



In vitro anti-oxidant, anti-microbial and anti-inflammatory activities of five Indian cultivars of mango (*Mangifera indica* L.) fruit peel extracts

Katike Umamahesh¹, Bandikari Ramesh^{1,2}, Bathal Vijaya Kumar³, Obulam Vijaya Sarathi Reddy^{1*}¹Department of Biochemistry, Sri Venkateswara University, Tirupati – 517 502²State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Shizishan Street, Wuhan 430070, China³Department of Biotechnology, Sri Venkateswara University, Tirupati - 517 502, India

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ABSTRACT

Introduction: All parts of the mango plant contain secondary metabolites that possess several beneficial properties. In the present study, evaluation of *in vitro* anti-oxidant, anti-microbial and anti-inflammatory activities of five Indian cultivars of mango fruit peel extracts were carried out.

Methods: Different solvent (hexane, ethyl acetate, and methanol) extracts of five Indian cultivars of mango fruit peels were prepared by using Soxhlet, and the aqueous extract was prepared by maceration. *In vitro* anti-oxidant activities of these extracts were determined by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), hydrogen peroxide (H₂O₂) and nitric oxide (NO) radical scavenging methods. Their anti-microbial and anti-inflammatory activities were determined by agar-well diffusion and HRBC (human red blood cell) methods, respectively.

Results: All the five mango peel extracts showed good anti-oxidant activities, especially the methanolic extract of peel of *Sindhura* cultivar showed better IC₅₀ values of DPPH (21.62±1.82 µg/mL), ABTS (21.33±1.94 µg/mL), H₂O₂ (19.87±2.61 µg/mL) and NO (57.29±2.17 µg/mL) radical scavenging activities than other extracts. It also possessed higher contents of phenolics (169.18±3.28 mg of GAE/g) and flavonoids (26.18±1.84 mg of QE/g) than the other extracts. All peel extracts of five cultivars of mango fruit revealed good anti-microbial activities against bacterial and fungal cultures and also possessed significant anti-inflammatory activity.

Conclusion: The present study revealed that all the mango peel extracts have potential antioxidant activity, as well as better anti-microbial and anti-inflammatory activities.

Implication for health policy/practice/research/medical education:

Mango peel extracts possess significant anti-oxidant, anti-microbial and anti-inflammatory activities. Hence eating mango fruit along with its peel would impart more health benefits.

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Introduction

The intermediate oxygen species are an integral part of cell signaling and metabolic pathways in living organisms and play a major role in some diseases (1). Anti-oxidants act primarily as oxidative stress reducing compounds (2). These are available in dietary supplements and are rich in fruits (3). These natural compounds are produced due to the response of stress conditions (4). In recent years, these

are used as food preservatives (5) to avoid the toxic effects of synthetic compounds (6).

Since time immemorial the pathogenic microorganisms are commonly known as agents for human infections (7). Many scientists have reported that microorganisms are the causal agents of food-borne diseases and food spoilage (8,9), which raises the necessity for anti-microbial agents (10). The anti-microbial properties of plants have been

*Corresponding author: Obulam Vijaya Sarathi Reddy,
Email: ovsreddy@yahoo.com

extensively investigated worldwide as they contain potent phytochemical constituents having antimicrobial properties (11).

Inflammation is a defense mechanism in the human body when it has a prompt response to injuries caused by trauma of chemical or microbial agents (12). The non-steroidal drugs are used for the management of inflammatory conditions, which have several disadvantages (13). The plants are potential sources for newer compounds with significant activities (14).

Mango (*Mangifera indica* L.) is the national fruit of India (15). Seed and peel are important products of mango fruit. Of these 7%-10% by weight of fruit is peel and is discarded as waste (16). The peel has more poly-phenol contents than that of flesh (17). Many researchers reported that the mango peels have bioactive compounds and exhibit different bioactivities such as anti-diabetic (18), anti-proliferating (19) and anti-inflammatory activities (20). This paper presents data on the anti-oxidant, anti-microbial and anti-inflammatory activities of peel extracts of 5 Indian mango cultivars.

Materials and Methods

Preparation of peel powders

Alphonso, *Sindhura*, *Malgoa*, *Rumani* and *Banisha* cultivars of ripened mangoes were brought from a local market in Tirupati. The peels were collected, dried and grounded individually into powders (21). The peel powder samples were kept at 4°C for further analysis.

Aqueous extracts of mango peel powders

Individually, the peel powders were macerated with distilled water by stirring periodically and filtrate was collected and stored at 4°C for further use (21).

Preparation of Soxhlet solvent extracts

A 100 g specimen from each of 5 mango cultivar peel powder was placed in the extraction chamber of Soxhlet separately, and bioactive compounds were extracted by polarity gradient solvents such as hexane, ethyl acetate and methanol. The extracts from each solvent were collected individually and stored at 4°C for further use.

