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Antimicrobial activity of essential oils

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Natural products have been studied aiming to understand their biological properties. Thus, this study aimed to investigate the antimicrobial activity of twenty-seven essential oils (EOs) used in aromatherapy procedures, a natural therapy with great emphasis currently used against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* strains. The agar dilution method was carried out and minimal inhibitory concentration against 50% and 90% of strains ($MIC_{50\%}$ and $MIC_{90\%}$ values) were reported. The *S. aureus* strains were highly susceptible with $MIC_{90\%}$ from 0.21 mg/mL to black pepper (*Piper nigrum*) and tea tree (*Melaleuca alternifolia*) to 26.52 mg/mL with copaiba (*Copaifera officinalis*) EO. Cinnamon (*Cinnamomum cassia*) and clove (*Syzygium aromaticum*) EOs were effective against *E. coli* (2.0 mg/mL) while the *S. aromaticum* EO was against *P. aeruginosa* (8.29 mg/mL). Thus, the higher susceptibility of Gram-positive bacteria when compared with Gram-negative strains was found, and a large variability in the potential antibacterial has also been observed.

Keywords: antibacterial; aromatherapy; EO; minimal inhibitory concentration

Introduction

Herbs, and their essential oils (EOs), have been used since the beginning of human history for flavored foods and beverages; they have been empirically used to disguise unpleasant odors, attract other individuals and control health problems, contributing to the welfare humans and animals, thus demonstrating the cultural and economic importance use of these products (1).

The EOs are typically liquid, clear and unusually colored, complex and the present compounds are volatile, characterized by a strong odor and synthesized by aromatic plants during secondary metabolites, which act to protect the plant against microorganisms and insects. They can be synthesized in several plant organs such as buds, flowers, leaves, stems, branches, seeds, berries, roots, wood or bark, being stored in secretory cells, cavities, channels, epidermal cells or trichomes (2). Temporal and spatial variations in the total content of secondary metabolites products from plants occur at different levels and, despite the existence of a genetic control, the expression may undergo changes resulting from biochemical, physiological, ecological and evolutionary interactions that represent an important interface between chemistry and the environment surrounding the plants (3).

As for industrial production, EOs are obtained by steam distillation, which is on the rise in food and pharmaceutical applications, and pressurized supercritical fluid, especially carbon dioxide (4).

EOs have several biological properties, such as larvicidal action (5), antioxidant (6), analgesic and anti-inflammatory (7), fungicide (8) and antitumor activity (9).

The *in vitro* antimicrobial activity of EO has been researched extensively against a variety of microorganisms (10). Nevertheless, the emergence of multidrug-resistant bacteria poses a challenge to treating infections, so the need to find new substances with antimicrobial properties for use in the fight against these microorganisms is evident (11, 12). Historically, most antibiotics come from a small set of functional molecular structures whose lives were extended by generations of synthetic reorganizations and arrangements (13). Moreover, the food, pharmaceutical and cosmetic industries have shown great interest in the antimicrobial properties of EOs, as the use of natural additives has received importance as a trend in the replacement of synthetic preservatives (14).

The objective was to establish *in vitro* the antimicrobial activities of EOs that are normally used in natural therapies against *S. aureus*, *E. coli* and *P. aeruginosa* strains isolated from human clinical specimens and one standard ATCC (American Type Culture Collection) of each bacterial species; this was

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Table 1. Density (mg/mL) and essential oils chemical compounds (%) obtained by chromatography–mass spectrometry (GC–MS) from the supplier of the essential oils (By Samia Aromatherapy/São Paulo/Brazil).

