Nutritional analysis and in-vitro antioxidant activity of apple (Malus domestica)

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Abstract

The objective of this study was to screen and assess the nutritional composition, phenolic, anthocyanin contents and antioxidant activity of various genotypes of apple growing in Rawalakot Azad Kashmir Pakistan. Vitamin C content, acidity %, reducing sugar and non reducing sugars were 11.4-14.2 mg/100 g, 0.21-0.44%, 9.66-7.23 mg/100 g and 1.63-2.66 mg/100 g. The aqueous extracts also showed high phenolic content (1.93-12 mg/g gallic acid equivalent), total antioxidant activity (65-155.3 µg/ml) and total monomeric anthocyanin (4.1-7.96 mg/L), respectively. The genotypes of apples, Golden delicious, Kala Kalu, Star King, Star King delicious, Chotta and Genotype-A, B and C, possess high radical scavenging activity in the 2,2-diphenyl-1-picrylhydrazyl assay. The evaluation of these genotypes not only provides important information regarding nutritional status of screened genotypes for the commercial exploitation, but also navigates the existing biodiversity in Malus domestica germplasm in these regions. The present results also suggest that different genotypes of apple are potential source of antioxidants and have therapeutic importance.

Key words: Apple, nutritional analysis, anthocyanin content, total phenolics, antioxidant activity.

Introduction

Apple is one of the most important tree fruit of the world, currently the world’s apple production is 64.25 million tons from an area of 421,767 hectares 1. The total area under apple cultivation in Pakistan is 112.6 thousand hectares with total production of 348.3 thousand tons 1. Apple has prominent position among the commercial fruits and the fifth largest fruit commodity of Pakistan; however, its cultivation is limited to the northern hilly areas of Punjab, Pukhtunkhwa, Baluchistan and temperate areas of Azad Jammu and Kashmir 2. Some important varieties grown in these regions are Kashmiri, Kashmiri Amri, Kandhuri, Kulu, Kalat special, Red Beauty of Bath, Golden delicious, Banki and Sky Spur. Northern areas of Pakistan including temperate region of Azad Jammu and Kashmir is blessed with diversity of environments which are conducive to growing apple, pear and other temperate fruit crops 3, 4.

Apple production is not only a profitable enterprise for the growers but also a good source of foreign exchange and the richest sources of human diet. It constitutes a rich source of monosaccharides, minerals, dietary fiber, vitamins and various biologically active compounds, such as phenolic compounds, which are responsible for most of the antioxidant activity of the fruits 5. The antioxidant activity of fruits are related to compounds such as vitamins and phenolics, which play an effective role in free radical scavenging activity, which varies among species and cultivars 6. Apple fruits from various species have different physico-chemical characteristics, such as color, dry matter, sugar, acidity, pH and total contents of soluble solids, phenolic and antioxidants 7-9. These parameters provide an important information to the consumers for recognition of nutritious fruits 9.

Apple is a major fruit crop of Azad Jammu and Kashmir. It is expected that crop cultivars with higher antioxidant ability have better stress resistance, nutritional quality and storage characteristics 10. The red coloration of apple skin is mainly due to anthocyanins that are reported to possess health benefits. Bioactive phytochemicals, such as anthocyanins in fruits and vegetables showed great interest to consumers and researchers due to their wide range of health benefits 11. Apple fruits contributed desirable levels of several antioxidant compounds and are thus good candidates for investigation as dietary intervention against chronic diseases 12. The fruit of apple and its juice decrease the possibility of incidences of prostate cancer, anti-influenza viral activity, which are involved in LDL oxidation and decrease the risk of chronic diseases such as cardiovascular disease and cancer. Apples contain several health-beneficial constituents including dietary fiber, sugars, vitamins and phenolic compounds 13. The red-skin apple is reported to contain anthocyanins such as cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, cyanidin-7-O-arabinoiside, cyanidin-3-O-rutinoside, cyanidin-3-O-xylloside, and cyanidin-3-O-glucose. The intensity of anthocyanins in apples is very important since it affects the market value 14 and additional health benefits.

