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ORIGINAL ARTICLE

# Comparative Evaluation of *in vitro* Antioxidant Activities and High-Performance Liquid Chromatography Fingerprinting of Fruit Peels Collected from Food Processing Industry Wastes

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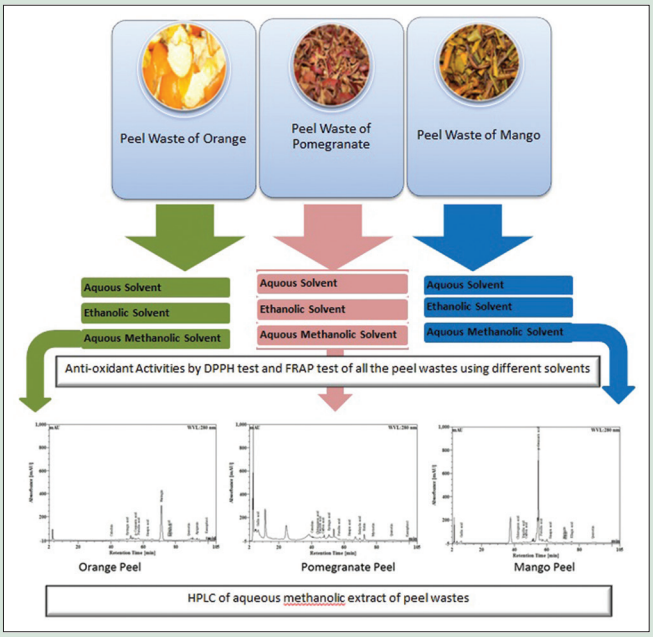
## ABSTRACT

**Background:** An enormous quantity of fruit peel is obtained from food processing industry as leftover materials which cause environmental pollution if not used judiciously. **Objective:** The present study was focused to explore antioxidant activities and detect bioactive compounds of three different fruit peel wastes (orange, mango, and pomegranate) collected from fruit processing centers. **Materials and Methods:** Peel extracts were primarily investigated for total phenolic and total flavonoid content (TFC) in three different solvent systems, namely aqueous–methanolic (20:80, v/v), ethanolic, and aqueous. These were examined for *in vitro* antioxidant potential by 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and ferric reducing antioxidant power (FRAP) test. Peel waste extracts were further characterized by high-performance liquid chromatography. **Results:** Pomegranate peel (PP) wastes exhibited significantly ($P < 0.01$) high concentration of total phenolic content (TPC) and TFC followed by mango peel (MP) wastes and orange peel (OP) wastes. DPPH and FRAP tests revealed significantly ($P < 0.01$) high antioxidant activity in aqueous–methanolic and aqueous extract of PP. The degree of antioxidant activities in each type of solvent was in the order of PP >MP >OP. Pearson's correlation coefficient analysis revealed a strong association between antioxidant activity and TPC. High concentration of gallic acid, salicylic acid, chlorogenic acid, rutin, and catechin was observed in aqueous–methanolic (20:80, v/v) extracts of PP, and these might be the reason behind the higher antioxidant activities of PP. **Conclusion:** Results of this study clearly suggest that PP waste contains strong antioxidant molecules and might be used as additive in commercial feed to ameliorate oxidative stress in animals.

**Key words:** Antioxidant activity, flavonoid, high-performance liquid chromatography, peel waste, phenolic acid

## SUMMARY

- Three fruit peel (pomegranate, mango, and orange) wastes were tested for their total phenolic and flavonoid contents. *In vitro* antioxidant activities were also measured by 2,2-diphenyl-1-picrylhydrazyl hydrate and ferric reducing antioxidant power test. Aqueous–methanolic extracts of pomegranate peel (PP) wastes exhibited potential free radical scavenging activities
- Further high-performance liquid chromatography characterization of aqueous–methanolic extract of PP wastes revealed high concentration of powerful antioxidant phenolic acids and flavonoids that could be responsible for its high antioxidant activities. Therefore, PP extracts might be used in animal feed industry for their bioactive roles.




**Abbreviations Used:** HPLC: High-performance liquid chromatography; DAD: Diode-array detector; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; PP: Pomegranate peel wastes; MP: Mango peel wastes; OP: Orange peel wastes.

