SVOA Microbiology

ISSN: 2634-534X

ScienceVolks Open Access

Research Article

Antibacterial Potential of the Essential Oil of *Myrciaria pilosa* Sobral & Couto (Myrtaceae) Against Gram-Negative Pathogenic Bacteria

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https://doi.org/10.58624/SVOAMB.2025.06.022

Received: November 10, 2025

Published: November 26, 2025

Citation: da Cruz RP, Almeida-Bezerra JW, Mota ML, Martins IM, Pereira-de-Morais L, Gondim CNFL, Filho AM, Bento AJM, da Silva NC, da Silva MP, Costa AR, Gomes HMS, Sa LS, de Matos YMLS, Sampaio NFL, Correia LM, Souza WFS, Grangeiro AALC, Gonçalo MABF, da Costa JGM, Rodrigues FFG. Antibacterial Potential of the Essential Oil of Myrciaria pilosa Sobral & Couto (Myrtaceae) Against Gram-Negative Pathogenic Bacteria. *SVOA Microbiology 2025,* 6:6, 197-203. doi:10.58624/SVOAMB.2025.06.022

Abstract

Bacterial resistance to conventional antibiotics represents a serious threat to public health, encouraging research into plant-derived therapeutic alternatives. In this context, the present study evaluated the antibacterial activity of the essential oil extracted from fresh and dried leaves of *Myrciaria pilosa* against Gram-negative strains. The oil was obtained by hydrodistillation, and the Minimum Inhibitory Concentration (MIC) was determined using the broth microdilution method following standardized protocols. The results demonstrated significant activity against most tested strains. Notably, *Proteus vulgaris* proved to be highly sensitive (MIC = 16 µg/mL). The oil obtained from dried leaves exhibited higher potency, reducing the MIC for *Klebsiella pneumoniae* (64 µg/mL) and *Pseudomonas aeruginosa* (16 µg/mL). Thus, the essential oil of *M. pilosa* shows relevant antibacterial potential, particularly when obtained from dried leaves, and stands out as a promising candidate for future studies aiming at the development of drugs against infections caused by resistant Gram-negative bacteria.

Keywords: *Terpenes, Cambuí, Caatinga, Microbial Resistance.*

1. Introduction

The Caatinga is a type of seasonally dry tropical forest that extends across all states of Northeastern Brazil and into Northern Minas Gerais, occupying an area that corresponds to approximately 11% of the national territory. This phytogeographic domain is characterized by irregular rainfall and long dry seasons throughout most of the year. Therefore, sustainable strategies are necessary to promote socioeconomic development and to enhance the value of this region, consequently ensuring the conservation of its vast biodiversity, represented by unique fauna and flora [1,2,3].

In the northern portion of the Caatinga lies the Chapada do Araripe, considered an important area for the conservation of the natural resources of this phytogeographic domain. It is estimated that this region harbors around 173 native plant species with biological potential and significant medicinal, cultural, nutritional, and economic value [4,5,6,7].

Extractivism is highly prevalent in this region, since native fruit-bearing species generate employment and income for local populations living around the Chapada do Araripe. Some of the most important species for extractive purposes include *Caryocar coriaceum* Wittm (pequi) [8], *Passiflora laurifolia* L. (maracujá peroba) [9], *Himatanthus drasticus* (Mart.) Plumel (janaguba) [10], and *Dimorphandra gardneriana* Tul. (faveira) [11].

Another example of an extractive resource from the Chapada do Araripe is the species *Myrciaria pilosa* Sobral & Couto (Myrtaceae), an endemic Brazilian plant popularly known as "cambuí" (Figure 1). Its fruits are edible globose berries with a juicy pulp that turn black to purplish when ripe [12,13]. The characteristic citrus-like flavor is highly appreciated by the local population, making the fruit a target for extractivists either for fresh consumption or for the production of wine, liqueur, cachaça, sweets, jams, flour, popsicles, ice cream, and other byproducts marketed in organic fairs throughout the Cariri region.

Figure 1. *Myrciaria pilosa Sobral & Couto (Myrtaceae) in an area of the Chapada do Araripe – CE.*

In folk medicine, the leaves of *Myrciaria pilosa* are traditionally used in the form of teas for the treatment of diabetes, diarrhea, cramps, edema, and stomach pain [14,15]. It has been scientifically demonstrated that the vegetative parts of *M. pilosa* produce, through their secondary metabolism, an essential oil rich in bioactive compounds, such as the sesquiterpenes guaiol and (E)-β-caryophyllene [16]. Essential oils are promising volatile substances that exhibit important biological and pharmacological properties, including insecticidal, anti-inflammatory, cytotoxic, antioxidant, and antimicrobial activities [17,18,19].

The search for natural products with antimicrobial activity has increased over the years. The indiscriminate use of conventional drugs in the treatment of infections has led to the emergence of resistance mechanisms in several types of microorganisms. The survival and spread of pathogenic strains represent a major challenge for public health, as they increase hospital costs and mortality rates [20,21,22].

