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Chemical Profile and Antioxidant Activity of Essential Oils and Polyphenolic Compounds of *Lippia Graveolens* from Different Mexican Localities

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Abstract

Extraction of polyphenolic fraction and essential oils (EOs) from *Lippia graveolens* (Mexican oregano) leaves were explored using innovative and conventional extraction techniques such as ultrasound assisted extraction (UAE) and steam distillation, respectively. The metabolites were further analyzed to evaluate their bioactivity as antioxidants and the identification of the chemical profile was performed using a UPLC-ESI-Q/TOF-MS^2 and GC-MS. The antioxidant assays on EOs showed potential as inhibitors of lipid oxidation, and the polyphenolic fraction obtained higher inhibition activity against DPPH$^•$ radical. The lower IC_{50} for DPPH$^•$ radical corresponded to the phenolics from "San Pedro" with 545.40 ± 47.2 μg mL^{-1}, and the higher inhibition of lipid oxidation appertained to EOs (99.06 ± 3.91%). Furthermore, the GC-MS tentative characterization identified sixty-one compounds for EOs, and UPLC-ESI-Q/TOF-MS^2 showed six identified compounds, the tentative compounds englobe flavonoids, terpenes, sesquiterpenes xanthines, and dicarboxylic acid. The present study demonstrated that Mexican crops of *L. graveolens* possess essential oils and polyphenolic fractions with strong antioxidant activity, which might be useful in the food and pharmaceutical industries.

Keywords: *Essential oil; Polyphenolic fraction; Antioxidant activity; Mexican oregano.*

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1. Introduction

Oregano is the common name for one of the most world´s commercially spices which includes 60 plant species from different families: *Lamiaceae* (European oregano), *Rubiaceae*, *Scrophulariaceae*, *Verbenaceae* and others [1]. In this regard, the Mexican oregano (*Lippia graveolens*) plant is recognized as one of the two economically relevant genera of *Verbenaceae* family (*Lanata* and *Lippia*) by its applications in daily life, mainly used as flavoring, but also used in traditional medicine [2]. The *L. graveolens* plant is native to the northern region of Mexico and it is consolidated a source of income for marginalized communities in the region, and its importance reaches up to 50% of wage income [3].

Additionally, the literature has established the oregano plant as a source of bioactive fractions such as polyphenolic rich fraction and essential oils (EOs), fractions with potential applications as: health auxiliary, main

component in food coating and auxiliary in green pesticides for aquiculture and agriculture [4-6]. Otherwise, recent research has focused on the potential of EOs as auxiliary agents against diseases (cancer cells, oxidative stress, antibiotic resistant bacteria, etc.) obtaining promising data [7, 8]. Also, the essential oils market shows tremendous growth and forecasts reach US $ 3,226.2 million by 2025 [9].

The EOs consists in complex mixtures made up of volatile components (esters, alcohols, aldehydes, ketones, hydrocarbons, and phenols) among many other constituents that have high volatility at ambient pressure and temperature [10, 11]. The chemical composition of EOs could be modified by the extraction process applied for fraction recovery, but it is mainly composed by monoterpenes which consist of a 10-carbon chain ($C_{10}H_{16}$) that can be alicyclic, monocyclic or bi-cyclic, and can comprise unsaturated hydrocarbons and / or functional groups (*e.g.* alcohols), and sesquiterpenes ($C_{15}H_{24}$) a more diverse group among terpenoid-type compounds, they can present a lactone ring and present a variety of forms such as: linear, monocyclic, bi-cyclic and tricyclic [12]. In context, the main molecules related to oregano species englobes thymol and carvacrol (monoterpenes) that confer organoleptic properties and bioactivity [13, 14].

In recent years, the studies about the phytochemicals of *L. graveolens* have been developed focusing on the essential oils fraction and to a lesser extent in polyphenolic fraction, despite the fact that according to literature in *L. graveolens* plants mainly presents flavonoids and phenolic acids, both commonly found conjugated with sugar [15-17]. The relevance of *L. graveolens* as a source of polyphenolic compounds belongs on the several scientific researches that have associated flavonoids and phenolic acids with multiple auxiliary effects against some chronic diseases such as hypertension, diabetes, among others [18, 19].

Due to the possibility of extracting functional biomolecules, and the limited information available in the literature about the phytochemical composition and antioxidant capacity of *L. graveolens* crops, the aim of this study was to evaluate the antioxidant potential of different extracts and essential oils from *L. graveolens* crops from the Northeast of Mexico using conventional and emerging economically viable technologies. In addition, phenolic compounds and EOs were sequentially fractionated using UPLC-ESI-Q/TOF-MS^2 (Ultra-Performance Liquid Chromatography coupled to Electrospray Ionization-Quadrupole/Time of Flight-Mass Spectrometry) and GC-MS (Gas Chromatography coupled to mass spectrometry) to identify and characterize their chemical constituents. Under our knowledge, the present study is the first antioxidant evaluation that integrates polar and no-polar fraction from crops of *L. graveolens* in the Northeast of Mexico.

2. Material and Methods

2.1. Plant Material

The plant material consisted in complete leaves, were obtained from the following different locations established in the northeast region of Mexico according to "CONACYT-CONAFOR Production Database": Higueras (Latitude, 25º57'38.0", Longitude, 100º02'30.3", Higueras, Nuevo León, Mexico), Puerto Carretas (Latitude, 24º03'09.4", Longitude, 99º57'20.0", Arramberri, Nuevo León, Mexico), La Tortuga (Latitude, 25º51'33.6", Longitude, 101º15'58.2", Ramos Arizpe, Coahuila, Mexico) and Margaritas del Norte (Latitude, 26º28'08.1", Longitude, 102º51'29.3", San Pedro de las Colonias, Coahuila, Mexico). The branches were defoliated manually, following by a washing procedure using distilled water and then dried in an oven at 50 °C for 24 hours. For the polyphenolic extraction, the oregano leaves were crushed in a blender. The particle size was estimated in a range between 2-5 mm. Finally, the resulting material was stored using plastic bags in dark conditions at room temperature until use.