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## INTRODUCTION

Food processing industry discards huge amount of fruit peels as waste materials containing plethora of bioactive compounds with antioxidant, antimicrobial, antitumor, myoprotective, and immune-stimulatory effects.[1] Recently, antioxidant extracts from fruit/vegetable peels have attracted researchers[2-4] to produce functional foods that might be used against oxidative damage of living cells.

India, the second largest fruit producer in the world, recorded 286.2 million ton fruit production[5] in 2015–2016; it is bestowed with variety of fruits grown in its diverse climatic zones. Indian fruit beverage



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industry is called "Sunrise Industry" due to its fast growth rate and is quite obvious that in the near future, large quantity of fruit peels would be available for recycling processes. At present, there are very limited available literatures on comparative evaluation of antioxidant activity of fruit peels collected as industry leftover materials. In this backdrop, objective of the present study was to characterize and compare three different fruit peel wastes (orange, mango, and pomegranate) from food processing industry for phenolic and flavonoid concentration, *in vitro* antioxidant activities, and high-performance liquid chromatography (HPLC) fingerprinting of phenolic acids/flavonoids present in these materials that might be recycled as additives in animal feed.

## MATERIALS AND METHODS

### Collection of samples

Peel wastes of orange, mango, and pomegranate [Table 1] were collected from a jelly and juice manufacturing center located at Narendrapur situated in Kolkata, India. These waste materials were collected in sterilized plastic bins during February–May 2018. Average moisture contents of the peel wastes were recorded during collection with a moisture meter (HE53, Mettler Toledo, USA) at the day of collection. Total titratable acidity and sugar content were measured by methods as described by AOAC (2000).[6]

### Preparation of samples for extraction

After collection, peels were washed with distilled water and then air-dried under shade for 5 days. The peels were chopped into small pieces with a sharp scissor and then made it into coarse powder using an electrical grinder. The dried powder of peels was packed into air-tight containers in refrigerated condition (4°C) for preparation of extracts.

#### *Preparation of extracts*

One gram of dried powder was extracted with 25 ml of three different solvents: aqueous–methanol (20:80, v/v), ethanol, and water in 25°C. The mixtures were kept in an orbital shaker for 4 h with 110 rpm. The extracts were filtered through Whatman No. 1 filter paper. The residues were re-extracted again with the same solvents under the same condition. The two extract fractions were pooled, and the final volume was adjusted to 50 ml. The peel waste extracts were then placed in dark bottles in refrigerator (4°C) for further analysis.

#### *Estimation of total phenolic and flavonoid content*

Total phenolic content (TPC) was measured using Folin–Ciocalteu method[7] with slight modifications. The TPC was measured against the serially diluted standard curve of gallic acid and expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight). Total flavonoid content (TFC) was measured according to Pal *et al.*[8] Results were expressed in mg of quercetin equivalent (mg of QE/g of dry weight).

#### *Determination of free radical scavenging activity (RSA) by 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) test*

*In vitro* antioxidant activity was determined by DPPH assay following Szabo *et al.*[9] RSA % was calculated using the following equation:

RSA % = (1− [absorbance of sample/absorbance of blank]) × 100

RSA % values were used to calculate inhibition concentration at 50% ($IC_{50}$) values that denote the effective concentration of a sample required to decrease the absorbance at 517 nm by 50%. All measurements were performed in triplicate.

#### *Determination of ferric reducing antioxidant power*

The ferric reducing antioxidant power (FRAP) of various peel extracts was performed based as per Benzie and Strain,[10] with slight modification. Absorbance of serially diluted standard $FeSO_4$ and $7H_2O$ (0.001M) was recorded after incubating it with 2 ml of the FRAP solution for 30 min at 37°C in dark chamber. Absorbance of the blue color product (ferrous tripyridyl triazine complex) was taken at 593 nm using a spectrophotometer (Shimadzu 1800-UV, Japan). FRA*P* values of peel waste extracts were obtained from the standard curve and were expressed as μM Fe (II)/mg dry material.

