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Relationship Between the Chemical Composition and Transport Properties with the Antimicrobial Activity of Essential Oil from Leaves of Mexican Lippia (*Aloysia citriodora*) Extracted by Hydro-Distillation

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Abstract: Mexican lippia (*Aloysia citrodora*) essential oil (LEO) was extracted by hydro-distillation from whole or ground leaves for up to 2h, then analyzed by GC–MS. Results exhibited that the extraction by hydro-distillation at 1h using ground leaves had a higher yield than other tested conditions. A total of thirty-one components were identified in the studied LEO, characterized by high levels of monoterpenes (> 40%) and moderate levels of sesquiterpenes (< 25%); citral, limonene, and spathulenol were its principal compounds. Extracted LEO displayed better antimicrobial activity against Gram-positive bacteria and better activity in the vapor phase than by direct contact (aqueous media) since its principal compounds and chemical structure exhibited better diffusivity coefficients in air and good transport properties like partition coefficient and solubility. Therefore, according to our results, Mexican lippia should be further studied for its application to diverse foods.

Keywords: *Aloysia citriodora*; essential oil; chemical composition; chemical structure; antimicrobial activity; transport properties.

1. Introduction

Lippia (*Aloysia citriodora*), also known as lemon verbena, cedron, or hierba luisa, is one of the 200 species belonging to the Verbenaceae family [1]. It has been cultivated in America, Asia, Europe, and Africa. *A. citriodora* is an evergreen shrub ranging from 1 to 3 m tall, with ridged and rough branches and 7-10 cm marginate lanceolate leaves. Traditionally, it is used as an antispasmodic, diuretic, digestive, cardiotonic, and sedative remedy [2].

Among the substances of natural origin that can be found are essential oils (EOs), which are economically and scientifically important. Plants produce EOs as secondary metabolites, and they exhibit several biological properties, especially antimicrobial, due to the presence of different compounds, such as phenolic compounds, phenolic acids, terpenoids, and alkaloids [3]. However, there has been limited research on these compounds' structure-function relationship. As a result, the importance of the chemical composition of plant-derived compounds regarding their antimicrobial activity is still not well understood.

Lippia EO (LEO) generally stands out for having terpenes (limonene, neral, and geranial) and phenolic compounds (verbascoside and isoverbascoside). In particular, the antimicrobial activity of LEO is attributed to the quantitative presence of at least 18 oxygenated monoterpenes and sesquiterpenes, in addition to the effect of synergistic interactions amongst the various components against bacteria (Gram-positive and Gram-negative) and fungi [4].

In addition, many authors have focused on the antimicrobial activity of different EOs; however, although there is extensive knowledge about minimal inhibitory concentrations (MICs) for different microorganisms, the intention to relate physicochemical and transport properties of a substance with its antimicrobial activity is a topic of interest [5]. To generate precise knowledge of the MICs and properties of EOs, to find the best method of application, and allow a balance between sensory acceptability and antimicrobial efficacy and, thus, to be utilized as natural preservatives.

Therefore, the present work aims to evaluate the chemical composition and antimicrobial activity by direct contact or vapor phase of LEO (cultivated in Mexico) extracted by hydro-distillation and relate it to the chemical structure of major compounds and estimated transport properties using a predictive model.

2. Materials and Methods

2.1. Plant materials.

Dried leaves of lippia were purchased from Redmexplant (Puebla, Mexico). Two types of samples were utilized for extraction, whole and ground leaves (using a commercial blender (Nutribullet 600 series, China) for 20 s and sieving in an 80 µm mesh).

2.2. Essential oil extraction.

A Clevenger-type apparatus was utilized to obtain studied LEOs by hydro-distillation [6]. Distillation is traditionally utilized to isolate volatile compounds such as EOs from plant material. EOs recovery is facilitated by distilling two immiscible liquids (water and essential oil); then, the water and oil vapor mixture is condensed where the EO is separated [7]. The extraction conditions evaluated were for ground and whole leaves (50 g) in a ratio of plant material to distilled water of 1:30 w/v for up to 2 h. Extracted LEOs were separated from the aqueous layer and subsequently dried over anhydrous sodium sulfate and stored at 4°C in sealed vials before analysis. EO yield was calculated as follows [8]:

$$\text{Yield (\%)} = \frac{Mass\ of\ essential\ oil}{Mass\ of\ the\ dried\ leaves} \text{ x } 100 \qquad \text{Eq. 1}$$

