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Research paper

Chemical profile of the essential oil of *Lippia origanoides* Kunth and antibiotic resistance-modifying activity by gaseous contact method

Brenda Nayranne Gomes dos Santos[a], Mariely Mendes Furtado[a], Eliézer Erbe de Freitas[b], Laís Rocha Lima[b], Patrícia Virna Sales Leão[b], Felipe Araújo de Alcântara Oliveira[b], Maria das Graças Freire de Medeiros[c], Edlane Martins de Andrade[d], Rita Celiane Alves Feitosa[d], Samia Jessica da Silva Tavares[d], Henrique Douglas Melo Coutinho[e], Josie Haydée Lima Ferreira[b], Humberto Medeiros Barreto[b,*,1]

[a] *Post-graduate Program in Pharmaceutical Sciences, Federal University of Piauí, Teresina, PI, Brazil*
[b] *Laboratory of Research in Microbiology, Federal University of Piauí, Teresina, PI, Brazil*
[c] *Laboratory of Research and Technological Innovation and Entrepreneurship of Medicines and Correlates, Federal University of Piauí, Teresina, PI, Brazil*
[d] *CECAPE College, Av. Padre Cícero, 3917 - São José, Juazeiro do Norte, CE 63024-015, Brazil*
[e] *Laboratory of Microbiology and Molecular Biology, Universidade Regional do Cariri, Crato, CE, Brazil*

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ABSTRACT

Introduction: *Lippia origanoides* is an aromatic shrub used as a culinary seasoning, as well as for therapy of gastrointestinal and respiratory diseases in traditional medicine of Brazilian Northeast. This study aimed to evaluate the antibacterial and resistance-modifying activity of the essential oil of *L. origanoides* (EOLO) and one of the major compounds, carvacrol.

Methods: EOLO was extracted by hydro distillation and carvacrol was obtained commercially. Multidrug-resistant *Staphylococcus aureus* and *Acinetobacter baumannii* strains were tested for its antibiotic susceptibility in the presence or absence of the EOLO or carvacrol by gaseous contact.

Results: Chemical analysis of EOLO demonstrated the presence of thymol, carvacrol and *p-cimene*. Both EOLO and carvacrol were active against both Gram-positive and Gram-negative strains. Moreover, volatile compounds from EOLO, as well as volatile carvacrol were able to potentiate the antimicrobial activity of the polymyxin B and norfloxacin against *S. aureus*. Gaseous contact of EOLO and carvacrol enhanced the activity of piperacillin and cefepime against *A. baumannii*. *S. aureus* and *A. baumannii* are important etiological agents of respiratory infections. Therefore, inhalation of EOLO concomitant with the administration of the antibiotics tested could be a promising clinical application in the treatment of infections caused by resistant *S. aureus* and *A. baumannii*.

Conclusions: Inhalation of EOLO could be used as a complementary procedure for potentiate the activity of conventional antibiotics in the treatment of respiratory infections caused by drug-resistant *S. aureus* and *A. baumannii* strains.

Introduction

Bacterial infections are among the leading causes of morbidity and mortality throughout the world, affecting individuals of all ages. Although antibiotic therapy has revolutionised the treatment of infectious diseases caused by bacteria, antibacterial drugs have been used extensively, which has contributed to the emergence and spread of resistant bacterial strains (Rad et al., 2022). The prevalence of antibiotic-resistant bacteria and their incidence in hospital-acquired infections has increased dramatically in recent years, making this a major public health problem worldwide (Furlan et al., 2021). In addition, the pandemic caused by the severe acute respiratory syndrome virus coronavirus 2 (SARS-CoV-2) has been one of the most significant challenges of our time, burdening healthcare systems by increasing patient

* Corresponding author.
E-mail address: hmbarreto@ufpi.edu.br (H.M. Barreto).
[1] ORCID ID: 0000-0001-5054-7555

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admissions and contributing to an increased risk of healthcare-related infections and the transmission of multidrug-resistant bacteria (Jeon et al., 2022). Because of the 2019 coronavirus disease pandemic (COVID-19), a rising number of patients admitted to hospitals have received empiric antibiotic therapy, increasing illnesses caused by resistant pathogens (Kariyawasam et al., 2022).

Staphylococcus aureus is a common bacterial pathogen that causes a wide range of infections, including skin infections and invasive infections such as pneumonia and other respiratory tract infections, surgical site, prosthetic joint, and cardiovascular infections, as well as nosocomial bacteremia (Cheung et al., 2021). *Acinetobacter baumannii* is an opportunistic pathogen that is important as an aetiological agent in intensive care units for a variety of illnesses (Lima et al., 2021). Both *S aureus* and *A baumannii* have a significant potential for antibiotic resistance, which contributes to the high occurrence of infections caused by both pathogens in the hospital environment (Lima et al., 2021; Wu et al., 2021). As a result, these pathogens were added to the list of priority bacteria to help guide efforts in the research and development of new antibacterial drugs (WHO, 2017).

