

Comparative Analysis of Phenolic and Flavonoid Content in Mango Varieties: Evaluating Antioxidant and Antimicrobial Activity

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Abstract

This study reports the antioxidant and antimicrobial attributes of different solvent extracts derived from selected mango varieties. The crude concentrated extracts (CCEs) obtained from Sindhri and Langra exhibited yields ranging from 6.12-23.89 g/100g and 6.64-24.86 g/100g per dry matter, respectively. These CCEs of Sindhri and Langra revealed a significant amount of total phenolic content over the range of 14.43-44.20 GAE (mg/100g) and 14.99-47.57 GAE (mg/100g), respectively. Similarly, the total flavonoid content in CCEs of Sindhri and Langra ranged from 9.85-30.54 CE (mg/100g) and 10.38-36.98 CE (mg/100g), respectively. The recovered CCEs of mango exhibited notable DPPH radical scavenging capacity with IC₅₀ values ranging from 6.78-18.53 µg/mL for Sindhri and 6.11-16.72 µg/mL for Langra. These extracts also exhibited strong ability inhibitory effects (37% to 79%) on the peroxidation process in the linoleic system. Aqueous ethanol (80%) exhibited the highest antimicrobial activity against a variety of pathogenic microorganisms, including *Bacillus pumilus*, *Escherichia coli*, *Fusarium oxysporum* and *Aspergillus niger*, while distilled water CCEs displayed the lowest potential. The number of phenolic components detected exceeds those previously reported through HPLC analysis. The findings of this study strongly support the potential utilization of aerial parts of mango as valuable sources of antioxidant and antimicrobial agents.

Keywords: *Mangifera indica*, Antioxidant, Antimicrobial, Bioactive agents, HPLC

Highlight:

- A significant amount of total phenolic content was reported from both varieties.
- Antioxidant potential and antimicrobial activities of different solvent extracts of selected varieties of mango
- Aqueous ethanol (80%) exhibited the highest antimicrobial activity against various pathogenic microorganisms.

1.0. Introduction

Free radicals have unpaired electrons, making them highly reactive, unstable and short-lived molecules. As a result, these frequently form bonds with other molecules to achieve stability (Carsono et al., 2022). Free radicals can form when molecules are attacked, leading to breaking chemical bonds and generating these reactive species. Excessive free radical generation and insufficient antioxidant production can lead to oxidative stress (Kashif et al., 2024). Oxidative stress is implicated in numerous diseases, including stroke, cancer, myocardial infarction and diabetes (Anees et al., 2023). Phytochemicals, chemical compounds in plants, possess protective properties (Naz et al., 2023). These phytochemicals are responsible for the antioxidant potential of plants. Antioxidants capture free radicals through chelation, function as scavengers of free radicals and use various mechanisms to prevent lipid oxidation (Kashif et al., 2024). Antioxidants can stop or hinder oxidative damage to molecules. Two main methods of radical deactivation by antioxidant molecules, hydrogen atom transfer and single electron transfer, lead to the same results. The equilibrium between these two mechanisms, which virtually always coexist in all samples, is defined by the antioxidant's pH and structure (Munteanu & Apetrei, 2022).

Plants serve as valuable sources of antioxidants, and herbs have been utilized for medicinal purposes since ancient eras. Each plant has its unique bioactivity (Kashif et al., 2024). Plants are also recognized for their antioxidant properties and benefits to human health (Kruk et al., 2019). Natural sources of antioxidants are preferred due to their affordability and organic nature. In the literature, synthetic antioxidants are noted to be expensive and associated with several adverse effects. Conversely, conventional extracts from medicinal plants are highly effective and have minimal side effects, making them the preferred choice (Gautam et al., 2022). Medicinal plants are abundant worldwide, and there is a wide variety of them in Pakistan. People commonly use them in various forms of herbal medicine to treat many diseases. Pakistan's climate, geographical features, traditional zones and flora are diverse, contributing to the country's rich variety of medicinal plants

(Tufail et al., 2020). Medicinal plants have been utilized in healthcare systems since ancient times and have proven effective in treating various ailments (Kashif et al., 2024; Keshav et al., 2019).

The substantial volume, environmental impact and decay of medicinal plant food waste present a significant issue (Jeswani et al., 2021). It is imperative to reduce, reuse and recycle food waste to protect the environment, conserve food resources and promote the sustainability of food systems. Many parts of plants, including leaves, fruits and flowers are edible and frequently consumed worldwide (Picot-Allain et al., 2021). Each plant has medicinal potential, which can be explored as a source of molecules of interest to the medicinal industry, even if not every part of every plant has healing properties (Naz et al., 2023). One example of a plant with potential therapeutic benefits is *Mangifera indica* (Linn.), or mango. *Mangifera indica* belongs to the Anacardiaceae family and is significant in traditional and Ayurvedic medicine (Nashvia et al., 2022). This fruit is rich in a diverse array of chemical components, including phenolics such as xanthenes (predominantly mangiferin), gallates, benzophenones, flavonoids and derivatives of ellagic acid (Asunción-Alvarez et al., 2020).

Mango also contains anthocyanins and carotenoids as phytochemicals. In addition, it is rich in various vitamins, including folic acid, ascorbic acid and niacin. Mango fruits also contain many volatile compounds, such as monoterpenes, esters, lactones and sesquiterpenes, contributing to their sweet and fruity aroma (Kawa-rygielska et al., 2020). Mangoes are frequently consumed fresh but can also be processed to produce juices and pulps, generating substantial waste (Jahurul et al., 2015). Peels are a significant by-product, rarely used in processed food, but can potentially serve as valuable ingredients, as they contain substantial bioactive components. Mango leaves have medicinal applications for treating conditions such as diarrhea, dysentery and colitis (Coelho et al., 2019). Mango bark's aqueous extract has a long history of use in ethnomedicine for addressing various ailments, including diarrhea, diabetes and anemia. It is rich in phenolic substances such as tannins, phenolic acids, benzophenones, xanthenes and flavonoids (Vazquez-Olivo et al., 2019).