2.3. Gas chromatography/mass spectrometry (GC/MS) analysis.

LEOs were analyzed by a gas chromatographer (6850 Series Network, Agilent Technologies, Santa Clara, CA), enabled with a mass selective detector (5975C VL) and a triple-axis detector (Agilent Technologies). Components were separated by an HP-5MS (5% phenyl – 95% polydimethylsiloxane) capillary column (30 m by 0.35 mm, 0.25 µm film thickness). Helium was used as a carrier gas at a constant flow mode of 1.5 mL/min. The column temperature was initially 60 °C for 10 minutes, increasing every 5 minutes until reaching 240 °C, and maintained at 240 °C for 50 minutes. The injector temperature was 240 °C. The retention index was calculated by a homologous series of n-alkanes (Sigma, St. Louis,

MO). Compounds were identified by comparing the obtained mass spectra with those reported in the US National Institute of Standard Technology (NIST) Library and the Shimadzu retention index isothermal equation [9].

2.4. Antimicrobial activity.

2.4.1. Bacterial strains.

From the Food Microbiology Laboratory of the Universidad de las Américas Puebla (UDLAP, Cholula, Mexico), bacterial strains (*Listeria monocytogenes* Scott A and *Escherichia coli* ATCC 25922) were attained and then sustained on Tryptic Soy Agar (TSA; Difco, BD, Sparks, MD) slants at 5 °C.

Cultures were prepared by inoculating tested bacteria (*L. monocytogenes* or *E. coli*) into 10 mL of Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) and incubated at 35 ± 1 °C for 24 h. To ensure the strains' viability, cultures were reinoculated into TSB and incubated for another 24 h before use. Inoculum cell concentrations were adjusted to 10^7 CFU/mL with a hemocytometer [9] and verified by plating on TSA plates.

2.4.2. Activity in the aqueous medium.

It was evaluated using the microdilution method with slight modifications [10]. The analyses were carried out in a microdilution plate with 96 wells (sterilized, 300 µL capacity, MicroWell, NUNC, Thermo-FisherScientific, Waltham, MA). Briefly, all wells were filled with 100 µL of TSB. Dilutions of LEOs were prepared from a stock solution of 250 µL/mL of studied LEOs and transferred into the wells of microplates. Then, 20 µl of the bacterial suspensions at 10^7 CFU/mL concentrations were added to each well to achieve a final 105 CFU/mL concentration. One row was used as a positive control, adding only the TSB medium and the pathogen. One row was used as a negative control with EO plus TSB medium. In each plate, each EO concentration was tested against two bacteria in triplicate.

The microplates were sealed with Parafilm™ to avoid interference with EO volatilization from the other plates. Plates were incubated at 35±1 °C for 24 or 48 h. After this incubation, a TSA plate count was performed by taking 10 µL of each well. The lowest concentration of EO without growth of bacteria (<1 CFU/mL) after 24 h at 35°C was defined as the minimum inhibitory concentration (MIC).

Then, from all wells that showed inhibition of the tested bacteria after 24 h, 10 µL were reinoculated onto TSA plates and incubated at 35±1 °C for 24 h. If no colonies were observed on the plate surfaces following incubation, the concentration was considered bactericidal. The lowest concentration of EO demonstrating bactericidal activity across all replicates was considered as its minimum bactericidal concentration (MBC)

2.4.3. Activity in vapor phase.

The vapor phase evaluation was performed by the inverted plate method [11]. Briefly, 10 µL of each bacteria containing approximately 10^5 CFU/mL were inoculated over the surface of TSA agar plates and allowed to dry under sterile conditions. Afterward, different volumes of studied LEOs (10-200 µL) were poured on sterile filter papers (Whatman No. 1, diameter 45 mm) and put on the inner surface of the top lid. The inoculated plates were tightly sealed with Parafilm™ to prevent leakage of the vapors. Finally, a control was carried out without the

application of LEO. Plates were incubated at 35 ± 1 °C for 24 or 48 h. The minimum essential oil concentration (µL/L**air**) that inhibited the visible growth after 24 h was defined as the MIC, and after 48 h as the MBC, which were calculated taking into account the volume of essential oil (µL) added and the calculated volume of air (L) between the agar surface and the plate lid.

2.5. Estimation of transport properties.

Computer-aided molecular design is a useful tool for predicting the properties of organic compounds when experimental data are not available. These methods can use structural characteristics such as the number of atoms, types of bonds, topological indices, molecular descriptors, functional groups, etc. Estimating properties by group contribution methods is based on an "additive principle", which states that the functional groups of the molecular structure of a pure substance have an additive relationship to specific substance properties [12,13].

