Vol. 2 No. 3 (2025)
ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081
Journal of Medical & Health Sciences Review

EXTRACTION, PHYTOCHEMICAL ANALYSIS, AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF *PSIDIUM GUAJAVA* (GUAVA) LEAVES

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ARTICLE INFO:

Keywords:
Psidium guajava, guava leaves, phytochemical screening, TPC, TFC, DPPH, ABTS, FRAP, antioxidant activity

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Article History:
Published on July 26, 2025

ABSTRACT

Psidium guajava (guava) is a tropical plant renowned for its ethnomedicinal properties. This study aimed to conduct a comprehensive investigation of the phytochemical constituents and antioxidant potential of guava leaf extracts using multiple *in vitro* models. Leaves collected from the Charsadda region were sequentially extracted using n-hexane, chloroform, and methanol. The extracts were evaluated for qualitative and quantitative phytochemistry, including total phenolic content (TPC) and total flavonoid content (TFC). Antioxidant activity was assessed using four different assays: DPPH, ABTS, Ferric Reducing Antioxidant Power (FRAP), and a Phosphomolybdenum assay for total antioxidant capacity (TAC). The methanol extract yielded the highest extractive value (9.85%) and contained the highest levels of TPC (185.42 ± 4.86 mg GAE/g) and TFC (92.75 ± 3.12 mg QE/g). Phytochemical screening confirmed the presence of flavonoids, phenols, terpenoids, steroids, and saponins in the methanol extract. In all antioxidant assays, the methanol extract demonstrated superior, dose-dependent activity, with an IC_{50} of 46.18 ± 1.45 µg/mL in the DPPH assay, significantly lower than the other extracts. A strong positive correlation ($R^2 > 0.95$) was observed between TPC/TFC and antioxidant activity. The study conclusively establishes that the methanol extract of *P. guajava* leaves is a rich source of natural antioxidants, primarily phenolics and flavonoids, validating its traditional use and potential for nutraceutical development.

1. INTRODUCTION

Reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals, and peroxides, are natural byproducts of cellular metabolism (Rahal et al., 2014). At controlled levels, they play a role in cell signaling and homeostasis. However, their overproduction leads to oxidative stress, a state implicated in the pathogenesis of numerous chronic disease, including cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions (Pham-Huy et al., 2008; Nordberg & Arnér, 2001). This oxidative damage occurs when ROS overwhelm the body's intrinsic antioxidant defense systems, causing oxidation of lipids, proteins, and DNA (Iqbal et al.,2015). The growing awareness of the detrimental effects of synthetic antioxidants has spurred intensive research into natural and safer alternatives, particularly from plant sources (Niciforović et al., 2010). Plants are a rich reservoir of bioactive compounds like phenolics, flavonoids, terpenoids, and alkaloids, which possess potent antioxidant activities capable of neutralizing free radicals and chelating pro-oxidant metals (Bouaziz et al., 2015).

Psidium guajava L., commonly known as guava, is a small tree belonging to the Myrtaceae family, native to tropical America but widely cultivated in regions including South Asia (Gutiérrez et al., 2008). It is extensively used in traditional medicine systems across the world. Different parts of the plant, especially the leaves, are employed to treat a variety of ailments such as gastroenteritis, dysentery, diabetes, hypertension, wounds, and inflammation (Gutiérrez et al., 2008). These ethnomedicinal uses are supported by scientific studies that have demonstrated the plant's antimicrobial, antidiabetic, anti-inflammatory, antispasmodic, and analgesic properties (Sanches et al., 2005; Wan Nur Zahidah et al., 2013).

The pharmacological activities of *P. guajava* are largely attributed to its diverse phytochemical composition. Previous investigations have reported the presence of flavonoids (e.g., quercetin), tannins, triterpenoids, phenolic acids, and essential oils in its leaves (Begum et al., 2004; Morais-Braga et al., 2016). However, the phytochemical profile and bioactivity of plants can be significantly influenced by geographical location, climate, soil conditions, and extraction methodologies (Baloch et al., 2024). While studies on guava leaves from other regions like Selangor (Malaysia) and Hyderabad (Pakistan) exist, there is a scarcity of detailed phytochemical and antioxidant profiling of specimens from the Khyber Pakhtunkhwa region of Pakistan.

