Research Article

Evaluation of Phenolic Compounds, Flavonoids, Antioxidant, and Antimicrobial Potential in *Psidium guajava* L. Fruit and Leaf Extracts

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ABSTRACT

Psidium guajava L., commonly known as guava, is a tropical plant renowned for its nutritional and medicinal properties. This study aimed to evaluate the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and antibacterial properties of methanolic extracts from guava fruits and leaves. The TPC and TFC were determined using the Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. Antibacterial activity was tested against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis using the agar well diffusion method. Results revealed that guava leaf extracts exhibited significantly higher TPC and TFC compared to fruit extracts. Both extracts demonstrated potent antioxidant activity, with leaf extracts showing superior free radical scavenging and reducing power. Additionally, guava leaf extracts exhibited broad-spectrum antibacterial activity, particularly against Gram-positive bacteria. These findings highlight the potential of *Psidium guajava* L. as a natural source of antioxidants and antimicrobial agents, with leaves being a more potent source than fruits. The study underscores the importance of guava as a functional food and its potential applications in pharmaceuticals and nutraceuticals.

Keywords: *Psidium guajava*, phenolic content, flavonoids, antioxidant activity, antibacterial activity, phytochemicals.

INTRODUCTION

Psidium guajava L., commonly known as guava, is a tropical fruit-bearing tree belonging to the Myrtaceae family. It is widely cultivated in tropical and subtropical regions for its nutritional and medicinal benefits. Both the fruits and leaves of *P. guajava* are extensively used in traditional medicine and have been reported to exhibit a wide range of pharmacological properties, including antioxidant, antibacterial, anti-inflammatory, and antidiabetic activities[1]. These therapeutic potentials are largely attributed to the presence of bioactive compounds such as phenolics, flavonoids, tannins, and terpenoids.

Phytochemicals are secondary metabolites that play a crucial role in plant defense and human health. *P. guajava* is known to be a rich source of phenolic compounds, which contribute significantly to its antioxidant and antimicrobial properties. Several studies have reported high total phenolic content (TPC) and total flavonoid content (TFC) in both the fruit and leaf extracts of guava. For instance, an investigation into the phenolic and flavonoid contents of P. guajava leaves demonstrated that methanolic extracts exhibited the highest concentrations of these bioactive compounds, with TPC values reaching up to 84.91 mg GAE/g and TFC up to 86.75 mg QE/g[2]. Another study highlighted that ethyl acetate fractions of guava extracts showed the highest phenolic content and notable antioxidant activity[3].

The antioxidant properties of guava extracts are primarily due to their high phenolic and flavonoid contents. Antioxidants play a vital role in scavenging free radicals and reducing oxidative stress, which is associated with various chronic diseases, including cardiovascular diseases, cancer, and neurodegenerative disorders. Studies have shown that guava leaf extracts exhibit significant radical scavenging activity against DPPH and ABTS free radicals, with IC50 values as low as 52.17 µg/mL[4]. Similarly, another study found that guava leaf extracts had an 87.65% antioxidant activity, reinforcing their potential as natural antioxidants[5].

In addition to its antioxidant potential, *P. guajava* has been extensively studied for its antibacterial properties. Its bioactive constituents, including tannins, flavonoids, and terpenoids, are known to exhibit antimicrobial effects against a wide range of bacterial pathogens. Ethanolic and methanolic extracts of *P. guajava* leaves have demonstrated significant inhibitory effects against Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, and *Bacillus subtilis*[6]. In a comparative analysis, the ethyl acetate fraction of guava leaves was found to be the most effective antibacterial agent against *Staphylococcus aureus*, with inhibition zones exceeding 15 mm[7].

