Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Evaluation of the Chemical Composition and Oral Antimicrobial Activity of the Essential Oil from the Leaves of *Pimenta pseudocaryophyllus* (Gomes) Landrum

Leila Teresinha Maranho, Elaine Cristina Rosas, Thaís Andrade Costa, João Luiz Coelho Ribas, Flares Baratto-Filho, Marilisa Carneiro Leão Gabardo

Department of Industrial Biotechnology, Universidade Positivo, Curitiba, Paraná 81280-330, Brazil

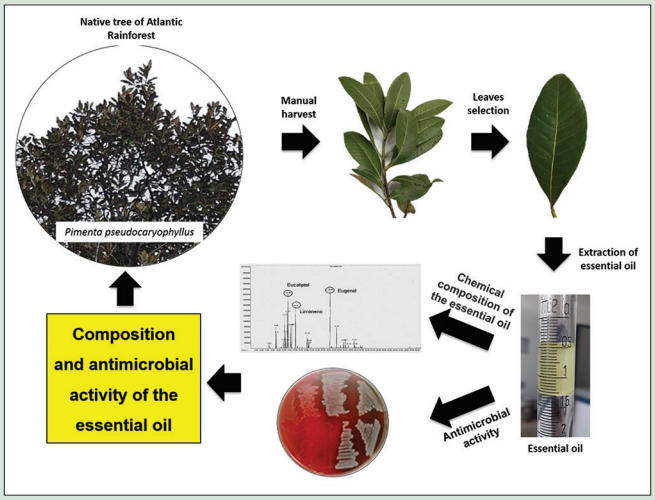
ABSTRACT

Background: *Pimenta pseudocaryophyllus* is a native species from Brazil, whose leaves are used in the traditional culture as medicinal plants, being reported with antimicrobial and antifungal properties. **Objectives:** The aim of this study is to analyze and characterize the chemical composition of the essential oil (EO) extracted from the leaves of *P. pseudocaryophyllus*, as well as to evaluate its potential for antimicrobial activity against pathogenic bacteria inhabiting the human oral cavity. **Materials and Methods:** The extraction was performed by steam distillation and the chemical composition was analyzed using gas chromatography-mass spectrometry (GC-MS). The antimicrobial potential of the EO against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus faecalis* was evaluated by agar well diffusion method. Different dilutions of the EO (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039%, v/v) were tested against the micro-organisms in disk diffusion tests to evaluate the minimum inhibitory concentration. **Results:** The extraction yielded 0.65% of EO in relation to fresh leaves of *P. pseudocaryophyllus.* GC-MS analysis identified eugenol, eucalyptol, and limonene as the major compounds of its. The material showed antimicrobial activity, especially against *E. faecalis,* with moderate activity against *S. aureus,* both at concentrations from 1.25% to 10%. **Conclusion:** The results suggested that this EO presents potential of activity against the oral micro-organisms here evaluated.

Key words: Antimicrobial activity, essential oil, eugenol, minimum inhibitory concentration, phytotherapy

SUMMARY

- *Pimenta pseudocaryophyllus* have been used for the treatment of many physical disorders
- *P. pseudocaryophyllus* leaves are used as a medicinal plant, with reported antimicrobial and antifungal properties
- Eugenol, eucalyptol, and limonene as the major compounds of this plant
- This plant showed antimicrobial activity against *Enterococcus faecalis* and *Staphlylococcus aureus.*



Abbreviations Used: BA: Blood agar; BHI: Brain Heart Infusion; DMSO: Dimethyl sulfoxide; EO: Essential oil; MH: Mueller-Hinton Broth; GC-MS: Gas chromatography-mass spectrometry; MIC: Minimum inhibitory concentration.

Correspondence:
Prof. Marilisa Carneiro Leão Gabardo, Universidade Positivo, Rua Professor Pedro Viriato Parigot de Souza, 5300, Cidade Industrial, Curitiba, Paraná 81280-330, Brazil.
E-mail: marilisagabardo@gmail.com

DOI: 10.4103/pr.pr_83_19

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

In dentistry, a variety of micro-organisms present in the biofilm is found in both the oral cavity and the dental work environment. In the mouth, for example, *Enterococcus faecalis* is the bacteria identified as the main cause of failure in endodontic treatment and diverse types of micro-organisms may compose the microbiota of persistent apical lesions.[1,2] In hospitals and dental clinics, the major problem with contamination is related to opportunistic bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, commonly found in equipment, and that may represent a potential source of cross-contamination, resulting in many health problems.[3-6] Biofilms formed by pathogenic bacteria are associated with a wide range of diseases, from device-related infections.[5,7]

