Research, Society and Development, v. 11, n. 9, e6811931478, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i9.31478

Inhibitory and bactericidal activities of *Lippia origanoides* essential oil against *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* multidrug resistant

Atividades inibitória e bactericida do óleo essencial de *Lippia origanoides* frente a *Escherichia coli, Klebsiella pneumoniae* e *Pseudomonas aeruginosa* multidrogas resistentes

Actividades inhibitorias y bactericidas del aceite esencial de *Lippia origanoides* frente a *Escherichia coli, Klebsiella pneumoniae* y *Pseudomonas aeruginosa* multirresistente

Received: 06/07/2022 | Reviewed: 06/18/2022 | Accept: 06/22/2022 | Published: 07/03/2022

Ludimila Gomes Pinheiro
ORCID: https://orcid.org/0000-0001-8936-6874
Federal University of Ceará, Brazil
E-mail: ludimilagomes_ce@hotmail.com
Raquel Oliveira dos Santos Fontenelle
ORCID: https://orcid.org/0000-0002-8865-5954
Vale do Acaraú State University, Brazil
E-mail: raquelbios@yahoo.com.br
Tigressa Helena Soares Rodrigues
ORCID: https://orcid.org/0000-0002-8044-3121
Vale do Acaraú State University, Brazil
E-mail: tigressa_helena@uvanet.br
Vicente de Paulo Teixeira Pinto
ORCID: https://orcid.org/0000-0002-8785-2171
Federal University of Ceará, Brazil
E-mail: pintovicente@gmail.com
Francisco Cesar Barroso Barbosa
ORCID: https://orcid.org/0000-0002-3444-6997
Federal University of Ceará, Brazil
E-mail: fcbbarbosa@yahoo.com.br

Abstract
The objective of this study was to evaluate the antimicrobial potential of essential oil (EO) from *L. origanoides* against Gram-negative bacteria with multidrug resistance (MDR) phenotype and to correlate the results obtained with the genetic profile of these microorganisms. The oil was extracted from the leaves of this plant by steam dragging and condensation with Clevenger equipment, the chemical components were identified and quantified by gas chromatography coupled with mass spectrometry and gas chromatography coupled with the flame ionization detector. Twenty clinical isolates and one standard strain of each species were analyzed: *E. coli*, *K. pneumoniae* and *P. aeruginosa* (n=63). The antibacterial potential was evaluated by broth microdilution tests. Thymol is the prevalent component (87.37%). The EO at a concentration of 312 μg/mL was effective in inhibiting 52% of *E. coli* strains and about 38% of *K. pneumoniae*. On the other hand, for *P. aeruginosa* a concentration of 2,500 μg/mL was needed to obtain growth inhibition of approximately 38% of the strains. Among the resistance genes detected, the highest prevalence was $bla_{\text{CTX-M 1/2}}$, however, even with the presence of these genes, an antimicrobial action of this EO was observed. Therefore, the results of this study suggest that the EO of *L. origanoides* presented an efficient antibacterial action with potential use in the fight against infections by MDR microorganisms.
Keywords: Phytotherapics; Natural products; Infections; Verbenaceae; β-lactamases.

Resumo
O objetivo deste estudo foi avaliar o potencial antimicrobiano do óleo essencial (OE) de *L. origanoides* contra bactérias Gram-negativas com fenótipo de multirresistência (MDR) e correlacionar os resultados obtidos com o perfil genético desses microrganismos. O óleo foi extraído das folhas desta planta por arraste de vapor e condensação com equipamento Clevenger, os componentes químicos foram identificados e quantificados por cromatografia gasosa acoplada à espectrometria de massas e cromatografia gasosa acoplada ao detector de ionização de chama. Foram

analisados vinte isolados clínicos e uma cepa padrão de cada espécie: *E. coli*, *K. pneumoniae* e *P. aeruginosa* (n=63). O potencial antibacteriano foi avaliado por testes de microdiluição em caldo. O timol é o componente prevalente (87,37%). O OE na concentração de 312 μg/mL foi eficaz na inibição de 52% das cepas de *E. coli* e cerca de 38% de *K. pneumoniae*. Por outro lado, para *P. aeruginosa* foi necessária uma concentração de 2.500 μg/mL para obter inibição do crescimento de aproximadamente 38% das cepas. Dentre os genes de resistência detectados, o de maior prevalência foi o $bla_{\text{CTX-M 1/2}}$, porém, mesmo com a presença desses genes, observou-se ação antimicrobiana deste OE. Portanto, os resultados deste estudo sugerem que o OE de *L. origanoides* apresentou uma ação antibacteriana eficiente com potencial de uso no combate a infecções por microrganismos MDR.
Palavras-chave: Fitoterápicos; Produtos naturais; Infecções; Verbenaceae; β-lactamase.