Preliminary phytochemical screening

The preliminary phytochemical analysis was carried out for detection of different chemical groups of compounds (22). The extract yields were calculated by using the below formula (23).

$$Y(\%) = \frac{W_2 - W_1}{W_0} \times 100$$

W_0 - weight of the initial dried sample, W_1 - weight of the container alone and W_2 - weight of the extract with container.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity

It was determined by Burits and Bucar method (24). One mL of different concentrations of each extract (25-100 µg) except hexane extract (200-1000 µg) in methanol was added to 4 mL of 0.04% DPPH solution. They were kept at room temperature under dark condition for 30 minutes and the intensity was read against a blank at 517 nm. The percentage of free radical inhibition (I %) was calculated using the following equation. $I\% = \{(A_c - A_s) / A_c\} \times 100$. Where, A_c - absorbance of control against blank (containing all the reagents except the test sample) and A_s - absorbance of test sample against blank.

ABTS (2,2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

The anti-oxidant activity was determined using stable ABTS⁺ radical method of Re et al (25).

H₂O₂ radical scavenging activity

The H₂O₂ scavenging ability of extracts was determined by the method of Vijayabaskaran et al (26).

Nitric oxide radical scavenging activity

Nitric oxide (NO) scavenging activity was measured by the method of Marcocci et al (27).

Total phenolics content (TPC)

It was determined calorimetrically by Folin-Ciocalteu method (28,29).

Total flavonoids content

It was determined colorimetrically by aluminum chloride method as described by Woisky and Salatino (30).

Test microorganisms and growth media

The bacterial cultures such as *Klebsiella pneumonia* MTCC109, *Escherichia coli* MTCC448, *Pseudomonas aeruginosa* MTCC741, *Bacillus subtilis* MTCC2394 and *Enterobacter aerogenes* MTCC111 were obtained from GITAM University, Visakhapatnam, and grown on nutrient media at 37°C and maintained on nutrient agar slants at 4°C. The fungal cultures such as *Saccharomyces cerevisiae* MTCC170, *Candida albicans* MTCC227, *Aspergillus fumigatus* MTCC1811 and *Filobasidiella neoformans* MTCC1347 were obtained from IMTECH, Chandigarh and maintained on different media as suggested by IMTECH for the study on antimicrobial activity.

Anti-bacterial activity

It was determined by agar well diffusion method against test microorganisms (31). A 24 h old bacterial broth cultures were used for testing the anti-bacterial activity (32). The extracts were dissolved in dimethylsulphoxide

(DMSO) and single concentrations (1000 µg/mL) of extracts were used for carried out anti-microbial activity. The triplicate average diameter of inhibition zones (in mm) was measured and recorded as anti-bacterial activity.

Anti-fungal activity

It was expressed as the diameter of inhibition zones (in mm) produced by the extracts against test fungal flora. The experiment was repeated thrice and the average value was recorded (33).

In vitro anti-inflammatory activity

A blood sample was collected from a healthy volunteer and anti-inflammatory activity was estimated by human red blood cell (HRBC) stabilization method (34). The hemolysis percentage was estimated by assuming the hemolysis produced in the presence of distilled water as 100%. The percent of protection was calculated by the following formula:

$$\text{Percentage of protection} = [1 - (\text{OD Sample} / \text{OD Control})] \times 100\%$$

Statistical analysis

The experiments were carried out in triplicate and results were expressed as mean \pm standard deviation (SD). The statistical analysis was carried out by using one-way analysis of variance (ANOVA) and the *P* values were considered significant at *P* < 0.05.

Results

Phytochemical screening

Preliminary screening of peel extracts of 5 mango cultivars showed positive results for the presence of secondary metabolites like steroids, saponins, terpenoids, polyphenols, glycosides and flavonoids (data are not presented). High amounts of bioactive compounds like steroids, carbohydrates, glycosides and polyphenols were observed in methanol extract as compared to other extracts. Hexane extracts showed fewer compounds than the others (data are not presented).

Yield of mango peel extracts

The yields of peel extracts are presented in Table 1. The highest extract yield was observed in aqueous peel extract of all 5 mango cultivars, among these aqueous peel extract of *malgoa* cultivar showed the highest yield, and the lowest yield was observed in hexane peel extract of *Rumani* cultivar.

DPPH radical scavenging activity

All peel extracts showed a concentration-dependent antiradical activity by reducing the stable purple to yellow colour. The scavenging effects of aqueous, methanol, ethyl acetate and hexane extracts of 5 cultivars of mango fruit

Table 1. Yield (%) of solvent extracts of fruit peels of five mango cultivars

Mango cultivar	Hexane extract	Ethyl acetate extract	Methanol extract	Aqueous extract
<i>Sindhura</i>	17.6	18	40.3	43.3
<i>Malgoa</i>	11.3	24.6	33.6	50
<i>Alphonso</i>	10.3	23.3	32.6	42.6
<i>Rumani</i>	8.6	20.6	28.3	40.6
<i>Banisha</i>	15.6	13.3	36.6	37.3

Note: The mean of triplicate values is represented in the table.

peels on the DPPH radical are shown in Figure 1 (A, B, C and D, respectively). The methanolic peel extract of *Sindhura* mango cultivar had higher scavenging effect than all other extracts. The IC₅₀ value of methanolic peel extract of *Sindhura* cultivar gave better activity at low concentration (21.62 \pm 1.82 µg/mL). The IC₅₀ values of different solvent extracts of *Sindhura*, *Malgoa*, *Rumani*, *Banisha* and *Alphonso* on the DPPH radical scavenging