Several mechanisms involved in antibiotic resistance have already been described, such as the production of enzymes that cleave the antibacterial, enzymatic inactivation of the antibacterial, substitution or deviation of the active site, alteration of the target site (by mutation or enzymatic alteration), protection of the active site, decreased permeability of the bacterial outer membrane, and efflux pumps (Varela et al., 2021). Efflux pumps are transmembrane proteins that can extrude harmful chemicals such as antibacterials, resulting in a drop in the intracellular concentration of the antibacterial agent and increased bacterial survival (Du et al., 2018). Several studies have been conducted to look for efflux pump inhibitors (EPI) that could be associated with conventional antibiotics to improve their action against resistant bacteria (Costa et al., 2016 and 2021; Ribeiro et al., 2019; Rezende-Júnior et al., 2020; Faillace et al., 2021; Leal et al., 2021; Silva et al., 2021; Rodrigues et al., 2022).

The usefulness of medicinal plants in modern medicine cannot be overstated because they have been used for ages in traditional medicine to treat a variety of ailments. These plants contain a variety of bioactive chemicals with medicinal properties, including antioxidant (Sinan et al., 2021), antitumoral (Ahmed et al., 2020; Akkol et al., 2020; Fernández et al., 2021; Mitra et al., 2022) and antimicrobial (Hossain et al., 2021) effects, among others. Furthermore, the usage of medicinal plants might provide a natural alternative to pharmaceutical medications, which frequently have undesirable side effects (Akkol et al., 2021). With the rising frequency of chronic diseases and the rising cost of healthcare, the value of medicinal plants as a source of safe and effective healthcare cannot be underestimated (Iqbal et al., 2020). As a result, there is considerable scientific interest in studying the possible medicinal effects of these plants and developing novel medications based on their active constituents.

Lippia origanoides Kunth (Verbenaceae) is a medicinal plant found in South and Central American countries. Previous research has shown that essential oil of *L origanoides* (**EOLO**) and its major constituents have antimicrobial activity against a variety of pathogens, including bacteria (Ribeiro et al., 2021), fungi (Oliveira et al., 2007; Barreto et al., 2014a), viruses (Meneses et al., 2009), and protozoa (Borges et al., 2012; Melo et al., 2020). EOLO also showed an inhibitory effect on mono- and polymicrobial biofilms of *Streptococcus mutans*, *Lactobacillus rhamnosus*, and *Candida albicans* (Oliva et al., 2023). These studies demonstrate the pharmacological relevance of EOLO as a potential therapeutic agent.

A previous study conducted by our research group showed that EOLO potentiated the activity of the aminoglycosides Neomycin and Amikacin against a clinical isolate of methicillin-resistant *S aureus*, indicating that this natural product is a source of secondary metabolites that could be used as adjuvants in antibiotic chemotherapy of infections caused by this pathogen (Barreto et al., 2014b). In the present study, we use the gas contact method to investigate if the volatile compounds from the EOLO could potentiate the action of different antibiotics against *S aureus* and *A baumannii* strains carrying efflux pump genes, aiming for a prospective practical application of this natural substance as an adjuvant in antibiotic therapy of the respiratory infections caused by these pathogens.

Methodology

Collection of plant material and essential oil extraction

Leaves of *L origanoides* H.B.K. (EOLO) were collected in the month of May 2013, in the José de Freitas city (latitude 04º45'23" South and longitude 42º34'32" West), Piauí, Brazil, and a voucher specimen of the species was deposited in the herbarium Graziela Barroso, University Regional of Piauí, and given the registration number TEPB 09205. Permission to collect the botanical material used in the present study was granted by National System of Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN), Genetic Heritage Management Council, Ministry of Environment of Brazil under registration number: A3D296E. For essential oil extraction, the leaves of the plant were dried at room temperature. Then, they were shredded and placed in a 5.0-L flask along with 2.5 L of distilled water, followed by hydrodistillation using a Clevenger-type apparatus for 4 hours. The essential oil obtained was stored under refrigeration (4 °C) until time of analysis.