2.2. EOs Extraction Equipment

For the extraction of EOs, a steam distillation system was required, with an operating time of one hour from displaying the condensation in the refrigerant. The process consists of three stages: The first stage consists in the water vapor generation, it was completed by adding one liter of distilled water in a flat-bottomed flask ball, the heat was supplied by direct flame, the second stage comprises the plant material (50 g of oregano leaves) in a 3-mouth ball, in this stage the flow of steam that passes through the plant material extract the compounds, the steam is withdrawn through a connector to a Liebig condenser (40 cm) connected to a chiller system that implements ethylene glycol as a counter-current refrigerant (4 °C), and finally the condensed product was deposited in a separation funnel (100 ml) , to carry out a separation by decantation, the oil obtained was stored in a freezer at (4 °C) in the dark until analysis.

2.3. Ultrasound Assisted Extraction (UAE)

The extraction of phenolic compounds was carried out using the methodology proposed by Castro-López, *et al.* [20], with some modifications. Each sample (5 g) was extracted by adding 60 mL of 35:65 (v/v) ethanol: water at room temperature, maintaining the 1:12 solid: liquid ratio. All extractions were made in dark brown reagent bottles and were immersed for 40 min at room temperature (25 °C) in an ultrasound bath (Model 2510, Sonics and Materials, Branson, MO, USA) to a 40 KHz power (100%). Subsequently, the extracts used were filtered by a vacuum pump using Whatman # 41 filter paper, the extraction solution was removed from the filtered extracts by drying in an oven at 50 °C for 48 h.

2.4. Purification of Extracts

The purification was carried out by a stationary phase (Amberlite XAD-16) and a mobile phase (ethanol). First, the dried extract was resuspended in 40 mL of bi-distilled water, 20 mL of sample was added to stationary phase and bi-distilled water was used as the eluent to discard unwanted compounds, then, ethanol was used as the eluent to obtain the purified polyphenolic compounds. The solvent was evaporated (50 °C for 48 hrs.) and the polyphenols were recovered as fine powder.

2.5. Antioxidant Activity

The purified samples were resuspended in distilled water and a single concentration (500 µg mL^{-1}) of the samples was considered to evaluate the lipoperoxidation and Folin-Ciocalteu. In addition, the essential oil samples were diluted in absolute ethanol, a single dilution (1: 100 v/v) was considered to evaluate the antioxidant activity assays. For additional tests ($DPPH^{\bullet}$), initial solutions were prepared at 1000 µg mL^{-1} for phenolic samples, and 1000 mg mL^{-1} for EOs.

2.6. $DPPH^{\bullet}$ Radical Scavenging Assay

The methodology was carried out with little modifications according to the methodology proposed by Brand-Williams, *et al.* [21], the electron donation capacity of the samples was evaluated from a purple color solution of radical $DPPH^{\bullet}$, using methanol as a solvent (60 mM). Subsequently, 2950 µL of $DPPH^{\bullet}$ radical were added to each test tube for every 50 µL of sample or standard curve (gallic acid). The reaction solution was subjected to an incubation period in the dark for 30 minutes, subsequently, the absorbance of the samples was recorded at a wavelength of 517 nm.

Inhibition capacity was calculated using the Equation (1) and the result was expressed by IC_{50} value (concentration necessary to reach 50% of radical inhibition):

$$\text{Inhibition (\%)} = [(A_{Control} - A_{Sample})/A_{Control}] * 100 \quad (1)$$

where $A_{Control}$ represents the absorbance value of the control and A_{Sample} represents the absorbance value obtained for each specific sample.

2.7. Reducing Capacity (Folin Ciocalteu)

The reducing capacity was determined using the Folin-Ciocalteu reagent according to the methodology proposed by Georgé, *et al.* [22]**,** with slight modifications. The Folin-Ciocalteu assay is an electron transfer method and gives reducing capacity which is expressed as phenolic content. First, 250 µL of diluted sample, 250 µL of Folin Ciocalteu reagent and 250 µL of sodium carbonate (75 g L^{-1}) were added. The resulting reaction solution was homogenized and incubated at 40 °C for 30 minutes in a water bath. Subsequently, 2000 µL of distilled H_2O were added, and it was analyzed at a wavelength of 750 nm. Finally, the results were reported as gallic acid µg equivalents per milliliter (µg GAE mL^{-1}) according to linear regression using a calibration curve.

2.8. Lipid Peroxidation Inhibition (LPO) Assay

The test was determined according to that described by Zou, *et al.* [23]**,** by means of slight modifications. A linoleic acid solution was prepared by mixing 0.6 g of linoleic acid and 1.5 g of Tween 20 dissolved in 8 mL of ethanol. Next, 100 µL of a linoleic acid solution and 1500 µL of the acetate buffer (0.02 M, pH 4) were mixed with 50 µL of the sample to be analyzed (control = 50 µL of distilled water). The samples were homogenized and incubated at 37 °C by water bath for a minute. After incubation time, the oxidative solution consisting of 750 µL of 50 M $FeCl_2$ solution (0.01 g of $FeCl_2$ and 0.017 g of EDTA diluted to 100 mL with distilled water) was added to induce lipid oxidation and incubated for 24 h 37 °C. Finally, 250 µL aliquots were withdrawn to analyze the oxidation in a time of 0 and 24 h. To each aliquot, 1 mL of NaOH (0.1 M, in 1:10, ethanol: water) was added to stop the oxidation process, then 2.5 mL of ethanol (1:10, ethanol: water) was placed to dilute the sample. Finally, the absorbance of the samples was measured at 232 nm, using ethanol (1:10, ethanol: water) as a blank. The percent inhibition of linoleic acid oxidation was calculated with the following Equation 2:

$$\text{Lipid oxidation inhibition (\%)} = [(A - B)/A] * 100 \quad (2)$$

where A is the difference between the absorbance of distilled water (as control) after 24 h and 0 h of incubation, and B is the difference between the absorbance of each extract sample after 24 h and 0 h of incubation.

