ANTIMICROBIAL ACTIVITY AND CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF *Malvaviscus arboreus* Cav, *Pimenta dioica* (L.) Merr., *Byrsonima crassifolia* (L.) Kunth AND *Psidium guajava* L.

[ACTIVIDAD ANTIMICROBIANA Y COMPOSICIÓN QUÍMICA DE LOS ACEITES ESENCIALES DE *Malvaviscus arboreus* Cav, *Pimenta dioica* (L.) Merr., *Byrsonima crassifolia* (L.) Kunth Y *Psidium guajava* L.]

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SUMMARY

The essential oils of leaves of *Malvaviscus arboreus* Cav., *Pimenta dioica* (L.) Merr., *Byrsonima crassifolia* (L.) Kunth and *Psidium guajava* L., obtained by hydrodistillation were evaluated as potential antimicrobial agents against *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 4028) and *Bacillus cereus* (ATCC 11778). Disk diffusion, microdilution and bioautography methods were used for antimicrobial activity evaluation; subsequently, the possitive extracts were analyzed by gas chromatography coupled to a mass spectrometry detector (GC-MS) to obtain the chemical profile. The essential oils of the four species showed antimicrobial activity by the agar diffusion method against the three strains of microorganisms. As for the bioautography, it was found that the component at a frontal reference (Rf) 0.42 from fraction four of the *P. dioica* essential oil, inhibited the growth of *B. cereus*, *S. typhimurium* and *S. aureus*. In the microdilution test, *B. cereus* was the most susceptible microorganism to all essential oils tested. Eugenol was the major component of *P. dioica* and *P. guajava* with abundance percentages of 94.86% and 33.84%, respectively.

Keywords: Essential oils; antimicrobial activity; *Malvaviscus arboreus* Cav.; *Pimenta dioica* (L.) Merr.; *Byrsonima crassifolia* (L.) Kunth; *Psidium guajava.*

RESUMEN

Los aceites esenciales de las hojas de *Malvaviscus arboreus* Cav., *Pimenta dioica* (L.) Merr., *Byrsonima crassifolia* (L.) Kunth y *Psidium guajava* L., obtenidos por hidrodeltilación, fueron evaluados como antimicrobianos potenciales contra *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 4028) y *Bacillus cereus* (ATCC 11778). Para la evaluación de la actividad antimicrobiana se emplearon los métodos de difusión en disco, bioautografía y microdilución, subsecuentemente cada extracto fue analizado por cromatografía de gases/masas (CG-MS) para la obtención del perfil químico. Los aceites esenciales de las cuatro especies presentaron actividad antimicrobiana por el método de difusión en agar contra las tres cepas de microorganismos. En cuanto a la bioautografía, se detectó que la fracción 4 en el aceite esencial de *P. dioica*, con una referencia frontal (R_f) de 0.42 cm, inhibió el crecimiento de *B. cereus*, *S. typhimurium* y *S. aureus*. En la microdilución se determinó que *B. cereus* fue el microorganismo más susceptible a la presencia de los aceites esenciales de *M. arboreus*, *P. dioica*, *B. crassifolia* y *P. guajava*. El eugenol fue el principal componente de *P. dioica* y *P. guajava* con porcentajes de abundancia de 94.86% y 33.84% respectivamente, determinado por CG-MS.

Palabras claves: Aceites esenciales; actividad antimicrobiana; *Malvaviscus arboreus* Cav.; *Pimenta dioica* (L.) Merr.; *Byrsonima crassifolia* (L.) Kunth; *Psidium guajava.*