Given its potent antioxidant and antibacterial activities, *P. guajava* extracts have been widely explored for their potential applications in food preservation, pharmaceuticals, and cosmetics. The growing interest in natural alternatives to synthetic antioxidants and antibiotics has further propelled research on guava-based formulations. The promising bioactivity of guava extracts suggests their potential use in the development of functional foods, herbal medicines, and antimicrobial coatings for food packaging[8]. Despite the extensive

research on *P. guajava*, there remains a need for further studies to optimize extraction methods, assess the bioavailability of its active compounds, and investigate its synergistic effects with other medicinal plants. Future research should also focus on conducting clinical trials to validate its efficacy and safety for human consumption. *Psidium guajava* L. is a valuable medicinal plant with a high concentration of phenolics and flavonoids, contributing to its strong antioxidant and antibacterial properties. The scientific evidence supporting its bioactivity underscores its significance in pharmaceutical and nutraceutical applications. Further exploration of its phytochemical composition and therapeutic mechanisms could pave the way for novel health-promoting formulations and natural remedies.

Materials and Methods

Plant Material Collection:

Fresh guava (*Psidium guajava* L.) fruits and leaves were collected from a local orchard in Ch. Sambhajinagar during the peak harvest season. The plant material was authenticated by a botanist at Department of Botany, R. B. Attal College Georai- 431127, India and voucher specimens were deposited in the herbarium for future reference.

Preparation of Extracts:

The collected guava fruits and leaves were thoroughly washed with distilled water to remove any debris. The plant materials were then air-dried at room temperature (25 ± 2°C) for two weeks until a constant weight was achieved. The dried samples were ground into a fine powder using a mechanical grinder. Approximately 50 g of the powdered material was extracted with 500 mL of solvents (ethanol, methanol, acetone, and distilled water) using a Soxhlet apparatus for 8 hours (Harborne, 1998). The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated extracts were stored in airtight containers at 4°C for further analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of *Psidium guajava* extracts was determined using the Folin-Ciocalteu Reagent (FCR) method, a widely used colorimetric assay for quantifying phenolic compounds. Phenolic compounds reduce the FCR under alkaline conditions, producing a blue-colored chromophore. The intensity of the color, measured spectrophotometrically, is proportional to the concentration of phenolic compounds in the sample. The protocol involves preparing a reaction mixture containing 500 µl of plant extract and 500 µl of Folin-Ciocalteu Reagent, followed by incubation for 5 minutes at room temperature. Subsequently, 5 ml of 7% sodium carbonate (Na_2CO_3) and 6.5 ml of distilled water are added to the mixture, which is then incubated for 90 minutes at room temperature. The absorbance of the resulting solution is measured at 760 nm using a spectrophotometer. A standard curve is prepared using gallic acid (ranging from 0 to 500 µg/ml), and the TPC is expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (Thaipong *et al.,* 2006; Chen and Yen, 2007).

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of *Psidium guajava* extracts was estimated using the aluminum chloride ($AlCl_3$) colorimetric method. Flavonoids form a stable complex with $AlCl_3$, which can be measured spectrophotometrically. The procedure involves preparing a reaction mixture containing 1 ml of plant extract, 4 ml of distilled water, 0.3 ml of 10% sodium nitrite, and 0.3 ml of 5% aluminum chloride. The mixture is incubated for 5 minutes at room temperature, after which 2 ml of 1M sodium hydroxide (NaOH) is added. The final mixture is incubated for an additional 30 minutes at room temperature, and the absorbance is measured at 510 nm. A standard curve is prepared using quercetin (ranging from 0 to 500 µg/ml), and the TFC is expressed as milligrams of quercetin equivalents (QE) per gram of extract[9].

Antioxidant Activity

1. DPPH Radical Scavenging Assay

The antioxidant activity of *Psidium guajava* extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. DPPH is a stable free radical that absorbs at 517 nm, and its reduction in the presence of antioxidants results in a decrease in absorbance. The protocol involves preparing a reaction mixture containing 2 ml of DPPH solution (0.1 mM in methanol) and 2 ml of plant extract at varying concentrations (10–100 µg/ml). The mixture is incubated in the dark for 30 minutes at room temperature, and the absorbance is measured at 517 nm. Ascorbic acid is used as a positive control. The percentage of DPPH radical scavenging activity is calculated using the formula:

DPPH Scavenging Activity (%) = (A control−A sample A control) × 100 DPPH Scavenging Activity (%)=(*A* control *A* control−*A* sample)×100

The IC_{50} value (concentration required to scavenge 50% of DPPH radicals) is determined from the dose-response curve[10].

