

Chemical profile and microbiological activity of essential oil from *Persea venosa*: an endemic species from Brazilian Flora

Leonardo de Assunção Pinto^{1,2,14}, Julia Chaves Scaffo^{2,7}, Francisco Paiva Machado^{2,8}, Ricardo Santos Esteves^{2,9}, Victor Moebus^{3,10}, Renata Freire Alves Pereira^{4,11}, Caio Pinho Fernandes⁵, Fabio Aguiar Alves^{4,6}, Luiz Antônio Moura Keller^{3,12} & Leandro Rocha^{2,13}

Key words:

- β-caryophyllene
- Antibacterial
- Antifungal
- Lauraceae

Abstract

The *Persea venosa* belongs to the Lauraceae family, whose essential oils exhibit diverse bioactivity, including against significant pathogens such as bacteria and fungi of medical interest and/or contribute to food spoilage. Consequently, essential oils are recognized as viable alternatives for developing sustainable antimicrobials. In this study, essential oil was extracted by hydrodistillation from the leaves of *P. venosa* and analyzed in gas chromatography coupled with mass spectrometry. Microbiological assays were conducted at concentrations up to 2,048 µg/mL against *Staphylococcus aureus*; *S. epidermidis*; *Escherichia coli*; *Enterobacter cloacae*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*; *Aspergillus flavus*; *A. parasiticus*, and *A. westerdijkiae*. The extraction yielded 0.10% (w/w) in which sesquiterpene hydrocarbons were identified (90.23%), with β-caryophyllene being the predominant substance at 38.59%. This composition aligns with the literature. The highest tested concentration exhibited inhibitory effects against all bacterial strains. The *P. aeruginosa* and *S. epidermidis* exhibited a minimum inhibitory concentration of 1,024 µg/mL. Bactericidal activity was only observed in *S. epidermidis* in a high concentration, the maximum tested. No antifungal activity was detected at any tested concentration. The present study highlights the antimicrobial effect of *P. venosa* essential oil (although only in high concentrations), being the first report of the activity related to the species.

Palavras-chave:

- β-cariofileno
- Antibacteriano
- Antifúngico
- Lauraceae

Resumo

A *Persea venosa* pertence à família Lauraceae, cujos óleos essenciais apresentam bioatividade diversa, incluindo contra patógenos significativos, como bactérias e fungos de interesse médico e/ou que contribuem para a deterioração de alimentos. Consequentemente, os óleos essenciais são reconhecidos como alternativas viáveis para o desenvolvimento de biopesticidas sustentáveis. No presente estudo, o óleo essencial foi extraído por hidrodestilação das folhas de *P. venosa* e analisado em cromatografia gasosa acoplada à espectrometria de massas.

7th Fluminense Symposium on Natural Products - conservation, sustainability, and innovation

¹ Federal University of Rio de Janeiro, Center for Health Sciences, Rio de Janeiro, RJ, Brazil. ORCID: 0000-0001-9411-9819

² Fluminense Federal University, School of Pharmacy, Niterói, RJ, Brazil.

³ Fluminense Federal University, School of Veterinary Medicine, Niterói, RJ, Brazil.

⁴ Fluminense Federal University, Niterói, RJ, Brazil.

⁵ Federal University of Amapá Foundation, Macapá, AP, Brazil. ORCID: 0000-0002-6538-7360

⁶ Palm Beach Atlantic University, Lloyd L. Gregory School of Pharmacy, West Palm Beach, FL, USA. ORCID: 0000-0001-6235-9367

⁷ ORCID: 0000-0001-6214-7778

⁸ ORCID: 0000-0002-5332-9972

⁹ ORCID: 0000-0002-6611-6722

¹⁰ ORCID: 0000-0003-2340-4166

¹¹ ORCID: 0000-0002-7917-901X

¹² ORCID: 0000-0002-3857-593X

¹³ ORCID: 0000-0003-0484-1918

¹⁴ Author for correspondence: leonardoap@id.uff.br

Cite as: Pinto LA, Scaffo JC, Machado FP, Esteves RS, Moebus V, Pereira RFA, Fernandes CP, Alves FA, Keller LAM & Rocha L (2025) Chemical profile and microbiological activity of essential oil from *Persea venosa*: an endemic species from Brazilian Flora. *Rodriguésia* 76: e00362025. DOI: 10.1590/2175-7860202576044

Area Editor: Dr. Davyson Moreira

Received on March 27, 2025. Accepted on July 07, 2025.

