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Evaluation of the Composition of Bioactive Compounds and Antioxidant Activity in Fourteen Apricot Varieties of North India

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Abstract

Variation in the content of bioactive compounds and antioxidant activity was evaluated in fourteen apricot varieties grown in north India. In the present study, the bioactive composition of apricot varieties of north Indian Kashmir was assessed by HPLC-MS/MS and their contribution towards free radical scavenging was assessed using DPPH, FRAP and ABTS assays. Studies revealed that content of bioactive compounds in apricot varieties was found to vary significantly ($p \leq 0.05$) among cultivars. Content of total phenols was significantly ($p \leq 0.05$) higher in Rakausilk variety (72.5±3.12 mg/100 g GAE) followed by Viva-gold (71.2±2.11 mg/100 g GAE) and Rakhchekarpo (69.6±1.65 mg/100 g GAE). Halman apricots exhibited the highest content of total carotenoids (12.2±2.13 mg/100 g) followed by Nugget (10.5±1.11 mg/100 g). HPLC analysis of apricot samples revealed the presence of fifteen phenolic acids in Halman, Venatchaa, Rakausilk and New Castle varieties; nine phenolic acid in Rakchekarpo and Sterling apricots; twelve phenolic acids in Shakanda, Nugget, Shakarpara and CITH-1 varieties; fourteen phenolic acid in Viva gold and Turkey varieties; seven phenolic acid in Khante and nineteen phenolic acids in CITH-2 apricots. Strong correlation existed between total phenolics and antioxidant activity for DPPH assay ($r = 0.91$) and ABTS radical inhibition ($r = 0.86$) while as moderate correlation existed for FRAP ($r = 0.76$). For DPPH and FRAP assays; EC_{50} values among apricot varieties were significantly ($p \leq 0.05$) lower in Rakausilk (69.2, 123.6 mg/ml) followed by Viva Gold (74.3, 129.5 mg/ml) and Rakhchekarpo (77.4, 132.4 mg/ml). For $ABTS^{\cdot+}$ inhibition the EC_{50} values among apricot varieties were in the order of $EC_{50(Rakausilk)} < EC_{50(Khante)} < EC_{50(Viva\ Gold)}$. The present study reported the first time presence of p-coumaric acid-O-hexoside, isorhamnetin-O-glucouronide, Kaempferol-3-O-acetylhexoside, quercetin-3-O-rhamnoside, sinapic acid-O-hexoside, apigenin-7-O-glucoside, vitexin and luteolin-7-O-rutinoside compounds in apricot varieties of north India.

Keywords: apricot varieties, bioactive compounds, antioxidant activity, LCMS/MS characterization, principle component analysis

1. Introduction

There is strong evidence that a diet rich in fruits and vegetables has a positive effect on human health, offering protection against degenerative diseases of ageing, such as heart disease, cardiovascular disease, Alzheimer's disease, cataracts and several forms of cancer (Marina et al., 2013; Kang, Ascherio, & Grodstein, 2005; Liu et al., 2001; Joshipura et al., 2001; Gandini et al., 2000). Fruits and vegetables contain a wide range of substances that are suggested to be part of these health-enhancing effects. In addition to the major food constituents such as protein, fat, carbohydrate and micronutrients such as vitamins, minerals and trace elements, fruit and vegetables contain other compounds that may have a positive effect on human health. These phytochemicals include groups of compounds such as carotenoids, flavonoids, polyphenols and other phenolic acids and glucosinolates (Kris-Etherton, Harris, & Appel, 2002; Cho, Howard, Prior, & Morelock, 2008). These phytochemicals are

collectively called as bioactive compounds and are thought to be potential antioxidants. Antioxidants are a diverse group of compounds that act against oxidative damage induced in the body. Antioxidants quench reactive free radicals, prevent the oxidation of other molecules and play a significant role in the prevention of degenerative diseases like cancer, cardiovascular diseases, cataract formation, the aging process, inflammatory diseases and a wide range of neurological disorders (Biglari, AlKarkhi, & Easa, 2008; Choe & Min, 2009). The action of these antioxidants compounds is related to the attenuation of oxidative events that could contribute to the pathophysiology of these diseases (Alonso et al., 2004). The effectiveness of antioxidants to scavenge free radicals depends on the bond dissociation energy of the bond involved in hydrogen donation, pH related to the acid dissociation constant, reduction potential and delocalization of the antioxidant radicals (Choe & Min, 2009).

An overproduction of radicals may be triggered by radiation, pollutants, cigarette smoke, alcohol, exercise and stress. The immune system also produces radicals as a defense against pathogens. In the normal case there is a balance between the formation and elimination of free radicals. Oxidative stress occurs when the level of free radicals or reactive oxygen species is higher than the antioxidant defense system can cope with (Finkel & Holbrook, 2000). If not neutralized by antioxidants, the radicals may react with cellular components such as lipids, proteins and nucleic acids. Lipids may be especially prone to damage due to a process known as lipid peroxidation. When radicals react with polyunsaturated fatty acids in cellular membrane phospholipids, the fatty acids themselves become radicals that can react with other fatty acids, starting a chain reaction that may cause severe damage. This type of damage can cause cell death and eventually ageing or diseases in the organism. Due to the incomplete efficiency of the human endogenous defense system and the influence of external factors, the importance of bioactive compounds obtained from diet has been well established, which can help overcome such deficiencies and also promote protection, prevention or reduction of these effects caused by oxidative stress (Huang, Ou, & Prior, 2005). In addition to the effects on human health when ingested in the diet, bioactive compounds are believed to affect storability of the fruits and vegetables that contain them. A product with a high concentration of antioxidants is well protected against oxidation, and may thereby retain its quality longer. Thus, increasing the concentrations of the presumed beneficial compounds (biotechnologically or by controlling and optimizing pre-harvest factors or growing the right cultivars) in fruits and vegetables may not only have positive health effects for the people who eat them, but may also extend shelf life and increase stress tolerance, leading to lower postharvest losses of produce.