Therefore, this study was designed to systematically extract and screen the phytochemical constituents of *P. guajava* leaves collected from Charsadda, Pakistan, using solvents of varying polarity. A comprehensive quantitative analysis of phenolics and flavonoids was conducted. Furthermore, the antioxidant potential of these extracts was quantitatively evaluated using multiple *in vitro* assays (DPPH, ABTS, FRAP, and Phosphomolybdenum) to establish a robust scientific basis for its traditional use and to explore its potential as a source of natural antioxidants.

2. Materials and Methods

2.1. Plant Material Collection and Identification

Fresh, mature leaves of *Psidium guajava* (Guava) were collected from the botanical garden of Bacha Khan University, Charsadda. The plant was authenticated by a botanist at the Department of Botany, Bacha Khan University. All other chemicals and solvents used were of analytical grade.

2.2. Preparation of Plant Material

The collected leaves were thoroughly washed with tap water to remove dust and adherent impurities, followed by a final rinse with

distilled water. They were then shade-dried at room temperature (25-30°C) for three weeks to constant weight. The completely dried leaves were ground into a coarse powder using a mechanical grinder. The powder was stored in an airtight container at 4°C until further use.

2.3. Sequential Solvent Extraction

The powdered plant material (250 g) was subjected to sequential maceration with solvents of increasing polarity: n-hexane, chloroform, and methanol. The process involved soaking the powder in 1.5 L of n-hexane for 72 hours with occasional shaking. The mixture was filtered using Whatman No. 1 filter paper, and the marc (the residual plant material) was air-dried to evaporate the residual solvent. The dried marc was then successively macerated with chloroform and finally with methanol, each for 72 hours, following the same procedure. The filtrates from each solvent were concentrated under reduced pressure at 40°C using a rotary evaporator (Heidolph, Germany). The yields of the crude extracts were calculated using the formula:

Percentage Yield=Weight of extract obtainedWeight of dry powder taken×100Percentage Yield=Weight of dry powder takenWeight of extract obtained×100

The concentrated extracts were stored in sterile vials at 4°C for subsequent analysis.

2.4. Phytochemical Screening

Standard qualitative chemical tests were conducted on the three extracts to identify the presence of major phytochemical classes, following established protocols with minor modifications (Iqbal et al., 2015; Jamuna et al., 2014).

1. **Test for Alkaloids (Wagner's Test):** To 2 mL of each extract, 6 drops of Wagner's reagent (iodine in potassium iodide) were added. The formation of a reddish-brown precipitate indicated the presence of alkaloids.
2. **Test for Flavonoids (Alkaline Reagent Test):** To 2 mL of the extract, a few drops of 2M sodium hydroxide (NaOH) were added. The formation of a yellow color that turned colorless upon the addition of dilute acid indicated the presence of flavonoids.
3. **Test for Phenolic Compounds (Ferric Chloride Test):** To 2 mL of the extract, 3-4 drops of 5% $FeCl_3$ solution were added. A bluish-black coloration confirmed the presence of phenols.
4. **Test for Terpenoids (Salkowski Test):** 2 mL of each extract was mixed with 2 mL of chloroform, and 1 mL of concentrated sulfuric acid was carefully added to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.
5. **Test for Steroids (Liebermann-Burchard Test):** About 100 mg of the extract was dissolved in 2 mL of chloroform. Acetic anhydride (2 mL) was added, followed by 1-2 drops of concentrated sulfuric acid down the side of the test tube. A color change from red to blue to green indicated the presence of steroids.
6. **Test for Saponins (Foam Test):** 2 mL of the extract was diluted with 6 mL of distilled water in a test tube and shaken vigorously for 2 minutes. The formation of a stable, persistent foam layer of about 1-2 cm indicated the presence of saponins.

2.5. Quantitative Phytochemical Analysis

2.5.1. Determination of Total Phenolic Content (TPC)

The TPC of the extracts was determined using the Folin-Ciocalteu method (Bouaziz et al., 2015). Briefly, 0.5 mL of each extract (1 mg/mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and allowed to stand for 5 minutes. Then, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added. The mixture was incubated in the dark for 90 minutes at room temperature. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. A standard calibration curve was prepared using

gallic acid (0-100 µg/mL). The TPC was expressed as milligrams of Gallic Acid Equivalents per gram of dry extract (mg GAE/g extract).