### High-performance liquid chromatography fingerprinting of polyphenols

Fingerprinting of aqueous–methanolic extracts of peels was performed using HPLC method[11] using Dionex UltiMate 3000, Thermo Scientific, USA, equipped with quaternary pump (LPG 3400 SD) for solvent delivery, 20 μl loop for injection and PDA detector (DAD 3000) and Chromeleon 6.8 system manager as data processor. The separation was achieved using reverse-phase column, Acclaim™ 120 $C_{18}$ column (250 mm × 4.6 mm, 5 μm). Individual peel extracts were further diluted with aqueous–methanol (20:80, v/v) at a concentration of 1 mg/ml and filtered through 0.2 μm PVDF filter. Standard polyphenols such as gallic acid, salicylic acid, catechin, chlorogenic acid, caffeic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, salicylic acid, naringin, rutin, ellagic acid, myricetin, quercetin, apigenin, and kaempferol were prepared in aqueous–methanol (20:80, v/v) at concentration 1 mg/ml as stock solution. Further dilutions were made for calibration of each standard. The mobile phase contains methanol (solvent A) and 1% acetic acid solution (solvent B), and the column was thermostatically controlled at 28°C. The gradient elusion was 10% A and 90% B with flow rate 1 to 0.7 ml/min in 27 min, from 10% to 40% A and 90% to 60% B with flow rate 0.7 ml/min in 28 min, 40% A and 60% B with flow rate 0.7 to 0.6 ml/min in 5 min, from 40% to 44% A and 60% to 56% B with flow rate 0.6 to 0.3 ml/min in 5 min, and 44% A and 56% B with flow rate 0.3 to 0.6 ml/min in 5 min. The mobile phase composition backs to initial condition of 10% A and 90% B and allowed to run for another 8 min, before another injection of sample. The detection of compounds was performed using detector at 280 nm. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area, and the content was calculated using the calibration curve of the respective standard sample.

### Statistical analysis

Data of antioxidant indicators from peel extracts ($n$ = 3 for each peel extract) in each solvent were analyzed for test of significance at 5% and

**Table 1:** Physiochemical attributes of peel wastes

| Fruit | Cultivar | Used for | Peeling method | Residue collected | Moisture at collection (%) | Acidity (%) | Total sugar (%) | Abbreviated as |
|---|---|---|---|---|---|---|---|---|
| Orange (*Citrus reticulata*) | *Nagpur Mandarin* | Squash production | Hand peeling | Peel | 75.57 | 0.48 | 18.52 | OP |
| Mango (*Mangifera indica*) | *Totapuri* | Squash production | Hand peeling | Peel | 65.69 | 0.35 | 39.17 | MP |
| Pomegranate (*Punica granatum*) | *Ruby* | Juice production | Hand peeling | Mixture of peel, albedo and membrane | 59.96 | 1.22 | 67.9 | PP |

OP: Orange peel wastes; MP: Mango peel wastes; PP: Pomegranate peel wastes

1% levels by ANOVA.[12] Multiple comparisons of means were measured by SPSS is a software package for statistical analysis by (IBM, USA). Pearson's correlation coefficients ($P < 0.05$) between TPC, TFC, and antioxidant assays were calculated by Microsoft Excel, 2007. Free radical scavenging activities of extracts were analyzed by non-linear regression curves generated by GraphPad Prism v. 7 (GraphPad Software, Callifornia, USA).

## RESULTS AND DISCUSSION

### Total phenolic content and total flavonoid content of peel waste

Total phenolic and TFC of mango (MP), orange (OP), and pomegranate peel (PP) wastes in three different solvents are presented in Table 2. Among all the solvents, aqueous–methanol was found to be most efficient for extracting polyphenols. TPC in different peel wastes in all three types of solvents was in the order of $PP_{Aq\text{-}Meth} > PP_{Aqueous} > MP_{Aq\text{-}Meth} > PP_{Ethanol} > MP_{Ethanol} > MP_{Aqueous} > OP_{Aq\text{-}Meth} > OP_{Ethanol} > OP_{Aqueous}$. These findings are in similar line with Singh *et al.*[13] who also reported highest level of TPC in PP in aqueous–methanolic extract among common Indian fruits and vegetables. High TPC in PP indicates high degree of accumulation of bioactive materials in PP. High level of TPC was also found in aqueous extract of PP that was in accordance with Viuda-Martos *et al.*[14] The present report further revealed no significant difference ($P < 0.01$) of TPC remained between $PP_{Aq\text{-}Meth}$ and $PP_{Aqueous}$. This might be due to the fact that PP contained polyphenols like gallic acid in high proportion that mostly dissolves in aqueous solvent and was in agreement with Galanakis *et al.*[15] who reported low activity coefficient of gallic acid in water.

