

COMPOUNDS WITH *in vitro* ANTIBACTERIAL ACTIVITY FROM HYDROSOL OF *Lippia palmeri* AND MORPHOMETRIC CHANGES ON *Listeria monocytogenes*

COMPUESTOS CON ACTIVIDAD ANTIBACTERIANA *in vitro* DE AGUA AROMÁTICA DE *Lippia palmeri* Y CAMBIOS MORFOMÉTRICOS SOBRE *Listeria monocytogenes*

Joel Said García-Romo¹, María Susana Yépez-Gómez¹, Maribel Plascencia-Jatomea¹, Hisila Del Carmen Santacruz-Ortega², Armando Burgos-Hernández¹, Jesús Ramon García De León¹, Francisco Javier Cinco-Moroyoqui¹, Jesús Borboa-Flores^{1*}

¹ Departamento de Investigación y Posgrado en Alimentos. Universidad de Sonora, Hermosillo, Sonora, México

² Departamento de Investigación en Polímeros y Materiales. Universidad de Sonora, Hermosillo, Sonora, México.

ABSTRACT

Foodborne diseases have increased, and with it, the interest to discover new natural antimicrobials; the use of aromatic plants, due to the extraction and use of the essential oil (EO), are widely studied and with great biological activity. However, studies with hydrosols (HS) from aromatic plants are limited. The aim in this study was to evaluate the antibacterial activity of HS from *Lippia palmeri* plant against the bacterial pathogen *L. monocytogenes*. HS was obtained by hydrodistillation and fractionation thereof by open column chromatography. The antibacterial activity was performed by disk diffusion. The inhibitory concentration (IC₅₀) was estimated using the PROBIT survival analysis. Ampicillin was used as control. To evaluate the diameter and cellular damage, optical microscopy and epifluorescence were used, respectively. The characterization was performed by spectroscopy. HS showed IC₅₀ of 224 µL/mL and from HS two fractions were obtained. The fraction with the highest activity showed an IC₅₀ of 125 mg/mL and rosmarinic acid and a phthalate derivative were identified from this fraction. HS, showed bioactivity against *L. monocytogenes*. Therefore, this would be a candidate for use as an active ingredient in the disinfection of food and areas in contact with them.

Keywords: *Lamiaceae*, bioactive compounds, pathogenic bacteria, antibacterial effect.

RESUMEN

Las enfermedades transmitidas por alimentos han incrementado, así como el interés por descubrir nuevos antimicrobianos naturales; las plantas aromáticas y uso del aceite esencial (EO), es ampliamente estudiado por su gran actividad biológica. Sin embargo, los estudios con agua aromática (HS) proveniente de plantas aromáticas son limitados. Se planteó como objetivo, evaluar la actividad antibacteriana de HS proveniente de la planta *Lippia palmeri* contra el patógeno bacteriano *L. monocytogenes*. HS se obtuvo por hidrodestilación y el fraccionamiento del mismo, por cromatografía de columna abierta. La actividad antibacteriana se realizó por difusión en disco. La concentración inhibidora (IC₅₀) se estimó utilizando el análisis de supervivencia PROBIT.

Se empleó ampicilina como control. Para evaluar el diámetro y daño celular, se utilizó microscopía óptica y de epifluorescencia, respectivamente. La caracterización se realizó por espectroscopía. HS mostró IC₅₀ de 224 µL/mL y a partir de HS se obtuvieron dos fracciones. La fracción con mayor actividad mostró una IC₅₀ de 125 mg/mL y se identificó ácido rosmarínico y un derivado de ftalato a partir de esta fracción. HS, mostró bioactividad contra *L. monocytogenes*; por lo tanto, este podría ser un candidato para uso como ingrediente activo en la desinfección de alimentos y áreas en contacto con ellos.

Palabras Clave: *Lamiaceae*, compuestos bioactivos, bacteria patógena, efecto antibacteriano.

