincense (*Boswellia* sp., Burseraceae). Phytochemistry. 62: 537–541.
13. Di Pasqua, R., Hoskins, N., Betts, G., Mauriello, G. (2006). Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. J Agr Food Chem. 54: 2745–2749.
14. Fernández, B.M.N. (1999). Análisis de la dinámica de comunidades vegetales con relación a la evolución del paisaje, en la zona semiárida de Coxcatlán, Puebla. UNAM. México D.F.
15. Harbone, J. B. (1988). Introduction to ecological biochemistry. Editorial Academia Press. USA. 354 pp.
16. Hashemi, M. Mohammad Hashemi, Ehsani, a., Jazani N.H., Aliakbarlu,J., Mahmoudi R. (2013). Chemical composition and in vitro antibacterial activity of essential oil and methanol extract of *Echinophora platyloba* D.C against some of food-borne pathogenic bacteria. Vet Res Forum. 4: 123 – 127.
17. Hernández-Hernández, J.D., Román-Marín, L.U., Cerda-García-Rojas, C.M., Joseph-Nathan, P. (2005). Verticillane derivatives from *Bursera suntui* and *Bursera kerberi*. J Nat Prod. 68: 1598-1602.
18. Hoet, S., Stévigny, C., Hérent, M. F., Quetin-Leclercq, J. (2006). Antitrypanosomal compounds from leaf essential oil of *Strychnos spinosa*. Planta Med. 72: 480–482.
19. Inouye, S., Watanabe, Y., Nishiyama, Y., Takeo, K., Akao M., Yamaguchi, H. (1998). Antisporulating and respiration-inhibitory effects of essential oils on filamentous fungi. Mycoses. 41: 403-410.
20. Jolad, S.D., Wiedhopf, R.M., Cole, J.R. (1977). Cytotoxic agents from *Bursera morelensis* (Burseraceae): deoxypodophyllotoxin and a lignan, 5'-desmethoxydeoxypodophyllotoxin. J Pharm Sci. 66: 892–893.
21. Jutiviboonsuk, A., Zhang, H., Tan, T.G., Ma, C., Van Hung, N., Manh Coung, N.M., Bunyapraphatsara, N., Soejarto, D.D., Fong, H.H.S. (2005). Bioactives constituents from roots of *Bursera tonkinensis*. Phytochemistry. 66: 2745-2751.
22. Kim, E.H., Kang, S.Y., Park, B.I., Kim Y.H., Lee, Y.R., Hoe, J.H., Choi, N.Y., Ra J.Y., An S.Y., You Y.O. (2016). Chamaecyparis obtusa suppresses virulence genes in *Streptococcus mutans*. Evid Based Complement Alternat Med. ID 2396404
23. Kordali, S., Kotan, R., Mavi, A., Fakir, A. Ala, A., Yildirim, A. (2005). Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish. *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. J Agr Food Chem. 53: 9452- 9458.
24. Kosalec, I., Pepeljnjak, S., Kustrak, D. (2005). Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., Apiaceae). Acta Pharm. 55: 377–385.
25. Lennette, H.E., Balows, A., Hausler, J.W., Shadomy, H.J. (1987). Manual de Microbiología Clínica. Medica Panamericana. Buenos Aires.
26. Leyva M. A., Martínez J. R., Stashenko E. E. (2007). Composicion química del aceite esencial de hojas y tallos de *Bursera graveolens* (Burseraceae) de Colombia. Scientia et Technica. XII: 201-202.
27. Magiatis P., Skaltsounis A. L., Chinou I., Haroutounian S. A. (2002). Chemical composition and in vitro antimicrobial activity of the essential oils of three greek *Achillea* species. Z Naturforsch. 57: 287-290.
28. Manzano S. P., Miranda, M., Gutiérrez, Y., García, G., Orellana, T., Orellana, A. (2009). Antinflammatory effect and chemical composition of *Bursera graveolens* Triana & Planch. branch oil (palo santo) from Ecuador. Rev Cubana Plant Med. 14: 45-53.
29. Mohadjerani, M., Hosseinzadeh, R., Hosseini, M. (2016). Chemical composition and antibacterial properties of essential oil and fatty acids of different parts of *Ligularia persica* Boiss. Avicenna J Phytomed. 3: 357-65.
30. Parveen, M., Hasan, M. K., Takahashi, J., Murata, Y., Kitagawa, E., Kodama, O., Iwahashi, H. (2004). Response of *Saccharomyces cerevisiae* to a monoterpene: evaluation of antifungal potential by DNA microarray analysis. J Antimicrob Chemot. 54: 46–55.