Flavonoids are important class of polyphenols, and biological activities of them are diverse including antioxidant, hepatoprotective, anticancer, anti-inflammatory, and antiviral activity. In the present study, TFC of peel extracts stood in the order of: $PP_{Aqueous} > PP_{Aq\text{-}Meth} > MP_{Ethanol} > OP_{Ethanol} > MP_{Aq\text{-}Meth} > PP_{Ethanol} > OP_{Aq\text{-}Meth} > OP_{Aqueous} > MP_{Aqueous}$. No significant difference ($P < 0.01$) was observed between $PP_{Aqueous}$ and $PP_{Aq\text{-}Meth.}$ However, TFC of the present study remained little bit lower than that obtained by Singh *et al.*[13] in his study on peels of pomegranate, kinnow orange, and mango. Varietal difference of the fruits and the extraction process might be responsible for this variation. The present results suggested that PP extract might be used as nutraceuticals due to high flavonoid contents.

### *In vitro* antioxidant assays of peel waste extracts

#### *2,2-diphenyl-1-picrylhydrazyl hydrate assay*

The DPPH RSA of three peel wastes (OP, MP, and PP) along with standard ascorbic acid in different solvents is presented in inhibition curves [Figure 1]. Principle of DPPH assay is based on single electron transfer (SET) reaction in which deep purple color of DPPH changes to colorless on reduction with antioxidants.[16] $PP_{Aqueous}$ showed maximum scavenging activity followed by $PP_{Aq\text{-}Meth}$ and $MP_{Aq\text{-}Meth}$. Least antioxidant activity was observed in $OP_{Ethanol}$. Previous works[13] reported similar antioxidant profile (PP > MP > OP) in aqueous–methanol extracts. In the present study, MP Ethanol showed maximum antioxidant activity among all the ethanolic extracts. Safdar *et al.*[17] observed that methanol extracted kinnow mandarin peel demonstrated higher scavenging activity than peels extracted with ethanol solvent. The present study confirmed the same with Nagpur OPs. However, all the OP samples showed very less antioxidant activities compared to PP and MP, which might be attributed to relative presence and reducing strength of polyphenols in three peel extracts. Inhibition curves of $PP_{Aqueous}$, $PP_{Aq\text{-}Meth}$, and $MP_{Aq\text{-}Meth}$ clearly showed dose-dependent scavenging activity.

$IC_{50}$ values (concentration to scavenge 50% of free radicals) of peel waste samples in different solvents were derived from regression curves and presented in Table 3. In the same reaction condition, $IC_{50}$ values of ascorbic acid (not shown in table) were recorded as 0.119, 0.139, and 0.1 mg/ml, respectively, in aqueous–methanolic, ethanolic, and water solvent. Lower $IC_{50}$ values indicate higher antioxidant activities. Lowest $IC_{50}$ value was observed in $PP_{Aqueous}$ followed by $PP_{Aq\text{-}Meth,}$ i.e., these extracts demonstrated highest antioxidant activities without any significant ($P < 0.01$) difference.

### Ferric reducing antioxidant power assay

FRAP assay is also a SET type reaction in which a single electron is donated by antioxidants to reduce the colorless ferric ($Fe^3$) ion to blue-colored ferrous ($Fe^2$) ion. More is the FRA*P* value, more is the antioxidant power. Results of FRAP assay of three peel waste extracts are presented in Table 4. PP extracts showed maximum antioxidant activities compared to other peel extracts in each solvent. Hierarchal order of antioxidant activity was PP > MP > OP. Earlier works[16] reported similar FRA*P* values from kinnow mandarin extracts from Pakistan. Efficiency of solvents for demonstration of antioxidant activity of peel wastes was in the order of aqueous methanol > water > ethanol. Aqueous–methanol extract of PP waste exhibited maximum antioxidant activity.