The concentrations of bioactive compounds in fruit and vegetables are certainly not constant and are affected by pre and postharvest factors as well as genetic factors. In some cases, the effect of a certain factor on a certain compound is very clear, but in many cases, changes in one factor may lead to higher or lower concentrations of a certain compound, or no change in the concentration. Some bioactive compounds are ubiquitous among fruit and vegetables but may vary in concentration between species or cultivars, whereas other compounds are specific for a certain family or even species. Genetic factors thus have a large influence on the content of bioactive compounds in fruit and vegetables. Variation between cultivars of the same species may also be large (Karav & Eksi, 2012; Howard, Pandjaitan, Morelock, & Gil, 2002). Climatic conditions also have a strong influence on the concentration of bioactive compounds. Climatic factors vary with growing site during the season and from year to year. Temperature, both in terms of total or average temperature and the extremes during the growth period, may influence the chemical composition (Lefsurd, Kopsell, Kopsell, & Curran-Celentano, 2005). Light is also known to affect the concentrations considerably; higher the light reception by the fruit or vegetable, more the concentrations of vitamin C, carotenoids and flavonoids (Lefsurd & Kopsell, 2006). Considerable variations also exist in concentration of bioactive compounds during growth and maturation of fruit and vegetables. This variation is probably especially evident in fruit ripening, where the carotenoid or flavonoid provide the color of the ripe fruit (Kalt, 2005).

Fresh apricot is considered as one of the most delicious temperate fruit and is a rich source of vitamin A, vitamin C, iron, potassium, calcium, phosphorous, essential trace minerals and fiber. It also contains good amount of phytochemicals, such as carotenoids, flavonoids, lycopene and other antioxidant compounds which contribute substantially to their antioxidant potential (Vinha, Barreira, Castro, & Machado, 2013; Erdogan-Orhan & Kartal, 2011). The major apricot producing countries of the world are Turkey, Iran, Pakistan, Uzbekistan and Italy (Hegedus et al., 2010; Zujko &Witkowska, 2011). In India apricots are grown commercially in the hills of Himachal Pradesh, Jammu and Kashmir, Utter Pradesh and to a limited extent in the north-eastern hills. Although different apricot varieties have been investigated by many researchers in the world (Sass-Kiss, Kiss, Milotay, Kerek, & Toth-Markus, 2005; Ruiz, Egea, Gil, & Tomas-Barberian, 2005; Akin, Karabulut, & Topcu, 2008; Drogoudi, Michailidis, & Pantelidis, 2008), a detailed investigation on the chemical compositions of apricots from North India, has not yet been carried out. Considering the influence of climatic conditions,

geographical location and type of cultivar on the chemical composition and antioxidant activity, the present research was aimed to evaluate the bioactive profile and antioxidant activity of apricot varieties from north India so as to enable the recommendation for consumption and propagation of the varieties which have potential health promoting effects and good grower returns.

2. Materials and Methods

2.1 Chemicals and Reagents

Standards phenolic acids, flavonoids, DPPH radical and dehydroascorbic acid were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Standard carotenoids were obtained from HIMEDIA Laboratories (Mumbai, India). Folin Ciocalteu reagent was purchased from Merck (Germany). All the analytical grade chemicals and reagents were purchased from Loba Chemie (Mumbai, India). HPLC grade methanol, acetronitrile, dichloromethane and water were purchased from Fisher Scientifics (Pittsburgh, PA, UK).

2.2 Raw Material

The apricot varieties studied in the present work were collected from Central Institute of Temperate Horticulture (CITH), Kashmir, India. Fourteen apricots varieties namely *Halman*, *Rakhchekarpo*, *Khante*, *Shakanda*, *Nugget*, *Venatchaa*, *Shakarpara*, *Rakauslik*, *Sterling*, *CITH-1*, *CITH-2*, *Viva Gold*, *Newcastle*, and *Turkey* grown in north India were taken up for the present study and evaluated for their bioactive contents and antioxidant activities using different assays. The apricots varieties were harvested at their commercial maturity stages during the month of June to August. Water, soil, fertilizers and other cultivar requirements were the same for each apricot variety. The apricots after harvesting were stored under refrigerated conditions (3±1 ^{o}C, RH 80%); solvent extracted and evaluated for bioactive contents and antioxidant activities. Triplicate samples were taken for each parameter.

2.3 Analysis of Bioactive Compounds

For determination of total phenols and flavonoids, homogenized sample (10 g) of apricot fruit in triplicates was extracted three times with 80% methanol. The extracts obtained were centrifuged for 20 min and the supernatants collected were dried under nitrogen. Total phenols were determined by Folin-Ciocalteu assay, which is an electron transfer based assay according the method described by waterhouse (2002). Total flavonoid content was measured by the aluminum chloride spectrophotometric assay as per the method of Chen, Lin, and Hsieh (2007). Ascorbic and dehydroascorbic acid estimation was done by HPLC system of JASCO, Japan (model, LC-Net II/ADC), fitted with an automatic degassing unit, UV-2070 detector, PU-2080 pump and a HiQ-Sil C18 column (size 4.6 mm × 250 mm) using the method of Wimalasiri and Wills (1983).Total carotenoids as beta carotene equivalents were determined using the previously described method with slight modifications (Kimura & Rodriguez-Amaya, 2004).

2.4 Extraction and Analysis of Phenolic Compounds

Known weight (500 g) of apricot sample of each variety was extracted three times with 80% methanol using sample to solution ratio of 1:2. The extracts so obtained after filtration were pooled together and concentrated under reduced pressure at temperature not exceeding 40 ^{o}C using rotary vacuum evaporator. This concentrated extract was designated as whole concentrate (WC). Known weight of sample from whole extract was extracted three times for phenolics using ethyl acetate. The ethyl acetate extracts were combined, passed over anhydrous sodium sulfate for 30 min. and filtered through Whatmann filter paper no. 42. The Combined ethyl acetate extracts were then evaporated to dryness under reduced pressure at temperature not exceeding 40 ^{o}C using rotary vacuum evaporator and stored in a desiccator at low temperature prior to analysis by HPLC.

2.4.1 HPLC Analysis of Apricot Phenolics

For quantification and identification purposes of phenolic compounds in fresh apricot samples, a 1.0% solution (w/v) in HPLC grade methanol was made for ethyl acetate extracts of all the samples. The samples were then filtered first through Whatman filter paper no. 42 and then through 0.22 mm membrane filters (Millipore). Separation of phenolic compounds was carried out by HPLC method. Aliquot of 20 µL sample of apricot sample was injected for estimation purposes in a C-18 column. Prior to analysis, the analytical column was thoroughly washed with methanol followed by mobile phase for 1 h. HPLC analysis of the samples was carried out with a Jasco HPLC system, Jasco Corporation (Tokyo, Japan) equipped with a C-18 reverse phase HYPERSIL (Chromatopack, Mumbai, India) column (250 mm × 4.6 mm) and a UV detector which was set at a wavelength of 280 nm for identification phenolic acids and 360 nm for identification of flavonoids. The HPLC gradient was methanol in 0.1% formic acid/H_2O as follows: 5 to 15% in 15 min, 15 to 30% from 15 to 35 min, 30 to 40% from 35 to 40 min, 40 to 50% from 40 to 50 min, 50 to 60% from 50 to 55 min, 60 to 75% from 55 to 60 min,

and finally reaching 95% in 65 min at a flow rate of 1.0 ml/min. Identification of the phenolic compounds present in apricot samples was done on comparison of the retention times (Rt) with standard samples and by LCMS/MS analysis. The quantification of individual phenolic compounds was done with the help of standard curve obtained by plotting percentage peak area versus concentration.