Rodriguésia

Revista do Instituto de Pesquisas Jardim Botânico do Rio de Janeiro
<<http://rodriguesia.jbrj.gov.br>>.

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Ensaaios microbiológicos foram conduzidos em concentrações de até 2.048 µg/mL contra: *Staphylococcus aureus*; *S. epidermidis*; *Escherichia coli*; *Enterobacter cloacae*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*; *Aspergillus flavus*; *A. parasiticus* e *A. westerdijkiae*. A extração rendeu 0,10% (p/p), onde hidrocarbonetos sesquiterpênicos foram identificados (90,23%), com β-cariofileno sendo a substância majoritária com 38,59%. A composição alinha-se a literatura. A maior concentração testada exibiu efeitos inibitórios contra todas as cepas bacterianas. As *P. aeruginosa* e *S. epidermidis* exibiram concentração inibitória mínima de 1,024 µg/mL. Atividade bactericida foi observada apenas em *S. epidermidis* em alta concentração, a máxima testada. Nenhuma atividade antifúngica foi detectada em nenhuma concentração avaliada. O presente estudo destaca o efeito antimicrobiano do óleo essencial de *P. venosa* (embora em altas concentrações), sendo o primeiro relato da atividade pela espécie.

Introduction

Brazil has the greatest floral biodiversity on the planet, with approximately 15% of global biodiversity concentrated within its borders and about 0.5% of the estimated known plant species being endemic to the country (Valli *et al.* 2018). The Atlantic Forest biome has significant potential for biotechnological exploration but faces severe environmental challenges, as only 7% of its original area remains intact (Valli *et al.* 2021).

Within this biome lies the Restinga ecosystem, a coastal environment characterized by marine factors such as high salinity, unstable substrate, and elevated temperatures. These edaphoclimatic conditions may impact the chemical composition of the local plant species. Studies conducted within Rio de Janeiro's Jurubatiba National Park reveal a diverse metabolic landscape featuring significant bioactivities. These findings highlight the importance of such sites for research and development (Rocha *et al.* 2023). However, as the biome, these ecosystems are constantly threatened by deforestation despite the government's conservation efforts. Consequently, the Restingas have endemic species and a rich biochemical diversity in the verge of been forever lost (Lourenço Jr. *et al.* 2021; Valli *et al.* 2018).

The Lauraceae family is one of the most prevalent families in the Restinga ecosystem, which is renowned for its diverse secondary metabolites, including steroids, terpenoids, and alkaloids. This family encompasses over 50 genera and more than 3,000 species, distributed across tropical regions worldwide. In Brazil, over 300 species are recognized, along with more than 200 endemics. The *Persea* genus within this family comprises approximately 200 species, with *P. americana* Mill., commonly known as avocado, being extensively studied. Species like *Persea venosa* Nees & Mart., commonly referred to as 'pau-de-andrade' or 'canela-sebo' have been utilized in traditional medicine due to their medicinal properties (Esteves *et al.* 2023).

Essential oils are complex mixtures produced by plants' secondary metabolism extracted through hydrodistillation from various plant parts. Typically consists of monoterpenes, sesquiterpenes, or phenylpropanoids. Over 3,000 essential oils have been described so far, with approximately 300 widely utilized in perfume, food, and pharmaceutical industries due to their diverse biological activities. Most essential oils are classified as Generally Recognized as Safe (GRAS) by regulatory agencies, such as the FDA (2017) (Chamorro *et al.* 2012; Awadalla *et al.* 2017; Haro-González *et al.* 2021).

One of the most demanded bioactivities associated with plant derivatives is their antimicrobial property. According to leading health organizations, including the World Health Organization (WHO 2024) and the Centers for Disease Control and Prevention (CDC 2019), bacteria and fungi pose significant threats as infectious agents, toxin producers, and potential food degraders. Conventional methods for preventing microbiological contamination often employ synthetic additives. However, these raised concerns due to their links to health and environmental harm. These toxic substances can alter the physiology of both consumers and non-target animals, potentially leading to oncogenesis, and cause an increasing disturbance in the resistance of infectious agents to these substances (Anand *et al.* 2013; Nasrollahzadeh 2022). Consequently, natural products, among them essential oils, have emerged as promising alternatives to control microbiologic contamination with a lower impact on the environment and human health (Gyawali & Ibrahim 2014; Singh *et al.* 2021).

