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Chapter

Chemical Composition and Antibacterial Activity of the Essential Oil of *Mesosphaerum suaveolens* (Lamiaceae)

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Abstract

Mesosphaerum suaveolens (Lamiaceae) is a medicinal plant commonly used in Brazil for the treatment of diseases related to the digestive tract and respiratory diseases, so we hypothesized that the essential oil of this species may have antibacterial activity. Thus, we aimed to evaluate the *in vitro* antibacterial and modulatory activity of the essential oil of *M. suaveolens* as well as to characterize its chemical composition. The identification of the constituents was performed by gas chromatography-flame ionization detector (GC-FID) and the antibacterial and modulating activity by the plate microdilution method. We found the oil had sesquiterpene β-caryophyllene as the major component. This compound may account for the antibacterial activity against *Staphylococcus aureus* strains, since the essential oil had a MIC of 64 μg/mL for the standard strain and 256 μg/mL for the multiresistant strain, demonstrated that the oil does not exhibit drug modulating activity. Thus, *M. suaveolens* oil has bioactive compounds which can be used in the preparation of drugs.

Keywords: bacteria, *Hyptis suaveolens*, bamburral, β-caryophyllene, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

1. Introduction

Bacterial infections are major problems in medicine due to the indiscriminate use of antibiotics that eventually select resistant microorganisms, which in turn proliferate [1]. Among the bacteria that cause infections stand out *Pseudomonas aeruginosa* (Pseudomonadaceae), *Escherichia coli* (Enterobacteriaceae) and *Staphylococcus aureus* (Staphylococcaceae) [2].

The bacterium, *P. aeruginosa*, is a gram-negative bacterium that is responsible for causing hospital infections, especially in patients who have compromised immune systems, and in rarer cases, it can lead to pneumonia, resulting in the death of 60% of infected [3, 4]. Although strains of *E. coli* colonize the human digestive tract, in large quantities they are capable of causing intestinal problems such as diarrhea. While *S. aureus* causes several acute infections such as pneumonia, osteomyelitis, endocarditis, myocarditis, pericarditis, and meningitis [1].

It has been reported that the mechanisms of bacterial resistance include the efflux pumps, which expel the antibiotic, in addition, the bacteria are capable of altering the target of the antibiotic for mutation or enzymatic inactivation and alteration of the permeability of the bacterium to the drug [5]. Thus, antibiotics alone cannot inhibit bacterial growth so that substances that modulate their effect are necessary in order to potentiate the action of the drug [6, 7].

These substances capable of modulating standard drugs can be found in plants, since these have constituents with antibacterial actions derived from their secondary metabolism, mainly the aromatic herbs, because their essential oils have diverse biological and pharmacological activities [8, 9]. Among the botanical families most

