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MICROBIOLOGY

Chemical composition and biological activities of the essential oils from *Lippia alba* and *Lippia origanoides*

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Abstract: There is an increasing interest in essential oils extracted from Verbenaceae plant species as potential sources of biologically active compounds that could provide a starting point for designing novel phyto-pharmaceuticals in aquaculture. The present study was aimed to investigate the chemical composition, antioxidant activity, acute toxicity and antimicrobial effects against *Vibrio parahaemolyticus* of essential oils extracted from *Lippia alba* and *L. origanoides*. Approximately 23 components were identified and quantified by gas chromatography-mass spectrometry and flame ionization detection in each species' essential oil. The most predominant compounds were geranial (23.0%), limonene (17.0%) and neral (15.5%) in *L. alba*, and thymol (47.2%), p-cymene (16.0%) and E-caryophyllene (11.3%) in *L. origanoides*. The essential oils have antibacterial activity against *Vibrio parahaemolyticus* presenting Minimum Inhibitory Concentration (MIC) and Bactericidal Concentration (MBC) values between 156-625 µg mL^{-1}. The essential oils also show antioxidant potential estimated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays, presenting IC_{50} of 60.16 mg mL^{-1} and 0.22 mg mL^{-1} for *L. alba* and *L. origanoides* EO, respectively. Both oils were classified as toxic to *Artemia salina* nauplii. Therefore, these essential oils may be useful for controlling pathogenic bacteria important to the aquaculture industry.

Key words: *Lippia alba*, *Lippia origanoides*, minimum bactericidal concentration, minimum inhibitory concentration, oxygenated monoterpenes.

INTRODUCTION

The bacteria of the genus *Vibrio*, which includes over 100 species, are predominantly associated with a variety of aquatic habitats, estuaries and coastal waters. In humans, these microorganisms are responsible for mild and inconvenient gastroenteritis to severe and life-threatening septicaemia and skin and soft tissue infections (Janda et al. 2015). The species *V. parahaemolyticus* is a leading cause of bacterial gastroenteritis transmitted by seafood worldwide (Ortiz-Jiménez 2018).

In the shrimp industry, infectious diseases caused by *Vibrio* species pose major challenges facing the world, causing considerable economic losses (Srinivasan & Ramasamy 2017). On Pacific white shrimp farms (*Litopenaeus vannamei*), for example, the main opportunistic pathogens belonging to the *Vibrio* genus are *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus*, known to cause serious outbreaks (Tepaamorndech et al. 2019). The bacterium *V. parahaemolyticus* is the pathogen that causes acute hepatopancreatic necrosis syndrome, responsible for severely

damaging shrimp production and consequently economic income (Wangman et al. 2018). The infection caused by this bacterium in shrimp can cause inactivity of the animals, slow growth, empty stomach and medium intestine, and atrophy associated with the pallor of the hepatopancreas. In the first 20 to 30 days after population with post-larvae, this disease can cause up to 100% mortality (Elshopakey et al. 2018).

The global emergence of bacterial resistance to antibiotics has become a major problem for healthcare, and therefore, new alternatives to antibiotics are needed to overcome this complication, especially natural compounds, herbs and phytochemicals (Prabu et al. 2018). In fact, herbal products may be more effective and economical than chemotherapeutic agents, and offer a viable solution to much pathogen control (Harikrishnan et al. 2011).

The family Verbenaceae consists of about 175 genera and 2,300 species of trees, shrubs, lianas and herbs distributed mainly in tropical and subtropical regions around the world (Cavalcanti et al. 2010). This taxon includes the species *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson and *L. origanoides* Kunth. The first is a medicinal small bush used as tranquilizer and gastroprotective agent (Gomes et al. 2018) and antispasmodic (Carvalho et al. 2018) in Central and South America. The species *L. origanoides* is a shrub with odorous leaves native from northeastern Brazil (Veras et al. 2017). The essential oil of the plant has potential use as an antiparasitic drug (Hashimoto et al. 2016) and antifungal and antibacterial agent (Pinto et al. 2016).

Natural products derived from plants have been of great interest in traditional medicine, as sources of potential alternative agents in the prevention and treatment of many infectious diseases (Khiya et al. 2018) and in the scavenging of free radicals (El Euch et al. 2019). Although many natural products have been reported to exhibit relevant biological activities, they may also cause some neurotoxic, hepatotoxic or other damage effects (Shirmohammadli et al. 2018). Therefore, tests on toxicity are also important, given that exposure to toxic agents can result in health impairment (Kampke et al. 2018).

This study, therefore, aimed to assess the chemical composition of *L. alba* and *L. origanoides* essential oils, evaluate their antioxidant activity, acute toxicity in *Artemia salina* and antibacterial effects against *Vibrio parahaemolyticus* were also investigated.

MATERIALS AND METHODS

Plant material

Accessions of *L. alba* and *L. origanoides* were collected in Parnaíba, Piauí, Brazil (03° 05' 12.5"S; 41° 47' 01.2"W), in March 2018. Both species were identified using the keys provided by Salimena & Múlgura (2015), and vouchers were deposited in the Herbarium of the Federal University of the Delta of Parnaíba - UFDPar, under numbers HDELTA5466 and HDELTA5469, respectively. All chemicals were of analytical grade and purchased from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO. USA) unless otherwise specified. This study was approved by the Brazilian Genetic Heritage Management Council (CGEN) (Process number A00721D).