Essential oil	Density (mg/mL)	Compounds in essential oils (%)
Bergamot (*Citrus aurantium bergamia*)	871	Limonene (35.24), linalina acetate (30.40), linalool (18.45), β-pinene (5.42), γ-terpinene (3.74), sabinene (0.92), α-pinene (0.89), myrcene (0.81), for-cymene (0.45)
Black pepper (*Piper nigrum*)	846	Limoneno (23.80), δ-3-carene (21.97), α-pinene (12.89), β-caryophyllene (11.34), β-pinene (3.91), sabinene (3.78), α-felandeno (3.76), myrcene (2.88), para-cymene (1.38), linalool (1.24), terpinolene (1.17), β-selineno (1.11), 1.8 cineole (0.98), α-terpinene (0.97), α-humulene (0.77), α-copaene (0.71), eugenol (0.56), terpinen-4-ol (0.47), camphene (0.21), safrole (0.17)
Brazil's spearmint (*Mentha arvensis*)	849	Menthol (54.48), menthone (19:12), pulegone (5.57), isopulegol (2.02)
Cardamom (*Elettaria cardamomum*)	869	n/d
Cedar (*Cedrus atlantica*)	891	Widreno (27.75), α-cedrol (22.14), α-Cedrenus (19.84), α-muuroleno (4.55), widrol (3.79)
Cinnamon (*Cinnamomum cassia*)	1008	Eugenol (72.13), eugenila acetate (3.87), β-caryophyllene (3.48), benzyl benzoate (3.24), linalool (1.23), para-cymene (0.76), α-pinene (0.63), α-humulene (0.61), α-phellandrene (0.49), 1.8 cineole (0.27), limonene (0.22), camphene (0.21), β-pinene (0.21)
Clary sage (*Salvia sclarea*)	857	Linalina acetate (66.77), linalool (22.67), geranyl acetate (3.29), β-caryophyllene (1.15), myrcene (0.18), limonene (0.15), 1.8 cineole (0.12)
Clove (*Syzygium aromaticum*)	988	Eugenol (83.63), β -caryophyllene (12.39), alpha-humulene (3.05), eugenol acetate (0.93)
Copaiba (*Copaifera officinalis*)	884	β-Caryophyllene (44.47), β-bisabolene (8.0), germacrene B (8.0), α-copaene (7.98), germacrene d (5.95), α-humulene (5.40), δ-cadinene (4.57)
Cypress (*Cupressus sempervirens*)	840	α-Pinene (52.26), δ-3-carene, α-terpinolene (2.65), α-terpinila acetate (2.63), limonene (2.60), myrcene (2.40), terpinen-4-ol (1.40), sabinene (1.24), β-pinene (1.14), α-tujeno (0.96), α-fenchene (0.81), γ-terpinene (0.79), p-cymene (0.70), geranyl acetate (0.41), α-terpinene (0.35), 1.8 cineole (0.34), camphene (0.28)
Eucalyptus (*Eucalyptus globulus*)	883	1.8 Cineole (80.17), α-pinene (11.25), diacetone alcohol (4.32), p-cymene (2.28), α-terpineol (0.85), terpinen-4-ol (0.60), β-pinene (0.53)
Fennel (*Foeniculum vulgare*)	919	*trans*-Anethole (95.66), linalool (2.91), estragol (0.39), α-pinene (0.13)
Geranium (*Pelargonium graveolens*)	848	Citronellol (31.58), geraniol (25.47), fermiato of citronelita (12.74), fermiato of geranyl (6.71), linalool (6.33); isomenthone (4.35), rose oxide (0.89), citronelita acetate (0.48)
Ginger (*Zingiber officinalis*)	850	α-Zingiberene (22.85), curcumene (18.96), β-sesquifilandreno (13:12), β-bisabolene (11.58), α-farnesene (4.28), camphene (1.77), β-phellandrene (1.58), 1.8 cineole (1.35), α-pinene (0.43) *trans*-β-farnesene (0.30), myrcene (0.20)
Lavender (*Lavandula officinalis*)	853	1.8 Cineole (45.97), p-cymene (4.19), 1-terpinen-4-ol (2.30), alpha-pinene (1.48), limonene (1.46), gamma-terpinene (1.17), terpinolene (1.04)
Lemongrass (*Cymbopogon schoenanthus*)	858	Geranial (48.57), neral (32.86), geranyl acetate (3.98), β-caryophyllene (1.59), linalool (1.23), camphene (1.19), caryophyllene oxide (0.67), eugenol (0.48), limonene (0.23), α-pinene (0.20), *trans*-β-ocimene (0.12)
Marjoram (*Origanum majorana*)	841	1.8 Cineole (48.05), linalool (22.69), limonene (8.10), α-pinene (4.42), β-pinene (4.05), isobornyl acetate (2.82), para-cymene (2.21),,estragol (1.02), γ -terpinene (0.96), camphene (0.74), wiridiflorol (0.73), myrcene (0.51), borneol (0.49) *trans*-linalool oxide (0.24), *cis*-linalool oxide (0.21)
Nutmeg (*Myristica fragans*)	889	α-Pinene (18.35), myristicin (17.65), β-pinene (12.29), sabinene (10.15), terpinen-4-ol (8.21), γ-terpinene (4.18), limonene (3.63), para-cymene (3.15), α-terpinolene (2.91), safrole (2.68), 1.8 cineole (2.16), terpinolene (1.84), methyl eugenol (1.59), α-terpineol (1.52), δ-3-carene (1.41), elemicin (0.74), eugenol (0.53)
Orange (*Citrus aurantium dulcis*)	820	Limonene (96.25), myrcene (1.81), linalool (0.49), α-pinene (0.49), sabinene (0.32), β-phellandrene (0.27)
Palmarosa (*Cymbopogon martinii*)	874	Geraniol (57.49), geranyl acetate (13.56), linalool (1.71), beta-caryophyllene (1.07), ocimene (0.27)
Patchouli (*Pogostemon patchouli*)	1009	Patchoulol (25.21), δ-guaieno (11.49); gurjunene-α (11.26); seicheleno (9.61), α-guaieno (9:56), benzyl alcohol (6.73), vidreno (3.12), aromadendrene (2.81), α-cedrol (2.63), β-patchouleno (1.57)