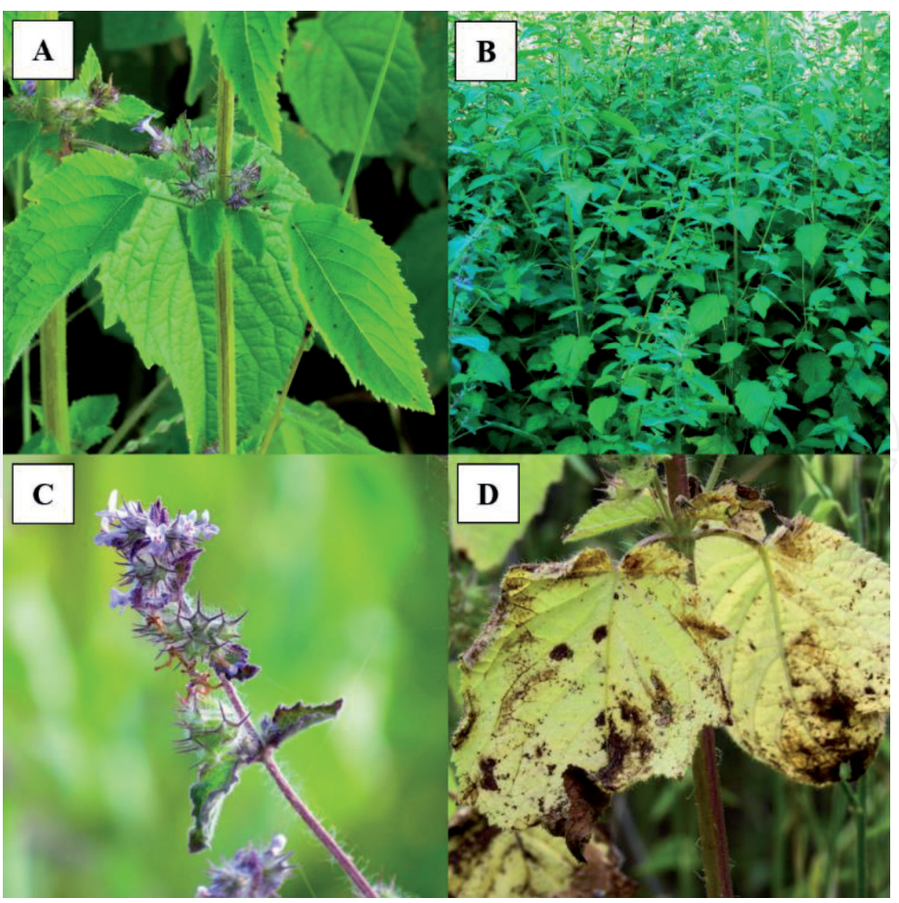

Figure 1.
Mesosphaerum suaveolens*. (a) Leaves and stem. (b) Population of* M. suaveolens *in Quixelô—CE, Brazil. (c) Highlight of flowers. (d) Leaves in senescence.*

rich in aromatic plants is Lamiaceae, which is well known for its representatives as sources of essential oils used in cooking, aromatherapy and medicine [10, 11]. Among the species of this family, the species *Mesosphaerum suaveolens* (L.) Kuntze (**Figure 1**), known in Brazil as "bamburral" and "alfazema-brava," is popularly used in the treatment of diseases related to gastrointestinal and respiratory tract [12], so that we hypothesize that the species is abundant in phytochemical constituents, which present biological activity against strains of pathogenic microorganisms, such as bacteria. This hypothesis is supported by the pharmacological and biological activities of these species already evidenced in the literature, such as antioxidant activity [13], neuroprotective [14], gastro-protective [15], antitumor [16], antinociceptive [17], anti-inflammatory [18], antifungal [19], anti-bacterial [20], insecticide [21], larvicide [8], and allelopathic action [22].

Thus, due to increasing bacterial resistance to drugs and the search for new bioactive sources, this research aims to evaluate the *in vitro* antibacterial and modulatory activity of *M. suaveolens* essential oil as well as to characterize its chemical compounds.

2. Results

2.1 Chemical composition of essential oil

The essential oil of *M. suaveolens* presented a total of 44 phyto-constituents, with β-caryophyllene (20.37%) being the major constituent (**Figure 2**). Following this, the oil presented as secondary compounds were sabinene (15.94%) and espatulenol (11.09%). As constituents traces (<1%), 26 constituents were found (**Table 1**).

2.2 Minimal inhibitory concentration (MIC)

According to **Table 2**, the essential oil of *M. suaveolens* showed no activity against gram-negative bacteria (*P. aeruginosa* and *E. coli*), both standard strains and multiresistant strains, since they have MIC ≥1024 μg/mL. However, the oil presented antibacterial action against *S. aureus* with MIC of 64 μg/mL for the standard strain (ATCC) and 256 μg/mL for the multiresistant strain.

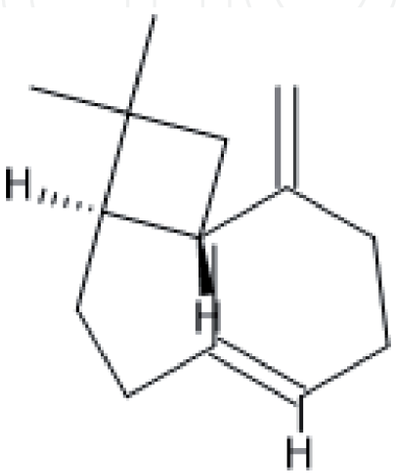

Figure 2.
Chemical structure of sesquiterpene β-caryophyllene.

