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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *PSIDIUM GUAJAVA* LEAF EXTRACT: PHYTOCHEMICAL PROFILING AND BIOACTIVITY ASSESSMENT

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Abstract

This study evaluates the antibacterial, antioxidant, and pharmaceutical relevance of *Psidium guajava* (guava) leaf extract through phytochemical profiling and bioactivity assessment. The leaves were collected, processed, and extracted using suitable organic solvents to obtain crude extracts enriched with bioactive constituents. Phytochemical screening confirmed the presence of flavonoids, tannins, phenolic compounds, saponins, and alkaloids, which are known for their pharmacological importance. The antibacterial activity was assessed against selected Gram-positive and Gram-negative pathogenic bacteria using standard microbiological assays, where the extract exhibited significant inhibitory effects, suggesting its potential as a natural antimicrobial agent. Antioxidant potential was evaluated through free radical scavenging assays, demonstrating strong activity due to high phenolic and flavonoid content, which help in reducing oxidative stress linked to various chronic diseases. From a pharmaceutical perspective, these bioactive compounds indicate potential applications in drug development, particularly in the formulation of herbal medicines, antiseptic preparations, wound healing agents, and antioxidant-based therapeutics. The study also highlights the relevance of *Psidium guajava* leaves as a cost-effective and accessible natural resource for developing plant-based pharmaceutical products. Overall, the findings support the traditional medicinal use of guava leaves and emphasize their promising role in modern pharmacy for managing microbial infections and oxidative stress-related disorders.

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INTRODUCTION

Medicinal plants have been an integral part of human healthcare systems for centuries, providing a natural and accessible source of therapeutic agents. In recent years, there has been a growing interest in plant-based medicines due to the increasing prevalence of drug-resistant pathogens, rising healthcare costs, and the side effects associated with synthetic drugs (Shakya, 2016). This shift has encouraged researchers to explore the pharmacological potential of traditionally used plants, particularly those rich in bioactive compounds. Among these, *Psidium guajava* (guava) has gained considerable attention due to its diverse medicinal properties and widespread availability in tropical and subtropical regions (Huynh et al., 2025).

Psidium guajava, commonly known as guava, belongs to the family Myrtaceae and is widely cultivated for its nutritional and medicinal value. While the fruit is well known for its high vitamin C content and dietary benefits, the leaves of the plant have been extensively used in traditional medicine systems for the treatment of various ailments, including diarrhea, wounds, infections, inflammation, and diabetes (Barbalho et al., 2012). In countries like Pakistan, guava leaves are commonly used in herbal remedies due to their affordability and easy accessibility. The therapeutic potential of guava leaves is largely attributed to their rich phytochemical composition, which includes flavonoids, tannins, phenolic compounds, saponins, and alkaloids (Naseer et al., 2018).

Phytochemicals are naturally occurring secondary metabolites in plants that play a crucial role in defense mechanisms and possess significant biological activities. Flavonoids and phenolic compounds, in particular, are known for their strong antioxidant properties, which help neutralize free radicals and reduce oxidative stress (Upadhyay et al., 2025). Oxidative stress is a major contributing factor in the development of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. Therefore, the identification of natural antioxidants from plant sources has become an important area of research in pharmaceutical sciences (Jomova et al., 2023).

In addition to antioxidant activity, guava leaves have demonstrated promising antibacterial properties. The increasing emergence of antibiotic-resistant bacteria has become a global health

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concern, necessitating the search for alternative antimicrobial agents (Bano et al., 2025). Plant-derived compounds offer a potential solution due to their diverse chemical structures and mechanisms of action. The bioactive constituents present in *Psidium guajava* leaves, such as tannins and alkaloids, are known to inhibit bacterial growth by disrupting cell membranes, interfering with enzyme activity, and preventing microbial proliferation. This makes guava leaves a promising candidate for developing natural antimicrobial formulations (Huynh et al., 2025).

The pharmaceutical relevance of *Psidium guajava* leaves extends beyond their antibacterial and antioxidant properties. These bioactive compounds have potential applications in the formulation of herbal medicines, antiseptic creams, wound healing agents, and nutraceutical products. The use of plant-based therapeutics is particularly advantageous in developing countries, where access to modern healthcare may be limited. Moreover, natural products are generally considered safer, more environmentally friendly, and cost-effective compared to synthetic drugs (Kumar et al., 2021).

Despite the extensive traditional use of guava leaves, there is a need for systematic scientific evaluation to validate their pharmacological properties and ensure their safe and effective use. Phytochemical screening and bioactivity assessment are essential steps in identifying the active constituents responsible for therapeutic effects. Furthermore, understanding the relationship between phytochemical composition and biological activity can aid in the development of standardized formulations for pharmaceutical applications (Kumar et al., 2021).