(*Continued*)

Table 1. (*Continued*).

Essential oil	Density (mg/mL)	Compounds in essential oils (%)
Pine (*Pinus sylvestris*)	874	Bornyl acetate (32.74), camphene (21.67), α-pinene (10.95), limonene (4.42), 1.8 cineole (3.15), borneol (3.11), β-pinene (1.82), β-caryophyllene (1.53), terpinolene (1.01), myrcene (0.54), geranyl acetate (12:34), camphor (0.22), para-cymene (0.14), γ-terpinene (0.12)
Rosemary (*Rosmarinus officinallis*)	885	1.8 Cineole (31.57), camphor (20:42), α-pinene (15.78), camphene (4.93), limonene (3.76), geraniol (2.43), myrcene (2.02), linalool (1.70), para-cymene (1.66), γ-terpinene (1.14), α-terpinolene (0.99), bornyl acetate (0.41), borneol (0.15)
Tahiti lime (*Citrus limonum*)	840	Limonene (62.34), γ-terpinene (11.96), β-pinene (10.23), β-bisabolene (2.68), α-pinene (1.97), geraniol (1.84), myrcene (1.49), para-cymene (1.18), neral (1.04), *trans*-α-bergamotene (1.02), α-tujeno (0.50)
Tea tree (*Melaleuca alternifolia*)	858	1-Terpinen-4-ol (53.40), p-cymene (8.9), gamma-terpinene (5.34), 1.8 cineole (3.18), alpha-pinene (1.40), terpinolene (1.05), limonene (0.70)
Vetiver (*Vetiveria zizanioides*)	977	n/d
Ylang ylang (*Cananga odorata*)	904	*trans*-β-Caryophyllene (12.92), linalool (11.38), germacrene-d (11.21), benzyl acetate (10.34), geranyl acetate (9.87).

Note: n/d, information not obtained.

performed by the dilution of EOs onto Mueller Hinton agar (MHA) and the minimal inhibitory concentration (MIC) against each and the $MIC_{50\%}$ and $MIC_{90\%}$ values were recorded.

Experimental

Essential oils

Twenty-seven samples of EOs, from the supplier By Samia Aromatherapy (São Paulo-SP, Brazil) in amber glass vials with a capacity of 10 mL, were selected according to their frequent use in aromatherapy procedures. These EOs were: bergamot (*Citrus aurantium bergamia*), black pepper (*Piper nigrum*), Brazil's spearmint (*Mentha arvensis*), cardamom (*Elettaria cardamomum*), cedar (*Cedrus atlantica*), cinnamon (*Cinnamomum cassia*), clary sage (*Salvia sclarea*), clove (*Syzygium aromaticum*), copaiba (*Copaifera officinalis*), cypress (*Cupressus sempervirens*), eucalyptus (*Eucalyptus globulus*), fennel (*Foeniculum vulgare*), geranium (*Pelargonium graveolens*), ginger (*Zingiber officinalis*), lavender (*Lavandula officinalis*), lemongrass (*Cymbopogon schoenanthus*), marjoram (*Origanum majorana*), nutmeg (*Myristica fragans*), orange (*Citrus aurantium dulcis*), palmarosa (*Cymbopogon martinii*), patchouli (*Pogostemon patchouli*), pine (*Pinus sylvestris*), rosemary (*Rosmarinus officinallis*), Tahiti lime (*Citrus limonum*), tea tree (*Melaleuca alternifolia*), vetiver (*Vetiveria zizanioides*) and ylang ylang (*Cananga odorata*). The samples were kept at room temperature and the chemical characterization of the oils samples were provided by supplier, including the gas chromatography–mass spectrometry (GC–MS) analysis (Table 1). Density values from each studied oil were performed using methodology recommended by Fonseca and Librandi (15) in Eppendorf tubes, which were weighed (P1) on an analytical balance and then weighed again (P2) after the addition of 1 mL (V) of oil. The density (*D*) was calculated using the formula below.