In this context, the indiscriminate use of antibiotics to eliminate pathogens plays an important role, associated with treatment ineffectiveness and the increase of persistent infections, as a consequence of antimicrobial resistance.[5,8] Thus, alternative compounds may present potential activity against oral pathogenic micro-organisms. Studies with natural substances against *E. faecalis*[9-11] and *P. aeruginosa*[8,11-13] were reported, and an interesting candidate for tests is *Pimenta*

Cite this article as: Maranho LT, Rosas EC, Costa TA, Ribas JL, Baratto-Filho F, Gabardo MC. Evaluation of the chemical composition and oral antimicrobial activity of the essential oil from the leaves of *Pimenta pseudocaryophyllus* (Gomes) Landrum. Phcog Res 2020;12:65-70.

pseudocaryophyllus (Gomes) Landrum, which belongs to *Myrtaceae* family.

Myrtaceae comprises one of the largest and most important families of Brazilian flora, being their members predominantly found in the Atlantic Forest. The species of the genus *Pimenta* are noteworthy for medicinal interest and are recognized for their potential to produce essential oil (EO) with pharmacological effects.[14,15]

The EO from *P. pseudocaryophyllus* has a complex chemical composition and includes phenylpropanoids, monoterpenes, aldehydes, and monoterpene alcohols.[14] Pharmacological properties were popularly propagated and attributed to this species by the consume of the infusion of their leaves in the form of teas, which is considered suitable for the treatment of flu, colds, arthritis, gonorrhea, bloody diarrhea, dysentery, fevers, and syphilis, besides it is used as digestive and regulator of menstruation. It also shows anthelmintic activities and displays an anti-inflammatory, antimicrobial, and antifungal potential.[14,16,17] In rats, low toxicity of the dry leaf extract of the P. *pseudocaryophyllus*, (E)-methyl isoeugenol chemotype was found.[18]

Hence, the aim of this study was to analyze and characterize the chemical composition of the EO extracted from the leaves of *P. pseudocaryophyllus*, as well as to evaluate its potential for antimicrobial activity against pathogenic bacteria inhabiting the human oral cavity.

MATERIALS AND METHODS

Sampling and material preparation

The sampling of *P. pseudocaryophyllus* was performed in an area of Mixed Ombrophilous Forest (Araucária Forest) located on private property in the city of Curitiba, Paraná, Brazil, around the geographical coordinates 25°C 35' 01.2" S and 49°C 15' 43.7" W, at an altitude of approximately 900 m. The material was collected with authorization from the Biodiversity Information and Authorization System-*Sistema de Autorização e Informação em Biodiversidade* (SISBIO/IBAMA), number 48580. The plants were taxonomically identified, and the species was confirmed with the website www.theplantlist.org. A dried sample was deposited in the institutional Herbarium as number 1457.

Leaves from different individual specimens were randomly collected [Figure 1] during April and May 2014. The material was conducted to the Botanical Laboratory and was visually sorted to discard foreign organic and inorganic materials, as well as dry leaves or leaves attacked by insects and/or fungi, resulting in 1200 g of leaves adequate for EO extraction.

Figure 1: Sample of *Pimenta pseudocaryophyllus* leaves

Extraction of essential oil

The EO was extracted from fresh leaves (200 g) that were cut into pieces with scissors and subjected to 6 h of extraction by steam dragging in Clevenger-type equipment as modified by Wasicky.[19] This procedure was repeated on six different batches.

Chemical composition analysis of the essential oil

The EO obtained from the fresh leaves was analyzed by gas chromatography-mass spectrometry (GC-MS), adapted from the method of Matos *et al.*[20] Analyses were carried out on a HP chromatograph model 6890, under the following experimental conditions: capillary column HP 19091S-433 (5% phenyl-methyl-silicone [HP-5MS] with 30 cm × 250 cm i.d.); film thickness, 0.25 μm; initial column temperature, 40°C (3°C min^{-1}) to 200°C (3°C min^{-1}), 200°C–280°C (10°C min^{-1}); injector temperature, 280°C, split ratio 200:1; carrier gas, helium (56 kPa); flow ratio, 0.8 mL/min; ionization energy, 70 eV; and injected volume, 0.2 μL of EO.

The evaluation of the chromatogram [Figure 2] was performed according to retention times and comparison with mass spectra in the library provided with the equipment (Using Aqc Method). Triplicates were performed.