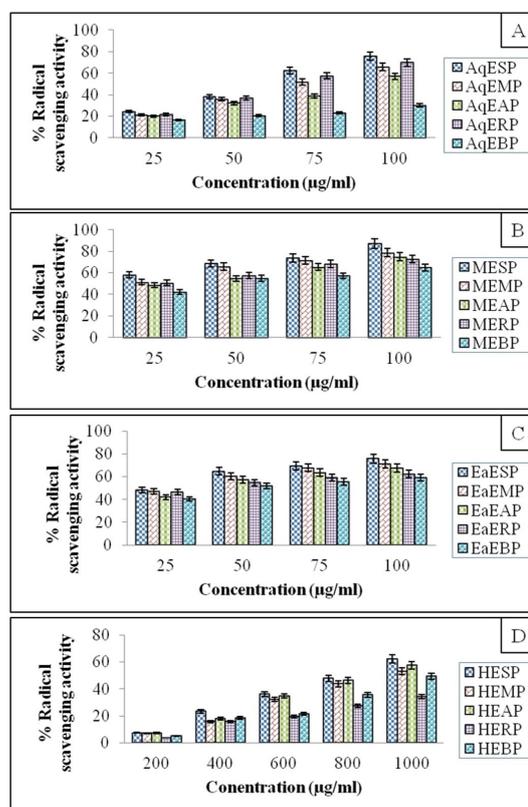


Figure 1. DPPH Radical scavenging activity: A- Aqueous; B-Methanolic; C-Ethyl acetate; D-Hexane extracts of peels of 5 mango cultivars. HESP - Hexane extract of *Sindhura* peel; EaESP - Ethyl acetate extract of *Sindhura* peel; MESP - Methanol extract of *Sindhura* peel; AqESP -Aqueous extract of *Sindhura* peel; HEMP - Hexane extract of *Malgoa* peel; EaEMP - Ethyl acetate extract of *Malgoa* peel; MEMP - Methanol extract of *Malgoa* peel; AqEMP -Aqueous extract of *Malgoa* peel; HERP - Hexane extract of *Rumani* peel; EaERP - Ethyl acetate extract of *Rumani* peel; MERP - Methanol extract of *Rumani* peel; AqERP - Aqueous extract of *Rumani* peel; HEBP - Hexane extract of *Banisha* peel; EaEBP - Ethyl acetate extract of *Banisha* peel; MEBP - Methanol extract of *Banisha* peel; AqEBP - Aqueous extract of *Banisha* peel; HEAP - Hexane extract of *Alphonso* peel; EaEAP - Ethyl acetate extract of *Alphonso* peel; MEAP - Methanol extract of *Alphonso* peel; AqEAP -Aqueous extract of *Alphonso* peel.

activity showed in the order of high scavenging activity at low concentration as MESP > MEMP > MERP > MEAP > MEBP > EAESP > EAEMP > EAEAP > EAERP > EAEBP > AqESP > AqEMP > AqERP > AqEAP > AqEBP > HESP > HEAP > HEMP (Table 2), respectively.

ABTS radical scavenging activity

The peel extracts of 5 mango cultivars on the ABTS radical scavenging activity are shown in Figure 2 (A, B, C and D, respectively). Results indicated that the IC₅₀ values of methanolic peel extract of *Sindhura* cultivar had the best activity at low concentration (21.33±1.94 µg/mL) when compared to all other extracts of mango cultivar peels. The IC₅₀ values of hexane, ethyl acetate, methanol and aqueous extracts of 5 Indian mango cultivar peels on the ABTS radical scavenging activity were in order of MESP > MEMP > MERP > MEAP > MEBP > EAESP > EAEMP > EAEAP > EAERP > EAEBP > AqESP > AqEMP > AqERP > AqEAP > AqEBP > HESP > HEAP > HEMP > HEBP (Table 2), respectively.

H₂O₂ radical scavenging activity

The scavenging activity of aqueous, methanol, ethyl acetate and hexane peel extracts of 5 mango cultivars on the H₂O₂ radical are shown in Figure 3 (A, B, C and D, respectively). Results indicated that the IC₅₀ value

of methanolic peel extract of *Sindhura* cultivar had the best activity at low concentration (57.29±2.17 µg/mL). The IC₅₀ values of hexane, ethyl acetate, methanol and aqueous extracts of *Sindhura*, *Malgoa*, *Rumani*, *Banisha* and *Alphonso* on the H₂O₂ radical showed high activity at low concentrations in order of MESP > MEMP > MEAP > MERP > MEBP > EAESP > EAEMP > EAEAP > EAERP > AqESP > AqEMP > AqERP (Table 2), respectively.

Nitric oxide radical scavenging activity

All peel extracts showed a concentration-dependent antiradical activity by donating protons to nitrite radical. The scavenging effects of aqueous, methanol, ethyl acetate and hexane peel extracts of 5 mango cultivars on the NO radical are shown in Figure 4 (A, B, C and D, respectively). Results indicated that the methanolic peel extract of *Sindhura* cultivar gave the best activity at low concentration (21.33 ± 1.94 µg/mL) when compared to all other extracts of mango cultivar peels. The IC₅₀ values of hexane, ethyl acetate, methanol and aqueous extracts of *Sindhura*, *Malgoa*, *Rumani*, *Banisha* and *Alphonso* on the NO radical showed in order of MESP > MEMP > MEAP > MERP > MEBP > EAESP > EAEMP > EAEAP > EAERP > EAEBP > AqESP > AqEMP > AqERP, respectively (Table 2). The IC₅₀ value of hexane was not measured in the range of 200-1000 µg/mL.

