# CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF OREGANO (*Lippia palmeri* S. WATS) ESSENTIAL OIL

# COMPOSICIÓN QUÍMICA Y ACTIVIDAD ANTIMICROBIANA DEL ACEITE ESENCIAL DE ORÉGANO (*Lippia palmeri* S. WATS)

**Ma. Magdalena Ortega-Nieblas[1], Ma. Refugio Robles-Burgueño[2], Evelia Acedo-Félix[2], Alberto González-León[2], Adriana Morales-Trejo[3] and Luz Vázquez-Moreno[2]***

[1]Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora. Rosales y Blvd. Luis Encinas s/n, Edificio 7G. 1819, Hermosillo, Sonora, México. [2]Centro de Investigación en Alimentación y Desarrollo A. C. Km 0.6 Carretera a La Victoria 83304, Apdo. postal 1735. Hermosillo, Sonora, México. Tel. 01 (662) 289-2400 Ext. 219 Fax: 01(662) 2800058. [3]Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California. Carretera a Delta s/n 21705, Ejido Nuevo León, Baja California, México.

* *Autor para correspondencia* (lvazquez@ciad.mx)

## SUMMARY

**The chemical composition and antimicrobial activity of *Lippia palmeri* S. Wats essential oil extracted from plants collected of two localities (Álamos and Puerto del Orégano) in the State of Sonora, México, was examined. Essential oils (EO) were obtained from oregano leaves by steam distillation, analyzed by gas chromatography coupled with a mass spectrometer, and their antimicrobial activity against human pathogens investigated by disc diffusion. Álamos and Puerto del Orégano essential oils (AEO and POEO) presented 50 and 60 constituents, respectively. The components were classified as monoterpenes, sesquiterpenes and phenolics. AEO most abundant components (>2%) included p-cymene, thymol, isoaromandrene, carvacrol, γ-terpinene, p-thymol, longipinene-epoxide and eudesmol; while for POEO were carvacrol, thymol, p-cymene, caryophyllene, thymol acetate, α-bisabolene, γ-terpinene, myrcene and α-caryophyllene. These results implicate that chemotypes involved were a p-cymene/thymol in AEO and carvacrol in POEO. In general, EO antimicrobial activity against four Gram-positive and six Gram-negative bacteria varied according to the plant origin. However, both POEO and AEO showed the strongest activity against *Escherichia coli* O157:H7 and *Staphylococcus aureus*. This is the first report of *L. palmeri* essential oil characterization, and our results support the notion that these oils could be useful in food flavoring and preservation.**

**Index words:** *Lippia palmeri*, antimicrobial activity, essential oil composition, oregano Verbenaceae.

## RESUMEN

**Se estudió la composición química y la actividad antimicrobiana del aceite esencial de *Lippia palmeri* S. Wats, colectada en dos localidades del Estado de Sonora, México. Los aceites esenciales (AE) fueron obtenidos de las hojas del orégano por hidrodestilación, analizados por cromatografía de gases acoplada con espectrómetría de masas, y su actividad antimicrobiana fue investigada por difusión en disco. Los aceites esenciales de Álamos (AEA) y del Puerto del Orégano (AEPO) presentaron 50 y 60 constitu-yentes, respectivamente. Los componentes fueron clasificados como monoterpenos, sesquiterpenos y fenólicos. Los componentes más abundantes en AEA (>2%) incluyeron p-cimeno, timol, isoaromandreno, carvacrol, γ-terpineno, p-timol, longipineno-epóxido y eudesmol; mientras que para AEPO fueron carvacrol, timol, p-cimeno, cariofileno, acetato de timol, α-bisaboleno, γ-terpineno, mirceno y α-cariofileno. Estos resultados implican que los quimiotipos involucrados fueron: p-cimeno/timol en AEA, y carvacrol en AEPO. En general, la actividad antimicrobiana de los AE contra cuatro bacterias Gram-positivas y seis bacterias Gram-negativas varió con respecto al origen de la planta. Sin embargo, ambos AE mostraron mayor actividad contra *Escherichia coli* O157:H7 y *Staphylococcus aureus*. Este es el primer reporte de la caracterización aceite del aceite esencial de *L. palmeri*, y estos resultados apoyan el concepto de que pueden ser utilizados para saborizar y preservar alimentos.**