2.9. Liquid Chromatography Coupled to Mass Spectrometry (UPLC-ESI-Q/TOF-MS^2)

The system used was an ultra-performance liquid chromatograph (UPLC), coupled to an autosampler and a binary pump equipped with a 10 µL loop (partial loop injection mode). The qualitative identification of the polyphenols present in the sample was performed using a PHENYL chromatographic column (2.1 mm x 100 mm, 1.7 µm, WATERS, UK) operated at 40 °C, following the chromatographic methodology proposed by Castro-López, *et al.* [20], two mobile phases were implemented for the separation of the compounds, as mobile phase A: water + 0.1% (v/v) formic acid and as solvent B: acetonitrile, using a constant flow of 0.3 mL / min. A sample volume of 3 µL was used by an automatic sampler with a fast scanning time of 10 minutes. The chromatographic method consists of a flow of 97% A for 1.10 minutes, followed by gradients from 5% B to 15% B between 1.10 to 4.40 minutes, 15% B is maintained for 4.60 minutes, finally returns to the conditions initial (3% B) in 1 minute, to balance the column. The purified phenolic samples selected for the analysis were those that expressed a higher activity against $DPPH^{\bullet}$ radical with lower IC_{50} values (Table 1).

2.10. Mass Spectrometry Method

MS detection was performed in a quadrupole-time-of-flight (Q-TOF ™, Waters, Mil-ford, MA, USA) orthogonal accelerated Q-TOF mass spectrometer, equipped with an electrospray ionization source (ESI). The full screen mass spectra detection was carried out in the negative ion mode in a mass range from 50–1200 m/z and using a capillary voltage of 3.0 kV, a dry gas temperature of 210° C, a dry gas flow of 8.0 L min^{-1}, a nebulizer pressure of 2.0 bar, and spectra rate of 1 Hz. Moreover, MS^2 experiments were performed using a ramp collision energy of 15–35 V with argon as collision gas and scan every second. Comparison of the observed MS^2 spectra with those found in the literature and Databases (Mass Bank of North America, Mass Bank Europe, and PubChem) was the main tool for identification of the compounds.

2.11. Gas Chromatography Coupled to Mass Spectrometry (GC-MS)

The GC-MS analysis used the methodology described by Sethi, *et al.* [24] with slight modifications. Which was carried out in Agilent 7890B equipment with 5975C triple-axis mass selective detector, nitrogen was used as carrier gas. 2 µL of each sample will be injected, where the temperature of the injector and the temperature of the ion source were 250 °C and 220 °C respectively. The oven temperature was initially maintained at 70 °C for 3 minutes and then increased to 170 ° C, the rate of increase was 10 ° C / min and this temperature was maintained for 10 minutes and finally the temperature increased to 250 °C for 20 minutes. The mass spectrum was recorded at 70ev with a 0.5 second scan interval and a mass range of 50-650 uma. Comparison of the observed MS spectra with those found in Databases from Agilent 7890B was the main tool for identification of the compounds.

2.12. Experimental Design and Statistical Analysis

All experiments were conducted at three levels of measurements and results reported as mean ± standard deviation (SD). The data were analyzed by using one-way analysis (Factor = sample location) of variance (ANOVA) followed by Tukey's HSD Test with p = 0.05. Statistical analysis was performed using Minitab 17 Statistical Software (Minitab, Inc., State College, Pennsylvania, PA, USA).