Table 2. IC₅₀ values of DPPH, ABTS, H₂O₂, NO, total phenolics and total flavonoid contents of different solvent extracts of fruit peels of 5 mango cultivars

Solvent extract	IC ₅₀ DPPH (µg/mL)	IC ₅₀ ABTS (µg/mL)	IC ₅₀ (H ₂ O ₂) (µg/mL)	IC ₅₀ (NO) (µg/mL)	TPC (mg of GAE/ g)	TFC (mg of QE/g)
AqESP	65.34±2.71	28.29±3.28	54.58±2.93	80.12±1.73	87.38±2.91	15.64±2.81
AqEMP	69.15±1.64	30.32±2.17	57.07±1.62	96.52±1.37	62.13±2.66	12.82±1.95
AqEAP	95.11±2.18	51.11±1.84	78.28±1.48	-	58.47±1.53	11.58±2.31
AqERP	67.28±1.59	31.79±2.14	59.66±2.62	87.56±1.82	84.38±2.92	14.71±2.19
AqEBP	169.83±2.39	84.88±1.63	-	-	48.93±1.77	8.72±1.69
MESP	21.62±1.82	21.33±1.94	19.87±2.61	57.29±2.17	169.18±3.28	26.18±1.84
MEMP	24.41±1.26	23.32±1.64	21.96±1.82	69.57±1.82	102.42±2.16	19.46±1.26
MEAP	25.82±1.97	25.82±1.72	23.19±1.48	76.21±1.42	92.57±1.92	16.96±1.19
MERP	24.75±1.83	24.75±1.28	21.29±2.21	78.94±2.74	151.36±2.36	23.82±2.04
MEBP	45.62±2.92	44.04±2.17	25.56±1.67	93.15±1.36	79.16±1.28	13.75±1.36
EaESP	25.82±2.57	24.27±2.69	21.68±2.91	79.95±2.97	148.28±2.74	22.52±1.92
EaEMP	26.7±2.18	25.93±2.26	22.4±2.18	86.35±1.74	93.19±1.93	17.04±1.28
EaEAP	43.63±2.92	41.87±1.75	24.36±1.89	89.12±2.88	84.27±2.08	15.19±1.08
EaERP	45.62±2.36	44.48±1.92	23.27±2.46	92.25±1.62	131.62±2.46	20.39±2.36
EaEBP	48.26±1.97	46.81±2.62	24.6±2.71	-	65.28±1.48	13.26±1.75
HESP	835.7±1.29	817.99±2.59	847.45±3.94	-	15.24±1.39	2.68±0.92
HEMP	943.75±1.46	914.07±2.31	932.4±2.62	-	11.39±1.07	2.04±0.57
HEAP	874.12±2.84	865.8±2.94	871.45±2.91	-	9.17±1.06	1.37±0.52
HERP	-	-	956.02±2.17	-	5.28±1.35	0.91±0.24
HEBP	-	950.57±2.13	-	-	7.25±1.25	1.07±0.36

HESP - Hexane extract of *Sindhura* peel; EaESP - Ethyl acetate extract of *Sindhura* peel; MESP - Methanol extract of *Sindhura* peel; AqESP - Aqueous extract of *Sindhura* peel; HEMP - Hexane extract of *Malgoa* peel; EaEMP - Ethyl acetate extract of *Malgoa* peel; MEMP - Methanol extract of *Malgoa* peel; AqEMP - Aqueous extract of *Malgoa* peel; HERP - Hexane extract of *Rumani* peel; EaERP - Ethyl acetate extract of *Rumani* peel; MERP - Methanol extract of *Rumani* peel; AqERP - Aqueous extract of *Rumani* peel; HEBP - Hexane extract of *Banisha* peel; EaEBP - Ethyl acetate extract of *Banisha* peel; MEBP - Methanol extract of *Banisha* peel; AqEBP - Aqueous extract of *Banisha* peel; HEAP - Hexane extract of *Alphonso* peel; EaEAP - Ethyl acetate extract of *Alphonso* peel; MEAP - Methanol extract of *Alphonso* peel; AqEAP - Aqueous extract of *Alphonso* peel.