Unfortunately, in the region of Azad Jammu and Kashmir most of the valuable species of genus Malus are endangered and genetically vulnerable due to biotic and abiotic stress. Up till now, no systematic studies of local apple cultivars and landraces for the selection of desirable types in term of nutritional basis have
been conducted. Thus, there is a need to assess, differentiate and select existing germplasm already adapted in this region for commercial exploitation. A more detailed knowledge of the variability of the composition of the different genotypes will be beneficial in the future selection of apple genotypes with improved nutritional quality and suitable use of apple products. In view of the above considerations, the exploration and assessment of indigenous apple genotypes are necessary for better management and maintenance. The present study was carried out on evaluation of biodiversity and nutritional status of primitive varieties, wild and local genotypes of apple germplasm which will contribute and increase our knowledge about the genus and enlarge the gene pool available for future apple breeding programs. The present study was therefore aimed 1) to characterize indigenous Malus genotypes, grown in the areas of Azad Jammu and Kashmir through the evaluation of physico-chemical traits; 2) to assess fruits of local genotypes and varieties on the basis of nutritional composition and to evaluate its antioxidant activity, phenolic content and anthocyanins for its use as a therapeutic agent.

Experimental

The present study was conducted to characterize apple (Malus spp.) germplasm from different locations of District Rawalakot Azad Jammu and Kashmir, Pakistan. Fifteen genotypes were selected and tagged from each location. These genotypes were Amnari, Kala Kalu, Kashmiri, Golden delicious, Red delicious, Banki, Star Crimson, Star King delicious, Star King, Royal Galla, Spartan (Polynizer), Chota (Crab apple), Genotype-A, Genotype-B and Genotype-C. Selected accessions were visited at the stage of flowering, fruit setting and fruit maturity. Apple descriptor developed by IBPGR was used to assess in situ observation during survey and information were added. Fruit at maturity stage was harvested, assessed morphologically and chemical analysis was done at the Department of Horticulture, Faculty of Agriculture, Rawalakot during the year 2010-2011. Meteorological data was collected during the study.

Estimation of vitamin C: Vitamin C was determined by using 2,6-dichlorophenol indophenol method 13. Of sample 5 ml was taken in 100 ml of conical flask and 5 ml of meta phosphoric acid solution (4%) was added and titrated with 2,6-dichlorophenol indophenol dye till the appearance of light pink colour as end point.

Determination of acidity: The titratable acidity was determined according to method given in A. O. A. C. 16. Of sample 20 g was taken in 100 ml conical flask and then 2-3 drops of phenolphthalein were added and titrated against NaOH (0.1 N) until a light pink colour appeared. Acidity was calculated by the following formula:

\[
\text{Acidity} \% = \frac{0.1 \times \text{equivalent wt. of acid} \times \text{normality of NaOH} \times \text{ml of NaOH} \times 100}{\text{Wt. of the sample}}
\]

Determination of reducing sugar: Of Fehling solution A (CuSO₄ solution) 5 ml and 5 ml of Fehling solution B (sodium potassium tartarate solution) were taken in 100 ml conical flask and 2-3 drops of methylene blue were added. The mixture was boiled for 2-3 min by using hot plate and the mixture was titrated with juice sample till the appearance of brick-red end point.

Determination of non reducing sugar: Of juice sample 20 ml was taken in 100 ml of beaker and 5 ml of conc. HCl was added and boiled for fifteen min and then cooled. The pH of mixture was adjusted to 7.0 by using NaOH (0.1 N), 5 ml of Fehling solution A (CuSO₄ solution) and 5 ml of Fehling solution B (sodium potassium tartarate solution) were taken in 100 ml conical flask and 2-3 drops of methylene blue were added. The mixture was boiled for 2-3 min by using hot plate and titrated against hydrolyzed juice till the appearance of brick red end point.