INTRODUCTION

Essential oils from aromatic and medicinal plants have been known to possess a broad range of biological activities, being antimicrobial one of its main medicinal properties reported (Viuda-Martos *et al*., 2008; O'Bryan *et al*., 2008). These activities are strongly associated by their chemical composition which is determined by the environmental and agronomic conditions as well as the genotype of each plant (Marotti *et al.*, 1993; Cosentino *et al.*, 1999). The interest in the application of essential oils to pathogenic microorganisms control has been increased in recent years (Rojas-Grau *et al*., 2006; Oussalah *et al*., 2004; Gutierrez *et al*., 2008). Essential oils are part of plant metabolites and they are usually composed of volatile terpenes. These compounds can be located in different parts of the plant such as flowers, seeds, leaves and fruits. They constitute 0.1% to 1% of plant dry weight, and they are usually less dense than water, with a high refractive index (Lopez, 2004). The concentration and composition of the essential oil metabolites varies due to environmental factors (Toncer *et al*., 2010; Gazim *et al*., 2010), soil nutrients (Powell and Raffa, 1999), the part of the plant used (Pala-Paul *et al*., 2005), the drying conditions (Mejia *et al*., 2007), the method of extraction (Quintero *et al*., 2004), among other factors.

Malvaviscus arboreus Cav, *Pimenta dioica* (L.) Merr., *Byrsonima crassifolia* (L.) Kunth and *Psidium guajava* L are four plants with a traditional use as condiment and medicinal purposes. *M. arboreus* (tulipancillo, in Spanish) has been used to treat whooping cough, as an urinary antiseptic and as a diuretic; *P. guajava* is used to treat gastrointestinal diseases, chills and stomach pain (UNAM, 2009). On the other hand, *P. dioica* is used traditionally as anti-hemetic (Germosén-Robineau, 2005) whereas *B. crassifolia* is used against skin infections and gastrointestinal disorders, it is also used as digestive, emenagoge, febrifuge and the bark infusion is used as an antidiarrheal, to treat inflammation, to treat scabies and as wound healing (Centurion *et al*., 2004; Gonzalez, 2007). Although there are reports of traditional medicinal use of these species, there are no reports on the study of their antimicrobial activity. Therefore, the objective of this study was to evaluate the antimicrobial activity of essential oils from the leaves of *M. arboreus*, *P. dioica*, *B. crassifolia* and *P. guajava* against *Staphylococcus aureus*, *Salmonella typhimurium* and *Bacillus cereus*, as well as the chemical profile obtained by GC-MS.

MATERIALS AND METHODS

Plant material

Plant species were obtained from a local market called "Jose Maria Pino Suarez" in Villahermosa, Tabasco, Mexico on September of 2010. The leaves were dried at room temperature and protected from light. Then, samples were ground in a hammer mill and sieved through a mesh number 60 (particle size of 0.250 mm). The sieved product was packaged in polyethylene bags stored in PET containers in a dry place for further analysis.

Essential oil extraction

A sample of 500 g of plant material was extracted by hydrodistillation in a Clevenger type equipment for 7 hours at 100 ^{o}C and the essential oil was obtained by condensation from the oil/water mixture. Hexane was added to separate the essential oil from water, and the oil was placed in amber vials and stored at 4 ^{o}C.

Gas chromatography-mass spectrometric (GC-MS) analysis

Essential oil components obtained by steam distillation were determined on an Agilent Technologies 6890N Gas Chromatography apparatus coupled with a mass selective detector 5975B, using the following chromatographic conditions: split injection of 1 µL of 10 µg of sample in hexane; HP5 MS phenyl column (30 m x 0.25 µm), flow rate 1 mL/min (helium as carrier gas); samples were analyzed with the column held initially at 60 ^{o}C for a minute after injection, then increased to 260 ^{o}C with a gradient of 10 ^{o}C/min heating program. Identification of components in the extract was performed by computer searches in commercial references libraries. The fragmentation patterns of the mass spectra were compared with those from the NIST05 libraries (Pala-Paul *et al*., 2005, Pala-Paul *et al*. 2007, Raseetha *et al*., 2009).

Bacterial strains

Cultures of *Staphylococcus aureus* (collection number 25923), *Salmonella typhimorium* (4028) and *Bacillus cereus* (11778) were obtained commercially on the American Type Culture Collection (ATCC) and were maintained on tryptose soy agar (TSA) at 4 ^{o}C.