3. Results

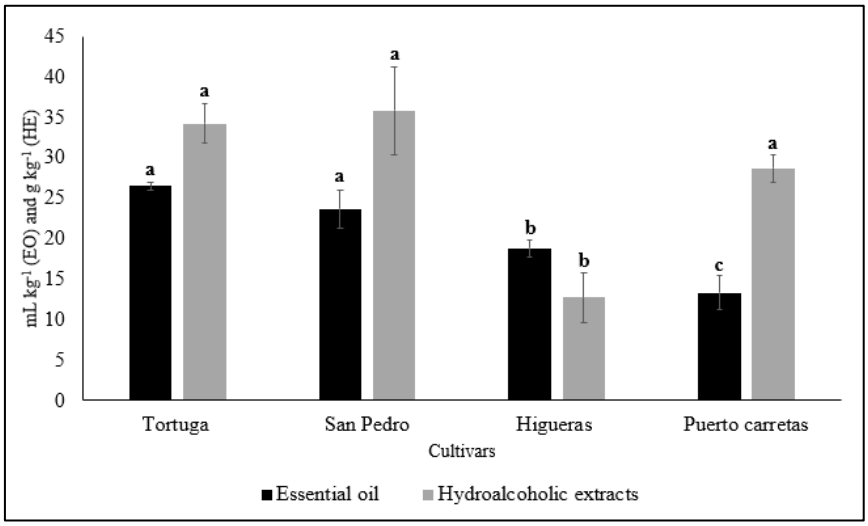
3.1. Extraction Yields of Process

The extraction yield shows statistical differences between locations (p<0.05) on the following descending order: Tortuga > San Pedro > Higueras > Puerto Carretas (Figure 1). The higher EO extraction yields (26.5 ± 0.500 – 13.33 ± 2.081 mL kg^{-1}) englobes "Tortuga" and "San Pedro" locations. Otherwise, the extraction yield for essential oil is similar to data reported by Morshedloo, *et al.* [25], who evaluated different tissues of *Origanum vulgare* such as leaves, roots and stem, the study showed the highest yield for leaves with 23.6 ± 1.5 mL kg^{-1} and lower yields for additional tissues. In addition, other authors reported an extraction interval between 22.0 – 47.0 mL kg^{-1} for *L. graveolens* and *Origanum onites L.* Our data supports the previously fact that leaves have a strong potential as an extraction plant material for recover essential oil, and the yields belongs on the literature interval [26, 27]**.** Otherwise, the polyphenolic extraction yields (Figure 1) showed a statistical difference (a<0.05) between locations on the following descendent order: San Pedro > Tortuga > Puerto Carretas > Higueras, respectively. The "San Pedro" and "Tortuga" locations (35. 80 ± 5.393 – 34.25 ± 2.367 g kg^{-1}) showed the higher extraction yield, the "Puerto Carretas" and "Higueras" locations reported lower values (28.67 ± 9.701 – 12.73 ± 3.065, g kg^{-1}). The variation is not the aim of this study, but it may be the result of extraction and environmental conditions such as: wind, solar energy and water [28, 29].

3.2. Antioxidant Activity of Different Extracts of *L. Graveolens*

Several studies have established that the reactions involving antioxidant activity are complex, they should not be evaluated by a single method [30]. For this reason, scavenging capacity of free radicals, reduction power and inhibitory effects on lipid oxidation of four *L. graveolens* (essential oil and phenolics) locations were evaluated.

Figure-1. Extraction yield of essential oil (mL kg^{-1}) and purified polyphenolic compounds (g kg-1) obtained from different *L. graveolens* locations



The antiradical activity of plant extracts is associated mainly with polyphenolic content, on increased concentration, the number of hydroxy groups on the reaction medium is higher and also the hydrogen donation possibility to free radical. The antiradical activity of essential oil (Table 1) shows statistical difference between locations (p<0.05) on the following ascending order Tortuga > San Pedro > Higueras > Puerto Carretas. The data show a higher antioxidant activity than that reported for *O. vulgare* (european oregano) evaluated by Pezzani, *et al.* [31], who reported an inhibition interval between 2.85 ± 1% and 3.68 ± 1% using 7 a 9 mg mL^{-1} of EOs, meanwhile our results have 50% inhibition of radical with similar concentrations (8.27 to 55.30 mg mL^{-1}). Otherwise, evaluations in *O. vulgare L.* and *P. longiflora* carried out by Han, *et al.* [32] and Cid-Pérez, *et al.* [33], respectively, reported lower IC_{50} values (0.332 ± 0.040 mg mL^{-1} y 83.70 ± 4.12 µg mL^{-1}) that our study does.

Furthermore, the antiradical activity of the purified polyphenolic compounds ranged from 537.29 µg mL^{-1} to 1496.01 µg mL^{-1}, and it was better than the obtained for EOs samples which ranged from 8.27 mg mL^{-1} to 55.30 mg mL^{-1}, registering for phenolics a lower concentration necessary to reach 50% of the inhibition of the DPPH$^{\bullet}$ radical. The IC_{50} values for purified phenolics (Table 1) show significant differences (p<0.05) between the locations in the following ascending order: San Pedro > Puerto Carretas > Tortuga > Higueras. The hydroalcoholic extract with the best activity against DPPH$^{\bullet}$ radical belongs to "San Pedro" location with an IC_{50} value of 545.40 µg mL^{-1}, a slightly elevated value compared to the data obtained on alcoholic extracts (151.90 ± 6.65 µg mL^{-1}) from *P. longiflora* another Mexican oregano [34].

Table-1. Antioxidant activity (DPPH$^{\bullet}$, Reducing power, and LPO) displayed by EOs and purified polyphenolic compounds obtained from leaves of different *L. graveolens* sample locations

Sample locations	**Antioxidant activity**					
	EOs			**Hydroalcoholic extracts**		
	DPPH$^{\bullet}$ IC_{50} (mg mL^{-1})	**Reducing power** (µg GAE mL^{-1})	**LPO** (% inhibition)	**DPPH$^{\bullet}$ IC_{50}** (µg mL^{-1})	**Reducing power** (µg GAE mL^{-1})	**LPO** (% inhibition)
Higueras	25.66 (±1.86) ^{B}	164.62 (±9.04) ^{A}	99.06 (±3.91) ^{A}	1195.7 (±55) ^{A}	57.22 (±3.66) ^{B}	76.91(±5.09) ^{B}
Puerto Carretas	55.31 (±1.203) ^{A}	63.48 (±6.98) ^{BC}	98.80 (±1.07) ^{A}	567.4 (±45.3) ^{C}	91.20 (±7.47) ^{A}	91.3 (±1.43) ^{A}
Tortuga	8.28 (±0.1021) ^{D}	78.19 (±5.07) ^{B}	95.32 (±0.42) ^{A}	989.17 (±15.77) ^{B}	62.56 (±8.65) ^{B}	66.42 (±3.67) ^{B}
San Pedro	12.84 (±0.610) ^{C}	51.15 (±4.75) ^{C}	55.81 (±2.52) ^{A}	545.40 (±47.2) ^{C}	102.611 (±1.23) ^{A}	73.75 (±7.63) ^{B}

IC_{50} values correspond to the sample concentration achieving 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. In each column, different letters mean significant (p<0.05) differences between the sample location's samples.

The evaluation by Folin-Ciocalteu showed higher reducing power for the locations with lower IC_{50} values on antiradical activity by DPPH$^{\bullet}$ radical, which could be the result of higher phenolic compounds presents on the sample and their hydroxyl group. Similarly, other studies have observed a higher reducing power (by Folin-Ciocalteu and FRAP assays) with the samples presenting a higher activity against DPPH$^{\bullet}$ radical [33, 34].

