









# Prospecting the antibacterial activity of *Vachellia farnesiana* (L.) against strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

## Prospecção da atividade antibacteriana de *Vachellia farnesiana* (L.) contra cepas de *Staphylococcus aureus* e *Pseudomonas aeruginosa*

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### Abstract

**Objective:** this study aimed to determine the antibacterial potential of the ethanolic extract of *Vachellia farnesiana* against strains of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* (ATCC 10145). **Method:** to analyze the antimicrobial activity, Antimicrobial Susceptibility Tests (AST) were performed using crude ethanolic extracts from the flowers, branches, roots, and pods of the plant *V. farnesiana* (L.) Wight & Arn. For these tests, a broth microdilution method was used to determine the minimum inhibitory concentration (MIC), and plating was employed to assess the minimum bactericidal concentration (MBC). **Results:** the growth reduction results were statistically significant for both strains compared to the control, especially for the roots' ethanolic extract. However, *S. aureus* was more sensitive than *P. aeruginosa*. The antimicrobial effect shown by the crude extract was bacteriostatic. **Conclusion:** thus, given the antibacterial potential *V. farnesiana* demonstrated, it is necessary to determine which biological compost of the extract has bacteriostatic activity and, in addition, to expand the research against other bacterial species of medical interest.

**Keywords:** antimicrobial susceptibility testing; biological activity; yellow acacia; ethanolic extract.

### Resumo

**Objetivo:** determinar o potencial antibacteriano do extrato etanólico de *Vachellia farnesiana* contra cepas de *Staphylococcus aureus* (ATCC 25923) e *Pseudomonas aeruginosa* (ATCC 10145). **Método:** para isto, foram realizados Testes de Sensibilidade a Antimicrobianos (TSA) usando extratos etanólicos brutos das flores, ramos, raízes e vagens de *V. farnesiana* (L.) Wight & Arn. Utilizamos o método de microdiluição em caldo, para determinar a concentração inibitória mínima (CIM), e o método de plaqueamento, para avaliar a concentração bactericida mínima (CBM). **Resultados:** os resultados de redução de crescimento microbiano foram estatisticamente significativos para ambas as cepas em comparação com o controle, destacando-se o extrato etanólico das raízes. A cepa de *S. aureus* mostrou-se mais sensível quando comparada à *P. aeruginosa*; e o efeito antimicrobiano observado foi bacteriostático. **Conclusão:** tendo em conta a atividade antibacteriana de *V. farnesiana* demonstrada neste trabalho torna-se necessário determinar qual composto biológico do extrato etanólico apresenta ação bacteriostática, além disso, recomendamos ampliar as pesquisas contra outras espécies bacterianas de interesse médico.

**Palavras-chave:** teste de sensibilidade a antimicrobianos; atividade biológica, acácia amarela; extrato etanólico.

### INTRODUCTION

The use of plants for therapeutic purposes has been present in various nations since ancient times. The search for new drugs has grown significantly with the advent of new technologies, which have improved techniques for extracting medicinal compounds from plants. Therefore, extracts have been obtained from plants and used mainly to investigate their biological effects in scientific research. These extracts are natural volatile fractions in various anatomical portions of plants, such as bark, leaves, stems, roots, fruits, and flowers<sup>1,2</sup>.

From this perspective, several studies on medicinal plants with antibacterial activity have been conducted worldwide. Brazil has one of the greatest biodiversities on the planet, which has

considerably boosted research in the health area. The country plays a crucial role in the development of new antimicrobials<sup>3,4</sup> and contributes to the appreciation of the region and local flora. In this context, *Vachellia farnesiana* (L.), commonly known as acacia, is of particular interest due to its bioactive, phenolic, and flavonoid compounds that may contribute to the development of new antimicrobials. It belongs to the Fabaceae family, the third largest family of angiosperms, comprising 751 genera and approximately 19,500 species. These plants have a global distribution and are found in temperate, tropical, arid, and savannah biomes<sup>5</sup>. In folk medicine, *V. farnesiana* is traditionally used to treat various infectious conditions, such as gastrointestinal injuries, wounds, cough, and typhoid fever<sup>6</sup>.