2.6.2 LCMS/MS Analysis of Apricot Samples

The chemical constituents of apricot phenolic extracts were further identified by LCMS/MS analysis. Mass spectra were recorded by atmospheric pressure chemical ionization in the negative mode using a Varian Ion Trap MS (410 Prostar Binary LC with 500 MS IT PDA detectors) equipped with a C-18 reverse phase stainless steel column (30 cm x 0.46 cm). All samples were filtered through a 0.45 μm filter (Millipore Coop.) before injection. The capillary voltage was kept at 80 V, and the air (nebulizing gas) pressure was 35 psi. Full scan data acquisition was performed by scanning from m/z 100 to 900. The presence of major phenolic compounds was confirmed by their molecular ion peak and base peak. The HPLC gradient was methanol in 0.1% formic acid/H_2O as follows: 5 to 15 % in 7.5 min, 15 to 30% from 7.5 to 17.5 min, 30 to 40% from 17.5 to 20 min, 40 to 50% from 20 to 25 min, 50 to 60% from 25 to 27.5 min, 60 to 70% from 27.5 to 30 min, and finally reaching 95% in 32.5 min at a flow rate of 0.5 ml/min.

2.7 Antioxidant Activity

Fifty gram homogenized sample of apricot varieties was extracted three times using methanol. The extracts were combined and filtered through Whatmann filter paper no. 42 followed by centrifugation at 4 ±1 ^oC for 20 min at 14,000 rpm. The supernatants so collected were kept at 2 ±1 ^oC till further use. DPPH radical scavenging activity of extracts was measured according to the method of Brand-Williams, Cuvelier, & Berset (1995) with some modifications. The reducing power of extracts was determined by evaluating the transformation of Fe^{3+}–Fe^{2+} according to the method of Oyaizu (1986). For ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfoniz acid) radical cation decolorization assay the procedure followed the method of Arnao, Cano, and Acosta (2001) with some modifications.

2.7.1 Determination of EC50 for Antioxidant Assays

A portion (20 mL) of the methanolic extract was completely dried under nitrogen in order to get exact weight of the extract. A1% solution of the dried extract was made in methanol so as to get a working concentration of 0.01 mg/mL. Aliquots of 1-10 mL of working solution were taken in a series of test tubes and 2 mL of DPPH solution was added to each test tube. The test tube containing only DPPH solution served as control. The test tubes containing the samples were shaken vigorously and kept in dark at room temperature for 30 min, followed by measurement of absorbance at 517 nm. The EC_{50} (DPPH) value, which represents the concentration of extract that gives 50% reduction in DPPH absorbance, was determined by linear regression analysis of absorbance versus concentration. Similar procedure was followed for determining the EC_{50} values for FRAP and ABTS assays.

2.8 Statistical Analysis

Mean values, standard deviation, analysis of variance (ANOVA) were computed using a commercial statistical package SPSS 10.1 (USA). The data was compared using Duncan's multiple range tests at 5% significance level.

3. Results and Discussion

3.1 Total Phenols and Flavonoids

The content of total phenols and flavonoids of apricot varieties is shown in Table 1. It is evident from the data that content of both total phenols and flavonoids was significantly ($p \leq 0.05$) influenced by variety. Data analysis indicated that content of total phenols and flavonoids of apricot varieties ranged from (24.2±1.31)-(72.5±3.12) mg/100 g GAE and (12.2±1.11)-(36.1±2.11) mg/100 g CE. Statistical analysis of the data revealed that total phenols and flavonoids were significantly ($p \leq 0.05$) higher in Rakausilk variety followed by Viva-gold and Rakhchekarpo respectively. Comparison of the data revealed no significant ($p \geq 0.05$) difference in phenolic content among cultivars Halman, Shakanda, Venatchaa, Khante, Nugget and Shakarpara respectively. The differences in total phenols and flavonoids among the apricot cultivars may be related to the genotype and maturity at harvest (Karav & Eksi, 2012). Another study also reported significant variation in content of total phenolics among Malatya apricot varieties (Akin, Karabulut, & Topcu, 2008). The phenolic content of other fresh fruits and their varieties has also been reported in the literature (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002; Gill, Tomas-Barberan, Hess-Pierce, & Kader, 2002). The ranges of the total phenolics reported were as low as 9.1 mg/100 g of fresh weight in white-flesh nectarines and as high as 17.90 mg/100 g of fresh weight in one genotype of Ribes L. (black currants). Base on the comparison of the phenolic contents; Rakausilk,

Viva-gold and Rakhchekarpo apricot varieties of north India may be considered as a good source of total phenolics and may be recommended for inclusion in daily diets.

3.2 Total Ascorbic Acid (TAA) and Dehydroascorbic Acid (DHA) Content

Content of total ascorbic acid and dehydroascorbic acid of apricot varieties is shown in Table 1. Data analysis indicated that content of total ascorbic acid as well as DHAA was also significantly ($p \leq 0.05$) influenced by variety. Among apricot varieties studied, highest total ascorbic acid content was recorded in Halman and Venatchaa followed by Khante, Rakausilk and Nugget varieties. Data analysis showed that TAA and DHA content of the apricot varieties ranged from (10.4±0.86)-(22.3±1.11) mg/100 g and (0.8±0.04)-(3.5±0.72) mg/100g respectively.