Identification of chemical components

The volatile constituents were analysed in a Shimadzu GC-17A gas chromatograph coupled to a GCMS-QP5050A mass spectrometer equipped with a J&W Scientific DB-5 HT capillary column (95% methyl polysiloxane and 5% phenyl, 30 m length, 0.25 mm internal diameter, and 0.1 μm thick fixed phase film). The analysis parameters were as follows: The injector temperature was set at 220 °C; the interface temperature was set at 240 °C, and the column was programmed to operate at 60 °C, with a temperature rise rate of 3 °C/min until the temperature reached 240 °C. The carrier gas used was helium, maintained at a constant flow rate of 1.0 mL/min. After adjusting all the equipment parameters, 1 μL of the volatile fraction (dichloromethane) was injected. Analysis with the mass detector was performed in 'scan' mode, with an acquisition time of 60.35 minutes and a solvent cutoff time of 2 minutes. The mass spectra were acquired in the 40 to 650 Daltons range by the electron ionisation method with an ionisation energy of 70 eV (voltage 1.5 kV, quadrupole type analyzer) and an ion source at 200 °C. The volatile constituents were mostly identified by comparing the mass spectra obtained with the records of the Wiley229 computer library, as well as by comparing their retention indices (IR) with those available in the literature (Adams, 2007).

Bacterial strains and chemicals

All tests were performed with the bacterial strains *S aureus* SA1199-B (kindly provided by Dr Glenn W. Kaatz, John Dingell VA Medical Center, Detroit MI) and *A baumannii* HUT41. SA1199-B strain overexpresses the *norA* gene encoding the NorA efflux pump that can efflux hydrophilic fluoroquinolones and other drugs, such as DNA-intercalating dyes, and quaternary ammonium compounds (Kaatz and Seo, 1995). *A baumannii* HUT-41 is a multidrug-resistant strain isolated from culture of respiratory tract specimen of a patient attended in an urgency hospital from Teresina, Piauí, Brazil (Lima et al., 2021). Bacterial strains were maintained on Nutrient Agar (Himedia, India) slant at 4 °C, and prior to assay cells were grown overnight at 37 °C in Brain Hearth Infusion (BHI, Himedia, India). Carvacrol was obtained from Sigma Chemical Co (St. Louis, MO). The culture media were purchased from HIMEDIA (India), and dimethyl sulfoxide (DMSO) was purchased from Merck (Germany).

Identification of the AdeABC-AdeRS efflux system genes in the A baumannii HUT-41 strain

Bacterial DNA was extracted from *A baumannii* HUT-41 strain by boiling. PCR was performed using Taq DNA Polymerase (Ludwig Biotec). The primes used were: OXA-51F: TCCAAATCACAGCGCTTCA AAA; OXA-51R: TGAGGCTGA ACAACCCATCCA; AdeA F: GAGGTGGC AAGACTCAAAGTTC; AdeA F: GAGGTGGCAAGACTCAAAGTTC; AdeA R: GCTAGAGCCTGACGATACTGAGC; AdeB F: TACCGGTATTACCTTT GCCGGA; AdeB R: GTCTTTAAGTGTCGTAAAAGCCA; AdeC F: ACAAT CGTATCT CGTGGACTC; AdeC R: TAGAAACTGGGTTATTGGGGT; AdeR F: ACTACGATATTGGCGACATT; AdeR R: GCGTCAGATTAAGCAAG ATT; AdeS F: TTGGTTAGCCACTGTTATCT; AdeS R: AGTGGACGTTAG GTCAAGTT (Pannek et al., 2006; Ni et al., 2016; Pagdepanichkit et al., 2016). PCR was performed in a 25 μL reaction mixture containing 1 μL primer, 1 μL Taq polymerase (Ludwig Biotec), 3 μL DNA template, 3.0 mM $MgCl_2$, 2.5 μL 10× buffer, 0.2 mM dNTPs, and nuclease-free water. Amplification conditions consisted of denaturation at 94 °C for 5 minutes and 30 cycles of denaturation at 94 °C for 1 minute, annealing at 56 °C for 30 seconds and extension at 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. PCR products were detected in 2% agarose gel.

Evaluation of the antibacterial activity

In petri dishes containing Mueller-Hinton agar medium, seeding was done to obtain a carpet of bacteria. EOLO and carvacrol were diluted in DMSO to obtain solutions containing 50%, 25%, 12.5%, and 6.25% of these compounds, and 100% of the substance was also used. An absorbent paper disk, like those used in the antibiogram test, was placed on the lid of each plate, and it was moistened with 10 μL of the test substance. The plates were incubated in an oven for 24 hours at 37 °C. A millimetre ruler was used to measure the inhibition zones. All tests were performed in triplicate, and only DMSO was used in control plates. Inhibition zones (including the diameter of disc) were measured, and values < 12 mm were considered as nonactive against bacteria (Alzoreky and Nakahara, 2003).