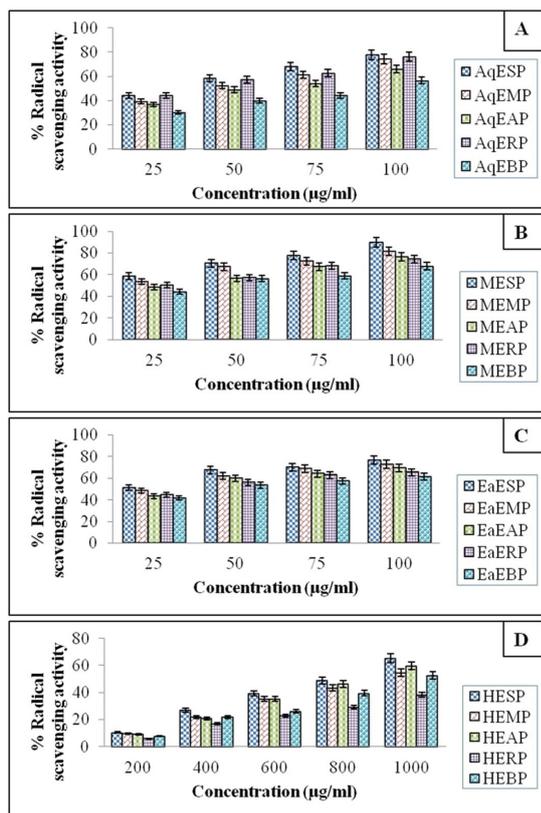


Figure 2. ABTS Radical scavenging activity: A- Aqueous; B-Methanolic; C-Ethyl acetate; D-Hexane extracts of peels of 5 mango cultivars. HESP - Hexane extract of *Sindhura* peel; EaESP - Ethyl acetate extract of *Sindhura* peel; MESP - Methanol extract of *Sindhura* peel; AqESP -Aqueous extract of *Sindhura* peel; HEMP - Hexane extract of *Malgoa* peel; EaEMP - Ethyl acetate extract of *Malgoa* peel; MEMP - Methanol extract of *Malgoa* peel; AqEMP -Aqueous extract of *Malgoa* peel; HERP - Hexane extract of *Rumani* peel; EaERP - Ethyl acetate extract of *Rumani* peel; MERP - Methanol extract of *Rumani* peel; AqERP - Aqueous extract of *Rumani* peel; HEBP - Hexane extract of *Banisha* peel; EaEBP - Ethyl acetate extract of *Banisha* peel; MEBP - Methanol extract of *Banisha* peel; AqEBP - Aqueous extract of *Banisha* peel; HEAP - Hexane extract of *Alphonso* peel; EaEAP - Ethyl acetate extract of *Alphonso* peel; MEAP - Methanol extract of *Alphonso* peel; AqEAP -Aqueous extract of *Alphonso* peel.

Total phenolics and flavonoids content

The TPC of mango peel extracts are shown in Table 2. The results revealed that the phenolics content of methanolic peel extract of *Sindhura* cultivar had the highest total TPC as compared to the extracts of other cultivars peels. The total flavonoids of mango peel extracts are shown in Table 2. The results revealed that higher flavonoids were observed in methanolic extract of *Sindhura* peel (26.18 ± 1.84 mg of QE/g) than the other extracts.

Anti-bacterial activity of mango peel extracts

All the peel extracts showed anti-bacterial activity against the tested pathogenic bacteria (Table 3). The hexane peel extracts of *Sindhura* and *Banisha* cultivars have shown better activity against *E. coli* (8 mm and 8 mm respectively). Methanolic peel extracts of *Sindhura* and hexane peel extract of *Malgoa* cultivars had better activity against *B. subtilis* (9 mm and 9 mm, respectively). Ethyl acetate peel extract of *Sindhura* had activity against *P. aeruginosa* (12

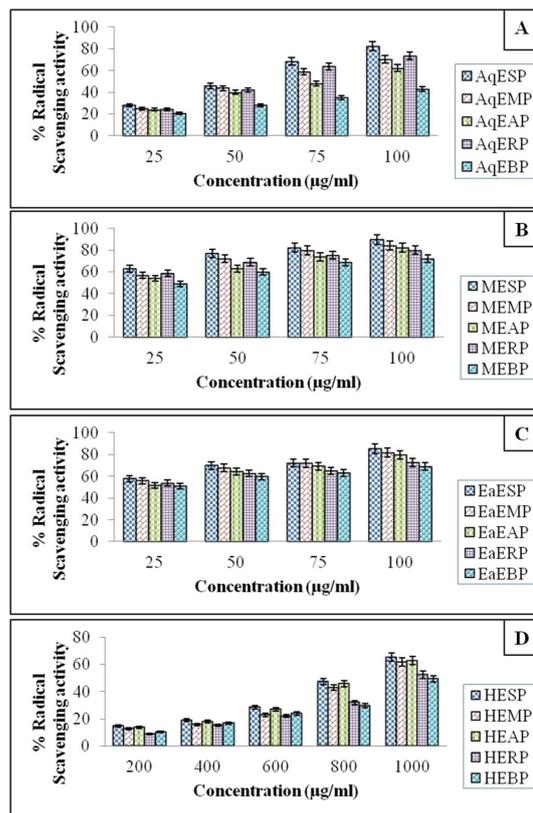


Figure 3. H_2O_2 Radical scavenging activity: A- Aqueous; B-Methanolic; C-Ethyl acetate; D-Hexane extracts of peels of 5 mango cultivars. HESP - Hexane extract of *Sindhura* peel; EaESP - Ethyl acetate extract of *Sindhura* peel; MESP - Methanol extract of *Sindhura* peel; AqESP -Aqueous extract of *Sindhura* peel; HEMP - Hexane extract of *Malgoa* peel; EaEMP - Ethyl acetate extract of *Malgoa* peel; MEMP - Methanol extract of *Malgoa* peel; AqEMP -Aqueous extract of *Malgoa* peel; HERP - Hexane extract of *Rumani* peel; EaERP - Ethyl acetate extract of *Rumani* peel; MERP - Methanol extract of *Rumani* peel; AqERP - Aqueous extract of *Rumani* peel; HEBP - Hexane extract of *Banisha* peel; EaEBP - Ethyl acetate extract of *Banisha* peel; MEBP - Methanol extract of *Banisha* peel; AqEBP - Aqueous extract of *Banisha* peel; HEAP - Hexane extract of *Alphonso* peel; EaEAP - Ethyl acetate extract of *Alphonso* peel; MEAP - Methanol extract of *Alphonso* peel; AqEAP -Aqueous extract of *Alphonso* peel.

mm). The ethyl acetate extracts of peels of *Rumani* and *Sindhura* showed better activity against *E. aerogenes* (10 mm and 9 mm, respectively). The aqueous peel extract of *Rumani* had better activity against *K. pneumonia* (8 mm).