Table 1. Content of bioactive compounds (mg/100 g) in apricot varieties grown in north India

Variety	TP	TF	TAA	RAA	DHAA	TC
Halman	51.8±1.70 ^{e}	26.1±1.12 ^{c}	22.3±1.11 ^{e}	20.4±1.13 ^{f}	1.9±0.44 ^{c}	12.2±2.13 ^{e}
Rakhchekarpo	69.6±1.65 ^{f}	34.3±1.21 ^{d}	15.4±1.12 ^{b}	13.4±1.11 ^{c}	2.0±0.41 ^{c}	8.2±1.11 ^{c}
Khante	45.1±1.86 ^{d}	23.4±1.40 ^{c}	21.3±1.31 ^{d}	19.6±1.14 ^{f}	1.7±0.35 ^{c}	9.4±1.02 ^{c}
Shakanda	53.6 ±1.65 ^{e}	26.3±1.31 ^{c}	15.3±1.11 ^{b}	13.2±1.10 ^{c}	2.1±0.44 ^{d}	7.5±1.04 ^{b}
Viva Gold	71.2±2.11 ^{f}	36.1±2.11 ^{d}	18.2±1.20 ^{c}	16.9±1.11 ^{e}	1.3±0.14 ^{b}	9.4±1.03 ^{c}
CITH-1	24.2±1.31 ^{a}	13.2±1.11 ^{a}	15.1±1.21 ^{b}	13.8±1.13 ^{c}	1.3±0.11 ^{b}	8.8±0.82 ^{c}
CITH-2	38.2±1.52 ^{c}	18.3±1.41 ^{b}	16.2±1.13 ^{b}	15.1±1.11 ^{d}	1.1±0.13 ^{a}	7.5±0.64 ^{b}
Newcastle	31.4±1.22 ^{b}	14.3±1.11 ^{a}	11.4±0.92 ^{a}	10.5±0.85 ^{b}	0.9±0.06 ^{a}	6.3±0.45 ^{a}
Turkey	36.7±1.73 ^{c}	15.4±1.31 ^{a}	10.4±0.85 ^{a}	9.6±0.76 ^{b}	0.8±0.04 ^{a}	6.8±0.55 ^{b}
Nugget	42.7±1.30 ^{d}	19.5±1.21 ^{b}	20.4±1.12 ^{d}	17.6±1.31 ^{e}	2.8±0.61 ^{e}	10.5±1.11 ^{d}
Venatchaa	51.3±2.14 ^{e}	23.2±1.41 ^{c}	22.1±1.31 ^{e}	19.6±1.20 ^{f}	2.5±0.55 ^{e}	8.2±1.11 ^{c}
Shakarpara	42.3±2.13 ^{d}	19.1±1.14 ^{b}	19.3±1.10 ^{c}	16.8±1.14 ^{e}	2.6±0.48 ^{e}	6.4±0.84 ^{a}
Rakauslik	72.5±3.12 ^{f}	35.1±1.51 ^{d}	21.3±1.11 ^{d}	17.8±1.11 ^{e}	3.5±0.72 ^{f}	5.3±0.75 ^{a}
Sterling	28.6±1.51 ^{b}	12.2±1.11 ^{a}	10.4±0.86 ^{a}	7.1±0.76 ^{a}	3.3±0.43 ^{f}	5.8±0.65 ^{a}
$LSD_{0.05}$	3.6	3.2	1.1	1.1	0.30	1.2

Note. Values are mean ±SD (n = 3); LSD = least significant difference ($p \leq 0.05$).

Values within varieties in a column with different superscript lowercase letter (a–f) are significantly different.

TP = total phenols; TF = total flavonoids; TAA = total ascorbic acid; RAA = reduced ascorbic acid; DHAA = dehydroascorbic acid; TC = total carotenoids; CITH = central institute of temperate horticulture.

Interestingly the DHA content of Turkey, Newcastle and CITH-2 apricot varieties was significantly ($p \leq 0.05$) lower than the other varieties .The variation in TAA content observed in the present study is in agreement with other earlier studies. Thompson and Trenerry (1995) reported that the ascorbic acid content of Turkish apricot varieties, particularly Hacihaliloglu, Hasanbey, Bursa and Igdir was about 10 mg/100 g of fresh weight. Akin et al. (2008) also reported significant ($p \leq 0.05$) variation in ascorbic acid content among the Malatya apricot varieties and the values ranged from 20.6-96.8 mg/100 g. The significant difference in TAA content among the apricot varieties observed in the present study can be attributed to the influences of the factors like genotypic differences and maturity at harvest and intensity of light received during the growing season (Lee & Kader, 2000; S. Kim, D. Kim, Y. Kim, & C. Kim, 2015). Higher the intensity of light during the growing season, the greater is the ascorbic acid content in plant tissue.

3.3 Total Carotenoids

Total carotenoids of the apricot varieties were found to be significantly ($p \leq 0.05$) different and influenced largely by variety (Table 1). Among apricot varieties, Halman exhibited the highest content of total carotenoids (12.2±2.13 mg/100 g) followed by Nugget (10.5±1.11 mg/100 g). Cultivar variation in total carotenoids ranged from (5.3±0.75)-(12.2±2.13) mg/100 g. Statistical analysis of the data indicated that total carotenoids of Shakarpara, Rakausilk and Sterling apricots differed marginally ($p \geq 0.05$) with respect to each other, but were significantly ($p \leq 0.05$) lower compared to Nugget and Venatchaa varieties. Similar observation was recorded for Rakhchekarpo and Khante apricots. The varietal influence on carotenoid variation has also been observed in other studies. Total carotenoids in Bursa, Alyanak and Tokaloglu apricot varieties were 10.87, 11.52 and 9.02

mg/100 g of fresh weight respectively (Thompson & Trenerry, 1995). Among the Malatya apricot varieties, Hasanbey and Kabaasu were found to have the highest total carotenoids of the order of 9.73 and 8.80 mg/100 g of fresh weight (Akin et al., 2008). Ruiz, Egea, Gil, and Tomas-Barberian (2005) in another study reported that the carotenoid content of Spanish apricot cultivars ranged from 1.36-38.52 mg/100 g on fresh weight basis. In comparison to the above data on total carotenoids, the apricots varieties from north India can be considered a good source of carotenoids.