The bacterial group characterized as ESKAPE (vancomycin-resistant *Enterococcus faecium* (VRE); methicillin-resistant *Staphylococcus aureus*; extended-spectrum β-lactamase (ESBL) and carbapenemase-producing *Klebsiella pneumoniae*; multidrug-resistant *Acinetobacter baumannii*; multidrug-resistant *Pseudomonas aeruginosa*, and ESBL-producing *Enterobacter* spp.) represents

a significant threat in the context of hospital-acquired infections, as it has an elevated level of antimicrobial resistance, commonly resulting from the misuse of widely used antibiotics (De Oliveira *et al.* 2020). There is an increasingly urgent need to discover new antimicrobial agents effective against resistant bacterial strains and this study highlights the Atlantic Forest unique ecosystems as promising sources of novel antimicrobials.

The fungal genus *Aspergillus* presents a strong association with human food supplies, and it is frequently linked to both superficial and invasive infections. Species like *A. flavus* and *A. parasiticus* are particularly concerning as they produce toxins known as aflatoxins, with Aflatoxin B1 (AFB1) being a serious health risk due to its oncogenic effects on liver tissues. This accentuates the need to find bioactive substances capable of reducing those damages without harming the consumers or the environment (Zinedine *et al.* 2019; Frisvad *et al.* 2019).

Although a few studies on the species have been published since 2006 (Linsingen *et al.* 2006), only one study was published in the past five years (Esteves *et al.* 2023), being also the only investigation focusing on specimens from the Restinga ecosystem. Esteves *et al.* (2023) characterized the phytochemical composition of the species under the specific edaphoclimatic conditions of the Restinga, revealing notable differences in essential oil profiles compared to populations from other regions, such as the mountains in the interior of Brazil (Silva *et al.* 2019). While Esteves *et al.* (2023) evaluated the insecticidal activity of the plant, its bioactivity remains largely unexplored, particularly antimicrobial properties that are so important nowadays. The present study, therefore, aimed to determine if the leaf essential oil of *P. venosa* can be an asset in the development of future formulations by characterizing its composition (thus validating its diversity under Restinga-specific edaphoclimatic stressors) and evaluating unprecedented antimicrobial efficacy against clinically and food-relevant pathogens.

This research focus on interconnected challenges caused by humanity, including antimicrobial resistance from the overuse or misuse of synthetic drugs in medical and food sectors, toxicity of these substances, and biodiversity loss. It emphasizes integrating human technology with nature's inherent characteristics as a key mission of this century. By demonstrating *P. venosa*'s bioactivity, the study may frame biodiversity as a reservoir of solutions for global health crises and advocating interdisciplinary strategies that align environmental protection with pharmacological innovation.

Materials and Methods

Plant material

The fresh leaves of *P. venosa* were collected in the Restinga of Jurubatiba National Park, Carapebus, RJ, Brazil (-22°12'42,36984"S, 41°35'20,5954"W). The species was identified, and a voucher specimen was deposited at the herbarium of Universidade Estadual do Rio de Janeiro - Faculdade de Formação de Professores (UERJ-FFP), under registration: M.G.Santos 2336. The collection and research of the plant material were authorized by SisBio/ICMBio (13659-20) and SisGen (A491A56).

Essential oil extraction

Fresh leaves of *P. venosa* (1,246 g) were homogenized with distilled water using a high-speed homogenizer. Then, the mixture was transferred to a 5.0 L round-bottom flask and subjected to hydrodistillation in a Clevenger-type apparatus for 4 h. The essential oil was dried with sodium sulfate anhydrous and stored in an amber glass vial at 4 °C (Esteves *et al.* 2023).