Extraction of essential oils

The fresh leaves of *L. alba* and *L. origanoides* were collected separately and dried at room temperature before being further processed. After the drying process, 600 g of dry leaves were mixed with 3.5 L of distilled water, in a hydrodistillation in a Clevenger-type apparatus. After the water started boiling, the process was

maintained for four hours. At the end of the process, 3 g of *L. alba* EO (0.5% yield) and 9.6 g of *L. origanoides* EO (1.6% yield) were obtained. Then the EO obtained by the hydrodistillation process was stored in an amber glass bottle at 4 ° C until later use (adapted from Yen & Lin 2017).

A stock solution of each EO was prepared by dilution with 50% ethanol P.A., 1% Tween 80 USP and 49% distilled water, obtaining the final concentration of 100 $mg.mL^{-1}$.

Chemical characterization of essential oils

Chemical characterization was carried out by gas chromatography-mass spectrometry (GC-MS) analysis performed on an Agilent GC-7890B/MSD-5977A (quadrupole) instrument with electron impact at 70 eV HP-5MS methylpolysiloxane column (30 m × 0.25 mm × 0.25 µm; Agilent, Santa Clara, CA, USA), helium carrier gas with flow rate 1.00 mL min^{-1} (8.8 psi) and constant linear velocity of 36.8 cm s^{-1}, injector temperature of 250°C, detector temperature of 150°C, transfer line temperature 280°C. Chromatographic furnace was set to an initial temperature of 70°C, with a heating ramp of 4°C min^{-1} to 180°C and an increase of 10°C min^{-1} to 250°C at the end of the run (34.5 min). The same parameters described above were employed for gas chromatography-flame ionization detection (GC-FID) analysis, using a Shimadzu GC-2010 Plus chromatograph.

The retention indices (RI) of the chemical components in *L. alba* and *L. origanoides* essential oils were calculated by injecting a mixture of standards containing a homologous series of C7-C30 alkanes in an HP-5MS column (Hu et al. 2019). The analytes were identified by comparing their mass spectra data with those from the National Institute of Standards and Technology (NIST) mass spectral library (Naumkin et al. 2012), and MS data from the literature (Adams 2007). The relative concentration of each compound in the essential oil was quantified based on the peak area integrated in the analysis program.

Antibacterial activity

Plant essential oils were tested for antimicrobial activity against bacterial strains of *Vibrio parahaemolyticus* provided by the Oswaldo Cruz Institute (OCI18950) and *V. parahaemolyticus* isolated from the hemolymph of *farm*-reared *Litopenaeus vannamei* shrimps from northeastern Brazil, after a high mortality event. The hemolymph of farmed shrimp samples were collected from ventral sinus by a puncture of the first abdominal segment with a 1-mL syringe containing ice-cold anticoagulant solution (citrate-EDTA) (Vargas-Albores et al. 1993). Then, the hemolymph was inoculated on agar plates with thiosulphate-citrate-bile salts-sucrose (TCBS) at 37°C for 24 h. Green or blue-green colonies were identified as *V. parahaemolyticus*-positive and transferred to tryptic soybean agar (TSA) plates containing 2% NaCl (Vieira et al. 2009).

The bacteria had their identification confirmed using the OMNILOG GEN III system (Biolog Inc., USA) according to the manufacturer's instructions. Presumptive *V. parahaemolyticus* colonies were inoculated in BUG™ Agar (Biolog Inc., USA) with 2% NaCl and incubated at 34°C for 24 h. After incubation, a single colony was picked from a plate and transferred into a 10 mL-inoculation fluid (IF-B) (Biolog Inc., USA). The inoculated IF-B was dispensed into a GEN III microplate. The microplate was incubated at 33°C for 24 h. The readings were carried out by the OmniLog® Data Collection software using a semiautomated Biolog MicroStation™ system microplate reader.

The antibacterial assay was performed using the microdilution method in nutrient broth, adapted from Clinical and Laboratory Standards Institute (CLSI 2012). The strains were

maintained on Mueller Hinton agar (Merck) and incubated at 34 ± 2°C. The bacterial suspensions were adjusted with sterile saline solution until the concentration of 1.5×10^8 CFU mL^{-1}. Then, the bacterial suspension broth was dispensed into a 96-well microplate for test with different EOs concentrations (10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 $mg.mL^{-1}$), with a final volume of 100 µL. Said concentrations were obtained in the 96-well plate as follows: 180 µL of the nutrient broth with bacteria were pipetted into the well in the first row (A) of the 96-well microplate, and in the other wells (B to H) 100 µL were dispensed on each. Subsequently, 20 µL of the stock solution (100 $mg.mL^{-1}$) of OE was pipetted into well A (totaling 200 µL in the well). Then, from well "A" to well "H", the solutions were diluted in series (dilutions by 2) with nutrient broth, pipetting 100 µL from one well to another. At the end, 100 µL of solution from well H was discarded, and the final volume of all wells was maintained at 100 µL.

A comparison between both plant essential oils and positive control antibiotics (oxytetracycline, enrofloxacin and sodium salt of ampicillin) was performed. Sterility control (nutrient broth without addition of bacterial inoculum) and growth controls (nutrient broth with bacterial inoculum and EO dilution solution) were included on each microtiter plate. All measurements were taken in *triplicate*. All microdilution plates were incubated at 34 ± 2°C for 24 hours under aerobiose conditions.

Bacterial growth was confirmed by adding 20 µL of 3% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution and incubating for 1 hour at the same temperature. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of essential oil that completely inhibited bacterial growth.

The minimum bactericidal concentration (MBC) was determined from the MIC by plating 10 µL-aliquots from those wells with no growth onto Petri dishes containing Mueller Hinton agar (2% NaCl) incubated at 34 ± 2°C for 24 h. The MBC is the lowest concentration of each essential oil that shows no bacterial growth on the agar. The bactericidal and bacteriostatic effect of the essential oils were determined using the ratio MBC/MIC (Marmonier 1990).