Resumen
El objetivo de este estudio fue evaluar el potencial antimicrobiano del aceite esencial (AE) de *L. origanoides* frente a bacterias Gram negativas con fenotipo multirresistente (MDR) y correlacionar los resultados obtenidos con el perfil genético de estos microorganismos. El aceite fue extraído de las hojas de esta planta por arrastre de vapor y condensación con equipo Clevenger, los componentes químicos fueron identificados y cuantificados por cromatografía de gases acoplada a espectrometría de masas y cromatografía de gases acoplada a detector de ionización de llama. Se analizaron veinte aislados clínicos y una cepa estándar de cada especie: *E. coli*, *K. pneumoniae* y *P. aeruginosa* (n=63). El potencial antibacteriano se evaluó mediante pruebas de microdilución en caldo. El timol es el componente predominante (87,37%). El AE a una concentración de 312 μg/mL fue eficaz para inhibir el 52% de las cepas de *E. coli* y aproximadamente el 38% de *K. pneumoniae*. En cambio, para *P. aeruginosa* fue necesaria una concentración de 2.500 μg/mL para obtener una inhibición del crecimiento de aproximadamente el 38% de las cepas. Entre los genes de resistencia detectados, el más prevalente fue $bla_{\text{CTX-M 1/2}}$, sin embargo, aun con la presencia de estos genes, se observó una acción antimicrobiana de este AE. Por lo tanto, los resultados de este estudio sugieren que el AE de *L. origanoides* presentó una acción antibacteriana eficiente con potencial uso en el combate a infecciones por microorganismos MDR.
Palabras clave: Hierbas medicinales; Productos naturales; Infecciones; Verbenáceas; β-lactamasas.

1. Introduction

Gram negative bacilli are organisms present in various environments and that participate in the balance of the habitats to which they are related, but when subjected to unfavorable situations they have the remarkable ability to take advantage of survival strategies. Multidrug-resistant bacteria are a matter of preoccupation in the global health area, as they narrow or even eliminate the existing therapeutic options (Fernández, *et al*., 2016).

These microorganisms can resist by several mechanisms such as: enzymatic products, alteration of membrane proteins, in addition to that, the genetic material can be easily transmitted between bacterial cells through specific cell structure (Ting, *et al.*, 2018). The action of β-lactamases on Gram negative bacteria is more favorable and efficient, since they are distributed in the periplasmic space, inactivating the antimicrobial before it has contact with the target of action present in the cytoplasmic membrane (Pragasam, *et al*., 2016).

Essential oils are a complex of volatile organic chemicals produced by secondary metabolites responsible for the biological activities of plants. Terpenes, aromatic and aliphatic compounds such as alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenolic ethers are among the most prevalent components in these substances (Puškárová, *et al.*, 2017).

From the semi-arid region of northeastern Brazil, *Lippia origanoides* Kunth (Verbenaceae) is an aromatic plant with insecticidal, antibacterial, larvicide, acaricide, and anti-inflammatory properties, among others (Baldim, *et al*., 2019). Thymol and carvacrol, commonly found in the essential oil of *Lippia* leaves, target the cell membrane, affecting electrostatic balance and other vital functions. The efficiency of these extracts is related exactly to the complexity of the compounds that behave synergistically (Khan & Ahmad, 2011).

Given the above, the purpose of this study was to evaluate the antimicrobial potential of the essential oil from *L. origanoides* against Gram-negative bacteria with multidrug resistance (MDR) phenotype and to correlate the results obtained with the genetic profile of these microorganisms.

2. Materials and Methods

The research, guided by Resolution N° 466/12 of the Brazil National Health Council, complies with ethical aspects and obtained a favorable opinion by the Research Ethics Committee of the State University of Vale do Acaraú, Sobral, Ceará, Brazil (4,633,262/ 2021). In order to follow the criteria established by Law N° 13.123/15 and its regulations, *Lippia origanoides* was registered in the database of the National System for the Management of Genetic Heritage and Associated Traditional Knowledge under N° AEBF288.

2.1 Obtaining essential oil

Fresh leaves of *L. origanoides*, HUVA exsicate n° 23.372, were cleaned with flowing water followed by distilled water. The oil was obtained by hydrodistillation, this procedure consisted of steam dragging and condensation with Clevenger equipment. For approximately 3 hours of boiling, the hydrolate is obtained (Ehlert, *et al*., 2006).

2.2 Determination of chemical composition

2.2.1 Gas chromatography coupled to mass spectrometry (GC-MS)

The qualitative analysis of the oils was performed by GC-MS using an Agilent model GC-7890B/MSD-5977A (quadrupole), with electron impact at 70 eV, HP-5MS methylpolysiloxane column (30m x 0,25mm x 0,25µm, Agilent), helium carrier gas with flow rate 1,00 mL/min (8,8 psi) and constant linear velocity of 36,8 cm/s, injector temperature 250 °C, detector temperature 150 °C, transfer line temperature 280 °C. The identification of the compounds was performed by analyzing the fragmentation patterns displayed in the mass spectra with those present in the database provided by the equipment (NIST version 2.0 of 2012 – 243,893 compounds) and literature data (Moita, *et al*., 2022).