Evaluation of resistance-modifying activity by gaseous contact

The evaluation of resistance-modifying activity was performed by the gaseous contact method. On petri plates containing Mueller-Hinton agar, seeding was done to obtain a carpet of bacteria. The antibiotic discs were placed on the agar: Imipenem (IMP), Amikacin (AMI), Polymyxin B (PolB), Cefazolin (CFZ), Cefepime (CPM), Norfloxacin (NOR), Gentamicin (GEN), Piperacillin/tazobactam (PPT), and Ticarcillin/clavulanic acid (TAC). An absorbent paper disk similar to an antibiogram disk was placed on the lid of each plate, and it was moistened with 10 μL of the highest concentration of EOLO or carvacrol. Other plates containing the drug discs but without oil or carvacrol were also prepared, and afterwards, all plates were incubated for 24 hours at 37 °C. A millimetre ruler was used to measure the inhibition halos of the antibiotics in the presence and absence of the test substances to determine synergism or antagonism. All tests were performed in triplicate, and only DMSO was used in control plates.

Statistical analysis

All tests were performed in triplicate, and results were expressed as the arithmetic mean ± standard deviation. The statistical analysis was performed by analysis of variance (ANOVA) followed by the Sidak post-test, utilising Graphpad Prism version 6.0 software. The significance calculation was performed using a two-step ANOVA and using Sidak's multiple comparison test. Differences were considered significant when $P < 0.05$.

Table 1
Constituents identified in *L origanoides* Kunth essential oil.

Retention time	Constituents	RI $_{(lit.)}$	RI $_{(calc.)}$	Area (%)
7516	*β*-mircene	988	988	2.54
8943	1,8-cineole	1026	1030	1.07
9203	*p*-cymene	1020	1021	18.87
9938	γ-terpinene	1054	1056	1.19
14 757	4-Terpineol	1175	1174	1.64
17 166	Thymol	1289	1284	23.89
17 121	Carvacrol	1298	1299	21.78
17 129	Thymil-methyl eter	1232	1230	3.57
22 249	Thymil acetate	1340	1346	3.27
23 674	*α*-copaene	1374	1379	1.36
25 515	*trans*-caryophyllene	1417	1422	8.28
26 937	*α*-humulene	1452	1456	2.92
32 097	Caryophyllene	1417	1598	1.04
-	Others	-	-	6.79
Total 98.21%				

Results and discussion

Essential oils are complex mixtures of hydrocarbons and oxygenated hydrocarbons derived from the isoprenoid routes and primarily comprise monoterpenes and sesquiterpenes, produced by glandular trichomes found on the surface of plant organs, particularly flowers and leaves (Bizzo et al., 2009). They are taken from different plant parts while accounting for quantitative and qualitative traits and variations that may be related to the function of each plant component as well as edaphoclimatic factors like climate, fertiliser, soil type, and location (Burt, 2004; Carvalho-Filho et al., 2006).

The findings of the chromatographic analyses carried out on the EOLO sample are displayed in Table 1. Several components were identified, accounting for 98.21% of the total oil. Thymol (23.89%), carvacrol (21.78%), and p-cymene (18.87%), which together made up more than 60% of the contents, were the three main components. The EOLO can be classified into three chemotypes based on the main ingredient discovered in its chemical composition: chemotype A, rich in p-cymene; chemotype B, rich in carvacrol; and chemotype C, high in thymol (Stashenko et al., 2010). This classification confirms that the EOLO examined in the present study resembles chemotype C, with thymol predominating.

The results obtained in the present study contrast with those previously published, which reported carvacrol as the majority constituent, followed by *p*-cymene and thymol (Santos et al., 2004; Escobar et al., 2010). On the other hand, previous studies identified carvacrol, as majority compound, followed by tymol and *p*-cimene (Sarrazin et al., 2015a and 2015b). Another study reported a different chemotype of EOLO, showing 1,5 cineole as the majority compound, followed by carvacrol and *p*-cymene (Ribeiro et al., 2021). *p*-Cymene is obtained from the aromatisation of γ-terpinene, and from there, p-cymene may give rise to several other monoterpenes by hydroxylation, giving rise to thymol, or by hydroxylation at the C-2 of the ring, in the case of carvacrol (Martín-Luengo et al., 2008). Carvacrol, eugenol, and thymol-rich essential oils in particular exhibit significant antibacterial activity (Burt, 2004).