Evaluation of antimicrobial activity

The antimicrobial activity was evaluated in a Microbiology Laboratory. The biological activity of the oil obtained from the leaves was tested on oral pathogenic micro-organisms. The agar well diffusion was the method elected, including test against oral micro-organisms and the minimum inhibitory concentration (MIC), assayed by the dilution method (macrodilution) in Mueller-Hinton (MH) Broth.[21] The inhibition of bacterial growth was confirmed by subsequent culture on blood agar (BA) plates according to the standard protocols.[22-24]

Agar well diffusion

Concentrations of 1.00, 0.75, 0.50, 0.25, and 0.00 g/L (v/v) of the EO diluted in MH agar were done, with nine replicates for each concentration.

Micro-organisms were commercially obtained on impregnated discs, which included *E. faecalis* (NEWPROV-0033), *P. aeruginosa* (NEWPROV-0053), and *S. aureus* (NEWPROV-0023). Suspensions of these micro-organisms were prepared in brain heart infusion (BHI) broth at 36°C for 24 h, which were adjusted according to the McFarland scale of 0.5 (equivalent to 1.5×10^8 cells/mL). Confirmation of inoculum concentration was obtained by spectrophotometer optical density analysis (Shimadzu, Kyoto, Japan) at 600 nm.

Next, 1 mL of each micro-organism suspension was inoculated in 140 mm diameter Petri dishes filled with 50 mL of MH agar, using a sterile swab in the horizontal, vertical, and diagonal directions. Subsequently, nine equidistant perforations were drilled in the medium with a 5 mm diameter steel cylinder for the formation of the wells, which were filled with 20 μL of the respective concentrations of EO to be tested. Afterward, the plates were kept in the refrigerator for 30 min to avoid the volatilization of the oil and to perfuse in the agar. The plates were then kept in an incubator at 36°C for 24 h, followed by the analysis and measurement of halo inhibition using a caliper with a readability of 0.05 mm (Vonder, Curitiba, Brazil).

As positive controls, discs impregnated with chloramphenicol at 30 μg per disk for *P. aeruginosa* and *S. aureus* and vancomycin at 30 μg per disk for *E. faecalis* were used.

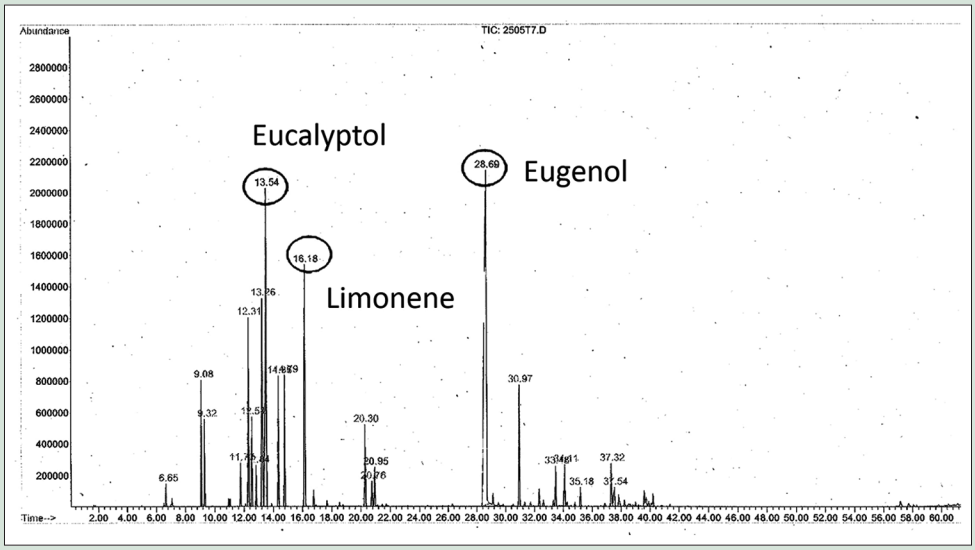


Figure 2: Chromatogram obtained for the essential oil from fresh leaves of *Pimenta pseudocaryophyllus*

Data were subjected to Shapiro-Wilk and Levene tests. Assumptions of normality and homogeneity of the variances were made and were compared statistically by *t*-tests and analysis of variance, followed by Tukey's test at 5% of probability.