**Palabras claves**: *Lippia palmeri*, actividad antimicrobiana, composición del aceite esencial, orégano Verbenaceae.

## INTRODUCTION

*Lippia palmeri* S. Wats (oregano) belongs to the Verbenaceae family. The oregano plant is a bushy, highly branched shrub from the semiarid zones of Sonora, Baja California and Sinaloa, México (Shreve and Wiggin, 1964). Essential oils (EO) of some *Lippias* species have characteristics comparable to European oregano, *Origanum vulgare* (González-Güereca *et al*., 2007), is used as flavoring power for food products and alcoholic beverages (Aligiannis *et al.,* 2001; Silva-Vázquez and Dunford, 2005), and has also medicinal properties (Pascual *et al.,* 2001). *L. graveolens* EO contains 45 chemical compounds and the main components are carvacrol, thymol, eugenol, cimene, pinene and linalool, among others (Vernin *et al.,* 2001). Some of these components possess antibacterial, antiviral, antifungal and

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insecticidal properties (Carson and Riley, 1995; Botelho *et al.,* 2007; Oyedemi *et al.,* 2009). *L. alba* Mill and other *Lippias* have medicinal properties attributed to the high content of terpenes, that include limonene, myrcene, durene and p-cymene (Pascual *et al.,* 2001).

Essential oil from many aromatic spices (oregano, thyme, salvia, parsley, clove, coriander, garlic and onion) show antimicrobial activity, that varies depending on species, sub-species and plant varieties (Friedman *et al.,* 2002; Burt, 2004; Benkeblia, 2004; Celikel and Kavas, 2008; Martino *et al.,* 2009).

Variability in amount and composition of essential oil in some plants, particularly in *Origanium vulgare* and *Lippias* depend on their developmental stage, climate factors, altitude, harvest season and plant handling (Kokkini *et al.,* 1997; Dunford and Silva, 2005). Several reports show that the highest concentration occurs when leaves are harvested during the plant flowering stage. In México, most handling of the wild population of oregano is according to the Mexican Official Norm (Carabias, 1997).

The chemical composition and some biological activities of essential oils of several *Lippias* and *Origanium* species have been reported; however, this is not known for the Mexican *Lippia palmeri*. The aim of this work was to characterize and to determine the antimicrobial activity of the essential oil of *Lippia palmeri* S. Wats harvested from two different (a hot arid and temperate climate) locations in the State of Sonora, México.

## MATERIALS AND METHODS

### Plant material

Oregano leaves from *Lippia palmeri* S. Wats were obtained from Puerto del Orégano, a hot arid site, located at 52 km West of Hermosillo, Sonora, with geographical coordinates of 29° 02' 52.5" LN and 110° 40' 15.8" LW, at an altitude of 280 m, with a maximum and minimum temperatures of 48 and 14 °C, respectively, and an average annual precipitation of 180 mm. The second location, Álamos, Sonora is a temperate site, with a mean precipitation of 347 mm, altitude of 1700 m, 27° 01' 01' 18'' LN and of 108° 56' 34'' LW, with maximum and minimum temperatures of 30 and 16 °C, respectively (INEGI, 2009). Plant collection was in October 2008, right before flowering. Samples were registered under voucher specimen No. 01655 at the Herbarium of the University of Sonora, where taxonomy was confirmed.

### Extraction and isolation

Oregano leaves were dried at room temperature and shade, separated from stems and stored in paper bags. The moisture content was determined by weight difference and ash content as described by AOAC (2002).

Each batch of essential oils from Álamos and Puerto de Orégano (AEO and POEO, respectively), was obtained from 100 g of leaves by water steam distillation using a Clevenger type apparatus (Winzer®), during 4 h with 1.5 L of distilled water. Essentials oils (EO) were separated from the aqueous phase using a separation funnel, dried over anhydrous sodium sulfate and stored at 4 °C until gas chromatography–mass spectrometry (GC-MS) analyses. EO content was expressed in mL 100 $g^{-1}$ of dried leaves. Density or specific gravity was determined by the method described in AOAC (2002) using a pycnometer. Refraction index was obtained using a Bausch & Lomb Abbe-31® refractometer, oil color determined using a Minolta colorimeter Chromameter CR200®, and free fatty acid content was determined by alkali titration according to method described in AOCS (2009). All determinations were done by triplicates.