2.2.2 Gas chromatography coupled to the flame ionization detector (CG-DIC)

The quantitative analysis was performed by CG-DIC using a Shimadzu model CG-2010 Plus, RTX-5 methylpolysiloxane column (30m x 0.25mm x 0.25µm), injection mode with 1:30 flow division, gas nitrogen carrier with flow 1.0 mL/min (84,1 kPa) and constant linear velocity of cm/s, injector temperature 250 °C, detector temperature 280 °C. Identification of compounds was performed by comparing their retention indices with those of known compounds, obtained by injecting a mixture of standards containing a homologous series of C_7-C_{30} alkanes and literature data (Moita, *et al*., 2022)

2.3 Bacterial strains

Sixty isolates, one more standard strain from each specimen (*E. coli*, *K. pneumoniae*, *P. aeruginosa*), were used in the experiments. These are part of a biological collection formed at Laboratory of Microbiology and Parasitology of the FAMED (FUC/Sobral) and come from hospitals located in the city of Sobral/CE, Brazil.

The reactivation of specimens stored in a freezer at -80 ºC was carried out by adding a 50 µl aliquot of the culture in a test tube containing 5,0 mL of Brain Heart Infusion broth, which was subsequently incubated for 18 hours at 35 ºC. After growth, the inoculum was seeded on a plate with MacConkey Agar using a sterile bacteriological loop and incubated again at 35 ºC for 24 h. After growth, the purity of the sample was confirmed by observing the morphological and dyeing characteristics by Gram stain (Morais, *et al*., 2019).

2.4 Broth Microdilution Test

The determination of the Minimum Inhibitory Concentration (MIC) of *L. origanoides* oil was performed in triplicate according to microdilution methodology in 96-well polystyrene plates standardized in accordance with the document M07-A11

10 th edition, Methods for Diluition Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, a standard developed through the conceptual process of Clinical and Laboratory Standards Institute (CLSI, 2018).

The wells of columns 1 to 11 were filled with 100 µL culture medium, after 100 µL the essential oil at an initial concentration of 2,500 µg/mL was added to column 1 and following the microdilution process, the other desired decreasing concentrations were obtained. Finally, each well received 100 µL of bacterial suspension adjusted to approximately $5x10^7$ CFU/mL according to the 0.5 MacFarland scale. The last column was reserved for the controls: negative (only culture medium), positive (bacterial suspension plus culture medium), toxicity (bacterial culture plus diluent - Tween 80 at 0.02%). Lines D and H were turbidity control, in this case, culture medium more essential oil in their respective dilutions.

Then, the microplate was incubated at 35 °C for 24 h and after this period it was analyzed by an ELISA reader (BIO Trak II – Plate Reader®) with a wavelength of 620 nm to quantify bacterial growth through turbidity (Fernandes, *et al*., 2016).

2.5 Minimum Bactericidal Concentration (MBC)

The determination of the Minimum Bactericidal Concentration (MBC) was performed according to the method proposed by Hafidh *et al*., (2011). After determining the MIC, 10 µl from the wells where there was no visible microbial growth were transferred to Petri dishes containing Muller Hinton Agar medium, which were then incubated at 35 °C for 24 h in the aerobic growth oven. MBC was considered the lowest concentration of the compound where there is no cell growth on the surface of the inoculated agar (99.9% microbial death).

2.6 Resistance profile analysis by the presence of ESBL

The Vitek 2 system carried out ESBL phenotypic identification and antimicrobial susceptibility tests (BioMérieux). This automated system utilizes antibioticcontaining cards (AST N105 card) as suggested by the Clinical and Laboratory Standards Institute - CLSI (CLSI, 2018). Previous studies carried out by the present research group investigated the presence of some resistance genes in these bacterial samples. For genotypic characterization, the genomic DNA of the bacteria was extracted with the Easy DNA™ kit (Invitrogen, USA). The DNA obtained was evaluated for its stability by electrophoresis performed at 120V for 40 minutes in a 0.8% agarose gel with ethidium bromide, 10 µl of genomic DNA plus 2 µl of bromophenol blue. The quantification of the extracted product was performed in a spectrophotometer (Gene Quant, Amersham, USA). This genetic material was subjected to conventional Polymerase Chain Reaction (cPCR) to detect the *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{GES}}$, *bla*$_{\text{IMP}}$ and *bla*$_{\text{KPC}}$ genes with parameters described by (Rocha, *et al*., 2019; dos Santos, *et al*., 2018). The data, referring to the resistance profile due to the presence of ESBL genes, were associated with the values obtained for MIC and MBC to try to relate the different inhibition values with the presence of certain genes.

2.7 Statistical analysis

The difference between the means of the replicates was evaluated by One-way ANOVA test with Bonferroni post-test, with the support of the GraphPad® Prism version 5.04 for Windows (GraphPad Software, San Diego California USA). Differences with $p < 0.05$ were considered significant.

3. Results and Discussion

The constituents of the essential oil of *L. origanoides* found are shown in the Table 1. It was observed that the chemical compound that presented the highest concentrations, being the major constituent was thymol (87.37%).

Table 1. Chemical composition of the essential oil of *Lippia origanoides*. [1]Calculated IK values. [2]IK values of the literature (Adams, 2007).