Compounds	Molecular formula	RI[a]	RI[b]	Oil
				%
α-Thujene	$C_{10}H_{16}$	989	931	1.09
Sabinene	$C_{10}H_{16}$	976	976	15.94
β-Pinene	$C_{10}H_{16}$	980	980	2.01
α-Phellandrene	$C_{10}H_{16}$	1006	1005	1.38
α-Terpinene	$C_{10}H_{16}$	1019	1018	1.05
Limonene	$C_{10}H_{16}$	1031	1031	5.19
1-8-Cineole	$C_{10}H_{18}O$	1037	1033	3.04
γ-Terpinene	$C_{10}H_{16}$	1060	1061	2.47
Terpinen-4-ol	$C_{10}H_{18}O$	1178	1177	6.62
δ-Elemene	$C_{15}H_{24}$	1335	1338	1.17
β-Caryophyllene	$C_{15}H_{24}$	1421	1418	20.37
γ-elemene	$C_{15}H_{24}$	1435	1433	1.04
α-humulene	$C_{15}H_{24}$	1453	1454	1.17
Germacrene D	$C_{15}H_{24}$	1481	1480	5.21
Bicyclogermacrene	$C_{15}H_{24}$	1501	1488	7.02
Spathulenol	$C_{15}H_{24}O$	1576	1576	11.09
Caryophyllene oxide	$C_{15}H_{24}O$	1580	1581	3.18
Cubenol	$C_{15}H_{26}O$	1641	1642	1.07
Monoterpene hydrocarbons	**$C_{10}H_n$**			**29.13**
Sesquiterpene hydrocarbons	**$C_{15}H_n$**			**35.98**
Phenylpropanoids	**$C_nH_nO_n$**			**25.00**
Total identified (%)				**90.11**

Relative proportions of the essential oil constituents were expressed as percentages.
[a]Retention indices experimental (based on homologous series of n-alkane C_7–C_{30}).
[b]Retention indices from literature.

Table 1.
Main constituents (>1%) of Mesosphaerum suaveolens *essential oil.*

Strains	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*
Strains standards (ATCC)	≥1024	≥1024	64
Multi-resistant Strains	≥1024	≥1024	256

Table 2.
Minimal inhibitory concentration (μg/mL) of essential oil of Mesosphaerum suaveolens *against conventional bacterial (ATCC) and multiresistant strains.*

2.3 Modulation of drugs

According to **Figure 3**, it was demonstrated that the essential oil of *M. suaveolens* does not have the capacity to modulate the antibiotics, gentamicin, imipinem, and norfloxacin, since there was no significant difference between the control group and the treatments.

3. Discussion

Although the leaves of *M. suaveolens* are used in folk medicine for the treatment of diseases related to the gastrointestinal and respiratory tract [12], it has been demonstrated that the volatile terpenes of the species are not related to this action, since in the in the present study, this product did not present antibacterial action at concentrations of clinical relevance for two of the three strains used [23].

However, it is possible to observe that there is antibacterial action against the standard strains of *S. aureus* multiresistants. This can be explained by the mechanisms of action that some natural products have, such as the ability to disintegrate their cytoplasmic membranes, as well as destabilization of the proton motive force, electron flow, active transport and cellular content coagulation [24]. In addition, activity against *S. aureus* can be linked to the major compound of the study oil, β-caryophyllene, since this sesquiterpene exhibits antibacterial activity, especially against Gram-positive bacteria [25].

Thus, the oil has a source of β-caryophyllene, such sesquiterpene is found to be the majority compound; however, the oil of this species shows heterogeneity according to internal (genetic) and external factors (origin, mode of collection, collection period, etc.) [26]. To avoid large variations in the chemical composition of the oil, the collections should be standardized, such as collection times, period of the year, as well as to identify if the individual is under herbivorous attack [8].

This variation in the essential oil explains why some works show the antibacterial action of the essential oil, as Tesch et al. [27], where the oil showed activity against *E. coli* ATCC 25922 (MIC 350 μL/mL), *Klebsiella pneumoniae* ATCC 23357 (MIC 300 μL/mL), *Salmonella* Typhi CDC57 (MIC 400 μL/mL). In this study, the natural product presented eucalyptol ($C_{10}H_{18}O$) and fenchone ($C_{10}H_{16}O$) as the main compounds.

In addition to antimicrobial activities, the products of plant origin can have a drug modulating action, and although *M. suaveolens* does not present such action, it is seen that in members of the Lamiaceae family, some species present such action. Among the species is *Origanum vulgare* L., where its essential oil has a modulating action of the tetracycline drug against bacterial strains of *S. aureus* IS-58, which had the TetK tetracycline efflux protein [28].