$$D = \frac{P_2 - P_1}{V} = \frac{mg}{mL}$$

Bacterial strains

The bacterial strains from the American Type Culture Collection (ATCC) standard strains (*E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853), as well as ten *S. aureus*, ten *E. coli* and nine *P. aeruginosa* isolated from human clinical specimens, were selected from strains stored at −80°C in the Department of Microbiology and Immunology of Biosciences Institute of UNESP, Botucatu-SP. Prior to use, the strains were plated on blood agar medium to check viability and purity, and maintained on nutrient agar for use in bacterial susceptibility assays.

Antimicrobial activity of EOs by the agar dilution method and MIC

Susceptibility tests for determining the MIC of EOs were carried out following the agar dilution method, adapted from CLSI (16) protocol, the plate discs were prepared and antimicrobial assays were performed during one day. Each EO was diluted alone in MHA plus 0.5% Tween 80 at 45°C in Petri dishes and equivalent concentrations of 0.025, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 1.5, 2.0, 2.5 and 3.0% v/v were established. The strains were grown at 35°C/18–24 hours in brain heart infusion (BHI) and standard suspensions were performed in sterile saline (0.85%) using scale 0.5 of MacFarland aiming a

bacterial concentration about 1.5×10^8 colony forming units (CFU)/mL. The inoculation of thirty-two strains, from standardized suspensions, was made using a Sterr multi-inoculator using suspensions standardized at 0.5 MacFarland, with a second dilution performed onto BHI to obtain inoculum of an approximate concentration of 10^5–10^6 CFU/mL. After Petri dishes were inoculated and incubated at 35°C/18–24 hours, bacterial growth was assessed and the MIC values were recorded for each strain. The conversion of values from % v/v to mg/mL, using the density values of each oil, and their calculations of the $MIC_{50\%}$ and $MIC_{90\%}$ for each tested bacterial strains were performed.

Statistical analysis

The results obtained were used to compare three or more independent testing treatments via the Kruskal–Wallis test. For meaningful analysis ($p \leq 0.001$), we apply the Student–Newman–Keuls test for multiple comparisons tests between treatments.

Results and discussion

The research about antimicrobial activity, the action mechanism and potential use of volatile plant oils has received prominence in recent decades in parallel with advances in traditional approaches to protecting the health of humans, animals and food against the presence of pathogenic and spoilage microorganisms. Thus, investigations on the antimicrobial activity of plant extracts against different pathogens have been performed worldwide (17); our results have importance because they provide information about this subject.

The density (mg/mL), chemical compounds and their percentages in the total composition of the each EO (twenty-seven samples) were found; results are presented in Table 1. We emphasize that the data about chemical analysis of the oils studied were received from the company By Samia Aromatherapy who supplied the EO samples. All of the oils studied showed a density above 800 mg/mL, with orange oil having the lowest value (820 mg/mL) and patchouli (1009 mg/mL), cinnamon (1008 mg/mL) and clove (988mg/mL) presenting the highest density values. Although the chemical characterization of the oils plays a role in studies of this nature, according to some authors, it cannot be concluded that the major component is the biologically active compound of this study, so the effect can be attributed to a constituent or lesser extent a synergy between existing compounds in the oil (18–21). In general, the EO showed diversity in their chemical characterization, but these are in agreement with the literature in question.

A total of twenty-seven oils were assayed by the agar dilution method. The $MIC_{90\%}$ values against bacterial strain tested (Table 2) show that *S. aureus* strains were susceptible to a high number of EOs, and eight of the twenty-seven oils tested showed inhibitory activity with $MIC_{90\%}$ values below 0.30 mg/mL (e.g. eucalyptus, lemongrass, patchouli, black pepper, clary sage, tea tree, vetiver, ylang ylang).

With the tests using the agar dilution methodology at the concentrations tested, it was not possible to achieve MIC values for *P. aeruginosa* strains, except for cinnamon; these results corroborate those reported by Hammer et al. (21), who determined the MIC against *E. coli* and *S. aureus* but failed to find results for *P. aeruginosa* strains.

