Phenolic profile and antioxidant capacity of some selected summer fruits from Pakistan

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HIGHLIGHTS

- Estimated the phenolic contents and antioxidant activity of some selected summer fruits
- Unripe mango, guava and melon extracts showed excellent reducing potential
- Highest TPC (9.35 g/100g of dry fruit material)

\textbf{ABSTRACT}

This study was conducted to estimate the phenolic contents and antioxidant activity of some selected summer fruits i.e. guava, apple, melon and unripe mango from Pakistan. The methanol extract of each fruit was prepared using orbital shaker. Total phenolic contents (TPC) and total flavonoid contents (TFC) of fruits extracts were measured as gallic acid and catechin equivalent, respectively. Highest TPC (9.35 g/100g of dry fruit material, measured as gallic acid equivalent) was determined from melon extract while minimum TPC (6.12 g/100g of dry fruit material measured as gallic acid equivalent) was measured from guava extract. Unripe mango extract showed excellent DPPH radical scavenging activity (IC\textsubscript{50} 4.99 µg/mL) and percentage inhibition of linoleic acid oxidation (68.1%). Overall unripe mango, guava and melon extracts showed excellent reducing potential. Statistical analysis revealed significant differences in the antioxidant potential of different fruits extracts.

\textbf{Key words:} Unripe mango extract; Guava extract; Apple and melon extract; Total phenolic contents; DPPH

1. \textbf{Introduction} The antioxidants are the principal ingredients in food that not only enhance the quality but also increase the stability of fats and fatty foods (Vertuani \textit{et al.}, 2004; Scheibmeir \textit{et al.}, 2006). It is difficult for body of human to detoxify and to protect from oxidative damage that caused by Reactive Oxygen Species (ROS), when their production increased by some internal and external factors. Therefore antioxidants are supplied by external source to assist internal antioxidant system, and are provided through vegetables, fruits and from other plant materials (Lenucci \textit{et al.}, 2006). Antioxidants consumed in foods reinforce to vital health promoting function (Scheibmeir \textit{et al.}, 2006). The increased intake of...
dietary antioxidants maintained normal physiological function of a living system (Ou et al., 2002). It is now broadly accepted that certain classes of plant-based compounds including phenolic acids, flavonoids and other polyphenols play preventive role against the incidence of some common diseases like cancer, cardiovascular and neurodegenerative disorder. The sum of rose flowers is used by the food industry for making jams and liqueurs and also rose extracts have been suggested to be potential sources of antioxidants which can be used in food preservation (Baydar et al., 2013). Some phenolic compounds including phenolic acids and flavonoids have been widely distributed in fruits and vegetables and among them flavonols are of particular importance in the human diet as antioxidants (Scheibmeir et al., 2006). Fruits and vegetables are excellent source of natural antioxidants. The use of antioxidant prevents consumer from chemical contaminations and required no safety tests because food component is generally regarded as safe (Heo et al., 2005). The World Health Organization recommended that for balanced and healthy nutrition, daily use of at least fifth portion of diet should be of fruits and vegetables (Kaur and Kapoor, 2002). Mango was observed a good source of phytosterols as campesterol, β-sitosterols, stigmasterol and tocopherols (Soong and Barlow, 2004). Berries and fruits contain flavonoids and phenolic acids that showed antioxidant activity. Main subgroups of flavonoid in berries and fruits are anthocyanins, proanthocyanins, flavonols, and catechins. The bioactive compounds includes antioxidants present in citrus fruits are important to human nutrition (Arima et al., 2002). The apple and orange extracts showed a remarkable antioxidants potential (Gorinstein et al., 1999) and high phenolic contents. The phenolic contents 839 mg/kg in apple vary from 0.15 – 2.5 % (Escarpa et al., 1998), whereas fresh apple juice contains only 10% and orange contains 217 mg / 100 g. The objective of this study was to estimate the total phenolic and total flavonoids contents, antioxidant property and DPPH free radical scavenging capacity of some summer fruits i.e. Guava, Apple, Unripe Mango and Melon from Pakistan.

2. Materials and methods

2.1. Collection of fruits samples

Four summer fruits i.e. unripe mango, melon, apple and guava were collected from the fruit and vegetable section, Ayub Agriculture Research Institute, Faisalabad and from local market. The fruits species were further indentified and authenticated by Dr Qasim Ali, Assistant Professor, Department of Botany, Government College University Faisalabad.

2.2. Drying and grinding of fruits samples

The fruit samples were washed, chopped and placed in a hot air oven at a temperature of 35°C. The dried samples were ground to make fine powder (80 mesh) using Commercial Blender (TSK-949, Westpoint, France).