Essential oil chemical characterization

The essential oil was characterized with a semi-quantitative method using a GC-MS QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph equipped with a single quadrupole mass spectrometer. The total chromatographic run time was 75 min, with analytes eluting within a retention time window of up to 38 min. The chromatographic conditions were as follows: an injector temperature of 260 °C, using helium as the carrier gas, a constant flow rate of 1 mL/min, and a split ratio of 1:40. The oven temperature initially started at 60 °C and then increased to 290 °C at a rate of 3 °C/min. The essential oil was dissolved in dichloromethane (1,000 ppm) and injected (1 µL) into a DB-5 column (0.25 mm ID, 30 m in length, 0.25 µm film thickness). The mass spectrometry conditions were 70 eV electron ionization (electron impact) and a scan rate of 1 scan/s in positive mode. The arithmetic index (AI) was calculated by interpolating the retention times of a mixture of aliphatic hydrocarbons (C7-C40, Sigma-Aldrich, USA) under the same chromatographic conditions as the sample. Compounds were considered identified only if the experimental AI agreed with published values (Adams 2007) within ±10 index units and the spectral (40 to 500 *m/z*) similarity in both the Adams and National Institute of Standards and Technology (NIST) libraries was ≥ 90%.

Bacterial strains

Strains were obtained from the Laboratory of Molecular Epidemiology and Biotechnology of Fluminense Federal University (UFF), Brazil. The

bacterial strains comprised *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *E. coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 13883, and *S. aureus* USA300 were stored in Brain Heart Infusion (BHI) broth with 10% of glycerol at -80 °C.

Minimum Inhibitory Concentration (MIC)

Strains were cultivated on Tryptic Soy Agar (TSA) plates and subsequently transferred to a 0.9% NaCl solution, which was then adjusted to a 0.5 McFarland standard. Essential oils, previously solubilized in 1% DMSO, were applied in 96-well plates with BHI broth and underwent a 2-fold serial dilution in concentrations ranging from 2.04 mg/mL to 0.25 mg/mL, with the final addition of the inoculum according to the 0.5 McFarland scale (1.5×10^8 CFU/mL). Positive controls were diluted with vancomycin for Gram-Positive bacteria and ciprofloxacin for Gram-Negative bacteria. The plates were then incubated at 37 °C for 24 h. Following this, the Minimum Inhibitory Concentration (MIC) was determined by adding 20 μ L of resazurin to each well. It is noteworthy that all tests were performed in biological triplicates, following the standard broth microdilution method (CLSI M07-A10), and a control group was realized with DMSO.

Minimum Bactericidal Concentration (MBC)

After MIC incubation, aliquots of 10 μ L from the wells were applied to Petri plates containing TSA, divided into fields corresponding to each concentration of the microdilution, including a positive control. The plates were then incubated for 24 h at 37 °C. MBC endpoint is defined according to the last quadrant without bacterial growth, indicating the elimination of 99.9% of bacteria.

Antifungal assays

The strains used were *A. flavus* RC 2054, *A. parasiticus* NRRL 2999, and *A. westerdijkiae* NRRL

3174, all reference strains known to produce aflatoxin B1 (AFB1). The methodology was adapted from Pinto *et al.* (2023), with the addition of essential oil diluted in DMSO at concentrations of 256, 512, 1,024, and 2,048 μ g/mL in 25 mL of Kasvi's Sabouraud Dextrose Agar (SDA) and then needle-inoculating the strains with 10 μ L of the spore suspension. The plates were incubated at 27.5 ± 2.5 °C for 96 h, with daily observation and diameter measurement. Plates containing SDA medium without essential oil served as the control, as well as plates containing the DMSO in the same concentration as the present in the assays. The experiment was realized in biological triplicate.

Statistical analyses

Data evaluation was performed using Analysis of Variance (ANOVA). Tukey's test was applied with a Confidence Interval of 95% for comparison among different product presentations and concentrations evaluated while considering exposition time variability. The analyses were conducted using the GraphPad Prism (version 8) program.

Result and Discussion

The extraction of the fresh leaves of *P. venosa* yielded 0.10% (w/w) of essential oil with a greenish transparent aspect. The chemical composition revealed 25 identified substances and 8 unidentified ones. The predominant class of compounds was non-oxygenated sesquiterpene (90.23 %), with β -caryophyllene as the major substance (38.59%), followed by α -copaene (7.57%), α -humulene (6.91%), and Germacrene D (6.60%) (Fig. 1). The complete list of substances is shown in Table 1 below.