Thus, the *S. aureus* strains showed high susceptibility to natural products, which again confirms the results from the literature (22, 23), or in other words, Gram-positive species are more sensitive to natural products that Gram-negative bacteria.

These data are important for the treatment of infections caused by these bacteria; *S. aureus* is described as one of the main agents responsible for infection, as its virulence and ability to acquire antimicrobial resistance results in a serious problem throughout the world for hospitals and health professionals (24).

Pseudomonas aeruginosa is a Gram-negative bacterium that produces water-soluble pigments, which is widely distributed in soil and water, and is a hospital pathogen that grows in damp areas such as sinks, bathtubs and showers; it is also considered a resistant bacterium (25). Despite the fact that *E. coli* is a Gram-negative bacteria as well as *P. aeruginosa*, this bacterium showed sensitivity to fourteen oils at the highest concentrations tested. These results corroborate with those reported by Duarte et al. (26), who concluded that the *C. martini* (palmarosa) EO and its major component, geraniol, may be useful for the treatment of diarrhoea caused by *E. coli*.

EOs mainly include two biosynthetic groups, all characterized by low molecular weight, including aromatic and aliphatic constituents, and terpenes and terpenoids (27).

As a typical lipophilic compound, EOs cross the cell wall and cytoplasmic membrane and the cytotoxic activity appears to be linked to disruption of the structures of the different layers of polysaccharides, fatty acids and phospholipids, due to its mechanism of action that hits multiple targets at the same time (22). Permeability, composition and charge of the outer structures of the microorganisms mainly determined these differences; the lipophilic character of terpenes is associated with the antimicrobial mechanism (28). Numerous reports have been made about the mechanisms of antimicrobial action of the oils, and some cases have been partly elucidated,

Table 2. Minimal inhibitory concentration 50% ($MIC_{50\%}$) and 90% ($MIC_{90\%}$) (mg/mL) found on essential oils samples against ATCC standard and *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* strains isolated from clinical human specimens.

Essential oil	*S. aureus* (*n*=11)*, $CIM_{50\%}$–$CIM_{90\%}$	*E. coli* (*n*=11)*, $CIM_{50\%}$–$CIM_{90\%}$	*P. aeruginosa* (*n*=10)**, $CIM_{50\%}$–$CIM_{90\%}$
Bergamot (*Citrus aurantium bergamia*)	10.50–19.81^{w}	>26.13–>26.13	>26.13–>26.13
Black pepper (*Piper nigrum*)	0.21–0.21^{a}	>25.38–>25.38	>25.38–>25.38
Brazil's spearmint (*Mentha arvensis*)	1.90–2.26^{l}	5.52 -5.52	>25.47–>25.47
Cardamom (*Elettaria cardamomum*)	7.58–7.58^{s}	>26.07–>26.07	>26.07- >26.07
Cedar (*Cedrus atlantica*)	1.78–2.76^{nl}	22.27–26.73$^{k–}$	>26.73–>26.73
Cinnamon (*Cinnamomum cassia*)	1.00–1.14^{ok}	2.00–2.00^{b}	25.00 – 30.0^{ba}
Clary sage (*Salvia sclarea*)	0.29–0.29^{hf}	>25.71–>25.71	>25.71–>25.71
Clove (*Syzygium aromaticum*)	0.67 -1.21^{k}	1.11–2.00^{a}	4.60–8.29^{a}
Copaiba (*Copaifera officinalis*)	24.07–26.52^{z}	>26.52–>26.52	>26.52–>26.52
Cypress (*Cupressus sempervirens*)	>25.2–>25.2	>25.20–>25.20	>25.20–>25.20
Eucalyptus (*Eucalyptus globulus*)	0.22–0.22^{c}	11.00–14.35^{h}	>26.49–>26.49
Fennel (*Foeniculum vulgare*)	7.81–7.81^{us}	13.08–20.22^{l}	>27.57–>27.57
Geranium (*Pelargonium graveolens*)	0.20- 0.31^{ge}	3.90–4.24^{ec}	>25.40–>25.40
Ginger (*Zingiber officinalis*)	3.23–4.93^{qp}	>25.5–>25.5	>25.50–>25.50
Lavender (*Lavandula officinalis officinalis*)	2.37–4.27^{r}	21.3–25.59^{mL}	>25.59–>25.59
Lemongrass (*Cymbopogon schoenanthus*)	0.15–0.22^{i}	1,98–2.10^{gc}	>25.74–>25.74
Marjoram (*Origanum majorana*)	4.21–4.21^{p}	4.21–4.21^{dc}	>25.23 ->25.23
Nutmeg (*Myristica fragans*)	13.96–13.96^{yx}	18.52–18.52^{j}	>26.67- >26.67
Orange (*Citrus aurantium dulcis*)	12.50–16.5^{x}	>24.63–>24.63	>24.63–>24.63
Palmarosa (*Cymbopogon martinii*)	0.48–0.59^{m}	1.90–2.09^{fc}	>26.22- >26.22
Patchouli (*Pogostemon patchouli*)	0.25–0.25^{f}	>30.27–>30.27	>30.27–>30.27
Pine (*Pinus sylvestris*)	2.58–2.58^{ji}	>26.22–>26.22	>26.22–>26.22
Rosemary (*Rosmarinus officinallis*)	6.40–7.26^{t}	17.70–22.12^{i}	>26.55–>26.55
Tahiti lime (*Citrus limonum*)	10.0–14.91^{v}	>25.2–>25.2	>25.2–>25.2
Tea tree (*Melaleuca alternifolia*)	0.21–0.21^{b}	4.29–4.29^{c}	>25.74–>25.74
Vetiver (*Vetiveria zizanioides*)	0.24–0.24^{e}	>29.31–>29.31	>29.31–>29.31
Ylang ylang (*Cananga odorata*)	0.23–0.23^{d}	>27.12–>27.12	>27.12–>27.12

