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Exploring the antibacterial potential of *Platymiscium pinnatum*

Explorando el potencial antibacteriano de *Platymiscium pinnatum*

Daniela Alejandra Mora-Estupiñan[1], Giovanni Chaves-Bedoya[1], Luz Yineth Ortiz Rojas[1*]

Resumen

La resistencia a los antibióticos es una preocupación creciente a nivel mundial, lo que hace necesario explorar agentes antimicrobianos alternativos. *Platymiscium pinnatum* (Jacq), un árbol perteneciente a la familia *Fabaceae*, ha tenido tradicionalmente un valor medicinal, especialmente en forma de infusiones de hojas para tratar infecciones de la piel y de los ojos. Este estudio profundiza en el potencial antibacteriano del extracto etanólico obtenido de la corteza de *P. pinnatum*. Utilizando el reconocido método de difusión en disco de agar Kirby-Bauer, se evaluó la actividad antibacteriana del extracto contra bacterias Gram-positivas y Gram-negativas. Los resultados indicaron halos de inhibición en varias cepas Gram-positivas, siendo *Staphylococcus aureus* la que demostró la mayor susceptibilidad. Esto señala el potencial del extracto como agente terapéutico contra infecciones causadas por bacterias Gram-positivas. Por el contrario, la bioactividad del extracto fue comparativamente limitada contra cepas Gram-negativas prominentes, como *E. coli* y *Pseudomonas aeruginosa*. La actividad diferencial entre bacterias Gram-positivas y Gram-negativas se puede deber al modo de acción o los compuestos específicos dentro del extracto. Las distintivas propiedades antibacterianas del extracto de la corteza de *P. pinnatum* sugieren su potencial como fuente para el desarrollo de nuevos agentes antimicrobianos. Estos hallazgos son cruciales para trazar el curso de futuros análisis fitoquímicos y estudios para comprender los componentes bioactivos específicos responsables de los efectos observados.

Palabras Clave: Infecciones bacterianas, recursos biológicos, plantas medicinales, remedios naturales.

Abstract

Antibiotic resistance is a growing concern worldwide, necessitating the exploration of alternative antimicrobial agents. *Platymiscium pinnatum* (Jacq), a tree belonging to the *Fabaceae* family, has traditionally held medicinal value, particularly in the form of leaf infusions for treating skin and eye infections. This study delves into the antibacterial potential of the ethanolic extract obtained from the bark of *P. pinnatum*. Employing the renowned Kirby-Bauer agar diffusion method, the antibacterial activity of the extract against Gram-positive and Gram-negative bacteria was assessed. The results indicated inhibition zones in several Gram-positive strains, with *Staphylococcus aureus* showing the highest susceptibility. This points to the potential of the extract as a therapeutic agent against infections caused by Gram-positive bacteria. In contrast, the bioactivity of the extract was comparatively limited against prominent Gram-negative strains, such as *E. coli* and *Pseudomonas aeruginosa*. The differential activity between Gram-positive and Gram-negative bacteria may be due to the mode of action or specific compounds within the extract. The distinctive antibacterial properties of the *P. pinnatum* bark extract suggests its potential as a source for the development of new antimicrobial agents. These findings are crucial in charting the course for future phytochemical analyses and studies to understand the specific bioactive components responsible for the observed effects.

Keywords: Bacterial infections, biological resources, medicinal plants, natural remedies

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[1] Fitobiomol Research Group. Francisco de Paula Santander University. Gran Colombia Avenue No. 12E-96, Colsag, Cúcuta, Colombia. E-mail: luzyinethor@ufps.edu.co

1. Introduction

The rapid emergence of antibiotic resistance poses a critical public health threat worldwide. Indiscriminate and excessive antibiotic use has resulted in resilient bacterial strains, thereby complicating infection management and heightening the need for innovative antibacterial solutions [1]. To address this, researchers are progressively turning to plant extracts as potent sources of antimicrobial compounds, potentially complementing or substituting traditional antibiotics.

Historically, plants have played a pivotal role in traditional medicine, with numerous species exhibiting antimicrobial traits useful for infection treatment [2]. These plants harbor an array of bioactive compounds, including alkaloids, terpenoids, flavonoids, and phenols, which curtail bacterial growth via varied mechanisms [3].

Recent studies on plant extracts have spotlighted compounds with considerable antibacterial prowess, propelling the hunt for plant-centric remedies to combat antibiotic resistance [4]. Understanding the mechanisms through which these compounds act can lead to the development of more targeted and effective therapeutic strategies [5].