Broth dilution method for assessing minimum inhibitory concentration

P. aeruginosa (NEWPROV 0053), *S. aureus* (NEWPROV 0023), and *E. faecalis* (ATCC 29212), purchased in the lyophilized form, were reconstituted in BHI broth and incubated for 24 h at 35°C ± 2°C before use. For bacterial isolation, aliquots were inoculated on BHI agar plates and incubated in the same described conditions. After 24 h, four to five isolated colonies were selected and transferred to a tube containing 10 mL of MH broth. This suspension was adjusted to match the turbidity to that of a McFarland 0.5 standard solution (equivalent to 10^8 cells/mL for most pathogens).[22-24]

A 20% (v/v) solution of the EO from *P. pseudocaryophyllus* in dimethyl sulfoxide (DMSO) was used as a master solution to evaluate the antimicrobial activity. Nine doubling dilutions of this solution were prepared in DMSO from 10% (v/v) to 0.039% (v/v).[22-24]

An aliquot of 100 µL of each serial dilution was added to 1 mL of MH broth containing the equivalent of 10^8 cells/mL of each test micro-organism. To verify the effect of DMSO in the dilutions, two additional tubes were used as controls, one of them with only the test micro-organisms in MH broth with DMSO and the other without DMSO. The tubes were incubated at 35°C ± 2°C for 18, 24, 48, and 72 h. At each time point, the tubes were examined for turbidity, and the cultures were then inoculated with a platinum loop onto BA plates and incubated for 24 h to ascertain the MIC. The tests were performed in duplicates for each micro-organism tested.[22-24]

RESULTS

Chemical composition of the essential oil

The mean yield of EO extracted from fresh leaves of *P. pseudocaryophyllus* was 1.3 mL (±0.2 mL) for each 200 g of extracted fresh leaves.

The GC-MS analysis revealed 30 compounds in the sample, of which 28 were identified, as shown in Table 1. A qualitative diversity was observed in the chemical composition of the obtained essential, and the three major constituents found were eugenol (31.5%), eucalyptol (14.2%), and limonene (9.3%).

Table 1: Composition of the essential oil from fresh leaves of *Pimenta pseudocaryophyllus*

Chemical compounds	Fresh leaf		
	RT	Area	Percentage
Eugenol	28.69	29.01	98
Eucalyptol	13.53	13.04	99
Limonene	16.17	8.56	97
β-Cymene	13.25	7.96	95
α-Phellandrene	12.31	5.35	93
Caryophyllene	30.97	4.08	99
3-Carene	14.79	3.68	94
Other	9.08	2.80	94
Carene	12.54	2.35	94
α-Pinene	9.32	1.93	96
γ-Elemene	34.10	1.54	80
Spathulenol	37.32	1.43	90
Germacrene D	33.47	1.25	99
4-Carene	12.84	1.10	96
α-Terpineol	20.95	1.10	91
β-Myrcene	11.70	1.07	76
Globulol	37.54	1.01	91
Silano	20.76	0.77	72
α-Caryophyllene	32.33	0.56	96
Ledol	37.85	0.50	91
3-Hexanol	6.65	0.48	90
Copaene	29.12	0.44	95
α-Cadinol	40.21	0.43	99
Linalyl butyrate	16.76	0.41	80
Hexanol	7.07	0.24	83
Other	38.24	0.22	55
Azulene	32.62	0.21	93
Naphthalene	33.31	0.19	99
β-Pinene	10.95	0.19	91
Cis-β-terpineol	17.68	0.16	96

RT: Retention time (min); Percentage: Probability of correct identification; Area: Equivalent to the amount of substance. Source: Using Aqc Method

Evaluation of antimicrobial activity

The diffusion tests in agar showed no formation of halos at the 0.00 g/L

concentration for any of the treatments and also at the 0.25 g/L concentration in the presence of *P. aeruginosa*.

For the three tested bacteria, the halo was significantly higher in the control treatments, demonstrating the effectiveness of the antibiotic used in relation to the EO.

In *S. aureus* and *E. faecalis*, there was a significant reduction of the halo size at the 0.25 g/L concentration and at the 0.75 g/L and 0.50 g/L concentrations, the halo remained statistically equal, with these means being lower than those recorded at the 1.00 g/L concentration. In *E. faecalis*, the means of the halo size at concentrations of 1.00, 0.75, and 0.50 g/L were significantly higher than that observed at 0.25 g/L [Table 2].

At concentrations of 1.00, 0.75, and 0.50 g/L, the size of the halos formed in the presence of the three bacteria differed statistically, being higher in *S. aureus*. At the 0.25 g/L concentration, the inhibition halo was significantly higher in *S. aureus* than in *E. faecalis*. The statistical comparison between the control treatments showed that the halo formed in *S. aureus* was higher than in *E. faecalis* and *P. aeruginosa* [Table 2].