3.4 Phenolic Acid and Flavonoid Composition

The variation in phenolic acid composition of apricot varieties in shown in Table 2. The data analysis indicated significant ($p \leq 0.05$) difference in phenolic acid content among apricot varieties. Statistical analysis of the data revealed that among apricot varieties, highest total phenolic acid concentration (10.5 mg/kg, fw) was recorded in Rakausilk variety followed by Viva gold (9.4 mg/ml, fw) and Shakanda/Halman (7.2/7.0 mg/kg, fw) respectively. HPLC analysis of apricot samples showed the presence of fifteen phenolic acids in Halman, Venatchaa, Rakausilk and New Castle varieties; nine phenolic acid in Rakchekarpo and Sterling apricot; twelve phenolic acids in Shakanda, Nugget, Shakarpara and CITH-1 varieties; fourteen phenolic acid in Viva gold and Turkey varieties; seven phenolic acid in Khante and nineteen phenolic acids in CITH-2 apricots. Figure 1 shows the representative HPLC chromatogram of Halman (A) and Newcastle (B) apricots and their peak identification (D and E) by LCMS/MS. In Halman variety, out of the fifteen phenolic acids, six were hydroxybenzoic acid (HBA) derivatives at total concentration of 1.88 mg/kg, fw and eight were hydroxycinnamic acid (HCA) derivatives at total concentration of 4.9 mg/kg, fw. Ellagic acid reported as other phenolic acid was present at a concentration of 0.24 mg/kg. In Rakchekarpo variety, out of the nine phenolic acids, seven were HBA derivatives at total concentration of 2.91 mg/kg and two were HCA derivatives at total concentration of 0.74 mg/kg.

Table 2. Phenolic acid content (mg/kg fw) of apricot varieties grown in north India

Phenolic acids	Apricot varieties														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	LSD
(a) Hydroxybenzoic Acids(HBA'S)															
i. Gallic acid	$0.10^{a,1}$	$0.24^{c,2}$	$0.61^{e,3}$	$0.69^{f,3}$	$0.45^{d,4}$	$0.11^{a,2}$	$0.09^{a,1}$	$0.85^{h,5}$	$0.18^{b,2}$	$0.78^{g,4}$	$0.21^{b,2}$	$0.08^{a,1}$	$0.08^{a,1}$	$0.11^{a,1}$	0.03
ii. p-hydroxybenzoic acid	$0.61^{f,1}$	$0.68^{g,5}$	-	$0.41^{e,2}$	$0.32^{d,2}$	$0.57^{f,6}$	$0.28^{c,3}$	$0.88^{h,5}$	$0.24^{c,3}$	$0.68^{g,3}$	$0.18^{b,2}$	$0.14^{a,2}$	$0.11^{a,1}$	$0.10^{a,1}$	0.04
iii. m-hydroxybenzoic acid	-	$0.56^{e,4}$	-	$0.38^{c,2}$	$0.24^{b,1}$	$0.46^{d,5}$	$0.21^{b,2}$	$0.83^{g,5}$	$0.16^{a,2}$	$0.73^{f,3}$	$0.12^{a,1}$	$0.22^{b,3}$	$0.25^{b,2}$	$0.15^{a,2}$	0.04
iv. Protocatechuic acid	$0.37^{e,3}$	-	$0.54^{f,2}$	$0.31^{c,1}$	$0.21^{b,1}$	$0.33^{d,4}$	$0.18^{a,2}$	$0.66^{g,4}$	$0.21^{b,2}$	$0.57^{f,2}$	$0.15^{a,1}$	$0.24^{b,3}$	$0.33^{d,3}$	$0.28^{c,3}$	0.03
v. Vanillic acid	$0.29^{b,2}$	-	-	-	$0.42^{c,3}$	$0.26^{b,3}$	$0.37^{c,4}$	$0.46^{d,2}$	$0.11^{a,1}$	$0.40^{c,1}$	-	$0.31^{b,4}$	$0.27^{b,2}$	-	0.05
vi. Gentisic acid	$0.44^{c,4}$	$0.36^{b,3}$	-	$1.31^{f,4}$	-	$0.41^{b,5}$	-	$0.41^{b,2}$	-	-	$0.11^{a,1}$	$0.65^{e,5}$	$0.58^{d,4}$	$0.58^{d,4}$	0.05
vii. Syringic acid	-	-	-	-	$0.41^{c,3}$	-	$0.35^{b,4}$	$0.51^{c,3}$	-	$0.42^{c,1}$	$0.18^{a,2}$	$0.30^{b,4}$	$0.30^{b,3}$	$0.24^{a,3}$	0.06
iii. Homoveratic acid	-	$0.18^{a,1}$	-	-	-	-	-	$0.25^{h,1}$	-	-	-	$0.33^{c,4}$	$0.24^{b,2}$	-	0.03
ix. α-resorcylic acid	$0.07^{a,1}$	$0.64^{d,5}$	-	-	-	$0.04^{a,1}$	$0.36^{c,4}$	-	-	-	-	$0.08^{a,1}$	$0.10^{b,1}$	-	0.03
x. β-resorcylic acid	-	$0.25^{b,2}$	$0.26^{b,1}$	-	-	-	-	-	$0.09^{a,1}$	-	-	$0.11^{a,1}$	-	$0.08^{a,1}$	0.03
LSD	0.04	0.04	0.04	0.05	0.03	0.05	0.06	0.08	0.05	0.06	0.04	0.04	0.03	0.04	
Total HBA	1.88^{b}	2.91^{c}	1.41^{a}	3.1^{d}	2.05^{b}	2.18^{b}	1.84^{b}	4.85^{e}	0.99^{a}	3.58^{d}	0.95^{a}	2.46^{c}	2.26^{b}	1.54^{a}	0.06
% HBA	26.9	79.9	24.8	42.9	40.2	32.1	44.5	46.3	57.6	38.0	46.8	39.2	45.1	34.8	
(b) Hydroxycinnamic Acids (HCA'S)															
i. p-Coumaric acid	$0.56^{c,2}$	$0.45^{b,2}$	-	$0.52^{c,2}$	$0.68^{e,4}$	$0.53^{c,2}$	-	$0.64^{d,2}$	$0.34^{a,3}$	$0.58^{c,3}$	$0.28^{a,3}$	$0.65^{d,5}$	-	$0.60^{d,4}$	0.06
ii. o-Coumaric acid	$0.51^{c,2}$	-	-	-	-	$0.47^{c,2}$	$0.64^{d,3}$	$0.72^{e,3}$	$0.31^{a,2}$	$0.64^{d,3}$	$0.26^{a,2}$	$0.44^{a,1}$	$0.48^{c,3}$	$0.38^{b,3}$	0.0
iii. m-Coumaric acid	$1.61^{f,5}$	-	-	$0.54^{c,2}$	$0.41^{b,2}$	$1.58^{f,4}$	$0.38^{b,2}$	$1.83^{h,5}$	-	$1.71^{g,5}$	$0.21^{a,2}$	$1.41^{e,6}$	$1.65^{f,4}$	$1.12^{d,4}$	0.10
iv. Caffeic acid	$0.19^{a,1}$	-	$1.84^{d,3}$	-	$0.56^{c,3}$	$0.17^{a,1}$	-	$0.41^{h,1}$	-	$0.35^{b,1}$	-	$0.34^{b,3}$	-	$0.26^{a,2}$	0.10
v. Ferulic acid	$0.13^{a,1}$	-	$0.84^{e,1}$	$1.42^{f,3}$	$0.84^{e,5}$	$0.11^{a,1}$	$0.79^{e,4}$	$0.56^{d,2}$	$0.08^{a,1}$	$0.49^{c,2}$	$0.08^{c,2}$	$0.48^{c,4}$	$0.22^{b,2}$	$0.27^{b,2}$	0.06
vi. Chlorogenic acid	$0.77^{d,3}$	$0.29^{b,1}$	$1.21^{f,2}$	$0.36^{c,1}$	$0.26^{b,1}$	$0.74^{d,3}$	$0.22^{b,1}$	$0.88^{e,4}$	-	$0.81^{d,4}$	-	$0.18^{a,2}$	-	$0.12^{a,1}$	0.07
vii. Cinnamic acid	$0.17^{c,1}$	-	-	$0.46^{d,2}$	-	-	-	-	-	$0.74^{e,4}$	$0.11^{b,1}$	$0.06^{a,1}$	$0.09^{a,1}$	-	0.04
viii. Sinapic acid	$0.92^{d,4}$	-	-	$0.35^{b,1}$	-	$0.83^{c,3}$	-	-	-	-	-	$0.15^{a,1}$	$0.20^{a,2}$	-	0.06
LSD	0.06	0.04	0.1	0.08	0.1	0.09	0.09	0.08	0.06	0.07	0.05	0.09	0.08	0.06	
Total HCA	4.9^{d}	0.74^{a}	3.9^{c}	3.6^{c}	2.8^{b}	4.4^{c}	2.0^{b}	5.0^{d}	0.73^{a}	5.3	0.94^{a}	3.7^{c}	2.6^{b}	2.8^{b}	0.8
% HCA	69.9	20.3	68.4	50.5	53.8	65.1	49.0	48.1	42.5	56.5	46.3	59.1	52.7	62.2	
(c) Other Phenolic Acid															
Ellagic acid	0.24^{c}	-	0.39^{e}	0.48^{f}	0.31^{d}	0.19^{b}	0.27^{c}	0.58^{g}	-	0.51^{f}	0.14^{a}	0.11^{a}	0.11^{a}	0.13^{a}	0.03
% Other Phenolic acid	3.4	-	6.8	6.6	6.1	2.8	6.5	5.5	-	5.4	6.9	1.8	2.2	2.9	
Total Phenolics acids	7.0^{d}	3.7^{b}	5.7^{c}	7.2^{d}	5.2^{c}	6.8^{d}	4.1^{b}	10.5^{e}	1.7^{a}	9.4^{e}	2.0^{a}	6.3^{d}	5.0^{c}	4.4^{b}	1.1