From both sustainability and biodiversity perspectives, utilizing plant extracts as antibacterial resources carries notable benefits. Many of these plants thrive abundantly, particularly in tropical and subtropical zones known for their rich species diversity [6]. Moreover, being potentially more environmentally benign and less predisposed to induce resistance than their synthetic counterparts, plant extracts offer an eco-friendly alternative [7]. As a result, harnessing antibacterial agents from these extracts seems a strategic move in combating antibiotic resistance. They might either supplement or replace conventional antibiotics, leading to more efficient and sustainable medical treatments [6].

Platymiscium pinnatum (Jacq.) Dugand, locally known as "peraco" in Norte de Santander (Colombia) and also referred to as "guayacán trébol", "granadillo", "cristobal", and "cachimbo", is a tree species in the Fabaceae family. It can grow up to 30 m in height and 90cm in diameter. With its hard, heavy, and high-quality wood resistant to insects and fungal attacks, this tree is highly sought after in construction and carpentry [8].

While direct studies on the antimicrobial potential of *Platymiscium pinnatum* (Jacq) are limited, investigations into related species have shown promising results. For example, the wood extract of *Platymiscium gracile* has demonstrated antibacterial effectiveness against species such as *Bacillus cereus* and *Staphylococcus aureus*. In a detailed exploration of this species, a variety of extracts underwent separation and purification, yielding a number of notable compounds. From the n-hexane extract, three compounds were identified: scoparone (6,7-dimethoxycoumarin), homopterocarpin, and oleanolic aldehyde acetate. Additionally, the dichloromethane extract contained compounds such as calycosin, medicarpin, 8-hydroxyhomopterocarpin, 8-methoxyhomopterocarpin, 3,4-dimethoxycinnamaldehyde, and 3,4,5-trimethoxycinnamaldehyde. The ethyl acetate extract also revealed the presence of liquiritigenin and isoliquiritigenin. This marks the first discovery of several of these compounds in P. gracile, highlighting its potential as a rich source of diverse biochemicals including coumarin, chalcone, flavanone, triterpene, cinnamic aldehyde derivatives, and pterocarpans, with some present in high concentrations. [9]. Given the probable shared chemical characteristics within the *Platymiscium genus*, *P. pinnatum* might harbor similar antimicrobial attributes. This research aims to discern the antibacterial efficacy of ethanol extracts derived from *P. pinnatum* against four pathogenic bacteria and explore their implications in infectious disease treatment. We hypothesize that these extracts demonstrate significant antibacterial prowess. To validate this hypothesis, we embarked on a comprehensive experimental journey, spanning extraction, chemical extract profiling, and *in vitro* susceptibility tests against bacterial strains: *Bacillus sp.*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

2. Material and methods

2.1. Collection of plant material

The bark of *P. pinnatum* was collected from trees located in the Peracos neighborhood (7°57´09´´**N** 72°29´57´´ **W**), accessible via the old road to Puerto Santander, in the municipality of Cúcuta, Norte de Santander, Colombia. The specific area of collection was chosen due to its natural abundance of *P. pinnatum* trees and its accessibility for sampling. Figure 1 presents a photographic representation of the collected bark material from *Platymiscium pinnatum* (Jacq). The photograph vividly captures the specific morphological components of the plant, such as the stem, bark, heartwood, and sapwood. Each component in the image is distinctly visible, and labeled accordingly for clarity and easy reference.

2.2. Preparation of ethanolic extract of *P. pinnatum* (Jacq)

Bark samples from *P. pinnatum* (Jacq) were carefully processed into thin, fine flakes. The precisely weighed flakes were then transferred to graduated flasks. Absolute ethanol (Merck, Germany) was added according to a specific weight / volume ratio. The concoction was thoroughly agitated for a period of 48 hours, ensuring that the process occurred in total darkness to protect the integrity of the active compounds.