Figure 1 shows the mean inhibition halo values in the tests against Gram-positive *S aureus* SA1199-B and Gram-negative *A baumannii* HUT41 strains. Both EOLO and carvacrol presented intrinsic antibacterial activity against the species tested. Additionally, it was found that the antimicrobial activity varied with EOLO and carvacrol concentration, with higher inhibition halos in the highest tested concentration. The Gram-positive bacteria *S aureus* SA1199-B was sensitive to both EOLO and isolated carvacrol in concentrations ranging from 100% to 12.5%, although isolated carvacrol was more effective in dilutions ranging from 50% to 12.5%. Both EOLO and carvacrol were

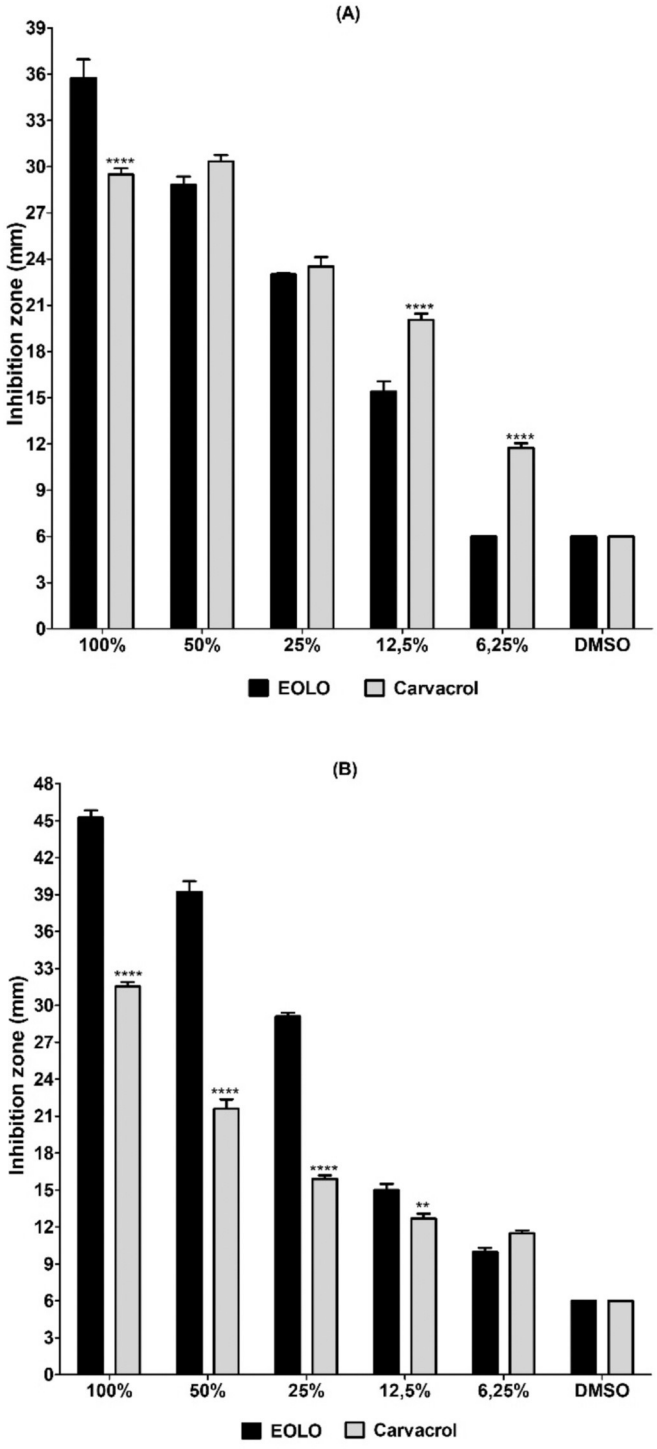

Fig. 1. Antimicrobial activity of the essential oil from *L origanoides* or Carvacrol against *S aureus* (A) and *Acinetobacter baumannii* (B). Results are the means of three simultaneous assays. (****) Statistically significant values with $P < 0.0001$. (**) Statistically significant values with $P < 0.0097$.

effective against the Gram-negative bacteria *A baumannii HUT41*, although EOLO had greater activity than carvacrol in all concentrations tested, pointing to a potential synergism between carvacrol and other components of EOLO against this strain.

This distinct response of EOLO to isolated carvacrol that has been verified in each strain may be connected to the differing chemical makeup of Gram-positive and Gram-negative bacterial cell walls and show that Gram-positive strains seem to have cell walls that are more permeable to carvacrol than Gram-negative ones. No zone of bacterial growth inhibition was produced in the DMSO assays (6 mm corresponds to the disk diameter), indicating that this solvent did not show antibacterial activity against both *S aureus* SA1199-B and *A baumannii* HUT41.

The antimicrobial activity of carvacrol has already been demonstrated in the literature (Didry et al., 1994; Griffin et al., 1999; Bagamboula et al., 2004). The literature shows that carvacrol and thymol increase cellular permeability and they can disintegrate outer membrane of Gram-negative bacteria, releasing lipopolysaccharides (Burt et al., 2005). Therefore, it is believed that these compounds are mainly responsible for the intrinsic antimicrobial activity of EOLO.