Note. 1 = Halman; 2 = Rakhchekarpo; 3 = Khante; 4 = Shakanda; 5 = Nugget; 6 = Venatchaa; 7 = Shakarpara; 8 = Rakausilk; 9 = Sterling; 10 = Vivagold; 11 = CITH1; 12 = CITH 2; 13 = Newcastle; 14 = Turkey; LSD = least significant difference ($p \leq 0.05$).

Values within columns among phenolic acids with different superscript lowercase letters (a-h) differ significantly ($p \leq 0.05$).

Values within rows among varieties with different superscript numericals (1-6) differ significantly ($p \leq 0.05$).

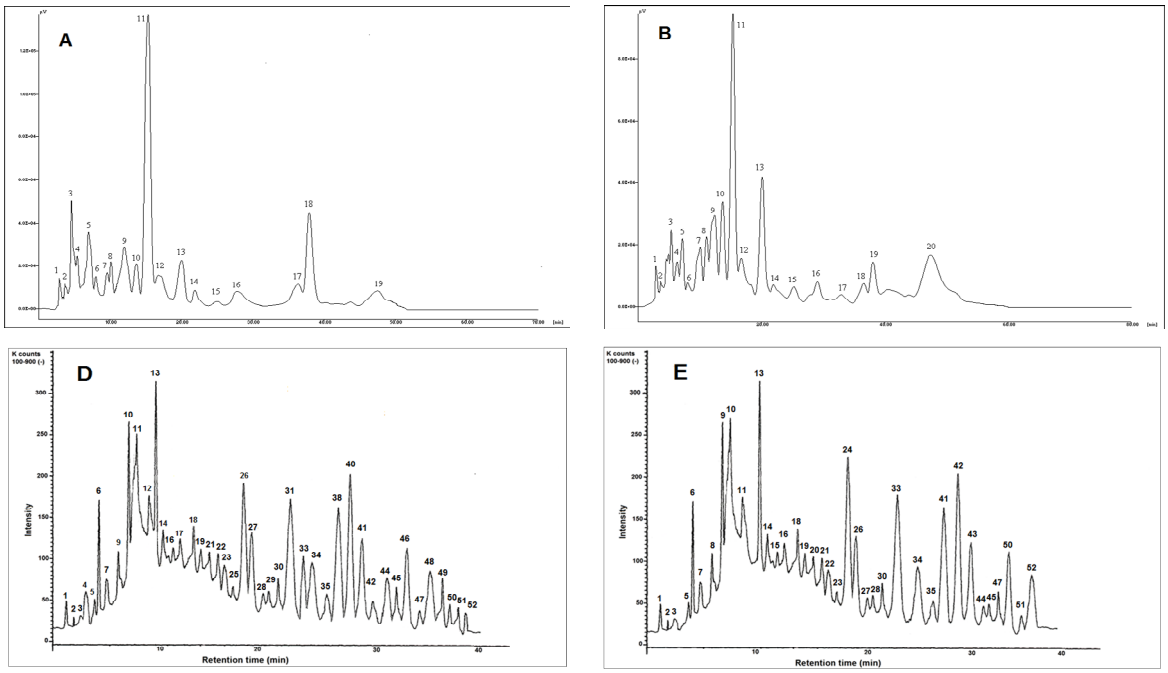

Figure 1. Representative HPLC chromatogram of Halman (A) and Newcastle (B) apricots and their peak identification (D and E) by LCMS/MS. (A): 1 = ellagic acid; 2 = gallic acid; 3 = chlorogenic acid; 4 = protocatechuic acid; 5 = p-hydroxybenzoic acid; 6 = cinnamic acid; 7 = caffeic acid; 8 = vanillic acid; 9 = p-coumaric acid; 10 = gentisic acid; 11 = m-coumaric acid; 12 = ferulic acid; 13 = o-coumaric acid; 14 = not detected; 15 = α-resorcyclic acid; 16 = not detected; 17 = not detected; 18 = sinapic acid; 19 = not detected; (B): 1 = ellagic acid; 2 = gallic acid; 3 = protocatechuic acid; 4 = p-hydroxybenzoic acid; 5 = m-hydroxybenzoic acid; 6 = cinnamic acid; 7 = vanillic acid; 8 =syringic acid; 9 = not detected; 10 = gentisic acid; 11 = m-coumaric acid; 12 = ferulic acid; 13 = o-coumaric acid; 14 = not detected; 15 = α-resorcyclic acid; 16 = hemovoratic acid; 17 = not detected; 18 = not detected; 19 = sinapic acid; 20 = not detected