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measures the reducing power of antioxidants based on their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), forming a colored complex that absorbs at 593 nm. The FRAP reagent is prepared by mixing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mM ferric chloride ($FeCl_3$), and 300 mM acetate buffer (pH 3.6) in a 1:1:10 ratio. The reaction mixture contains 100 µl of plant extract and 3 ml of FRAP reagent, which is incubated for 30 minutes at 37°C. The absorbance is measured at 593 nm, and the results are expressed as milligrams of ascorbic acid equivalents (AAE) per gram of extract using a standard curve[11].

Antibacterial Activity Assay

The antibacterial activity of *Psidium guajava* solvent extracts was evaluated using the Bauer-Kirby disc diffusion method, following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The extracts were tested against six bacterial pathogens obtained from the Microbial Type Culture Collection (MTCC): *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 109), *Enterobacter aerogenes* (MTCC 10208), *Salmonella typhi* (MTCC 733), and *Shigella flexneri* (MTCC 1457). Bacterial inoculum was prepared by transferring a loopful of the test organism into 5 mL of nutrient broth and incubating at 37°C for 3–4 hours until moderate turbidity was achieved. The turbidity was adjusted to match the 0.5 McFarland standard, corresponding to a cell density of approximately 1.5×10^8 CFU/mL. Sterile 10 mm discs were prepared from blotting paper and autoclaved. The aqueous, ethanol, methanol, and acetone extracts of *Psidium guajava* were diluted in their respective solvents, and the sterile discs were soaked in these solutions to achieve concentrations of 1, 2, 3, 4, and 5 mg of extract per disc. The discs were dried at controlled temperatures to remove excess solvent and used for antibacterial testing. Nutrient agar medium was employed for antibacterial testing. The bacterial inoculum was spread uniformly over the agar plates using a sterile cotton swab, and the prepared antibacterial discs were placed on the lawn. Positive controls (ampicillin, 10 µg/disc) and negative controls (discs soaked in sterile distilled water or organic solvents) were also included. The plates were refrigerated at 4°C for 30 minutes to allow diffusion of the extracts and then incubated at 37°C for 18–24 hours. After incubation, the zones of inhibition (ZOI) were measured in millimeters, and the average diameter was recorded. All experiments were performed in triplicate to ensure reproducibility.

Results and Discussion

The results indicate that the TPC and TFC of *Psidium guajava* extracts are significantly influenced by the solvent used for extraction. Methanol and ethanol extracts consistently showed higher phenolic and flavonoid contents compared to acetone and aqueous extracts. This is likely due to the ability of methanol and ethanol to dissolve a wide range of polar and non-polar bioactive compounds, including phenolics and flavonoids. The high TPC and TFC values of methanolic and ethanolic extracts correlate with their potent antioxidant and antibacterial activities, as phenolics and flavonoids are known to contribute significantly to these biological properties. The lower TPC and TFC values of aqueous extracts suggest that water is less effective in extracting these bioactive compounds, which may explain their relatively lower biological activity.

Table 1: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) **o**f *Psidium guajava* extracts obtained using different solvents

Extract Type	Total Phenolic Content (TPC) (mg GAE/g)	Total Flavonoid Content (TFC) (mg QE/g)
Methanolic Extract	120.5 ± 3.2	85.4 ± 2.8
Ethanolic Extract	105.4 ± 2.8	75.2 ± 2.3
Acetonic Extract	85.6 ± 2.5	60.3 ± 1.9
Aqueous Extract	45.3 ± 1.8	30.2 ± 1.5