Total phenolic content: The total phenol content as gallic acid equivalent was determined by the method of Singleton et al. 17. The total phenolic content was determined by adding 0.5 ml of aqueous extract to 2.5 ml of 10% Folin-Ciocalteu’s reagent (v/v) and 2 ml of 7.5% sodium carbonate solution. The reaction mixture was incubated at 45°C for 40 min and absorbance was measured at 765 nm in a spectrophotometer. Gallic acid was used as a standard phenol. The mean of three readings was used and the total phenolic content was expressed as mg of gallic acid equivalents/g extract.

Total antioxidant activity: The total antioxidant potential of the extracts was estimated using the phosphomolybdenum reduction assay 19. The total antioxidant activity was analyzed by using 4 mM ammonium molybdate, 0.6 M H₂SO₄ and 28 mM sodium phosphate. In control, 0.3 ml of water, 0.7 ml H₂SO₄, 1 ml ammonium molybdate and 1 ml buffer were added. For the sample, 0.3 ml of water was replaced by the extract. Test tubes were kept in water bath for 90 min. at 95°C and their absorbance was recorded at 695 nm in a spectrophotometer.

Antioxidant activity by DPPH radical scavenging: The antioxidant activities of the apple extracts were determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical according to the method of Hatano et al. 13. Briefly 0.25 mM solution of DPPH radical (0.5 ml) was added to the sample solution in ethanol (1 ml) at different concentrations (50-1000 μg/ml). The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated using the following equation:

\[
\%	ext{DPPH scavenging} = \frac{[A_o - A_\text{sample}]}{A_o} \times 100,
\]

where, A₀ is the absorbance of the control reaction and Aₙ is the absorbance of the sample itself.

Statistical analysis: Data was statistically analyzed using analysis of variance and mean was compared using least significant difference (LSD) test where necessary 20. The software package Statistica was used for the analysis of data.
## Results and Discussion

**Nutritional analysis:** The results of nutritional analyses of different genotypes are shown in Table 1. Statistically significant differences (P<0.05) were found in vitamin C, acidity percentage, reducing and non-reducing sugars, anthocyanins and total phenolics. In terms of vitamin C, the maximum amount of vitamin C, 14.01 mg/100 g was found in Golden delicious and also similar results 14, 13.83, 13.75 and 13.3 mg/100 g were observed in Starking delicious, Golden delicious, Kashmiri and Genotype-B, respectively. The vitamin C content in fruits can be influenced by various factors such as genotypic differences, preharvest climatic factors, maturity and harvesting methods. In the present study, fruit samples were collected from different geographical and ecological zones during ripening stage. Fluctuation in day temperature at different localities might be responsible for variability in vitamin C contents. Our agreements are closely related to the arguments of Shakir et al. 21.

