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Evaluation of the Antimicrobial Properties of Citrus sinensis, Ficus religiosa, and Psidium guajava Extracts Against Various Pathogenic Bacteria

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Abstract

This study evaluates the antimicrobial properties of Citrus sinensis, Ficus religiosa, and Psidium guajava extracts against Escherichia coli, Bacillus cereus, and Klebsiella pneumoniae. The extracts were prepared using ethanol, ethyl acetate, and distilled water, and their antimicrobial efficacy was assessed through the zone of inhibition method. Among the solvents, ethanol extracts at 80% concentration exhibited the highest antibacterial activity, showing significant inhibition against E. coli (6mm) and K. pneumoniae (5mm). Ethyl acetate extracts were particularly effective against B. cereus, with the 100% concentration demonstrating the highest inhibition (5mm). Phytochemical analysis confirmed the presence of tannins, suggesting their role in the observed antimicrobial effects. These results indicate that ethanol and ethyl acetate are effective solvents for extracting bioactive compounds from these plants. However, further research is required to explore additional bioactive constituents, investigate the effectiveness of other solvents, and assess antimicrobial activity against a broader spectrum of pathogens. Additionally, in vivo studies and resistance evaluations are necessary to determine the therapeutic potential and clinical applicability of these plant extracts in combating bacterial infections.

Keywords: *Plant extracts, antimicrobial activity, Escherichia coli, Bacillus cereus, Klebsiella pneumonia.*

1. Introduction

The increasing prevalence of antimicrobial resistance (AMR) has become a significant global health challenge, necessitating the search for alternative and natural antimicrobial agents. Medicinal plants have been widely recognized for their therapeutic potential in combating microbial infections, offering a promising approach to overcoming the limitations associated with synthetic antibiotics (Oladunjoye et al., 2022). Among the diverse range of medicinal plants, *Citrus sinensis* (sweet orange), *Ficus religiosa* (sacred fig), and *Psidium guajava* (guava) have been extensively studied for their bioactive compounds, which exhibit potent antibacterial properties (Upadhyaya et al., 2022). The extracts of these plants contain phytochemicals such as flavonoids, tannins, phenolic compounds, and essential oils that are known for their ability to inhibit the growth of pathogenic bacteria, including antibiotic-resistant strains (Marouf et al., 2022). This study aims to analyze the antimicrobial activity of these plant extracts against different pathogenic bacteria, contributing to the growing body of research on plant-based antimicrobials.

1. Importance of Medicinal Plants in Antimicrobial Research

Traditional medicinal plants have been an integral part of human healthcare for centuries, offering natural remedies for bacterial infections. Recent advancements in biotechnology and nanotechnology have facilitated the development of plant-based antimicrobial agents, which are increasingly being explored for their efficacy against drug-resistant pathogens (Cuong et al., 2022). The biosynthesis of metallic nanoparticles using plant extracts has further enhanced the antibacterial potential of these natural compounds (Begum et al., 2022). Research on medicinal plants such as *Citrus sinensis*, *Ficus religiosa*, and *Psidium guajava* has demonstrated their broad-spectrum antibacterial activity against common pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Sitarek et al., 2020). The bioactive constituents of these plants interact with bacterial cell membranes, causing structural damage, inhibition of enzyme activity, and disruption of metabolic pathways, thereby preventing bacterial growth and survival (Saeed et al., 2020).

2. Potential Applications of Citrus sinensis, Ficus religiosa, and Psidium guajava Extracts

The antimicrobial properties of *Citrus sinensis*, *Ficus religiosa*, and *Psidium guajava* have significant applications in the fields of medicine, pharmaceuticals, and food preservation. These plant extracts can be incorporated into topical formulations for treating skin infections, wound healing, and oral healthcare products such as mouthwashes and toothpaste (Ugboko et al., 2020). Additionally, their potential as natural food preservatives can help in reducing the reliance on chemical additives, ensuring food safety and extending shelf life (Domokos et al., 2019). Recent studies have also explored the potential of these extracts in biosynthesizing silver and copper nanoparticles, which exhibit enhanced antimicrobial activity and biocompatibility (Rajan et al., 2015). Furthermore, the development of plant-based hand sanitizers and disinfectants has gained attention, particularly in the wake of the COVID-19 pandemic, as a means to combat microbial contamination effectively (Alghamdi, 2021).

