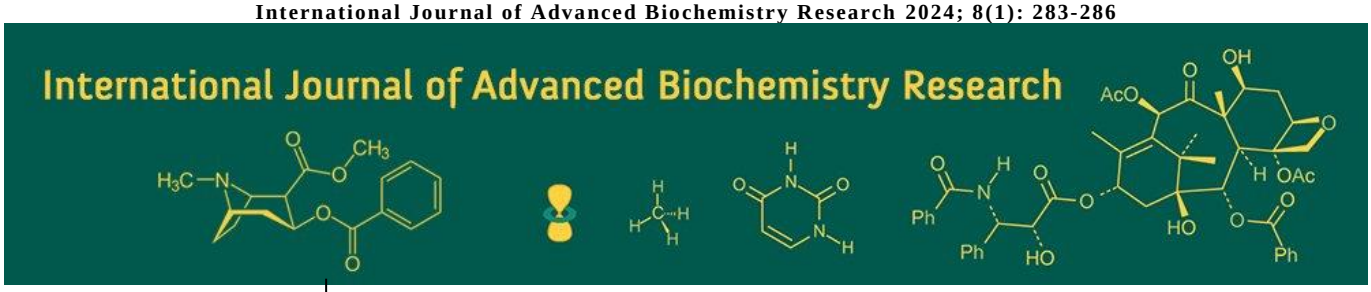

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Bioactive compounds and antioxidant activity of mango peel (*Mangifera indica* L.) produced by different methods of extraction and solvents

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Abstract
Mango, a prominent tropical fruit, holds significant global importance, with India leading in its production. The processing of mango, primarily for pulp and amchur powder preparation, generates peel as a by-product, constituting approximately 20% of the whole fruit. Currently, mango peel is considered a waste product, posing challenges for its disposal. In this study, an effort was made to harness the potential of mango peel by examining its proximate composition, phenolic compounds, flavonoids, carotenoid, and antioxidant activity. The analysis involved three solvents (distilled water, ethanol, and hydroalcoholic) and two extraction methods (conventional solvent extraction and microwave-assisted extraction). The goal was to explore the valuable components within mango peels, providing insights into its potential applications and addressing the environmental concerns associated with its disposal. Highest extraction like total phenolic content contents 32.77 mgGAE/100 gm, flavonoid content 20.13 mgQE/gm, carotenoid 8.25 mg/100 gm and antioxidant activity 77.32% was recorded in microwave assisted extraction. Therefore, hydro alcohol (70:30) is recommended for extraction of phenolic compounds, their secondary metabolites, and antioxidant capacity from the mango peel further isolation and utilization.

Keywords: Bioactive compounds, antioxidant activity, *Mangifera indica* L., solvents

Introduction
Mango (*Mangifera indica* L. Anacardiaceae) is one of the most important tropical fruits in the world and currently ranked 5th in total world production among the major fruit crops. Mango, being a seasonal fruit, undergoes processing for various products like puree, nectar, leather, pickles, canned slices, and chutney. These items have garnered global popularity and have gained significance in both the US and European markets. Approximately 20% of the mango harvest is utilized for the production of these processed goods. In the process of mango processing, by-products like peel and kernel are generated. Currently, the peel is discarded as waste since it is not employed for any commercial purposes, leading to environmental pollution. This waste should be considered a specialized residue due to its high levels of residual phenolics, which can potentially have adverse environmental impacts, primarily by inhibiting seed germination properties of polyphenols. Consequently, the industry faces increasing costs for waste treatment. While there have been numerous studies on the composition and potential utilization of mango seed kernel, research on peels is limited. Phenolic compounds play a crucial role in determining the color and flavor of various foods and beverages, and their consistent intake is linked to positive effects on human health. Certain phenolic compounds found in mango act as antioxidants, potentially lowering the risk of cardiovascular diseases. Additionally, compounds like gallic acid and quercetin are believed to possess properties that can combat allergies, inflammation, hypertension, arthritis, and carcinogenesis. India holds the dominant position as the primary producer of mangoes, and the processing of peeled raw mangoes is undertaken to produce amchur, while ripe mangoes are processed to create mango pulp and fruit bars. Consequently, both raw and ripe peels are abundantly generated as by-products in the mango processing industry. In this current investigation, the focus is on identifying valuable bioactive compounds like polyphenols, carotenoids, antioxidant in the peels of mango. The objective is to explore the potential value of these peels.