### GC-MS analysis of *Lippia palmeri* essential oil

AEO and POEO composition was analyzed by GC-MS using a gas chromatograph Varian 3900® coupled to a Varian Saturn 2100T® ion trap mass spectrometer, DB-5MS column (30 m x 0.25 mm, film thickness 0.25 mm). Operating conditions were as follows: Carrier gas used was ultra pure helium degree 5.0 at a flow rate of 1 mL $min^{-1}$; column temperature was held constant at 50 °C for 10 min, then heated to 290 °C at 5 °C $min^{-1}$ and held constant at 290 °C for 10 min; injector temperature was 300 °C; volume injected, 1 µL of EO in methanol (1:10); split ratio, 1:100. The MS operating parameters were as follows: ionization potential, 70 eV; electronic ionization source temperature, 300 °C; total ion monitoring was done in a scan mass range from 40-400 m/z (D'antuono *et al.,* 2000). The identification of GC peaks corresponding to the components of the EO was based on direct comparison of the retention times and mass spectra data with those standards compounds when available, computing matching with the NIST 2000 library, and by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Pino *et al.*, 1997; Derwich *et al.*, 2010). Relative percentage amounts of separated compound were calculated from peak areas of the total ion chromatograms.

### Antimicrobial activity

Microorganisms tested were obtained from the collection of the accredited microbiology laboratory at Centro de Investigación en Alimentación y Desarrollo, A. C. (Hermosillo, Sonora. México). Antibacterial activity of EOs against Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Listeria ivanovii* and *Listeria monocytogene*s) and Gram-negative bacteria (*Salmonella* Senftemberg, *Salmonella* Choleraesuis, *Salmonella* Typhimurium, *Escherichia coli* K88, *Escherichia coli* O157:H7 and *Escherichia coli* ATCC25922) was determined using a modified technique (paper disc diffusion method) described by Prabuseenivasan *et al.,* (2006). Briefly, bacteria grown in Mueller-Hinton broth at 37 °C for 18 h was adjusted to $10^5$ UFC $mL^{-1}$ with sterile saline solution and streaked on agar plate using sterile cotton swabs for a uniform lawn growth. Essential oils were diluted with 10 % dimethylsulfoxide (DMSO) containing 0.5 % Tween® 80 (v/v) and sterilized by filtration through a 0.45 $\mu$m membrane filter. Oil dilutions tested were 1:1 (6.6 mg/disc), 1:5 (2.6 mg/disc) and 1:10 (1.3 mg/disc). Fifteen microliters of each dilution of essential oils were loaded on sterile filter paper discs (5 mm in diameter, Whatman® No. 1) allowed to dry in an open sterile petri dish and applied on top of agar plates. Plates were left for 30 min at room temperature and then incubated at 37 °C for 18-24 h. After incubation, growth inhibition halos were recorded. Tests were carried out in duplicate and mean value calculated. Controls included discs impregnated with 15 $\mu$L of DMSO (as vehicle control) and streptomycin (25 $\mu$g/disc).

The sensibility to each of oil was classified according to growth inhibition diameter and to specifications described by Celikel and Kavas (2008): not sensitive, if the total diameter was under 8.0 mm; sensitive for 9-14 mm; highly sensitive for 15-19 mm; and extremely sensitive to inhibition for diameters larger than 20 mm.

### Statistical analysis

In order to establish possible differences in the physical chemical characteristics of *Lippia palmeri* essential oils, a variance analysis was performed with a significance level $\alpha = 0.05$, and mean comparison was done by the Tukey's test, using the JMP package version 5.0.1 (SAS Institute, 2002).

For antimicrobial activity, the experimental design was a completely randomized with factorial arrangement of 2 x 3, where factor A was essential oil with two levels (AEO and POEO), and factor B were dilutions with three levels (1:1, 1:5 and 1:10 dilutions). Data were analyzed by ANOVA/GLM at $P < 0.05$. Mean comparison was done by Tukey-Kramer multiple-comparison test using the NCSS package (2001).

