International Journal of Microbial Science, ISSN (online), 2582-967X, Volume 6, Issue 2, June 2025, pp. 25-30
Available at https://internationaljournalofmicrobialscience.com/

Original Article

Leaves of *Psidium gaujava* Collected from Village Ganegaon Dumala, Taluka Shirur, District Pune, Maharashtra State of India can Inhibit the Growth of Pathogenic *Pseudomonas aeruginosa*

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Article Info

Article history:
Received: April 13, 2025
Accepted: May 26, 2025
Published: June 7, 2025

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Keywords: Microalgae, Biostimulant,Phytochrome,Stress tolerant,Chlorophyll,Bioactive Component.

Abstract

In this study, the antimicrobial activity and phytochemical constituents of *Psidium gaujava* plant extract were evaluated. The present study investigates the antimicrobial activity of leaf extracts of *Psidium guajava* against the human pathogenic bacterium *Pseudomonas aeruginosa.* Different extracts were obtained by Soxhlet extraction with ethanol and butanol. The efficacy of these extracts was tested against *Pseudomonas aeruginosa* through an agar well diffusion method. Based on the outcome of our results, the ethanolic extract showed significant microbial activity against *Pseudomonas aeruginosa* compared to the butanolic extract. The maximum zone of inhibition (18mm) was obtained with the ethanolic extract of *Psidium guajava* against *Pseudomonas aeruginosa.* Phytochemical analysis of the extracts revealed the presence of flavonoids, sterols, and tannins. The presence of these biologically active chemicals in *Psidium guajava* may justify their widespread use in traditional medicine

1. Introduction:

Pseudomonas aeruginosa (P. aeruginosa) causes many types of healthcare-associated infections, including pneumonia, bloodstream infections, urinary tract infections, and surgical site infections [1]. *Pseudomonas aeruginosa* is one of the leading causes of nosocomial pneumonia [2]. Ventilator-associated pneumonia by *Pseudomonas aeruginosa* in hospitalized patients is associated with high mortality [3]. Over the past decade, there have been unrecognized increases in the number of infections with highly drug-resistant *Pseudomonas aeruginosa* occurring in children. This increase in drug resistance in *P aeruginosa* involves multiple antibiotic classes, including our last line of effective preventative strategies, which is critical [1]. The use of medicinal plants dates back to ancient times and may even be considered the origin of modern medicine. Compounds of plant origin have been and still are an important source of compounds for drugs [4]. Products of plants are easily available, cheap, and have the slightest tendency to develop resistance, due to which they could be proven beneficial for health [5]. Herbal medicines are experiencing widespread adoption worldwide as people increasingly turn to plant-derived bioactive compounds to treat various diseases, from bacterial infections to cancer [6]. The guava (*Psidium guajava*) has long been valued for its diverse range of pharmacological properties. The plant has bioactive chemicals in its leaves, fruit, and bark, which enhance its potential for therapeutic use [5]. Guava leaves are a rich source of various health-promoting micro- and macronutrients as well as bioactive compounds [7]. The guava tree is an evergreen small tree. The guava leaves are 2 to 6 inches long and 1 to 2 inches wide, aromatic when crushed, and appear dull green, with stiff but coriaceous leaves and pronounced veins. There are bioactive components in guava leaves that can fight pathogens, regulate blood glucose levels, and even aid in weight loss. The leaves of guava contain an essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and some other fixed substances [8]. Many studies have described the antimicrobial activity of different extracts from the leaves of *P. guajava* [8,9].

In the present study, the antimicrobial activity and phytochemical constituents of *Psidium gaujava* plant extract were evaluated. We investigated the antimicrobial activity of leaf extracts of *Psidium guajava* leaves against the human pathogenic bacteria *pseudomonas aeruginosa.* Different extracts were obtained by Soxhlet extraction with ethanol and butanol. The efficacy of these extracts was tested against *Pseudomonas aeruginosa* through an agar well diffusion method.