The chemical potential of *Persea venosa* remains almost unexplored due to the restricted geographic distribution of the species. Nevertheless, two authors have reported the chemical profile of the essential oil derived from the leaves. More recently, Esteves *et al.* (2023) described the presence

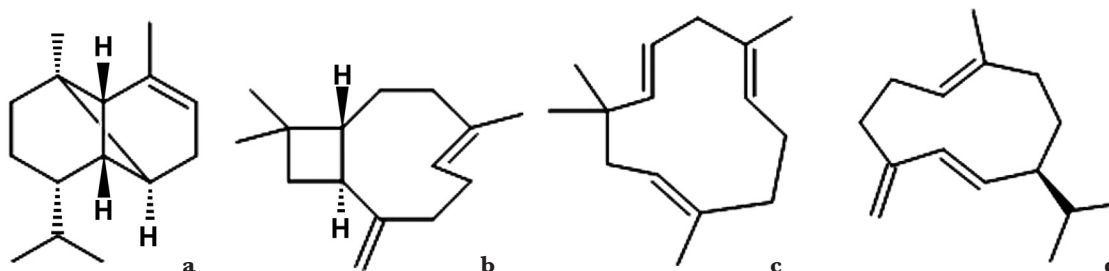


Figure 1 – a-d. Major metabolites of the *Persea venosa* essential oil – a. α -copaene (7.57%); b. β -caryophyllene (38.59%); c. α -humulene (6.91%); d. Germacrene D (6.60%).

Table 1 – Chemical profile of the essential oil from leaves of *Persea venosa* by GC-MS.

	Rt	AI _{Rep}	AI _{Lit}	Substances	Relative abundance (%)
1	8.427	1026.87	1026	1,8-cineole	0.31
2	22.031	1362.05	1373	α -ylangene	0.47
3	22.294	1368.32	1374	α -copaene	7.57
4	22.896	1382.66	1389	β -elemene	1.51
5	24.095	1411.65	1417	β -caryophyllene	38.59
6	24.528	1422.37	-	N.I.	1.05
7	24.751	1427.88	1437	α -guaiene	0.51
8	24.850	1430.33	1439	aromadendrene	0.62
9	25.339	1442.43	1449	amorpha-4,11-diene	0.57
10	25.526	1447.06	1452	α -humulene	6.91
11	25.698	1451.31	1458	<i>allo</i> -aromadendrene	4.97
12	26.350	1467.44	1478	γ -muurolene	3.87
13	26.574	1472.98	1480	germacrene D	6.60
14	26.693	1475.93	-	N.I.	0.50
15	26.884	1480.65	1489	β -selinene	0.98
16	26.992	1483.33	1495	γ -amorphene	1.48
17	27.146	1487.14	1500	bicyclogermacrene	3.08
18	27.318	1491.39	1500	α -muurolene	1.70
19	27.425	1494.04	1509	α -bulnesene	0.82
20	27.748	1502.12	-	N.I.	0.78
21	27.875	1505.40	1513	γ -cadinene	1.60
22	28.084	1510.79	1522	δ -cadinene	3.99
23	28.218	1514.25	1528	<i>cis</i> -calamene	1.25
24	28.800	1529.28	1537	α -cadinene	0.82
25	28.973	1533.75	1545	selina-3,7(11)-diene	0.65
26	29.590	1549.68	1559	germacrene B	1.67
27	30.336	1568.94	-	N.I.	0.78
28	30.495	1573.04	1582	caryophyllene oxide	3.46
29	30.684	1577.92	-	N.I.	0.41
30	32.183	1617.48	-	N.I.	0.56
31	32.832	1635.09	1638	<i>epi</i> - α -cadinol	0.16
32	33.326	1648.49	-	N.I.	0.98
33	37.827	1773.91	-	N.I.	0.77
Total identified substances					94.16
Non-oxygenated monoterpene					0.31
Non-oxygenated sesquiterpene					90.23
Oxygenated sesquiterpenoids					3.62

*N.I. = non-identified; Rt = retention time; AI_{Rep} = arithmetic index reported; AI_{Lit} = arithmetic index from literature (ADAMS 2017).

of β -caryophyllene (43.78%), α -humulene (8.27%), caryophyllene oxide (8.92%), and α -copaene (5.90%) as the major components, as well as presenting the non-oxygenated sesquiterpene fraction as the most predominant fraction of the essential oil (Esteves *et al.* 2023). Silva *et al.* (2019) reported the major components as spathulenol (27.8%), humulene epoxide II (11.3%), caryophyllene oxide (7.6%), cadalene (7.1%), and α -copaene (5.5%) (Silva *et al.* 2019).