The gas contact method has been used to examine whether the volatile components in essential oils could enhance the effects of industrialised antibiotics, suggesting a potential clinical use of inhaling essential oils in the antibiotic therapy of bacterial respiratory infections (Freitas et al., 2013; Oliveira et al., 2014; Silva et al., 2016). Figure 2 shows the mean inhibition halo values obtained for different antibacterial agents in the absence or presence of the volatile compounds from EOLO (Fig. 2A) or volatile carvacrol isolated (Fig. 2B) against *S aureus* SA1199-B. A *S aureus* strain is considered resistant to norfloxacin if it produces an inhibition halo with $\leq$12.00 mm, and it is considered sensitive if it produces inhibition halo with $\geq$17.00 mm (CLSI, 2013).

Results found in the present study showed that SA1199-B produced a mean inhibition halo with 9.01 mm, indicating that it is resistant to norfloxacin. In fact, SA1199B is a known resistant strain that overexpress the proton-motive force-dependent efflux pump NorA belongs to major facilitator superfamily, which extrude hydrophilic fluoroquinolones, including norfloxacin (Kaatz and Seo, 1995). However, the mean inhibition halos produced for SA1199-B increased from 9.01 to 21.65 mm in the presence of volatile compounds from EOLO, showing that the volatile components from EOLO have reverted the phenotype of the SA1199-B from resistance to sensitive. A possible mechanism for explaining this modulating effect could be the NorA inhibition by volatile compounds from EOLO. As previously reported, oxygenated monortepenes such as nerol, 3,7-dimethyl-1-octanol, and estragol act as NorA inhibitors, probably by dissipating the proton-motive force (Coêlho et al., 2016).

Although the volatile carvacrol had enhanced the activity of norfloxacin against the SA1199-B strain, increasing the mean inhibition halo from 9.01 to 11.03 mm. it was not able to revert the phenotype. However, we cannot rule out the possibility of carvacrol to act synergistically with other EOLO components in the norfloxacin resistance-modifying activity against SA1199-B.

Polymyxin B it is not conventionally used in the treatment of Gram-positive bacteria (CSLI, 2013), however, we verified that the volatile compounds of EOLO increased the mean inhibition halo of this antibiotic against SA1199B from 9.27 to 14.67 mm. Furthermore, the mean inhibition halo of polymyxin B against SA1199B increased from 9.27 to 12.49 mm in the presence of volatile carvacrol. Polymyxin B is a cationic polypeptide that disrupt the bacterial plasma membrane structure, enhancing cell permeability (Stachelek et al., 2021). Due to their lipophilicity, the terpenes and terpenoids volatilised from EOLO also intercalate into the plasma membrane (Inoue et al., 2004; Souza et al., 2013), increasing its permeability, which would explain the increased antibacterial activity of polymyxin B against *S aureus* SA1199B in the presence of the volatile constituents.

Figure 3 shows the mean inhibition halo values obtained for different antibacterial agents in the absence or presence of the volatile compounds from EOLO (Fig. 2A) or volatile carvacrol isolated (Fig. 2B) against *A baumannii* HUT-41. This strain showed resistance to piperacillin (mean inhibition halo of 8.54 mm; cut-off $\leq$17.00 mm), cefepime (mean inhibition halo of 6.56 mm; cut-off $\leq$14.00 mm) and norfloxacin (mean inhibition halo of 6.47 mm; cut-off for ciprofloxacin $\leq$15.00 mm) (CLSI, 2013). The EOLO potentiated the activity of piperacillin (increasing the mean inhibition halo from 8.54 to 10.22 mm), as well as polymyxin B