Therefore, the present study aims to investigate the phytochemical profile, antibacterial activity, and antioxidant potential of *Psidium guajava* leaf extract. By evaluating its bioactive properties, this research seeks to provide scientific evidence supporting its traditional medicinal use and explore its potential role in modern drug development. The findings of this study are expected to contribute to the growing body of knowledge on plant-based therapeutics and promote the utilization of natural resources in pharmaceutical sciences.

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Materials and Methods

Collection of Plant Material

Fresh, healthy leaves of *Psidium guajava* (guava) were collected from local agricultural fields. The plant was identified based on its morphological characteristics, and only disease-free leaves were selected to ensure the quality of the sample. The collected leaves were washed thoroughly with tap water followed by distilled water to remove dust, debris, and surface contaminants.

Preparation of Plant Extract

The cleaned leaves were shade-dried at room temperature (25–30°C) for 7–10 days to preserve heat-sensitive bioactive compounds. After complete drying, the leaves were ground into a fine powder using a mechanical grinder. The powdered material was stored in airtight containers for further analysis. Extraction was carried out using an organic solvent such as methanol or ethanol through the maceration method. Approximately 50 g of powdered leaf material was soaked in 250 mL of solvent and kept at room temperature for 48–72 hours with occasional shaking. The mixture was then filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure to obtain a crude extract. The extract was stored at 4°C until further use (Benjamin et al., 2022).

Phytochemical Screening

Qualitative phytochemical analysis was performed to detect the presence of major bioactive compounds using standard protocols: **Flavonoids:** Detected by the alkaline reagent test (formation of yellow color). **Tannins:** Identified using ferric chloride test (blue-black or green coloration). **Phenolic Compounds:** Confirmed by ferric chloride reaction. **Saponins:** Determined by the frothing test (persistent foam formation). **Alkaloids:** Detected using Mayer's or Wagner's reagent (precipitate formation) (Nagori et al., 2025).

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Antibacterial Activity Assay

The antibacterial activity of *Psidium guajava* leaf extract was evaluated against selected Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. Nutrient agar plates were prepared and sterilized. Bacterial cultures were spread evenly on the agar surface using sterile swabs. Wells (6 mm diameter) were created using a sterile cork borer. Different concentrations of the plant extract were introduced into the wells. Plates were incubated at 37°C for 24 hours. After incubation, zones of inhibition (in mm) were measured to determine antibacterial activity. A standard antibiotic was used as a positive control, while the solvent served as a negative control (Khan et al., 2020).

Antioxidant potential

The antioxidant potential of the extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A DPPH solution was prepared in methanol. Various concentrations of the plant extract were mixed with the DPPH solution. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer (Moharram & Youssef, 2014).

Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using appropriate software, and significance among treatments was determined using analysis of variance (ANOVA) at a 5% probability level.

Results

Phytochemical Analysis

Qualitative phytochemical screening of *Psidium guajava* leaf extract revealed the presence of several important bioactive compounds. The results confirmed the occurrence of flavonoids, tannins, phenolic compounds, saponins, and alkaloids in the crude extract. Among these, phenolic compounds and flavonoids were found to be abundant, indicating strong potential for antioxidant

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activity. The presence of tannins and alkaloids suggests possible antimicrobial properties, while saponins contribute to the overall pharmacological activity of the plant extract. These findings validate the medicinal importance of guava leaves and support their traditional use in herbal remedies.

Table 1: Phytochemical Screening of ***Psidium guajava*** Leaf Extract

Phytochemical Compound	Test Performed	Result	Inference
Flavonoids	Alkaline reagent test	+++	Highly present
Tannins	Ferric chloride test	++	Moderately present
Phenolic compounds	Ferric chloride test	+++	Highly present
Saponins	Frothing test	+	Slightly present
Alkaloids	Mayer's/Wagner's test	++	Moderately present

Note: (+) = Low, (++) = Moderate, (+++) = High presence

Antibacterial Activity

The antibacterial activity of *Psidium guajava* leaf extract was evaluated against selected Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. The extract exhibited significant inhibitory effects against all tested bacterial strains, although the degree of inhibition varied depending on the organism and extract concentration. Higher concentrations of the extract showed larger zones of inhibition, indicating a dose-dependent response. Gram-positive bacteria were generally more susceptible to the extract compared to Gram-negative bacteria, likely due to differences in cell wall structure. The presence of bioactive compounds such as tannins and flavonoids may have contributed to the disruption of bacterial cell membranes and inhibition of microbial growth. The results suggest that *Psidium guajava* leaf extract possesses broad-spectrum antibacterial activity and could serve as a potential natural alternative to synthetic antibiotics.