4. Materials and methods

4.1 Collection of botanical material

Fresh leaves of *M. suaveolens* were collected in the municipality of Quixelô located in the state of Ceará (Brazil) under coordinates −6°14′22.40″S, −39°16′14.29″W in March 2015 (**Figure 4**). The collection area is characterized as being part of the Caatinga, a seasonally dry tropical forest. The leaves were dried in an oven at 30°C. The plant material was identified and a voucher specimen was deposited in the Herbarium Caririense Dárdano of Andrade-Lima – HCDAL under #12.104.

4.2 Extraction of essential oil

After drying, the leaves were packed in a volumetric flask containing 4 L of distilled water and subjected to constant boiling for 2 hours. Then the essential oil was collected and stored in an amber bottle under constant refrigeration until the conduction of the chemical analyzes and microbiological tests [8].

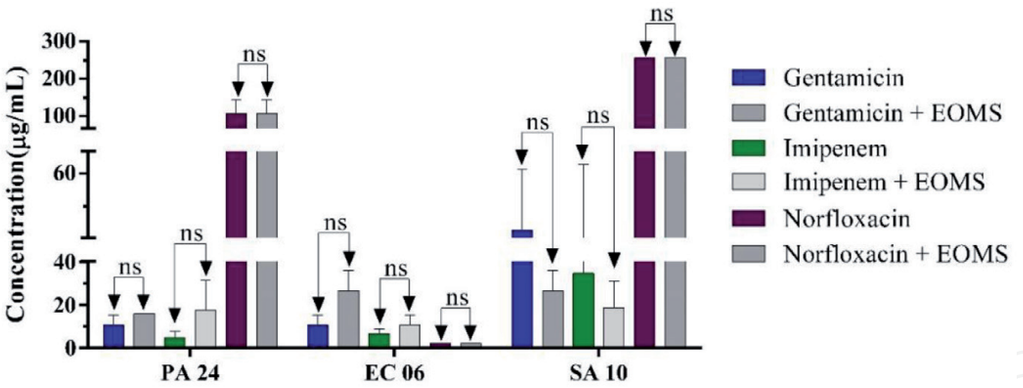

Figure 3.
Minimum inhibitory concentration of antibiotic modulation in combination with essential oil of Mesosphaerum suaveolens. *PA 24,* Pseudomonas aeruginosa *24; EC 06,* Escherichia coli *06; SA 10,* Staphylococcus aureus *10; EOMS, essential oil of* Mesosphaerum suaveolens.

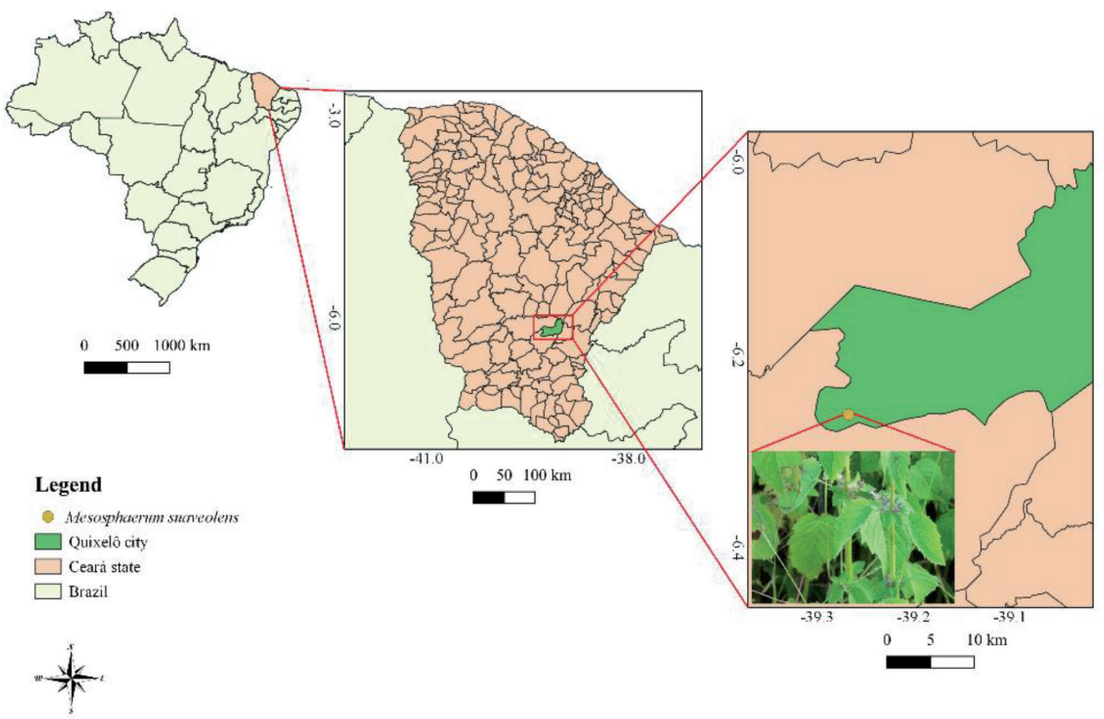

Figure 4.
Map of the collection of the species Mesosphaerum suaveolens *in the municipality of Quixelô—CE, Brazil.*