Agar diffusion method

Agar diffusion method was performed according to the procedure described by Moulari *et al.* (2006). An inoculum of each bacterial strain (1 x 10^6 bacteria

mL^{-1}) was applied on a homogeneous dispersion in Mueller Hinton medium plates. The essential oil samples were applied on Whatman filter paper disks at a concentration of 10 mg mL^{-1}. Each disk, containing the sample to be tested, was brought into contact with an inoculated medium and, after incubation for 24 h at 37 ^{o}C, the diameter of the clear zone around the disk (inhibition diameter) was measured.

Bioautography on TLC plates

Direct bioautography with *S. aureus*, *S. typhimorium* and *B. cereus* was performed on silica gel GF254 TLC Aluminum plates. Briefly, 10 µl of each extract solution (20 µg mL^{-1}) were applied on silica gel plates separately. TLC plates were development using *n*-hexane-acetone (80:20 v/v) as solvent system. The plates were then dried under an extraction hood. After drying for complete removal of solvent, the TLC plates were placed in 9 cm x 9 cm sterile square Petri dishes, to which an inoculum of the bacteria (1 x 10^6 bacteria mL^{-1}) in TSA were added. The medium with the inoculum was distributed over the developed TLC plates uniformly. After media solidification, the plates were incubated at 37^{o}C for 24 h. Finally, the plates with a solution of 3,5-triphenyl-2H-tetrazolium chloride (MTT). The inhibition zones were revealed and visualized by spraying the plates with MTT solution followed by their incubation at 37 °C for 1 hour. Active compounds in the extract were detected as clear spot against a dark red background due to the transformation of MTT in its reduction form, formazan (Moulari *et al*., 2006).

Statistical design

For antimicrobial activity by agar diffusion method, we conducted a completely randomized design with a 4x3 factorial distribution: four plant species (*M. arboreus*, *P. dioica*, *B. crassifolia* and *P. guajava*) and three microorganisms (*Staphylococcus aureus*, *Salmonella typhimurium* and *Bacillus cereus*) with a total of 12 treatments and three replicates of each one were performed.

Determination of minimum inhibitory concentration

The extracts showing an inhibition zone in the bioautography assay were chosen to test the minimum inhibitory concentration (MIC) with the agar dilution method reported by Andrews *et al*. (2001). Additionally, the minimum bactericidal inhibition was measured. The test microorganisms (*S. aureus, S. typhimurium* y *B. cereus*) were grown for 24 h before the bioassay in MH broth at 37^{o}C. Then, the optical density of the culture was adjusted (1 x 10^8 cell ml^{-1}) using the 0.5 Mac Farland turbidity standard. Microdilution tests were performed in a sterile 96-well micro-plate. All extracts were initially tested at 20 mg ml^{-1} and were serially diluted two-fold to 0.0156 mg ml^{-1}, after which 100 µg of bacterial culture (final concentration 1 x 10^6 cell ml^{-1}) were added to each well. The antibiotic amikacin was included as a positive control in each assay. Two additional wells were used as culture medium control without inoculum and as a growth control with the inoculum. The microplates were incubated overnight at 37^{o}C. To indicate bacterial growth, 100 µl of a solution of 1% MTT was added to each well and the plates were incubated at 37^{o}C for one hour. Bacterial growth in the well was indicated by a red color, whereas clear wells indicated growth inhibition by the essential oil. The minimum bactericide concentration (MBC) was determined by making a replication with a 96 teeth brush on a MH medium plate, incubating it at 37 °C for 24 h and observing the presence or absence of growth (Taylor *et al*., 1983).