## RESULTS AND DISCUSSION

Yield and some physical-chemical characteristics of oregano EO from both localities are shown in Table 1. Puerto del Orégano plants had 6 % of essential oil yield, while Álamos plants contained 5 %. *Oreganum vulgare* oil, *L. sidoides* and *Lippia julliana* have similar yields (Juliani *et al.,* 2002; Nunes *et al.,* 2005; Wogiatzi *et al.,* 2009). These percentages were higher than those reported for *L. multiflora* (0.16 %), *L. alba* (0.6 %), *L. graveolens* (3.5 %), and *L. laxibracteata* (3 %) (Juliani *et al.,* 2000; Salgueiro *et al.,* 2003; Gómez *et al.,* 2007; Owolabi *et al.,* 2009). The moisture content of POEO was significantly lower than AEO, due to the fact that POEO comes from a most arid habitat. No significant differences ($P < 0.05$) were found in EO refractive index (RI), density or specific weight, free acidity and color. RI for POEO and AEO were 1.4813 and 1.4770, respectively, values which are slightly lower than those reported (1.4817-1.4918) for *L. alba* (Ricciardi and Ricciardi, 2000) and for *L. graveolens* (Analiz *et al.,* 2000) and *O. vulgare* (Albado *et al.,* 2001). The studied EO exhibited slightly lower density than those from *Origanum* and other *Lippia* species (Ricciardi and Ricciardi, 2000; Analiz *et al.,* 2000; Albado *et al.,* 2001).

**Table 1. Characteristics of *Lippia palmeri* essential oil from two locations of the State of Sonora, México.**

| Characteristics | AEO | POEO |
|---|---|---|
| Essential oil yield (%) | 5.00 a | 6.00 b |
| Humidity (%) | 7.12 b | 3.15 a |
| Ashes (%) | 7.58 b | 8.43 a |
| Refraction index at 25 °C | 1.47 a | 1.48 a |
| Specific density (g $mL^{-1}$) | 0.88 a | 0.87 a |
| Free acidity index (%) | 2.80 a | 2.30 a |
| Color | 2.4R 50A a Yellow | 2.4R 50A a Yellow |

Means (n = 3) with the same letter in a row indicate there are not significant differences (Tukey, 0.05). AEO = Álamos essential oil; POEO = Puerto del Orégano essential oil.

Analysis of EO by GC-MS led to the identification and quantification of a total of 50 compounds AEO while for POEO presented 60 compounds, accounting for 99.9 and 99.6 % of total components (Table 2). Identification was done by direct comparison of the retention times and mass spectral data of compounds with their respective reference compounds, library matching and by comparison of the fragmentation patterns of mass spectra with reports in the literature. The identification of the compound is fairly strong since was done by recording total ion count and not

by selected ions (m/z), with chromatograms reported by NIST library. In AEO, 11 components were the most abundant (1.0-22.3 %) and constituted 90.4 % of the oil. These components included p-cymene (22.3 %), followed by thymol (21.3 %), iso-aromandrene (16.7 %), carvacrol (8.8 %), γ-terpinene (6.7 %), p-thymol (5.9 %), longipinene epoxide (2.6 %), α-eudesmol (2.5 %), α-bisabolene epoxide (1.3 %) and limonene-6-ol (1.2 %).

In POEO, 12 compounds were the most abundant (1.1-24.5 %) representing 88.1 % of total oils, which included carvacrol (24.5 %), thymol (15.1 %), p-cymene (14.2 %), caryophyllene (9.9 %), thymol acetate (5.5 %), α-bisabolene (4.8 %), γ-terpinene (4.2 %), myrcene (3.8 %), α-caryophyllene (2.2 %), linalool (1.2 %), and terpinene-4-ol (1.1 %).