The partition coefficient (LogKow) and Solubility parameters (Sol. Par.) were predicted directly by the group-contribution method proposed by [12] using Eqs. 2 & 3

Octanol-water partition coefficient (logKow)

$$logKow = \sum_{i}^{n} n_i F_i + \sum_{j}^{n} n_j F_j + 0.097 \qquad \text{Eq. 2}$$

Total Solubility parameter (298 K) [$MPa^{1/2}$]

$$Sol.Par. = \left(\sum_{i}^{n} n_i F_i + \sum_{j} n_j F_j + 74954.1 \right)^{0.383837} - .56.14 \qquad \text{Eq. 3}$$

To calculate the diffusion coefficient at infinite dilution for binary mixtures with water Tyn and Calus correlation (Eq. 4) was chosen, approximating the units [12]

$$D°_{AB} = 8.93\ X\ 10^{-8} \frac{{V_B}^{0.267}}{{V_A}^{0.433}} \frac{T}{\eta_B} \qquad \text{Eq. 4}$$

where D°AB is the mutual diffusion coefficient of solute A at very low concentrations in solvent B, cm^2/s; T is temperature, K; ηB is the viscosity of solvent B, cP; VA and VB are molar volumes of solute and solvent at the normal boiling temperature.

To get boiling volume, Tyn and Calus, (Eq. 5), was used, as boiling point is related to the critical volume of a substance. The critical volume of each substance was estimated by using Constantinou and Gani groups contribution method eq (Eq. 6). Functional groups used for log Kow and Solubility Parameter approximations were the same for critical volume.

$$V_B = 0.285 {V_c}^{1.048} \qquad \text{Eq. 5}$$

Critical Volume [V_c, cm^3/mol]:

$$V_c = 0.00435 + \sum_{i}^{n} n_i F_i + W \sum_{j}^{n} n_j F_j \qquad \text{Eq. 6}$$

The modified Fuller equation (Eq. 7) was used to estimate the diffusion coefficient of binary mixtures with low-pressure air (DAB) [12]:

$$D_{AB} = \frac{0.00143 T^{1.75}}{P M_{AB}^{1/2} \left[(E_v)_A^{1/3} + (E_v)_B^{1/3} \right]^2} \qquad \text{Eq. 7}$$

3. Results and Discussion

3.1. Extraction and chemical composition of LEO.

Results showed that the content of LEO isolated was between 0.13 and 0.25% (v/w). The highest content of the LEO was obtained using whole leaves (0.25%). These results are similar to those reported by [14–17], who obtained yields of LEO between 0.2 to 0.5%. Distillation time is an influential factor related to the quantity and quality of the active compound. Different distillation times (1, 1.5, 2, or 3 h) were investigated by [18]; after 2 h, the highest content of essential oil (0.73%) was obtained; however, the maximum contents of neral, limonene, and geranial were obtained after 1 h . In our study, the lowest content was found in the ground leaves (0.13%) at 2 h, which may be due to less contact of the plant material with water because ground leaves floated. In addition, the lowest yield can also be explained by the decomposition of the volatile components in EOs when exposed to heat for a long time [19].

As determined by the conducted GC-MS analyses, the simultaneous use of mass spectral matching allowed for the identification of 31 compounds, accounting for more than 90 % of the oil obtained from the studied leaves of *A. citriodora*. In general, EO compounds can be classified into two main groups, terpenes (monoterpenes and sesquiterpenes) and terpenoids. Terpenes encompass several different chemical functionalities, which include: aldehyde (citral and citronellal), phenol (thymol and carvacrol), alcohol (linalool, geraniol, carveol, citronellol, terpineol, menthol, borneol, and bisabolol), ketone (carvone and camphor), ether (eucalyptol), and hydrocarbon (cymene, pinene, limonene, and phellandrene) groups; in contrast, terpenes are secondary metabolites of plants formed by isoprene units and exhibit high volatilities [20].

As shown in Table 1, the LEOs from whole or ground leaves were characterized by high levels of monoterpenes (> 40 %) and moderate levels of sesquiterpenes (< 25 %). In the two tested samples (whole and ground leaves), citral (17-19%) was the principal component, which, together with limonene (8-14%) and spathulenol (11-12%), represented more than one-third of the extracted oils. Other major components identified included mainly estragole (10-11 %), eugenol-methyl (10-11%), α-curcumene (5 %), and citronellal (3-5%). It has been reported that these compounds may have different biological activities such as antimicrobial, antioxidant, anesthetic, and sedative, as well as anti-inflammatory [2].