2.5.2. Determination of Total Flavonoid Content (TFC)

The TFC was estimated using the aluminum chloride colorimetric method (Jamuna et al., 2014). 1 mL of each extract (1 mg/mL) was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution. After 5 minutes, 0.3 mL of 10% $AlCl_3$ was added. At the 6th minute, 2 mL of 1M NaOH solution was added, and the total volume was made up to 10 mL with distilled water. The mixture was vortexed, and the absorbance was measured immediately at 510 nm. A standard calibration curve was prepared using quercetin (0-100 µg/mL). The TFC was expressed as milligrams of Quercetin Equivalents per gram of dry extract (mg QE/g extract).

2.6. Evaluation of *In Vitro* Antioxidant Activity

2.6.1. DPPH Radical Scavenging Assay

The free radical scavenging activity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Tagashira and Ohtake (1998) with slight modifications. Briefly, a 0.1 mM methanolic solution of DPPH was prepared fresh. Each extract was dissolved in methanol to prepare a stock solution of 1000 µg/mL. Serial dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL.

An aliquot of 0.2 mL of each sample concentration was mixed with 3.8 mL of the DPPH solution. The mixture was vortexed thoroughly and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm. A control was prepared by mixing 0.2 mL of methanol with 3.8 mL of DPPH solution (*Acontrol*). A blank for each sample was also prepared, containing 0.2 mL of the sample solution and 3.8 mL of methanol (*Ablank*), to correct for the color of the extract itself. Ascorbic acid was used as the standard reference antioxidant. The experiment was performed in triplicate.

The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\text{Scavenging Activity}\,(\%) = \left[\frac{(A_{control} - (A_{sample} - A_{blank}))}{A_{control}}\right] \times 100$$

Where:

- $A_{control}$ is the absorbance of the DPPH solution mixed with methanol (without extract).
- A_{sample} is the absorbance of the DPPH solution mixed with the extract.
- A_{blank} is the absorbance of the extract mixed with methanol (without DPPH).

The IC_{50} value (the concentration required to scavenge 50% of the DPPH radicals) was determined from the non-linear regression curve of the percentage inhibition plotted against the concentration.

2.6.2. ABTS Radical Cation Scavenging Assay

The ABTS+ scavenging activity was determined according to a standard method. The ABTS+ radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for 12-16 hours. This solution was then diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. 0.2 mL of each extract at different concentrations was mixed with 3.8 mL of the ABTS+ solution. After 6 minutes of incubation in the dark, the absorbance was measured at 734 nm. The percentage inhibition was calculated similarly to the DPPH assay, and the IC_{50} value was determined. Trolox was used as a standard.

2.6.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed as described by Benzie & Strain (1996). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 ratio. 0.1 mL

of the extract was mixed with 3 mL of the FRAP reagent and incubated at 37°C for 30 minutes. The increase in absorbance was measured at 593 nm. A standard curve was prepared using ferrous sulfate ($FeSO_4 \cdot 7H_2O$) (100-1000 µM), and the results were expressed as micromoles of Ferrous Equivalents per gram of extract (µM Fe^{2+} E/g extract).

2.6.4. Total Antioxidant Capacity (Phosphomolybdenum Assay)

The total antioxidant capacity was evaluated by the phosphomolybdenum method (Prieto et al., 1999). 1 mL of each extract (1 mg/mL) was combined with 3 mL of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 minutes. After cooling to room temperature, the absorbance of the solution was measured at 695 nm against a blank. Ascorbic acid was used as a standard, and the total antioxidant capacity was expressed as milligrams of Ascorbic Acid Equivalents per gram of extract (mg AAE/g extract).

2.7. Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). The IC_{50} values were calculated using non-linear regression analysis in GraphPad Prism software (Version 10.0). Statistical significance between the extracts was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with a p-value of < 0.05 considered statistically significant. Correlation analysis was performed using Pearson's correlation coefficient.

3. Results

3.1. Extraction Yield

The sequential extraction of *P. guajava* leaf powder with different solvents yielded crude extracts with varying weights and percentages. The results, presented in Table 1, show that the extraction yield was highly dependent on solvent polarity. The non-polar n-hexane gave the lowest yield (1.52%), followed by the intermediate polarity chloroform (2.35%). The polar methanol solvent produced the highest yield of 9.85%, suggesting that the majority of the soluble constituents in guava leaves are of a polar nature.

Table 1. Yield of *P. guajava* leaf extracts from sequential solvent extraction.