Medicinal plants contain inherent antioxidants such as phenols, flavonoids and tannins, which are vital in disease prevention. With the growing demand for new natural antioxidants sourced from plants, the abundant presence of these compounds in medicinal plants holds substantial promise for their utilization in pharmaceutical products. Medicinal plants possess valuable properties, including antioxidants, antimicrobials and potential anticancer activities. This study aimed to assess the levels of biologically active compounds and antioxidant capacity across various Mango species in different solvents and concentrations. These solvents encompassed absolute methanol, aqueous methanol, absolute ethanol, aqueous ethanol, chloroform and distilled water extracts derived from various parts of mango varieties (including leaves, bark and peel) through maceration.

2.0. Materials and Methods

2.1. Chemicals and Reagents

Folin-Ciocalteu reagent, aluminum chloride, ammonium thiocyanate, sodium carbonate (anhydrous), linoleic acid, ascorbic acid, gallic acid, ferrous chloride, sodium nitrite, BHT (butylated hydroxytoluene), and DPPH (2, 2-diphenyl-2-picrylhydrazyl) were purchased from Sigma Chemicals. Additional analytical-grade reagents, including ethanol, chloroform and methanol, were obtained from Merck Chemical Company (Darmstadt, Germany). Some chemicals were also purchased from Oxoid Ltd. (Hampshire, United Kingdom) to prepare culture media for antimicrobial tests.

2.2. Sample collection and its pretreatment

Different aerial parts (leaves, peel and bark) of selected *Mangifera indica* (mango) varieties (Sindhri and Langra) were collected from the native district of Multan, South Punjab, Pakistan. A taxonomist from the Department of Botany at the University of Agriculture Faisalabad, Pakistan, assisted in verifying and identifying the mango samples. These samples were chopped into smaller pieces, and finally, the air-dried pieces were stored in plastic bags at -4°C.

2.3. Extraction from plant materials

Different parts (leaves, peel and bark) of selected mango varieties (Sindhri and Langra) were washed and rinsed with tap water to remove potential contaminants. The cleaned samples were shade-dried and ground into powder using an electric grinder (mesh 80). 10 g of ground sample was subjected to extraction using 100 mL of various solvents and concentrations, including absolute ethanol, absolute methanol, aqueous ethanol, aqueous methanol, chloroform and distilled water by maceration (16 hours at 26°C).

The recovered green extracts were filtered thrice through Whatman No.1 filter paper to remove any insoluble residue. The resultant extracts were pooled in a new glass vial. Excessive solvents from the crude extract were removed using a rotary evaporator at 45°C, producing concentrated crude extracts (CCEs). These CCEs were then stored at -4°C for further analysis.

2.4. Estimation of antioxidant activity of CCEs

The following tests assessed the antioxidant activity of selected parts of different mango varieties.

2.4.1. Estimation of total phenolic content in CCEs

Selected parts of mango were used to determine the total phenolic content using the method described by Yesil-Celiktas et al. (2007). Briefly, CCEs (1.0 mg/mL) were dissolved in 7.5 mL of distilled water, 1.5 mL of 20% sodium carbonate and

0.5 mL of Folin-Ciocalteu reagent. The mixture was then incubated at 40°C for 20 minutes. The absorbance of the solution was measured at 755 nm using a UV-Vis spectrophotometer. The results were expressed as gallic acid equivalents (GAE)/100g of the tested sample.

2.4.2. Estimation of total flavonoid content in CCEs

Selected parts of mango were used to determine the total flavonoid contents following the method reported by Kashif et al. (2024). Briefly, 5.0 mL of distilled water was mixed with CCEs (100 mg/mL) in the presence of 0.3 mL of 5% sodium nitrate. After incubation, the resultant solution was reacted with 2.0 mL of 1.0 M sodium hydroxide and 0.6 mL of 10% aluminum chloride. The absorbance was measured at 510 nm. The concentration of total flavonoid content was determined in catechin equivalents (CE)/100g using a catechin calibration curve.

2.4.3. 2, 2-diphenyl-2-picrylhydrazyl radical scavenging analysis

The capacity to neutralize free radicals such as DPPH was assessed using the methodology reported by Tepe et al. (2005). 50 µL sample of each extract with concentration (0.10-5.0 mg/mL) was added to 5.0 mL of a 0.004% DPPH solution in methanol. The resultant solution was incubated for 20 minutes, and the absorbance of the sample and the blank was recorded at 517 nm using Equation 1.

$$I(\%) = \left[\frac{A_b}{A_s} \right] \times 100 \quad (1)$$

Where A_s and A_b represent the absorbance of the sample and the blank, respectively.

2.4.4. inhibition of peroxidation by CCEs

The antioxidant capacity of the recovered CCEs from mango samples was evaluated by assessing their ability to inhibit oxidation of linoleic acid using the methodology given by Azab et al. (2017). For each green extract, 5.0 mg was dissolved in a mixture of 10.0 mL of ethanol (99.8%), 0.13 mL of linoleic acid and 10 mL of sodium phosphate buffer (pH = 7, 0.2 M). Distilled water was added to reach a final volume of 25.0 mL. The degree of oxidation was calculated by measuring the absorbance of the solution after incubation at 40 °C (Yen et al., 2000). To the sample solution (0.2 mL), 10.0 mL of 75% ethanol, 0.2 mL of 30% ammonium thiocyanate and 0.2 mL of ferrous chloride solution in 3.5% HCl were sequentially added. The final solution was then incubated at room temperature for 3 minutes while stirring and analyzed using a Spectrophotometer at 500 nm. A control treatment (without plant extract) was also performed, and percentage inhibition was calculated using Equation 2.

$$I(\%) = \left[\frac{A_c - A_s}{A_c} \right] \times 100 \quad (2)$$

Where A_c and A_s represent the absorbance of the control and sample. This study used positive controls ascorbic acid ($C_6H_8O_6$) and butylated hydroxytoluene (BHT).