The predominant fraction (oxygenated sesquiterpenoids) identified in the essential oil obtained by Silva *et al.* (2019) differs from the major fraction described by Esteves *et al.* (2023) and the one observed in this study (non-oxygenated sesquiterpenes). Esteves *et al.* (2023) and the current study were collected in a coastal Restinga region in Rio de Janeiro state (Brazil) and had the same extraction methodology, justifying the similar results observed. Silva *et al.* (2019), however, collected their material in the mountains of the state of Minas Gerais (Brazil) and, even though the method was also the same, the environmental pressures and geographical conditions of each collection region may explain the difference between the major compounds (Verma & Shukla 2015). Abiotic stresses characteristic of Restinga ecosystems, such as elevated temperatures, saline soils, and periodic water deficit, have been shown to induce β -caryophyllene accumulation in *Persea* spp. and related Lauraceae species under analogous conditions (Ceballos *et al.* 2019; Han *et al.* 2022; Manasathien *et al.* 2025). This adaptive response underscores the ecological and chemotaxonomic significance of β -caryophyllene in plant species in harsh edaphoclimatic environments.

The β -caryophyllene, the predominant substance identified, is approved by the U.S. Food and Drug Administration (FDA) for direct use in foods intended for human consumption. Although the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) indicates that β -caryophyllene may be fatal if swallowed or aspirated and that it has the potential to cause allergic skin reactions, eye irritation, or corrosion, recent toxicological evaluations demonstrate consistently, both *in vitro* and *in vivo*, that β -caryophyllene exhibits low toxicity. So, the Organization for Economic Co-operation and Development (OECD) classifies it as a Category 5 substance, indicating toxicity only at doses exceeding 2,000 mg/kg. Therefore, despite some potential toxic effects, the concentrations of β -caryophyllene commercially used are safe for humans (Francomano *et al.* 2019; PubChem 2025; ADMETLab 3.0 2025).

The *P. venosa* essential oil showed a minimum inhibitory concentration (MIC) of 2,048 μ g/mL in *S. aureus*, *E. coli*, *E. cloacae*, and *K. pneumoniae*. While

in *S. epidermidis* and *P. aeruginosa* the MIC was 1,024 μ g/mL. In addition, the essential oil didn't show a minimum bacterial concentration (MBC) at the tested concentration, except for *S. epidermidis*, which exhibited an MBC of 2,048 μ g/mL. The inhibition profile is presented in Table 2.

Several studies investigating the antimicrobial potential of essential oils derived from various *Persea* species have reported significant inhibitory effects. For example, essential oils obtained from *P. odorantissima*, *P. gamblei*, and *P. duthiei* have demonstrated activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* serovar Enterica, and *Pasteurella multocida*, with minimum inhibitory concentration (MIC) values ranging from 3.9 to 7.81 μ L/mL (Joshi *et al.* 2010). Nasri *et al.* (2022) similarly evaluated the antimicrobial activity of *Persea americana* essential oil against *S. aureus* and *S. epidermidis*, reporting minimum inhibitory concentration (MIC) values between 1 and 10 μ g/mL (Nasri *et al.* 2022). Assuming an approximate oil density of 0.90 g/mL (*P. americana*), these values align with the results obtained in the present study.

Overall, the present study findings are consistent with the literature on the antibacterial effect of essential oils, which usually exhibit MIC values in the range of 1,000 μ g/mL (Tegos *et al.* 2002). The sesquiterpenes present in the *P. venosa* essential oil may interact with the bacterial membrane. At low concentrations, the amount of the compounds is insufficient to induce alterations in membrane structure, allowing bacterial maintenance and membrane integrity. In contrast, at elevated concentrations of essential oil, the monoterpenes, especially the oxygenated ones, due to their intermediate polarity, may reach a state of saturation within the bilayer. This saturation results in lipid disorganization, which consequently increases membrane permeability and can eventually lead to disruption of the bacterial membrane (Nazzaro *et al.* 2013; Qie *et al.* 2025). Although antibacterial activity was observed, the high concentration required to achieve efficacy poses a challenge to the therapeutic applicability.

No fungistatic or fungicidal activity of the *P. venosa* essential oil was observed against the *Aspergillus* strains at concentrations greater than 2,048 μ g/mL. The result aligns with the observations of Tan (2022), with many essential oils rich in β -caryophyllene often displaying a MIC much higher than the maximum of 2,048 μ g/mL tested (Tan *et al.* 2022). Other studies, however, report inhibition of *Aspergillus*, as the previous work by Nouara (2024) demonstrated that *P. americana* essential oil inhibited mycelial growth of 43.29–53.12% at 500 μ g/mL against *A. carbonarius*, *A. flavus*, *A. ochraceus*, and *A. terreus* (Nouara & Nouara 2024).