Antioxidant activity

Antioxidant activity of e*ssential oils* were determined by the radical scavenging activity method using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as free radical (Brand-Williams et al. 1995). A stock solution of each EO was prepared by diluting 100 mg of oil in 1 mL of P.A. ethanol. Then, aliquots of 30 µL of each essential oil ethanolic solutions at different concentrations, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 and 0.0312 $mg.mL^{-1}$, were added to 200 µL ethanolic DPPH solution (100 µM) in 96-well microplates. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as reference synthetic antioxidant compound at molar concentrations of 15.63, 7.81, 3.91, 1.95 and 0.98 µM. The absorbance was measured at 517 nm immediately after the addition of the compounds to be tested (time 0) and after incubation at room temperature for 30 min under dark conditions. Readings for each sample on the xMark™ Microplate Absorbance Spectrophotometer, using the software Microplate Manager®.

The radical sequestering activity was calculated by the following equation: I (%) = $[1-(A_{sample} / A_{control})] \times 100$, where $A_{control}$ is the absorbance of the control reaction (ethanolic DPPH solution) and A_{sample} is the absorbance in the presence of the tested compound at different concentrations. Based on the calculated I (%) values, the sample concentration providing 50% inhibition of free DPPH radicals (IC_{50}) calculated graphically using a calibration curve in the

linear range, representing the EO concentration against the corresponding elimination effect. Tests were carried out in triplicate.

Acute toxicity

Artemia salina cysts (INVE Aquaculture, Belgium) were kept at 5°C until further analysis. The toxicity of EOs was determined according to an adapted method from Meyer et al. (1982) using freshly hatched nauplii. Dried cysts were incubated in natural seawater (salinity 35 $g.L^{-1}$; dissolved oxygen 5.3 mg L^{-1}; pH 8.2; and temperature 27.5°C) previously filtered through a 0.22 µm porosity filter under light conditions and continuous aeration. After 24 hours the *A. salina* nauplii were separated from the eggshells using a Pasteur pipette. All water used in the experiment was also prefiltered in a 0.22 µm porosity filter.

A stock solution of each EO was prepared in prefiltered sea water with 0.1% Tween 80 U.S.P., obtaining the final concentration of 100 $mg.mL^{-1}$. Toxicity assays were performed in 96-well microplates with 200 µL of EO solution in seawater at different concentrations and 10 nauplii to each well, in order to determine dose-response relationship. The EOs were diluted in seawater with 0.1% Tween 80 U.S.P. Each horizontal row of the 96-well plate corresponded to the EO tested concentrations of 2000, 1000, 500, 250, 125, 62.5, 31.2, and 15.6 µg mL^{-1}. Each vertical row contained quadruplicate samples and controls. In addition to the tests performed with EO of *L. alba* and *L. origanoides*, 2 control groups were also used: control A - containing only nauplii and filtered seawater (to demonstrate that the volume of seawater used in the experiment allowed nauplii to survive for 24 hours); control B - containing nauplii and Tween 0.1% filtered seawater (to demonstrate that the concentration of Tween used did not influence the mortality of nauplii during the experiment).

After 24 hours of incubation, under normal photoperiod conditions and ambient temperature (27 to 30°C), the number of live and dead larvae in each well was counted using a stereoscopic microscope. Larvae that showed no internal or external movement during 30 seconds of observation were considered dead. In both control groups the survival of nauplii of *A. salina* was 100% after 24 hours of incubation.

Statistical analysis

The software Statistical Package for the Social Sciences (SPSS) 20.0 was used for statistical analysis by applying the analysis of unidirectional variance (ANOVA) followed by Tukey's HSD (honestly significant difference) test with $\alpha<0.05$ to determine statistical differences. Stundet t-test was used for independent samples ($\alpha<0.05$). The determination of the average lethal concentration (LC_{50}) was performed by the Probit regression analysis method with 95% confidence limits (Finney 1971).

RESULTS AND DISCUSSION

Chemical characterization of essential oils

The chemical composition of the essential oils extracted from two *Lippia* species are described in Table I. Major compounds in EO samples from *L. alba* and *L. origanoides* were oxygenated monoterpenes, 56.6% and 52.9%, respectively, and by a low content of sesquiterpene hydrocarbons, 6.8% and 12.4%, and oxygenated sesquiterpenes, 8.0% and 1.4%, respectively.

In both EOs twenty-three compounds were identified, being geranial (23.0%), limonene (17.0%) and neral (15.5%) the most abundant compounds in *L. alba*, and thymol (47.2%), p-cymene (16.0%) and E-caryophyllene (11.3%) the most predominant components in *L. origanoides*. The remaining compounds are listed in Table I.

Table I. Chemical composition of essential oils from *Lippia alba* and *L. origanoides*.