Materials and Methods

Estimation of total phenolics, flavonoids

The dried extracts underwent a thorough phytochemical analysis, including assessments of total phenols and flavonoids, following the methodology outlined by Jan *et al.* (2022) [10]. The determination of total phenols employed the Folin-Ciocalteu assay, and the results were expressed in milligrams of gallic acid equivalents per gram (mg GAE/g). In a concise procedure, 0.5 ml of the sample or gallic acid standard, 2.5 ml of Folin-Ciocalteu's reagent (10%), and 2.5 ml of $Na2CO_3$ (7.5%, w/v) were combined. The mixture was stirred and incubated at 45 °C for 30 minutes, and the absorbance was measured at 765 nm using a spectrophotometer (UV-1601, Shimadzu).

The method outlined by Dewanto *et al.* (2002) [5], involving aluminum chloride, was utilized to determine the total flavonoid content in the sample extract. Specifically, 2 ml of the sample extract was mixed with 1.25 ml of distilled water and 75 µl of a 5% sodium nitrite ($NaNO_2$) solution. After a 5-minute interval, 150 µl of 10% aluminum chloride ($AlCl_3.H_2O$) was introduced. The mixture underwent a 6-minute incubation, followed by the addition of 500 µl of 1 M NaOH and 275 µl of distilled water. Subsequently, the solution was thoroughly mixed, and its intensity was measured at 510 nm, using water as a blank reference. The total flavonoid content was determined through a standard calibration curve of Quercetin and expressed as milligrams of Quercetin equivalents (QE) per gram of the sample.

Antioxidant studies

The antioxidant activity of the extracts was assessed through both the DPPH and reducing power methods, following the procedure outlined by Gaurav *et al.* (2020) [11] for the DPPH analysis. In a concise summary, a DPPH solution (0.01 mM) was prepared using methanol as the reagent. Using a 96-well plate, 20 µl of the sample or standard was combined with 180 µl of the DPPH reagent. The plate was then incubated in darkness for 30 minutes at room temperature, and the absorbance was subsequently recorded at 517 nm. The scavenging effect was calculated using equation 1.

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Total carotenoid content (TCar)

The determination of carotenoid content in the persimmon extracts was conducted spectrophotometrically, employing the approach outlined by Nagata and Yamashita (1992) [12], with the extracting solvent serving as the blank. The calculation of total carotenoid content was performed using a specific equation.

TCar (mpg β-carotene/100 ml) = 0.216A663 - 1.22A645 - 0.304A505 + 0.452A453

Where

A663, A645, A505 and A452 represent the absorbances measured at 663 nm, 645 nm, 505 nm and 453 nm, respectively. The TCar was expressed as mg of βcarotene equivalents per 100 g of sample.

Statistical analysis

The experimental design followed a completely randomized approach with three replicates. All recorded data were presented as mean values accompanied by their respective standard deviations (SD). The comparisons between the mean values were tested using Duncan's new multiple-range test at a level of 0.05

Results and Discussion

Table 1: Effect of extraction solvent and extraction method on total phenolic content (mg GAE/g) of mango peel

Product	Protein	Carbohydrate	Crude fiber	Moisture	Crude fat	Ash
Mango peel	2.08±0.02	25.10±0.60	6.70±0.10	72.50±0.50	2.12±0.02	1.18±0.14