2.5. Determination of Alkaloids

10 g of dried powder from each sample was weighed and placed into a 250 mL flask. Subsequently, 100 mL of deionized water was added to the flask. The mixture was shaken for 16 hours and then filtered. For Marme's test, approximately 3 mL of the extracted solution was mixed with several drops of Marme's reagent, which consists of potassium iodide (20 g in 20 mL distilled water) and Cadmium chloride (10 g in 50 mL distilled water). Diluted sulfuric acid was added to the mixture and heated to 40°C for 10 minutes, forming a reddish-brown precipitate.

2.6. Antimicrobial activity

2.6.1. Microorganisms tested

The solvent extracts recovered from mango samples were individually tested against a set of pathogenic microorganisms, including *Escherichia coli* (gram-negative bacteria), *Bacillus pumilis* (gram-positive bacteria) and fungal strains (*Fusarium oxysporum* and *Aspergillus niger*). The pathogenic microorganism strains were obtained from the Department of Biological Sciences, International Islamic University, Islamabad, Pakistan. Nutrient agar medium and potato dextrose agar (Oxoid, UK) were used to grow the bacterial and fungus strains at 37°C and 30°C, respectively.

2.6.2. Disc diffusion method

The antimicrobial potential of the solvent extracts from the tested samples was measured using disc diffusion, as described by Elfi Susanti & Mulyani (2022). Discs were soaked with each solvent extract (100 mg/mL) and placed on agar plates inoculated with pathogenic microorganisms. Positive controls (*amoxicillin* and *flumequine*) and negative controls (without extract) were also processed using the same protocols.

The measurement of the minimum inhibition concentration (MIC) was carried out using the micro-dilution method. MIC represents the exact leaf, bark, and peel extract concentration required to inhibit microorganism growth completely in vitro conditions. Each concentrated solvent extract from the tested samples was diluted in a 5-100 mg/mL range in 96 well plates. A growth control (without plant extract) and sterility control (with plant extract) were also included under similar conditions. A diluted solution of selected plant extracts (20 µL) was added to the culture medium (160 µL) of nutrient broth for bacterial strains and sabouraud dextrose broth for fungal strains. The inoculation of broth culture (20 µL; with 5×10^5 CFU) of each

tested microorganism was performed in the 96-well plate. The plates were incubated at 37°C for 24 hours for bacterial strains and at 30°C for 48 hours for fungal strains. The presence of a white pellet at the bottom of the well indicated microorganism growth. MIC was determined as the dilution where no microorganism growth occurred.

2.7. HPLC Analysis

The plant peel was separated using the method described by Öztürk et al. (2007) for sample preparation. Phenolic compounds were analyzed using high-performance liquid chromatography (HPLC). Mango peel (29 g) was mixed with 40 mL of distilled water to produce a juice. The mixture was then centrifuged at 2000g for 15 minutes. After centrifugation, the juice was filtered through a 0.25 mm filter to remove any suspended debris, resulting in a pure juice extract. 15 µL aliquot of the filtered peel sample was injected into an analytical column (Shim-Pak CLC-ODS (C-18), 250 × 4.6 mm; 5 µm particle size), and detection was performed at 280 nm. Phenolic compounds were identified using external standards by comparing them with known standards under identical conditions.

3.0 Results and Discussion

3.1. Yield of CCEs

The yield (g/100g) of various green extracts obtained from selected parts of different mango varieties using different solvents and concentrations is shown in Table (1). The yield of plant extracts with antioxidative ingredients depends on the solvent used, with yields ranging from leaves (6.12-12.27 g/100g), peel (15.63-23.89 g/100g) and bark (6.91-16.21 g/100g) per dry matter for the Sindhri variety. For the Langra variety, yields ranged from leaves (6.64-11.89 g/100g), peel (16.51-24.86 g/100g) and bark (7.29-17.86 g/100g) per dry matter. Aqueous ethanol recovered the highest yield, whereas distilled water produced the lowest in both mango varieties. The results of this study indicated that the extract yield varied significantly ($p < 0.05$) depending on the plant component and the solvent used. The highest extract yield was obtained with aqueous ethanol, demonstrating the solvent's superior efficiency in recovering antioxidant constituents (Oktay et al., 2003). A comparison between the two mango varieties revealed that Langra plant samples had a higher yield than Sindhri.

The percentage yield values recovered in this study are lower than those reported in previous research on mangoes (Ling et al., 2009; Sultana et al., 2012). The differences in extract yield may be due to variations in the availability of extractable components, mango variety, fruit maturity and prevailing agroclimatic conditions (Hsu et al., 2006).

Table. 1: Extract yields from selected parts of the Sindhri and Langra mango plant.