RESULTS AND DISCUSSION

The essential oils of *P. dioica*, *B. crassifolia*, *P. guajava* and *M. arboreus* showed antimicrobial activity in the agar diffusion method (Table 1) for both *S. aureus* (8.00 mm to 17.06 mm) and *B. cereus* (13.46 mm to 18.16 mm). Also, *S. typhimurium* showed sensitivity to *M. arboreus* and *P. dioica* essential oils similar to the amikacina control. These results coincide with the ones found by Alzamora *et al*. (2001) with the *Eucalyptus globulus* (Myrtaceae family) essential oil obtained by steam distillation with inhibition zones of antibacterial activity of 13, 12 and 12 mm on *S. aureus, S. typhi* and *S. typhimurium*, respectively.

On the other hand, Sahadeo y Vilas (2011) reported that the *P. dioica* essential oil presented an inhibition zone of 19 y 35 mm for *B. cereus* and *S. aureus*, respectively. The inhibition zones obtained in the present study: 13 and 17 mm for Gram positive (*B. cereus* and *S. aureus*) and 19 mm for Gram negative (*S. typhimurium*) were bigger (Table 1) than the ones found by Girova *et al*. (2010) for *P. dioica* essential oil that inhibited Gram positive bacteria growth with inhibition zones of 12-13.7 mm and 10-11 mm for Gram negative bacteria. It has been reported antibacterial activity of extracts of Malpighiaceae family species on *S. aureus, E. faecalis* (Suffrendin *et al.,* 2004) and *Byrsonima crassifolia* root extracts presented antimicrobial activity on *S. aureus, E. coli* and *S. typhi* (Martinez-Vasquez *et al.,* 1999).

Table 1. Antimicrobial activity of essential oils using the agar diffusion method.

Essential oil (50 µg mL^{-1})	Inhibition zone (mm)		
	S. aureus	*S. typhimurium*	*B. cereus*
P. dioica	17.06^{c} ±2.90	19.46^{c} ±5.10	13.46^{c} ±2.20
B. crassifolia	15.30^{c} ±5.20	0.00	18.16^{c} ±3.20
P. guajava	8.00^{a} ±1.70	0.00	13.80^{b} ±1.20
M. arboreus	10.00^{a} ±2.00	21.80^{c} ±0.80	14.20^{b} ±1.50
(Amikacine 1 µg mL^{-1})	23.83^{d} ±2.70	22.83^{d} ±2.70	28.96^{d} ±2.20

Data are expressed as mean ± standard deviation (n=3). Different letters indicate statistically significant differences (Tukey, α=0.05).

The bioauthography results confirm that the fraction 4 of the leaf essential oil *of P. dioica* have metabolites with antimicrobial activity, with a R_f of 0.42 cm, against the three studied microorganisms (*B. cereus*, *S. typhimurium*, *S. aureus*) meanwhile the fraction 3 presented such activity on *S. aureus* with a R_f of 0.34 (Table 2). *M. arboreus* essential oil presented one inhibition zone on *S. aureus* growth without definition of specific fractions (Figure 1). Moreover, the essential oil of *P. guajava* presented three compounds (fractions 2, 3 and 4), with inhibition R_f of 0.5, 0.56 y 0.68 cm, respectively. On the other hand, essentials oils from *B. crassifolia* and *M. arboreus* leaves showed activity against *S. aureus* without defined fractions. Borges-Argaez *et al*. (2010) reported that the fraction 4 of *P. dioica* leaf essential oil obtained by steam distillation, Rf of 0.5 cm, inhibited *S. aureus* growth, data similar to the one obtained in the present study. Reports were not found in the literature related to antimicrobial activity of the other species.

Table 2. Essential oil active fractions and their frontal references (R_f in cm).