The lipoperoxidation data identified higher values of inhibition on EOs, in agreement with our results, previous studies have shown the essential oil potential from oregano (*Origanum vulgare*) even with lower concentrations (10 µg mL^{-1}) [35]. In the other hand, the results obtained for purified polyphenolic compounds identified "Puerto Carretas" loca-tion with better inhibition activity close to inhibition values registered for EOs. Contrary to the values obtained by DPPH$^{\bullet}$ assay, the "San Pedro" location showed a lower activity against lipoperoxidation in both extractions products (EOs and hydroalcoholic extract) which could be resulted by interaction between the components present in the sample [36].

3.3. Phytochemical Constituents Profile of *L. graveolens locations*

The analysis of compounds carried out on UPLC-ESI-Q/TOF-MS^2 and GC-MS in samples of *L. graveolens* showed the presence of bioactive compounds that could be related with beneficial properties of extracts. The hydroalcoholic extracts selected for the analysis were those that expressed a higher activity against DPPH$^{\bullet}$ radical and a higher reducing power (Table 1).

3.3.1. Essential Oils (EOs)

The evaluation of EOs samples by GC-MS allowed the partial tentative identification (Table 2) of compounds belonging to the class of terpenes and oxygenated compounds. The information revealed unique signals for location, in "San Pedro" location twenty-nine compounds was detected (fourteen unique), "Tortuga" showed twenty-three compounds (eleven unique), "Puerto carretas" demonstrated fifteen compounds (six unique) and "Higueras" showed twenty-nine compounds (thirteen unique). In 2018, Barbieri, *et al.* [37] published a complete analysis of *L. graveolens* essential oil and identified as major terpenes and sesquiterpenes: p-cymene, eucalyptol, γ-terpinene, thymol, carvacrol, β-caryophyllene, α-humulene and caryophyllene oxide, all of them present in our study. Also, β-pinene, terpinene-4-ol, α-terpineol and thymol acetate have been identified by Dos Santos, *et al.* [38]. Finally, the presence of oleic acid is reported in oregano essential oil obtained by cold pressed technology [39].

The identified compounds englobe molecules reported on literature for oregano species such a thymol and carvacrol, the compounds were found as monoterpene, but also in derivatives forms as: thymol methyl ether, thymol acetate, isothymol methyl ether and carvacryl acetate. Other similar compounds correspond to sesquiterpenes molecules well reported in previously studies. The presence and absence of certain compounds between different locations could be the result of volatility capacity. For example, the β-Cymene was identified in "San Pedro" and "Higueras" crop, and its absence in other crops could be the result of the high volatility and a low compound boiling point (175.1 °C). Also, the absence of other compounds like "Carvacrol" with a higher boiling point (237-328 °C) could be the product of growing conditions such as: soil conditions, pests, geographical location, and climate [40].

The data showed the presence of other relevant bioactive compounds beyond thymol and carvacrol like β-elemene, a compound found mainly in a traditional Chinese medicinal herb (Curcuma wenyujin) with an interesting activity by inhibition of tumoral prolifera-tion and apoptosis induction [41]**.** Also, β-elemene has negative effects on tu-mor-associated macrophages (TAMs) which have been reported to promote tumorigenesis [42]. Furthermore, its activity as auxiliary against atherosclerosis and other inflammation diseases has been studied in previously reports [43, 44]. Other compounds as β-cymene and humelene have showed a different bioactivity as auxiliary in inflammatory, cancer and microbial ailments [45, 46].

Table-2. Mass spectral data and tentative identification of present compounds in EOs of *L. graveolens*

Sample location	N^0 peak	Retention time (min)	m/z Experimental [M-H]-	m/z Calculated [M-H]-	Tentative assignment	Molecular formula	MS^2 Fragmention	Class
San Pedro de las Colonia	1	1.199	136.1252	136.23	β-Pinene	$C_{10}H_{16}$	93	Monoterpene
	2	1.279	134.1095	134.22	β-Cymene	$C_{10}H_{14}$	91	Non-oxygenated Monoterpene
	3	1.338	154.1357	154.25	Eucalyptol	$C_{10}H_{18}O$	43	Monoterpene
	4	1.396	154.1357	154.25	Terpineol, cis-β	$C_{10}H_{18}O$	43	Monoterpene
	5	1.518	154.1357	154.25	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	$C_{10}H_{18}O$	43	Monoterpene
	6	1.693	154.1357	154.25	Terpinen-4-ol	$C_{10}H_{18}O$	71	Monoterpene
	7	1.741	154.1357	154.25	α-Terpineol	$C_{10}H_{18}O$	59	Monoterpene
	8	1.937	444.1127	-	-	-	73	Not identified
	9	2.296	150.1044	150.22	Carvacrol	$C_{10}H_{14}O$	91	Monoterpene
	10	2.603	164.0837	164.2	Eugenol	$C_{10}H_{12}O_2$	77	Monoterpene
	11	2.684	222.1983	222.36	4-epi-cubedol	$C_{15}H_{26}O$	43	Sesquiterpenes
	12	2.781	204.1878	204.35	β-elemene	$C_{15}H_{24}$	81	Sesquiterpene
	13	2.954	222.1983	222.37	α-acorenol	$C_{15}H_{26}O$	93	Sesquiterpenes
	14	3.111	204.1878	204.35	Caryophyllene	$C_{15}H_{24}$	43	Sesquiterpenes
	15	3.268	204.1878	204.35	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1α,7β,8aα)]-	$C_{15}H_{24}$	41	Sesquiterpenes
	16	3.431	204.1878	204.35	Humulene	$C_{15}H_{24}$	93	Sesquiterpenes
	17	3.672	204.1878	204.35	β-copaene	$C_{15}H_{24}$	91	Sesquiterpenes
	18	3.814	180.1514	180.29	1H-Inden-1-ol, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-	$C_{12}H_{20}O$	43	Sesquiterpenes
	19	4.006	204.1878	204.35	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	$C_{15}H_{24}$	41	Sesquiterpenes
	20	4.11	222.1983	222.36	cubedol	$C_{15}H_{26}O$	43	Sesquiterpenes
	21	4.443	264.2089	264.4	Nerolidyl acetate	$C_{17}H_{28}O_2$	43	Sesquiterpenes
	22	4.525	222.1983	222.36	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4β)]-	$C_{15}H_{26}O$	59	Sesquiterpenes