By analyzing the antimicrobial activity of *Citrus sinensis*, *Ficus religiosa*, and *Psidium guajava* extracts against different pathogenic bacteria, this study aims to provide valuable insights into the efficacy of these natural agents in addressing bacterial infections. The findings could contribute to the development of alternative therapeutic strategies, promoting the use of plant-derived antimicrobials in healthcare and pharmaceutical applications.

2. Literature Review

Medicinal plants have long been recognized for their antimicrobial properties, offering natural alternatives to synthetic antibiotics. Citrus sinensis, Ficus religiosa, and Psidium guajava are known for their bioactive compounds that exhibit antibacterial activity against various human pathogens (Ugboko et al., 2020; Domokos et al., 2019). Studies highlight their effectiveness in inhibiting bacterial growth through mechanisms such as enzyme inhibition and membrane disruption (Marasini et al., 2015; Saeed et al., 2020). Additionally, advancements in plant-based nanoparticle synthesis have enhanced their antimicrobial efficacy (Rajan et al., 2015).

Summary of Literature Review

Author's	Work Done	Findings
El-Sherbini, M. S. (2022)	Discusses the potential of combining planetary health and traditional medicine to combat antimicrobial resistance.	The paper proposes a synergistic approach using traditional medicine to address the growing issue of antimicrobial resistance.
Patel, I. (2022)	Investigates the effects of non-ionizing radiation from radio waves on the antimicrobial potential of medicinal plants.	Radio wave exposure negatively impacts the antimicrobial efficacy of medicinal plants, suggesting environmental influence.
Podoprigora, I. V., et al. (2022)	Explores the antibacterial activity of medicinal plants against uropathogenic Escherichia coli.	Several medicinal plants demonstrated significant antibacterial effects against E. coli, offering potential treatment options.
Oza, R., et al. (2022)	Reviews the biosynthesis of copper oxide (CuO) nanoparticles using plant extracts and their potential applications.	Plant extract-mediated CuO nanoparticle synthesis holds promise for various environmental and biomedical applications.
Venugopal, D., et al. (2022)	Focuses on the green synthesis of bioactive metallic nanoparticles and their characterization and applications.	Bioactive metallic nanoparticles show broad-spectrum applicability, including antimicrobial and therapeutic potential.
Alghamdi, H. A. (2021)	Discusses the need for herbal disinfection techniques and formulations, focusing on	Herbal disinfectants, especially hand sanitizers, offer an eco-friendly and effective alternative

	health-friendly hand sanitizers.	to chemical-based sanitizers.
Kowalczyk, T., et al. (2020)	Investigates the synergistic actions of bioactive compounds from plant extracts against skin-infecting microorganisms.	Synergistic effects from plant extracts provide enhanced antimicrobial activity against skin infections.
Naseer, R., et al. (2020)	Valorizes locally available waste plant leaves for tannase and gallic acid production via solid-state fermentation.	Waste plant leaves serve as valuable substrates for the sustainable production of tannase and gallic acid.
Oranusi, S. U., et al. (2020)	Reviews the antimicrobial importance of medicinal plants in Nigeria.	Medicinal plants in Nigeria exhibit considerable antimicrobial properties, offering potential for drug development.
DEZMIREAN, D., et al. (2019)	Reviews traditional medicinal plants used worldwide against Staphylococcus aureus strains.	A wide variety of traditional medicinal plants are effective in combating S. aureus, highlighting their therapeutic potential.
Harper, S. L., et al. (2015)	Examines plant extract-synthesized silver nanoparticles and their potential biocompatible applications.	Silver nanoparticles synthesized from plant extracts show promise in biomedical applications due to their biocompatibility.
Aryal, P., et al. (2015)	Evaluates the antibacterial activity of traditionally used medicinal plants against human pathogenic bacteria.	Traditional plants exhibit significant antibacterial properties against a wide range of human pathogens.
Khan, T. (2015)	Investigates radical scavenging, protease activities, and phenolic composition in bark extracts from 21 medicinal plants.	Medicinal plant barks show promising antioxidant and protease activities, with a rich phenolic profile.
Elumalai, K. (2012)	Screens selected indigenous plants for antibacterial activity.	Several indigenous plants demonstrated strong antibacterial activity against common bacterial strains.