Table 3 describes the results of the MIC tests. There was no inhibition of *P. aeruginosa* at any of the dilutions tested. For *S. aureus*, after 48 h of incubation, there was a moderate inhibition of bacterial growth in the dilutions corresponding to the following concentrations: 10%, 5%, 2.5%, and 1.25%. After 72 h, there was complete inhibition of bacterial growth at 10%, with moderate growth at the other concentrations (5%, 2.5%, and 1.25%). There was complete growth inhibition of *E. faecalis* after 48 h of incubation at 10, 5, and 2.5% and moderate growth at 1.25%. After 72 h, there was total inhibition at 10%, 5%, 2.5%, and 1.25%, demonstrating the antimicrobial activity of the EO from *P. pseudocaryophyllus*.

DISCUSSION

This study aimed to analyze and characterize the chemical composition of the EO extracted from the fresh leaves of *P. pseudocaryophyllus* and to evaluate its antimicrobial activity against *S. aureus*, *P. aeruginosa,* and *E. faecalis*, micro-organisms commonly found in the mouth and biofilms.

The EO extracted presented a yield of 0.65% in relation to the fresh leaves. A value of 1.0% yield was reported to EO from fresh leaves of the same species[25] and the difference could be related to the geographic origin of the samples. Other study found 1.9% and 2.3% yield of EO from dried leaves of *P. pseudocaryophyllus*.[26] In this case, the difference observed is due to the previous dehydration of the leaves, besides the sample origin.

Analysis of the composition of the EO from fresh leaves of *P. pseudocaryophyllus* identified components that match those previously found in other species of the family *Myrtaceae*.[27]

A previous pharmacognostic study analyzed the oil from the leaves of *P. pseudocaryophyllus* using various methods such as phytochemical screening, colorimetric reactions, and protein precipitation, which revealed the presence of phenolic compounds, tannins, and flavonoids.[16]

The chemical analyzes in the present work identified eugenol (31.5%) as the predominant compound, as reported by other studies with *P. pseudocaryophyllus*.[15,25] Eucalyptol (14.2%) and limonene (9.3%) are also found in considerable amount in the present work. Together, the three major components identified in the extracted EO totalized 55% of the sample. Overall, satisfactory antimicrobial activities against the micro-organisms studied were observed.

Authors reported that eugenol and 4-methyl-eugenol were the major constituents of the EO from the leaves of *P. pseudocaryophyllus* and that eugenol is responsible for most of the antiseptic and antimicrobial activities attributed to the species.[15] In a later study, others concluded that eugenol represented the main component in the EO of *Tynanthus micranthus* as well as *P. pseudocaryophyllus* and that both of these oils possessed antimicrobial and antifungal activities.[25] These authors also suggested that eugenol could be useful as an antimicrobial agent in agriculture, the food industry and in the preparation of pharmaceutical products. In dentistry, eugenol is used in the manufacture of dentifrice because of its powerful potential as an antimicrobial agent.[25]

The antimicrobial activity of plant extracts can be measured by sequential dilution of the test substances to identify the lowest concentration that inhibits the growth of micro-organisms. In sensitivity tests using the broth dilution method, the MIC is the lowest concentration of

Table 2: Mean±standard deviation referring to the inhibition halo sizes formed in three species of bacteria submitted to five concentrations of essential oil and in the presence of antibiotic (control)

Concentration (g/L)	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*	Variance
1.00	15.38 ± 1.30^{bA}	8.41 ± 0.79^{bC}	11.23 ± 0.62^{bB}	*F*: 122.50
0.75	13.11 ± 0.91^{cA}	2.92 ± 3.60^{cC}	9.28 ± 0.54^{bB}	*F*: 75.25
0.50	12.44 ± 1.30^{cdA}	2.99 ± 3.57^{cC}	9.14 ± 0.62^{bB}	*F*: 54.80
0.25	9.61 ± 3.98^{d}	-	4.08 ± 3.90^{c}	*t*: 3.61
0.0	-	-	-	-
Control	27.98 ± 0.89^{aA}	10.91 ± 0.81^{aC}	22.83 ± 0.87^{aB}	*F*: 938.00
F	264.80	30.51	135.30	

F: ANOVA; *t*: *t*-test; *P*: Significance ($P<0.001$). Different letters indicate statistical difference by Tukey's test. Lowercase letters: Comparisons in the same column; Capitalization: Comparisons on the same line

Table 3: Minimum inhibitory concentration of the essential oil from fresh leaves of *Pimenta pseudocaryophyllus* against different micro-organisms