Anti-fungal activity of mango peel extracts

All peel extracts showed anti-fungal activity against tested fungal cultures (Table 4). Among these, the ethyl acetate peel extract of *Sindhura* gave better activity against *C. albicans* (10 mm), aqueous peel extract of *Sindhura* showed better activity against *S. cerevisiae* (10 mm), ethyl acetate extract of *Sindhura* peel gave better activity against *A. fumigatus* (15 mm) and ethyl acetate peel extract of *Sindhura* revealed better activity against *F. neoformans* (12 mm).

In vitro anti-inflammatory activity

The extracts of 5 cultivars of mango peels showed significant stabilization towards HRBC membranes. The

Table 3. Zone of inhibition of mango peel extracts on bacterial cultures

Solvent extract	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aerogenosa</i>	<i>Enterobacter aerogenes</i>	<i>Klebsiella pneumonia</i>
AqESP	7	8	6	4	6
AqEMP	5.33	6.67	6.4	5.68	4.67
AqEAP	5	4	8	6	4
AqERP	9	6	10	7	8
AqEBP	5	4	7	4	5
MESP	6	9	10	6	8
MEMP	6.67	7.33	7	5	7.33
MEAP	5	6	7	4	6
MERP	7	8	8	6	7
MEBP	5	6	7	4	5
EaESP	7	9	12	9	7
EaEMP	5.2	6.5	7.33	6.83	4.7
EaEAP	4	5	9	7	5
EaERP	6	7	6	10	7
EaEBP	4	6	10	8	4
HESP	8	4	3	6	3
HEMP	6.6	9.67	5.4	4.57	3.17
HEAP	5	3	4	3	-
HERP	4	5	6	5	4
HEBP	8	7	8	7	6

Note: The mean of triplicate values is represented in the table.

HESP - Hexane extract of *Sindhura* peel; EaESP - Ethyl acetate extract of *Sindhura* peel; MESP - Methanol extract of *Sindhura* peel; AqESP - Aqueous extract of *Sindhura* peel; HEMP - Hexane extract of *Malgoa* peel; EaEMP - Ethyl acetate extract of *Malgoa* peel; MEMP - Methanol extract of *Malgoa* peel; AqEMP - Aqueous extract of *Malgoa* peel; HERP - Hexane extract of *Rumani* peel; EaERP - Ethyl acetate extract of *Rumani* peel; MERP - Methanol extract of *Rumani* peel; AqERP - Aqueous extract of *Rumani* peel; HEBP - Hexane extract of *Banisha* peel; EaEBP - Ethyl acetate extract of *Banisha* peel; MEBP - Methanol extract of *Banisha* peel; AqEBP - Aqueous extract of *Banisha* peel; HEAP - Hexane extract of *Alphonso* peel; EaEAP - Ethyl acetate extract of *Alphonso* peel; MEAP - Methanol extract of *Alphonso* peel; AqEAP - Aqueous extract of *Alphonso* peel.

percentage protection of aqueous peel extract of *Sindhura* at concentration 200 µg/mL had higher stabilizing activity than that of other cultivars extracts, even at both concentrations (100 and 200 µg) (Table 5). However, the percentage of protection was found to be increased at an increased concentration of the extracts.

Discussion

The present study revealed that mango peel had secondary metabolites like steroids, saponins, terpenoids, flavonoids and polyphenols, which corroborates with the reports of Rakholiya et al (35). The study also showed that the methanolic peel extracts had high yield based on the dissolution of phytochemicals in the methanol solvent (36), and the results are in accordance with the reports of Putri et al (37) who reported the yields of various extracts of mango peel.

The *in vitro* anti-oxidant studies would give better leads to carry out the biological activities in animal models. DPPH is casually used for evaluation of anti-oxidant activity (38). The change of purple to yellow colour was observed due to reduction of hydrogen or electron donor (39). In the present study, the methanolic peel extract of *Sindhura* cultivar gave better anti-oxidant activity than the other cultivar extracts. These results are similar to the reports of few researchers who studied the DPPH activity

on peel extracts of mango (40,41).

ABTS is a general assay to determine the anti-oxidant capacity in food products (42). The ABTS⁺ has high reactive nature with phenolics, thiols and vitamin C (43). In this assay, ABTS radical cation was formed by adding sodium persulfate. In this assay blue color changed to colorless form due to interaction of some bioactive compounds and its intensity was measured at 734 nm (25). In the present study, the methanolic peel extract of *Sindhura* had better activity than the other extracts. These results are in accordance with the results of few researchers who measured ABTS activity on peel extracts of mango (40,41).