### Correlation between total phenolic content, total flavonoid content, and antioxidant assays

Babbar *et al.*[18] earlier suggested TPC and TFC of fruit residues are correlated to its antioxidant ability. In the present study, strong correlations [Table 5] were observed between TPC and antioxidant assays (FRAP, $IC_{50}$) in aqueous methanol extracts, suggesting that phenolic contents are responsible for antioxidant activities. Significantly high ($P < 0.01$) correlation was found among $IC_{50}$ and TFC in aqueous methanol extract of OP, suggesting that flavonoids may be the principal constitutes for its antioxidant activities. Results further revealed that for determination of TFC in ethanolic solvents, DPPH assay is a better method than FRAP.

### Phenolic acids/flavonoids identified by high-performance liquid chromatography

Phenolic acids and flavonoids detected and quantified by HPLC-DAD in three peel wastes (OP, MP, and PP) are presented in

**Table 2:** Total phenolic content and total flavonoid content of orange peel wastes, mango peel wastes, and pomegranate peel wastes

| Variables | Aqueous | | | Ethanol | | | Aqueous methanol (20:80, v/v) | | | *P* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MP | OP | PP | MP | OP | PP | MP | OP | PP | Solvent | Peel wastes | S×P* |
| TPC (mg of GaE/g DW) | $18.3\pm0.32^{e}$ | $8.55\pm0.14^{g}$ | $71.9\pm0.49^{a}$ | $23.4\pm0.39^{d}$ | $9.19\pm0.13^{g}$ | $26.9\pm1.12^{c}$ | $31.5\pm0.64^{b}$ | $13.6\pm0.16^{f}$ | $74.3\pm2.11^{a}$ | <0.01 | <0.01 | <0.01 |
| TFC (mg of QE/g DW) | $1.59\pm0.021^{f}$ | $1.73\pm0.12^{ef}$ | $7.88\pm0.32^{a}$ | $6.83\pm0.13^{b}$ | $3.22\pm0.063^{c}$ | $2.18\pm0.13^{de}$ | $2.31\pm0.086^{d}$ | $1.82\pm0.03^{ef}$ | $7.86\pm0.22^{a}$ | 0.03 | <0.01 | <0.01 |

Values are means±SEM, $n$=3 per treatment group. Means in a row without a common superscript letter differ ($P$<0.01) as analyzed by two-way ANOVA and the Duncan test. *S×P=Solvent×peel wastes interaction effect. SEM: Standard error of mean; TPC: Total phenolic content; TFC: Total flavonoid content; OP: Orange-peel wastes; MP: Mango peel wastes; PP: Pomegranate peel wastes