In Khante variety, the three HBA and HCA were present at a total concentration of 1.41 and 3.9 gm/kg while as ellagic acid was present at a concentration of 0.39 mg/kg. Similarly in Shakanda variety, the five HBA detected were present at a total concentration of 3.1 mg/kg and six HCA were present at concentration of 3.6 mg/kg. Ellagic acid was present at a concentration of 0.48 mg/kg. In case of Nugget, Venatchaa, Shakarpara, Rakausilk and Sterling apricots; the hydroxybenzoic acids were present at a concentration of 0.99-4.85 mg/kg while as HCA were present in the concentration range of 0.73-5.0 mg/kg. Data analysis further indicated that m-coumaric acid was present at concentration of 1.71 mg/kg in Viva gold, 1.41 mg/kg in CITH-2, 1.65 mg/kg in New Castle and 1.12 mg/kg in Turkey apricot respectively. Further ellagic acid detected in Viva gold, CITH-2, New Castle and Turkey apricot varieties was in the concentration range of 0.11-0.51 mg/kg. The results of the present study regarding the phenolic acid composition in apricots are in the agreement with the earlier studies. Hussain, Chatterjee, Variyar, Sharma, Dar, and Wani (2013) reported the presence of major phenolic acids like gallic acid, chlorogenic acid, ferulic acid and others in Halman apricots. Principle component analysis was performed on the data obtained from HPLC analysis of phenolic acids to make it more interpretable and give a clear distinction of varietal grouping. PCA accounted for about 28.23% of total variation in the samples (17.34% for PC2 and 10.89% for PC3) as shown in Figure 2. It is seen from the PCA plot that caffeic acid, chlorogenic acid, m-coumaric acid, ferulic acid and gentisic acid are the main effects on the separation and grouping of the apricot varieties. The varieties of Khante, Halman, Venatcha, Rakausilk, Viva Gold, Newcastle and Shakanda contained higher levels of caffeic acid, chlorogenic acid, m-coumaric acid, ferulic acid and gentisic acid compared to other varieties.

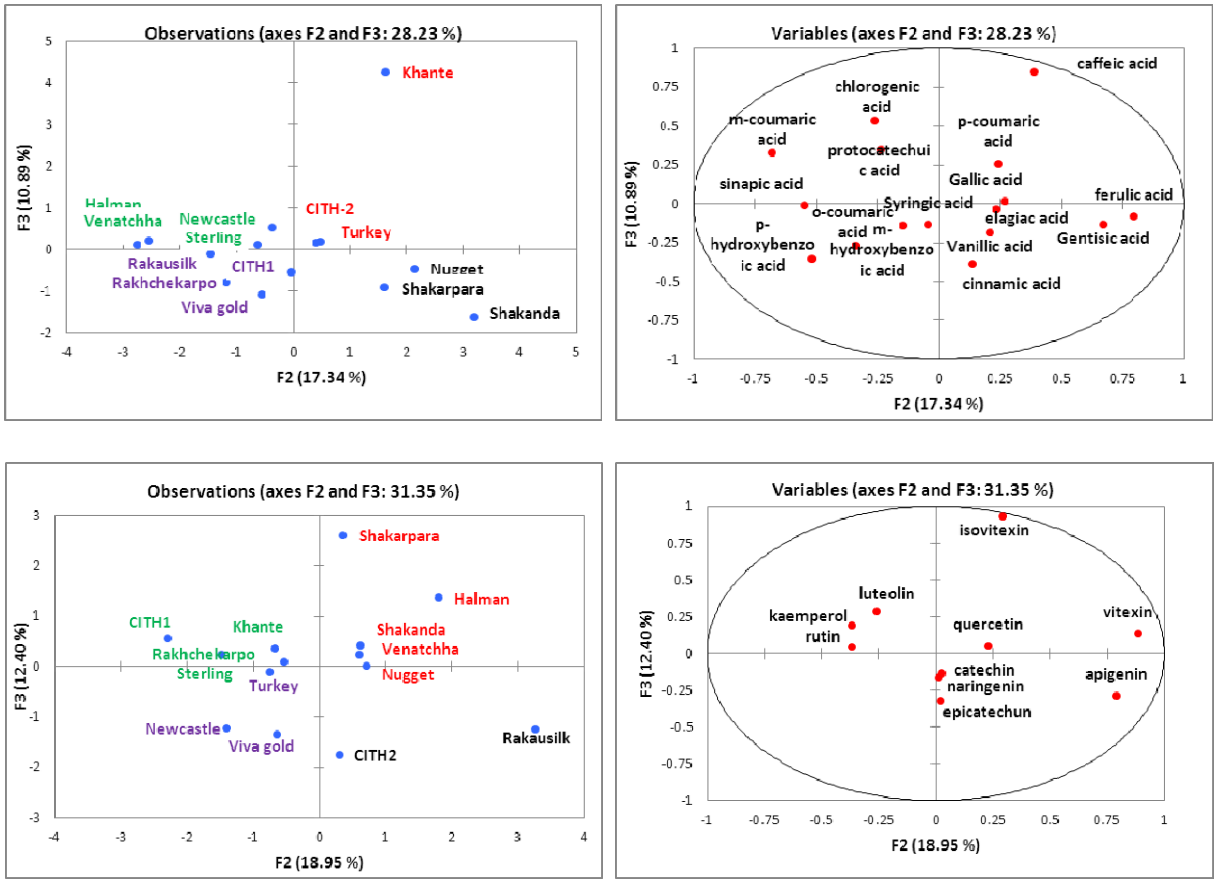

Figure 2. Principal component analysis plots of phenolic acids and flavonoids of apricot varieties