Leaf essential oils	*S. aureus*	*S. typhimurium*	*B. cereus*
P. dioica	Fraction 3 and 4 R_f : 0.34 and 0.42	Fraction 4 R_f : 0.42	Fraction 4 R_f : 0.42
M. arboreus	A	NA	NA
B. crassifolia	A	NA	NA
P. guajava	Fraction 2, 3 and 4 R_f :0.5, 0.56 and 0.68	NA	NA

A= Active, NA= Not active

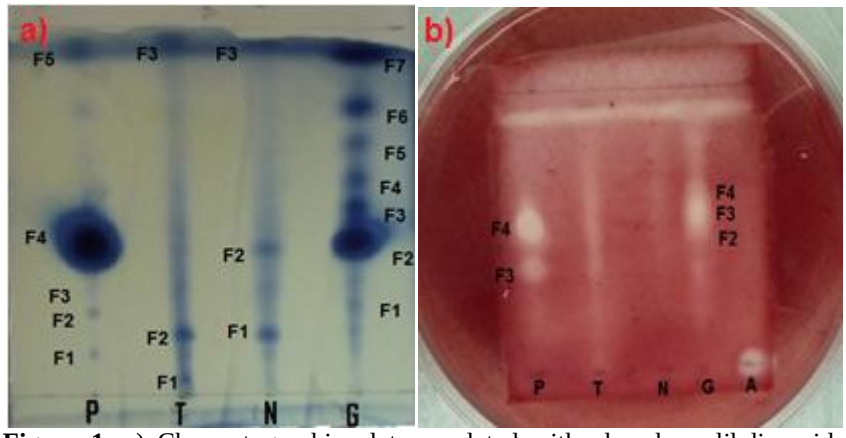

Figure 1. a) Chromatographic plate revelated with phosphomolibdic acid; b) Chromatographic plate of bioautography bioassay. (P: *Pimenta dioica*, T: *Malvaviscus arboreus*, N: *Byrsonima crassifolia*, G: *Psidium guajava*, A: Amikacina).

The most sensitive microorganism to the presence of *P. dioica*, *P. guajava*, *B. crassifolia* and *M. arboreus* essentials oils was *B. cereus* (Table 3). *P. guajava* essential oil presented the lowest MIC (5 mg mL^{-1}) followed of *P. dioica* (10 mg mL^{-1}), *B. crassifolia* and *M. arboreus* (both at 20 mg mL^{-1}). In the other hand *P. dioica* (10 mg mL^{-1}), *B. crassifolia* (20 mg mL^{-1}) and *M. arboreus* (20 mg mL^{-1}) inhibited *S. aureus* growth, meanwhile *S. typhimurium* was the least sensitive microorganism, although *P. dioica* and *P. guajava* essential oils inhibited its growth at concentrations of 20 and 10 mg mL^{-1}, respectively.

Table 3. Minimal Inhibitory Concentration (mg mL^{-1}) of leaf essentials oils.

Essential oil	MIC (mg mL^{-1})		
	S. typhimurium	*S. aureus*	*B. cereus*
P. dioica	20	10	10
P. guajava	10	NA	5
B. crassifolia	NA	20	20
M. arboreus	NA	20	20

MIC= Minimal Inhibitory Concentration, NA= No activity.

It has been reported that the leaf *P. dioica* essential oil obtained by steam distillation presented a MIC of 6.25 mg mL^{-1} on *S. aureus* with the plate microdilution method (Borges-Argaez *et al*., 2010). In the present study, the MIC value was 10 mg mL^{-1} obtained on the same microorganism, probably due to that the essential oil was obtained by hydrodistillation and it had different concentration of some components with antimicrobial activity. Although MIC of *B. crassifolia* leaf essential oil reports have not been found, the result obtained in this paper (20 mg mL^{-1}) was expected because diverse species and plant structures of *Byrsonima* have antimicrobial activity, such as Rivero-Cruz *et al*. (2009) found that the dichloromethanic extract of *B. crassifolia* bark had activity with a MIC of 63 mg mL^{-1} on *S. aureus* using the microdilution method. Even though reports have not been found on *P. guajava* leaf essential oil, some reports exist that demonstrate antimicrobial activity of extracts obtained with other solvents and using the same method. Sanches *et al*. (2005) reported that the *P. guajava* extracts obtained by ethanol:water maceration of leaf, root and bark presented a MIC of 0.5, 0.25 y 0.12 mg mL^{-1}, respectively, on *S. aureus*. Barbieri *et al*. (2002) reported a MIC of 0.25 mg mL^{-1} on *S. aureus* with ethanol-water (90:10) extract of *P. guajava* leaves. Finally, data on *M. arboreus* essential oil were not found in the consulted literature.