31. Peters, C.M., Purata, S.E., Chibnik, M., Brosi, B.J., López, A.M., Ambrosio, M. (2003). The life and times of *Bursera glabrifolia* (H.B.K.) Engl. In Mexico: a parable for Ethnobotany. Econ Bot. 57: 431-441.
32. Queiroga, CL., Duarte, M.C., Ribeiro, B.B., de Magalhaes, P.M. (2007). Linalool production from the leaves of *Bursera aloexylon* and its antimicrobial activity. Fitoterapia. 78: 327-328.
33. Ricci, D., Fraternale, D., Giamperi, L., Bucchini, A., Epifanio, F., Burini, G. Y Curini, M. (2005). Chemical composition, antimicrobial and antioxidant activity of the essential oil of *Teucrium Marum* (Laminaceae). Phytochemistry. 98: 195-200.
34. Rzedowski, J. (1978). Vegetación de México. Ed. Limusa. México.
35. Rzedowski, J. (1986). Vegetación de México. Editorial Limusa. México.
36. Rzedowski J., Ortiz E. (1988). Estudios Quimiotaxonómicos de *Bursera* (Burseraceae). II. Una especie nueva de origen híbrido de la Barranca de Tolango, estado de Hidalgo, Acta Bot Mex. 001: 11-19.
37. Rzedowski, J., Guevara-Féfer, F. (1992). Familia Burseraceae. Fasc. 3, Flora del Bajío y de Regiones Adyacentes. Instituto de Ecología A.C., Centro Regional del Bajío, Pátzcuaro, México.
38. Santana-Rios, G., Orner, G. A., Amantana, A., Provost, C., Wu, S.Y., Dashwood, R. H. (2001). Potent antimutagenic activity of white tea in comparison with green tea in the *Salmonella assay*. Mutat Res-Gen Tox En. 495: 61–74.
39. Sosa, S., Balick, M.J., Arvigo, R., Esposito, R.G., Pizza, C., Altinier, G., Tubaro, A. (2002). Screening of the topical anti-inflammatory activity of some Central American plants. J Ethnopharmacol 81: 211-215.
40. Syamasundar, K.V., Mallavarapu, G.R. (1995). Two triterpenoid lactones from the
41. resin of *Bursera delpechiana*. Phytochemistry. 40: 337–339.
42. Tangarife C.V., Correa R.J., Zapata L.B., Durán C., Stanshenko E. y Mesa A.C. (2011). Anti-*Candida albicans* activity, cytotocity an interaction with antifungal drugs of essential oils and extracts from aromatic and medicinal plants. Infectio. 15: 160-167.
43. Turina, A. V., Nolan, M. V., Zygadlo, J. A., Perillo, M. A. (2006). Natural terpenes: self-assembly and membrane partitioning. Biophys Chem. 122: 101–113.
44. Ultee, A., Bennik, M. H., Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl Environ Microb. 68: 1561–1568.
45. Ultee, A., Kets, E. P., Alberda, M., Hoekstra, F. A., Smid, E. J. (2000). Adaptation of the food-borne pathogen *Bacillus cereus* to carvacrol. Arch Microbiol. 174: 233–238.
46. Vanden Berghe D, Vlietinck AJ. 1991. Screening methods for antibacterial and antiviral agents from higher plants. In: Dey P, Harborne JB, Hostettman K, eds. *Methods in Plant Biochemistry Assays for Bioactivity*. 6. San Diego, AcademicPress, pp. 47–69.
47. Yasunaka, K., Abe, F., Nagayama, A., Okabe, H., Lozada-Pérez, L., López-Villafranco, E., Estrada, M.E., Aguilar, A., Reyes-Chilpa, R. (2005). Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthones. J Ethnopharmacol. 97: 293-299.
48. Ye, X.Y., Wang, H.X., Ng, T.B. (1999). First chromatographic isolation of an antifungal thaumantin-like protein from French bean legumes and demonstration of its antifungal activity. Biochem Bioph Res Co. 263: 130.134
49. Zúñiga, B., Guevara-Fefer, P., Herrera, J., Contreras, J.L., Velasco, L., Pérez, F.J., Esquivel, B. (2005). Chemical composition and anti-inflammatory activity of the volatile fractions from the Bark of eight Mexican *Bursera* species. Panta Med. 71: 825-828.