In this sense, the identification of molecules with bioactive potential is of utmost importance, as they may contribute to the development of new drugs and help combat resistant strains. From a biological standpoint, *M. pilosa* remains underutilized, as little is known about its pharmacological applications and benefits. Based on these premises, the objective of this study was to investigate the antibacterial potential of the essential oil from *M. pilosa* leaves against pathogenic Gram-negative strains.

2. Materials and Methods

2.1 Plant material collection

Fresh and healthy leaves of *Myrciaria pilosa* were collected in the Chapada do Araripe (coordinates: 7°33'29.2" S, 39° 18'22.1" W), in the municipality of Jardim, Ceará, Brazil. Additionally, flowering branches were pressed, dehydrated, and incorporated as a voucher specimen in the Herbarium Caririense Dárdano de Andrade-Lima (HCDAL) at the Regional University of Cariri (URCA), where it was deposited under record number #17.027.

The botanical material was registered in accordance with Brazilian regulations, obtaining a registration in SisGen (National System for the Management of Genetic Heritage and Associated Traditional Knowledge) under number A81DD6E, and authorization in SISBIO/ICMBio (Biodiversity Authorization and Information System) under process 82348-1.

2.2 Essential oil extraction

The essential oil was obtained by hydrodistillation, adapting the methodology described by Matos [23]. For each extraction, 200 g of plant material (fresh or dried) were placed in a glass flask containing 2 L of distilled water. The system was heated to boiling and maintained for 2 hours. The organic phase containing the essential oil was separated from the aqueous phase using a Clevenger-type condenser. The oil was collected with a glass Pasteur pipette and immediately stored in amber vials under refrigeration (-10°C) until analysis, as a measure to preserve its chemical integrity.

2.3 Antibacterial activity

2.3.1 Bacterial strains, reagents, and preparation of solutions

The following Gram-negative bacterial strains were used for minimum inhibitory concentration (MIC) testing: *Pseudomonas aeruginosa* ATCC 00027, *Proteus vulgaris* ATCC 13315, *Campylobacter jejuni* ATCC 33560, and *Klebsiella pneumoniae* ATCC 4352. All microorganisms were obtained from the National Institute for Quality Control in Health (INCQS) of the Oswaldo Cruz Foundation – Fiocruz, Ministry of Health.

The strains were initially cultured on Heart Infusion Agar (HIA) and incubated at 37°C for 24 hours in a bacteriological incubator at the Laboratory of Natural Products Research (LPPN/URCA). After the growth period, bacterial suspensions were transferred to sterile test tubes, where the inoculum was standardized to 1×10^8 CFU/mL, corresponding to 0.5 on the McFarland scale (measured by nephelometric turbidimetry). During the experiments, the strains were grown in Brain Heart Infusion (BHI) broth at 10%, ensuring optimal conditions for bacterial development.

The essential oil obtained from fresh and dried leaves of *M. pilosa* was initially weighed (10 mg) and subsequently diluted in dimethyl sulfoxide (DMSO) and sterile distilled water to a final concentration of 1,024 µg/mL. In parallel, a sodium resazurin solution (Sigma-Aldrich®) was prepared by dissolving 2 mg of the indicator in 50 mL of sterile distilled water. The solution was stored at 4°C in the dark to preserve its redox properties.

2.3.2 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined by the broth microdilution method. Initially, wells of a microtiter plate were filled with 900 µL of BHI broth (10%) and 100 µL of the standardized bacterial suspension (1×10^8 CFU/mL). Subsequently, twofold serial dilutions of *M. pilosa* essential oil were performed by transferring 100 µL of the stock solution to the first well and proceeding with successive dilutions up to the penultimate well, obtaining a final concentration range from 512 to 8 µg/mL. The last well was reserved as a positive growth control (medium + inoculum). The plates were incubated at 37°C for 24 hours, and afterward, 20 µL of resazurin solution was added to each well as a redox indicator by color change. After 1 hour, visual readings were performed, and the MIC was defined as the lowest concentration that inhibited bacterial growth [24]. All assays were carried out in triplicate, and the mean values with their respective standard deviations (±SD) were calculated.

3. Results

According to Table 01, both essential oils of *Myrciaria pilosa* exhibited antibacterial activity against pathogenic Gram-negative strains at clinically relevant concentrations [25], which showed an MIC higher than the maximum concentration evaluated in this in vitro study. It is noteworthy that the essential oil extracted from dried leaves displayed lower MIC values compared to fresh leaves for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Among the tested strains, *Proteus vulgaris* was the most susceptible, with an MIC of 16 µg/mL.

Table 1. Minimum inhibitory concentration (MIC) of the essential oil from fresh leaves (EOFLMP) and dried leaves (EODLMP) of Myrciaria pilosa against bacterial strains.