Compound	$^{1}IK_{calc}$	$^{2}IK_{lit}$	Area (%)
α-Pinene	941	939	0,54
Myrcene	998	990	0,44
p-Cymene	1030	1024	4,45
Eucalyptol	1038	1031	0,65
Terpinen-4-ol	1183	1177	0,52
α-Terpineol	1198	1188	0,21
Thymol methyl ether	1240	1235	0,36
Thymol	1296	1290	87,37
β-Caryophyllene	1423	1419	3,11
Aromandendrene	1443	1441	0,36
α-Caryophyllene	1458	1454	0,18
γ-Muurolene	1480	1479	0,06
Bicyclogermacrene	1497	1500	0,29
δ-Cadinene	1527	1523	0,21
Spathulenol	1582	1578	0,15
Caryophylene oxide	1586	1583	0,98
Total Composition			99,89

Source: Elaborated by the authors.

Thymol is one of the monoterpenic phenols significantly present in essential oils of plants included in the Verbenaceae family (*Aloysia triphylla*, *Laosaurus gracilis*, *Lasius grandis*, *Lippia origanoides*). Biologically, it has an antioxidant, anti-inflammatory, local anesthetic, healing and antiseptic action, with an emphasis on antibacterial and antifungal properties (Amalraj, *et al*., 2020). Alcohols are also common chemical components present in essential oils, with a structure of intense binding affinity with various molecules such as proteins or glycoproteins. Thus, they demonstrate a high potential to permeate cell walls, leading to the loss of cytoplasmic material (Pérez Zamora, *et al*., 2018).

All isolates used in this study were beta-lactamase producers according to the susceptibility profile automatically traced at the time that they were identified. Among the genes investigated, $bla_{\text{CTX-M1/2}}$ was the most prevalent in all samples. However, the presence of this resistance gene did not prevent the bactericidal action of the oil in question. The result with the *E. coli* (EC 42) that harbored $bla_{\text{CTX-M1/2}}$ and bla_{TEM} genes was the same when compared to the standard sample ATCC 25922 that did not have such genes, showing an inhibitory and bactericidal action on concentration of 312 µg/mL for both.

The essential oil had an inhibitory and bactericidal action in 10 (50%) of the *E. coli* samples analyzed at a concentration of 625 µg/mL. At this same concentration, a bactericidal effect was observed in 8 (40%) isolates of *K. pneumoniae*. Regarding *P. aeruginosa* isolates, the OE proved to be less effective for most, since it showed a bactericidal effect for only 6 (30%) nosocomial isolates and for the standard strain, but with the highest tested concentration of the compound (2,500 µg/mL) for 5 (71.4%) of these, and in 2 (33.3%) isolates and in the standard strain the MIC was equal to CBM (2,500 µg/mL). Furthermore, in 4 (20%) strains the OE, at the concentrations tested, did not show an inhibitory effect. The values can be seen in tables 2, 3 and 4. The greater resistance of *P. aeruginosa* can be attributed to the excellent ability to form biofilm and the action of efflux pumps, intrinsic characteristics of this species (Moradali, *et al*., 2017).

Table 2. MIC, MBC of *L. origanoides* essential oil against *E. coli* isolates and ATCC 25922, and presence of the investigated resistance genes.

E. coli samples	Essential oil of *L. origanoides* µg/mL		ESBL genes
	MIC	MBC	
EC 03	625	625	
EC 04	625	625	
EC 08	625	625	CTX-M 1/2
EC 10	625	625	
EC 11	625	625	
EC 12	625	625	CTX-M 1/2
EC 16	312	312	
EC 18	625	625	CTX-M 1/2
EC 22	625	625	
EC 23	312	1,250	CTX-M 1/2
EC 29	625	625	
EC 31	625	625	
EC 32	312	312	
EC 33	312	312	
EC 34	312	312	CTX-M 1/2
EC 37	312	625	
EC 38	312	312	
EC 39	312	312	
EC 40	312	625	CTX-M 1/2 TEM SHV
EC 42	312	312	CTX-M 1/2 TEM
ATCC 25922	312	312	-
Geometric mean	461	536	

Source: Elaborated by the authors.

Table 3. MIC, MBC of *L. origanoides* essential oil against *K. pneumoniae* isolates and ATCC 70063, and presence of the investigated resistance genes.

K. pneumoniae samples	Essential oil of *L. origanoides* µg/mL		ESBL genes
	MIC	MBC	
KP 01	312	312	
			CTX-M 1/2
KP 02	312	312	TEM
			SHV
KP 05	312	312	
KP 06	312	312	
KP 07	312	312	
KP 09	312	625	
KP 13	312	312	
KP 14	1,250	1,250	
KP 17	1,250	1,250	
KP 19	1,250	1,250	
KP 20	1,250	1,250	
KP 21	1,250	1,250	
KP 24	625	625	
KP 25	625	625	
KP 26	625	625	
KP 28	625	625	
KP 35	625	625	
KP 36	625	1,250	CTX-M 1/2
KP 41	625	625	CTX-M 1/2
KP 49	312	625	
ATCC 70063	2,500	2,500	
Geometric mean	744	803	

Source: Elaborated by the authors.

Table 4. MIC, MBC of *L. origanoides* essential oil against *P. aeruginosa* isolates and ATCC 15442, and presence of the investigated resistance genes.