In terms of acidity the maximum acidity (0.44 %) was recorded in Ammri and minimum (0.21%) in Golden delicious, Star Crimson and Chota. The present findings are supported by the studies of Nogueira et al. 22, who reported titrable acidity values between 0.28-0.38% for apple cultivars grown in Brazil. The maximum amount of reducing sugar 9.66 mg/100 g was found in Kala Kalu and minimum 7.23 mg/100 g in Royal Gala. For non reducing sugar, maximum 2.66 mg/100 g was observed in Ammri closely similar to 2.50 mg/100 g in Royal Gala and minimum 1.63 mg/100 g was observed in Star Crimson. Sugar is an important component of fruits which relates with sweetness and is basic component of fruit quality. These differences among the genotypes might be attributed to prevailing environmental conditions, harvesting of fruits at different time of maturity/ripening and genetic variability in genotypes. Prevailing temperatures and rainfall distribution over growing areas definitely affected the growth and composition of fruits especially during late stage of fruit development. Consistency in rainfall during fruit development increases the size of fruits as well as juice contents whereas restricted supply of water during late stage of fruit growth enhances soluble solids as well as sugar contents. Our arguments are supported by the findings of Ali et al. The results are also in line with the findings of Brown and Walker 10 and Chen et al. 11 who reported the genotypic variations for fruit quality in apricots and pear, respectively. Total phenolic contents were measured as gallic acid equivalent (mg/g) among fifteen different varieties/genotypes. In terms of total phenolic content the most promising cultivars seemed to be Ammri (12 mg/g GAE) whereas, Royal Gala contained the least phenolic content (1.93 mg/g GAE). The total phenolic content, anthocyanin and antioxidant activity vary considerably depending on the part of the fruit and of the apple cultivar analyzed. Phenolic compounds widely distributed in plants, attract significant scientific interest due to their bio-functional health-promoting properties. Fruits are potential sources of natural phenolic antioxidants used as food additives for the prevention of lipid oxidation and thus prolongation of food self-life. Many fruits have been characterized due to their phenolic profile and antioxidant activity. The results obtained in this study have shown that aqueous extracts of apples from Azad Kashmir are rich source of phenolic content. Yuri et al. 14 have reported the phenolic content of 8.96 ± 0.57 mg/g in flesh of apple. The high phenolic content of apple in this study may also be due to the attack of disease which results in the accumulation of phenolic compounds. Total anthocyanin contents (mg/l) were measured from whole apple (including peel) from different locations of Rawalakot Azad Kashmir. The maximum amount of anthocyanin was present in genotype Golden delicious (7.96 mg/l) while, minimum was present in Kashmiri (4.1 mg/l). Anthocyanins are the phytochemicals which belong to the flavonoid groups and are predominant in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa, and cereals. The flavonoids, perhaps the most important single group of phenolics in foods, comprise a group of over 4000 C₆, aromatic plant compounds with multiple substitution patterns. The primary function in this group includes the anthocyanins (e.g. cyanidin, pelargonidin, petunidin), the flavonols (quercetin, kaempferol), flavones (luteolin, apigenin), flavan-3-ols (catechin, epicatechin, galloycatechin), and sometimes classified separately, the isoflavones (genistein, daidzein). Phytochemicals in this class are frequently referred to as bioflavonoids due to their multifaceted roles in human health maintenance and anthocyanins in food are typically ingested as components of complex mixtures of flavonoid components. Daily intake is estimated from 500 mg to 1 g, but can be several g/day if an individual is consuming flavonoid supplements (grape seed extract, *Ginkgo biloba*, or pycnogenol) 28. The free-radical scavenging and antioxidant capacities of anthocyanin pigments