**Table 2. Constituents of leaves *Lippia palmeri* essential oil from two localities in the State of Sonora, México, analyzed by gas chromatography – mass spectrometry.**

| RT† (min) | Compounds | Amount in oil (%) | | Molecular ion (m/z) | ††††Main fragment ions (m/z) |
|---|---|---|---|---|---|
| | | AEO†† | POEO††† | | |
| 3.69 | 2-Ethyl-furan | 0.02 | 0.02 | 96 | 55,67,42,89 |
| 4.37 | Prenal | 0.17 | 0.03 | 84 | 83,55,67,42 |
| 5.59 | Borneol | 0.07 | 0.34 | 154 | 67,95,81,55,110,93 |
| 7.60 | 2-Methoxy diethylamine | nd | 0.12 | 103 | 99,67,41,115,69,55 |
| 10.60 | β-Pinene | 0.12 | 0.22 | 136 | 93,79,41,105,68 |
| 11.06 | α-Thujene | 0.07 | 0.31 | 136 | 91,93,79,77,41,51 |
| 11.46 | α -Pinene | 0.32 | 0.23 | 136 | 93,91,77,92,79, |
| 12.16 | Origanene | 0.22 | 0.13 | 152 | 94,92,77,91,55,103 |
| 12.43 | α-Terpinolene | 0.24 | 0.18 | 136 | 93,122,105,121,79.77 |
| 13.70 | o-Cymene | 0.23 | 0.22 | 134 | 77,119,115,41 |
| 13.81 | 3-Carene | 0.34 | 0.01 | 136 | 91,79,113,77,122,80 |
| 14.01 | β-Phellandrene | 0.01 | 0.08 | 136 | 93,91,77,41,50,135 |
| 14.46 | 1-Nonen 3-ol | 0.33 | 0.24 | 142 | 57,43,41,55,88,105 |
| 14.61 | 2-Ethyl-oxole | nd | 0.03 | 128 | 57,43,41,108,67,55 |
| 14.82 | Myrcene | 0.16 | 3.82 | 136 | 93,41,69,91,67,79 |
| 15.30 | 3-Octanol | 0.08 | 0.02 | 130 | 119,116,99,43 |
| 15.52 | α -Phellandrene | 0.14 | 0.03 | 136 | 91,93,77,92,51 |
| 15.60 | α-Terpinene | 0.30 | 0.13 | 136 | 93,91,77,40,79,103 |
| 16.03 | Terpinolene | 0.06 | 0.18 | 136 | 93,121,136,77,39,79 |
| 16.19 | Cymene | 0.03 | 0.05 | 134 | 119,134,91,94,65,121 |
| 16.50 | p-Cymene | 22.37 | 14.25 | 134 | 91,119,67,65,41 |
| 16.63 | Limonene | nd | 0.74 | 136 | 68,119,93,91,67,79 |
| 16.72 | Caryophyllene oxide | 0.81 | nd | 220 | 41,79,43,69,94,81 |
| 17.88 | t-Terpinene | nd | 0.13 | 136 | 93,91,77,136,121,92 |
| 18.44 | Cis-b-Terpineol | nd | 0.10 | 154 | 43,93,121,71,79,81 |
| 19.21 | Dehydro-p-Cymene | nd | 0.07 | 132 | 118,115,91,65,131 |
| 19.29 | Durenol | 0.27 | 0.16 | 150 | 117,132,115,91,50,82 |
| 19.68 | Linalool | 0.44 | 1.20 | 136 | 93,71,55,41,69,77 |
| 19.91 | α-Bisabolene epoxide | 1.31 | 0.11 | 220 | 71,43,93,121,69 |
| 20.43 | 3-Allyl cyclohexene | nd | 0.02 | 122 | 81,78,77,53,41 |
| 20.96 | Phellandrene | nd | 0.05 | 136 | 77,91,136,79,94,80 |
| 21.95 | 2-Pinene 4-one | 0.1 | 0.04 | 159 | 93,41,79,57,137 |
| 22.08 | 3-Thujene 2-one | 0.09 | 0.24 | 150 | 95,67,41,55,77,91 |
| 22.51 | Terpinene 4-ol | 0.37 | 1.15 | 154 | 71,93,43,68,55,88 |
| 22.79 | p-Cymene 8-ol | 0.08 | 0.04 | 150 | 135,119,91,65,55 |
| 23.01 | α-Terpineol | 0.12 | 0.19 | 154 | 59,43,121,93,91,136 |
| 24.03 | Thymol methylether | 0.27 | 0.12 | 164 | 150,91,43,71,41 |
| 24.40 | α-Pinene epoxide | nd | 0.50 | 152 | 41,93,69,93,121 |
| 24.64 | Linalool acetate | 0.12 | nd | 136 | 93,43,41,91,121,79 |
| 24.91 | Geraniol | 0.45 | 0.52 | 154 | 81,41,68,43,44,70 |
| 25.81 | Carvacrol | 8.76 | 24.57 | 150 | 135,107,91,51,43 |
| 26.15 | Thymol acetate | nd | 5.5 | 192 | 135,150,107,91,63 |
| 26.34 | Thymol | 21.39 | 15.11 | 150 | 135,107,151,43,91 |
| 26.43 | p-Thymol | 5.94 | nd | 150 | 135,107,91,43,151 |
| 27.71 | Eugenol | nd | 1.34 | 164 | 149,103,77,55,121 |