	23	5.041	220.1827	220.35	Caryophyllene oxide	$C_{15}H_{24}O$	43	Epoxide
	24	5.421	220.1827	220.35	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R,3E,7E,11R)]-	$C_{15}H_{24}O$	43	Sesquiterpenes
	25	5.625	220.1827	220.35	Isoaromadendrene epoxide	$C_{15}H_{24}O$	41	Epoxide
	26	5.743	222.1983	222.37	Cubenol	$C_{15}H_{26}O$	41	Sesquiterpenes
	27	5.915	220.1827	220.35	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	$C_{15}H_{24}O$	41	Sesquiterpenes
	28	6.202	222.1983	222.36	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]-	$C_{15}H_{26}O$	59	Sesquiterpenes
	29	6.544	220.1827	220.35	trans-Z-α-Bisabolene epoxide	$C_{15}H_{24}O$	43	Epoxide
Tortuga	1	1.188	136.1252	136.23	β-Pinene	$C_{10}H_{16}$	93	Monoterpene
	2	1.432	136.1252	136.23	3-Carene	$C_{10}H_{16}$	93	Monoterpene
	3	1.547	154.1357	154.13	1.4,6,6-Trimethyl-bicyclo[3.1.1]heptan-2-ol	$C_{10}H_{18}O$	85	Monoterpene
	4	1.576	152.1201	152.23	2,7-Octadien-4-ol, 2-methyl-6-methylene-, (S)-	$C_{10}H_{16}O$	85	Monoterpene
	5	1.432	154.1357	154.25	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	71	Monoterpene
	6	1.798	154.1357	154.25	Terpinen-4-ol	$C_{10}H_{18}O$	71	Monoterpene
	7	2.311	150.1044	150.22	Carvacrol	$C_{10}H_{14}O$	91	Monoterpene
	8	2.465	150.1044	150.2176	Thymol	$C_{10}H_{14}O$	91	Monoterpene
	9	3.448	150.1408	150.26	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	$C_{11}H_{18}$	93	Monoterpene
	10	3.649	204.1878	204.35	Humulene	$C_{15}H_{24}$	93	Sesquiterpenes
	11	3.965	204.1878	204.35	β-Bisabolene	$C_{15}H_{24}$	69	Sesquiterpenes
	12	4.341	180.115	180.24	Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	$C_{11}H_{16}O_2$	77	Sesquiterpenes
	13	5.332	220.1827	220.35	Caryophyllene oxide	$C_{15}H_{24}O$	43	Epoxide
	14	5.692	220.1827	220.35	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R,3E,7E,11R)]-	$C_{15}H_{24}O$	43	Sesquiterpenes
	15	6.823	220.1827	220.35	Isoaromadendrene epoxide	$C_{15}H_{24}O$	41	Sesquiterpenes
	16	7.402	220.1827	220.35	cis-Z-α-Bisabolene epoxide	$C_{15}H_{24}O$	43	Epoxide
	17	8.593	666.1691	-	-	-	73	Not identified
	18	9.869	536.5896	537	1-Heptatriacotanol	$C_{37}H_{76}O$	43	Alcoholic compound
	19	10.888	250.2296	250.4	5,8,11-Heptadecatrien-1-ol	$C_{17}H_{30}O$	79	Alcoholic compound
	20	10.925	318.2558	-	-	-	91	Not identified
	21	11.657	292.2402	292.5	Methyl 5,9,12-octadecatrienoate	$C_{19}H_{32}O_2$	67	Ester
	22	13.671	772.2986	-	-	-	91	Not identified
	23	15.629	324.2453	324.5	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	43	-
Puerto Carretas	1	0.995	193.8859	195.9	2-Propanone, 1,1,3,3-tetrachloro-	$C_3H_2Cl_4O$	43	Oxygenated compounds
	2	1.276	140.1201	140.22	1,8-Nonadien-3-ol	$C_9H_{16}O$	57	Alcoholic compound
	3	1.401	136.1252	136.23	γ-Terpinene	$C_{10}H_{16}$	93	Monoterpene
	4	1.774	154.1357	154.25	Terpinen-4-ol	$C_{10}H_{18}O$	71	Monoterpene
	5	1.868	164.1201	164.24	Thymol methyl ether	$C_{11}H_{16}O$	91	Monoterpene
	6	2.8	150.1044	150.21	Thymol	$C_{10}H_{14}O$	91	Monoterpene
	7	3.178	204.1878	204.35	Caryophyllene	$C_{15}H_{24}$	43	Sesquiterpenes
	8	3.487	204.1878	204.35	Humulene	$C_{15}H_{24}$	93	Sesquiterpenes
	9	3.706	204.1878	204.35	α-Farnesene	$C_{15}H_{24}$	41	Sesquiterpene hydrocarbons
	10	3.873	204.1878	204.35	γ-Elemene	$C_{15}H_{24}$	41	Sesquiterpenes
	11	4.565	222.1983	222.36	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4β)]-	$C_{15}H_{26}O$	59	Sesquiterpenes
	12	5.063	220.1827	220.35	Caryophyllene oxide	$C_{15}H_{24}O$	43	Epoxide
	13	5.222	592.1503	-	-	-	73	Not identified
	14	8.134	666.1691	-	-	-	73	Not identified
	15	28.99	282.2558	282.5	Oleic Acid	$C_{18}H_{34}O_2$	55	Monounsaturated fatty acid