4.3 Phytochemical analysis of essential oil by gas chromatography (GC-FID)

For gas chromatography (GC), the Agilent Technologies 6890 N GC-FID system, equipped with DB-5 capillary column with the following specifications: 30 m of length, 0.32 mm and 0.50 µm of film thickness was used, which was connected to an FID detector. The temperature ramp consisted of: Initial temperature of 60°C for 1 min and was raised to 3° C/min until reaching 180°C [8].

4.4 Identification of the components

As for the identification, the terpenes were identified as to the of retention index (RI). In addition, they were compared with two spectral libraries, Nist and Wiley, and data in the literature [23].

Bacteria	Origin	Resistance profile
Escherichia coli 06	Urine culture	Cephalothin, cephalexin, cefadroxil, ceftriaxone, cefepime, ampicillin-sulbactam
Pseudomonas aeruginosa 03	Uroculture	Amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, meropenem, cefepime
Staphylococcus aureus 10	Rectal swab culture	Cefadroxil, cephalexin, cephalothin, oxacillin, penicillin, ampicillin, amoxicillin, moxifloxacin, ciprofloxacin, levofloxacin, ampicillin-sulbactam, amoxilin/ac. Clavulanic, erythromycin, clarithromycin, azithromycin, clindamycin

Source: Laboratory of Microbiology and Molecular Biology—LMBM—regional University of Cariri—URCA.

Table 3.
Isolated clinical bacterial strains used for MIC and modulation tests with their antibiotic resistance and origin profile.

4.5 Antibacterial activity

4.5.1 Bacterial strains, culture media and drugs

For the antibacterial tests, standard strains were used to determine minimum inhibitory concentration (MIC), being *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Staphylococcus aureus* ATCC 25923. While for the modulation and also MIC tests, strains resistant cells (**Table 3**), being *Escherichia coli* 06, *Pseudomonas aeruginosa* 03 and *Staphylococcus aureus* 10.

As for the culture medium for the antibacterial assays, Brain Heart Infusion (BHI) was prepared according to the measures recommended by the manufacturer. While for *in vitro* modulation assays, the drugs used were Gentamicin from class aminoglycoside, Norfloxacin, belonging to the classes of fluoroquinolones and Imipenem of the carbapenem class.

4.5.2 Minimal inhibitory concentration (MIC)

It was followed the methodology employed in the work Bezerra et al. [3] for the determination of the Minimum Inhibitory Concentration (MIC). In this study, concentrations ranging from 1 to 1024 μg/mL of the essential oil of *M. suaveolens* against pathogenic bacteria were evaluated. For that, the inoculants of the strains were mixed with BHI (10%), being distributed in microdilution plates with the natural product. After 24 hours of microbial growth at a temperature of 37°C, the MIC was evaluated with the addition of resazurin.

4.5.3 Effect modulator of antibiotics

To assess the modulating effect of essential oil, sub-inhibitory concentrations (MIC/8) of the product against multidrug-resistant bacteria were used. For that, concentrations of standard antibiotics (1–1024 μg/mL) were added to microdilution plates containing BHI (10%) and bacteria inoculum, as well as volatile *M. suaveolens* terpenes in sub-inhibitory concentrations. After 24 hours in a bacteriological oven (37°C), a resazurin solution was added to determine the MIC [7].

4.6 Statistical analysis

The results were analyzed in the GraphPad Prism program, version 6, in which the data were analyzed by Anova One-way and followed by post hoc Tukey test and were considered significant when $p < 0.05$.

5. Conclusion

The essential oil of *Mesosphaerum suaveolens* exhibits antibacterial activity against strains of *Staphylococcus aureus* so that its phytochemicals can be used in the formulation of new drugs. Further studies on toxicity should be performed in order to ascertain the tolerable concentrations that can be used of this oil.

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Conflict of interest

The authors declare no conflict of interest.

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