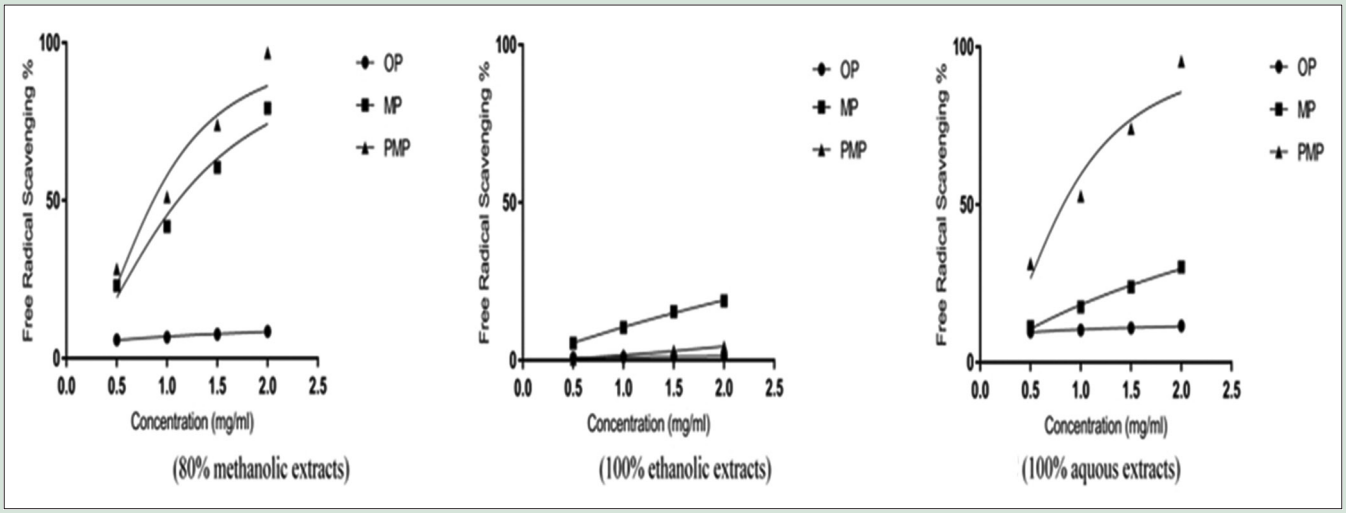


**Figure 1:** 2,2-diphenyl-1-picrylhydrazyl hydrate scavenging activities of peel wastes in different solvents

**Table 3:** Inhibition concentration$_{50}$ (mg/ml) values of peel waste extracts by 2,2- diphenyl-1-picrylhydrazyl hydrate method

| | Aqueous | | | Ethanol | | | Aqueous methanol (20:80, v/v) | | | *P* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MP | OP | PP | MP | OP | PP | MP | OP | PP | Solvent | Peel wastes | S×P* |
| $IC_{50}$ | 3.56±0.042$^{d}$ | 32.6±2.3$^{b}$ | 0.935±0.013$^{d}$ | 5.06±0.302$^{d}$ | 139±12.2$^{a}$ | 19.2±1.22$^{c}$ | 1.21±0.024$^{d}$ | 25.5±0.21$^{bc}$ | 0.973±0.011$^{d}$ | <0.01 | <0.01 | <0.01 |

Values are means±SEM, *n*=3 per treatment group. Means in a row without a common superscript letter differ (*P*<0.05) as analyzed by two-way ANOVA and the DUNCAN test. *S×P=Solvent×peel wastes interaction effect. SEM: Standard error of mean; OP: Orange peel wastes; MP: Mango peel wastes; PP: Pomegranate peel wastes; $IC_{50}$: Inhibition concentration$_{50}$

**Table 4:** Ferric reducing antioxidant power values (μM Fe (II)/mg) of peel waste extracts

| | Aqueous | | | Ethanol | | | Aqueous methanol (20:80, v/v) | | | *P* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MP | OP | PP | MP | OP | PP | MP | OP | PP | Solvent | Peel wastes | S×P1 |
| FRAP | 242.04±15.5$^{d}$ | 23.32±0.49$^{g}$ | 1322.96±20$^{b}$ | 67.24±2.69$^{f}$ | 17.48±0.38$^{g}$ | 100.57±1.9$^{e}$ | 568.92±4.03$^{c}$ | 28.96±0.25$^{g}$ | 1630.96±15.6$^{a}$ | <0.01 | <0.01 | <0.01 |

Values are means±SEM, *n*=3 per treatment group. Means in a row without a common superscript letter differ (*P*<0.05) as analyzed by two-way ANOVA and the DUNCAN test. *S×P=Solvent×peel wastes interaction effect. SEM: Standard error of mean; OP: Orange-peel wastes; MP: Mango peel wastes; PP: Pomegranate peel wastes; FRAP: Ferric reducing antioxidant power

**Table 5:** Pearson correlation coefficients between total phenolic content, 2,2-diphenyl-1-picrylhydrazyl hydrate and ferric reducing antioxidant power assays

| Antioxidant assays | TPC | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 100% aqueous | | | 100% ethanol | | | Aqueous methanol (20:80, v/v) | | |
| | MP | OP | PP | MP | OP | PP | MP | OP | PP |
| DPPH ($IC_{50}$, mg/ml) | −0.765 | −0.485 | −0.521 | −0.209 | −0.145 | −0.531 | −0.933 | −0.989* | -0.983* |
| FRAP (μM Fe (II)/mg) | −0.216 | −0.456. | 0.994* | 0.810 | 0.944 | 0.912 | 0.996** | 0.976* | 0.996** |
| | TFC | | | | | | | | |
| DPPH ($IC_{50}$, g/ml) | −0.446 | −0.778 | −0.481 | −0.993* | −0.903 | −0.916 | −0.994* | −0.996** | −0.370 |
| FRAP (μM Fe (II)/mg) | 0.198 | 0.798 | 0.514 | 0.180 | 0.929 | 0.832 | 0.941 | 0.987* | 0.180 |