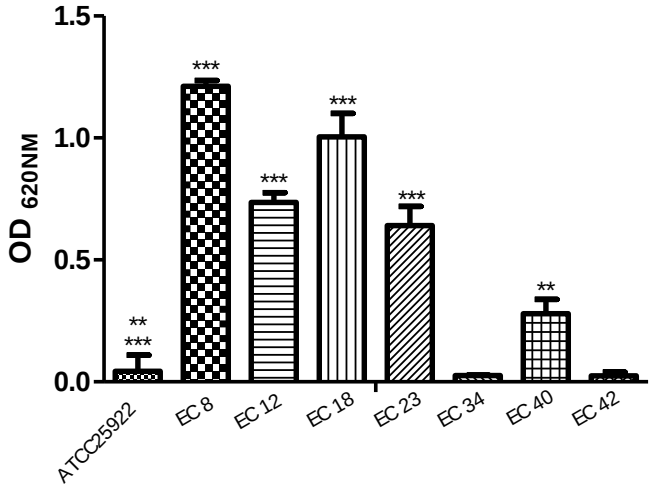
P. aeruginosa samples	Essential oil of *L. origanoides* µg/mL		ESBL/KPC/IMP genes
	MIC	MBC	
PA 02	625	2,500	SHV CTX-M 1/2 KPC
PA 04	625	2,500	
PA 06	312	625	CTX-M 1/2
PA 08	2,500	2,500	CTX-M 1/2 TEM GES
PA 09	1,250	-	CTX-M 1/2 TEM IMP-1
PA 12	2,500	-	CTX-M 1/2 GES
PA 13	-	-	CTX-M 1/2 GES
PA 14	1,250	-	CTX-M 1/2
PA 15	2,500	-	
PA 16	625	-	SHV
PA 17	312	1,250	SHV
PA 20	1,250	-	
PA 22	1,250	-	
PA 23	-	-	CTX-M 1/2
PA 28	2,500	-	
PA 30	2,500	-	GES
PA 31	-	-	
PA 32	-	-	
PA 33	2,500	2,500	TEM
PA 34	2,500	-	TEM
ATCC 15442	2,500	2,500	
Geometric mean	1,309	685	

Source: Elaborated by the authors.

Studies have shown that the chemical composition of essential oils are preponderant in the final results of the proposed objectives, and the knowledge generated promote to new discoveries and improvements. Pandini *et al*., (2017) used the essential oil of *Guarea kunthiana* abundant in α-zingiberene, which showed bactericidal activity against standard strains of *Salmonella enterica* ATCC 14028; *Proteus mirabilis* ATCC 25933 and *P. aeruginosa* ATCC 27853, but it was not efficient against *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 13883 even with a concentration of 7,000 µg/mL for both MIC and MBC.

In figures 1, 2 and 3, we can visualize the action of *L. origanoides* essential oil against the bacteria studied, showing that the presence of the resistance genes studied did not impair its inhibitory capacity. So, EO at a concentration of 312 µg/mL had a bactericidal effect against the *E. coli* (EC 34) that harbored $bla_{\text{CTX-M 1/2}}$ gene, as well as against ATCC 25922 that did not have these resistance genes, a similar behavior was observed in EC 42, which carries more than one ESBL gene (Figure 1).

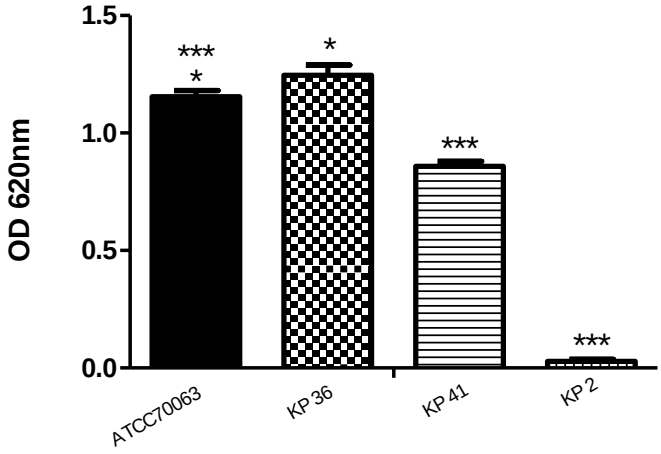
Figure 1. Growth of *E. coli* strains. ATCC 25922 and clinical isolates with at least one identified ESBL gene (EC8, EC12, EC23 and EC34), as well as clinical isolates with more than one identified ESBL gene (EC40 and EC42) and *L. origanoides* essential oil activity at 312 µg/mL. Statistically significant values *** ($p \leq 0.001$) and ** ($p \leq 0,01$) compared to control.



Source: Elaborated by the authors.

In relation to *K. pneumoniae* isolates, OE had a bactericidal effect even in those that contained ESBL genes, including a smaller MBC (312 µg/mL) to eliminate the KP 2 strain, which carried three of the analyzed resistance genes, than the bactericidal concentration required for the ATCC 70063 strain that did not have any ESBL gene ($p \leq 0.001$) (Figure 2).