2. Materials and Methods

2.1 Collection of plant materials: Leaves of *Psidium gaujava* were collected from village Ganegaon- Dumala, Taluka- Shirur, District- Pune, Maharashtra**.**

2.2 Preparation of extracts:

Collected leaves were washed thoroughly and chopped into small pieces by using a mortar and pestle, shade dried, and ground into a powder. About 5 grams of dried powder was extracted with 150 milliliters of various solvent systems, such as ethanol and butanol, using the Soxhlet extraction method [10].

2.3 Preparation of inoculums:

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring loopful of cells from the stock cultures to test tubes containing Mueller-Hinton broth for bacteria, which were then incubated without agitation for 24 hours at 37°C. To 5ml of Mueller-Hinton broth, 0.2 ml of culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 McFarland solution at 600nm, which is equivalent to 10^6– 10^8 CFU/ml [11].

2.4 Determination of antibacterial activity:

The antimicrobial activity of different plant extracts was determined using the agar well diffusion method. 0.1 ml of freshly grown culture of test organisms (10^6cfu/ml) was aseptically introduced and spread on the surface of sterile Muller-Hinton agar plates. Wells of 6 mm diameter were made in an agar plate with the help of a sterile cork-borer. 50 microliters of *Psidium guajava* leaves extract, and the same volume of extraction solvent for the negative control were filled in the wells with the help of a micro pipette. Standard reference antibiotics, such as penicillin and gentamicin, were used as positive controls for the test organisms. Plates were left at 4°C for some time until the extract had diffused into the medium with the lid closed, and then incubated at 37°C for 24 hours. The plates were observed for a zone of inhibition. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition against the tested bacterial pathogen, *Pseudomonas aeruginosa*. Each assay in this experiment was replicated three times [12].

2.5 Phytochemical analysis:

Different phytochemical tests were performed, as described below**.**

2.5.1. Test for steroids: 2 ml of acetic anhydride was added to 0.5 g extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples, indicating the presence of steroids [13**].**

2.5.2. Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration[13].

2.5.3. Test for flavonoids: Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed, indicating the presence of flavonoids [13].

2.5.4. Test for alkaloids: Meyer's test- To 2 mL of extract, 1 mL of Meyer's reagent was added. The pale-yellow precipitate indicated the presence of an alkaloid [14].

3. Results:

The present study, conducted on the leaves of *Psidium guajava,* revealed the presence of several medicinally active phytochemical constituents. The phytochemical characteristics of *P. guajava* leaves are summarized in Table 1, indicating the presence of flavonoids, sterols, and tannins**.**

Table 1: Preliminary phytochemical analysis of ethanolic and butanolic extracts of *Psidium gaujava* leaves

Sr. no.	test	***Psidium guajava***	
		Ethanolic	**Butanolic**
1	Alkaloid test	Negative	Negative
2	Flavonoid test	Negative	Positive
3	Sterol test	Positive	Positive
4	Tannin test	Positive	Negative

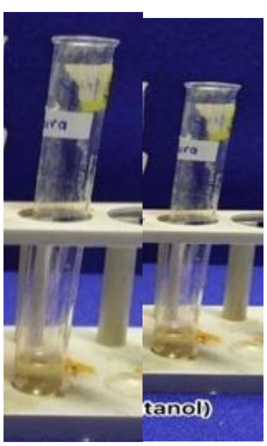

Figure 1(a) Figure 1(b)

Figure 1(a): Alkloid test of ethanolic extracts of *Psidium gaujava* leaves.

Figure 1(b): Alkloid test of butanolic extracts of *Psidium gaujava* leaves

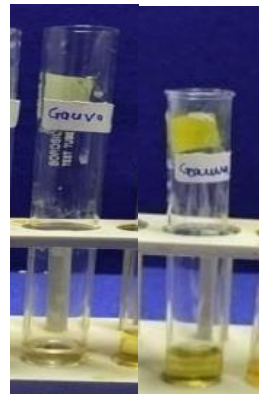

Figure 2(a) Figure 2(b)

Figure 2(a): Flavonoid test of ethanolic extracts of *Psidium gaujava* leaves.

Figure 2(b): Flavonoid test of butanolic extracts of *Psidium gaujava* leaves.