Although the compounds obtained from the whole or ground leaves were very similar, there is a difference in the percentage obtained. In general, the LEO extracted from the whole leaves presents a higher amount of the compounds, mainly terpenes and terpenoids, probably resulting from the increased contact surface between the plant material and the solvent (water) in a more efficient oil extraction [21]. Generally, when the plant is ground, there is a greater contact surface; however, in our study, this was not the case; this may be due to different factors such as the particle size of the leaves, the ratio of plant material to distilled water, the density, and surface tension [19].

Similar compounds, except for methyl eugenol and estragole, have been reported [22], while on lippia cultivated in Iran [18], they found geranial (27-30%), neral (20-23%), limonene (7-15%) and spathulenol (4-7%) as major compounds. For lippia cultivated in Argentina, geranial (17-36%), neral (14-27%), and limonene (10-30%) were reported [13]. These differences demonstrate this plant exhibits great genetic biodiversity; therefore, the class of chemical constituents and their concentration may vary due to variations in the time of harvest, climatic conditions, part of the plant, method of extraction, and drying and storage conditions

[2]. In addition, the season of the year in which the lippia is grown is important, a reduction in limonene was detected in autumn and winter, and an increase in citral in autumn and summer due to monoterpenes which facilitate their volatilization, being attractive to pollinators [23]. On the contrary, winter favored the increase of spathulenol and caryophyllene oxide because sesquiterpenes are larger, heavier, and less volatile and often have protective functions such as antimicrobial activity and fungi toxic action, while during winter, with a rainier season that encourages the development of phytopathogens [24, 25].

Table 1. Chemical composition of extracted lippia essential oils.

		Whole leaves		**Ground leaves**	
No	Component	%	RT	%	RT
	Monoterpenes				
1	α-pinene	0.41	6.71	0.31	6.57
2	Camphene	0.04	7.33	0.02	7.19
3	Sabinene	0.63	8.78	0.56	8.58
4	β-Pinene	0.11	9.73	0.03	9.72
5	Limonene	14.06	13.72	8.78	13.14
6	β-Ocimene	0.11	14.01	0.28	13.56
7	Linalool	0.82	16.17	-	-
8	Citral	19.96	23.03	17.20	22.25
	Monoterpenoids				
9	Citronellal	3.05	19.11	5.35	18.9
10	Cis-carvyl-acetate	-	-	0.09	24.73
11	Verbenone	0.65	25.27	0.45	25.01
	Sesquiterpenoids				
12	Farnesyl acetate	1.8	26.03	-	-
13	Alloaromadendrene	0.85	28.14	1.07	27.98
14	Cuparene	0.3	29.25	0.37	29.15
15	Caryophyllene oxide	0.4	30.12	0.47	30.06
16	Spathulenol	11.23	30.19	12.94	31.71
17	Tau-cadinol	1.52	32.66	2.26	32.58
18	Ledene oxide II	1.58	33.49	0.36	33.06
19	Corymbolone	0.1	35.8	-	-
	Sesquiterpenes				
20	β-bourbonene	-	-	3.02	25.94
21	α-curcumene	5.17	28.92	5.02	28.78
22	Germacrene	1.5	29.09	1.71	28.97
23	Aromadendrene oxide	1.47	34.75	1.43	34.48
	Other compounds				
24	Limonene epoxide	0.45	17.52	0.44	17.45
25	Estragole	11	20.83	10	20.29
26	Eugenol methyl	11.42	27.14	10.02	26.99
27	E-nuciferol	5.33	31.9	-	-
28	Cedren-13-ol-8	1.24	32.93	1.16	32.9
29	Isoaromadendrene epoxide	3.6	33.23	1.50	32.12
30	1-heptatriacotanol	0.16	36.05	0.42	35.39
31	Widrrol	0.12	41.93	0.41	42.11

RT= Retention time

3.2. Antimicrobial activity.

As observed in the previous section, the LEO extracted from whole leaves displayed a higher percentage of selected compounds, so the antimicrobial study was carried out only with this EO. The antimicrobial activity of LEO against *L. monocytogenes* was more noticeable in both application methods (Table 2) since Gram-negative bacteria are anticipated to have a higher tolerance to EOs than Gram-positive ones owing to the physical barrier created by the hydrophilic lipopolysaccharide layer in the outer cell wall membrane.