### Table 1. Average vitamin C content (mg/100 g), acidity (%), sugars (mg/100 g), total anthocyanin (mg/l) and total phenolics (mg/g) of fruits of different cultivars of apple.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Vitamin C</th>
<th>Acidity</th>
<th>Reducing sugar</th>
<th>Non reducing sugar</th>
<th>Total anthocyanins</th>
<th>Total phenolics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammri</td>
<td>11.4±1.1</td>
<td>0.44±0.02</td>
<td>8.76±1.1 §</td>
<td>2.66±0.12</td>
<td>6.51±0.96</td>
<td>7.96±0.57</td>
</tr>
<tr>
<td>Kala Kalu</td>
<td>14.2±1.6</td>
<td>0.24±0.03</td>
<td>9.66±1.3 §</td>
<td>1.90±0.15</td>
<td>4.06±1.1 §</td>
<td>5.01±0.14 §</td>
</tr>
<tr>
<td>Kashmiri</td>
<td>13.8±1.3</td>
<td>0.39±0.02</td>
<td>7.46±0.91 §</td>
<td>2.17±0.21</td>
<td>7.96±1.3 §</td>
<td>6.02±0.21 §</td>
</tr>
<tr>
<td>Golden delicious</td>
<td>14.1±1.3</td>
<td>0.21±0.03</td>
<td>7.35±0.82 §</td>
<td>2.36±0.25</td>
<td>6.43±1.4 §</td>
<td>5.02±0.12 §</td>
</tr>
<tr>
<td>Red delicious</td>
<td>15.1±0.9</td>
<td>0.43±0.02</td>
<td>7.83±0.61 §</td>
<td>2.40±0.31</td>
<td>5.77±0.9 §</td>
<td>3.38±0.3 §</td>
</tr>
<tr>
<td>Banki</td>
<td>11.66±0.8</td>
<td>0.33±0.03</td>
<td>7.83±1.3 §</td>
<td>2.01±0.41</td>
<td>4.73±0.34 §</td>
<td>4.33±0.14 §</td>
</tr>
<tr>
<td>Star Crimson</td>
<td>11.63±1.1</td>
<td>0.31±0.02</td>
<td>9.13±1.2</td>
<td>1.63±0.21</td>
<td>4.97±0.41 §</td>
<td>2.63±0.13 §</td>
</tr>
<tr>
<td>Star King delicious</td>
<td>14.02±1.3</td>
<td>0.21±0.02</td>
<td>8.53±0.39 §</td>
<td>1.91±0.87</td>
<td>7.11±1.3 §</td>
<td>5.18±0.21 §</td>
</tr>
<tr>
<td>Star King</td>
<td>12.3±0.8</td>
<td>0.31±0.01</td>
<td>9.06±0.78 §</td>
<td>1.83±0.56</td>
<td>5.73±0.9 §</td>
<td>8.54±1.1 §</td>
</tr>
<tr>
<td>Royal Gala</td>
<td>14.12±1.7</td>
<td>0.25±0.12</td>
<td>7.23±0.56 §</td>
<td>2.50±1.15</td>
<td>6.75±1.3 §</td>
<td>7.84±1.4 §</td>
</tr>
<tr>
<td>Spartan (Polymerizer)</td>
<td>12.6±0.9</td>
<td>0.27±0.03</td>
<td>9.02±1.1</td>
<td>2.08±0.23</td>
<td>6.75±1.1 §</td>
<td>4.89±0.9 §</td>
</tr>
<tr>
<td>Chota (Crab apple)</td>
<td>14.15±1.1</td>
<td>0.21±0.12</td>
<td>7.69±1.3</td>
<td>2.34±0.9</td>
<td>6.13±0.42 §</td>
<td>2.46±0.9 §</td>
</tr>
<tr>
<td>Genotype-A</td>
<td>13.1±1.2</td>
<td>0.29±0.02</td>
<td>8.01±1.2</td>
<td>2.12±0.98</td>
<td>6.75±1.3 §</td>
<td>7.84±1.4 §</td>
</tr>
<tr>
<td>Genotype-B</td>
<td>13.3±0.9</td>
<td>0.29±0.01</td>
<td>7.24±1.1</td>
<td>2.30±0.67</td>
<td>6.75±1.1 §</td>
<td>4.89±0.9 §</td>
</tr>
<tr>
<td>Genotype-C</td>
<td>12.8±0.78</td>
<td>0.25±0.05</td>
<td>8.30±0.89</td>
<td>2.37±0.81</td>
<td>6.23±0.9</td>
<td>4.42±0.6 §</td>
</tr>
<tr>
<td>Mean</td>
<td>12.98</td>
<td>0.29</td>
<td>8.21</td>
<td>2.17</td>
<td>6.51</td>
<td>5.37</td>
</tr>
</tbody>
</table>

LSD 0.05 for treatments = 0.76, LSD 0.05 for Locations = 0.34, LSD 0.05 for interaction = 1.32
are the most highly beneficial as human therapeutic targets, but, in fact, research clearly indicates that other mechanisms of action are also responsible for the observed health benefits 29, 30. Anthocyanin isolates and anthocyanin-rich mixtures of bioflavonoids may provide protection from DNA cleavage, estrogenic activity (altering development of hormone-dependent disease symptoms), enzyme inhibition, boosting production of cytokines (thus regulating immune responses), anti-inflammatory activity, lipid peroxidation, decreasing capillary permeability and fragility, and membrane strengthening 18, 31.