Figure 3(a) Figure 3(b)

Figure 3(a): Sterol test of ethanolic extracts of *Psidium gaujava* leaves.

Figure 3(b): Sterol test of butanolic extracts of *Psidium gaujava* leaves.

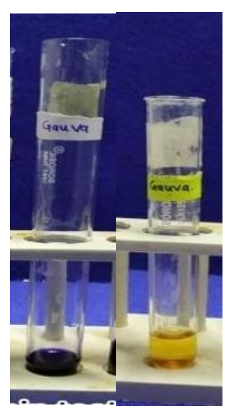

Figure 4(a) Figure 4(b)

Figure 4(a): Tannin test of ethanolic extracts of *Psidium gaujava* leaves.

Figure 4(b): Tannin test of butanolic extracts of *Psidium gaujava* leaves

This study further investigated the antimicrobial potential of the leaf extracts of *Psidium guajava* against the human pathogenic bacterium *Pseudomonas aeruginosa.* Leaf extracts were prepared using the Soxhlet extraction method with ethanol (Figure 5) and butanol (Figure 6).

Figure 5: The ethanolic extract of *Psidium guajava* leaves obtained by the Soxhlet extraction method.

Figure 6: The butanolic extract of *Psidium guajava* leaves obtained by the Soxhlet extraction method.

The antimicrobial effectiveness of these extracts was tested against *Pseudomonas aeruginosa* using the agar well diffusion method (Figures 7 and 8).

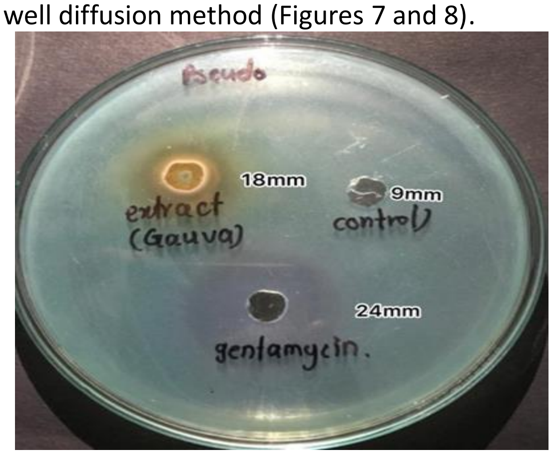

Figure 7: Antimicrobial activity of ethanolic extract of *Psidium guajava* leaves on *Pseudomonas aeruginosa*.

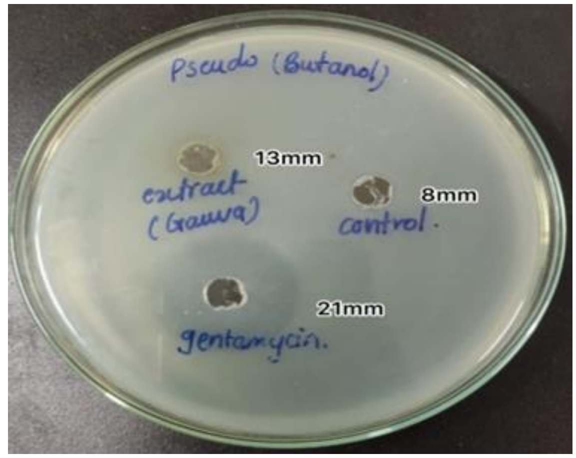

Figure 8: Antimicrobial activity of butanolic extract of *Psidium gaujava* leaves on *Pseudomonas aeruginosa*

Based on the experimental findings, the ethanolic extract exhibited significantly greater antimicrobial activity than the butanolic extract. The maximum zone of inhibition was 18mm, observed with the ethanolic extract of *Psidium guajava* against *Pseudomonas aeruginosa* (Figure 7).

4. Discussion

The present study highlights the phytochemical richness and antimicrobial potential of *Psidium guajava* leaves, supporting earlier reports on the medicinal value of this plant. Phytochemical screening in the current investigation confirmed the presence of flavonoids, sterols, and tannins, which are widely recognized for their biological activities. These results are in agreement with previous findings where *P.guajava* leaves showed positive tests for phenols, tannins, terpenoids, flavonoids, and glycosides [15]. Other studies have also confirmed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids in *P. guajava* extracts, which were identified as responsible for inhibiting microbial growth in vitro [16,17]. The consistent detection of these phytochemicals indicates that *P.guajava* leaves are a promising source of natural bioactive compounds.