Table 2. Antimicrobial activity of (extracted from whole leaves) lippia essential oil.

	Escherichia coli		***Listeria monocytogenes***	
	MIC	MBC	MIC	MBC
Direct contact (µL/ mL)	180	200	139.36	139.36
Vapor phase (mL/L_{air})	7.07	10.61	2.94	6.48

MIC= Minimum Inhibitory Concentration, MCB= Minimum Bactericidal Concentration

These results are similar to the antimicrobial activity previously reported [26] against *E. coli* with a MIC of 400 µg/mL. Similarly, a MIC of 390 µg/mL was reported against *Aeromonas* spp [27]. In addition, when evaluated by the diffusion agar method, the antimicrobial effect against *Staphylococcus aureus* and *Pseudomonas putida* a MIC of 1.25 µL/mL, and 2.5 µL/mL for *E. coli* and *S.* Typhimurium, respectively have been reported by [28] whereas [29] reported against *E. coli* and *S. aureus* MICs ranging from 2.84 to 8.37 mg/mL. Antimicrobial testing aims to evaluate the efficiency of inhibiting or inactivating a selected range of organisms in specified conditions; every method utilized to assess antimicrobial efficacy is influenced by factors that can affect the activity; for instance, the antimicrobial agent, the test medium and/or the test procedure, the culture medium, and inoculum size, among others [30].

Regarding application method, selected studies have conveyed that vapors generated by EOs exhibit superior antimicrobial effect than EOs applied in liquid form by direct contact, given that lipophilic molecules associate and form in the aqueous phase micelles which restrict the adhesion of EOs to microorganisms. In contrast, the vapor phase allows free attachment of EOs [31–33].

Furthermore, most of the antimicrobial activity of EOs derives from oxygenated terpenoids such as alcoholic and phenolic terpenes [34]. The antibacterial mechanism of these aromatic and aliphatic compounds is their lipophilic ability to partition in the lipophilic lipids of the mitochondria and cytoplasmic membrane as well as disturb the structures, resulting in leakage of bacterial cell contents [35]. Our work could be attributed to the presence of a high percentage of citral, limonene, and spathulenol and the possible additive or synergic effects amongst the various constituents of this EO. In addition, these compounds could have important pharmacological activities to be studied in the future [36–39].

3.3. Transport properties and chemical structure.

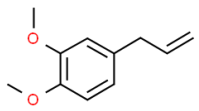
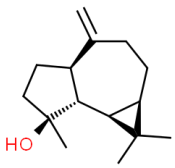
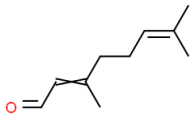
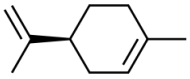
The chemical structure and estimated transport properties of major compounds of studied LEOs are reported in Table 3. Citral and limonene have the lowest molecular weight, which could indicate that they are the compounds that volatilize first.

All the compounds show better diffusivity coefficients in the air than in water, with citral and limonene having the highest value and being the predominant compounds in studied LEOs. It is important to mention that EOs contain both volatile and non-volatile fractions that can be composed of more than 30 compounds. However, although its bioactivity is generally attributed to one or two of these major compounds, there is a possibility that it may result from the combination of several constituents acting in synergism to exert a significant effect [40].

The volatile fraction can constitute up to 99% of the EO. It is characterized by monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives, aliphatic aldehydes, alcohols, and esters. In contrast, the non-volatile fraction can contain hydrocarbons, sterols, fatty acids, psoralens, carotenoids, waxes, coumarins, and flavonoids [41]. Therefore,

the presence of volatile compounds explains a lower MIC in the vapor phase, thanks to the volatile compounds that tested LEO.

Table 3. Estimated properties of principal compounds of lippia essential oil.

Compound	Chemical structure	Molecular weight (g/mol)	Log kow	Sol. Par. (298K), $(MPa)^{1/2}$	D°AB in water (298K), cm^2/s ($x10^{-6}$)	DAB in air (298K), cm^2/s
Methyleugenol		178.23	3.27	17.31	6.16E-06	0.05
Spathulenol		220.35	4.09	15.26	6.01E-06	0.04
Citral		152.24	3.03	18.59	6.41E-06	0.05
Limonene		136.24	4.25	16.47	6.58E-06	0.06

In addition, limonene had the best partition coefficient, while citral had the best solubility. The main antimicrobial action mechanism in the EOs relates to the permeability increase and subsequent plasma membrane disruption. Therefore, the antimicrobial activity of EOs is primarily attributed to their terpene content. Furthermore, in most cases, related to the hydrophobicity, which is directly correlated to the log Kow and their partition in the cytoplasmic microbial membranes [42]. Indeed, lipophilic compounds possess a high affinity for cell membranes, and their insertions induce changes in membrane physicochemical properties.