Strains	OEFLMP	OEDLMP
Campylobacter jejuni ATCC 33560	256 µg/mL	256 µg/mL
Klebsiella pneumoniae ATCC 4352	128 µg/mL	64 µg/mL
Pseudomonas aeruginosa ATCC 00027	32 µg/mL	16 µg/mL
Proteus vulgaris ATCC 13315	16 µg/mL	16 µg/mL

4. Discussions

Species of the genus *Myrciaria* are recognized for their richness in essential oils with biological properties [26], which, due to the presence of terpenes, the main metabolites of the Myrtaceae family, have broad applicability in the pharmaceutical, food, and cosmetic industries [27]. Thus, the growing interest in biological studies of species of this genus highlights the potential of this taxon as a source of bioactive molecules for the development of new natural products and innovative therapies [28].

The essential oil of *M. pilosa*, in addition to showing antibacterial activity against Gram-negative strains, also demonstrated significant effects against the Gram-positive bacterium *Staphylococcus aureus*, both in the standard strain (ATCC 6538) and in clinical isolates (UFPEDA 679 and UFPEDA 683). For all tested strains, the observed MIC was 5 µg/mL, a result that indicates higher efficacy compared to the drug chloramphenicol, whose MIC ranged from 10, 40, and 60 µg/mL, respectively [16].

Beyond its direct antibacterial activity against *S. aureus*, the essential oil of this aromatic species also exhibits antivirulence properties [16]. Among the inhibited virulence factors, hemolysin stands out, a cytotoxin produced by *S. aureus* that, under physiological conditions, induces the lysis of red blood cells. This process is fun-damental for the bacterium, as it releases essential nutrients, such as iron, which support pathogen growth and dissemination within the host. Moreover, hemolytic activity contributes to immune evasion, allowing *S. aureus* to establish persistent and difficult-to-treat infections [29]. The inhibition of hemolysin production by the natural product, even at sub-inhibitory concentrations, reveals a differentiated mechanism of action capable of attenuating virulence without directly compromising bacterial viability. This opens the possibility for the development of compounds that act as virulence modulators, reducing the risk of resistance and increasing the effectiveness of combined therapies.

Another virulence factor inhibited by *M. pilosa* essential oil is the production of staphyloxanthin, a golden carotenoid pigment typical of *S. aureus* [16]. This pigment neutralizes reactive oxygen species (ROS), protecting the microorganism from oxidative stress generated by the host's immune system, enabling survival against immune cells such as neutrophils and macrophages. In addition, it contributes to resistance to phagocytosis, thereby enhancing the pathogen's ability to cause persistent infections [30].

The essential oil of *M. pilosa* also showed relevant effects against bacterial biofilms, complex structures that provide bacteria with protection from the host's immune system and antimicrobial treatments [13,31]. The oil was able to inhibit the formation of new biofilms, preventing bacterial organization and surface adhesion, a crucial step for the persistence of chronic infections. Moreover, it demonstrated activity against pre-established biofilms, moderately disrupting the extracellular matrix and destabilizing bacterial colonies, which may facilitate antibiotic penetration and enhance therapeutic efficacy. This effect is particularly relevant in combating multidrug-resistant strains that frequently use biofilms as a defense mechanism, making the essential oil a promising candidate for combined infection-control strategies [13].

In addition to in vitro activities, Cruz et al. [13] demonstrated that the essential oil of *M. pilosa* also exhibits antibacterial activity and enhances the effects of antibiotics in vivo using the *Danio rerio* (zebrafish) model. These results reinforce the pharmacological potential of the essential oil, showing that its antibacterial effects go beyond in vitro assays, with significant activity in complex biological models.

The antibacterial activity observed for *M. pilosa* essential oil may be associated with multiple mechanisms of action on the bacterial membrane of Gram-negative strains. Hydrophobic compounds present in the oil, such as monoterpenes and sesquiterpenes, may insert into the lipopolysaccharide outer layer, altering its fluidity and organization, which leads to pore formation and increased permeability, resulting in the loss of ions and essential molecules. Furthermore, this interaction may disrupt membrane potential and ionic gradients, impairing vital processes such as cellular respiration and ATP synthesis. In parallel, the oil may induce oxidative stress by promoting the generation of reactive oxygen species that damage lipids, proteins, and nucleic acids, while also interfering with protein and nucleic acid synthesis, ultimately compromising bacterial replication and translation [32,33].

5. Conclusion

The findings of this study reinforce the therapeutic potential of *M. pilosa* essential oils as antimicrobial agents against pathogenic Gram-negative strains, highlighting that variations in leaf processing can influence their effectiveness. The greater susceptibility observed in *Proteus vulgaris* suggests that compounds present in these oils may be explored in the development of innovative antimicrobial strategies, contributing to alternatives against bacterial resistance and expanding the knowledge of the pharmacological value of this species.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The Regional University of Cariri (URCA).

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