INTRODUCTION

Listeria monocytogenes is a pathogenic bacterium with a large reservoir, it is found in soil, sewage, and mainly in a wide variety of food products such as meat and dairy products. This microorganism is the major pathogen causing abortions in pregnant women, and causes diseases such as meningitis, encephalitis and septicemia, with a high mortality rate (around 30 %) (Schlech, 1996; Klatt *et al.*, 1986). *L. monocytogenes* has the capacity to survive in a variety of hostile environments, such as low temperature, nutrient deprivation, oxidative stress, and antibiotics; therefore, throughout time its control has been a great challenge (Gahan and Hill, 2014). Until this day, great importance has been given to the study of the resistance of microorganisms to germicides; currently, emergency measures have been implemented for the control of *L. monocytogenes*, mainly through the addition of synthetic germicidal compounds; nevertheless, most of these represent huge problems for the health and the ecosystem (Gammariello *et al.*, 2008).

Some herbs that are used to give flavor and aroma to many types of food have antimicrobial activity. Essential oils from thyme (*Thymus vulgaris* L.), oregano (*Lippia palmeri*), and rosemary (*Rosmarinus officinalis* L.) have been shown to have inhibitory activity against food spoilage by bacteria. The aromatic water or hydrosol, a by-product from the extraction of essential oils from spices, is the water-soluble fraction con-

*Autor para correspondencia: Jesús Borboa-Flores
 Correo electrónico: jesus.borboa@unison.mx

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sisting of a mixture of terpenes, alcohols, aldehydes, phenols, acids, and aliphatic hydrocarbons. From this mixture, terpenes are especially responsible for medicinal uses that have been conferred to herbs, and phenols such as thymol and carvacrol have shown a strong antimicrobial action against the food-borne pathogens (Tornuk *et al.*, 2014).

There are studies where hydrosols from the family *lamiaceae* are suggested as a possible natural disinfectant with application in food safety, mainly in fresh vegetables, without affecting their sensory properties. Since in studies on tomato and cucumber surfaces inoculated with *Escherichia coli* O157:H7, a total inhibition of the bacteria was observed with these vegetables previously treated with oregano hydrosol (Ulukanli *et al.*, 2013). Recently, great importance has been given to the study of the biological properties of essential oils from spices, but there still are a very limited number of investigations on hydrosols. The aim of this study was to isolate and partially characterize bioactive compounds present in hydrosol from *L. palmeri*, commonly called oregano belonging to the generum *Origanum* and evaluate *in vitro* their antibacterial activity and morphometric impact on *L. monocytogenes*.

MATERIALS AND METHODS

Plant material and HS extraction

Leaves of *L. palmeri* plant were collected in the region of Alamos, Sonora, Mexico at 380 meters above sea level, at 27°01' N and 108° 56' W, during the spring. They were transported to the laboratory and dried in the dark at 15 °C for 3 weeks (Corella-Bernal and Ortega-Nieblas, 2013). Hydrosols were obtained by hydrodistillation employing 100 g of dry plant in 500 mL of distilled water, and the extraction was conducted for 3 h in a Clevenger hydrodistillator (AOAC, 1990). The concentration of the crude extract (HS) was regarded as 100 %; thereafter, volume/volume dilutions were made.

Bacterial strains

Listeria monocytogenes (ATCC 4766) was used at a concentration of 1E⁰⁷ UFC/mL (estimated by spectrophotometry 650 nm) (Castellanos *et al.*, 2009).

Antibacterial assay

In order to test the antibacterial activity of HS, the disc diffusion method was used (Cona, 2002), using the technique of massive inoculation of bacterial culture in nutrient agar (Difco, MD USA). On each plate, three Whatman filter paper discs (4 mm in diameter) were placed and impregnated with 10 µL of HS. Plates were incubated at 35 ± 2 °C for 12-24 h and diameter (mm) of growth-inhibition zones were measured with a Vernier caliper. Methanol (70 %) and Sensi-Disc Ampicillin (AM) 10 mg (Becton Dickinson and Company, MD USA) were used as negative control and as a reference antibiotic, respectively; also, positive control bacteria either without extract or antibiotic was also included (Balsalobre-Hernández and Hernández-Godoy, 2004). Inhibitory concentration 50

(IC₅₀) was calculated using the results from the antibacterial activity assay, using the NCSS statistical program (Survival/Reliability, Probit Analysis) (Osorio, 2005).