	1	1.113	136.1252	136.23	β-Thujene	$C_{10}H_{16}$	93	Monoterpene
	2	1.162	128.1201	128.21	1-Octen-3-ol	$C_8H_{16}O$	57	C8 aliphatic compounds
	3	1.25	134.1095	134.22	β-Cymene	$C_{10}H_{14}$	91	Non-oxygenated monoterpenes
	4	1.299	136.1252	136.23	γ-Terpinene	$C_{10}H_{16}$	93	Monoterpene
	5	1.358	154.1357	154.25	Terpineol, cis-β	$C_{10}H_{18}O$	43	Monoterpene
	6	1.566	212.1776	212.33	Tridecanedial	$C_{13}H_{24}O_2$	55	Aliphatic compounds
	7	1.663	154.1357	-	-	-	95	Not identified
	8	1.817	164.1201	164.24	Isothymol methyl ether	$C_{11}H_{16}O$	91	Derivative of thymol
	9	2.184	150.1044	150.22	Thymol	$C_{10}H_{14}O$	91	Monoterpene
	10	2.413	150.1044	150.22	Carvacrol	$C_{10}H_{14}O$	91	Monoterpene
	11	2.493	192.115	192.25	Thymol acetate	$C_{12}H_{16}O_2$	43	Derivative of thymol
	12	2.601	192.115	192.25	Carvacryl acetate	$C_{12}H_{16}O_2$	43	Derivative of carvacrol
Higueras	13	2.773	204.1878	204.35	(-)-β-Bourbonene	$C_{15}H_{24}$	81	Sesquiterpenes
	14	2.842	164.1201	164.24	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	$C_{11}H_{16}O$	79	Cyclic ketone.
	15	3.093	204.1878	204.35	Caryophyllene	$C_{15}H_{24}$	93	Sesquiterpenes
	16	3.391	204.1878	204.35	Humulene	$C_{15}H_{24}$	93	Sesquiterpenes
	17	3.523	204.1878	204.35	γ-Muurolene	$C_{15}H_{24}$	41	Sesquiterpenes
	18	3.645	204.1878	204.35	Germacrene D	$C_{15}H_{24}$	91	Sesquiterpenes
	19	3.793	204.1878	204.35	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1α,7β,8aα)]-	$C_{15}H_{24}$	41	Sesquiterpenes
	20	3.98	204.1878	204.35	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	$C_{15}H_{24}$	41	Sesquiterpenes
	21	4.166	222.1983	222.36	cubedol	$C_{15}H_{26}O$	43	Sesquiterpenes
	22	4.521	222.1983	222.37	6-epi-shyobunol	$C_{15}H_{26}O$	81	Elemane sesquiterpenoids
	23	4.992	220.1827	220.35	Caryophyllene oxide	$C_{15}H_{24}O$	43	Epoxide
	24	5.184	592.1503	-	-	-	73	Not identified
	25	5.545	222.1983	222.37	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	$C_{15}H_{26}O$	95	-
	26	6.146	220.1827	220.35	Isoaromadendrene epoxide	$C_{15}H_{24}O$	41	Epoxide
	27	8.234	498.356	-	-	-	73	Not identified
	28	9.62	150.1044	150.22	Cyclohexanone, 2-(2-butynyl)-	$C_{10}H_{14}O$	91	Cyclohexanone
	29	14.831	324.2453	234.5	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	43	-

3.3.2. Hydroalcoholic Extracts

The chemical profile of the polyphenolic compounds allowed the tentative identification of 5 compounds belonging to the flavonoid family (glycosylated compounds) and one dicarboxylic acid (Table 3). Peak 1 of the "San Pedro de las Colonias" location corresponds to isopropylmalic acid, a dicarboxylic acid with a parent ion value of m/z 175.0045 $[M - H]^-$ and a fragment ion value of m/z 131.0177 $[M - H]^-$ which would correspond to the loss of a $[M - H - CHO_2]$ fragment reported on data base. In Puerto Carretas location, peak 2 corresponds to Hesperetin-7-O-rutinoside with a parent ion m/z 609.131 $[M - H]^-$ and a fragment ion mass of m/z 285.033 that would correspond to the loss of a fragment $[M - H - C_{13}H_{25}O_9]$, the Hesperetin-7-O-rutinoside corresponds to a flavanone typically found in citrus fruits and it has showed a reduction of the osteoporosis risk in animal models [47], peak 3 Kaempferol-3-O-glucoside-2 " - p-coumaroyl m/z 593.1393 $[M-H]^-$ and a fragment mass m/z 285.0353, peak 4 Kaempferol-3-O-glucoside corresponds to m/z 447.0842 $[M - H]^-$ according to its next fragment with a mass value of 285.0340 $[M - H]^-$ the loss of 162 units of mass indicates the presence of a hexose unit. This compound has been previously related with hepatoprotective activity in HepG2 cells [48]. Also, it was identified in methanolic extract of *Erica multiflora* with preventive activity against obesity [49]. Likewise, the plants grown in "San Pedro de las Colonias" presented (peak 8) isoquercitrin, previously reported on *Origanum vulgare* spp. [50] with a parent ion m/z 464.0894 $[M - H]^-$ and a fragment corresponding to m/z 301.0680, which corresponds to the loss of a fragment $[M–H– C_6H_{11}O_5]$. Finally, peak 9 was identified as Kaempferol-3-O-glucoside with parent ion m/z 447.0842 $[M - H]^-$, a compound previously registered in the "Puerto Carretas" location. The analysis allowed to determine the partial composition of the polyphenolic compounds for the 2 localities that showed better antioxidant activities, compounds as: cirsimaritin, apigenin, acacetin, hesperetin and kaempferol, have already been previously related (*O. majoram* and *L. graveolens*) and methylxanthine (as 7-methylxanthine) in oregano characterization studies [16, 51].