Extracting solvents	Extraction yields (g/100g of dry weight)					
	Sindhri			Langra		
	Leaves	Peel	Bark	Leaves	Peel	Bark
Absolute ethanol	9.57 ± 0.29	20.54 ± 0.13	13.54 ± 0.17	9.89 ± 0.31	21.2 ± 0.17	14.74 ± 0.13
Aqueous ethanol	12.27 ± 0.18	23.89 ± 0.10	16.21 ± 0.21	11.89 ± 0.20	24.86 ± 0.14	17.86 ± 0.17
Absolute methanol	8.22 ± 0.32	19.29 ± 0.13	12.03 ± 0.14	9.57 ± 0.24	19.77 ± 0.18	13.18 ± 0.24
Aqueous methanol	11.05 ± 0.21	22.17 ± 0.11	15.35 ± 0.11	11.31 ± 0.26	23.61 ± 0.15	17.29 ± 0.21
Distilled water	6.12 ± 0.30	15.63 ± 0.15	6.91 ± 0.24	6.64 ± 0.26	16.51 ± 0.24	7.29 ± 0.22
Chloroform	7.87 ± 0.27	17.17 ± 0.12	10.17 ± 0.13	8.19 ± 0.24	18.01 ± 0.16	10.63 ± 0.18

Values are the average (mean ± SD) of three replicates, analyzed individually.

3.2. Total phenolic and flavonoid content.

Wojdyło et al. (2007) reported that plants with properties such as anti-lipid oxidation potential and anti-carcinogenic effects have gained significant attention in the food industry. Natural antioxidants, particularly phenolics, are commonly derived from plants (Awika et al., 2003). Several studies have shown that total flavonoids and phenolics in fruits and vegetables contribute to their antioxidant activity.

The total flavonoids and phenolics extracted using different solvents from mango are illustrated in Tables (2a & b). The total phenolics (TP) and total flavonoids (TF) extracted from Sindhri mango using various solvents ranged from 14.43-44.20 mg/100g GAE and 9.85-30.54 mg/100g CE. Similarly, TP and TF extracted from Langra mango ranged from 14.99-47.57 mg/100g GAE and 10.38-36.98 mg/100g CE, respectively. Aqueous ethanol extracts showed the highest amounts of TP and TF in both mango varieties. The TP and TF content variation depends on the solvents' effectiveness in extracting

antioxidants from the mango plant. The TPC from the plant samples varied significantly ($p < 0.05$) among the solvents tested. Ethanol is often preferred for antioxidant extraction due to its higher efficiency and lower toxicity (Jaffery et al., 2003). This study's total phenolic content values are consistent with previous research on mango plants. However, lower levels of TPC and TFC were noted in certain sections of the mango plant (Sultana et al., 2012).

Table. 2 (a): Total phenolic and total flavonoid content of Sindhri mango plant.

Extracting solvents	TPC (mg GAE /100g)			TFC (mg CE /100g)		
	Leaves	Peel	Bark	Leaves	Peel	Bark
Absolute ethanol	20.22 ± 0.02	35.84 ± 0.02	18.10 ± 0.01	18.77 ± 0.02	23.15 ± 0.01	11.52 ± 0.01
Aqueous ethanol	26.87 ± 0.01	44.20 ± 0.01	22.77 ± 0.01	22.97 ± 0.01	30.54 ± 0.01	16.95 ± 0.02
Absolute methanol	19.46 ± 0.03	34.34 ± 0.01	17.83 ± 0.02	17.22 ± 0.02	21.61 ± 0.02	10.70 ± 0.01
Aqueous methanol	23.13 ± 0.01	39.16 ± 0.01	20.81 ± 0.01	20.03 ± 0.01	27.70 ± 0.01	14.50 ± 0.02
Distilled water	19.30 ± 0.02	31.31 ± 0.02	14.43 ± 0.02	16.96 ± 0.02	18.13 ± 0.02	9.65 ± 0.01
Chloroform	18.41 ± 0.02	33.26 ± 0.02	15.98 ± 0.02	17.44 ± 0.02	19.87 ± 0.01	10.03 ± 0.02

Values are the average (mean ± SD) of three replicates, analyzed individually.

Table. 2 (b): Total phenolic and total flavonoid content of Langra mango plant.

Extracting solvents	TPC (mg GAE /100g)			TFC (mg CE/100g)		
	Leaves	Peel	Bark	Leaves	Peel	Bark
Absolute ethanol	28.57± 0.02	41.29± 0.01	19.81± 0.01	19.42± 0.01	29.68± 0.02	12.61± 0.01
Aqueous ethanol	34.30± 0.01	47.57± 0.01	25.21± 0.02	23.88± 0.02	36.98± 0.02	17.26± 0.01
Absolute methanol	27.19± 0.01	39.98± 0.02	17.08± 0.01	18.89± 0.01	28.09± 0.02	11.61± 0.02
Aqueous methanol	31.03± 0.02	43.86± 0.01	22.13± 0.02	20.94± 0.02	33.07± 0.02	15.72± 0.01
Distilled water	26.96± 0.01	30.16± 0.02	14.99± 0.02	18.36± 0.01	23.07± 0.01	10.38± 0.02
Chloroform	26.48± 0.01	33.55± 0.02	15.37± 0.01	18.48± 0.01	26.27± 0.01	11.23± 0.02

Values are the average (mean ± SD) of three replicates, analyzed individually.

3.3. DPPH radical scavenging assay:

The stable free radical, DPPH, is known for its deep violet colour, with absorption peaks ranging from 515-528 nm. When it absorbs a proton from hydrogen donors, such as phenolics, it changes colour from violet to yellow. This transition reflects the compound's ability to neutralize free radicals. The DPPH radical scavenging capacity is a widely recognized method to evaluate the antioxidant potential of leaf extracts or similar compounds, and it increases proportionally with higher concentrations of phenolic components or a greater degree of hydroxylation (Sánchez-Moreno et al., 1999).