Cell diameter

The effect of HS and its fractions on cell diameter of *Listeria monocytogenes* after 24 h of incubation at 35 ± 2 °C, was evaluated. Dimension parameters (length and width) were measured in 60 cells per observation field in the optical microscope. AM was used as a bacterial-growth inhibition positive control (100 % inhibition). An Olympus CX31RTSF optical microscope adapted with an Infinity 1 chamber was used. Images were processed using the Image-Pro plus 6.3 software (Media Cybernetics, USA) (Martínez-Camacho *et al.*, 2013). Inhibitory concentration 50 (IC₅₀) was determined with statistical program NCSS survival/reliability, probit analysis (Osorio, 2005).

Cell damage

A Leica DM 2500 epifluorescence microscope, equipped with Kit Live/Dead BacLight (BL) (Molecular Probes Inc., 2008) was used to assess bacterial cell damage using propidium iodide (PI) and fluorescein diacetate (FDA) as fluorochromes (Plascencia-Jatomea *et al.*, 2003).

Purification of bioactive compounds

In order to isolate and partially characterize bioactive groups, a biological activity-guided isolation procedure, using solvent-affinity properties was conducted (Rosas-Burgos *et al.*, 2009).

Column chromatography

HS contents were loaded on a silica gel chromatographic column (2.5 x 100 cm). The elution was carried out using 500 mL of various solvent systems (Table 1).

Table 1. Solvent systems used as mobile phase for column chromatography.

Tabla 1. Sistemas de solventes utilizados en fase móvil para la cromatografía en columna.

Solvents systems	Polarity Index*
Hexane	0
Hexane: Ethyl acetate 50:50	2.15
Hexane: Ethyl acetate 25:75	3.3
Ethyl acetate	4.4
Acetone	5.4
Methanol	6.6
Methanol: Water: Acetic acid 50:40:10	7.5
Water: Acetic acid 90:10	8.7
Water	9

* The polarity index is a measure of the relative polarity of a solvent and is useful for identifying suitable mobile phase solvents.

Thin layer chromatography (TLC)

TLC analyzed each fraction collected from column chromatography. A 10 μ L-aliquot was applied onto TLC aluminum sheets silica gel DC-Alufolien Kieselgel 60 F254, schichtdicke 0.2 mm (Merck). TLC were developed sequentially using all of the solvent systems in column chromatography and they were observed under UV light. Samples showing similar bands were considered parts of a same fraction and they were mixed and labeled (eg. FA1, FA2). Fractions were taken to dryness under reduced pressure and were suspended in methanol to be stored (Moreno-Félix *et al.*, 2013).

Ultraviolet-visible spectroscopy (UV-Vis)

UV-Vis spectra of fraction FA1 was obtained on an Agilent diode array spectrophotometer (model 8453, Shanghai), using a solution of 2 μ L extract in 10 mL ethanol and measurements were made within a wavelength-range from 200 to 700 nm at 25 °C, using a 1 cm quartz cuvette.

Fluorescence spectroscopy

Luminescence spectra of fraction FA1 was measured in a Perkin Elmer LS-50B Luminescence Spectrometer (Buckinghamshire, England), using a solution of 2 μ L extract in 10 mL ethanol, within a wavelength-range from 200 to 700 nm at 25 °C, using a 1 cm quartz cuvette.

Infrared spectroscopy (FT-IR)

FT-IR spectra were obtained in a Perkin Elmer FT-IR Spectrum GX (Waltham, MA, USA) with an average of sixteen scans within a spectral range of 4000–400 cm^{-1} . IR spectra of lyophilized FA1 sample were obtained in KBr pellets.

Proton nuclear magnetic resonance ($^1\text{H-NMR}$)

$^1\text{H-NMR}$ spectra were obtained using a Bruker Avance 400 nuclear magnetic spectrometer (Billerica, MA, USA) operated at 400 MHz. To carry out the determinations, ~1 mg of FA1 was dissolved in 0.5 mL of acetone- d_6 in NMR tubes. Tetramethylsilane (TMS) was used as a reference, the spectral window was 20 ppm and the NMR readings were conducted at 24 ± 1 °C.

Statistic analysis

An ANOVA was performed to establish significant differences among variables and a Tukey multiple range test ($p < 0.05$) was carried out for comparison of means using the SPSS (Field, 2013). All analyzes were performed in triplicate.

RESULTS AND DISCUSSION

Hydrosol yield

An average volume of 250 mL was obtained from 500 mL of water and 100 g of *L. palmeri* (50 % yield). Similar results were obtained by Sagdic *et al.* (2013), they used 100 g of sample and 500 mL of water and they extracted it for 1 h; the difference in the extraction time is probably because the authors used a flask with a greater capacity providing the sample of a greater surface contact with water vapor.