**Table 2** (continued).

| | | | | | |
|---|---|---|---|---|---|
| 28.00 | o-Cymene 5-ol | 0.19 | nd | 150 | 119,150,91,107,134 |
| 28.34 | Geraniol acetate | nd | 0.49 | 196 | 69,41,43,67,93,121 |
| 28.98 | Methyl eugenol | nd | 0.80 | 178 | 164,149,91,103,55 |
| 29.50 | b-Caryophyllene | 0.08 | 9.96 | 204 | 93,69,134,91,41,105 |
| 29.79 | b-Farnesene | 0.24 | 0.06 | 204 | 41,69,93,42,77,133 |
| 29.98 | Aromandrene | 0.21 | 0.41 | 204 | 161,91,105,119,107,41 |
| 30.43 | α-Caryophyllene | nd | 2.24 | 204 | 93,151,91,105,79,77 |
| 31.13 | γ-Terpinene | 6.69 | 4.23 | 136 | 93,121,78,105,91,43 |
| 31.34 | Longifolene | nd | 0.07 | 204 | 165,104,107,151,91,93 |
| 31.71 | b-Bisabolene | 1.04 | 0.44 | 204 | 93,121,165,105,91,43 |
| 33.49 | (-) Spathulenol | 0.83 | 0.85 | 204 | 43,159,105,131,93,187 |
| 33.62 | α-Bisabolene | 0.39 | 4.77 | 204 | 95,107,121,109,105,43 |
| 34.25 | iso-Caryophyllene | nd | 0.52 | 222 | 67,93,107,79,91,205 |
| 34.58 | b-Eudesmol | 0.13 | 0.15 | 222 | 149,93,108,67,79,81 |
| 34.90 | Alloaromandrene | 0.41 | 0.41 | 220 | 136,91,41,67,77,93 |
| 35.36 | α-Eudesmol | 2.49 | 0.91 | 222 | 67,41,93,77,81,91 |
| 35.70 | iso-Aromandrene | 16.7 | 0.62 | 222 | 91,93,107,41,105,77 |
| 39.38 | Longipinene epoxide | 2.58 | nd | 252 | 43,95,109,58,77,81 |
| 40.27 | 4-Carene | 0.16 | 0.07 | 136 | 113,91,121,81,77,135 |
| 42.86 | m-Cymene | 0.81 | nd | 134 | 135,91,121,79,150,119 |
| 43.01 | Limonen 6-ol | 1.22 | 0.08 | 236 | 93,67,109,68,177,50 |
| | **Total identified** | **99.94** | **99.63** | | |

†RT = retention time; nd= not detected ††AEO = Álamos essential oil; †††POEO = Puerto del Orégano essential oil. Composition was analyzed by a gas chromatograph Varian 3900 coupled to a Varian Saturn 2100T ion trap mass spectrometer; ††††Molecular and fragment ions (m/z) were determined by mass spectrometry, electronic ion ionization at 70 eV, total ion monitoring was done in a scan mass range from 40-400 m/z, and match comparison with the NIST-MS library.