H₂O₂ is not very reactive; sometimes it can be toxic due to the increase in hydroxyl radicals in the cells (44). In the present study, all extracts showed activity depending on concentration, and the results were in agreement with the reports of Nabavi et al (45) who reported the anti-oxidant activity of Iran medicinal plants such as *Diospyros lotus* and *Pyrus boissieriana*.

The nitrite ions diazotize with sulphanilamide acid and couple with naphthyl ethylene diamine forming pink colour, the anti-oxidant activity is shown by donating protons to the nitrite radical (46). In the present study, the low inhibition of NO radical scavenging activity was observed even at high concentration. The increased

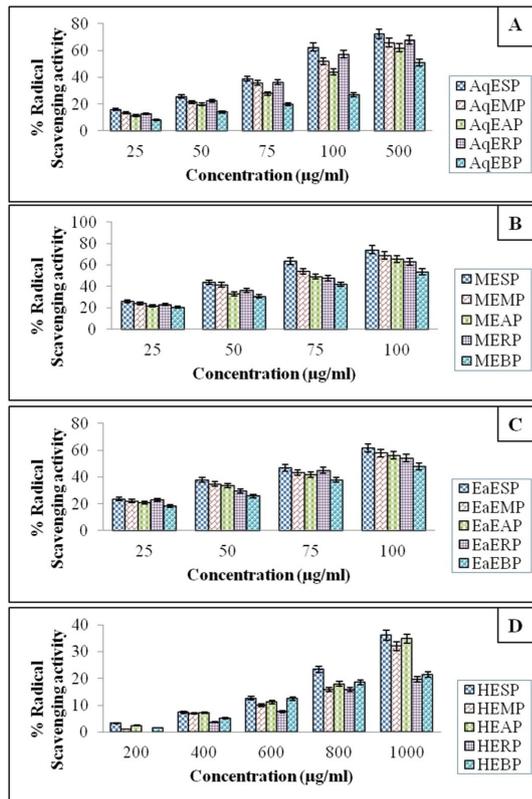


Figure 4. NO Radical scavenging activity: A- Aqueous; B-Methanolic; C-Ethyl acetate; D-Hexane extracts of peels of 5 mango cultivars. HESP - Hexane extract of *Sindhura* peel; EaESP - Ethyl acetate extract of *Sindhura* peel; MESP - Methanol extract of *Sindhura* peel; AqESP -Aqueous extract of *Sindhura* peel; HEMP - Hexane extract of *Malgoa* peel; EaEMP - Ethyl acetate extract of *Malgoa* peel; MEMP - Methanol extract of *Malgoa* peel; AqEMP -Aqueous extract of *Malgoa* peel; HERP - Hexane extract of *Rumani* peel; EaERP - Ethyl acetate extract of *Rumani* peel; MERP - Methanol extract of *Rumani* peel; AqERP - Aqueous extract of *Rumani* peel; HEBP - Hexane extract of *Banisha* peel; EaEBP - Ethyl acetate extract of *Banisha* peel; MEBP - Methanol extract of *Banisha* peel; AqEBP -Aqueous extract of *Banisha* peel; HEAP - Hexane extract of *Alphonso* peel; EaEAP - Ethyl acetate extract of *Alphonso* peel; MEAP - Methanol extract of *Alphonso* peel; AqEAP -Aqueous extract of *Alphonso* peel.

inhibition of radical scavenging activity was observed at increased concentration (47). The present study revealed that the methanolic extract had good radical scavenging activity than other extracts and these results are in concurrence with those of Boora et al (47) who reported that methanol or ethanol extract showed better activity when compared with aqueous extract of Zimbabwean plants.

The peel extracts of mango cultivars by methanol had more TPC due to the solvent polarity for the extraction of TPC (48). The high TPC was observed in ethanol and acetone extracts of mango fruit peel (16,19). The increased contents of phenolics and flavonoids were observed due to the dissolving capacity of residual substances, which interfere in the quantification of phenolics and flavonoids (36). The results were in accordance with Kim et al (19) who reported higher TPC and total flavonoids content values in peel extract than the flesh extract of ripe mango.

The agar well diffusion assay of the present study

Table 4. Zone of inhibition of mango peel extracts on fungal cultures

Solvent extract	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i>	<i>Aspergillus fumigatus</i>	<i>Filobasidiella neoformans</i>
AqESP	8	10	13	8
AqEMP	5.7	7.33	11	5.77
AqEAP	5	7	9	5
AqERP	6	9	10	7
AqEBP	4	5	7	4
MESP	6	8	12	7
MEMP	4.2	6.33	9	4.77
MEAP	4	5	6	4
MERP	5	7	8	6
MEBP	3	5	7	5
EaESP	10	9	15	12
EaEMP	6.2	5.7	12.7	7.3
EaEAP	8	6	10	10
EaERP	9	7	11	9
EaEBP	5	6	9	7
HESP	4	4	7	5
HEMP	1.7	1.33	4	2.33
HEAP	-	4	6	4
HERP	4	3	5	3
HEBP	5	6	5	6

Note: The mean of triplicate values is represented in the table.