Antibacterial assay

The HS obtained showed high antibacterial activity against *L. monocytogenes*, showing 54 % inhibition compared whit AM (100 %) at 12 hours of incubation to a maximum concentration of 500 $\mu\text{L/mL}$. HS showed antibacterial effect from very low concentrations (3.6 $\mu\text{L/mL}$) and is shown in Figure 1, the diameter of inhibition proportionally increased as the HS concentration was increased; however, this effect was only observed during the first 12 h of incubation, after which bacteria apparently adapted of HS.

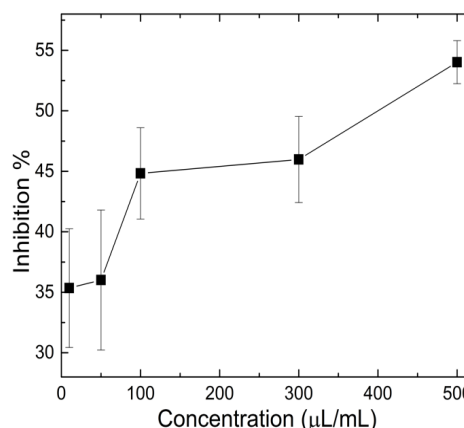


Figure 1. Antibacterial activity of hydrosol obtained from *L. palmeri* on *L. monocytogenes* after 12 h of incubation.

Figura 1. Actividad antibacteriana de hydrosol obtenida de *L. palmeri* sobre *L. monocytogenes* después de 12 h de incubación.

This behavior may have occurred because crude extracts obtained from herbs or spices have a heterogeneous composition, both bioactive and inactive compounds are present; inactive compounds might present an antagonistic effect, reducing the antimicrobial activity acting as a substrate (growth factor) to the microorganism (Toroglu, 2011). Loss of antibacterial activity might have also been due to sufficient concentration of the active compounds in order to exert an increased bioactivity. Bacterial growth inhibition could be attributed to a variety of compounds present in HS; therefore, at least one of them has an inhibitory effect against *L. monocytogenes*. The IC_{50} of HS was 223.65 ± 29.83 $\mu\text{L/mL}$.

In a similar study, the authors obtained a log 1-1.5 log reduction in UFC/g of *L. monocytogenes* with individual 20-min washes was obtained, also observed that when prolonging the treatment (washings) time to 60 min, the hydrosols from thyme, sage, and rosemary can completely inhibit to *L. monocytogenes* (Ozturk *et al.*, 2012).

Antibacterial activity of fractions

Fraction FA1 showed antibacterial activity against *L. monocytogenes*, showing 71 % bacterial growth inhibition compared whit AM (100 %) at 24 hours of incubation to a maximum concentration of 200 mg/mL. When comparing results of antibacterial activity obtained from FA1 and HS,

the antibacterial activity of HS was not observed after 12 h of incubation, whereas FA1 continued inhibiting bacterial growth beyond 12 h and up to 24 h, this is shown in Figure 2, and the IC_{50} of FA1 was 125 ± 11.54 mg/mL.

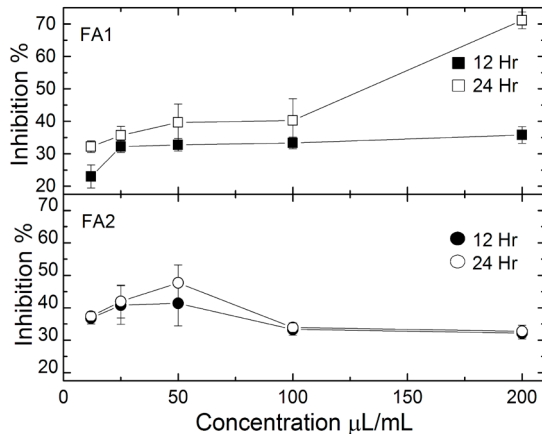


Figure 2. Effect of fractions FA1 and FA2 on growth of *L. monocytogenes* after 12 and 24 h of incubation.

Figura 2. Efecto de las fracciones FA1 y FA2 sobre el crecimiento de *L. monocytogenes* después de 12 y 24 h de incubación.