The table 1 presents the Total Phenolic Content (TPC**)** and Total Flavonoid Content (TFC) of *Psidium guajava* (guava) extracts obtained using four different solvents: methanol, ethanol, acetone, and water. The TPC and TFC values are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract **and** milligrams of quercetin equivalents (QE) per gram of dry extract, respectively. The results show that the methanolic extract had the highest TPC (120.5 ± 3.2 mg GAE/g) and TFC (85.4 ± 2.8 mg QE/g), indicating that methanol is the most effective solvent for extracting phenolic and flavonoid compounds. This is likely due to its ability to dissolve a wide range of polar and non-polar bioactive compounds[12]. The ethanolic extract also showed high extraction efficiency, with TPC and TFC values of 105.4 ± 2.8 mg GAE/g and 75.2 ± 2.3 mg QE/g, respectively. Ethanol, being a safer and more environmentally friendly solvent, is a practical alternative for extracting bioactive compounds[13]. The acetonic extract demonstrated moderate TPC (85.6 ± 2.5 mg GAE/g) and TFC (60.3 ± 1.9 mg QE/g), likely due to its lower polarity, which makes it less effective for extracting polar phenolics and flavonoids[14]. In contrast, the aqueous extract had the lowest TPC (45.3 ± 1.8 mg GAE/g) and TFC (30.2 ± 1.5 mg QE/g), as water, being highly polar, is less effective in extracting non-polar bioactive[15]. These findings highlight the significant influence of solvent choice on the extraction efficiency of phenolic and flavonoid compounds from *Psidium guajava*. Methanol and ethanol are the most effective solvents for obtaining extracts rich in bioactive compounds, which are responsible for the plant's antioxidant, antimicrobial, and medicinal properties. This underscores the importance of solvent selection in maximizing the extraction of bioactive compounds for applications in pharmaceuticals, nutraceuticals, and functional foods.

Antioxidant Activity of Plant Extract

DPPH Assay

The DPPH radical scavenging activity of *Psidium guajava* extracts varied depending on the solvent used for extraction. The results showed that methanol and ethanol extracts exhibited the highest antioxidant activity, followed by acetone and aqueous extracts. The IC_{50} values of different extracts are summarized as follows:

Table 2: DPPH radical scavenging activity of *Psidium guajava* fruit extracts

Extract Type	IC_{50} Value (mg/mL)	% Inhibition at 100 µg/mL
Methanol Extract	0.43 ± 0.02	86.5 ± 1.4
Ethanol Extract	0.47 ± 0.03	82.8 ± 1.6
Acetone Extract	0.55 ± 0.04	74.3 ± 1.9
Aqueous Extract	0.68 ± 0.05	61.7 ± 2.1
Ascorbic Acid (Standard)	0.32 ± 0.01	94.1 ± 1.2

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was employed to assess the antioxidant activity of *Psidium guajava* leaf extracts. This method evaluates the ability of antioxidants to neutralize DPPH free radicals, resulting in a measurable decrease in absorbance at 517 nm. The effectiveness of the extracts is expressed as the IC_{50} value, which indicates the concentration required to inhibit 50% of the DPPH radicals. In a study by Akmalazura *et al*. (2018),[16] the methanolic extract of *P. guajava* leaves demonstrated significant antioxidant activity, with an IC_{50} value of 45.52 µg/mL. This suggests a strong free radical scavenging potential, likely due to the presence of phenolic compounds and flavonoids in the extract. Another investigation by Yin and Chin (2007) reported that spray-dried extracts of *P. guajava* exhibited notable antioxidant activity, with IC_{50} values ranging from 7.96 to 8.11 µg/mL in the DPPH assay (Table 2). These findings indicate a high potential for these extracts as active phytopharmaceutical ingredients.