Compounds	Composition (%)		
	IK*	*L. alba*	*L. origanoides*
α-Thujene	938	-	1.3
α-Pinene	947	-	0.6
β-Pinene	981	-	0.3
Sabinene	986	1.2	-
Myrcene	996	5.5	2.6
δ-3-Carene	1013	-	0.2
α-Terpinene	1019	-	1.8
p-Cymene	1026	2.7	**16.0**
Limonene	1041	**17.0**	0.9
1,8-Cineole	1044	-	2.1
β-(*Z*)-Ocimene	1047	0.7	-
γ-Terpinene	1059	5.1	5.6
Cis-Sabinene Hydrate	1069	0.2	0.3
Linalool	1102	1.2	-
trans-Sabinyl acetate	1103	-	0.4
Ipsdienol	1147	-	1.6
Terpinene-4-ol	1186	-	0.4
α-Terpineol	1199	-	0.5
Citronellol	1234	5.5	-
Thymol, methyl ether	1236	-	2.4
Neral	1243	**15.5**	-
Carvone	1246	1.3	-
Geraniol	1256	8.7	-
Geranial	1272	**23.0**	-
Thymol	1300	-	**47.2**
Thymol acetate	1357	-	0.2
β-Cubeben	1389	0.4	-
β-Elemene	1400	0.5	-
E-Caryophyllene	1422	0.2	**11.3**
Aromadendrene	1442	-	0.6
α-Humulene	1456	-	0.5
γ-Muurolene	1490	3.5	-
α-Zingiberene	1501	0.4	-
γ-Cadinene	1516	0.1	-
δ-Cadinene	1525	0.4	-
Elemol	1549	5.8	-
E-Nerolidol	1565	0.5	-
Spatulenol	1577	-	0.3
Caryophyllene oxide	1586	-	1.1
Guaiol	1600	0.4	-
TOTAL		99.8	98.2
Monoterpene hydrocarbons		28.4	31.5
Oxygenated monoterpenes		56.6	52.9
Sesquiterpenes hydrocarbons		6.8	12.4
Oxygenated sesquiterpenes		8.0	1.4

***IK: Kovats Indices obtained in column RTX-5.**

Previous studies report the same major compounds in EOs extracted from *L. alba* and *L. origanoides* (Batista et al. 2018, Damasceno et al. 2018). However, variations on the concentration of major compounds in EOs from these two *Lippia* species can also be observed in the literature. For example, carvone and limonene are the major components of the EO obtained from *L. alba* (Teles et al. 2012), whereas carvacrol and thymol are the major components in the EO of *L. origanoides*, both collected at Bahia region, Brazil (Menezes et al. 2018). The individual variation in essential oil composition among plant species can also be influenced by other factors such as climatic variations, altitude, soil, crop area, harvest, processing and genetic varieties (Vaičiulytė et al. 2017, Farhat et al. 2019).

Antibacterial activity

The antibacterial activities of the essential oils are summarized in Table II. Both essential oils exhibited antimicrobial activity against *Vibrio parahaemolyticus* strains. Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) ranged from 156 to 625 μg mL^{-1} for these bacterial strains. The essential oil from *L. alba* showed the best antibacterial activity against *V. parahaemolyticus* strains. These results have also showed that both essential oils had a bactericidal effect against *V. parahaemolyticus* strains (OCI 18950 and isolated from farmed shrimp) as the MBC/MIC ≤ 4. According to Marmonier (1990) plant fractions are considered as bactericidal agents when the ratio MBC/MIC ≤ 4 and bacteriostatic agents when the ratio MBC/MIC > 4. Moreover, according to the classification proposed by Aligiannis et al. (2001) for plant materials, MIC values of ≤ 500 μg mL^{-1} are considered strongly inhibitory. Therefore, the essential oils extracted from these *Lippia* species against *V. parahaemolyticus* strains show strong antimicrobial potential.

The major components of these EOs are described in the literature as antibacterial compounds, such as E-caryophyllene (Yoo & Jwa 2019), geranial (Espina et al. 2017), neral (Liao et al. 2015), limonene (Costa et al. 2019), p-cymene (Miladi et al. 2017) and thymol (Cai et al. 2019). However, minority constituents in their composition should not be neglected, since possible synergistic or antagonistic interactions between components could influence their antibacterial potential and, as a result, their

Table II. Antimicrobial activity of essential oils from *Lippia alba* and *L. origanoides* against *Vibrio parahaemolyticus* (OCI 18950) and *V. parahaemolyticus* isolated from farmed shrimp hemolymph.

Species	*V. parahaemolyticus* (OCI 18950)			*V. parahaemolyticus* in shrimp		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MCI
	Essential oils (μg mL^{-1})					
L. alba	156	313	2.00	313	313	1.00
L. origanoides	313	313	1.00	313	625	2.00
	Antibiotics (μg mL^{-1})					
Ampicillin	625	625	1.00	10000	-	-
Enrofloxacin	0.15	0.31	2.07	0.15	0.31	2.07
Oxytetracycline	0.00060	0.0012	2.00	1.22	2.44	2.00

MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration.

biological activities would vary accordingly to their overall composition (Chaturvedi et al. 2018).

Possible mechanisms of antimicrobial action of *Lippia* EOs are associated with high hydrophobicity of monoterpenes, and include cell wall degradation, increased membrane fluidity and permeability, cytoplasmic membrane damage, disruption of membrane-incorporated proteins, respiration inhibition, alteration of ion transport processes and leakage of intracellular materials (Badawy et al. 2019).

The antibacterial activity of these essential oils was of similar magnitude to that of ampicillin against *V. parahaemolyticus* (strain OCI 18950). However, an augmented antibacterial effect of all essential oils against the *V. parahaemolyticus* isolated from farmed shrimp hemolymph was noticed when compared with ampicillin. It is noteworthy that the strain isolated from the hemolymph of farmed shrimp showed higher resistance to ampicillin and oxytetracycline when compared with the strain of *V. parahaemolyticus* provided by the Oswaldo Cruz Institute (OCI18950). In several countries, antibiotic resistance of environmentally isolated *Vibrio* involve ampicillin, penicillin and tetracycline, which is a major concern in shellfish farming (Elmahdi et al. 2016).

Antioxidant activity

The antioxidant potential of *Lippia alba* and *L. origanoides* essential oils were evaluated and results are presented in Table III. The lower the IC_{50} value, the greater the antioxidant potential of the tested substance.