2.3. Preparation of antioxidant extracts

Fifteen gram of each sample of ground fruit material was homogenized with 150 mL of absolute methanol (Merck, Germany) at room temperature and placed on orbital shaker (Irmeco, Germany) for 8 hours. The samples were filtered through filter paper and the extracts were concentrated at 45 °C under reduced pressure, using a rotary evaporator. The dried concentrated extracts were weighed to calculate the yield and were stored in refrigerator at 4 °C until use for analyses.

2.4. Antioxidant activity

The fruits extracts were investigated for total phenolic, total flavonoid contents and antioxidant activity using different assays and BHT was used as a positive control.

2.4.1. Measurement of total phenolic contents (TPC)

The TPC from fruits extracts were determined by Folin-Ciocalteu reagent (Chaovanalikit et al., 2004). Briefly, the extracts solutions of fruits 0.5 ml were mixed with 0.5 ml and 7.5ml of Folin-Ciocalteu reagent and deionized water, respectively. The mixtures were
placed for 10 minutes at room temperature and then
20% sodium carbonate (1.5 ml) was added. The
mixtures were heated in a water bath at 40°C for 20
min and kept in ice-bath. The absorbance was
measured at 755 nm using double beam
spectrophotometer. Total phenolic contents were
determined with the help of calibration curve of Gallic
acid (10-130 ppm) and the results were reported as
gallic acid equivalents (GAE) g/100g dry matter (Fig 1).

2.4.2. Measurement of total flavonoid
contents (TFC)

The total flavonoid contents of fruits extracts were
estimated by the method described by Dewanto et al.,
(2002). The extracts solutions of 1ml were mixed with 5
ml of distilled H2O and 0.3 ml of 5% NaNO2. Each
sample of solution of 10% AlCl3 (0.6 ml) and 2 ml of 1M
NaOH was added at 5 min duration and filled up with
distilled water. The solution absorbance was measured
with spectrophotometer at 510 nm wavelength and it
was repeated thrice. The TFC was determined using
catechin standard curve (100-1300 ppm) and total
flavonoids contents were reported as g/100 g of dry
fruit material, measures as catechin equivalent (Fig 2).

2.4.3. Antioxidant activity in term of
reducing power

The reducing power of fruit extracts were calculated
with the method explained by Yen et al. (2000). Briefly,
the solution (1.0 ml) of extract containing 2.5, 5, 7.5
and 10 mg was mixed with sodium phosphate (5.0 mL,
0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL,
1.0%). The solution was placed in incubator for 20 min
at 50°C. After that 10% trichloroacetic acid (5ml) was
mixed in a refrigerated centrifuge. The mixture of 980g
was centrifuged at 5°C for 10 min. The solution (5.0 ml)
diluted with distilled water of 5.0 ml and 0.1%
ferric chloride (1.0 ml).The absorbance was measured
at 700 nm. The method was repeated thrice in order to
average the results.

2.4.4. Antioxidant activity in term of percent
inhibition of linoleic acid peroxidation

The antioxidant activity of fruits extracts was estimated
by measurement of percentage inhibition of linoleic
acid peroxidation using method given by Iqbal et al.,
(2005). Each fruit extract sample of 5mg was added
into solution of 0.13 ml linoleic acid, 10 ml of 99.8%
ethanol and 0.2 M sodium phosphate buffer (10 ml)
with7 pH value.
The total mixture was diluted with distilled water up to
25ml. The solution incubated at temperature of 40°C
and then oxidation was calculated by using thiocyanate process (Yen et al., 2000) with 10 ml of
75% ethanol, 0.2 ml of 30% aqueous solution of
ammonium thiocyanate (0.2 ml sample solution, 0.2 ml
FeCl3 solution and 20 mm in 3.5% HCl) being added
sequentially.
The absorption of the mixtures recorded at wavelength
500 nm after 3 min of stirring. Synthetic antioxidants
such as BHT and ascorbic acid (200 ppm) was used as
positive control percent Inhibition.

2.4.5. DPPH radical scavenging activity

2,2 diphenyl 1, picryl hydrazyl (DPPH) free radical
scavenging capacity of fruits extracts was estimated
Different concentration of fruit extracts were mixed
with one milliliter of 90 μM DPPH solution and filled
with methyl alcohol (95%), to make final volume of
solution up to 4mL.
The absorbance of solutions and the blank were
recorded after one hour at a wavelength of 515 nm
using spectrophotometer. BHT was used as a positive
control. Three values of each sample were recorded. .
The scavenging of free radical in percent was calculated
using following formula;
Scavenging (%) = 100 x (A_{blank} - A_{sample}/A_{blank})

IC_{50} values, which represented the concentration of extracts that gave 50% scavenging of DPPH radicals, was calculated drawing graph between scavenging percentage and extract concentration.