Solvent	Weight of Extract (g)	Percentage Yield (%)
n-Hexane	3.80	1.52
Chloroform	5.88	2.35
Methanol	24.63	9.85

3.2. Qualitative Phytochemical Screening

The results of the qualitative phytochemical analysis of the n-hexane, chloroform, and methanol extracts are summarized in Table 2. The methanol extract tested positive for the widest range of phytoconstituents, including flavonoids, phenols, terpenoids, steroids, and saponins. The chloroform extract showed the presence of phenols and terpenoids, while the n-hexane extract contained steroids and terpenoids. Alkaloids were not detected in any of the three extracts. Terpenoids were the only class of compounds found to be present in all three extracts.

Table 2. Phytochemical constituents of *P. guajava* leaf extracts.

Phytoconstituent	n-Hexane Extract	Chloroform Extract	Methanol Extract
Alkaloids	-	-	-
Flavonoids	-	-	+

Steroids	+	-	+
Saponins	-	-	+
Phenols	-	+	+
Terpenoids	+	+	+
Note: + = Present; - = Absent			

3.3. Quantitative Phytochemical Analysis

The quantitative analysis of phenolics and flavonoids revealed significant differences among the extracts (Table 3, Figure 1). The methanol extract possessed the highest TPC (185.42 ± 4.86 mg GAE/g) and TFC (92.75 ± 3.12 mg QE/g), which were significantly greater ($p < 0.001$) than those of the chloroform and n-hexane extracts. The chloroform extract showed moderate levels (TPC: 45.18 ± 2.54 mg GAE/g; TFC: 21.65 ± 1.87 mg QE/g), while the n-hexane extract contained negligible amounts of these polar compounds.

Table 3. Total phenolic and flavonoid content of *P. guajava* leaf extracts.

Extract	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)
n-Hexane	12.35 ± 1.45 a	8.42 ± 0.95 a
Chloroform	45.18 ± 2.54 b	21.65 ± 1.87 b
Methanol	185.42 ± 4.86 c	92.75 ± 3.12 c
Values are mean ± SD (n=3). Different superscript letters (a, b, c) in the same column indicate significant differences ($p < 0.05$).		

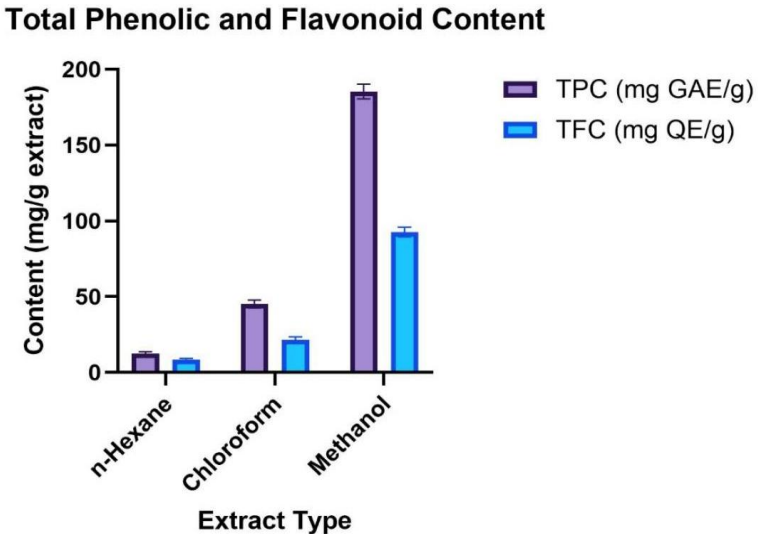

Figure 1. Total Phenolic and Flavonoid Content of Guava Leaf Extracts. A bar graph showing the significantly higher TPC and TFC in the methanol extract compared to the chloroform and n-hexane extracts.

3.4. *In Vitro* Antioxidant Activity

3.4.1. DPPH Radical Scavenging Assay

The methanol extract exhibited a strong, concentration-dependent radical scavenging activity, with an inhibition of 86.45% at the highest tested concentration (1000 µg/mL). Its IC_{50} value was calculated to be 46.18 µg/mL (Table 4, Figure 2). In contrast, the chloroform extract showed only moderate activity (41.73% inhibition at 1000 µg/mL; IC_{50} > 1000 µg/mL), and the n-hexane extract demonstrated very weak activity (16.84% inhibition). The standard antioxidant, ascorbic acid, showed the highest potency with an IC_{50} value of 25.95 µg/mL.