FA2 showed greater inhibitory activity at 50 mg/mL but not at 200 mg/mL as expected; this could be attributed to the presence of other compounds that might serve as nutrients to the bacteria, which in high concentration promote growth. Exposure of bacteria to FA2 at the highest concentration (200 mg/mL) resulted in higher bacterial growth (less inhibition) after 24 h (Figure 2). This suggests that FA1 might contain the group of compounds responsible for the antibacterial activity of *L. palmeri*, or that they are concentrated in this fraction. This phenomenon has been associated to possible antagonistic effect between different extracts resulting in the rising of bacterial growth. Crude extracts from herbs or spices, have a very complex composition, featuring active and inactive compounds; the active compounds are those exerting biological activity, including antibacterial activity; however, inactive compounds may serve as a substrate or growth factor to microorganisms, that is, show an antagonistic effect, because it decreases the antibacterial activity (Peèilyte; 2005; Toroglu, 2011).

Cell diameter

The average cell diameter of *L. monocytogenes* control was 10.20 ± 2.12 µm, the cell diameter of bacteria treated with fraction FA1 at a concentration of 200 mg/mL was 17.47 ± 1.42 µm (more than 7 µm larger than control bacteria), and the cell diameter of the bacteria treated with the antibiotic AM was 19.96 ± 1.94 µm (Figure 3).

Cell damage

Cells of *L. monocytogenes* treated with fraction FA1, at a concentration of 200 mg/mL, showed a red bright color

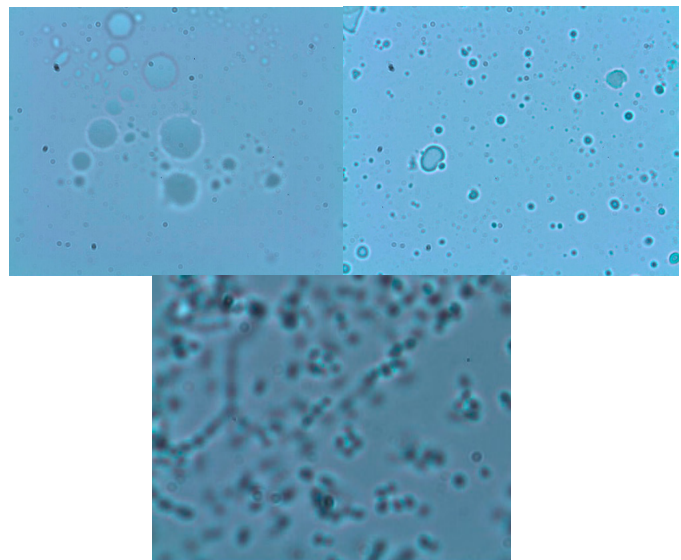


Figure 3. *L. monocytogenes* cells at 100x [A, negative control (10.20 ± 2.12 microns), B, exposed to fraction FA1 (17.47 ± 1.42 microns) and C, exposed to ampicillin (19.96 ± 1.94 microns)].

Figura 3. Células de *L. monocytogenes* a 100x [A, control negativo (10.20 ± 2.12 micrómetros), B, expuestas a fracción FA1 (17.47 ± 1.42 micras) y C, expuestas a ampicilina (19.96 ± 1.94 micras)].

inside, indicating interaction of PI with DNA. Small pores could be observed with a glowing red color (indication of damage), meaning that IP could pass through the cell membrane, reach the inside and interact with DNA; these results are shown in Figure 4.

Cells that did not have their membrane damaged showed brightness around their membrane and not inside the cell; this suggesting that there wasn't damage by the treatment. This could be due to possible resistance to compounds present in fraction FA1, forming a permeability barrier between the intracellular and extracellular environments. On the other hand, depending on tested bioactive fraction concentration, some cells could have not been exposed to

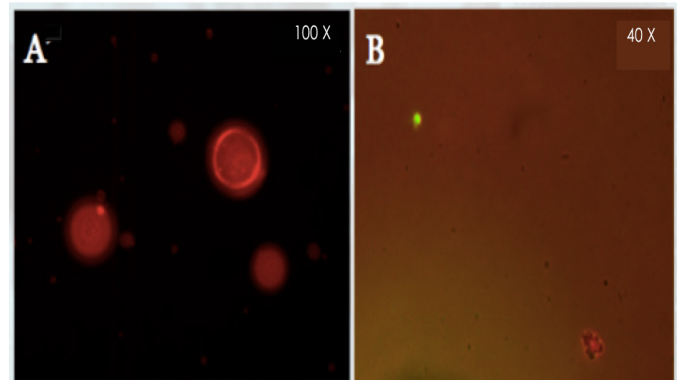


Figure 4. Damaged cell membrane of *L. monocytogenes*. A) Cells damaged after been treated with FA1 at 200 mg/mL using propidium iodide, B) Control using fluorescein diacetate viable cell membrane.