Table-3. Mass spectral data and tentative identification of present compounds in purified hydroalcoholic extracts of *L. graveolens*

Sample location	N° peak	Retention time (min)	*m/z* Experimental $[M-H]^-$	*m/z* Calculated $[M-H]^-$	Tentative assignment	Molecular formula	Error (mg/L)	MS^2 Fragmention	Class
Puerto Carretas	1	3.138	646.0934	-	Unknown	-	-	623.1132	Not identified
	2	3.327	610.131	610.1898	Hesperetin-7-O-rutinoside	$C_{28}H_{34}O_{15}$	-96.36	285.033	Glycosylated flavonoid
	3	3.876	594.1393	594.1373	Kaempferol-3-O-glucoside-2"-*p*-coumaroyl	$C_{30}H_{26}O_{13}$	3.36	285.0353	Glycosylated flavonoid
	4	4.219	448.0842	448.1006	Kaempferol-3-O-glucoside	$C_{21}H_{20}O_{11}$	-36.59	285.0340	Glycosylated flavonoid
	5	4.854	480.1096	-	Unknown	-	-	479.1096	Not identified
	6	5.025	580.124	-	Unknown	-	-	285.0343	Not identified
San Pedro de las Colonias	1	0.634	176.0045	176.0685	Isopropylmalic acid	$C_7H_{12}O_5$	-363.49	175.0044	Dicarboxylic acid
	2	0.814	392.1394	-	Unknown	-	-	175.0044	Not identified
	3	1.029	392.1404	-	Unknown	-	-	175.0044	Not identified
	4	1.723	390.1241	-	Unknown	-	-	389.1247	Not identified
	5	2.461	388.1839	-	Unknown	-	-	387.184	Not identified
	6	2.967	554.136	-	Unknown	-	-	553.1375	Not identified
	7	3.147	226.156	-	Unknown	-	-	225.1563	Not identified
	8	3.301	464.0894	464.095	Isoquercitrin	$C_{21}H_{20}O_{12}$	-12.06	463.0893	Glycosylated flavonoid
	9	3.85	448.0842	448.1006	Kaempferol-3-O-glucoside	$C_{21}H_{20}O_{11}$	-36.59	285.0340	Glycosylated flavonoid

3.4. Health Perspectives

Several studies have been elucidated the potential of botanical extracts used by traditional medicine for disease treatment by different technologies with the aim to understand the chemical components and the molecular mechanism involved. According to literature, the application of bioactive compounds could boost cellular mechanism and functions with positive impact against possible infection as lower incidence and severity [52]. The main family of bioactive compounds involve the polyphenolic compounds which has been well study and related with an important role in boosting cellular functions as antioxidant, antibacterial, anti-inflammatory auxiliary against some diseases [53-55]. In addition, the terpenoids show bioactive properties against microbial infections and other diseases [41, 42]. The oregano EO demonstrated antifungal activity, the application of *L. graveolens* EO against *Candida albicans* where showed an MLC (minimum lethal concentration) of 6.4µL mL^{-1} [56]. Also, the UV-protection activity was probed in methanolic extracts applied in SKH-1 mice, it showed an absorption value of 20.14 ± 1.86 µg cm^{-2} and a reduction of skin damage with respect of control group, according to the authors the activity could be related with the presence of polyphenolic compounds [57].

The application of EO comprises a complex procedure and the final product could be a health similar with the Myrtol® standardized, which englobes an herbal medical product (phytomedicine) from essential oils with antioxidative, anti-inflammatory, and antibacterial benefits while other studies confirmed its secretolytic and bronchospasmolytic effect [58]. Finally, the chemical diversity of EOs in aromatic plants improve the research activity to elucidate a new alternative in health field. In addition, the oregano EO application requires more detailed analysis for possible future applications.

4. Conclusion

The present study demonstrated the potential of leaves from Mexican crops of *L. graveolens* cultivated in the northern region of the country as a source of bioactive compounds with strong antioxidant activity, which has a possible future application in the development of novel products or treatment of diseases. The analysis of the plant material allowed obtaining similar or slightly higher EO extraction yields (26.5 ± 0.500 – 13.33 ± 2.081, mL kg^{-1}) than those previously reported in the literature, the analysis of antioxidant activity identified the essential oil from "Tortuga" (8.28 ± 0.1021, mg mL^{-1}) and the extract from "San Pedro de las Colonias" (545.40 ± 47.2, µg mL^{-1}) as the locations with the best antiradical activity, otherwise, lipoperoxidation shows the greatest potential of essential oils as auxiliary inhibitors (99.06 ± 3.91%). A partial identification of the samples was obtained with a total sixty-one compounds for EOs and six for hydroalcoholic extracts, the tentative compounds englobe flavonoids, terpenes,

sesquiterpenes, xanthines and dicarboxylic acid. Also, phytochemical diversity and variation are evident when locations are compared, which could be the result of the genetic constitution and climatic conditions. Despite these positive results more detailed studies are needed to confirm this possible correlation with genotypes, increasing the number of individuals, detecting more phytochemical diversity, and complementing with gene expression studies.

Declarations

Author contribution statement

Israel Bautista-Hernández, Mireya Vázquez-Aguilar, Cecilia Castro-López: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Alma D. Paz-González, Gildardo Rivera: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Guillermo C. G. Martínez-Ávila and Romeo Rojas: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Declaration of Interests Statement

The authors declare not conflict of interests.

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