2.4.6. Bleach-ability of β-Carotene in linoleic acid system

The extracts antioxidant activity was also estimated using bleach-ability of β-carotene in linoleic acid system as reported by Kulisic et al. (2004). β-carotene-linoleic acid mixture was prepared by dissolving (0.1 mg 3-carotene, 20 mg linoleic acid and 100 mg Tween 40 in 1.0 mL of chloroform). The chloroform was removed under vacuum in rotary evaporator at 50 °C. The 50 ml distilled H_{2}O saturated with oxygen (100 mL/min, 30 min,) was added into mixture. The reaction

**Figure 1:** Standard curve for gallic acid

**Figure 2:** Standard curve for catechin
mixture (5.0 ml) was distributed to test tubes with 200 µl of the extracts solutions of fruits, which was prepared at concentrations of 4.0 g/L. The absorbance was checked at 490 nm wavelength against a blank (contained an emulsion without β-carotene). At room temperature, emulsion was incubated for 50 hours and absorbance was noted at time intervals. The method was repeated with butylated hydroxy toluene and blank.

### 2.5. Statistical analysis

The experiments were performed in triplicate and data were recorded and presented as mean values ± standard deviation. Analysis of Variance (ANOVA) was performed using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Ok, USA) and probability value $p \leq 0.05$ was considered as statistically significant.

### 3. Results and discussion

#### 3.1. Extracts yield

The fruits extracts yield calculated on dry material basis are listed in the Table 1. Absolute methanol was used as extracting solvent. The extract yield of melon, apple, guava and unripe mango fruits were 21.3, 17.7, 19.4 and 10.7 g/100g of dry materials, respectively. Maximum extract yield was obtained from melon fruit (21.3 g/100g) while the minimum extract yield (10.7 g/100g) was obtained from unripe mango fruit. Analysis of variance (ANOVA) revealed a significant ($P<0.05$) different in the extract yield of different fruits.

#### 3.2. Antioxidant activity

##### 3.2.1. Total phenolic and total flavonoid contents

The phenolic compounds contributed to antioxidant activities of plant extracts (Liu, R.H, 2007). The total phenolic content of fruits samples are given in the Table 1. The TPC, extracted from melon, apple, guava and unripe mango was ranged from 6.12 to 9.35 g/100g dry matter, measured as gallic acid equivalent. The maximum TPC (9.35 g/100g) was extracted from melon and the minimum TPC (6.12 g/100g) was extracted from apple fruits. The different in the extract yields of different fruits were found significant at value of $P < 0.05$. The TFC of fruits are also given in Table 1. The TFC extracted from melon, apple, guava and unripe mango were ranged from 1.71 to 6.32 g/100g of dry matter, measured as catechin equivalent. The maximum TFC was determined from unripe mango and the minimum TFC was determined from melon. ANOVA showed a significant ($P < 0.05$) different in the TFC of fruits extracts. The unripe mango gave more TF than that of other fruits.

<table>
<thead>
<tr>
<th>Fruit Extracts</th>
<th>Yield (g/100g)</th>
<th>TPC$^a$</th>
<th>TFC$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melon</td>
<td>21.3 ± 0.2$^d$</td>
<td>9.35 ± 0.09$^d$</td>
<td>1.17 ± 0.06$^d$</td>
</tr>
<tr>
<td>Apple</td>
<td>17.7 ± 0.5$^b$</td>
<td>6.12 ± 0.24$^a$</td>
<td>5.10 ± 0.15$^b$</td>
</tr>
<tr>
<td>Guava</td>
<td>19.4 ± 0.4$^c$</td>
<td>6.45 ± 0.19$^b$</td>
<td>5.20 ± 0.10$^c$</td>
</tr>
<tr>
<td>Unripe Mango</td>
<td>10.7 ± 0.4$^a$</td>
<td>7.94 ± 0.15$^c$</td>
<td>6.32 ± 0.06$^c$</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three independent experiments. Different letters in superscript in the same column represent significant difference ($P < 0.05$).

$^a$ Total phenolic contents, g/100g of dry plant material, measured as gallic acid equivalent

$^b$ Total flavonoid contents, g/100g of dry plant material, measured as catechin equivalent
3.2.2. Reducing power

The reducing power of different fruits extract in term of absorbance curves are shown in Fig 3. The absorbance of fruits extracts increased in a concentration dependent manners. The melon, guava and unripe mango extracts demonstrated maximum absorbance at higher concentration as compare to apple fruit extract and thus showed better antioxidant potential.