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## 2 Antibacterial activity of *Vachellia farnesiana* (L.)

The World Health Organization has updated its list of priority pathogens for antimicrobial research<sup>7</sup>. Gram-positive and Gram-negative bacterial species comprise this list of microorganisms of concern, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are commonly found as pathogens on human skin and mucous membranes and are frequently associated with a wide range of infections, from localized cases to life-threatening systemic infections<sup>8,9</sup>.

The increase of antibiotic-resistant bacteria has become increasingly concerning as microorganisms are becoming adapted to current drugs. Consequently, there is a growing demand for developing new therapeutic agents to combat and control these pathogens. Thus, this study aims to investigate new therapeutic strategies against infections caused by *S. aureus* and *P. aeruginosa* by exploring the antibacterial potential of the ethanolic extract of *V. farnesiana* and contributing to the valorization and use of local biological resources.

### MATERIAL AND METHOD

#### Collection and obtaining of ethanol extract

The plant sample collection and herbarization procedures were conducted in accordance with the protocol described by Cartaxo et al. (2010)<sup>10</sup>. Specimens of *V. farnesiana* (L.) Wight & Arn were collected in the municipality of Tauá, Ceará, Brazil (S 5.997624, W 40.299998), located in the Inhamuns Region. The collection to acquire botanical material was authorized under permit number 29145-3 issued by the Biodiversity Authorization and Information System (SISBIO). The specimen was duly identified and deposited in the Prisco Bezerra Herbarium of the Federal University of Ceará through exsiccate n°. 56045.

To obtain crude ethanol extracts from the flowers, branches, pods, and roots of *V. farnesiana* were prepared following the methodology described by Turnes et al. (2014)<sup>11</sup>. Briefly, commercial ethanol (96° GL) was used as an organic solvent for cold extraction and simple filtrations. Then, the samples were kept in a water bath (50±2°C) for total evaporation of the solvent.

#### Bacterial strains and inoculum adjustment

Standard strains of *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 10145) were selected for this study. The isolates were stored at -80 °C in Trypticase Soy Broth (TSB; Himedia, India) supplemented with glycerol as a cryoprotectant. For microbial reactivation, the strains were cultured on Trypticase Soy Agar medium (TSA; Himedia, India) and incubated at 37 °C for 24 h. Subsequently, isolated colonies were transferred to 5 mL of TSB broth under constant agitation, maintaining the same time

and temperature conditions. Finally, the bacterial suspension was adjusted to 2 x 10<sup>6</sup> CFU/mL with a spectrophotometer aid (Sunrise-Tecam) at 620 nm according to the standard growth curve previously performed for each strain<sup>12</sup>.

#### Evaluation of Antimicrobial Activity

The antibacterial activity was performed by the microdilution method in sterile 96-well plates, as recommended by CLSI-2003, with adaptations. Aliquots of 100 µL of the adjusted bacterial inoculum were inoculated with 100 µL of crude extracts at concentrations ranging from 50 mg/mL to 0.39 mg/mL. The growth conditions were the same as previously mentioned. For positive control, 100 µg/mL ampicillin was used, and TSB was used as a negative control. The experiments were performed in triplicate.

Growth inhibition was assessed by reading the optical density at 620 nm. From the wells with a reduction in turbidity, 10 µL was removed for plating onto TSA and incubated at 37 °C for 24 h to determine the Minimum Bactericidal Concentration (MBC). After incubation, microbial growth was observed on the agar surface and counted manually.

#### Statistical Analysis

The data were compared using one-way statistical analysis of variance (ANOVA) with Bonferroni post-test in the GraphPad Prism® software, version 5.0 for Microsoft Windows®. Significant results were considered when  $p < 0.05$ .