The essential oil from both plant sources appeared quite different and allowed to identify two chemotypes: AEO as a p-cymene/thymol and POEO as a carvacrol chemotype. Similar chemical polymorphism has been reported for *O. vulgaris* L. ssp *hirtum* (Martino *et al.,* 2009; Kokkini *et al.,* 1997), O. *syriacum* L. (Lukas *et al.,* 2009) and *Lippia junelliana* (Juliani *et al.,* 2002).

When compounds of both EO were grouped by their chemical functionality, the major group corresponded to phenolic monoterpenes, followed by monoterpenes hydrocarbons and sesquiterpene hydrocarbons (Table 3). Total monoterpenes in AEO was 72.3 % and 75.6 % for POEO, while sesquiterpenes accounted 27.2 and 21.5 %, respectively. Monoterpenes and sesquiterpenes represented 99.4 and 97.1 % of the total component for the studied EO of both localities. These results make evident the composition dependency of essential oils with the geographical origin of *Lippia* genus (Prabuseenivasan *et al.,* 2006; Owolabi *et al.,* 2009).

*In vitro* studies have demonstrated antibacterial activity of spices and *Lippia* essential oils against some Gram-negative and Gram-positive bacteria (Salgueiro *et al.,* 2003; Burt, 2004; Botelho *et al.,* 2007; Gómez *et al.,* 2007; Paredes *et al.,* 2007; Owolabi *et al.,* 2009). Some species of *Lippia* EO are commonly used in folk medicine, as gastrointestinal and respiratory remedies, and for treatment of skin wounds. Their biological properties have been attributed to the content of carvacrol, thymol and p-cymene (Burt, 2004).

**Table 3: Grouped components of essential from *Lippia palmeri* leaves, based on their chemical functionality.**

| | Percentage in essential oil | |
|---|---|---|
| Constituents | AEO | POEO |
| Monoterpene hydrocarbons | 32.26 | 25.06 |
| Oxygenated monoterpenes | 3.64 | 5.23 |
| Phenolic monoterpenes | 36.36 | 45.29 |
| Sesquiterpene hydrocarbons | 19.06 | 19.50 |
| Oxygenated sesquiterpenes | 8.16 | 2.03 |
| Phenolics | 0.26 | 2.30 |
| Others | 0.19 | 0.22 |
| **Total identified** | **99.94** | **99.63** |

AEO = Álamos essential oil; POEO = Puerto del Orégano essential oil.

The antimicrobial activity of *L. palmeri* essentials oils was tested against Gram negative and Gram positive human pathogens (except for *S. epidermidis*). All of them cause diseases transmitted by food or food intoxications. According to growth inhibition, bacteria were classified from not sensitive to extremely sensitive (Table 4). Results revealed that the inhibition of POEO was stronger than that of AEO. All microorganisms were extremely sensitive to EO at the highest concentration; at other concentrations, the antimicrobial effect varied (*Listeria monocytogenes* was not sensitive with POEO at the 1:10 dilution).