Figure 2. Growth of *K. pneumoniae* strains. ATCC 70063 and clinical isolates with at least one ESBL gene identified (KP36 and KP41), as well as clinical isolates with more than one ESBL gene identified (KP2) and *L. origanoides* essential oil activity at 312 µg/mL. Statistically significant values * ($p \leq 0.05$) and *** ($p \leq 0,001$) compared to control.

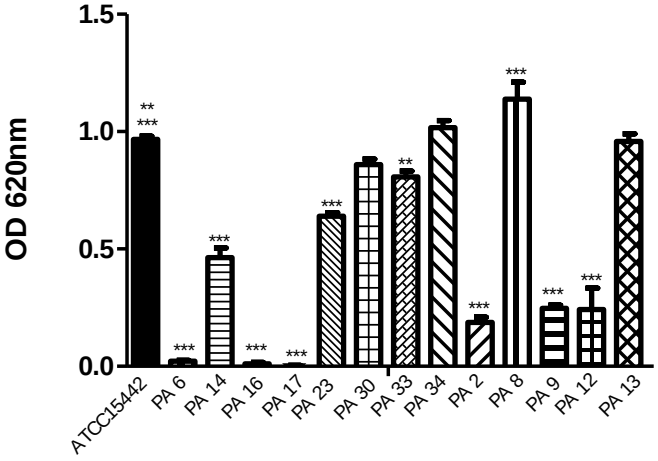


Source: Elaborated by the authors.

The results obtained with *P. aeruginosa* also followed the same behaviors (Table 4 and Figure 3). Concentrations of 625 µg/mL and 1,250 µg/mL of EO were required to obtain a bactericidal effect against PA06 and PA17 strains, respectively,

both with ESBL genes. However, to obtain a bactericidal effect against the ATCC 15442 strain that did not carry these genes, a concentration of two to four times higher was necessary ($p \leq 0.001$).

Figure 3. Growth of *P. aeruginosa* bacterial strains. ATCC 15442 and clinical isolates with at least one ESBL gene identified (PA6, PA14, PA16, PA17, PA23, PA30, PA33 and PA34), as well as isolates with more than one ESBL gene (PA2, PA8, PA9, PA12 and PA13), and *L. origanoides* essential oil activity at 1,250 µg/mL. Statistically significant values ** ($p \leq 0.01$) and *** ($p \leq 0,001$) compared to control.



Source: Elaborated by the authors.

Imane *et al.*, (2020) tested six essential oils: *Rosmarinus officinalis L., Zingiber officinale Roscoe, Melaleuca alternifolia Cheel, Cymbopogon winterianus, Salvia sclarea L.* and *Syzygium aromaticum* against multidrug-resistant bacteria such as *E. coli* ESBL and obtained good bactericidal results, emphasizing that the action of compounds does not depend on the antibiotic susceptibility profile. The vast chemical composition of substances stands out in biological action. The MIC and MBC of *S. aromaticum* against *E. coli* ESBL was 210 µg/mL, with the same result for strain ATCC 25922 and for *K. pneumoniae* ATCC 70063. These results are similar to those obtained in the present study, since the MIC and MBC obtained for *E. coli* strains, for example, were 312 µg/mL for ATCC 25922 and also for 35% of the resistance profile samples tested.

Ribeiro *et al.*, (2020) tested several essential oils against antibiotic resistant *E. coli, P. aeruginosa* and *K. pneumoniae* isolates and demonstrated better activity of cinnamon and Chinese oregano (*Cinnamomum cassia* and *Origanum compactum*) with the best MIC results, with 250 µg/mL for *E. coli* and *K. pneumoniae* samples and 500 µg/mL for *P. aeruginosa*. The other compounds tested had a MIC as from 1,000 µg/mL. The highlighted activities were related to the prevalence of cinnamaldehyde in Chinese cinnamon and carvacrol in oregano. The authors described that penetration of oils is more difficult in Gram negatives due to the presence of the outer membrane, which does not prevent the compounds from gaining access to the cells, even if slowly, through porins or by destructuring the unsaturated fatty acids that make up the external structure and therefore disordering the cell. The main mechanisms of action described are: membrane destabilization, enzymatic inhibitions, such as ATPase activity, interruption of cell division or failure of efflux pump activity.

Freitas *et al.*, (2020) tested essential oil extracted from the leaves of *Baccharis coridifolia* against *E. coli* and *P. aeruginosa* multiresistant and obtained divergent results from the findings in this study, since, for *P. aeruginosa* there was an inhibitory effect with a MIC of 128 µg/mL, while there was no effect for *E. coli*. The effects are attributed to sesquiterpenes such as germacrene D and α-caryophyllene as main components.

Veras *et al.*, (2017) analyzed the behavior of *L. origanoides* essential oil against some microorganisms and obtained MIC of 256 µg/mL for *K. pneumoniae* ATCC 10031 and 512 µg/mL for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 15442, values one more time, similar to the studies already mentioned and close to this research. The main constituents identified were thymol (84.9%), ethyl-methyl-carvacrol (5.33%), and p-cymene (3.01%).