Antioxidant component analysis

Type of solvent	Method of Extraction			
	Water	Ethanol	Hydro- alcohol	Mean
CSE	22.71	25.55	27.23	25.16
MAE	28.02	30.53	32.77	30.44
Mean	25.36	28.04	30.00	

Factors	$C.D_{0.05}$
Factor (A)	0.05
Factor (B)	0.05
Factor (A X B)	0.08

MAE: Microwave assisted extraction; CSE: Conventional solvent extraction

Phenols from mango peel was extracted via two different methods microwave assisted extraction and conventional solvent extraction using three diverse solvents such as water, ethanol and hydro- ethanolic extract 70:30 resulting in the formation of aqueous extract (AE), ethanolic extract (EE) and hydro- ethanolic extract (HEE) depicted in table no: 01. The microwave assisted extraction method also followed the trend (HA> >ET>AQ)highest phenols being in hydro alcoholic solvent (32.77 mgGAE/100 gm) using microwave assisted extraction. The presence of water in solvent increase the release of phenols as it improve the swelling of plant material and cause large surface area which helps in better interaction between solvent and material. Similar results were reported by (Xia *et al.*, 2015) [13] that mixture of water and ethanol act as better solvent as compared to pure water and pure ethanol as it extracts more phenolic contents.70% ethanol was used as best extraction solvent for total phenolic while the process decreases as the water content increases in aqueous solvent investigated by (Sharma *et al.*, 2020) [11], he also stated that by using 70 and 96% ethanol more phenolic compounds are extracted.

Table 2: Effect of extraction solvent and extraction method on total flavonoid content (mg QE/g) of mango peel

Type of solvent	Method of Extraction			
	Water	Ethanol	Hydro- alcohol	Mean
CSE	7.25	12.43	15.23	11.63
MAE	17.03	19.59	20.13	18.91
Mean	12.14	16.01	17.68	

Factors	$C.D_{0.05}$
Factor (A)	0.03
Factor (B)	0.03
Factor (A X B)	0.05

MAE: Microwave assisted extraction
CSE: Conventional solvent extraction

Flavonoids from mango peel was extracted via two different methods microwave assisted extraction and conventional solvent extraction using three diverse solvents such as water, ethanol and hydro- ethanolic extract 70:30 resulting in the formation of aqueous extract (AE), ethanolic extract (EE) and hydro- ethanolic extract (HEE) presented in table no:02. The microwave assisted extraction method also followed the trend (HA> >ET>AQ) the highest phenols being in hydro alcoholic solvent (20.13 mg QE/100 gm) using microwave assisted extraction. Regarding the solvent used for extracting flavonoids from mango peel, hydro alcohol and pure ethanol did not differ much in their recovery percentage as compared to aqueous extract. Thus flavonoids in plants are associated with their redox properties which allow them to act as a reducing agent, hydrogen donors and singlet oxygen quenchers. Flavonoids extracted from *sativum* peel using hydro alcoholic extracts shows better recovery of compounds than pure aqueous extract reported by (Kallel *et al.,* 2014) [14]. Our findings coincides with Seo *et al.,* 2014 [15] who suggested that among the three solvents used for extraction of flavonoids, water in combination with ethanol (70:30, 50:50, 30:70, and 10:90 (v/v) shows higher recovery of compounds than methanol extracts.

Table 3: Effect of extraction solvent and extraction method on carotenoid content (mg 100/g) of mango peel

Type of solvent	Method of Extraction			
	Water	Ethanol	Hydro- alcohol	Mean
CSE	0.30	7.21	6.44	4.65
MAE	0.42	9.58	8.25	6.08
Mean	0.36	8.39	7.34	