Mango extracts from selected parts have shown excellent radical scavenging activity, with IC₅₀ values ranging between Sindhri (6.78-18.53 µg/mL) and Langra (6.11-16.72 µg/mL) as detailed in Table (3). Aqueous ethanol extracts exhibited the lowest IC₅₀ value, indicating the most substantial free radical scavenging activity. The results revealed significantly ($p < 0.05$) higher radical-capturing potential in ethanol extracts than in other solvents. Compared to synthetic antioxidant butylated hydroxytoluene (BTH), the tested mango extracts demonstrated lower scavenging activity. The free radical scavenging values obtained in this study were lower than those reported by Sultana et al. (2012).

Table. 3: DPPH radical scavenging activity of selected parts of Sindhri and Langra mango plant.

Extracting solvents	IC ₅₀ Value (µg/mL)					
	Sindhri			Langra		
	Leaves	Peel	Bark	Leaves	Peel	Bark
Absolute ethanol	11.28 ± 0.03	9.71 ± 0.04	12.83 ± 0.04	10.02 ± 0.05	8.35 ± 0.05	11.39 ± 0.04
Aqueous ethanol	8.34 ± 0.04	6.78 ± 0.04	9.78 ± 0.02	7.52 ± 0.03	6.11 ± 0.03	8.57 ± 0.02
Absolute methanol	13.46 ± 0.04	10.52 ± 0.04	13.66 ± 0.03	11.47 ± 0.05	9.35 ± 0.03	12.22 ± 0.03
Aqueous methanol	10.53 ± 0.02	8.65 ± 0.02	11.45 ± 0.05	8.75 ± 0.02	7.42 ± 0.04	9.86 ± 0.05
Distilled water	17.67 ± 0.05	13.56 ± 0.06	18.53 ± 0.02	15.59 ± 0.04	12.73 ± 0.04	16.72 ± 0.04
Chloroform	15.08 ± 0.03	11.48 ± 0.04	15.76 ± 0.03	14.96 ± 0.04	11.07 ± 0.02	14.39 ± 0.03

Values are the average (mean ± SD) of three replicates, analyzed individually.

3.4. Antioxidant activity in the linoleic acid system

Linoleic acid, an unsaturated fatty acid, undergoes oxidation to produce peroxides. These peroxides subsequently oxidize Fe (II) into Fe (III), forming a complex with thiocyanate anion (SCN⁻). The concentration of this complex is measured using a UV-vis spectrometer at 500 nm. Higher absorbance values indicate increased peroxide formation, reflecting a lower antioxidant potential, as a greater concentration of peroxides signifies reduced antioxidant activity (Oktay et al., 2003).

The antioxidant potential of selected mango extracts in inhibiting lipid peroxidation is presented in Table (4). The extracts showed varying degrees of inhibition of linoleic acid oxidation, with Sindhri extracts ranging from 37-72% and Langra extracts ranging from 48-79%. Aqueous ethanolic extracts from both mango varieties demonstrated significantly better protection against peroxidation, likely due to their higher concentration of phenolic compounds. Notably, Langra extracts exhibited superior inhibition of linoleic acid oxidation compared to Sindhri extracts, suggesting a higher antioxidant capacity. However, when compared to synthetic antioxidants like BHT, all mango extracts tested showed lower inhibition of linoleic acid oxidation, with significantly less effectiveness ($p < 0.05$). The oxidation inhibition values obtained in this study were higher than previously reported in the literature (Sultana et al., 2012).

Table. 4: Antioxidant activity (% inhibition) of Sindhri and Langra mango plant.

Extracting solvents	% Inhibition					
	Sindhri			Langra		
	Leaves	Peel	Bark	Leaves	Peel	Bark
Absolute ethanol	44.51 ± 0.52	62.78 ± 0.27	56.41 ± 0.16	67.40 ± 0.32	69.09 ± 0.28	61.84 ± 0.51
Aqueous ethanol	62.61 ± 0.19	72.34 ± 0.71	66.09 ± 0.47	76.32 ± 0.57	78.90 ± 0.60	70.21 ± 0.26
Absolute methanol	41.51 ± 0.31	61.11 ± 0.53	51.14 ± 0.17	65.13 ± 0.43	67.07 ± 0.54	59.91 ± 0.16
Aqueous methanol	57.2 ± 0.39	69.83 ± 0.48	61.59 ± 0.31	70.53 ± 0.52	75.08 ± 0.36	66.53 ± 0.31
Distilled water	37.47 ± 0.28	46.08 ± 0.65	42.21 ± 0.54	58.46 ± 0.41	59.05 ± 0.45	48.16 ± 0.48
Chloroform	39.3 ± 0.41	48.81 ± 0.50	45.71 ± 0.18	61.80 ± 0.19	64.34 ± 0.27	54.71 ± 0.64

Values are the average (mean ± SD) of three replicates, analyzed individually.

3.5. Antimicrobial activity

Extracts from mango leaf, peel and bark demonstrated antibacterial activity against pathogenic microorganisms, as summarized in Tables (5&6). *Bacillus pumilus* showed the highest sensitivity among the tested microorganisms, with

inhibition zones ranging from 12-14 mm when exposed to extracts from the leaves, peel and bark of the Sindhri mango variety. These results were obtained using the disc diffusion method and determining minimum inhibitory concentration (MIC) values (Table 5a & b). For *Bacillus pumilus*, the leaf, peel and bark extracts exhibited the lowest MIC values, ranging from 221 to 245 µg/mL, 202 to 237 µg/mL and 222 to 362 µg/mL, respectively (Table 5a). In contrast, *Escherichia coli* demonstrated lower antimicrobial activity against the leaf, peel and bark extracts of Langra, with comparatively narrower inhibition zones of 2-11 mm, 2-12 mm and 2-7 mm and higher MIC values of 219-281 µg/mL, 198-270 µg/mL and 249-302 µg/mL, respectively (Table 5b). Additionally, bark extracts overtook leaf extracts in antibacterial efficacy against both bacterial and fungal strains.