**Table 4. Halos of inhibition of bacterial growth caused by *Lippia palmeri* essential oils.**

| | Dilutions (mg/disc) | | | | | |
|---|---|---|---|---|---|---|
| | AEO | | | POEO | | |
| | 1:1 (6.6) | 1:5 (2.6) | 1:10 (1.3) | 1:1 (6.6) | 1:5 (2.6) | 1:10 (1.3) |
| Bacteria | Inhibition zones (mm) | | | | | |
| *Staphylococcus aureus* ATCC 25923†,††,††† | 30.0±2.1 b | 19.5±0.7 c | 20.5±0.7 c | 37.0±0.0 a | 28.0±1.4 b | 21.0±1.4 c |
| *Staphylococcus epidermidis* ATCC 2228†,††† | 30.0±0.0 b | 23.5±2.1 c | 14.0±0.7 d | 38.0±0.0 a | 29.0±1.4 b | 21.0±1.4 c |
| *Listeria ivanovii* ATCC 19119†,††,††† | 21.5±2.1 a | 13.0±0.7 b | 10.5±0.7 b | 26.0±1.4 a | 21.0±1.4 a | 10.5±1.4 b |
| *Listeria monocytogenes A*TCC 7644†,††,††† | 24.0±1.4 a | 9.5±0.7 c | 9.0±0.0 c | 20.0±0.0 b | 10.0±0.0 c | 0.0±0.0 d |
| *Salmonella senftemberg* ATCC 8400††† | 22.0±1.4 ab | 20.0±0.0 ab | 10.0±0.0 cd | 25.3±3.5 a | 16.5±0.7 bcd | 12.0±1.4 cd |
| *Salmonella choleraesuis* ATCC 14029††† | 20.5±2.1 a | 11.5±0.7 ab | 11.0±0.0 b | 22.5±2.8 a | 12.5±0.7 ab | 11.0±0.0 b |
| *Salmonella typhimurium* ATCC 14028†,††,††† | 22.5±0.7 a | 20.5±0.7 a | 12.5±0.7 b | 23.0±0.0 a | 14.0±1.4 b | 12.5±1.4 b |
| *Escherichia coli* K88†,††,††† | 26.0±0.0 a | 11.5±0.7 c | 13.0±1.4 c | 29.0±0.3 a | 18.5±1.4 b | 11.0±0.0 c |
| *Escherichia coli* O157:H7††,††† | 25.0±1.1 ab | 23.5±0.7 b | 19.0±1.4 bc | 30.0±0.0 a | 21.0±2.8 b | 14.0±2.1 c |
| *Escherichia coli* ATCC 25922††† | 25.0±0.0 a | 19.0±2.1 b | 12.5±0.7 c | 25.0±0.0 a | 20.0±3.2 b | 12.0±0.0 c |

Numbers are means and standard deviations of diameter halos in millimeters (duplicates). AEO = Álamos essential oil; POEO = Puerto del Orégano essential oil. †Significant differences between Eos; ††Significant interactions. †††Significant differences between dilutions (Tukey, 0.05). Means with the same letter in a row indicate that there are not significant differences (Tukey, 0.05). Bacteria sensibility clasification by Celikel and Kavas (2008). Not sensitive = halo of inhibition < 8.0 mm; sensitive = 9-14 mm; highly sensitive = 15-19 mm; and extremely sensitive, > 20 mm.

Differences were significant only for *S. aureus, S. epidermidis, L. inovani, L. monocytogenes,* S. *typhimurium* and *E. coli K88*. Interactions (EO and dilutions) were significant in all bacteria except for *S. epidermidis, S. senftemberg*, *S. Choleraesuis* and *E.coli ATCC 25922*. *(Table 4)*. These results showed that antimicrobial activity against *S. aureus, L. inovani, L. monocytogenes, S. typhimurium E. coli K88* and *E. coli* O157:H7 decrease with reduction of EO concentration. The observed reduced response of *L. monocytogenes* has also been reported for EO obtained from thyme, sage, myrtle, orange and laurel (Celikel and Kavas, 2008).

It is important to emphasize that *Escherichia coli* O157:H7 and *Staphylococcus aureus* are two of the pathogen of greatest concern in the industry of meat products, in which *L. palmeri* EO could be used as natural preservative.

In this study the variability of antimicrobial activity could be attributed to differences in composition of oregano oil, and in bacterial target sites. The strong antimicrobial activity of POEO (compared to AEO) might be linked to its higher phenolic monoterpene content, and to thymol acetate, eugenol and methyl eugenol concentration. Other reports have attributed the antimicrobial effectiveness of oregano essential oils to thymol, eugenol and carvacrol (Analiz *et al.,* 2000; Aligiannis *et al.,* 2001; Salgueiro *et al.,* 2003; Gómez *et al.,* 2007). The mechanism of action of these compounds was related to the hydrophobicity of EO components that enable them to break down the lipids of bacterial cell membranes, then increasing ion permeability, and lipid and protein leakage, which in turn could cause cell lysis (Aligiannis *et al.,* 2001; Oyedemi *et al.,* 2009).

## CONCLUSIONS

The composition of essential oil from *Lippia palmeri* S. Wats varies the region of collection. AEO from a temperate zone was a p-cymene/thymol chemotype, while POEO from a most arid region was a carvacrol chemotype. Phenolic monoterpenes were the most abundant terpene group in both oils. With different potency, the oils studied have the ability to inhibit bacterial growth of pathogens, making *L. palmeri* essential oils useful for the food industry as flavoring agent and natural preservative.

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