While Morais *et al.*, (2016) working with essential oil of *L. origanoides* collected from São Gonçalo do Abaeté, in the state of Minas Gerais, Brazil, found MIC of 2,000 µg/mL for samples of *P. aeruginosa* ATCC 9027 and *E. coli* ATCC 11229, the plants compound originating from this region had isoborneol (14.66%), bornyl acetate (11.86%), α-humulene (11.23%) as prevalent components. The yield and chemical composition of essential oils are determined by a combination of natural and genomic factors, being therefore variable and these variations will determine physical, chemical, biological and organoleptic properties, defining the usual and commercial purpose of the substance (Saraiva, *et al*., 2020).

A review published by Pérez Zamora *et al*., (2018) evidenced results of tests with essential oil of *L. origanoides* that presented MIC values of 256 µg/mL for *K. pneumoniae* and 512 µg/mL for *P. aeruginosa* and *E. coli*, results similar to those found in the present study. In addition, *L. origanoides* essential oil was active against strains of *Candida* sp., *Staphylococcus aureus, Streptococcus mutans, Providencia rettigeri, Enterobacter cloacae* and *Enterococcus faecalis*. The studies mentioned in the review above used microorganisms from standard strains and isolated from foods such as milk and cheese.

The secondary components of essential oils, even at low concentrations, can contribute to the overall efficiency of antimicrobial activity through synergistic interaction with other constituents. Thus, the main components, representing many times more than 85% of the total, are not responsible for the high performance of an antibacterial activity alone (Pandini, *et al*., 2017).

A limitation of this study would be the fact that we did not test separately the antimicrobial action of thymol, the main component of the EO of *L. origanoides*, against the pathogens analyzed.

4. Conclusion

The results of this research showed that the essential oil extracted from the leaves of *L. origanoides* has an inhibitory and bactericidal action against ESBL-positive Gram-negative pathogens, an action that can be attributed to the prevalence of thymol and other secondary terpenes in its composition, and that this action is not restricted by the presence of resistance genes that encode beta-lactamases conferring advantages in the virulence of microorganisms.

In this way, the antibacterial action against nosocomial isolates makes the compound promising for improving the performance of currently inactive drugs or even for the development of new drugs aimed at the treatment of nosocomial infections, suggesting complementary studies.

References

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream: Allured publishing corporation. 456, 544-545.

Amalraj, A., Haponiuk, J. T., Thomas, S. & Gopi, S. (2020). Preparation, characterization and antimicrobial activity of polyvinyl alcohol/gum arabic/chitosan composite films incorporated with black pepper essential oil and ginger essential oil. *International Journal of Biological Macromolecules*, 151, 366-375.

Baldim, I., Tonani, L., Kress, M. R. Z. & Oliveira, W. P. (2019). *Lippia sidoides* essential oil encapsulated in lipid nanosystem as an anti-*Candida* agente. *Industrial Crops and Products*, 127, 73-81.

Clinical and Laboratory Standards Institute. 2018. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, approved standard (10th ed.) M07-A11. Clinical and Laboratory Standards Institute, Wayne, PA.

dos Santos, R. A., Pinto, V. P. T. & Barbosa, F. C. B. (2018). Antimicrobial Susceptibility and Genetic Characterization of Extended Spectrum Beta-Lactamase (ESBL)-Producing Nosocomial Strains of *Escherichia coli* Isolated from a Brazilian Teaching Hospital. *Int. J. Curr. Microbiol. App. Sci.,* 7(8), 422-434.

Ehlert, P. A. D., Blank, A. F., Arrigoni-Blank, M. D. F., Paula, J. W. A. D., Campos, D. D. A. & Alviano, C. S. (2006). Hydrodistillation time in essential oil extraction from seven species of medicinal plants. *Rev. Bras. Pl. Med*., 8(2), 79-80.

Fernandes, S.R., Ferreira, H.D., Chaul, L.T., Sá, S., Alves, V.F., Tresvenzol, L.M.F., Ferri, P.H., Santos, P.A., Paula, J.R. & Fiuza, T.S. (2016). Composição química do óleo essencial e avaliação da atividade antimicrobiana das folhas de *Trembleya phlogiformis* DC. *Rev. Bras. Pl. Med*, 18(4), 828-836.

Fernández, J., Bert, F. & Nicolas-Chanoine M. H. (2016). The challenges of multi-drug-resistance in hepatology. *Journal of hepatology*, 65(5), 1043-1054.

Freitas, P. R., de Araújo, A. C. J., Barbosa, C. R. S., Muniz, D. F., Rocha, J. E., de Araújo Neto, J. B., Silva, M. M. C., Pereira, R. L. S., Silva, L. E., Amaral, W., Deschamps, C., Tintino, S. R., Ribeiro-Filho, J. & Coutinho, H. D. M. (2020). Characterization and antibacterial activity of the essential oil obtained from the leaves of *Baccharis coridifolia* DC against multiresistant strains. *Microbial pathogenesis*, 145, 104223.

Hafidh, R. R., Abdulamir, A. S., Vern, L. S., Bakar, F. A., Abas, F., Jahanshiri, F. & Sekawi, Z. (2011). Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. *The open microbiology journal*, 5, 96-106.