Figura 4. Daño de membrana celular de *L. monocytogenes*. A) Células dañadas después de haber sido tratadas con FA1 a 200 mg/mL usando yoduro de propidio, B) Control, utilizando diacetato de fluoresceína en viabilidad de membrana celular.

bioactive compounds at all (Bass *et al.*, 1983). Control cells showed a smaller diameter and a green bright color inside, therein by action of the FDA signal enzyme activity of living cells (Hoet *et al.*, 2013).

A study of Yokoyama *et al.* (1997), where they observed in microspores surrounding areas stained with IP suggesting cell viability, where IP was unable to penetrate into the cells indicating no cell-damage; these same cells were stained with FDA showing an enzymatic activity (green brightness) and thereby ensuring cell viability.

Results from cell damage assays provided information that could be contributory to attempt to visualize a possible mechanism of action of the compounds present in the fraction FA1 obtained from *L. palmeri* HS.

Membrane damage possibly occurred by the action of electronegative groups present in compounds from bioactive fraction FA1, which may have interacted with the membrane destabilizing its electrostatic charges. This may have caused hyperpolarization of cell membrane, directly affecting the entry and exit of molecules by the imbalance of active and passive transport, which is known as damage to cell permeability (Peña *et al.*, 2013).

Cell permeability maintain balanced ionic gradients, these regulate cell volume; if cell permeability has been damage, ions uncontrollably get in and out, leading to increased intracellular water; increased intracellular water causes a disorder in the cytoplasmic material altering normal cell function (Chung and Chen, 2008; Palma-Guerrero *et al.*, 2009; Martínez-Camacho *et al.*, 2013).

Ultraviolet-visible spectroscopy (UV-Vis)

Figure 5 shows the absorption spectra fraction FA1 dissolved in ethanol at different concentrations, being the most concentrated sample solution the one in which the greater UV absorption occurs. The absorption spectrum of fraction FA1 shows an unstructured band centered at 274 nm and a band in the high-energy region ($\lambda_{max} = 225$ nm) as well as two low absorption bands at 300 and 326 nm. The absorption band at 274 nm is likely due to the presence of aromatic compounds present in the fraction FA1.

Signals observed at 301 and 326 nm may be explained as follows. The first one maybe attributed to low probability transitions of the type $n \rightarrow \pi^*$, which are found when a conjugated system is attached to an element with available electrons such as oxygen, nitrogen, sulfur or halogens. Another possibility is that the presence of others compounds present at low concentrations (Pretsch *et al.*, 2009). Different types of biologically active phenolic compounds, showing maximums of absorption in the region of 200 to 350 nm including phenolic compounds, have been reported: vanillic acid (260-292 nm), caffeic acid (296-324 nm), acid ferulic (298-324 nm), rosmarinic acid (270-290 nm), espirosmanol (288 nm), carnosol (284 nm), among others (Atoui *et al.*, 2005).

Fluorescence spectroscopy

The emission spectra of fraction FA1 dissolved in ethanol are shown in the Figure 5. Two excitation wavelengths

were used, 223 and 274 nm. Upon excitation at 224 nm, the emission spectrum showed a band with several emission maximums at 310, 345, and 360 nm. When using excitation wavelength of 274 nm, an unstructured band centered at 320 nm was obtained. Features as the position at which the emission bands appears as well as their shapes suggest both, the presence of aromatic compounds and the possible existence of more than one compound.