Research Gap

Despite promising findings on the antimicrobial activity of Citrus sinensis, Ficus religiosa, and Psidium guajava extracts, several research gaps remain. The study examined a limited number of plant species and bacterial strains, highlighting the need for broader investigations. While tannins were identified, other bioactive compounds contributing to antimicrobial effects remain unexplored. Additionally, in vivo efficacy and potential bacterial resistance were not assessed, emphasizing the need for further research to fully understand these extracts' therapeutic potential.

3. Problem Statement

This study explores the antimicrobial efficacy of Citrus sinensis, Ficus religiosa, and Psidium guajava extracts against common bacterial pathogens. It highlights the need for further analysis of bioactive compounds and their potential therapeutic applications in combating microbial infections.

4. Methodology

The antimicrobial potential of Citrus sinensis, Ficus religiosa, and Psidium guajava extracts was evaluated against Escherichia coli, Bacillus cereus, and Klebsiella pneumoniae. The bacterial cultures were obtained in lyophilized form, reconstituted in sterile nutrient broth, and incubated under optimal conditions. Remaining cultures were preserved at -20°C for future use. To confirm proper identification, colony morphology and Gram's staining were performed, verifying the Gram-negative nature of E. coli and K. pneumoniae and the Gram-positive nature of B. cereus. The morphological characteristics of each strain are summarized in Table 1. Phytochemical analysis was conducted to detect bioactive compounds in the plant extracts. A ferric chloride test for tannins revealed a blue-black coloration, confirming their presence. These secondary metabolites may contribute to the antimicrobial properties observed in the study. To assess antimicrobial activity, extracts were prepared using ethanol, ethyl acetate, and distilled water. The agar well diffusion method was used to determine efficacy, where clear inhibition zones around the wells indicated bacterial growth suppression. Results were recorded as the zone of inhibition (mm), reflecting each extract's effectiveness. The findings highlight the potential of these plant species as sources of natural antimicrobial agents, warranting further investigation.

5. Result & Discussion

Test Microbial Strains: Three microbial cultures were obtained in lyophilized form. Each culture suspension was transferred into a sterile test tube containing nutrient broth and incubated at the required temperature. The remaining mixture was preserved at -20°C.

Table1 Colony morphology and Gram's reaction of pathogenic isolates

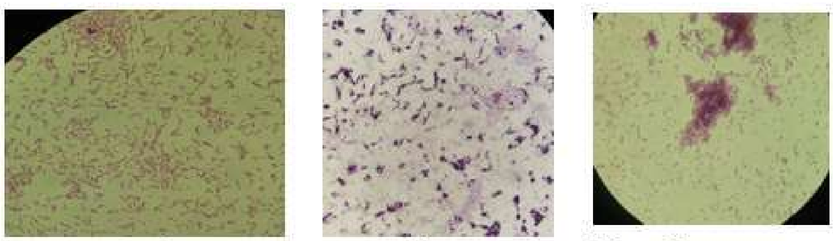
Strain	Colony Morphology	Gram's Reaction
E. coli	Cream, small circular, mucoid	Gram-negative
Bacillus cereus	Grey-whitish, large, granular colonies, less waxy	Gram-negative
Klebsiella pneumoniae	Large, mucoid, and white in color	Gram-negative

E.coli   Bacillus cereus

Klebsiellapneumoniae

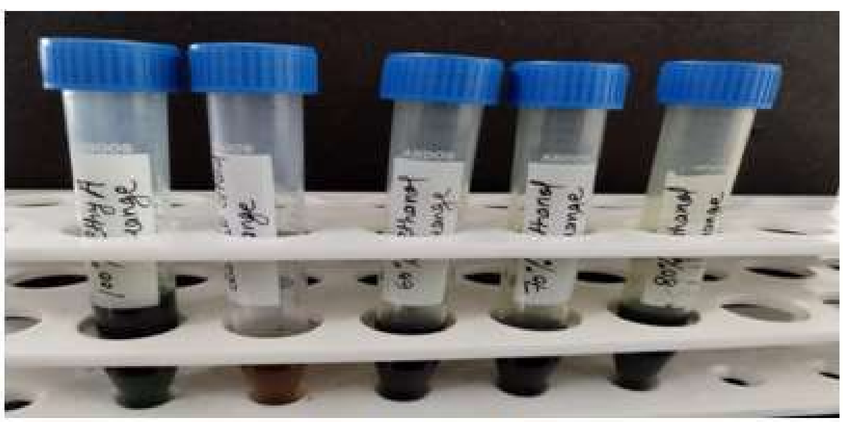
Fig 2 Showing the results of streaking of test microorganisms.