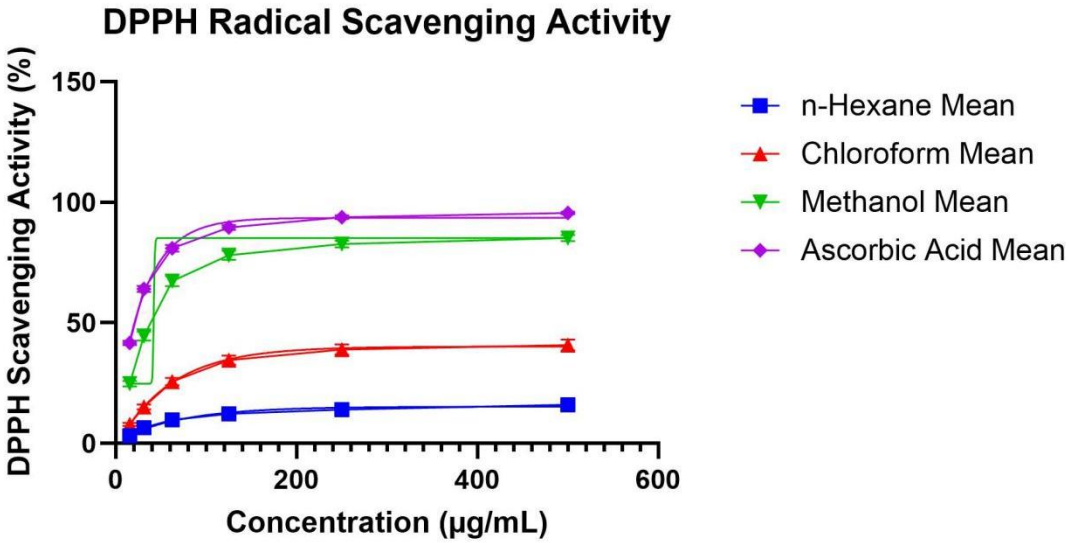

Figure 2. DPPH Radical Scavenging Activity of Guava Leaf Extracts and Ascorbic Acid. A line graph showing % Inhibition vs. Concentration (µg/mL). The methanol extract line shows a steep, dose-dependent increase, positioned between the ascorbic acid line (highest) and the nearly flat lines of the chloroform and n-hexane extracts.

3.4.2. ABTS Radical Cation Scavenging Assay

A similar trend was observed in the ABTS assay (Table 4). The methanol extract displayed potent activity with an IC_{50} value of 38.92 µg/mL, which was closer to the standard Trolox (IC_{50} = 22.15 µg/mL) than the other extracts. The chloroform and n-hexane extracts showed significantly lower activity.

3.4.3. Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the extracts, as determined by the FRAP assay, is presented in Table 4 and Figure 3. The methanol extract showed the highest reducing capacity (1245.6 ± 35.82 µM Fe^{2+} E/g), which was significantly greater ($p < 0.001$) than that of the chloroform (285.41 ± 18.95 µM Fe^{2+} E/g) and n-hexane (95.33 ± 12.44 µM Fe^{2+} E/g) extracts.