Post-agitation, the resulting ethanolic extract was filtered utilizing a vacuum pump (DOSIVAC, Buenos Aires, Argentina), in tandem with a funnel and a filter paper (Qual. Dia. 125mm, BOECO, Germany). The filtrate was subsequently concentrated under diminished pressure using a rotary evaporator (IKA®RV10, Wilmington, United States) as depicted in Figure 2. To maintain its stability, the concentrated extract was

Figura 1: Plant material collected from the bark of *P pinnatum* (Jacq), showing specific morphological components including stem, bark, heartwood, and sapwood. The figure illustrates the different parts of the plant used in this study, helping in understanding the source of the extracted material.

securely stored in amber-colored vials under a cool condition of 4°C. This preparation was later subjected to antibacterial scrutiny, targeting both Gram-positive strains (specifically *Bacillus sp* and *Staphylococcus aureus*) and Gram-negative strains (namely Pseudomonas aeruginosa and *Escherichia coli*).

2.3. Identification of secondary metabolites by gas chromatography coupled with mass spectrometry (GC-MS)

The ethanolic extract derived from the bark of *P. pinnatum* was subjected to gas chromatography-mass spectrometry for a comprehensive analysis of its chemical constituents. The GC-MS analysis was conducted on an AT 6890 Series Plus gas chromatograph coupled with an MSD 5973 mass spectrometer (Agilent Technologies, Santa Clara, United States). The chromatographic separation took place on a DB-5MS column (5 %-phenyl-poly(methylsiloxane), dimensions: 60 m x 0.23 mm x 0.25 μm). The injection of the sample was facilitated in Split mode (30:1) utilizing the Solid-Phase Microextraction (SPME) technique. The acquired mass spectral data were subsequently compared and identified against reference spectra from the Adams, Wiley, and NIST databases.

2.4. Antimicrobial Activity Assessment of Platymiscium pinnatum (Jacq) Ethanolic Extracts

To evaluate the antimicrobial properties of the *P. pinnatum* bark's ethanolic extracts, we employed the Kirby-Bauer disk diffusion method. This was conducted against four bacterial strains: Bacillus sp, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

2.5. Preparation of Test Samples

Extracts from *P. pinnatum* bark were diluted in varying ratios ranging from 1:0 (concentrated extract) to 1:5 (extract to ethanol). Sensi-disks, impregnated with these varied dilutions, were prepared to assess their antibacterial activity against the target bacteria. The inhibitory effects of the extracts were evaluated quantitatively by measuring the zones of inhibition. Each test was replicated thrice to ensure reliability and for statistical verification.

2.6. Controls and Treatment Groups

Positive Controls: Kanamycin (1 mg/mL) and Ciprofloxacin (ranging from 0.1 to 1 mg/mL). Negative Controls: Distilled water (T2) and ethanol (T3). Treatment Groups : T4: Concentrated extract; T5: 1:1 Ethanol extract; T6: 1:2 Ethanol extract; T7: 1:3 Ethanol extract; T8: 1:4 Ethanol extract; T9: 1:5 Ethanol extract

2.7. Incubation

Following the application of treatments, the plates were incubated at 37 °C for a 24-hour duration. Post-incubation, zones of inhibition were measured and recorded.

3. Results and Discussion

The GC-MS (Gas Chromatography-Mass Spectrometry) analysis of the ethanolic extract from the bark of P. pinnatum revealed several major compounds (Figure 3). Escoparone was identified as the predominant component, accounting for 48.2 % of the extract. Medicarpin contributed a significant 20.1 %, while homopterocarpin was present at 16.3 %. Methyl eudesmatewas found in smaller amounts, constituting 0.8 %. Additionally, several unidentified compounds (NI. Compound) were detected, with their relative amounts ranging from 1.6 % to 4.1 %, as detailed in Table 1.

In the GC-MS results presented , there are several instances of "NI. Compound M+", a notation indicative of unidentified compounds within the sample. Specifically, the "M+"signifies its molecular ion peak, reflecting the compound's molecular