Gram's staining



E.coli   *Bacillus cereus*   *Klebsiellapneumoniae*

Fig 3 Showing the result of Gram's staining of test Microorganism.



(a)Plant extract of *Citrus sinensis*

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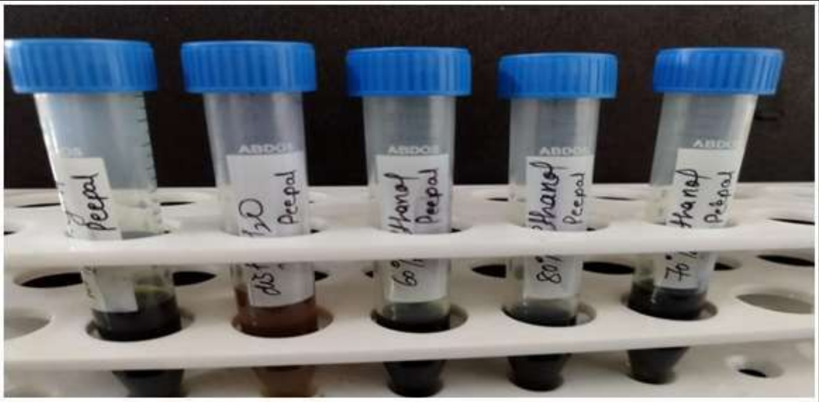
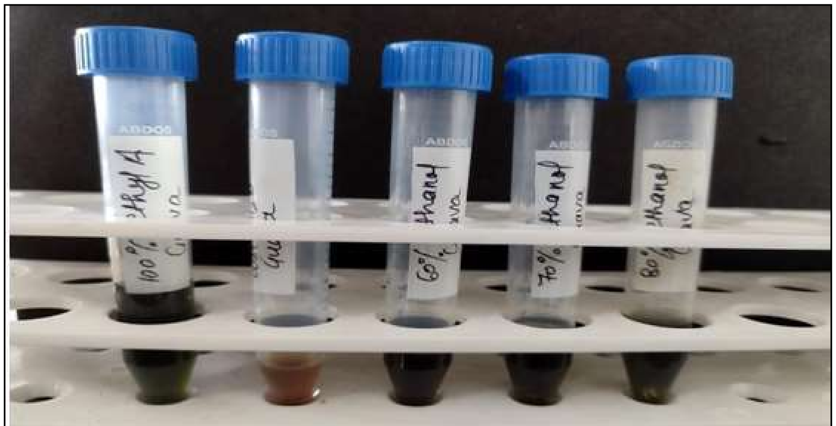


(b) Plant extract of *Ficusreligiosa*

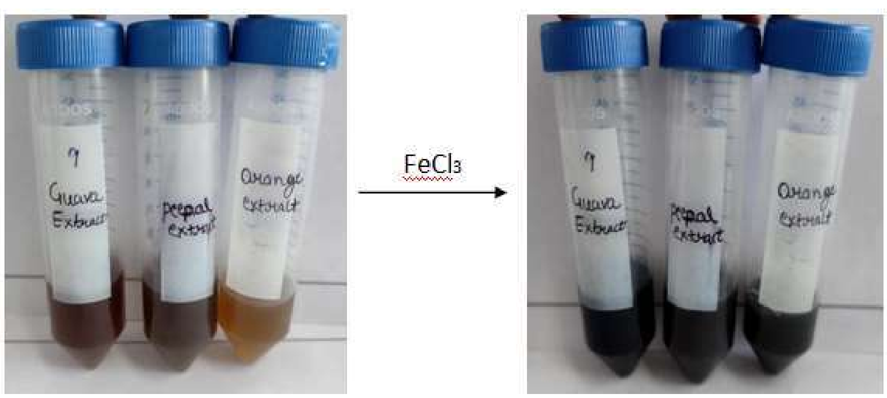


(c)Plant extract of *Psidiumguajava*

Fig 3 Plant extracts dissolved in different solvents.