Table 2 – Minimal inhibitory, bactericidal, and fungicidal concentration from *Persea venosa*'s essential oil in strains of clinical interest.

<i>Persea venosa</i> inhibition (µg/mL)		
Strain	MIC	MBC/MFC
<i>Staphylococcus aureus</i> (ATCC 25923)	2,048	> 2,048
<i>Staphylococcus aureus</i> (USA300)	2,048	> 2,048
<i>Staphylococcus epidermidis</i> (ATCC 122282)	1,024	2,048
<i>Escherichia coli</i> (ATCC 25922)	2,048	> 2,048
<i>Enterobacter cloacae</i> (ATCC 13047)	2,048	> 2,048
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	1,024	> 2,048
<i>Klebsiella pneumoniae</i> (ATCC 13883)	2,048	> 2,048
<i>Aspergillus flavus</i> (RC 2054)	> 2,048	> 2,048
<i>Aspergillus parasiticus</i> (NRRL 2999)	> 2,048	> 2,048
<i>Aspergillus westerdijkiae</i> (NRRL 3174)	> 2,048	> 2,048

MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration; MFC = Minimum Fungicidal Concentration.

Da Silva *et al.* (2024) also reported antifungal activity against *A. parasiticus* and *A. flavus* using pure β -caryophyllene, the major substance present in the *P. venosa* essential oil. The pure substance at a 1,024 µg/mL concentration reduced the colonies by 25–30% for 72 h. Other works suggest a modest fungistatic effect from β -caryophyllene being related to its ability to interfere with cell membrane synthesis or integrity through partial inhibition of ergosterol-related metabolic pathways or by modulating oxidative stress. However, based on the results of the present study, the concentration of β -caryophyllene in *P. venosa* essential oil may not be sufficient to produce a significant antifungal effect (Tan *et al.* 2022).

The absence of antifungal activity may result from challenges in penetrating the fungal cell wall or interacting with intracellular targets, such as ergosterol, a crucial component of the fungal cell membrane. While other substances of the essential oil have been reported to exhibit antifungal activity (even though low to moderate), the result in the current study also excludes the possibility of synergistic interactions among these constituents against *A. flavus* and *A. parasiticus*. The findings suggest that the antifungal activity of the essential oil components is highly concentration-dependent and differs significantly between plant species (Tan *et al.* 2022).

Further optimization of *P. venosa* essential oil as a raw material through diverse formulation approaches could enhance its antimicrobial potential for practical applications. Evaluating its synergistic interactions with established antibiotics or antifungals may also demonstrate improved

efficacy. Consequently, the essential oil could be developed as an adjuvant or active principle for antimicrobial agents in clinical settings, food preservation, or agricultural pesticides.

The *Persea venosa* showed β -caryophyllene as the major compound of the essential oil from its leaves. Consistent with existing literature, the essential oil inhibits clinically relevant bacterial strains only at pharmacologically challenging concentrations (> 1,000 µg/mL), and no activity was observed against the *Aspergillus* species. Nevertheless, this study constitutes the first report of antimicrobial properties for *P. venosa*, a Brazilian endemic species native to a unique and threatened ecosystem. The direct application of the crude essential oil may be limited; however, its potential for formulation development, with or without synergistic interactions with established antimicrobials, warrants further investigation. Thus, this study reinforces the *P. venosa* as a promising source for novel antimicrobial agents and the restinga as a reservoir of possible eco-friendly solutions.

Acknowledgements

We would like to thank the National Council for Scientific and Technological Development (CNPq), for financial support through projects 304179/2021-3 and Biodiversity and Sustainability Research Network: Formulation of Biopesticides from Brazilian Flora (406399/2022-0). We would also like to thank FAPERJ, for its support in financing the projects E-26/200.161/2025, E-26/200.675/2021 (262222), E-26/200.162/2025 (304867), E-26/210.598/2023 (285944), and E-26/200.915/2022 (268191).

Data availability statement

In accordance with Open Science communication practices, the authors inform that there is no data sharing of this manuscript.

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