3.2.3. DPPH radical scavenging activity

In the DPPH assay, the radical scavenging capacity of different fruits extracts increased in a concentration dependent manner. The extracts concentrations provided 50% scavenging (IC\textsubscript{50}) are given in Table 2. The IC\textsubscript{50} values of different fruits extracts were ranged from 4.99-7.01 μg/mL. Unripe mango extract showed excellent DPPH radical scavenging capacity (4.99 μg /mL), comparable with the activity of BHT, followed by melon extract (5.03 μg/mL). DPPH radical scavenging capacity of plant extracts could be explained by the presence of phenolic acids and flavonoids. The barks, E. jambolana Lam was found to offer the most efficient antioxidant activity. The effectiveness of these bark extracts towards inhibition of peroxidation was found to be greater than C. fistulaL. (Siddhuraju et al., 2002).

3.2.4. Percent inhibition of linoleic acid peroxidation

The antioxidant activity was noticed as the ability to prevent oxidation. Thus, oxidation prevention of linoleic acid was used to determine the antioxidant activity of the fruits extracts. The oxidation of Linoleic acid formed peroxides which oxidize Fe\textsuperscript{2+} to Fe\textsuperscript{3+}, the later forms complex with SCN\textsuperscript{-}, and concentration was measured using spectrophotometer at 500 nm absorbance. Antioxidant potential of different fruits extracts was estimated by inhibition of peroxidation in linoleic acid system using thiocyanate procedure (Yen et al., 2000) and results are reported in the Table 2. The fruit extracts demonstrated appreciable inhibition ranging from 26.1 to 70.0%. The maximum inhibition (70.0%) was found from melon and minimum (26.1%) was found from apple. Maisuthisakul and Pusak (2007), reported inhibition of peroxidation ranged from 26.4% to 30.05% from different fruits extracts.

3.2.5. Bleach-ability of β-carotene in linoleic acid system

The extraction method and solvent system affect antioxidant activities of different fruits when determined in term of β-carotene bleaching (Fig 4). The antioxidant effectiveness slowed down the depletion of color. A slight decrease in β-Carotene absorbance showed a lower rate of linoleic acid oxidation and gave higher antioxidant activity. The fruits extracts gave good antioxidant activity. The solvent extraction method exercised gave significant effect (p < 0.05) on the antioxidant activities of the fruits extracts. Synthetic antioxidant, BHT showed significant (P<0.05) higher antioxidant activity, than that of fruits extracts. No literature was found regarding antioxidant activity of fruits extracts using the β-carotene bleaching in linoleic acid.

Table 2: The antioxidants of fruits in term of DPPH radical scavenging capacity and percentage inhibition of linoleic acid peroxidation

<table>
<thead>
<tr>
<th>Fruit Extracts</th>
<th>DPPH IC\textsubscript{50} (μg/mL)</th>
<th>Inhibition(%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melon</td>
<td>5.03 ± 0.25$^a$</td>
<td>70.0 ± 2.7$^c$</td>
</tr>
<tr>
<td>Apple</td>
<td>5.07 ± 0.21$^a$</td>
<td>26.1 ± 1.7$^a$</td>
</tr>
<tr>
<td>Guava</td>
<td>7.01 ± 0.67$^b$</td>
<td>60.8 ± 2.8$^b$</td>
</tr>
<tr>
<td>Unripe Mango</td>
<td>4.99 ± 0.22$^a$</td>
<td>68.1 ± 1.6$^c$</td>
</tr>
<tr>
<td>BHT</td>
<td>4.80 ± 0.20$^a$</td>
<td>91.35 ± 2.70</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three independent experiments. Different letters in superscript in the same column represent significant difference (P < 0.05).

$^a$ Inhibition of linoleic acid peroxidation (%) provided by extract concentration 200 μg/mL

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4. Conclusions

In conclusion, this study reports the phenolic profile and antioxidant activity of summer fruits extracts. The results of the present study would certainly help to ascertain the potency of the fruit extracts as potential source of natural antioxidants. Among all extracts, methanol extracts of unripe mango and guava contained the high TPC and TFC and showed the excellent antioxidant and free radical scavenging activities. However, further research is needed to investigate these extracts in vivo using different diseased models and develop their application for pharmaceutical and nutraceuticals industries.

Figure 3: Absorbance of apple, guava, unripe mango and melon extracts in terms of ferric reducing power.

Figure 4: Fruit samples measured in terms of β-carotene bleaching in linoleic acid

References


Maisuthisakul and Pasuk. 2007. Antioxidant Properties and Phenolic Phytochemicals from Various Cultivars of Thai Mango Seed Kernels. School of Science, University of the Thai Chamber of Commerce, Bangkok. J.food sci & tech. 26-34.