Regarding the fungus strain *Fusarium oxysporum*, MIC values ranged from 242-282 µg/mL, 252-308 µg/mL and 259-302 µg/mL for leaf, peel and bark extracts, respectively, with inhibition zones between 2-8 mm, 2-6 mm and 2-7 mm. In comparison, *Aspergillus niger* exhibited inhibition zones of 2-9 mm, 2-13 mm and 2-10 mm, with MIC values between 237-296 µg/mL, 199-275 µg/mL and 228-289 µg/mL (Table 6a). The variability in the chemical composition of the extracts likely accounts for the differences in antibacterial activity across different components. Previous research has demonstrated that changes in the chemical composition of plant extracts can significantly impact their biological activities. In this study, *E. coli* was less sensitive to the mango extracts, showing lower inhibitory effects compared to the results documented by Osei-Djarbeng et al. (2020).

Table. 5 (a): Antibacterial activity of extracts of Sindhri mango plant.

Extracting solvents	Bacterial strains	Zone of inhibition (mm)			Minimum inhibitory concentration (µg/mL)		
		Leaves	Peel	Bark	Leaves	Peel	Bark
Absolute ethanol	<i>Bacillus pumilus</i>	2	3	2	302	277	316
	<i>Escherichia coli</i>	8	10	7	224	211	222
Aqueous ethanol	<i>Bacillus pumilus</i>	6	5	4	262	252	270
	<i>Escherichia coli</i>	12	14	9	221	202	248
Absolute methanol	<i>Bacillus pumilus</i>	2	3	2	318	268	312
	<i>Escherichia coli</i>	6	9	5	270	216	275
Aqueous methanol	<i>Bacillus pumilus</i>	3	4	2	262	249	312
	<i>Escherichia coli</i>	8	11	6	302	199	281
Distilled water	<i>Bacillus pumilus</i>	2	3	1	345	319	362
	<i>Escherichia coli</i>	4	8	3	245	237	274
Chloroform	<i>Bacillus pumilus</i>	3	4	2	269	280	312
	<i>Escherichia coli</i>	5	5	3	268	241	299
Positive control (Amoxicillin)	<i>Bacillus pumilus</i>	—	21	—	—	148	—
	<i>Escherichia coli</i>	—	25	—	—	98	—

Values are average (mean ± SD) of three replicates, analyzed individually.

Table. 5 (b): Antibacterial activity of extracts of Langra mango plant.

Extracting solvents	Bacterial strains	Zone of inhibition (mm)			Minimum inhibitory concentration (µg/mL)		
		Leaves	Peel	Bark	Leaves	Peel	Bark
Absolute ethanol	<i>Bacillus pumilus</i>	3	4	2	254	240	299

Aqueous ethanol	<i>Escherichia coli</i>	9	9	6	220	213	252
	<i>Bacillus pumilus</i>	7	7	5	242	235	268
	<i>Escherichia coli</i>	11	12	7	219	198	249
Absolute methanol	<i>Bacillus pumilus</i>	2	4	3	319	255	280
	<i>Escherichia coli</i>	5	11	4	258	202	262
Aqueous methanol	<i>Bacillus pumilus</i>	3	6	3	270	249	282
	<i>Escherichia coli</i>	8	10	5	238	208	272
Distilled water	<i>Bacillus pumilus</i>	1	3	2	356	286	305
	<i>Escherichia coli</i>	3	7	5	281	233	276
Chloroform	<i>Bacillus pumilus</i>	2	3	4	302	292	285
	<i>Escherichia coli</i>	3	5	2	281	270	302
Positive control (Amoxicillin)	<i>Bacillus pumilus</i>	–	21	–	–	148	–
	<i>Escherichia coli</i>	–	25	–	–	98	–

Values are average (mean \pm SD) of three replicates, analyzed individually.

Table. 6 (a): Antifungal activity of extracts of Sindhri mango plant.

Extracting solvents	Bacterial strains	Zone of inhibition (mm)			Minimum inhibitory concentration ($\mu\text{g/mL}$)		
		Leaves	Peel	Bark	Leaves	Peel	Bark
Absolute ethanol	<i>Aspergillus niger</i>	8	11	6	250	211	256
	<i>Fusarium oxysporum</i>	4	3	2	272	283	277
Aqueous ethanol	<i>Aspergillus niger</i>	9	13	10	237	199	229
	<i>Fusarium oxysporum</i>	3	4	2	282	279	300
Absolute methanol	<i>Aspergillus niger</i>	6	7	5	255	261	268
	<i>Fusarium oxysporum</i>	3	4	3	279	276	279
Aqueous methanol	<i>Aspergillus niger</i>	7	9	7	260	250	260
	<i>Fusarium oxysporum</i>	8	6	7	242	252	259
Distilled water	<i>Aspergillus niger</i>	2	3	2	290	281	275
	<i>Fusarium oxysporum</i>	2	3	3	298	298	292
Chloroform	<i>Aspergillus niger</i>	2	3	2	296	275	290
	<i>Fusarium oxysporum</i>	3	2	2	284	307	301

Values are average (mean \pm SD) of three replicates, analyzed individually.

Table. 6 (b): Antifungal activity of extracts of Langra mango plant.