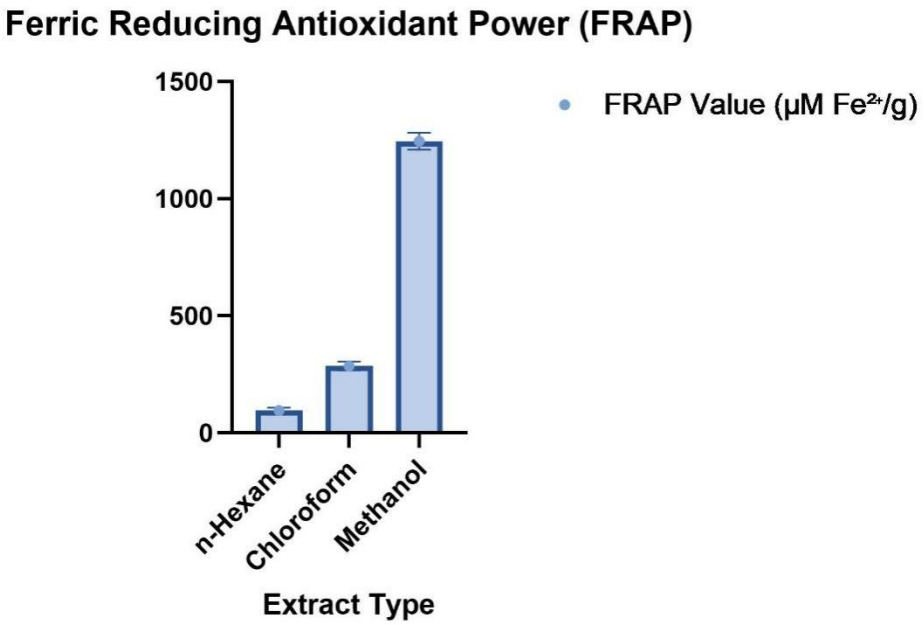

Figure 3. Ferric Reducing Antioxidant Power (FRAP) of Guava Leaf Extracts. A bar graph showing the FRAP values (µM Fe^{2+} E/g) for the three extracts. The bar for the methanol extract is substantially taller, indicating superior reducing power.

3.4.4. Total Antioxidant Capacity (Phosphomolybdenum Assay)

The phosphomolybdenum assay, which measures the total antioxidant capacity, confirmed the superior activity of the methanol extract (Table 4). Its total antioxidant capacity was 412.58 ± 15.75 mg AAE/g, which was several-fold higher than that of the other extracts.

Table 4. Antioxidant activity of *P. guajava* leaf extracts.

Sample	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP (µM Fe^{2+} E/g)	TAC (mg AAE/g)
n-Hexane	>1000	>1000	95.33 ± 12.44 a	45.12 ± 5.82 a
Chloroform	>1000	785.45 ± 42.18 b	285.41 ± 18.95 b	98.65 ± 8.41 b
Methanol	46.18 ± 1.45 c	38.92 ± 1.87 c	1245.67 ± 35.82 c	412.58 ± 15.75 c
Ascorbic Acid	25.95 ± 0.82 d	-	-	-
Trolox	-	22.15 ± 0.95 d	-	-
Values are mean ± SD (n=3). Different superscript letters (a, b, c, d) in the same column indicate significant differences ($p < 0.05$). (-) Not determined.				

3.5. Correlation between Phytochemicals and Antioxidant Activity

A strong and significant positive correlation was observed between the TPC and the antioxidant activities in all assays (DPPH: $R^2 = 0.978$, $p < 0.01$; ABTS: $R^2 = 0.985$, $p < 0.01$; FRAP: $R^2 = 0.991$, $p < 0.01$). Similarly, TFC also showed a strong positive correlation with the antioxidant activities (DPPH: $R^2 = 0.972$, $p < 0.01$; ABTS: $R^2 = 0.981$, $p < 0.01$; FRAP: $R^2 = 0.987$, $p < 0.01$). This indicates that the phenolic and flavonoid compounds are the primary contributors to the antioxidant potential of *P. guajava* leaves.

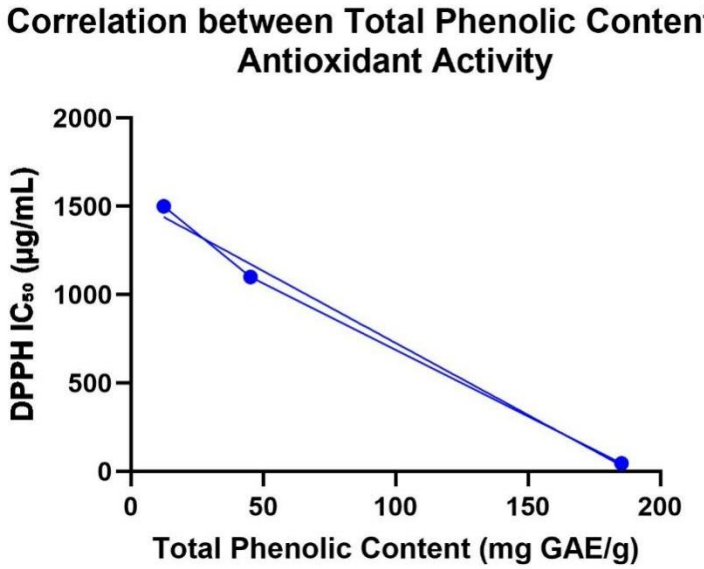

Figure 4. Correlation between Total Phenolic Content and DPPH Radical Scavenging Activity. A scatter plot showing the IC_{50} values (on a log scale) on the Y-axis and the TPC (mg GAE/g) on the X-axis. The three data points (n-Hexane, Chloroform, Methanol) show a clear negative linear trend, demonstrating that as TPC increases, the IC_{50} (required for 50% inhibition) decreases, meaning activity increases.

