RESEARCH PAPER

Phytochemical Screening, Antioxidant Capacity, and Antimicrobial Activity of Aqueous Leaf Extract of *Psidium guajava* L.

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Abstract

Background: Psidium guajava L. (Myrtaceae) has formed the base of traditional health systems of humans for the treatment of gastric illnesses, infection, and inflammation**.**

Aim: This study aimed to investigate the phytochemical profile, antioxidant potential, and antimicrobial property of the aqueous extract of leaves of P. guajava.

Methods: Preparation of the extract was done by Soxhlet extraction method. Qualitative analysis of phytochemical screening and quantitative determination of total phenol and flavonoid content were performed. The antioxidant activity was determined by DPPH free radical scavenging assay while the antimicrobial efficacy was checked against Staphylococcus aureus and Escherichia coli using agar well diffusion method.

Results: Results of qualitative analysis revealed the presence of alkaloids, carbohydrates, saponins, proteins, phenols, and flavonoids. However, glycosides were absent. Total phenol and flavonoid content were found to be 1.15 mg GAE/mg and 1.15 mg QE/mg, respectively. DPPH assay revealed a dose-dependent scavenging effect, showing 57.71% inhibition at a concentration of 100 µg/ml. Moreover, the extract showed marked antibacterial activity with maximum zones of inhibition being 17 mm against S. aureus and 19 mm against E. coli at 100 µg/ml.

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Introduction

Traditional healing has been greatly influenced by medicinal plants which have acted as the basis for the health systems of human beings ever since ancient times[WHO], 2013). Traditional medicine practices rely heavily on plant species for management of illnesses such as digestion disorders, chronic inflammatory and metabolic diseases (Fabricant & Farnsworth, 2001). Plants have significantly contributed towards pharmaceutical products as they act as lead compounds for drugs (Newman & Cragg, 2020).

Psidium guajava L. belongs to the family Myrtaceae. The plant is a medicinal and fruit bearing tree that grows in tropical and subtropical environments (Lim, 2012). Psidium guajava L. systematically belongs to the order of Myrtales, which grows effectively in warm environments and is

adapted to various agroclimatic belts (Morton, 1987). In traditional folk medicines including Ayurveda and Siddha, leaves of P. guajava are used to treat diarrhea, dysentery, diabetes and oral infections (Gutiérrez et al., 2008; Pandey & Shweta, 2015).

The biological activities of guava leaves can be attributed to the presence of different phytochemicals such as phenolics, flavonoids, alkaloids, tannins, and terpenoids (Harborne, 1998). Antioxidant capacity of flavonoids (quercetin) and phenols is that they act as powerful scavengers of reactive oxygen species (ROS) thereby protecting against oxidative stress that causes cancers, heart diseases, and aging (Halliwell & Gutteridge, 2015; Valko et al., 2007). Moreover, there has been an increased need for alternative antimicrobial agents due to the development of antimicrobial resistance globally (Ventola, 2015). Antimicrobial activity of plant phytochemicals results from their interference with cell membrane of microorganisms, protein precipitation, and enzyme inhibition (Burt, 2004; Cowan, 1999).

Whereas some previous studies have explored the biological activities of different organic solvent extracts of guava leaves (Möwes et al., 2025; Agada, 2024), continued testing of water-based extracts is important. Thus, the objective of the current study was to examine the phytochemistry, antioxidant capacity, and antimicrobial activity of water-based extract of P. guajava leaves.



Fig:1: ***Psidium guajava*** **Tree and dried leaves**

2. Materials and Methods

2.1. Sample Collection and Extract Preparation

The P. guajava leaves were gathered from the Chengalpet district and allowed to dry by air drying. The leaves after drying were grounded into a powdered form. Extraction was done using the Soxhlet apparatus. The finely powdered crude drug was subjected to continuous extraction in the Soxhlet apparatus along with the hydroalcoholic solvent. After extraction, the solvent was evaporated using a rotary evaporator.

2.2. Qualitative Phytochemical Analysis

Qualitative tests were conducted on the aqueous extract to determine the presence of secondary metabolites:

- Saponins (Foam test): 5.0 ml distilled water and crude extract were shaken and foam presence indicated the presence of saponins.

- Flavonoids (Alkaline Reagent test): 2.0 ml 2.0% NaOH was added to the extract, yellowish colouration indicated presence of flavonoids.

- Terpenoids: The extract was mixed with 2.0 ml chloroform and evaporated then concentrated H2SO4 was added and boiling was done, grey colouration indicated terpenoids.

- Glycosides: The extract was mixed with 4.0 ml glacial acetic acid, 1 drop of 2.0% FeCl3 and 1 ml concentrated H2SO4; brown ring indicated steroidal glycosides.

- Alkaloids (Dragendorff test): 5 mg of extract was treated with Dragendorff's reagent, orange red precipitate indicated alkaloids.

- Proteins (Biuret test): 5 mg extract was mixed with Biuret's reagent and heated; red/violet color indicated proteins.

- Amino Acids (Ninhydrin test): 5 mg extract was heated with 2 ml of 0.2% Ninhydrin; violet colour indicated amino acids.

- Carbohydrates (Fehling's test): 5 mg extract was heated with Benedict's reagent, reddish brown colouration indicated carbohydrates.

• Phenols: Filtered extract was boiled with 3-4 drops of 0.1% FeCl3; brownish green/blue colour indicated phenols.