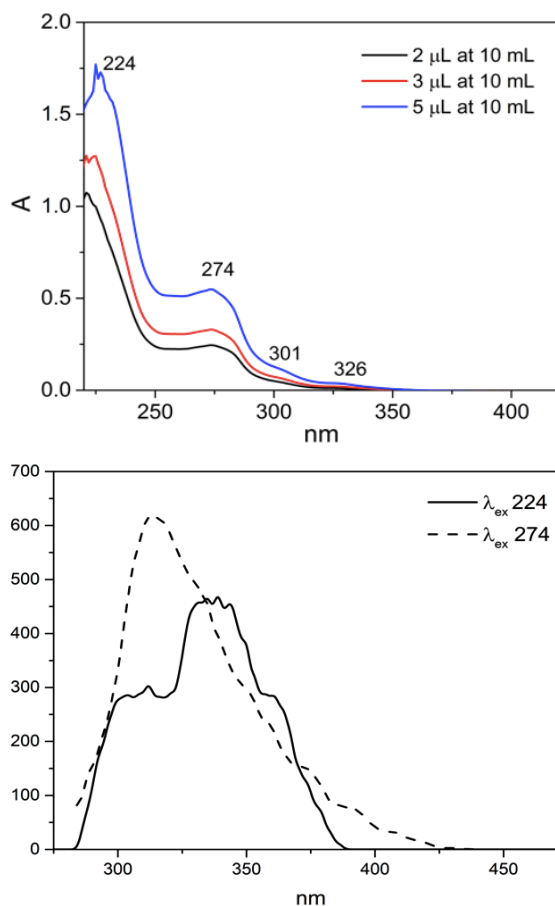


Figure 5. UV-Vis and fluorescence spectra of fraction FA1, at different dilution (2, 3 and 5 μ L at 10 mL) in ethanol.

Figura 5. Espectros de UV-Vis y de fluorescencia de la fracción FA1, a diferentes diluciones (2, 3 y 5 μ L a 10 mL) en etanol.

Infrared spectroscopy (IR)

Figure 6 shows the FTIR spectrum of fraction FA1 in KBr pellet. The signal at 3100 cm^{-1} corresponding to stretching vibration of C-H bonds is associated to aromatic groups, while signals at 2976 and 2936 cm^{-1} corresponds to stretching vibration of C-H bonds of saturated groups. Signals at 1731 and 1250 cm^{-1} to absorption band of corresponding to stretching vibration of C=O and C-O bonds, respectively; they are associated to the presence of ester groups. The presence of bands at 1600 cm^{-1} (stretch of vibration of C=C bonds) within the range of 900 to 650 cm^{-1} , suggests the presence of aromatics compounds. The spectrum shows a vibration band at 3500 cm^{-1} corresponding to stretching vibration of O-H bond and

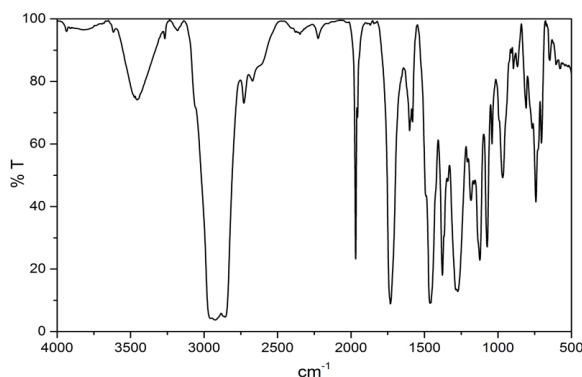


Figure 6. FTIR spectrum of fraction FA1 in KBr pellet.

Figura 6. Espectro de FTIR de la fracción FA1 en pastille de KBr.

the vibration band at 1040 cm^{-1} s associated to C-O bond stretching which indicate the presence of phenol or alcohol type of compounds (Silverstein *et al.*, 2007). The various bands observed in the IR spectrum suggest the presence of phenolic-type compounds (Domínguez, 1979; Pretsch *et al.*, 2009). Based on results obtained by electron spectroscopy, we can assume that fraction FA1 contains different phenolic compounds in addition to molecules that have ester groups in their structure.

Proton nuclear magnetic resonance ($^1\text{H-NMR}$)

Figure 7 shows the $^1\text{H-NMR}$ spectrum of fraction FA1 in acetone- d_6 . The number of signals shown in the $^1\text{H-NMR}$ spectrum suggests that more than one type of compound is present. In the $^1\text{H-NMR}$ spectrum, within the range of 0.9 and 1.8 ppm, a group of signals associated to aliphatic protons is observed.

The 4.23 ppm (t) signal is attributed to protons that are attached to oxygen atoms involved in ester bonds. The signals δ downfield = 6.5 - 7.8 ppm confirms the presence of protons belonging to aromatic groups.