The interactions between antimicrobial compounds and cell membranes are reported to affect lipid ordering and bilayer stability, resulting in a decrease in membrane integrity and an increase in passive proton flux across the membrane. In our study, all the major compounds show a value higher than 3, which has been reported as a good partition coefficient [43]. In addition, it has been shown that a high Log Kow did not always result in greater toxicity of the compounds. Compounds with a Log Kow higher than 4 (like spathulenol and limonene) are generally not toxic; because of their insolubility, they cannot reach a toxic concentration in the cell membrane [44]; therefore, future research could focus on the study of the toxicity of extracted LEOs. As expected, the solubility correlated well with the hydrophilicity of the compounds.

On the other hand, the composition and functional groups present in the active components and their synergistic interactions determine the activity of EOs. [45]. For instance,

previous studies state that terpenoids (such as phenolics) display better antimicrobial activity than hydrocarbons (such as limonene) [46, 47].

Furthermore, all the major compounds have an aromatic ring with the exception of citral; also, all compounds have double bonds. Antimicrobial activity has been reported to be due to aromatic nuclei with a polar functional group [48]. The presence of a delocalized electron system and the hydroxyl group present in spathulenol are highly reactive and form hydrogen bonds with the active sites of target enzymes, inactivating them [20], causing dysfunction or rupture of the cell membrane. Such a particular structure would allow compounds to act as proton exchangers, thereby reducing the gradient across the cytoplasmic membrane. Cell death is caused by the resulting collapse of the proton motive force and depletion of the ATP pool. [49]. The structural variations of the compounds and the hydroxyl group's position may influence the compound's effectiveness against microorganisms.

Indeed, the inhibitory effect of citral against microorganisms could be explained by its structural characteristics, as it is an α, β-unsaturated aldehyde with the carbonyl group adjacent to the α and β carbons [50]. Citral, like other aldehydes, can act as a direct alkylating agent. These alkylating agents can covalently bind cellular nucleophilic groups, modifying cellular processes and potentially toxic [51]. The antimicrobial effect can also be attributed to the ability of citral to alter and penetrate the lipid and protein structure of bacterial cell walls; this leads to protein denaturation and cell membrane destruction, followed by cytoplasmic filtration, lysis, and cell death [52, 53].

An additional relevant feature of antimicrobial activity is the mechanism of action [45]. It can vary with the tested strain of microorganisms or the type of EO studied. As already mentioned, *L. monocytogenes* is more susceptible to these coefficients for being Gram-positive bacteria. The lipophilic ends of lipoteichoic acid in the Gram-positive cell wall facilitate the penetration of hydrophobic compounds, such as EOs, into these bacteria [54]. However, thanks to their outer membrane, Gram-negative bacteria show greater resistance to the action of EOs given that outer membrane protein and lipopolysaccharides can limit the rate of diffusion of these hydrophobic compounds [55, 56]. The physical characteristics of major components, such as high volatility and low solubility, as well as interactions with the diffusion medium, have probably contributed to the observed differences.

4. Conclusions

Studied *A. citriodora* had high percentages of citral, limonene, and spathulenol, which would add a significant value to the characteristics of its oil. Moreover, obtained LEO was found notably rich in monoterpenes (> 40%), which are repeatedly reported to have numerous biological activities. Furthermore, LEO displayed better antimicrobial activity against Gram-positive when applied in the vapor phase, which can be related to the chemical structure of the studied essential oil principal compounds and their transport properties. All of the LEO major compounds have double bonds and, except for citral, an aromatic ring; they also have better diffusivity coefficients in the air than in water. The predominant compounds, such as citral and limonene, have the best partition coefficient and solubility, respectively. In addition, the importance of these properties (chemical structure of volatile compounds and their hydrophobicity) with regard to antimicrobial activity may contribute to a more efficient application and enhance their use as antimicrobial agents. Future research could use alternative techniques to extract studied EOs and evaluate other methods of application both *in vitro* and *in vivo*. Clearly, our results with Mexican lippia will encourage future studies that should focus

on studying certain bacteria, the corresponding type of food, and the application method to achieve the natural preservation of different food matrices.

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Conflicts of Interest

The authors declare no conflict of interest.

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