.2.3. Quantitative Analysis

. • TOTAL PHENOLIC CONTENT: Calculated using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). 20 µL of the extract (made to 3 mL with distilled water) was added with 0.5 mL of the Folin-Ciocalteu reagent and allowed to incubate at 45°C for 3 minutes. Afterwards, 2 mL of 20% Na2CO3 solution was added and the absorbance recorded at 650 nm.

• TOTAL FLAVONOIDS CONTENT: Assayed using a modified spectrophotometric technique (Karadeniz et al., 2005). 0.5 mL of the extract was mixed with 3 mL distilled water and 0.3 mL of 5% sodium nitrite, incubated for 5 minutes and then 0.6 mL of 10% aluminum chloride was added. After 6 minutes, 2 mL of 1M NaOH solution was added to make up 10 mL and the absorbance read at 510 nm.

.2.4. Antioxidant Assay (DPPH)

The DPPH radical scavenging experiment was carried out as per the procedure described by Szabo et al. (2007). The reaction mixture, which comprised of 0.5 ml of sample, 1 ml of methanol, and 1 ml of 0.5 mM of DPPH radical solution, was incubated for 100 minutes, after which the color change of DPPH (violet to light yellow) was read at 517 nm.

Quercetin served as the standard, and percentage inhibition was calculated as:

% Inhibition = [(Control O.D. - Sample O.D.) / Control O.D.] × 100.

2.5. Antimicrobial Activity

The effectiveness of antimicrobial action was carried out using the agar well diffusion technique in the Mueller-Hinton Agar (MHA) medium. The test organisms (S. aureus and E. coli) were standardized at 1.5 x 10^8 CFU/ml (0.5 McFarland standard). Extract concentrations of 50, 75, and 100 µg/ml were introduced into the wells. Positive control was used as streptomycin 20 µg; negative control was 100% DMSO. Plates were incubated at 37°C for 18-24 h and ZOI was measured in mm.

3. Results and Discussion

3.1. Phytochemical Profiling

The qualitative analysis of the aqueous extract of P. guajava leaves has shown the presence of several bioactive secondary metabolites, but no glycosides (Table 1). All these data correlate with other studies which showed that guava leaves contain phenolics, flavonoids, saponins, and alkaloids (Sofowora, 1993; Edeoga et al., 2005). Also, the presence of saponins and terpenoids proves biological significance of polar plant extracts in traditional medicine (Tona et al., 2004).

Table 1: Qualitative analysis of Phytochemicals in *P. guajava* Aqueous Extract

Phytochemicals	**Result DOCX**
Alkaloids	+
Carbohydrates	+
Glycosides	-
Saponins	+
Proteins	+
Phenols	+
Flavonoids	+

The quantitative results showed total phenol content of 1.15 mg GAE/mg and total flavonoid content of 1.15 mg QE/mg. Phenolics and flavonoids are well known for their excellent free radical

scavenging abilities (Harborne, 1998). The results clearly show that the aqueous extraction method is very efficient for the extraction of phenolics owing to the solubility of their hydroxyl groups in water, which is consistent with research carried out by Thaipong et al. (2006).

3.2. Antioxidant Activity

In terms of quantification, the extract had a total phenolic content of 1.15 mg GAE/mg and total flavonoid content of 1.15 mg QE/mg. Both phenolic and flavonoids are known for their strong capability in scavenging free radicals (Harborne, 1998). The results indicate that extraction using aqueous solvent was highly efficient in extracting phenolic components because of the solubility of its hydroxyl group in water, consistent with the findings of Thaipong et al. (2006).

Table 2: DPPH radical scavenging activity of *P. guajava* extract

Concentration (µg/ml)	Inhibition (%) DOCX	DPPH Standard (Quercetin %) DOCX
20	3.428	32.86
40	13.14	49.43
60	24.57	63.81
80	37.71	78.30
100	57.71	93.93

3.3. Antimicrobial Activity

This aqueous extract had a strong, dose-dependent inhibitory effect on both Gram positive and Gram negative bacteria (Table 3). The inhibition zones of S. aureus ranged from 14 mm to 17 mm, whereas the inhibition zones of E. coli were larger, with ranges from 17 mm to 19 mm, at concentrations of 50-100 µg/ml. The strong activity against E. coli indicates that the active compounds that can dissolve in water from the P. guajava plant have the capacity to penetrate or avoid the outer membrane of Gram negative bacteria. The observed antimicrobial action of the aqueous extract could be caused by the presence of tannins, flavonoids, and alkaloids.

Table 3: Antimicrobial activity of *P. guajava* extract

Organism	Streptomycin (20 µg) Control DOCX	50 µg/ml DOCX	75 µg/ml DOCX	100 µg/ml DOCX
Staphylococcus aureus	12 mm	14 mm	16 mm	17 mm
Escherichia coli	10 mm	17 mm	18 mm	19 mm

4. Conclusion

This current study scientifically proves the presence of potent bioactive secondary metabolites like phenols, flavonoids, and alkaloids in aqueous leaf extract of Psidium guajava L. The extract showed strong concentration dependent free radicals scavenging ability as well as broad spectrum antibacterial activity against S. aureus and E. coli. This shows the scientific basis behind the use of guava leaf extract in treating infections as well as oxidative stress related conditions. Further studies in vivo toxicity testing can be done for pharmacological application.

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