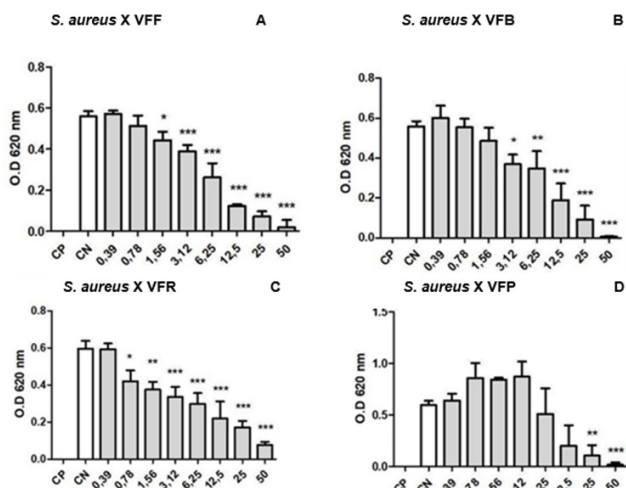
### RESULTS

The crude ethanolic extracts of the flowers, branches, pods, and roots of *V. farnesiana* showed a significant reduction in the microbial growth of *S. aureus* (ATCC 25923) when compared to the negative control, as shown in Figures 1A, 1B, 1C, and 1D. The extracts that exhibited the highest and lowest statistical significance of inhibitory activity at all concentrations tested when compared to the negative control were the root and pod, respectively.

The crude ethanolic extract of the root showed a reduction in the growth of *S. aureus* up to a dilution of 0.78 mg/mL, proving to be superior to the other extracts (Fig. 1C). The crude extracts of the flowers and branches showed significant inhibition results from the dilution of 1.56 mg/mL (Fig. 1A) and 3.12 mg/mL (Fig. 1B), respectively. The pod also obtained a significant result compared to the negative control, but only at higher concentrations (25 mg/mL and 50 mg/mL), as shown in Figure 1D.

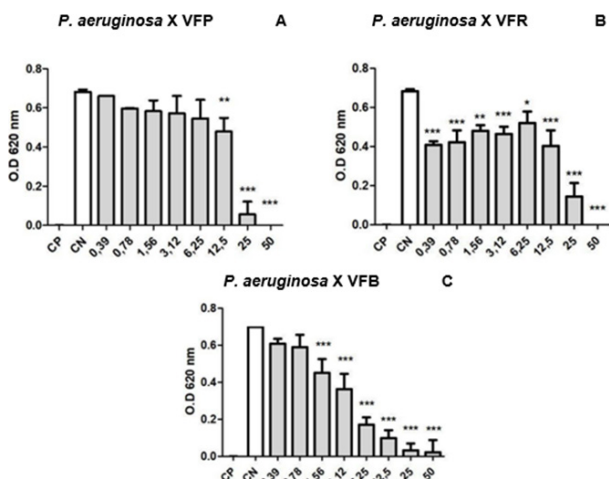
### 3 Antibacterial activity of *Vachellia farnesiana* (L.)

**Figure 1.** (A) Effect of *V. farnesiana* Flowers – VFF; (B) Effect of *V. farnesiana* Branches – VFB; (C) Effect of *V. farnesiana* Roots – VFR, and (D) Effect of *V. farnesiana* Pod – VFP on the planktonic growth of *S. aureus* (ATCC 25923). Data represent mean  $\pm$  standard deviation and do not differ significantly at 5% probability by the Bonferroni test.



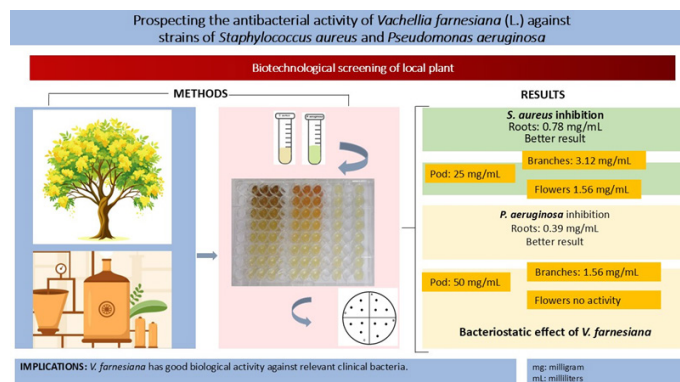
The evaluation of the antimicrobial activity against *P. aeruginosa* (ATCC 10145) showed significant results for the crude ethanolic extracts of the pods, roots, and branches of the plant *V. farnesiana*. There was no reduction in microbial growth when we used the crude extract of the flowers, as shown in Figures 2A, 2B, and 2C. In the pod fraction, a significant reduction in the *P. aeruginosa* growth was observed at 50 mg/mL (Fig. 2A). The root extract showed the best performance in reducing bacterial growth at all concentrations (Fig. 2B), and the crude ethanolic extract of the branches showed the second-best result with inhibitions starting at 1.56 mg/mL (Fig. 2C).