The antimicrobial activity of *P. guajava* leaf extracts observed in this study, particularly against *Pseudomonas aeruginosa*, further strengthens existing literature. In the present work, ethanol and butanol extracts were prepared antimicrobial efficacy was evaluated through agar well diffusion. The ethanolic extract demonstrated superior inhibitory activity, producing a maximum zone of inhibition of 18 mm, compared to the weaker activity of the butanol extract. This enhanced activity supports earlier research.

This enhanced activity of acetone, ethanol, and methanol extracts of *P. guajava* demonstrated notable inhibition zones against *P. aeruginosa* (13.5 ± 2.12 mm for acetone, 13 ± 1.41 mm for ethanol, and 12.5 ± 3.53 mm for methanol) [16]. Similarly, ethanolic extracts were previously reported to inhibit *P. aeruginosa, E.coli, S. pneumoniae*, and *K. pneumoniae* at 25 mg concentrations, with *S. aureus* being inhibited at 50 mg [16]. The present finding aligns strongly with these trends, emphasizing the efficiency of ethanol as an extraction solvent.

The improved antimicrobial activity of the ethanolic extract may be attributed to ethanol's ability to dissolve a wider range of polar and moderately polar phytochemicals. Flavonoids and tannins, in particular, are highly soluble in ethanol and are known to disrupt microbial cell membranes, interfere with DNA replication, and inhibit enzymes essential for bacterial survival. Since *Pseudomonas aeruginosa* is a highly resistant pathogen with strong adaptive mechanisms—including biofilm formation, efflux pumps, and enzyme-mediated resistance—the observed inhibition suggests the presence of potent bioactive compounds in *P. guajava* that may overcome some of these defense systems.

The comparatively lower activity of the butanolic extract could be due to the limited solubility of certain phytochemicals in butanol. This indicates that solvent polarity plays a crucial role in determining the antimicrobial efficacy of plant extracts. The findings of this study highlight the importance of using appropriate solvents to maximize the extraction of active constituents.

Overall, the results support the traditional medicinal use of *Psidium guajava* and demonstrate its promising potential as a natural antimicrobial agent, especially against drug-resistant pathogens like *Pseudomonas aeruginosa*. The consistency between the present findings and earlier studies [15,16,18] further validates the therapeutic relevance of this plant. However, additional studies, such as MIC and MBC determination, phytochemical quantification, compound isolation, and mechanistic analyses, are recommended to fully explore this work.

5. Conclusion

Psidium guajava leaves inhibit the growth of pathogenic organisms,*Pseudomonas aeruginosa*.

Author contributions:MG: Developed an idea and wrote the manuscript. SK: Verified the manuscript.

Competing interest: The Authors declare that no conflict of interest exists.

Ethical Statement: This work does not violate ethical guidelines.

Grant Support Details: The authors did not get funding from any agency to complete this work.

Acknowledgement: The authors are thankful to Dr. Bharat Shinde, Principal, and Dr. Lalasaheb Kashid, Vice Principal of Vidya Pratishthan's Art, Science and Commerce College, Baramati, Maharashtra, India, and also they like to thank the faculty members of the Department of Microbiology, Mrs. Nilima Pendharkar (HOD), Mrs. A. Neelima Devi, Dr. Amarsingh Bhosale, Miss. Kiran Deshmukh, Miss. Ujwala Khot, Miss. Tejswini Bankar, Miss. Komal Kokare, Miss. Trupti Mahadik for their everlasting support and inspiration in publishing this work.

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Cite this article as: Kamble SN, Garud MS. Leaves of *Psidium gaujava* Collected from Village Ganegaon Dumala, Taluka Shirur, District Pune, Maharashtra State of India can Inhibit the Growth of Pathogenic *Pseudomonas aeruginosa*. Int. J. Micro. Sci. 2025; 6(2):25-30.