Micro-organisms	Dilution of EO									IT (h)
	10% v/v	5% v/v	2.5% v/v	1.25% v/v	0.625% v/v	0.312% v/v	0.156% v/v	0.078% v/v	0.039% v/v	
Pseudomonas	+	+	+	+	+	+	+	+	+	48
aeruginosa	+	+	+	+	+	+	+	+	+	72
Staphylococcus	±	±	±	±	+	+	+	+	+	48
aureus	−	±	±	±	+	+	+	+	+	72
Enterococcus	−	−	−	±	+	+	+	+	+	48
faecalis	−	−	−	−	+	+	+	+	+	72

+: Abundant bacterial growth (>80%); ±: Moderate bacterial growth (40%-80%); −: Absence of bacterial growth; IT: Incubation time; % v/v: Concentration volume/volume of the EO from *Pimenta pseudocaryophyllus*; EO: Essential oil

the test antimicrobial substance that prevents visible growth of the micro-organism.[21]

Although the disk diffusion test is widely used, it is not considered the most appropriate method to measure antimicrobial activity. During the incubation period, the antimicrobial agent diffuses out of the disk impregnated with the antimicrobial solution into the culture medium. If an inhibition zone is formed around the disk, the test agent material is scored as effective; however, the result may be affected by the diffusion capacity of the agent in the culture medium.[28]

The oil from *P. pseudocaryophyllus* inhibited the growth of *S. aureus* at the higher concentrations (10%) and longer incubation times tested (72 h), while moderate inhibition was also noted at the lower concentrations (5.0%, 2.5%, and 1.25%) and times (48 h/72 h). In this study, there was no inhibition of *P. aeruginosa* at any of the concentrations of the tested oil. In contrast, a study demonstrated similar antimicrobial activities against both *P. aeruginosa* (ATCC 9027) and *S. aureus* (ATCC 6538) when EO from the leaves of two specimens of *P. pseudocaryophyllus* collected from different sites were tested.[15]

The reasons for choosing *P. aeruginosa* and *S. aureus* as test organisms in this study are based on the presence of these two bacterial species in the oral cavity and because they display a great capacity for biofilm formation.[3-6] They are commonly found in moist environments and even in dental equipment, thus, representing a potential source of cross-contamination.[3,4] The presence of biofilms formed by *P. aeruginosa* and *S. aureus* in fragments of dental handpieces have been reported.[3]

Water collected from 33 samples of dental apparatus from private clinics, public services, and a College of Dentistry were examined and *P. aeruginosa* was found in 21.2% of these samples.[4] This observation deserves more attention from dental professionals because it represents a risk of cross-infections in clinics. *P. aeruginosa* is known as an opportunist bacterium since it can invade the bloodstream, resulting in sepsis, which is a serious and potentially fatal complication.[28]

Another opportunist bacterium, *S. aureus*, may be transmitted through disrupted skin barriers or during invasive surgical procedures, posing a major problem in hospitals and dental clinics. The evaluation of the presence of *S. aureus* on different surfaces from the School of Clinical Dentistry demonstrated the presence of this micro-organism in 34% of the samples tested, suggesting that the surfaces became contaminated during the dental treatment.[6] These findings highlight the importance of biosecurity designed to minimize cross-contamination between the oral health team and patients in the dental clinic.

In this study, the EO from *P. pseudocaryophyllus* inhibited the growth of *E. faecalis*, confirming its antimicrobial potential. These results are particularly important since this bacterium is a common contaminant in endodontic treatments and displays resistance. In addition, this bacteria forms biofilm that can adhere to the inside of root canals and its presence has been noted in persistent periapical infections.[1,2] Moreover, *E. faecalis* can tolerate long periods of nutritional restriction and withstand extremes of salinity and pH, with the ability to remain viable in the walls of the root canal for up to 12 months after dental procedures.[29] The organism features several virulence factors that modulate the expression of genes in unfavorable conditions, increasing its adherence and resistance to antimicrobials and rendering this bacterium a potential risk to oral health.[30]

The antimicrobial activity of natural substances against *P. aeruginosa*[9-11] and *S. aureus*[9,11-13] has been investigated. The action of the EO from *P. pseudocaryophyllus* against *E. faecalis* illustrates the importance of evaluating the antimicrobial effects of medicinal plants as a support for dental treatments. In Addition, the development of a formulation for intracanal medications and irrigators to assist in the treatment and blocking of the action of virulence factors is essential to prevent bacterial adherence and invasion of the oral mucosal tissues.