**Figure 2.** (A) Effect of *V. farnesiana* Pod – VFP; (B) Effect of *V. farnesiana* Roots – VFR, and (C) Effect of *V. farnesiana* Branches – VFB on the planktonic growth of *P. aeruginosa* (ATCC 10145). Data represents standard deviation and does not differ significantly at 5% probability by the Bonferroni test.



In the samples that showed turbidity reduction, we performed plating. For both strains, there was colony growth, suggesting only a bacteriostatic effect. The results are summarized in Figure 3.

**Figure 3.** Summary of results.



### DISCUSSION

Our results demonstrated that the tested extracts moderately inhibited the planktonic growth of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 10145; however, lower concentrations exhibited only weak inhibition. These findings are relevant given the high clinical importance of *S. aureus* and *P. aeruginosa*, which are extremely pathogenic and capable of causing a wide range of acute and chronic infections. Additionally, these bacteria spread easily and are frequently associated with high levels of antimicrobial resistance<sup>13,14</sup>.

In this context, the search for new compounds effective against drug-resistant microorganisms is of great importance. Therefore, ethanolic plant extracts, such as those of *V. farnesiana*, may represent a promising strategy for controlling infections caused by *S. aureus* and *P. aeruginosa*.

Several parts of *V. farnesiana* showed a significant reduction in *S. aureus* ATCC 25923 growth in the broth microdilution assay, although continuous growth on agar plates indicated a bacteriostatic effect of the analyzed extracts. Similarly, a study conducted by Hernández et al. (2009) in Cuba evaluated the ethanolic root extract of *V. farnesiana* against several bacterial and fungal strains<sup>15</sup>. Their results showed inhibition of *S. aureus* only at 100 mg/mL; however, a bactericidal effect was also reported, partially corroborating our findings, particularly the superior bacteriostatic effect observed in the root extract for both *S. aureus* and *P. aeruginosa*.

Another study by Matanna et al. (2012) demonstrated that the ethanolic extract of *Vachellia* sp. leaves exhibited antibacterial activity against *S. aureus*, with a Minimum Inhibitory Concentration (MIC) of 156  $\mu$ g/mL<sup>16</sup>. Similarly, Bezerra et al. (2025) show an antibacterial activity of *V. farnesiana* leaves at concentration  $\geq$ 512  $\mu$ g/mL and a reduction of MIC in associate to antimicrobials<sup>17</sup>.

#### 4 Antibacterial activity of *Vachellia farnesiana* (L.)

These studies suggest effective inhibition of bacterial growth without elimination, consistent with a bacteriostatic mechanism. In contrast, our study identified a significantly lower MIC of 0.78 µg/mL for the crude ethanolic root extract of *Vachellia* sp., suggesting stronger antibacterial activity compared to leaf extracts from previous studies.

Notably, the antimicrobial activity against *S. aureus* was more pronounced than that observed for *P. aeruginosa*, corroborating the findings of Andrade et al. (2005) when evaluating *Vachellia podalyriifolia* flowers<sup>18</sup>. Interestingly, *Matricaria chamomilla* L. flowers showed limited activity against *S. aureus* but inhibited *P. aeruginosa* at 1 g/mL, a relatively high concentration for crude extracts<sup>19</sup>.

A recent study by Herrera-Moreno et al. (2023) evaluated the antibacterial activity of silver nanoparticles synthesized using *V. farnesiana* leaf extract<sup>20</sup>. The nanoparticles inhibited *Salmonella agona* at 128 ppm, with a bactericidal effect at 1024 ppm. These results highlight the potential variability in bioactive compounds across different plant parts, influenced by geographic origin and extraction method, which may explain differences in biological activity<sup>21</sup>.