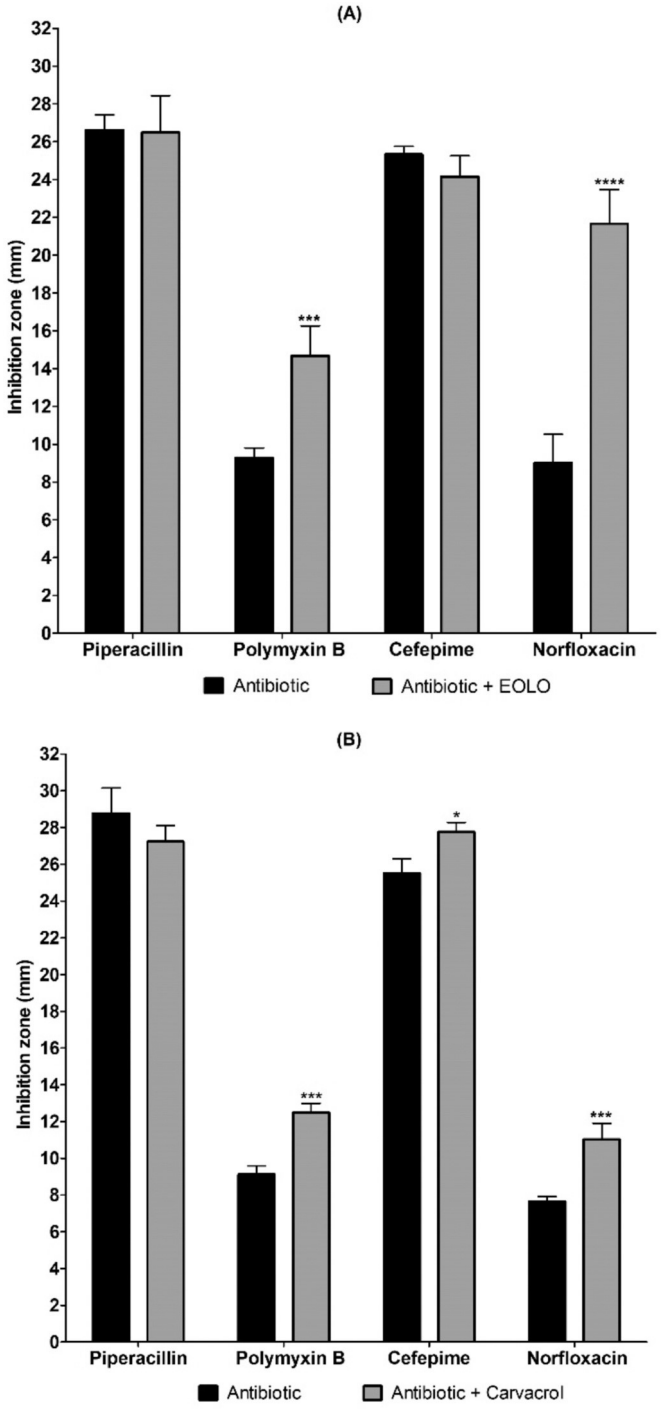

Fig. 2. Antimicrobial activity of the antibiotics tested against *S aureus* in the presence or absence of the essential oil from *L origanoides* (OELO) (A) or Carvacrol (B). Results are the means of three simultaneous assays. (****) Statistically significant values with $P < 0.0001$. (***) Statistically significant values with $P < 0.001$. (*) Statistically significant values with $P < 0.05$.