Phytochemical Analysis of Plant Extract

Test for Tannins: A few drops of ferric chloride reagent were added to 3 mL of the plant extract. The formation of a blue-black color indicated the presence of tannins.



Before After

Fig 4 Phytochemical analysis of medicinal plant.

Antimicrobial Activities of Different Plant Extracts: A total of 15 extracts prepared using ethanol, ethyl acetate, and aqueous solvents were tested for antimicrobial activity against selected pathogens. The results of antimicrobial activity for different extracts are presented in the respective tables.

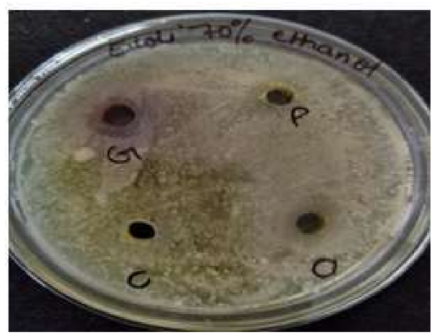
Zone of Inhibition of Ethanol, Ethyl Acetate, and Distilled Water Against Bacteria: The ethanol and ethyl acetate extracts demonstrated significant growth inhibition against the tested bacterial species.

Table 2 Showing the zone of inhibition of plant extract in conc. of Ethanol, Ethyl acetate and distilled water against the E. coli.

Zone of Inhibition (mm) Against *E. coli*					
Plant Extract	**Concentration**	**C. sinensis (O)**	**F. religiosa (P)**	**P. guajava (G)**	**Control (C)**
Ethanol	80%	6mm	4mm	4mm	NA
	70%	2mm	2mm	2mm	NA
	60%	2mm	NA	1mm	NA
Ethyl Acetate	100%	3mm	4mm	NA	NA
Distilled Water	NA	NA	NA	NA	NA

Result of zone of inhibition of E.Coli

80% ethanol



70% ethanol

60% ethanol

100% ethyl acetate

Distilled water

Fig 5 The zone of inhibition of plant extract conc. of Ethanol, Ethyl acetate and distilled water against the *E. coli.*

Zone of Inhibition of Plant Extracts Against *Bacillus cereus: The* ethanol and ethyl acetate extracts exhibited significant growth inhibition against *Bacillus cereus*, demonstrating their potential antimicrobial properties.

Table 3 Showing the zone of inhibition of plant extract in conc. of Ethanol, Ethyl acetate and distilled water against the *Bacillus cereus.*

Plant Extract	Concentration	C. sinensis (O)	F. religiosa (P)	P. guajava (G)	Control (C)
Ethanol	80%	3mm	4mm	2mm	1mm
	70%	3mm	1mm	2mm	1mm
	60%	2mm	1mm	1mm	NA
Ethyl Acetate	100%	5mm	NA	1mm	NA
Distilled Water	NA	1mm	1mm	NA	NA

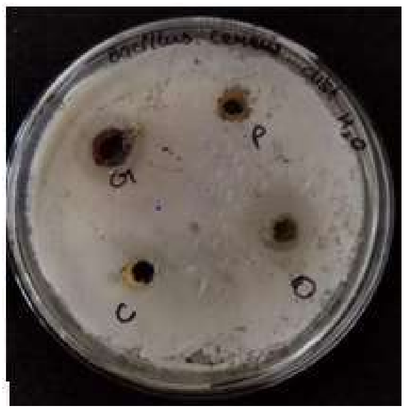
Result of zone of inhibition of Bacillus cereus

80% ethanol

70% ethanol

60% ethanol

100% ethyl acetate



Distilled water

Fig 6 The zone of inhibition of plant extract in conc. of Ethanol, Ethyl acetate and distilled water against the *Bacillus cereus.*

The ethanol extracts of C. sinensis and P. guajava demonstrated effective inhibition against Bacillus cereus at all tested concentrations. However, the 100% ethyl acetate extract exhibited the highest inhibition against Bacillus cereus.

Zone of Inhibition of Plant Extracts Against Klebsiella pneumonia: The ethanol and ethyl acetate extracts showed significant growth inhibition against Klebsiella pneumoniae, indicating their potential antimicrobial activity.