Antioxidant activity of *L. alba* EO, rich in limonene (27-77%), is reported in the literature to be similar to that of vitamin E, a product widely used as a natural and synthetic additive (Stashenko et al. 2004). Different *L. alba* chemotypes with high amounts of polyphenols, especially phenylpropanoids, may exhibit high antioxidant activities (Timóteo et al. 2015). Limonene and geranial, presented in major quantities in *L. alba* EO, as observed in this study, are common non-phenolic terpenoids of essential oils, whose kinetics of their antioxidant activity occur by co-oxidation with the substrate due to self-termination and cross-termination of the oxidative chain (Baschieri et al. 2017).

Table III. Antioxidant activity of the essential oils against the DPPH solution (100 µM). IC_{50} = concentration that provides 50% inhibition of the free radical DPPH (mg mL^{-1}).

Species	IC_{50}
Trolox	2.75 ± 0.14 ($\times 10^{-3}$)^{c}
Lippia alba	60.16 ± 0.28^{a}
Lippia origanoides	0.22 ± 0.02^{b}

Means followed by *different letters* in the *same column indicate significant differences using* the Tukey test (*P*<0.05).

Also in our study, the antioxidant activity of *L. origanoides* essential oil was significantly higher ($P < 0.05$) than *L. alba* EO, which may be explained by differences in the chemical compositions between the two *Lippia* species. The higher antioxidant potential of *L. origanoides* EO is associated with large amounts of thymol in its composition (Damasceno et al. 2018). Moreover, p-cymene, the second most abundant compound in *L. origanoides* EO, has been described as having relevant antioxidant activity, and enhancing the antioxidant potential of other constituents (Milos & Makota 2012). Both thymol and p-cymene were present in *L. origanoides* EO in major amounts (>63 %; Table I).

The high antioxidant activity presented by compounds such as thymol and essential oils rich in phenolic monoterpenes are due to the hydrogen donation capacity of the phenolic hydroxyl, forming a phenolic radical (Teles et al. 2014, Baschieri et al. 2017). Natural antioxidants can protect cells against reactive oxygen species and therefore can help neutralize tissue

damage mediated by oxidative stress (Ghorbani & Esmaeilizadeh 2017), which is very common during the management of captive shrimp and during infections.

Acute Toxicity

The brine shrimp lethality bioassay is considered a useful tool for testing biologically active compounds toxicity (Valadbeigi 2016). In our study, the estimated median lethal concentration (LC_{50}) of the essential oils required to kill 50% of the brine shrimp was lower than 1000 µg/mL (Table IV). According to the Meyer's toxicity index (Meyer et al. 1982), plant essential oils with an LC_{50} value < 1000 µg mL^{-1} are considered cytotoxic. Therefore, *Lippia alba* and *L. origanoides* essential oils exhibited toxicity against *A. salina* indicating that samples are biologically active. No mortality was found in control group and differences between groups were not statistically significant (P<0.05).

Previous reports of acute toxicity (Olivero-Verbel et al. 2009) corroborates the toxicological property of essential oils from different species of the genus *Lippia*, using *Artemia franciscana*. However, for *L. alba* and *L. origanoides* essential oils, their findings indicate LC_{50} values ranging from 8.87-20.13 µg mL^{-1} and 10.29-34.90 µg mL^{-1}, respectively. That is, higher toxic potential compared with our results. Highier toxicity was also recorded for *L. alba* EO against *A. salina* with an LC_{50} of 41.56 µg mL^{-1} (Queiroga et al. 2019). This discrepancy suggests that the difference in the toxicity of essential oils may be related to the chemical composition of the oils, which in turn is determined by genetic factors and varies qualitatively depending on climate, soil type, time and method of extraction, etc. (Fernandes et al. 2011, Oliveira et al. 2012).

Table IV. LC_{50} (µg mL^{-1}) values of *Lippia alba* and *L. origanoides* essential oils in *Artemia salina* nauplii.

Species	LC_{50} (µg mL^{-1})	Classification
Lippia alba	307.95 ± 17.18[a]	Toxic
Lippia origanoides	215.73 ± 22.13[a]	Toxic

Different letters in the same column indicate statistically significant differences by the T-student test (P<0.05).

Lippia alba essential oil, containing predominantly linalool, eucalyptol, γ-muurolene and E-caryophyllene in its composition, has also shown genotoxic effects in fish (*Oreochromis niloticus*) and mammals (*Mus musculus*) (Kampke et al. 2018). However, *L. origanides* EO, containing thymol as one of its major constituents, was used in a mouse peritoneal macrophage toxicity assay, and showed no toxicity against mammalian cells (cytotoxic concentration - CC_{50} > 100 µg mL^{-1}) (Borges et al. 2012). The toxicological property of thymol, major constituent in *L. origanides* EO, has already been observed (LC_{50} = 514 µg mL^{-1}) in the brine shrimp lethality assay (Meyer et al. 1982).

On the whole, this toxicity of *Lippia* essential oils is mostly attributed to the presence of phenols such as thymol, in *L. origanides* EO, aldehydes such as the two isomeric acyclic monoterpenes geranial and neral, in *L. alba* EO, and acyclic alcohols such as geraniol, linalool, and citronellol, in *L. alba* EO exclusively (Bruni et al. 2004, Sacchetti et al. 2005).

CONCLUSIONS

Essential oils extracted from *L. alba* and *L. origanoides* shared several components and exhibited similar bioactivities when examined for their antimicrobial and toxicity effects. *L. origanoides* EO showed higher potential to free radical scavenging activity than *L. alba* EO. These essential oils also showed promising antibacterial activity against *V. parahaemolyticus* strains. To our knowledge, this is the first report of the antibacterial

activity of *L. origanoides* essential oil against *V. parahaemolyticus*. Thereby, the studied EOs may be useful in controlling the pathogen *V. parahaemolyticus*, however, due to its exhibited toxicity against *A. salina*, further research is warranted into the usage of these EOs as candidates in the control of vibriosis infection. The *major compounds* identified in the essential oils appear to be directly involved in biological activity of these plants. In future research, it is important to isolate key components of essential oils to confirm their usefulness and to evaluate their antimicrobial, antioxidant and toxic potential separately, as well as conducting *in vivo* tests to attest the potential for use and safe dose in marine shrimp.