Extracting solvents	Bacterial strains	Zone of inhibition (mm)			Minimum inhibitory concentration (µg/mL)		
		leaves	fruit	bark	Leaves	peel	bark
Absolute ethanol	<i>Aspergillus niger</i>	7	14	6	261	193	264
	<i>Fusarium oxysporum</i>	6	5	4	261	272	279
Aqueous ethanol	<i>Aspergillus niger</i>	8	12	10	261	205	240
	<i>Fusarium oxysporum</i>	3	7	3	288	259	290
Absolute methanol	<i>Aspergillus niger</i>	6	6	7	259	267	252
	<i>Fusarium oxysporum</i>	4	7	5	277	255	269
Aqueous methanol	<i>Aspergillus niger</i>	8	8	6	267	264	267
	<i>Fusarium oxysporum</i>	9	9	9	247	248	244
Distilled water	<i>Aspergillus niger</i>	4	5	2	278	274	298
	<i>Fusarium oxysporum</i>	4	4	3	281	278	289
Chloroform	<i>Aspergillus niger</i>	3	5	3	291	273	294
	<i>Fusarium oxysporum</i>	3	4	2	297	277	299
Positive control (Amoxicillin)	<i>Aspergillus niger</i>	23	–	–	134	–	–
	<i>Fusarium oxysporum</i>	20	–	–	160	–	–

Values are the average (mean \pm SD) of three replicates, analyzed individually.

3.6. Qualitative analysis of alkaloids

The results of Marme's test revealed a distinct variation in alkaloid content among different parts of the *Mangifera indica* plant. The bark extract demonstrated the highest concentration of alkaloids, significantly more than the leaves. In contrast, no alkaloids were detected in the peel extract (Table 7). The absence suggests that the peel either does not synthesize alkaloids or contains them in quantities below the detection limit of Marme's test. Conversely, the high alkaloid content in the bark indicates its potential as a rich source of these bioactive compounds (Simon et al., 2012).

Table. 7: Alkaloids of Sindhri and Langra mango plant extracts.

Solvent Extract	Alkaloids					
	Sindhri			Langra		
	Leaves	Peel	Bark	Leaves	Peel	Bark
Distilled water	+	-	++	+	-	++

+ Alkaloids are present, - Alkaloids are absent

3.7. HPLC analysis

The peels of the tested *Mangifera indica* plant were analyzed for potent bioactive components, specifically phenolic compounds, using HPLC. Chlorogenic acid was identified as a significant aromatic acid, with a concentration of 112.65 ppm. Other detected aromatic acids including quercetin, benzoic, cinnamic, gallic, *p*-coumaric, caffeic, vanillic

and sinapic acids at the concentration of 16.85, 10.17, 6.12, 2.35, 1.71, 1.33, 1.19 and 0.71 ppm, respectively. The promising biological activities, such as antioxidant and antimicrobial potentials, might be attributed to the presence of these phenolic components in *M. indica* peels, as indicated by the results of correlation analysis. Phytochemicals like phenolic acids are well known for their strong biological properties due to the phenolic moiety (Öztürk et al., 2007).

Although the concentration of bioactive components is relatively low, the number of phenolic components detected in this study exceeds those previously reported through HPLC analysis by Safdar et al. (2017). The phytochemical constituents identified in the aqueous extracts via HPLC are presented in Fig. (1), with the corresponding constituents listed in Table (8).

Table. 8: Phytochemical components and concentration (ppm) in a crude extract of mango peel.

Compound name	Retention Time	Concentration in ppm
Quercetin	2.727	16.85 ± 0.04
Gallic acid	4.227	2.35 ± 0.02
Caffeic acid	12.140	1.33 ± 0.02
Vanillic acid	13.453	1.19 ± 0.02
Benzoic acid	14.760	10.17 ± 0.05
Chlorogenic acid	15.873	112.65 ± 0.04
p-coumaric acid	17.300	1.71 ± 0.01
Cinnamic acid	24.907	6.12 ± 0.02
Sinapic acid	26.120	0.71 ± 0.03

Values are average (mean ± SD) of three replicates, analyzed individually.

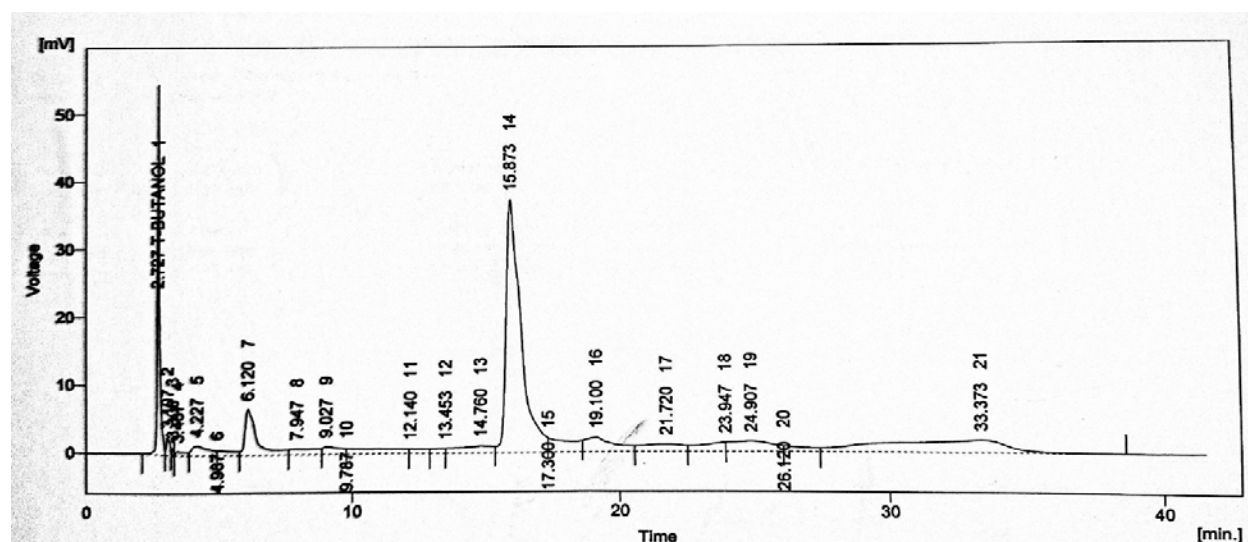


Figure 1: HPLC chromatogram of the crude extract obtained under optimum conditions.