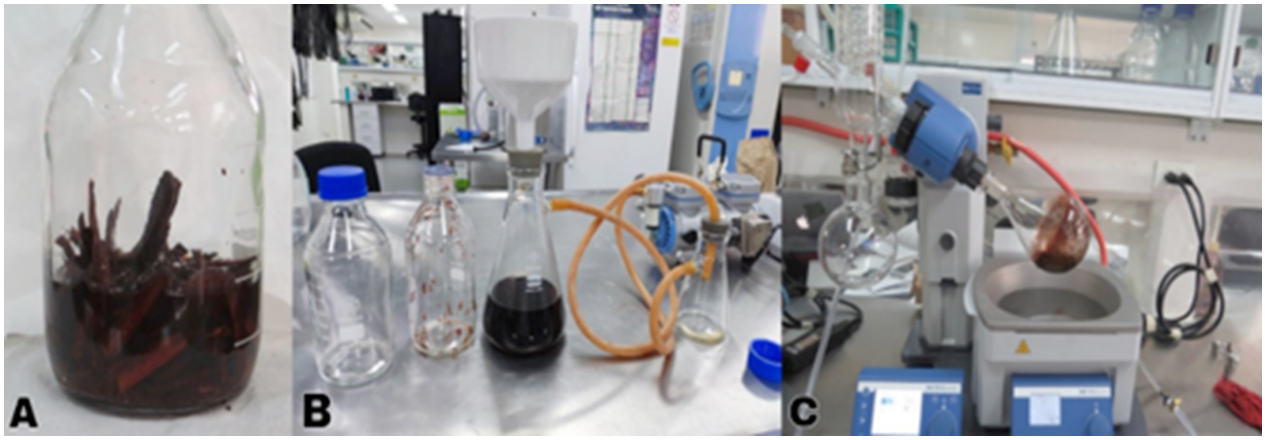

Figura 2: **A**. *P. pinnatum* plant sample immersed in ethanol, ready for the subsequent agitation period. **B.** Filtration of the plant sample using a vacuum pump, a funnel, and filter paper after completing the agitation process. **C**. Filtered sample to be further concentrated using a rotary evaporator.

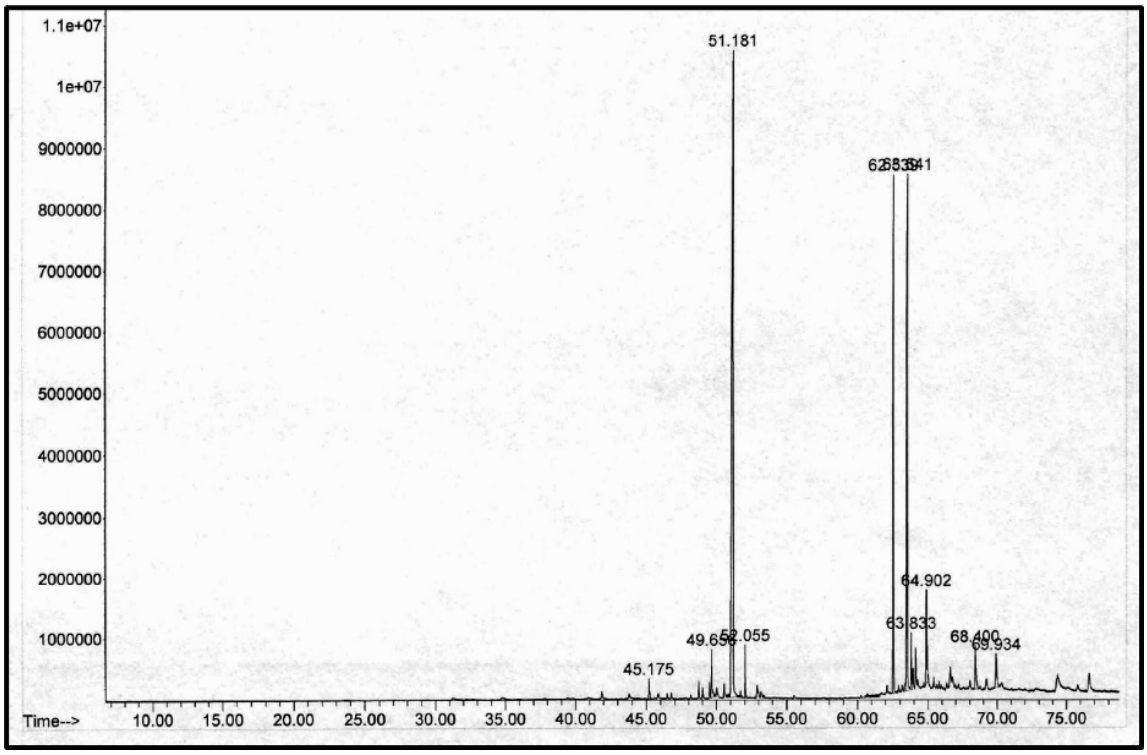

Figura 3: Chromatogram of the ethanolic extract obtained from the bark of *P. pinnatum* (peraco) analyzed using Gas Chromatography-Mass Spectrometry (GC-MS).

Tabla 1: Major compounds identified in the ethanolic extract obtained from the bark of P. pinnatum (Jacq) by Gas Chromatography-Mass Spectrometry (GC-MS).