Note:*ATCC and plus ten clinical isolated; **ATCC and plus nine clinical isolated. Values preceded by '>' were not considered in the statistical analysis because they did not show inhibitory capacity up to the maximum concentration tested in the trials. Different letters in columns represent statistical differences for antibacterial activities of essential oils (mg/mL) when $p \leq 0.001$.

e.g. the tea tree EO (*M. alternifolia*) and its major compound, terpinen-4-ol, which causes lysis and loss of membrane integrity due to output ions and cellular respiration inhibition (29, 30).

Cypress (*C. sempervirens*) was the only species that showed no antibacterial activity according the susceptibility assay performed. According to Hammer et al. (21), this oil also showed no activity against *E. coli* and *P. aeruginosa,* although it has some effect on the growth of strains of *S. aureus* with an MIC value of 2% v/v. However, the authors report that a single standard NCTC strain (National Collection of Type Cultures) was used, which may explain the different results obtained, while this study tested strains isolated from human clinical cases, and therefore distinct phenotypes itself. However, *C. arizonica*, from the same family as *C. sempervirens*, has been attributed weak antimicrobial activity for this EO because of the high hydrocarbon content (31).

EO contain complex mixtures of components and thus have multiple antimicrobial properties; most of this action appears to derive from oxygenated terpenoids, particularly phenolic terpenes, phenylpropanoids and alcohols; other constituents, e.g. hydrocarbons that typically showed low activities, can be used in combinations to increase their bioactivities (27).

In general, the oils of cinnamon and clove oils were those with the highest potential inhibitors against the three bacterial strains used. According to Prabuseenivasan et al. (32), these oils were able to inhibit the growth of both Gram-positive and Gram-negative species. Both oils showed eugenol, i.e. phenylpropanoid, to be the main compound.

The clove EO exhibited the best activity among the twenty-seven oils tested against both Gram-negative strains, but for the strains of *S. aureus,* this was black pepper oil with an $MIC_{90\%}$ of 0.21 mg/mL.

Most of the EOs used in this study has terpinen-4-ol, linalool and eugenol as part of their compounds.

The antimicrobial mechanism involved with linalool is related to its high water solubility and to its ability to penetrate the bacteria cell wall (33). One hypothesis is that linalool has the potential to act as either a protein denaturing agent or as a solvent dehydrating agent,

which may also contribute to its antimicrobial activity (34).

The antimicrobial activity of some EOs could be explained by the significant amount of linalool, which is an oxygenated monoterpenoid (35). Knobloch et al. (36) related that linalool had a significantly increased antimicrobial activity when compared to eugenol. Overall, the antibacterial activity of the EOs can be related to the content of many of the compounds identified in the oils, including eugenol (17, 37).

Although linalool presents important antioxidant and antimicrobial effects (38), it must be noted that the antimicrobial effect of an EO depends on all of its chemical components (35).

Thus, we concluded that *P. aeruginos*a strains were highly resistant to the EOs, while the *S. aureus* strains were considerably sensitive, although the potential use of EOs can be applied to both Gram-positive and Gram-negative bacteria.

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Conflict of interest

The authors have declared no conflict of interest

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