Leaf *P. dioica* essential oil showed bactericidal activity on *S. aureus* and *B. cereus* at 20 and 2.5 mg mL^{-1}, respectively, meanwhile *S. typhimurium* required an essential oil concentration of 20 mg mL^{-1} presenting bacteriostatic activity (Table 4). On the other hand, *P. guajava* needed essential oil concentrations of 5 and 10 mg mL^{-1} in order to present bactericidal activity on *B. cereus* and *S. aureus* whereas the same essential oil showed bacteriostatic activity on *S. typhimurium* at 20 mg mL^{-1}, the maximum concentration assayed in this study. *B. crassifolia* essential oil was the least effective on *S. aureus*, *S. typhimurium* and *B. cereus* elimination because it had bacteriostatic activity at 20 mg mL^{-1} and *M. arboreus* required a lower concentration (5 mg mL^{-1}) for *B. cereus* elimination, although *S. typhimurium* elimination required the maximum concentration assayed (20 mg mL^{-1}) and *S. aureus* showed a bacteriostatic activity at the same concentration.

Table 4. Minimal Bactericidal Concentration (mg mL^{-1}) of essential oils.

Leaf essential oil	MBC (mg mL^{-1})		
	S. aureus	*S. typhimurium*	*B. cereus*
P. dioica	20 (Bactericidal)	20 (Bacteriostatic)	2.5 (Bactericidal)
P. guajava	10 (Bactericidal)	20 (Bacteriostatic)	5 (Bactericidal)
B. crassifolia	20 (Bacteriostatic)	20 (Bacteriostatic)	20 (Bacteriostatic)
M. arboreus	20 (Bacteriostatic)	20 (Bactericidal)	5 (Bactericidal)

The CG-MS analysis of essential oils from the leaves of the studied plant species is showed in Table 5. *P. dioica* essential oil had nine compounds with retention times from 6.61 to 30.20 min; some of these substances are eugenol with 94.86%, alpha-terpineol with 2.45% and cinnamic acid methyl ester with 1.06% as main compounds. *P. guajava* essential oil presented 13 substances such as eugenol (33.84%), caryophylene (7.08%), caryophylene oxide (7.02%), beta-bisabolene (4.62%), longiborneol (4.33%) and nerolidol (3.48%).

Rao *et al*. (2010) found eugenol as the mayor component (95 %) in leaf *P. dioica* essential oil, being this value similar to the one found in this study. On the other hand, Jirovetz *et al.* (2007) reported that this species, found in Jamaica, had eugenol (76.02%), alpha–selinene (1.04%), 4-terpineol (0.19%), alpha-terpineol (0.09%) and beta–selinene (0.52%). Moreover, Nascimiento *et al.* (2000) determined the antimicrobial activity, with the disc diffusion method, of secundary metabolites such as eugenol (positive on *S. aureus, S. choleraesuis, Bacillus subtilis* and *Candida albicans*), cinnamic acid (positive on *S. aureus, Enterobacter aerogenes* and *E. coli*). Joseph y Priya (2011) reported that eugenol is one of the main substances in *P. guajava* leaf, besides of alpha-pinene, limonene, menthol and volatile compouds such as β-cariofilene, β-bisabolene, cariofilene, β-copaenoe and eucalipte in lower percentages. Most of these components also formed part of the chemical composition of *P. guajava* essential oil used in this study. On the other hand, de Lima *et al.* (2010) reported selin-11-en-4-α-ol (22.19%), 1,8-cineol (12.83%), *trans*-cariophylene (8.89%), cariophylene oxide (9.09%), guaiene (6.74%), α-selinene (5.79%) and cadinol (8.18%) as main components found in the essential oil of the Brazilian species. For these reason, it is confirmed that ethnogeographic differences establish a variation between the composition and chemical substances abundance (Toncer *et al*., 2010; Gazim *et al*., 2010; Powell y Raffa, 1999; Pala-Paul *et al.*, 2005; Amna *et al*., 2010; Mejia *et al*., 2007; Quintero *et al*., 2004).