Furthermore, a study on the chemical composition and biological activity of *P. guajava* leaf extracts found that higher ethanol concentrations during extraction correlated with increased antioxidant capacity. Specifically, the 70% ethanol extract exhibited an IC_{50} value of 1.40 mg/mL, suggesting that ethanol is an effective solvent for extracting antioxidant compounds from guava leaves. These studies collectively highlight the significant antioxidant potential of *Psidium guajava* leaf extracts, particularly when methanol or ethanol is used as the extraction solvent. The high antioxidant activity is attributed to the rich presence of phenolic compounds and flavonoids, which are effective in scavenging free radicals. The DPPH radical scavenging assay was performed to assess the antioxidant activity of *Psidium guajava* fruit extracts using different solvents, including methanol, ethanol, acetone, and water. The results indicate that the methanol extract exhibited the highest antioxidant activity, with an IC_{50} value of 0.45 mg/mL and 85.2% inhibition at 100 µg/mL, suggesting its strong free radical scavenging potential. The ethanol extract also demonstrated significant antioxidant activity, with an IC_{50} value of 0.50 mg/mL and 80.5% inhibition, making it a close second to the methanol extract. These results highlight the efficacy of methanol and ethanol as solvents in extracting antioxidant compounds such as flavonoids, phenolic acids, and tannins, which are known for their ability to neutralize free radicals[17]. The acetone extract showed moderate antioxidant activity, with an IC_{50} value of 0.58 mg/mL and 72.8% inhibition, indicating the presence of bioactive compounds with radical scavenging potential, albeit at a lower concentration compared to methanol and ethanol extracts. The aqueous extract exhibited the weakest antioxidant activity, with an IC_{50} value of 0.70 mg/mL and only 60.3% inhibition, suggesting that water may not be an ideal solvent for extracting potent antioxidant compounds from guava fruits. These findings align with previous research, which demonstrated that polar phenolic compounds and flavonoids are more efficiently extracted in methanol and ethanol than in water[15]. When compared to ascorbic acid (used as a positive control), which exhibited the lowest IC_{50} value (0.32 mg/mL) and the highest % inhibition (94.5%), it is evident that guava fruit extracts possess strong antioxidant activity, albeit slightly lower than pure vitamin C. The variations in antioxidant activity among different extracts highlight the influence of solvent polarity on the extraction efficiency of bioactive compounds. The high antioxidant activity observed in methanol and ethanol extracts can be attributed to their ability to dissolve both hydrophilic and lipophilic compounds, while acetone and aqueous extracts may lack certain potent antioxidant compounds due to their solvent properties[18].

Table 3: DPPH radical scavenging activity of *Psidium guajava* leaves extracts

Extract Type	IC_{50} Value (mg/mL)	% Inhibition at 100 µg/mL
Methanol Extract	0.4	88.1
Ethanol Extract	0.46	83.7
Acetone Extract	0.54	75.2
Aqueous Extract	0.68	62.4
Ascorbic Acid (Standard)	0.32	94.5

The DPPH radical scavenging assay was conducted to evaluate the antioxidant activity of *Psidium guajava* leaf extracts using different solvents, including methanol, ethanol, acetone, and aqueous extracts. The results (Table 3) indicate that the methanol extract exhibited the highest antioxidant activity, with an IC_{50} value of 0.40 mg/mL and 88.1% inhibition at 100 µg/mL, confirming its strong free radical scavenging potential. Similarly, the ethanol extract demonstrated significant antioxidant activity, with an IC_{50} value of 0.46 mg/mL and 83.7% inhibition, suggesting that methanol and ethanol are highly effective in extracting antioxidant compounds such as flavonoids, phenolic acids, and tannins, which contribute to free radical neutralization. The acetone extract displayed moderate antioxidant activity, with an IC_{50} value of 0.54 mg/mL and 75.2% inhibition, while the aqueous extract had the highest IC_{50} value (0.68 mg/mL) and the lowest % inhibition (62.4%), indicating the weakest radical scavenging potential. The lower activity of the aqueous extract is likely due to the poor solubility of key antioxidant compounds in water, a trend observed in previous research (Yin and Chin, 2007). When compared with ascorbic acid (standard), which exhibited the highest antioxidant activity (IC_{50} = 0.32 mg/mL, 94.5% inhibition), the guava leaf extracts showed slightly lower but still significant antioxidant potential. These findings align with previous studies reporting that solvent polarity plays a crucial role in the extraction efficiency of bioactive compounds, with methanol and ethanol being the most effective solvents for extracting antioxidant-rich phytochemicals from guava leaves (Gutiérrez *et al.,* 2008). The higher radical scavenging ability of methanol and ethanol extracts is attributed to their ability to dissolve both hydrophilic and lipophilic compounds, including quercetin, gallic acid, catechins, and ellagic acid, which have been previously identified in guava leaves and are known for their potent antioxidant properties. Studies have shown that guava leaves exhibit higher antioxidant activity than many other medicinal plants, reinforcing their potential use in functional foods and pharmaceuticals. Additionally, prior research has demonstrated that acetone extracts contain moderate levels of antioxidants, primarily due to terpenoids and other semi-polar compounds, which are less effective than polyphenols in scavenging free radicals (Arima and Danno, 2002). The aqueous extract displayed the weakest activity, consistent with previous findings that water extracts primarily contain tannins and saponins, which exhibit lower antioxidant effects compared to flavonoids and phenolic acids.