The variable effect on the studied micro-organisms may be explained by the fact that the inhibitory concentration of EO required for each bacterial species is different. The greater sensitivity of Gram-positive compared to Gram-negative bacteria may be related to differences in the bacterial cell wall structure, which hinders the efficacy of the EO for Gram-negative bacteria.[31] Many plants with potential antimicrobial activity have been shown to be effective only against strains of Gram-positive bacteria.[32,33] Researchers have postulated that the outer membrane of Gram-negative bacteria could act as a barrier against the active principles found in medicinal plants.[34] However, other studies have verified antimicrobial activity against both Gram-positive and Gram-negative bacteria.[35,36]

CONCLUSION

EO from fresh leaves of *P. pseudocaryophyllus* presented as the three major components eugenol, eucalyptol, and limonene. Although complementary studies are necessary, these compounds SE may be related to the observed antimicrobial activity against certain micro-organisms present in the human oral cavity and suggest the biological potential of this species of the *Myrtaceae* Family.

Acknowledgements

The authors are grateful to the Director of the Second Integrated Center for Air Defense and Air Traffic Control (Segundo Centro Integrado de Defesa Aérea e Controle de Tráfego Aéreo-CINDACTA II), as well as the Head of the Clinical Analysis Laboratory, for providing the facilities of the Microbiology Laboratory for the conduction of this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Dioguardi M, Di Gioia G, Illuzzi G, Arena C, Caponio VC, Caloro GA, *et al.* Inspection of the microbiota in endodontic lesions. Dent J (Basel) 2019;7. pii: E47.
2. Prada I, Micó-Muñoz P, Giner-Lluesma T, Micó-Martínez P, Collado-Castellano N, Manzano-Saiz A. Influence of microbiology on endodontic failure. Literature review. Med Oral Patol Oral Cir Bucal 2019;24:e364-e372.
3. Freitas VR, Van der Sand ST, Simonetti AB. *In vitro* biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus aureus* on the surface of high-speed dental handpieces. Rev Odontol UNESP 2010;39:193-200.
4. Moreira AC, Pereira AF, Menezes AR. Water contamination of odontological equipment by *Pseudomonas sp.* R Ci Méd Biol 2006;5:146-50.
5. Beloin C, Renard S, Ghigo JM, Lebeaux D. Novel approaches to combat bacterial biofilms. Curr Opin Pharmacol 2014;18:61-8.
6. Martins JR, Cappelari JF, dos Santos RB, Weigert KL, Gelatti LC, dos Santos O. Presence of *Staphylococcus aureus* on different dental clinic settings. Rev Fasem Ciências 2013;3:92-9.
7. Barbot V, Robert A, Rodier MH, Imbert C. Update on infectious risks associated with dental unit waterlines. FEMS Immunol Med Microbiol 2012;65:196-204.
8. Ciofu O, Rojo-Molinero E, Macià MD, Oliver A. Antibiotic treatment of biofilm infections. APMIS 2017;125:304-19.
9. Herrera DR, Durand-Ramirez JE, Falcão A, Silva EJ, Santos EB, Gomes BP. Antimicrobial activity and substantivity of *Uncaria tomentosa* in infected root canal dentin. Braz Oral Res 2016;30:e61.
10. Noushad MC, Balan B, Basheer S, Usman SB, Muhammed Askar MK. Antimicrobial efficacy of different natural extracts against persistent root canal pathogens: An *in vitro* study. Contemp Clin Dent 2018;9:177-81.
11. Hu Y, Keniry M, Palmer SO, Bullard JM. Discovery and analysis of natural-product compounds inhibiting protein synthesis in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2016;60:4820-9.
12. Tadtong S, Puengseangdee C, Prasertthanawut S, Hongratanaworakit T. Antimicrobial constituents and effects of blended eucalyptus, rosemary, patchouli, pine, and cajuput essential oils. Nat Prod Commun 2016;11:267-70.