3.8. Correlation study among TP, TF and biological potential.

The Pearson correlation analysis was conducted at a significance level of $P = 0.001$ (Tables 9-12). These results revealed a positive correlation between total flavonoids (TF) and total phenolics (TP) with both inhibition potential and antimicrobial properties in the Sindhri and Langra mango varieties. The correlation coefficient for inhibition potential and antimicrobial properties were $P = 0.917$ and $0.233-0.863$, $P = 0.953$ and $0.144-0.897$, $P = 0.965$ and $0.643-0.923$, $P = 0.998$ and $0.592-0.837$, respectively. Conversely, the IC_{50} values were negatively correlated with TPC and TFC values, with correlation coefficients of $P = -0.937$, $P = -0.913$, $P = -0.876$ and $P = -0.811$, respectively.

The aerial parts of mango plants from Multan, Pakistan, were investigated for their nutritional content and potential bioactive compounds, driven by increasing interest in their pharmacological benefits. This study represents the first characterization of these selected parts, confirming the presence of phenolic antioxidant compounds. These findings significantly contribute

to scientific knowledge by providing evidence of phenolic antioxidants in these parts, showing notable biological activities that were previously underexplored. The results of this study support the potential use of mango aerial parts in various nutraceutical formulations and functional foods, offering multiple health benefits.

Table. 9: Correlation analysis between biological activities and TP of Sindhri peel.

	TPC	IC ₅₀ value	Inhibition Potential	Antibacterial activity		Antifungal activity	
				<i>B. pumilus</i>	<i>E. coli</i>	<i>A. niger</i>	<i>F. oxysporum</i>
TPC	1						
IC ₅₀ value	- 0.937**	1					
Inhibition Potential	0.917**	-0.948**	1				
<i>B. pumilus</i>	0.863**	-0.910**	0.902**	1			
<i>E. coli</i>	0.233	-0.308	0.279	0.147	1		
<i>A. niger</i>	0.810**	-0.843**	0.784**	0.600**	0.538*	1	
<i>F. oxysporum</i>	0.624**	-0.636**	0.828**	0.702**	0.107	0.390	1

Table. 10: Correlation analysis between biological activities and TF of Sindhri peel.

	TFC	IC ₅₀ value	Inhibition Potential	Antibacterial activity		Antifungal activity	
				<i>B. pumilus</i>	<i>E. coli</i>	<i>A. niger</i>	<i>F. oxysporum</i>
TFC	1						
IC ₅₀ value	- 0.913**	1					
Inhibition Potential	0.953**	-0.948**	1				
<i>B. pumilus</i>	0.897**	-0.902**	0.910**	1			
<i>E. coli</i>	0.144	-0.279	0.308	0.147	1		
<i>A. niger</i>	0.771**	-0.784**	0.843**	0.600**	0.538*	1	
<i>F. oxysporum</i>	0.735**	-0.828**	0.636**	0.702**	0.107	0.390	1

Table. 11: Correlation analysis between biological activities and TP of Langra peel.

	TPC	IC ₅₀ value	Inhibition Potential	Antibacterial activity		Antifungal activity	
				<i>B. pumilus</i>	<i>E. coli</i>	<i>A. niger</i>	<i>F. oxysporum</i>
TPC	1						
IC ₅₀ value	- 0.876**	1					

Inhibition Potential	0.965**	-0.984**	1				
<i>B. pumilus</i>	0.923**	-0.828**	0.910**	1			
<i>E. coli</i>	0.750**	-0.622**	0.706**	0.878**	1		
<i>A. niger</i>	0.643**	-0.554*	0.669**	0.801**	0.540*	1	
<i>F. oxysporum</i>	0.813**	-0.854**	0.805**	0.671**	0.657**	0.162	1

Table. 12: Correlation analysis between biological activities and TF of Langra peel.

	TFC	IC ₅₀ value	Inhibition Potential	Antibacterial activity		Antifungal activity	
				<i>B. pumilus</i>	<i>E. coli</i>	<i>A. niger</i>	<i>F. oxysporum</i>
TFC	1						
IC ₅₀ value	0.811**	1					
Inhibition Potential	0.998**	-0.984**	1				
<i>B. pumilus</i>	0.837**	-0.910**	0.828**	1			
<i>E. coli</i>	0.625**	-0.706**	0.622**	0.878**	1		
<i>A. niger</i>	0.592**	-0.669**	0.554*	0.801**	0.540*	1	
<i>F. oxysporum</i>	0.825**	-0.805**	0.854**	0.671**	0.657**	0.162	1

Conclusion

In this study, the extraction from selected mango varieties using different solvents yielded various chemical compounds, resulting in distinct antioxidant and antimicrobial properties. These variations are influenced by the solvent type and the specific mango part used, which affect the extraction of particular bioactive compounds. We concluded that selecting the appropriate extraction technique is crucial for maximizing the yield of potent antioxidant compounds from mango plant material. We strongly recommend a comprehensive follow-up investigation focused on these bioactive compounds' structural elucidation, isolation and in vivo biological activities in selected mango parts.

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