**In vitro antioxidant activity**: Data collected on total antioxidant activity (µg/ml) as ascorbic acid equivalent were analyzed statistically (Fig. 1). The total antioxidant capacities of the hot water extracts of apple were quantitatively determined by the formation of phosphomolybdenum complex. This method is based on the reduction of Mo (VI) - Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex, which has maximal absorption at 695 nm. The results for antioxidant capacities of apple were expressed as water-soluble ascorbic acid equivalents and varied from 47.45-195.1 µg/ml. Genotype-A showed the highest antioxidant activity (195.10 µg/ml) whereas, the Star Crimson showed the lowest antioxidant activity (47.45 µg/ml). Keeping in view the mean values for antioxidant activity from three districts, Genotype-A was found to be the best (165.90 µg/ml) followed by Golden delicious (155.30 µg/ml) and Kashmiri (145.60 µg/ml).

![Figure 1](image1.png)

**Figure 1.** The variation in total antioxidant activity among different cultivars of apple at 100 µg/ml concentration assessed in phosphomolybdenum reduction assay. The results are means ± SD (n = 3).

As a rapid and simple measure of antioxidant activity, the DPPH radical scavenging activity has been widely used. The DPPH assay was based on the reduction of the stable DPPH radical to yellow coloured diphenyl picrylhydrazine in the presence of a hydrogen donor. The antioxidant activity of different genotypes of apple extracts is shown in Fig. 2. The antioxidant activity of genotype Ammri to Red delicious is shown in Fig. 2a. For measuring the antioxidant activity five different concentrations of extracts were used (1 = 50, 2 = 100, 3 = 250, 4 = 500 and 5 = 1000 µg/ml, respectively). The results revealed that the extract showed a concentration dependent scavenging of DPPH radical. Ammri showed the less antioxidant activity even at the higher concentration. Kala Kalu, Golden delicious and Red delicious reported to have sustainable hydrogen donating and radical scavenging activity. The results of antioxidant activity of Banki, Star Crimson, Star King delicious, Star King and Roya Gala are shown in Fig. 2b. Among these samples Star King delicious, Star King and Roya Gala showed good antioxidant activity. Whereas, Banki and Crimson displayed moderate antioxidant activity. The results of antioxidant activity of Spartan, Chota and genotypes A, B & C are shown in Fig. 2c. Spartan was found to have the least hydrogen donating and radical scavenging activity. Whereas, other genotypes (Chota, Genotype-A, B and C) were reported to have sustainable hydrogen donating and radical scavenging activity.

![Figure 2](image2.png)

**Figure 2.** Variation in antioxidant activity by DPPH radical scavenging (a) antioxidant activity of Ammri, Kala Kalu, Kashmiri, Golden delicious and Red delicious apple (b) antioxidant activity of Banki, Star Crimson, Starking delicious, Star King and Royal Gala apple (c) antioxidant activity of Spartan, Chota and genotypes A, B & C. The results are means ±SD (n = 3). 1, 2, 3, 4 and 5 represents the concentrations of 50, 100, 250, 500 and 1000 µg/ml, respectively.
The DPPH radical activity is a simple and widely used method to measure the ability of compounds as free radical scavenger or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods [27, 33]. The antioxidant activity of apple is partly due to the presence of high phenolic contents, vitamin C and anthocyanins. The highest antioxidant activity of Golden delicious is due to its high vitamin C and anthocyanin contents. Zardo et al. [33] also reported that Golden delicious showed higher antioxidant activity compared to other cultivars.

**Conclusions**

On the basis of the above results we have concluded that the genotypes of apples collected from different areas of Azad Jammu and Kashmir are the rich source of nutritional constituents. The high antioxidant activity of apple are due to the presence of vitamin C, anthocyanins and phenolics. The Golden delicious cultivar showed the highest antioxidant activity. In general, the variation among phytoc hemicals and antioxidant activity indicates that the genetic variability can be used to develop cultivars with enhanced health benefits.

**References**