13. Poma P, Labbozzetta M, Zito P, Alduina R, Ramarosandratana AV, Bruno M, *et al*. Essential oil composition of *Alluaudia procera* and *in vitro* biological activity on two drug-resistant models. Molecules 2019;24. pii: E2871.
14. Paula JA, Reis JB, Ferreira LH, Menezes AC, Paula JR. Pimenta genus: Botanical aspects, chemical composition and pharmacological potential. Rev Bras Plantas Med 2010;12:363-79.
15. Lima ME, Cordeiro I, Young MC, Sobra ME, Moreno PR. Antimicrobial activity of the essential oil from two specimens of *Pimenta pseudocaryophyllus* (Gomes) L. R. Landrum (*Myrtaceae*) native from São Paulo State – Brazil. Pharmacol on Line 2006;3:589-93.
16. Paula JA, Paula JR, Bara MT, Rezende MH, Ferreira HD. Estudo farmacognóstico das folhas de *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum – Myrtaceae. Rev Bras Farmacogn 2008;18:265-78.
17. Ferrari FC, Lemos Lima Rde C, Schimith Ferraz Filha Z, Barros CH, de Paula Michel Araújo MC, Antunes Saúde-Guimarães D. Effects of *Pimenta pseudocaryophyllus* extracts on gout: Anti-inflammatory activity and anti-hyperuricemic effect through xantine oxidase and uricosuric action. J Ethnopharmacol 2016;180:37-42.
18. Cardoso BS, Machado KB, de Paula JR, de Paula JAM, Cuvinel WM, Amaral VCS. Developmental toxicity evaluation of *Pimenta pseudocaryophyllus* (Gomes) Landrum, (E)-methyl isoeugenol chemotype, in Wistar rats. Birth Defects Res 2017;109:1292-300.
19. Wasicky R. A modification of Clevenger apparatus for essential oil extraction. Rev Farm Bioquim Univ S Paulo 1963;1:77-81.
20. Matos IL, Machado SM, Souza AR, Costa EV, Nepel A, Barison A, *et al*. Constituents of essential oil and hydrolate of leaves of *Campomanesia viatoris* Landrum.Quím Nova 2015;38:1289-92.
21. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71-9.
22. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard. 6th ed. Wayne: Clinical and Laboratory Standards Institute; 2003.
23. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests: Approved Standard. 8th ed. Wayne: Clinical and Laboratory Standards Institute; 2003.
24. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 15th Informational Supplement. Wayne: Clinical and Laboratory Standards Institute; 2005.
25. Custódio DL, Burgo RP, Moriel B, Barbosa AM, Rezende MI, Daniel JF, *et al*. Antimicrobial activity of essential oils from *Pimenta pseudocaryophyllus* and *Tynanthus micranthus*. J Braz Arch Biol Technol 2010;53:1363-9.
26. Yokomizo NK, Nakaoka-Sakita M. Antimicrobial activity and essential oils yield of *Pimenta pseudocaryophyllus* var. *Pseudocaryophyllus* (*Gomes*) Landrum, Myrtaceae. Rev Bras Plantas Med 2014;16:513-20.
27. Apel MA, Sobral M, Henriques AT. Chemical composition of the volatile oils of native Myrcianthes species from South Brazil. 2006;16:402-7.
28. Tortora GJ, Funke BR, Case CL. Microbiologia. 1st. ed. São Paulo: Artmed; 2005.
29. Sedgley CM, Nagel AC, Shelburne CE, Clewell DB, Appelbe O, Molander A. Quantitative real-time PCR detection of oral *Enterococcus faecalis* in humans. Arch Oral Biol 2005;50:575-83.
30. Valera MC, Silva KC, Maekawa LE, Carvalho CA, Koga-Ito CY, Camargo CH, *et al*. Antimicrobial activity of sodium hypochlorite associated with intracanal medication for *Candida albicans* and *Enterococcus faecalis* inoculated in root canals. J Appl Oral Sci 2009;17:555-9.
31. da Silva RH, Bastos JR, Mendes HJ, Castro RF, Camargo LM. Dental caries, community periodontal index and oral hygiene in a riverside community. RGO, Rev Gaúcha Odontol. Rev Gaúcha Odontol 2010;58:457-62.
32. Kelmanson JE, Jäger AK, van Staden J. Zulu medicinal plants with antibacterial activity. J Ethnopharmacol 2000;69:241-6.
33. Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jäger AK, *et al*. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol 1999;68:267-74.
34. Urzua A, Caroli M, Vasquez L, Mendoza L, Wilkens M, Tojo E. Antimicrobial study of the resinous exudate and of diterpenoids isolated from *Eupatorium salvia (Asteraceae)*. J Ethnopharmacol 1998;62:251-4.
35. Abd Aziz SM, Low CN, Chai LC, Abd Razak SS, Selamat J, Son R. Screening of selected Malaysian plants against several food borne pathogen bacteria. Int Food Res J 2011;18:1195-201.
36. Stefanovic O, Comie L. Synergistic antibacterial interaction between *Melissa officinalis* extracts and antibiotics. J Appl Pharma Sci 2012;2:1-5.