Domingo and Lopez-Brea (2003) described the antimicrobial potential of various chemical compounds in *V. farnesiana*, including phenols, which are associated with reduced *S. aureus* growth<sup>22</sup>. Additionally, Hernández et al. (2009) detected moderate amounts of phenolic compounds and tannins in ethanolic extracts, which may be responsible for the antimicrobial activity observed in our study<sup>15</sup>. Phytochemical analyses of the roots, flowers, branches, and pods of *V. farnesiana* collected in Ceará revealed constituents such as phenols, leucoanthocyanidins, catechins, flavonoids, triterpenes, and alkaloids. Phenolic acids are widely distributed across plant tissues, while root extracts contain a higher flavonoid content<sup>23</sup>.

Flavonoids are of significant interest in the pharmaceutical industry due to their antioxidant and antibacterial properties, particularly in the fight against resistant pathogens<sup>24</sup>. Their antibacterial mechanisms include disrupting cell membranes and inhibiting enzymatic activity. Some flavonoids bind to lipid components in bacterial membranes, compromising membrane integrity and leading to leakage of vital intracellular components<sup>25, 26</sup>.

Moreover, flavonoids may inhibit bacterial metabolic enzymes and interfere with DNA and protein synthesis, thereby limiting bacterial growth and proliferation<sup>27</sup>. Certain flavonoids have demonstrated binding affinity to bacterial DNA and phosphoethanolamine transferase, an enzyme linked to resistance in Gram-negative bacteria, highlighting their potential in antimicrobial resistance research<sup>28</sup>. The inhibitory role of flavonoids is further supported by their synergistic interactions with commercial antibiotics, reducing MIC values even against

resistant strains such as methicillin-resistant *S. aureus*<sup>29, 30</sup>.

Although the extract of *V. farnesiana* demonstrated notable antimicrobial activity against *S. aureus*, the investigation into its potential against *P. aeruginosa* remains essential, particularly considering other plant species have shown efficacy against this pathogen<sup>31</sup>. Notably, different types of extracts may vary in effectiveness, with the root extract of *V. farnesiana* appearing especially promising.

In the plating assay, residual bacterial growth was observed across all concentrations and extract types against both *S. aureus* and *P. aeruginosa*, indicating a bacteriostatic rather than bactericidal effect. Generally, antimicrobial activity increased with higher extract concentrations against *S. aureus*, indicating a dose-dependent effect. For *P. aeruginosa*, this pattern was only observed in the ethanolic extract of the branches.

Gram-positive bacteria such as *S. aureus* are generally more susceptible to antibiotics than Gram-negative species<sup>32</sup>. The limited permeability of the outer membrane in *P. aeruginosa* restricts the entry of small hydrophilic molecules, contributing to its resistance to exogenous compounds<sup>33</sup>.

Agencies such as the World Health Organization (2024) emphasize the importance of developing new antimicrobial agents due to the rise in resistance to conventional drugs<sup>7</sup>. From this perspective, natural plant extracts particularly the root extract of *V. farnesiana*, which showed the most promising results, represent a potential alternative for the treatment of bacterial infections.

The emergence of resistant and multidrug-resistant strains reinforced the need to explore the biotechnological potential of flora. The encouraging findings of this research suggest that the pods, flowers, branches, and especially the roots of *V. farnesiana* are valuable sources of compounds with therapeutic potential against pathogenic microorganisms through their demonstrated bacteriostatic action against strains of *S. aureus* and *P. aeruginosa*.

#### CONCLUSION

*Vachellia farnesiana* has great biological potential against important clinical bacteria, and your root extract represents the better fraction with antibacterial activity. Our results encourage further research to identify and purify the bioactive compounds of *V. farnesiana* associated with antimicrobial activity, besides expanding the analysis to other clinically relevant strains to develop new therapeutic alternatives for the treatment of challenging infections. In addition, the responsible exploration of local flora in the search for pharmacological alternatives contributes to biodiversity preservation and ecosystem health, promoting local sustainability and encouraging further medicinal research.

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