RT (min)	Tentative identification	Relative amount (%)
48.18	Methyl eudesmate	0.8
49.66	NI. Compound M^+ 240	2.0
51.18	Escoparone	48.2
52.06	NI. Compound M^+ 236	1.6
62.54	Homopterocarpin	16.3
63.54	Medicarpin	20.1
63.83	NI. Compound M^+ 270	2.1
64.90	NI. Compound M^+ 270	4.1
68.40	NI. Compound M^+ 286	2.8
69.93	NI. Compound M^+ 298	2.1

weight. The subsequent numbers (e.g., 270, 240) specify the m/z (mass-to-charge ratio) values for these molecular ion peaks, representing the exact mass of the primary ion generated during the mass spectrometry. To elucidate, an entry like "NI. Compound M+ 270"suggests the presence of an unidentified compound with a molecular ion peak at an m/z value of 270. The multiple unidentified compounds detected underscore the chemical complexity of the ethanolic extract from *P. pinnatum* bark, hinting at potential bioactive compounds that are yet to be explicitly identified or studied.

The majority of the compounds detected within the extract can be categorized under secondary metabolites. The extract contains terpenoids (Methyl eudesmate), isoflavonoids (homopterocarpin and Medicarpin), and coumarins (escoparone). Historically, these have been recognized for their broad-spectrum antimicrobial properties, and their efficacy against pathogens such as Escherichia coli, Salmonella, and Staphylococcus aureus has been documented in previous studies [10].

The ethanolic extract from *P. pinnatum* displayed noteworthy antibacterial activity against a range of both Gram-positive and Gram-negative bacteria. Notable zones of inhibition were observed across all extract dilutions tested against bacteria like Bacillus sp., *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Figure 4). An ANOVA (Analysis of Variance) further highlighted significant differences in inhibition between varied bacterial strains and treatments. These findings align with existing literature, suggesting that different extracts may exhibit higher antibacterial activity against different bacterial strains. In example a study on nine plant species highlighted *C. triflora* and *H. roeperianum* as demonstrating promising antimicrobial activities [11]. While the extract's efficacy didn't surpass that of a commercial antibiotic, the presence of moderate antibacterial compounds within the extract was evident. Application of the Tukey's HSD test elucidated three significantly different groups based on average inhibition in response to the *P. pinnatum* ethanolic extracts. Among them, *Staphylococcus aureus* exhibited the most considerable average inhibition, with Bacillus sp., *Escherichia coli*, and *Pseudomonas aeruginosa* following in its wake.

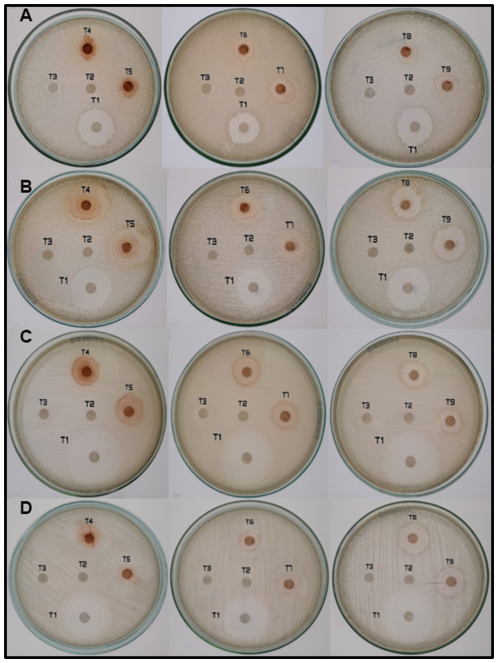

Figura 4: Comparative antibacterial activities of P. pinnatum ethanolic extract dilutions against four bacterial species. The figure contains four panels (A-D), each representing a distinct bacterial species: A) Bacillus sp., B) Staphylococcus aureus, C) Escherichia coli, and D) Pseudomonas aeruginosa. Each panel presents the results from three Petri dishes demonstrating the effects of the different treatments: T1) Kanamycin and Ciprofloxacin as positive controls, T2) distilled water as a negative control, T3) Ethanol, T4) the concentrated extract, and T5-T9) various dilutions of the concentrated extract, with T5-T9 representing dilutions 1:1, 1:2, 1:3, 1:4, and 1:5, respectively.