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REFERENCES

ADAMS RP. 2007. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, v. 456. IL: Allured publishing corporation, 811 p.

ALIGIANNIS N, KALPOUTZAKIS E, MITAKU S & CHINOU IB. 2001. Composition and antimicrobial activity of the essential oils of two Origanum species. J Agric Food Chem 49(9): 4168-4170.

BADAWY ME, MAREI GIK, RABEA EI & TAKTAK NE. 2019. Antimicrobial and antioxidant activities of hydrocarbon and oxygenated monoterpenes against some foodborne pathogens through *in vitro* and in silico studies. Pestic Biochem Phys 158: 185-200.

BASCHIERI A, AJVAZI MD, TONFACK JLF, VALGIMIGLI L & AMORATI R. 2017. Explaining the antioxidant activity of some common non-phenolic components of essential oils. Food Chem 232: 656-663.

BATISTA ES, BRANDÃO FR, MAJOLO C, INOUE LAKA, MACIEL PO, DE OLIVEIRA MR & CHAGAS EC. 2018. *Lippia alba* essential oil as anesthetic for tambaqui. Aquac 495: 545-549.

BORGES AR, DE ALBUQUERQUE AIRES JR, HIGINO TMM, DE MEDEIROS M DGF, CITÓ AMDGL, LOPES JAD & DE FIGUEIREDO RCBQ. 2012. Trypanocidal and cytotoxic activities of essential oils from medicinal plants of Northeast of Brazil. Exp Parasitol 132(2): 123-128.

BRAND-WILLIAMS W, CUVELIER ME & BERSET CLWT. 1995. Use of a free radical method to evaluate antioxidant activity. LWT 28(1): 25-30.

BRUNI R, MEDICI A, ANDREOTTI E, FANTIN C, MUZZOLI M, DEHESA M & SACCHETTI G. 2004. Chemical composition and biological activities of Ishpingo essential oil, a traditional Ecuadorian spice from *Ocotea quixos* (Lam.) Kosterm. (Lauraceae) flower calices. Food Chem 85(3): 415-421.

CAI R, ZHANG M, CUI L, YUAN Y, YANG Y, WANG Z & YUE T. 2019. Antibacterial activity and mechanism of thymol against *Alicyclobacillus acidoterrestris* vegetative cells and spores. LWT 105: 377-384.

CARVALHO PM, MACÊDO CA, RIBEIRO TF, SILVA AA, DA SILVA RE, DE MORAIS LP & BARBOSA R. 2018. Effect of the *Lippia alba* (Mill.) NE Brown essential oil and its main constituents, citral and limonene, on the tracheal smooth muscle of rats. Biotechnol Rep 17: 31-34.

CAVALCANTI SCH, NICULAU EDS, BLANK AF, CÂMARA CAG, ARAÚJO IN & ALVES PB. 2010. Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). Bioresour Technol 101(2): 829-832.

CHATURVEDI T, KUMAR A, KUMAR A, VERMA RS, PADALIA RC, SUNDARESAN V & VENKATESHA KT. 2018. Chemical composition, genetic diversity, antibacterial, antifungal and antioxidant activities of camphor-basil (*Ocimum kilimandscharicum* Guerke). Ind Crops Prod 118: 246-258.

CLSI - CLINICAL AND LABORATORY STANDARDS INSTITUTE. 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M07-A9, 9th ed., Wayne, PA, USA.

COSTA MDS, ROCHA JE, CAMPINA FF, SILVA AR, DA CRUZ RP, PEREIRA RL & TEIXEIRA AM. 2019. Comparative analysis of the antibacterial and drug-modulatory effect of d-limonene alone and complexed with β-cyclodextrin. European J Pharm Sci 128: 158-161.

DAMASCENO ETS, ALMEIDA RR, DE CARVALHO SYB, DE CARVALHO GSG, MANO V, PEREIRA AC & DE LIMA GUIMARÃES LG. 2018. *Lippia origanoides* Kunth. essential oil loaded in

nanogel based on the chitosan and p-coumaric acid: encapsulation efficiency and antioxidant activity. Ind Crops Prod 125: 85-94.

EL EUCH SK, HASSINE DB, CAZAUX S, BOUZOUITA N & BOUAJILA J. 2019. *Salvia officinalis* essential oil: Chemical analysis and evaluation of anti-enzymatic and antioxidant bioactivities. S African J Bot 120: 253-260.

ELMAHDI S, DASILVA LV & PARVEEN S. 2016. Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: a review. Food Microbiol 57: 128-134.

ELSHOPAKEY GE, RISHA EF, ABDALLA OA, OKAMURA Y, HARADA S, KISHIDA S & ITAMI T. 2018. Efficacy of dietary fermented vegetable product on immune response, up-regulation of immune-related genes and protection of kuruma shrimp (*Marsupenaeus japonicus*) against *Vibrio parahaemolyticus*. Aquac 497: 431-439.

ESPINA L, BERDEJO D, ALFONSO P, GARCÍA-GONZALO D & PAGÁN R. 2017. Potential use of carvacrol and citral to inactivate biofilm cells and eliminate biofouling. Food Control 82: 256-265.

FARHAT MB, SOTOMAYOR JA & JORDÁN MJ. 2019. *Salvia verbenaca* L. essential oil: Variation of yield and composition according to collection site and phenophase. Biochem Syst Ecol 82: 35-43.