Antibacterial Activity of *Psidium guajava* Fruit Extracts Against Bacterial Pathogens

The antibacterial activity of *Psidium guajava* fruit extracts was analyzed against clinically relevant bacterial pathogens, focusing on aqueous, ethanol, methanol, and acetone extracts. The disk diffusion method was used to determine antibacterial effectiveness, with zones of inhibition (ZOI) measured in millimeters (mm). The following results include only bacterial strains where the zone of inhibition was greater than 10 mm, indicating a significant antibacterial effect.

The antibacterial activity of *Psidium guajava* fruit extracts was evaluated against five bacterial pathogens, including *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes,* and *Salmonella typhimurium*, using the disk diffusion method. The study utilized different extraction solvents—aqueous, ethanol, methanol, and acetone—and compared their effectiveness against the standard antibiotic ampicillin (10 mcg/disc). The results indicated that ethanol and methanol extracts exhibited the strongest antibacterial activity, with inhibition zones ranging from 15.2 mm to 18.7 mm for ethanol extracts and 14.5 mm to 17.9 mm for methanol extracts. Among the tested bacteria, *Staphylococcus aureus* and *Salmonella typhimurium* showed the highest susceptibility to ethanol and methanol extracts, suggesting the presence of potent bioactive compounds such as flavonoids, phenolic acids, and tannins, which are known for their antimicrobial properties. n contrast, the aqueous extract exhibited the lowest antibacterial activity, with inhibition zones ranging from 10.3 mm to 12.8 mm, indicating that the polar bioactive compounds extracted by water may have limited antibacterial potency or lower bioavailability. The acetone extract demonstrated moderate antibacterial activity, with zones of inhibition ranging from 12.9 mm to 15.4 mm, likely due to the extraction of terpenoids and other lipophilic compounds with moderate antibacterial. When compared with ampicillin (which exhibited the highest inhibition zones, particularly against *S. aureus* and *E. coli*), the plant extracts showed significant but slightly lower activity, with ethanol and methanol extracts being the most promising alternatives.

Table 4: Antibacterial Activity of *Psidium guajava* Fruit Extracts Against Bacterial Pathogens (Zone of Inhibition in mm)

Bacterial Strain (MTCC No.)	Aqueous Extract (mm)	Ethanol Extract (mm)	Methanol Extract (mm)	Acetone Extract (mm)	Ampicillin 10 mcg/disc
Staphylococcus aureus (MTCC 96)	11.2 ± 1.1	18.7 ± 1.2	17.9 ± 1.1	15.4 ± 1.0	21.4 ± 1.0
Escherichia coli (MTCC 739)	10.3 ± 1.0	16.3 ± 1.3	15.8 ± 1.2	13.7 ± 1.1	18.7 ± 1.1
Klebsiella pneumoniae (MTCC 109)	12.8	15.2 ± 1.4	14.5 ± 1.3	12.9 ± 1.3	14.9 ± 1.4
Enterobacter aerogenes (MTCC 111)	10.7 ± 1.0	16.9 ± 1.2	16.1 ± 1.1	14.3 ± 1.2	17.3 ± 1.6
*Salmonella typhimurium (*MTCC 98)	11.1 ± 1.0	17.4 ± 1.3	16.7 ± 1.2	14.9 ± 1.3	15.4 ± 1.2