Factors	$C.D_{0.05}$
Factor (A)	0.04
Factor (B)	0.04
Factor (A X B)	0.06

MAE: Microwave assisted extraction; CSE: Conventional solvent extraction

Carotenoid from mango peel was extracted via two different methods microwave assisted extraction and conventional solvent extraction using three diverse solvents such as water, hydro- ethanolic and ethanol extract resulting in the formation of aqueous extract (AE), hydro- ethanolic extract (HEE) and ethanolic extract (EE) depicted in table no:03.The microwave assisted extraction method also followed the trend (ET> >HA>AQ) the highest caretenoids being in ethanolic solvent (9.58 mg/100 gm) using ultrasonic assisted extraction. Similarly according to (Bulut *et al.,* 2018) [16] highest carotenoids present in *Scenedesmus* spp were extracted from ethyl acetate then hydro alcohol and none were detected in water. Similarly by (Sun *et al.,* 2011) [17] reported that beta carotene extracted from mandarin (*Citrus succosa* Hort) using ethanol as solvent shows better recovery than conventional method. Saini and Keum 2018 reported similar results that Ultrasonic assisted extraction gives highest percentage of carotenoids using ultrasonic intensity (total power 664W) with a temperature of about 30 ºC and using 75% of ethanol. (Lakshmi *et al.,* 2021) [19] studied that the total carotenoid yield from muskmelon and watermelon was about (775.25 µg/g and 639.54 µg/g) from ultrasonic extraction as compared to microwave assisted extraction (590.85 µg/g and 474.72 µg/g). So they concluded that Ultrasonic extraction gives better results for carotenoid than microwave extraction using ethanol as solvent.

Table 4: Effect of extraction solvent and extraction method on antioxidant activity (%) of mango peel

Type of solvent	Method of Extraction			
	Water	Ethanol	Hydro- alcohol	Mean
CSE	43.07	65.15	70.09	59.43
MAE	55.25	74.12	77.32	68.89
Mean	49.16	69.63	73.70	

Factors	$C.D_{0.05}$
Factor (A)	0.05
Factor (B)	0.04
Factor (A X B)	0.08

MAE: Microwave assisted extraction; CSE: Conventional solvent extraction

DPPH from mango peel was extracted via two different methods microwave assisted extraction and conventional solvent extraction using three diverse solvents such as water, ethanol and hydro- ethanolic extract 70:30 resulting in the formation of aqueous extract (AE), ethanolic extract (EE) and hydro- ethanolic extract (HEE) depicted in table no:04. The microwave assisted extraction method also followed the trend (HA> >ET>AQ) the highest phenols being in hydro alcoholic solvent (77.32%) using microwave assisted extraction. (Mouratoglou *et al.,* 2016) [20] studied that TPC value of mango peel extract increases with increase in the microwave power and time which ultimately cause rise in DPPH content, this is because antioxidant activity of product relay on the polyphenolic concentration of the sample extracts to same limit. While increasing microwave power and liquid to solid ratio antioxidant activity increases this is mainly due to the rise in mass transfer which ultimately leads to increase in FRAP, TPC and DPPH. Rojas *et al.,* 2015 [21] reported that yield and extraction of phenolic compounds obtained from microwave assisted extraction have major antioxidant activity in DPPH and lipid oxidation inhibition assays (70.31 and 86.46%) as compared conventional method. Song *et al.,* 2021 [22] studied different extraction methods for evaluation of antioxidant activity from persimmon peel and revealed that microwave assisted extraction had an antioxidant activity 4 times higher than TMA extract.

Conclusion

This research has demonstrated that mango peel possesses a substantial concentration of bioactive compounds, including phenolic content, flavonoids, carotenoids, and antioxidant activity. Whether consumed as a whole fruit or in processed forms, integrating mango into one's diet can significantly contribute to the intake of these beneficial compounds. Mango peel, a by-product of the mango processing industry, emerges as a promising reservoir of bioactive compounds and enzymes such as protease, peroxidase, and polyphenol oxidase. This newfound source holds great potential as a functional food or as value-added ingredients in our dietary landscape. With proper processing, mango peel could yield valuable products that not only offset waste treatment expenses but also reduce the overall cost of the primary product.

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