*Correlation significant at 0.05 level, **Correlation significant at 0.01 level. DPPH: 2,2-diphenyl-1-picrylhydrazyl hydrate; FRAP: Ferric reducing antioxidant power; TPC: Total phenolic content; TFC: Total flavonoid content; OP: Orange peel wastes; MP: Mango peel wastes; PP: Pomegranate peel wastes

Table 6 and Figure 2. Phenolic acids are categorized into hydroxybenzoic acid and hydroxycinnamic acid derivatives based on their carbon backbones, whereas basic flavonoid structure consists of two benzene rings linked through a heterocyclic pyran ring. In the present study, among benzoic acid derivatives, gallic acid (3,4,5-trihydroxybenzoic acid) was detected in significantly high ($P < 0.05$) concentration in PP than MP, whereas it was not detected in OP. This was in agreement with Singh *et al.*[13] Concentration of salicylic acid (2-hydroxybenzoic acid) was next to gallic acid and detected only in PP. Among the cinnamic acid derivatives, chlorogenic acid and caffeic acid were significantly ($P < 0.05$) high in PP than MP. Szwajgier *et al.*[19] investigated antioxidant activities of several benzoic acid and cinnamic acid derivatives and observed that caffeic acid and its esters are potent antioxidants. Badhani *et al.*[20] reviewed structure–activity relationship in detail in respect to strong antioxidant property of gallic acid and its derivatives. He also predicted that gallic acid along with other cinnamic acid derivatives may show stronger antioxidant characteristic due to synergism. Therefore, high antioxidant activity of PP, as shown in the present DPPH and FRAP tests, could be due to the presence of relatively high concentration of gallic acid. Salicylic acid is also a potent antioxidant[21] that was found in PP.