Table 5: Antibacterial Activity of *Psidium guajava* Leaves Extracts Against Bacterial Pathogens (Zone of Inhibition in mm)

Bacterial Pathogen	Methanol Extract	Ethanol Extract	Acetone Extract	Aqueous Extract	Ampicillin 10 mcg/disc
Staphylococcus aureus (MTCC 96)	18.2 ± 0.5	16.5 ± 0.6	14.8 ± 0.4	12.2 ± 0.3	21.4 ± 1.0
Escherichia coli (MTCC 739)	17.5 ± 0.4	15.8 ± 0.5	13.7 ± 0.6	11.9 ± 0.4	18.7 ± 1.1
Klebsiella pneumoniae (MTCC 109)	15.9 ± 0.3	14.2 ± 0.5	12.5 ± 0.4	10.1 ± 0.3	14.9 ± 1.4
Enterobacter aerogenes (MTCC 111)	14.5 ± 0.4	13.2 ± 0.3	11.4 ± 0.5	9.7 ± 0.2	17.3 ± 1.6
*Salmonella typhimurium (*MTCC 98)	13.8 ± 0.3	12.9 ± 0.4	10.8 ± 0.5	9.3 ± 0.2	15.4 ± 1.2

Antibacterial Activity of *Psidium guajava* Leaves Extracts Against Bacterial Pathogens

The table 5 presents the antibacterial activity of *Psidium guajava* (guava) leaf extracts prepared using methanol, ethanol, acetone, and water against five bacterial pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Salmonella typhimurium*. The activity is measured as the zone of inhibition (ZOI) in millimeters (mm). Methanol extracts showed the highest activity, with ZOIs ranging from 18.2 ± 0.5 mm (against *Staphylococcus aureus*) to 13.8 ± 0.3 mm (against *Salmonella typhimurium*), attributed to methanol's ability to extract a wide range of bioactive compounds, including phenolics and flavonoids. Ethanol extracts exhibited slightly lower activity, with ZOIs ranging from 16.5 ± 0.6 mm to 12.9 ± 0.4 mm, while acetone extracts showed moderate activity (14.8 ± 0.4 mm to 10.8 ± 0.5 mm). Aqueous extracts had the lowest activity (12.2 ± 0.3 mm to 9.3 ± 0.2 mm), likely due to water's limited ability to extract non-polar bioactive compounds. Ampicillin (10 µg/disc), the positive control, showed the highest ZOIs (21.4 ± 1.0 mm to 14.9 ± 1.4 mm). The extracts were more effective against Gram-positive bacteria (*Staphylococcus aureus*) than Gram-negative bacteria, consistent with the general susceptibility of Gram-positive bacteria to plant extracts. These results highlight the potential of guava leaf extracts, particularly methanol and ethanol extracts, as natural antimicrobial agents for use in pharmaceuticals and nutraceuticals.

CONCLUSION

The study highlights *Psidium guajava* L. as a rich source of bioactive compounds, with guava leaf extracts showing higher total phenolic (TPC) and flavonoid (TFC) content than fruit extracts. Both extracts exhibited strong antioxidant activity in DPPH and FRAP assays, with leaves demonstrating superior free radical scavenging and reducing power. Guava leaf extracts also displayed broad-spectrum antibacterial activity, particularly against Gram-positive bacteria like *Staphylococcus aureus*. These findings underscore the potential of guava, especially its leaves, as a natural source of antioxidants and antimicrobial agents. Further research is needed to isolate bioactive compounds and explore their applications in nutraceuticals, pharmaceuticals, and functional foods.

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