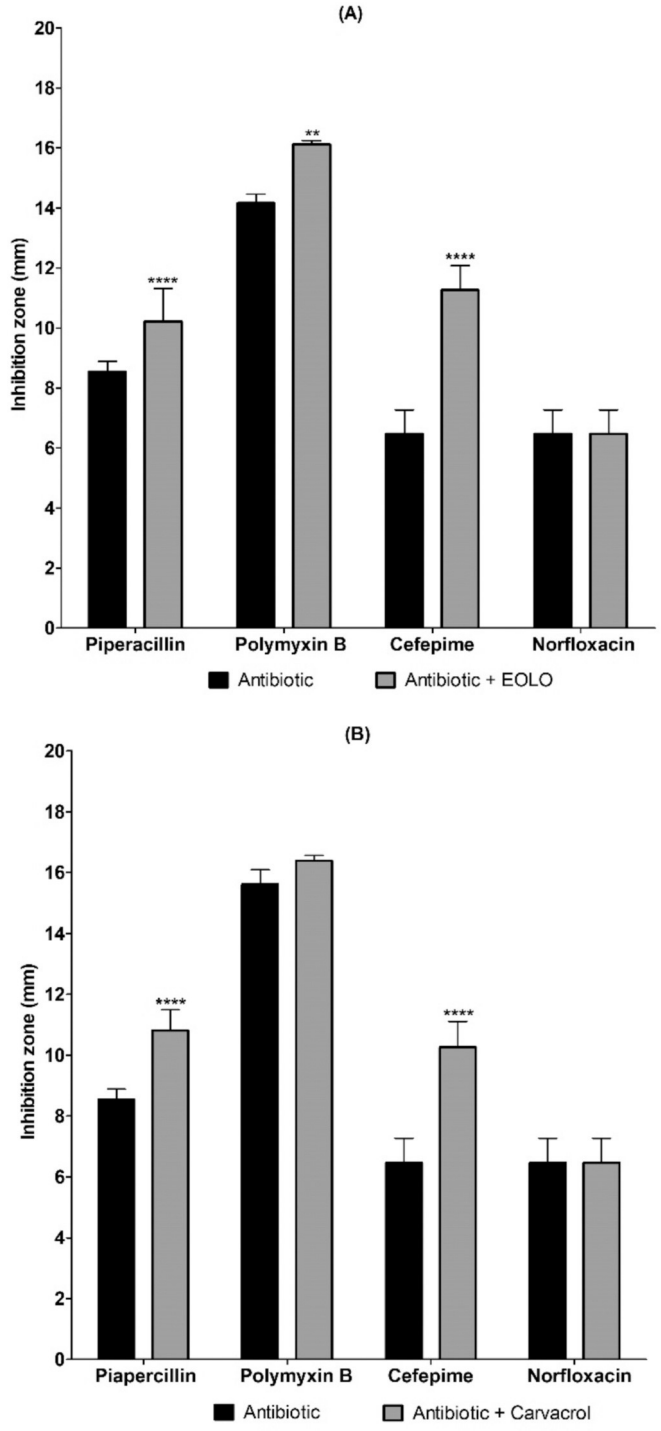

Fig. 3. Antimicrobial activity of the antibiotics tested against *A baumannii* in the presence or absence of the essential oil from *L origanoides* (OELO) (A) or Carvacrol (B). Results are the means of three simultaneous assays. (****) Statistically significant values with $P < 0.0001$. (**) Statistically significant values with $P < 0.0097$.

(increasing the mean inhibition halo from 14.16 to 16.12 mm, and cefepime (increasing the mean inhibition halo from 6.47 to 11.26 mm) against HUT-41, but it did not change the resistance phenotype of the *A baumannii* strain. Similar results were found for volatile carvacrol that enhanced the activity of piperacillin (increasing the mean inhibition halo from 8.54 to 10.81 mm) and cefepime (increasing the mean inhibition halo from 6.47 to 10.26 mm) against HUT-41.

Both piperacillin and cefepime are β-lactam antibiotics of primary choice for the treatment of infections caused by *A baumannii*; however, resistance to these antibiotics has become prevalent in recent years (Lima et al., 2021). The *A baumannii* HUT-41 tested in the present study carries the *adeABC*-adeRS gene encoding the tripartite system AdeABC-AdeRS, a proton-motive force-dependent efflux pump belongs to the resistance/nodulation/division superfamily, able to extrude a wide range of antibiotics including, β-lactams, fluoroquinolones, tetracycline, tigecycline, macrolide, chloramphenicol, and aminoglycosides (Xu et al., 2019; Leão et al., 2023). A possible mechanism evolved in the

antibiotic potentiating the activity of the β-lactams piperacillin and cefepime could be the inhibition of this system by volatile components from EOLO, including carvacrol.

The modulating effect of the EOLO was previously related the aminoglycosides amikacin and neomycin against clinical isolates of *S aureus* (Barreto et al., 2014b). However, this is the first report of the modulating effect of EOLO by gaseous contact. In general, results *in vitro* obtained in the present study suggest a possible clinical use of the EOLO by inhalation route as an adjuvant of norfloxacin or polymyxin B in the treatment of respiratory infections caused by *S aureus*. Inhalation of essential also could be effective in potentiate the antibiotic activity of piperacillin, polymyxin B, and cefepime applied in the treatment of respiratory infections caused by *A baumannii*. However, the clinical efficacy of these associations need be validated by future *in vivo* studies.

Conclusion

We verified that the essential oil extracted from the leaves of *L origanoides* and one of its major compounds, carvacrol, showed relevant intrinsic antibacterial activity when evaluated by direct diffusion contact method in agar using varied concentrations. Furthermore, the volatile components of the essential oil and carvacrol were able to potentiate the activity of different classes of antibiotics against *S aureus* and *A baumannii*. The combination of natural plant products in the form of complex mixtures, or isolated components, with conventional antibiotics could be an alternative form of control bacterial infections. In addition, these products associated with antibiotics tested in this study may represent a novel therapy of respiratory tract infections caused by resistant *S aureus* and *A baumannii* strains.

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Ethics approval/Clinical trial registration number/Informed consent

No applicable.

Author contributions

Brenda Nayranne Gomes dos Santos: Conceptualization. Mariely Mendes Furtado: Methodology. Eliézer Erbe de Freitas: Methodology. Laís Rocha Lima: Methodology. Patrícia Virna Sales Leão: Software. Felipe Araújo de Alcântara Oliveira: Software. Maria das Graças Freire de Medeiros: Resources. Edlane Martins de Andrade: First draft of the manuscript. Henrique Douglas Melo Coutinho: Project administration. Josie Haydée Lima Ferreira: Resources. Humberto Medeiros Barreto: Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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