HESP - Hexane extract of *Sindhura* peel; EaESP - Ethyl acetate extract of *Sindhura* peel; MESP - Methanol extract of *Sindhura* peel; AqESP -Aqueous extract of *Sindhura* peel; HEMP - Hexane extract of *Malgoa* peel; EaEMP - Ethyl acetate extract of *Malgoa* peel; MEMP - Methanol extract of *Malgoa* peel; AqEMP -Aqueous extract of *Malgoa* peel; HERP - Hexane extract of *Rumani* peel; EaERP - Ethyl acetate extract of *Rumani* peel; MERP - Methanol extract of *Rumani* peel; AqERP - Aqueous extract of *Rumani* peel; HEBP - Hexane extract of *Banisha* peel; EaEBP - Ethyl acetate extract of *Banisha* peel; MEBP - Methanol extract of *Banisha* peel; AqEBP -Aqueous extract of *Banisha* peel; HEAP - Hexane extract of *Alphonso* peel; EaEAP - Ethyl acetate extract of *Alphonso* peel; MEAP - Methanol extract of *Alphonso* peel; AqEAP -Aqueous extract of *Alphonso* peel.

revealed that the mango peel extract had anti-microbial activity. Based on cultivars and solvents, it showed the highest activities to different pathogenic cultures. The present results of anti-bacterial activity were similar to the report of Thambi et al (49) and the anti-fungal activity results were similar to report of Dorta et al (50). Our results support the hypothesis that the medicinal properties of the peels could be due to the presence of bioactive compounds.

The erythrocyte membrane is analogous to the liposomal membrane (51-53). The stabilization of the erythrocyte membrane resembles the stabilization of lysosomal membranes (52,53). In the present study, the peel extracts of mango fruit exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. These results are similar to reports of Saleem et al (54) who reported *in vitro* anti-inflammatory activity of *Gendarussa vulgaris* Nees leaf extracts. According to Knodler et al (20), mango peel has bioactive compounds

Table 5. Effect of mango peel extracts on HRBC membrane stabilization

Solvent extract	% of inhibition	
	100 µg/mL	200 µg/mL
AqESP	63.85±1.37	68.27±1.48
AqEMP	57.83±1.24	63.85±1.27
AqEAP	51.26±1.11	57.86±1.25
AqERP	56.62±1.22	62.65±1.35
AqEBP	45.24±0.97	51.24±1.10
MESP	57.83±1.24	63.85±1.37
MEMP	56.29±1.21	61.29±1.32
MEAP	52.16±1.12	59.27±1.28
MERP	58.36±1.26	63.18±1.36
MEBP	54.45±1.17	61.37±1.32
EaESP	49.27±1.06	56.24±1.21
EaEMP	42.96±0.928	49.62±1.07
EaEAP	51.8±1.11	58.29±1.25
EaERP	57.36±1.23	64.16±1.38
EaEBP	52.83±1.14	59.85±1.29
HESP	59.03±1.27	66.85±1.44
HEMP	51.27±1.11	56.39±1.21
HEAP	37.16±0.8	43.29±0.93
HERP	26.17±0.57	31.26±0.68
HEBP	32.67±0.71	38.27±0.82

HESP - Hexane extract of *Sindhura* peel; EaESP - Ethyl acetate extract of *Sindhura* peel; MESP - Methanol extract of *Sindhura* peel; AqESP - Aqueous extract of *Sindhura* peel; HEMP - Hexane extract of *Malgoa* peel; EaEMP - Ethyl acetate extract of *Malgoa* peel; MEMP - Methanol extract of *Malgoa* peel; AqEMP - Aqueous extract of *Malgoa* peel; HERP - Hexane extract of *Rumani* peel; EaERP - Ethyl acetate extract of *Rumani* peel; MERP - Methanol extract of *Rumani* peel; AqERP - Aqueous extract of *Rumani* peel; HEBP - Hexane extract of *Banisha* peel; EaEBP - Ethyl acetate extract of *Banisha* peel; MEBP - Methanol extract of *Banisha* peel; AqEBP - Aqueous extract of *Banisha* peel; HEAP - Hexane extract of *Alphonso* peel; EaEAP - Ethyl acetate extract of *Alphonso* peel; MEAP - Methanol extract of *Alphonso* peel; AqEAP - Aqueous extract of *Alphonso* peel.

like resorcinols which might be responsible for anti-inflammatory activity.

Conclusion

The methanolic peel extracts of *Sindhura* mango cultivar has shown higher anti-oxidant activity by bleaching DPPH, ABTS, H₂O₂ and NO radicals than the other solvent extracts of mango cultivars peels. The peel extracts of *Sindhura* cultivar gave the best activity against both bacteria and fungi, followed by *Rumani* cultivar, which has shown potency against *K. pneumonia*. Among the aqueous extracts, peel extract of *Sindhura* cultivar (at 200 µg/mL) has shown potent red blood cells protection. Hence, *Sindhura* peel should be exploited for its components in the development of products that will have health benefits.

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Authors' contributions

The ideology and design of the study were done by KU and OVSR. However, the experimental procedures were done by KU and BV under the guidance of OVSR. The statistical data and interpretation of the results were done by KU, BR and OVSR. All the authors contributed to preparation of the manuscript and agreed with publication of the final proof.

Conflict of interests

The authors declare that they have no conflict of interest.

Ethical considerations

Ethical issues (including falsification, double publication or submission, data fabrication, misconduct, redundancy and plagiarism) were carefully observed by authors.

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