Imane, N. I., Fouzia, H., Azzahra, L. F., Ahmed, E., Ismail, G., Idrissa, D., Mohamed, K-H., Sirine, F., L'Houcine, O. & Noureddine, B. (2020). Chemical composition, antibacterial and antioxidant activities of some essential oils against multidrug resistant bacteria. *European Journal of Integrative Medicine*, 35, 101074.

Khan, M. S. A. & Ahmad, I. (2011). In vitro antifungal, anti-elastase and anti-keratinase activity of essential oils of *Cinnamomum, Syzygium* and *Cymbopogon* species against *Aspergillus fumigatus* and *Trichophyton rubrum*. *Phytomedicine*, 19(1), 48-55.

Moita, E. C. S., Frota, V. M., Mendonça, L. S., Gomes, L. C. S., Fontenelle, R. O. S., Costa, M. F., Fernandes, J. A. B., Cunha, M. E. T., Gomes, G. A., Vale, J. P. C., Bandeira, P. N., Santos, H. S., Zocolo, G. J. & Rodrigues, T. H. S. (2022). Chemical composition and antifungal properties of apolar fraction of green propolis from northeastern Brazil. *Revista Brasileira de Farmacognosia*, 32(1), 139–143.

Moradali, M. F., Ghods, S. & Rehm, B. H. (2017). *Pseudomonas aeruginosa* lifestyle: a paradigm for adaptation, survival, and persistence. *Frontiers in cellular and infection microbiology*, 7, 39.

Morais, A. J. A., Brito, I. L. P., Fonseca, X. M. Q. C., Pinto, V. P. T. & Barbosa, F. C. B. (2019). Evaluation of antimicrobial activity of cinnamaldehyde against carbapenem-resistant *Acinetobacter baumannii* nosocomial isolates. *Int. J. Curr. Microbiol. App. Sci*, 8(5), 434-445.

Morais, S. R., Oliveira, T. L. S., de Oliveira, L. P., Tresvenzol, L. M. F., da Conceição, E. C., Rezende, M. H., Fiuza, T. S., Costa, E. A., Ferri, P. H. & Paula, J. R. (2016). Essential oil composition, antimicrobial and pharmacological activities of *Lippia sidoides* Cham. (Verbenaceae) from São Gonçalo do Abaete, Minas Gerais, Brazil. *Pharmacognosy Magazine*, 12(48), 262-270.

Pandini, J. A., Pinto, F. G. S., Scur, M. C. C., Santana, B., Costa, W. F. & Temponi, L. G. (2017). Chemical composition, antimicrobial and antioxidant potential of the essential oil of *Guarea kunthiana* A. Juss. *Brazilian Journal of Biology*, 78, 53-60.

Pérez Zamora, C. M., Torres, C. A. & Nuñez, M. B. (2018). Antimicrobial activity and chemical composition of essential oils from Verbenaceae species growing in South America. *Molecules*, 23(3), 544.

Pragasam, A. K., Vijayakumar, S., Bakthavatchalam, Y. D., Kapil, A., Das, B. K., Ray, P., Gautam, V., Sistla, S., Parija, S. C., Walia, K., Ohri, V. C., Anandan, S. & Veeraraghavan, B. (2016). Molecular characterisation of antimicrobial resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* during 2014 and 2015 collected across India. *Indian journal of medical microbiology*, 34(4), 433-441.

Puškárová, A., Bučková, M., Kraková, L., Pangallo, D. & Kozics, K. (2017). The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. *Scientific reports*, 7(1), 1-11.

Ribeiro, S. O., Fontaine, V., Mathieu, V., Zhiri, A., Baudoux, D., Stévigny, C. & Souard, F. (2020). Antibacterial and cytotoxic activities of ten commercially available essential oils. *Antibiotics*, 9(10), 717.

Rocha, F. R., Fehlberg, L. C. C., Cordeiro-Moura, J. R., Ramos, A. C., Pinto, V. P. T. & Barbosa, F. C. B. (2019). High frequency of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* nosocomial strains isolated from a teaching hospital in Brazil. *Microbial Drug Resistance*, 25(6), 909-914.

Saraiva, A. G. Q., Saraiva, G. D., Albuquerque, R. L., Nogueira, C. E. S., Teixeira, A. M. R., Lima, L. B., Cruz, B. G. & Sousa, F. F. (2020). Chemical analysis and vibrational spectroscopy study of essential oils from *Lippia sidoides* and of its major constituent. *Vibrational Spectroscopy*, 110, 103111.

Ting, S. W., Lee, C. H. & Liu, J. W. (2018). Risk factors and outcomes for the acquisition of carbapenem-resistant Gram-negative bacillus bacteremia: a retrospective propensity-matched case control study. *Journal of microbiology, immunology and infection*, 51(5), 621-628.

Veras, H. N., Rodrigues, F. F., Botelho, M. A., Menezes, I. R., Coutinho, H. D. & Costa, J. G. (2017). Enhancement of aminoglycosides and β-lactams antibiotic activity by essential oil of *Lippia sidoides* Cham. and the thymol. *Arabian Journal of Chemistry*, 10, S2790-S2795.