The significance of these findings lies in the potential therapeutic use of *P. pinnatum* extracts in treating infections caused by Staphylococcus aureus, a bacterium notorious for antibiotic resistance, including Methicillin-resistant *Staphylococcus aureus* (MRSA) [12]. The efficiency of *P. pinnatum* extracts

against Staphylococcus aureus underscores its potential as a novel antibacterial treatment, especially in the context of antibiotic-resistant infections.

On the discussion front, there's a mounting global concern over increasing antibiotic resistance, posing a significant threat to public health and undermining the efficacy of current antimicrobial treatments. The unregulated use of antibiotics, both in human and veterinary medicine, has expedited the emergence of multi-drug resistant bacterial strains, rendering infection treatments progressively challenging [13]. Thus, the quest for new antibacterial agents that can counter this escalating resistance is imperative.

Historically, natural products, especially plant extracts, have served as invaluable reservoirs of bioactive molecules, laying the foundation for many clinically relevant antibiotic classes [2]. These plants encompass a wealth of chemical compounds like alkaloids, terpenoids, flavonoids, and phenols, which possess antimicrobial properties. Their varied mechanisms of action could potentially counter existing antibiotic resistances [14]. Given that numerous plant species remain under-explored for their antimicrobial capabilities, there is tremendous potential to uncover novel therapeutic agents. This highlights the pressing need for continued investigation of natural resources, not just to identify but to harness these agents for more effective, targeted treatment strategies [15].

While the ethanolic extract of showcases antibacterial activity, it may not rival the potency of conventional antibiotics. Yet, it could emerge as a complementary alternative in treating Staphylococcus aureus-induced infections, especially when met with antibiotic resistance. The extract's effectiveness against *Staphylococcus aureus* can likely be attributed to the plant's secondary compounds like terpenoids, coumarins, flavonoids, and isoflavonoids, renowned for their broad-spectrum antimicrobial activity. While the precise antibacterial mechanism remains elusive, some researchers propose that these compounds, due to their lipophilic nature, induce alterations in bacterial outer membrane fatty acid composition, leading to increased permeability, ATP loss, ion leakage, culminating in cell lysis [16].

These findings could guide subsequent studies, focusing on the evaluation of alternative extracts or considering chemical alterations to boost their antimicrobial efficacy. The observed consistent susceptibility among the three bacterial strains suggests that the active compounds in P. pinnatum might exert a specific effect on *Staphylococcus aureus* or the used concentrations weren't sufficiently potent to inhibit the other bacteria significantly.

It's worth noting that the ethanol, employed as a solvent in the treatment dilutions, didn't impede bacterial growth. This observation reinforces the notion that the witnessed antibacterial activity in the treatments is attributable to bioactive compounds, not ethanol's effects, thereby bolstering the study's validity and facilitating more accurate inter-treatment comparisons. This lack of ethanol interference in bioactivity tests augments the credibility of findings regarding the antibacterial activity of evaluated treatments.

The study's results gain traction considering the clinical significance of Staphylococcus aureus, an opportunistic bacterium capable of triggering a plethora of infections, thereby emerging as a public health challenge. This bacterium also exhibits resistance to various antibiotic groups. Thus, the pronounced antibacterial activity of the *P. pinnatum* ethanolic extract against Staphylococcus aureus emerges as a promising find. While it didn't match a commercial antibiotic's efficiency used in the study, it could serve as a pivotal starting point in devising novel antimicrobial agents or as an adjunct strategy in treating Staphylococcus aureus-induced infections.

4. Conclusion

In the persistent pursuit of natural antimicrobial agents within pharmacognosy, this study offers essential insights. Our findings indicate that the ethanolic extract of P. pinnatum exhibits noteworthy antibacterial efficacy, particularly against the Gram-positive bacterium, *Staphylococcus aureus*. The phytochemical analysis, revealing the presence of secondary metabolites like flavonoids, isoflavonoids, terpenes, and coumarins, accentuates the potential of *P. pinnatum*. These compounds are distinguished for their broad-spectrum biological activities, inclusive of antimicrobial properties. Consequently, the ethanolic extract derived from the bark of *P. pinnatum* could emerge as a prospective contender in formulating novel antimicrobial agents tailored to combat infections triggered by Staphylococcus aureus. Nonetheless, before integrating this extract into clinical practice, it's paramount to rigorously assess its efficacy, safety, and any potential side effects across varied populations and under specific medical circumstances.

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