It has been reported that gram positive bacteria, *L. monocytogene*s and *S. pyogenes*, were less sensitive to inhibition of eugenol, α-terpineol and γ-terpinene at concentration between 0.25 to 0.50% than the two gram negative bacteria, *P. vulgaris* and *E. coli* at 0.50 to 0.75% (Oyedemi *et al.*, 2009; Tippayatum and Chonhenchob, 2007; Dorman and Deans, 2000). Finally, in the *M. arboreus* and *B. crassifolia* essential oils were identified only two and three compounds, respectively, in minimal concentrations (>0.001%) that indicate that both oils are poor in aromatic compounds. It is important to mention that reports were not found on chemical composition of these species in the consulted literature.

Table 5. Abundance (%) of mayor substances of essential oils of studied plant species.

Substance	*P. dioca*	*P. guajava*	*M. arboreus*	*B. crassifolia*	Retention time (min)
4-terpineol	>0.0001	-	-	-	11.62
Eucaliptol	>0.0001	-	-	-	6.6
Caryophylene	>0.0001	7.08	-	-	21.24
Cariyophylene oxide	0.72	7.02	-	-	27.51
α - caryophylene	-	1.25	-	-	24.20
Cinnamic acid methyl ester	1.06		-	-	20.10
Eugenol	94.86	33.84	>0.001	-	19.28
Selinene	-	4	-	-	
α - Selinene	>0.0001		-	-	
α-Terpineol	2.45	0.83	-	-	12.18
β - Selinene	>0.0001		-	-	23.84
Acoradiene	-	0.55	-	-	22.97
Cis α-bisabolene	-	2.02	-	-	24.65
β-bisabolene	-	4.62	-	-	24.89
Copaene	-	0.53	-	-	19.50
Longiborneol	-	4.33	-	-	30.25
Nerolidol	-	3.48	-	-	27.06
α-bisalobol	-	0.47	-	-	31.41
1-4- dichlorobencene	-	-	>0.001	>0.001	6.10
Curcumene	-	-	-	>0.001	30.16
Bencene 1-(1,5-dimethyl-4-hexenyl)-4-methyl	-	-	-	>0.001	23.82

(-) = Not present

CONCLUSIONS

Leaf essential oils of *P. dioca*, *P. guajava*, *M. arboreus* and *B. crassifolia* presented antimicrobial activity with the agar diffusion method against *S. aureus* and *B. cereus.* Essential oils of *P. dioica* and *P. guajava* were the most effective in *B. cereus* elimination, at a concentration of 2.5 mg mL^{-1}. Essential oil of *P. guajava* presented three active fractions for *S. aureus* growth inhibition. Fractióon 4 of the *P. dioica* essential oil inhited *B. cereus*, *S. typhimurium*, *S. aureu*s growth meanwhile fraction 3 was active againt *S. aureus*.

The main compound of *P. dioica* and *P. guajava* essential oils was eugenol, 94.86% and 33.84%, respectively. The present study allows knowing the potential antimicrobial activity of plant species that could have future applications as preservatives for perishable food such as meat or milk products.

Acknowledgement

To Fondo Mixto CONACYT - Gobierno del Estado de Tabasco for the research funding to the project titled "Caracterización de propiedades de antimicrobianos de extractos de plantas alimentarias tropicales" (FOMIX-2009-C17-120880).

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Submitted April 29, 2011– Accepted November 10, 2011
Revised received April 09, 2013