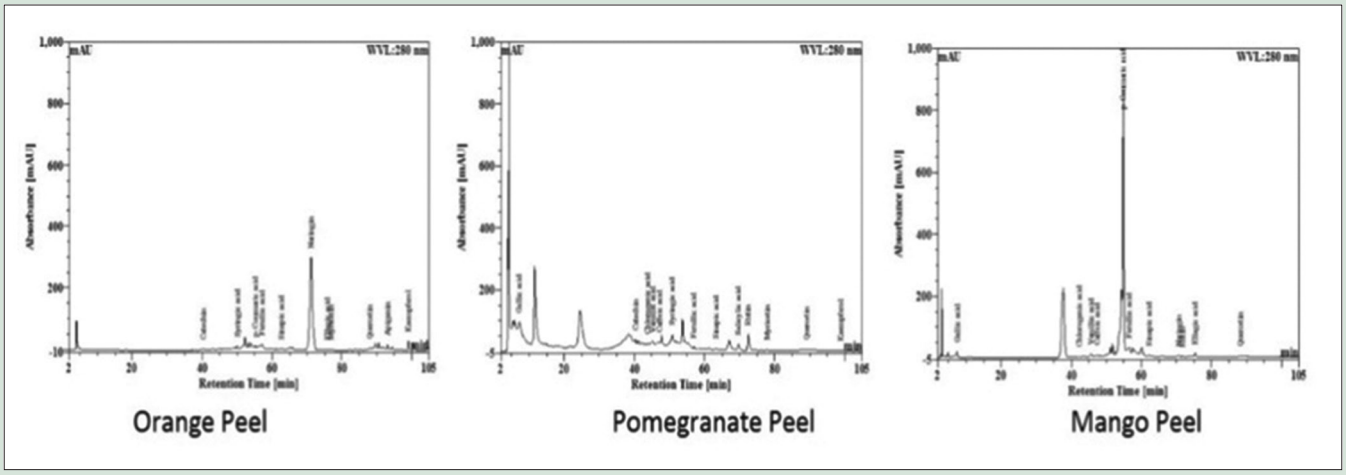


**Figure 2:** High-performance liquid chromatography chromatograms of aqueous–methanolic extracts of peel wastes

**Table 6:** HPLC analysis of phenolic acid and flavonoids (mg/g) present in aqueous-methanolic (20:80, *v/v*)

| | Extract of peel wastes | | |
|---|---|---|---|
| | MP | OP | PP |
| Phenolic acids | | | |
| Gallic acid | 1.07±0.193[b] | ND | 8.78±0.5[a] |
| Vanillic acid | 0.218±0.097[a] | ND | 0.206±0.046[a] |
| Syringic acid | ND | 0.0913±0.035[b] | 0.654±0.148[a] |
| Salicylic acid | ND | ND | 2.24±0.044[a] |
| Chlorogenic acid | 0.27±0.098[a] | ND | 0.43±0.26[a] |
| Caffeic acid | 0.107±0.041[a] | ND | 0.163±0.056[a] |
| p-Coumaric acid | 10.9±0.484[a] | 0.0309±0.004[b] | ND |
| Ferulic acid | 4.1±0.711[a] | 0.344±0.009[b] | 0.0456±0.006[b] |
| Sinapic acid | 0.0238±0.001[a] | 0.0114±0.002[b] | 0.027±0.003[a] |
| Ellagic acid | 0.512±0.028[a] | 0.159±0.0293[b] | ND |
| Flavonoids | | | |
| Catechin | ND | 0.174±0.056[b] | 1.14±0.08[a] |
| Naringin | 0.357±0.0254[b] | 24.1±0.313[a] | ND |
| Rutin | 0.341±0.142[b] | ND | 2.44±1.02[a] |
| Myricetin | ND | 0.137±0.00139[b] | 0.161±0.007[a] |
| Quercetin | 0.0404±0.006[b] | 0.0681±0.016[b] | 0.18±0.019[a] |
| Apigenin | ND | 0.225±0.019 | ND |
| Kaempferol | ND | 0.619±0.024[a] | 0.341±0.129[b] |

Values are means±SEM, *n*=3 per treatment group. Means in a row without a common superscript letter differ (*P*<0.05) as analyzed by one-way ANOVA and the Duncan test. ND: Not detected; HPLC: High performance liquid chromatography; SEM: Standard error of mean; OP: Orange peel wastes; MP: Mango peel wastes; PP: Pomegranate peel wastes

Apart from the antioxidant role, PP might also be used in feed additive as analgesic and antipyretic due to the presence of salicylic acid which is known for its cyclooxygenase-II pathway inhibitory action. Ellagic acid is known for its antiproliferative and antioxidant activities, and the present study showed that MP contains ($P < 0.05$) significant concentration of ellagic acid than OP, whereas it was not detected in PP. MP also contains significant concentration ($P < 0.05$) of p-coumaric acid, which acts as hepatoprotective and neuroprotective agents.[22]

Flavonoids belong to a diverse group of chemicals such as flavones, flavonols, flavanones, isoflavones, and flavan-3-ols. Antioxidant activities differ with each other due to the arrangement of functional groups surrounding the ring structure and glycosylation. The present study revealed that predominant flavonoid in PP is rutin – a flavone compound with widespread pharmacological benefits against various chronic diseases such as hypertension, hypercholesterolemia, diabetes, and arthritis. Catechin and quercetin are also observed in statistically significant ($P < 0.05$) amount in PP. Quercetin is a strong antioxidant with great pharmacological functions such as immune system modulation and anti-inflammation.[23,24] Pereira *et al.*[24] showed that naringin is abundantly found in industrial wastes of orange juice. The present study is in good agreement to it. Naringin has many potential health benefits and great effect on modulation of oxidative stress and inflammation.[25]

## CONCLUSION

Fruit peel wastes from food processing industry are a valuable waste with many bioactive compounds with strong antioxidant properties. PP waste showed highest antioxidant activity followed by MP wastes and OP wastes in DPPH and FRAP tests. HPLC fingerprinting of aqueous–methanolic extracts revealed significant presence of gallic acid, chlorogenic acid, rutin, and catechin in PP, whereas p-coumaric acid, ferulic acid, ellagic acid, and rutin were predominant polyphenols in MP. It was also observed that although OP had less antioxidant activity, it had fairly high concentration of naringin.

The present study revealed that vast scope exists for recycling of fruit peel wastes from fruit beverage industry. Recycling of fruit peel wastes for harnessing bioactive molecules will not only provide cheap source of valuable antioxidant molecules for using it as additive in animal feed but also will reduce environmental pollution at the same time. In high-density animal farming, where oxidative stress is likely to be more, feeding of peel waste might be beneficial for better production and overall well-being of animals. Further study in *in vivo* model is necessary to determine the best peel waste extract for using them as antioxidants in animal farming.

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## Conflicts of interest

There are no conflicts of interest.

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