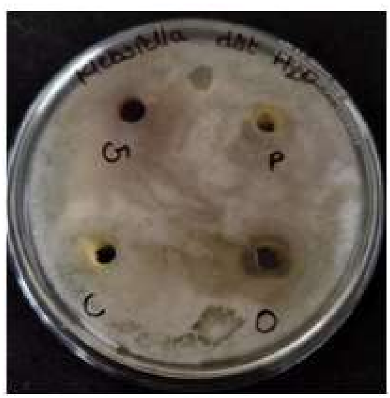
Table 4 Showing the zone of inhibition of plant extract in conc. of Ethanol, Ethyl acetate and distilled water against the Klebsiellapneumoniae.

Plant Extract	Concentration	C. sinensis (Orange - O)	F. religiosa (Peepal - P)	P. guajava (Guava - G)	Control (C)
Ethanol	80%	2mm	4mm	5mm	1mm
	70%	2mm	1mm	3mm	NA
	60%	1mm	NA	1mm	NA
Ethyl acetate	100%	2mm	NA	4mm	1mm
Distilled Water	-	1mm	NA	NA	NA

Result of zone of inhibition of *Klebsiellapneumoniae*

80% ethanol **70% ethanol**

70% ethanol **100% ethyl acetate**



Distilled water

Fig 7 The zone of inhibition of plant extract conc. of Ethanol, Ethyl acetate and distilled water against the *Klebsiellapneumoniae.*

The ethanol extract of the plant exhibited significant inhibition against Klebsiella pneumoniae at all concentrations, with the highest inhibition observed at 80% concentration.

Discussion

Three bacterial species were tested to evaluate the antimicrobial potential of plant extracts using the agar well diffusion method. The antimicrobial activity was assessed by measuring the zone of inhibition (mm), as presented in the tables. The extracts demonstrated varying degrees of effectiveness against the tested bacteria. The highest inhibition against E. coli was observed with the 80% ethanol extract (6mm), followed by another ethanol extract (5mm) and a lower inhibition (4mm). The lowest inhibition was recorded with the 60% ethanol extract (1mm) and the 70% ethanol extract (3mm). Control samples using ethanol, ethyl acetate, and distilled water showed no inhibition. For Bacillus cereus, the highest inhibition was recorded with the 100% ethyl acetate extract (5mm), while the lowest inhibition (1mm) was seen with the 60% ethanol extract. For Klebsiella pneumoniae, the highest inhibition was observed with the 80% ethanol extract (5mm) and the 100% ethyl acetate extract (4mm), while the lowest inhibition was recorded with the 60% ethanol extract (1mm). No inhibition was observed in the 60% ethanol extract, 100% ethyl acetate extract, and distilled water for certain plant samples 14.

6. Conclusion

This study evaluated the antimicrobial activity of Citrus sinensis, Ficus religiosa, and Psidium guajava extracts against Escherichia coli, Bacillus cereus, and Klebsiella pneumoniae. Extracts were prepared using ethanol, ethyl acetate, and distilled water, exhibiting varying degrees of antimicrobial efficacy. Ethanol extracts demonstrated the highest inhibition, with 80% ethanol showing maximum activity against E. coli (6mm) and K. pneumoniae (5mm). Ethyl acetate extracts were particularly effective against B. cereus, with the 100% concentration yielding the highest inhibition (5mm). Phytochemical analysis confirmed the presence of tannins, which may contribute to the observed antimicrobial effects. The study highlights ethanol and ethyl acetate as effective solvents for extracting bioactive antimicrobial compounds from these plants. However, distilled water extracts exhibited comparatively lower activity, suggesting solvent polarity plays a crucial role in compound extraction. While the findings suggest the potential of C. sinensis, F. religiosa, and P. guajava as natural antimicrobial agents, further research is necessary. Future studies should explore additional bioactive compounds, different solvent types, and a broader range of bacterial strains. Moreover, in vivo testing and bacterial resistance assessments are essential to fully understand these extracts' therapeutic potential in combating microbial infections.

Future Scope

- Future studies can investigate a wider range of plant species to expand the understanding of antimicrobial properties.
- Utilizing various solvents, such as methanol or acetone, could provide insights into the effectiveness of different extraction methods.
- Conducting animal model tests would help evaluate the therapeutic potential and safety of the plant extracts.
- Further research can focus on isolating and identifying other bioactive compounds, such as alkaloids or flavonoids, responsible for antimicrobial activity.

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