Chemical shifts of protons present in the compounds in fraction FA1 match those the FTIR spectra, where the presence of aromatic rings and ester groups are present.

The number of signals in the $^1\text{H-NMR}$ spectrum was obtained using the 2D COSY software. The signals at 7.55 and 7.65 ppm correspond to o-substituted aromatic compounds and they coincide with those reported by Saeed *et al.* (2007), suggesting the presence of phthalic acid esters. Three signals are shown within the ranges of 7.1 to 7.5 ppm and 6.4 to 6.9 ppm, with a second-order coupling. The shape of the signal indicates the presence of two aromatic rings with substitutions at positions 1, 2, and 4. These signals match those signals reported for rosmarinic acid (Kontogianni *et al.*, 2013; Exarchou *et al.*, 2003).

Results from chemical-structural partial-characterization performed by UV-Vis, fluorescence, IR and $^1\text{H-NMR}$ techniques, suggests that the compounds responsible for the biological activities of fraction FA1 was rosmarinic acid and phthalates.

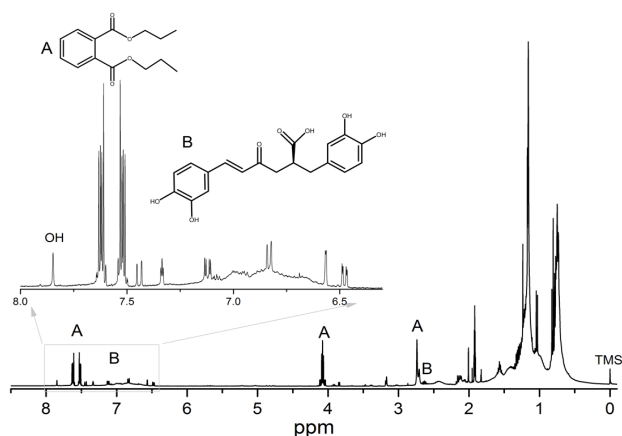


Figure 7. $^1\text{H-NMR}$ spectra of fraction FA1 in acetone- d_6 , A, indicates phthalate ester signals and B, indicates rosmarinic acid signals.

Figura 7. Espectro de $^1\text{H-NMR}$ de la fracción FA1 en acetona- d_6 , A, indica la señal del ester de ftalato y B, indica la señal del ácido rosmarinico.

Rosmarinic acid, found in a variety of plants, is a compound to which antioxidant properties and/or medicinal properties have been attributed due to their phenolic and acrylic functional groups. Considered as a phenolic antioxidant, rosmarinic acid has the ability to retard microbial invasion and putrefaction in fruits and vegetables (Pedersen, 2000). This organic acid is found in plants belonging to the Lamiaceae family and they are commonly used as spices as culinary herbs such as *L. palmeri*.

Phthalates or esters of phthalic acid are a group of chemical compounds used primarily as plasticizers (Mankidy *et al.*, 2013). However, there is evidence of a variety of organisms that can produce phthalates as a secondary metabolite in their systems, such as algae, fungi and different types of plants (Duty *et al.*, 2003). Although most phthalate esters are chemically synthesized to be used as plasticizers in the industry, there are recently conducted studies in which a number of biological activities such as antioxidant, antimutagenic, antibacterial and anti-hypoglycemic, have been reported for phthalates derivatives (Lee *et al.*, 2000; El-Syed, 2012; Quian *et al.*, 2012).

CONCLUSIONS

Hydrosol from *L. palmeri* has antibacterial activity against *L. monocytogenes*, exhibiting a concentration-response type of relationship, and an IC_{50} of $223.6\ \mu\text{L/mL}$. Fraction FA1, obtained from HS, is the fraction isolated from this plant that has the greatest antibacterial activity against *L. monocytogenes*, exhibiting an IC_{50} of $125\ \text{mg/mL}$. Fraction FA1 causes damage to cell membrane and therefore an increase in cell diameter of *L. monocytogenes*, suggesting damage in cellular permeability when this comes in contact with bioactive compounds from FA1. Based on chemical characterization studies, FA1 may contain rosmarinic acid and phthalate groups as the compounds mainly responsible

for the antibacterial activity; however, further studies are required for a full characterization of FA1 composition.

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