FERNANDES AP, RIBEIRO GE, RUFINO LRA, SILVA LMD, BORIOLLO MFG, OLIVEIRA NDMS & FIORINI JE. 2011. Efeito do extrato hidroalcoólico de *Pyrostegia venusta* na mutagênese "*in vivo*", e avaliação antimicrobiana, e interferência no crescimento e diferenciação celular "*in vitro*". RMMG 21: 264-274.

FINNEY DJ. 1971. Quantal responses to mixtures. Probit Analysis. Third Edition. Cambridge University Press, Cambridge, United Kingdom, p. 230-268.

GHORBANI A & ESMAEILIZADEH M. 2017. Pharmacological properties of *Salvia officinalis* and its components. J Tradit Complement Med 7(4): 433-440.

GOMES AF, GANZERA M, SCHWAIGER S, STUPPNER H, HALABALAKI M, ALMEIDA MP & DAVID JM. 2018. Simultaneous determination of iridoids, phenylpropanoids and flavonoids in *Lippia alba* extracts by micellar electrokinetic capillary chromatography. Microchem J 138: 494-500.

HARIKRISHNAN R, BALASUNDARAM C & HEO MS. 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquac 317(1-4): 1-15.

HASHIMOTO GSO, NETO FM, RUIZ ML, ACCHILE M, CHAGAS EC, CHAVES FCM & MARTINS ML. 2016. Essential oils of *Lippia sidoides* and *Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia. Aquac 450: 182-186.

HU J, WANG W, DAI J & ZHU L. 2019. Chemical composition and biological activity against *Tribolium castaneum* (Coleoptera: Tenebrionidae) of *Artemisia brachyloba* essential oil. Ind Crops Prod 128: 29-37.

JANDA JM, NEWTON AE & BOPP CA. 2015. Vibriosis. Clin Lab Med 35(2): 273-288.

KAMPKE EH, DE SOUZA BARROSO ME, MARQUES FM, FRONZA M, SCHERER R, LEMOS MF & GOMES LC. 2018. Genotoxic effect of *Lippia alba* (Mill.) NE Brown essential oil on fish (*Oreochromis niloticus*) and mammal (*Mus musculus*). Environ Toxicol Pharmacol 59: 163-171.

KHIYA Z, HAYANI M, GAMAR A, KHARCHOUF S, AMINE S, BERREKHIS F, BOUZOUBAE A, ZAIR T & HILALI FE. 2018. Valorization of the *Salvia officinalis* L. of the Morocco bioactive extracts: Phytochemistry, antioxidant activity and corrosion inhibition. J King Saud Univ Eng Sci 31(3): 322-335.

LIAO PC, YANG TS, CHOU JC, CHEN J, LEE SC, KUO YH & CHAO LKP. 2015. Anti-inflammatory activity of neral and geranial isolated from fruits of *Litsea cubeba* Lour. J of Funct Foods 19: 248-258.

MARMONIER AA. 1990. Introduction aux techniques d'étude des antibiotiques. Bactériologie Médicale, technique usuelles, p. 227-236.

MENEZES PMN, BRITO MC, DE PAIVA GO, DOS SANTOS CO, DE OLIVEIRA LM, DE ARAÚJO RIBEIRO LA & SILVA FS. 2018. Relaxant effect of *Lippia origanoides* essential oil in guinea-pig trachea smooth muscle involves potassium channels and soluble guanylyl cyclase. J Ethnopharmacol 220: 16-25.

MEYER BN, FERRIGNI NR, PUTNAM JE, JACOBSEN LB, NICHOLS DJ & MCLAUGHLIN JL. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45(5): 31-34.

MILADI H, ZMANTAR T, KOUIDHI B, AL QURASHI YMA, BAKHROUF A, CHAABOUNI Y & CHAIEB K. 2017. Synergistic effect of eugenol, carvacrol, thymol, p-cymene and γ-terpinene on inhibition of drug resistance and biofilm formation of oral bacteria. Microb pathog 112: 156-163.

MILOS M & MAKOTA D. 2012. Investigation of antioxidant synergisms and antagonisms among thymol, carvacrol, thymoquinone and p-cymene in a model system using the Briggs–Rauscher oscillating reaction. Food Chem 131(1): 296-299.

NAUMKIN AV, KRAUT-VASS A, GAARENSTROOM SW & POWELL CJ. 2012. NIST X-ray photoelectron spectroscopy database,

NIST standard reference database 20, version 4.1. US Department of Commerce, Washington. Gaithersburg.

OLIVEIRA ARMF, JEZLER CN, OLIVEIRA RA, MIELKE MS & COSTA LC. 2012. Determinação do tempo de hidrodestilação e do horário de colheita no óleo essencial de menta. Hort Bras 30(1): 155-159.

OLIVERO-VERBEL J, GUEETTE-FERNANDEZ J & STASHENKO E. 2009. Acute toxicity against Artemia franciscana of essential oils isolated from plants of the genus *Lippia* and *Piper* collected in Colombia. B LatinoAm Caribe PL 8(5): 419-427.

ORTIZ-JIMÉNEZ MA. 2018. Quantitative evaluation of the risk of *Vibrio parahaemolyticus* through consumption of raw oysters (*Crassostrea corteziensis*) in Tepic, Mexico, under the RCP2. 6 and RCP8. 5 climate scenarios at different time horizons. Int Food Res J 111: 111-119.

PINTO NDOF, RODRIGUES THS, PEREIRA RDCA, SILVA LMA, CÁCERES CA, DE AZEREDO HMC & CANUTO KM. 2016. Production and physico-chemical characterization of nanocapsules of the essential oil from *Lippia sidoides* Cham. Ind Crops Prod 86: 279-288.