4. DISCUSSION

The quest for natural antioxidants from plant sources has gained immense momentum due to their potential role in preventing oxidative stress-related diseases. This study provides a comprehensive analysis of the phytochemical profile and antioxidant capacity of *P. guajava* leaf extracts from Charsadda, Pakistan, using multiple complementary methods.

The extraction yield was found to be solvent-dependent, with methanol yielding the highest amount (9.85%). This is consistent with the findings of Jani et al. (2020), who reported a 9.65% yield for a methanol extract. This high yield can be attributed to the high polarity of methanol, which efficiently extracts a broad spectrum of polar compounds such as flavonoids, tannins, and phenolic acids, which are abundant in guava leaves (Kumar et al., 2021). The lower yields from n-hexane and chloroform indicate that non-polar and medium-polarity constituents are less prevalent in this plant material.

The phytochemical screening revealed a diverse profile of bioactive compounds. The methanol extract was the most enriched, containing flavonoids, phenols, steroids, saponins, and terpenoids. This aligns with the results of Baloch et al. (2024), who also found a high concentration of these compounds in polar extracts like ethanol and hydroalcoholic mixtures. The presence of terpenoids across all extracts, regardless of solvent polarity,

suggests the existence of both non-polar and polar terpenoid derivatives in guava leaves. The absence of alkaloids in our study is consistent with some reports (Jani et al., 2020) but contrasts with others (Baloch et al., 2024), which may be due to genetic, environmental, or seasonal variations affecting the plant's phytochemistry.

The quantitative analysis provided crucial numerical data to support the qualitative findings. The remarkably high TPC (185.42 mg GAE/g) and TFC (92.75 mg QE/g) in the methanol extract are indicative of a rich reservoir of these antioxidant compounds. These values are comparable or even superior to those reported for guava leaves from other regions, highlighting the quality of the plant material from Charsadda (Sampath Kumar et al., 2021).

The antioxidant potential was rigorously evaluated using four distinct mechanisms: hydrogen atom transfer (DPPH), single electron transfer (ABTS, FRAP), and a combined method for total capacity (Phosphomolybdenum). In every single assay, the methanol extract demonstrated superior and dose-dependent activity. Its strong performance in the DPPH (IC_{50} = 46.18 µg/mL) and ABTS (IC_{50} = 38.92 µg/mL) assays confirms its efficacy as a free radicalscavenger. The high FRAP value (1245.67 µM Fe^{2+} E/g) underscores its potent reducing power, which is a key mechanism in mitigating oxidative stress. The significantly lower activity of the n-hexane and chloroform extracts further corroborates the notion that the primary antioxidant principles in guava leaves are polar in nature.

The most compelling evidence comes from the strong positive correlation ($R^2 > 0.97$) between the TPC/TFC and the antioxidant activities across all assays. This statistically significant relationship strongly suggests that phenolic acids and flavonoids, such as the quercetin, gallic acid, and ellagic acid identified in other studies (Sampath Kumar et al., 2021), are the primary contributors to the observed antioxidant effects. This structure-activity relationship is well-established, as the hydroxyl groups on these compounds can donate hydrogen atoms or electrons to stabilize free radicals (Górniak et al., 2019).

5. CONCLUSION

The findings of this study conclusively demonstrate that *Psidium guajava* leaves from the Charsadda region are a rich source of various phytochemicals, with a particularly high concentration of phenolic and flavonoid compounds in the polar methanol extract. The comprehensive antioxidant profiling using DPPH, ABTS, FRAP, and Phosphomolybdenum assays consistently revealed that the methanol extract possesses significant, multi-mechanistic antioxidant activity. The strong correlation between the phytochemical content and bioactivity provides a clear scientific rationale for the observed effects. This work validates the traditional use of guava leaves and positions them as a high-potential, natural source of antioxidants for applications in the food preservation, nutraceutical, and pharmaceutical industries. Future work should focus on the bioassay-guided fractionation of the methanol extract to isolate and characterize the specific active compounds, followed by *in vivo* studies to confirm efficacy and safety.

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