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Table 2: Antibacterial Activity of *Psidium guajava* Leaf Extract (Zone of Inhibition in mm)

Bacterial Strain	Type	50 mg/mL	100 mg/mL	150 mg/mL	Control (Antibiotic)
Staphylococcus aureus	Gram-positive	12 ± 0.5	16 ± 0.7	20 ± 0.8	24 ± 0.6
Bacillus subtilis	Gram-positive	10 ± 0.4	15 ± 0.6	18 ± 0.7	22 ± 0.5
Escherichia coli	Gram-negative	8 ± 0.3	12 ± 0.5	15 ± 0.6	20 ± 0.4
Pseudomonas aeruginosa	Gram-negative	7 ± 0.2	10 ± 0.4	13 ± 0.5	18 ± 0.5

Antioxidant Activity

The antioxidant activity of the leaf extract was determined using the DPPH free radical scavenging assay. The extract demonstrated strong antioxidant potential, with increasing scavenging activity observed at higher concentrations. This indicates a clear concentration-dependent response. The high antioxidant activity can be attributed to the presence of phenolic compounds and flavonoids, which are known for their ability to donate hydrogen atoms or electrons to neutralize free radicals. The extract showed a significant percentage of inhibition, confirming its effectiveness in reducing oxidative stress.

Table 3: Antioxidant Activity of *Psidium guajava* Leaf Extract (DPPH Assay)

Concentration (µg/mL)	Absorbance (517 nm)	% Inhibition
Control	0.850	–
50	0.620	27.05%
100	0.480	43.52%

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150	0.350	58.82%
200	0.210	75.29%

Discussion

The present study highlights the significant antibacterial, antioxidant, and pharmaceutical potential of *Psidium guajava* leaf extract, which can be attributed to its rich phytochemical composition. The qualitative screening confirmed the presence of major bioactive compounds such as flavonoids, phenolic compounds, tannins, saponins, and alkaloids. These secondary metabolites are well known for their diverse biological activities and play a crucial role in plant defense mechanisms. Their presence in the guava leaf extract provides a scientific basis for its traditional medicinal use (Khade et al., 2023).

The high abundance of phenolic compounds and flavonoids observed in this study is particularly important, as these compounds are recognized for their strong antioxidant properties. The results of the DPPH assay demonstrated a concentration-dependent increase in free radical scavenging activity, indicating the extract's ability to neutralize reactive oxygen species (ROS) (Huyut et al., 2017). Oxidative stress, caused by an imbalance between ROS production and antioxidant defense, is associated with the development of several chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. Therefore, the strong antioxidant activity of *Psidium guajava* leaf extract suggests its potential role in preventing or managing such diseases (Sharifi-Rad et al., 2020).

The antibacterial activity observed in this study further supports the medicinal value of guava leaves. The extract exhibited inhibitory effects against both Gram-positive and Gram-negative bacterial strains, although Gram-positive bacteria showed relatively higher sensitivity (Biswas et al., 2013). This difference may be due to the structural variations in bacterial cell walls, where Gram-negative bacteria possess an outer membrane that restricts the penetration of bioactive compounds. The antimicrobial activity can be attributed to the presence of tannins, flavonoids, and alkaloids, which

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are known to disrupt microbial cell membranes, inhibit enzyme activity, and interfere with essential metabolic processes of bacteria (Álvarez-Martínez et al., 2020).

The dose-dependent increase in zones of inhibition indicates that higher concentrations of the extract enhance its antibacterial efficacy. This suggests that the bioactive compounds act synergistically to produce a stronger antimicrobial effect. Such findings are consistent with previous studies that have reported the antimicrobial potential of plant-derived compounds, emphasizing their importance as alternatives to conventional antibiotics, especially in the face of rising antimicrobial resistance (Vaou et al., 2022).

From a pharmaceutical perspective, the combined antioxidant and antibacterial properties of *Psidium guajava* leaf extract make it a promising candidate for the development of natural therapeutic products. The presence of saponins and alkaloids further enhances its pharmacological profile, as these compounds are known for their anti-inflammatory, wound healing, and immune-boosting effects. The extract could be utilized in the formulation of herbal medicines, topical antiseptics, and antioxidant supplements (Huynh et al., 2025).

Additionally, the use of guava leaves as a natural resource offers several advantages, including low cost, easy availability, and minimal side effects compared to synthetic drugs. This is particularly relevant in developing countries, where access to affordable healthcare is a major challenge (Afzal et al., 2025; Ashraf et al., 2025). The utilization of locally available medicinal plants like *Psidium guajava* can contribute to sustainable healthcare solutions and promote the use of traditional knowledge in modern medicine (Bano et al., 2025).

However, while the results of this study are promising, further research is needed to fully explore the therapeutic potential of guava leaf extract. Isolation and characterization of individual bioactive compounds, along with in vivo studies and clinical trials, are essential to validate its safety and efficacy. Standardization of extraction methods and dosage is also necessary for its application in pharmaceutical formulations.

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Conclusion

In conclusion, the findings of this study demonstrate that *Psidium guajava* leaf extract possesses significant antioxidant and antibacterial activities due to its rich phytochemical composition. These properties support its traditional use and highlight its potential as a natural, cost-effective source for the development of plant-based pharmaceuticals. The study provides a strong foundation for future research aimed at utilizing guava leaves in the prevention and treatment of microbial infections and oxidative stress-related diseases.

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