PRABU DL, CHANDRASEKAR S, AMBASHANKAR K, DAYAL JS, EBENEEZAR S, RAMACHANDRAN K & VIJAYAGOPAL P. 2018. Effect of dietary *Syzygium cumini* leaf powder on growth and non-specific immunity of *Litopenaeus vannamei* (Boone 1931) and defense against virulent strain of *Vibrio parahaemolyticus*. Aquac 489: 9-20.

QUEIROGA IMBN, SILVA GMDS, COSTA JSD, GUEDES JPDS, DANTAS CDO & CAVALCANTI MT. 2019. Characterization and application of *Lippia alba* (Mill) and *Cymbopogon citratus* DC Stapf. essential oils as natural sanitizers in coriander. Food Sci Technol Res 39(4): 993-998.

SACCHETTI G, MAIETTI S, MUZZOLI M, SCAGLIANTI M, MANFREDINI S, RADICE M & BRUNI R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food chem 91(4): 621-632.

SALIMENA FRG & MÚLGURA ME. 2015. Notas sobre o gênero *Lippia* (Verbenaceae) no Brasil. Bol Bot 33: 45-49.

SHIRMOHAMMADLI Y, EFHAMISISI D & PIZZI A. 2018. Tannins as a sustainable raw material for green chemistry: A review. Ind Crops Prod 126: 316-332.

SRINIVASAN P & RAMASAMY P. 2017. Morphological characterization and biocontrol effects of *Vibrio vulnificus* phages against Vibriosis in the shrimp aquaculture environment. Microb Pathog 111: 472-480.

STASHENKO EE, JARAMILLO BE & MARTÍNEZ JR. 2004. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) NE Brown, grown in Colombia, and evaluation of its *in vitro* antioxidant activity. J Chromatog A 1025(1): 93-103.

TELES S, PEREIRA JA, SANTOS CH, MENEZES RV, MALHEIRO R, LUCCHESE AM & SILVA F. 2012. Geographical origin and drying methodology may affect the essential oil of *Lippia alba* (Mill) NE Brown. Ind Crops Prod 37(1): 247-252.

TELES S, PEREIRA JA, DE OLIVEIRA LM, MALHEIRO R, LUCCHESE AM & SILVA F. 2014. *Lippia origanoides* HBK essential oil production, composition, and antioxidant activity under organic and mineral fertilization: Effect of harvest moment. Ind Crops Prod 60: 217-225.

TEPAAMORNDECH S, CHANTARASAKHA K, KINGCHA Y, CHAIYAPECHARA S, PHROMSON M, SRIARIYANUN M & VISESSANGUAN W. 2019. Effects of *Bacillus aryabhattai* TBRC8450 on vibriosis resistance and immune enhancement in Pacific white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol 86: 4-13.

TIMÓTEO P, KARIOTI A, LEITÃO SG, VINCIERI FF & BILIA AR. 2015. A validated HPLC method for the analysis of herbal teas from three chemotypes of Brazilian *Lippia alba*. Food Chem 175: 366-373.

VAIČIULYTĖ V, LOŽIENĖ K, TARAŠKEVIČIUS R & BUTKIENĖ R. 2017. Variation of essential oil composition of *Thymus pulegioides* in relation to soil chemistry. Ind Crops Prod 95: 422-433.

VALADBEIGI T. 2016. Chemical Composition and Enzymes Inhibitory, Brine Shrimp Larvae Toxicity, Antimicrobial and Antioxidant Activities of *Caloplaca biatorina*. Zahedan J Res Med Sci 18(11): e4267.

VARGAS-ALBORES F, GUZMÁN MA & OCHOA JL. 1993. An anticoagulant solution for haemolymph collection and prophenoloxidase studies of penaeid shrimp (*Penaeus californiensis*). Comp biochem physiol Mol Amp Integr Physiol 106(2): 299-303.

VERAS HN, RODRIGUES FF, BOTELHO MA, MENEZES IR, COUTINHO HD & COSTA JG. 2017. Enhancement of aminoglycosides and β-lactams antibiotic activity by essential oil of *Lippia sidoides* Cham. and the Thymol. Arab J Chem 10: 2790-2795.

VIEIRA CB, SOUSA OV, GESTEIRA TCV, DE CARVALHO FCT & DOS FERNANDES VIEIRA RHS. 2009. *Vibrio* spp. em hemolinfa de camarões *Litopenaeus vannamei* coletados em três fazendas de cultivo do Estado do Ceará. Bol Tec Cient CEPNOR 9 (1): 141-150.

WANGMAN P, LONGYANT S, TAENGCHAIYAPHUM S, SENAPIN S, SITHIGORNGUL P & CHAIVISUTHANGKURA P. 2018. PirA & B

toxins discovered in archived shrimp pathogenic *Vibrio campbellii* isolated long before EMS/AHPND outbreaks. Aquac 497: 494-502.

YEN HY & LIN YC. 2017. Green extraction of *Cymbopogon citratus* essential oil by solar energy. Ind Crops Prod 108: 716-721.

YOO HJ & JWA SK. 2019. Efficacy of β-caryophyllene for periodontal disease related factors. Arch Oral Biol 100:113-118.

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All authors participated in the planning of the experiment, read and approved the manuscript in its final version. The authors Souza ASQ, Canuto KM & Brito ES performed the extraction and analysis of the chemical composition of essential oils. The authors Santos Filho LGA, Reis RB, Castro KNC, Pereira AML developed the in vitro microbiological assays, toxicity test and antioxidant activity. Santos Filho LGA, Pereira AML and Diniz FM wrote the manuscript.