Flavonoid analysis by HPLC revealed the presence of ten flavonoids in apricot cultivars (Data not shown). Flavonoids identified using HPLC were catechin, epicatechin, quercetin, kaempferol, naringenin, apigenin, luteolin, vitexin, isovitexin and rutin. Data analysis indicated significant ($p \leq 0.05$) difference in the content of individual flavonoids among the apricot varieties. Quercetin was the most abundant flavonoid present in Sterling, Rakausilk, Halman, Khantey, Shakanda and Rakhchekarpo cultivars followed by catechin, epicatechin, kaempferol and luteolin. Quercetin was present at maximum concentration of 1.2 mg/kg in Sterling, 0.91 mg/kg in Rakausilk, 0.54 mg/kg in Halman, 0.75 mg/kg in Rakchekarpo, 0.41 mg/kg in Khante and 0.65 mg/kg in Shakanda cultivar respectively. Halman was the only apricot which showed the presence of all flavonoids except rutin. Data analysis further indicated that total flavonoids as sum of individual flavonoids determined by HPLC were significantly ($p \leq 0.05$) higher in Sterling (4.77 mg/kg) and Rakausilk (2.78 mg/kg) variety followed by Rakchekarpo (2.73 mg/kg) and Halman (2.47 mg/kg) variety. In order to get a general evaluation and comparison of the 14 apricot varieties of north India in terms of flavonoid composition, a principle component analysis was performed on the data obtained from HPLC analysis of individual flavonoids. The PCA accounted for about 31.35% of total variation in the samples (18.95% for PC2 and 12.40% for PC3) as shown in Figure 2. PCA explains the main difference among the cultivars and groups them according to their quantitative values of the individual flavonoids. PCA of the data showed that the varieties namely Shakarpara, Halman, Rakausilk and Sterling were completely distinguished from the other apricot varieties. The separation is based on the higher levels of isovitexin, vitexin, apigenin, quercetin, kaempferol, luteolin and rutin.

4.5 HPLC-CPAI-MS/MS Characterization of Apricot Phenolics

The phenolic compounds detected in this present work were tentatively characterized by means of comparing the $[M-H]^-$ ion peaks, together with the interpretation of the observed MS/MS spectra in comparison with those found in the literature. The HPLC-CPAI-MS/MS analysis of the apricot phenolics was carried out in negative mode. A total of 52 phenolic compounds have been tentatively identified by comparing retention times and MS data of the detected peaks with that reported in the literature. The major peaks observed in the mass

chromatogram have been assigned in Table 3. The identified compounds belonged to various classes including 24 phenolic acids and their derivatives and 33 flavonoids.

In the negative ion mode, hydroxybenzoic acids produce a deprotonated $[M-H]^-$ molecule and a $[M-H-44]^-$ fragment ion through loss of carbon dioxide from the carboxylic moiety. For instance, loss of CO_2 was observed for caffeic, ferulic, protocatechuic and gallic acid giving the $[M-H-44]^-$ as a characteristic ion at m/z 135, m/z 149, m/z 109 and m/z 125. Ferulic acid and syringic acid also show the loss of the CH_3 group, providing an [M-15] anion radical at m/z 178 and m/z 182 (Sanchez-Rabaneda, Jauregui, Casals, Andres-Lacueva, Izquierdo-Pulido, & Lamuela-Raventios, 2003). Syringic acid in addition to this undergoes loss of water molecule producing a major fragment ion at m/z 179 from its precursor ion. On the basis of this, peak Nos. 3, 5, 12, 15 and 23 were identified as gallic acid, protocatechuic acid, caffeic acid, syringic acid and ferulic acid respectively. Sinapic acid also undergoes loss of CH_3 group from the $[M-H]^-$ ion, thus provides a [M-H-15] anion radical at m/z 208. Hence the peak 51 was identified as sinapic acid. Chlorogenic acid shows the $[M-H]^-$ deprotonated molecule (m/z 353) and the ion corresponding to the deprotonated quinic acid at m/z 191 (Sun, Liang, Bin, Li, & Duan, 2007). Therefore the peak 4 (m/z 191) eluting at 4.5 min was assigned as chlorogenic acid. Mass fragmentation signals at m/z 167 and m/z 137 were identified as vanillic acid and 4-hydroxybenzoic acid by comparing their MS spectral data with those of authentic standards.

Table 3. Phenolic compounds identified in present work in apricot varieties using HPLC-APCI-MS/MS analysis

Peak No.	t_R (min)	MW	$[M-H]^-$ (m/z)	MS^2 $[M-H]^-$ (m/z)	Compound	Detected in
1	2.80	302	301	257, 227, 185	Ellagic acid	1, 3, 4, 5, 6, 7, 8 10, 11, 12, 13, 14
2	3.20	192	191	127, 173, 111	Quinic acid	1, 2, 3, 5, 6, 8, 9, 12, 13, 14
3	3.58	170	169	125	Gallic acid	DIAV
4	4.50	354	353	191, 179, 173	Chlorogenic acid	1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14
5	5.35	154	153	109	Protocatechuic acid	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
6	5.85	326	325	163, 145, 187, 265, 119	p-Coumaric acid-O-hexoside	1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 13, 14
7	6.25	138	137	93	p-hydroxybenzoic acid	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
8	7.17	138	137	93	m-hydroxybenzoic acid	2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
9	7.74	306	305	179, 125	Gallocatechin	DIAV
10	8.01	148	147	62	Cinnamic acid	1, 4, 10, 11, 12, 13
11	8.51	290	289	245, 205, 179	Catechin	DIAV
12	9.64	180	179	135, 161, 107	Caffeic acid	1, 3, 5, 6, 8, 10, 12, 14
13	10.01	480	479	359, 167, 389	Vanillic acid derivative	1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
14	10.22	168	167	91, 123	Vanillic acid	1, 5, 6, 7, 8, 9, 10, 12, 13
15	11.20	198	197	179, 135, 182	Syringic acid	5, 7, 8, 10, 11, 12, 13, 14
16	11.81	290	289	245, 205, 179	Epicatechin	DIAV
17	12.02	164	163	119, 163	p-Coumaric acid	1, 2, 4, 5, 6, 8, 9, 10, 11, 12, 14
18	13.22	442	441	289, 169	Epicatechin gallate	1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
19	13.82	154	153	109	Gentisic acid	1, 2, 4, 6, 8, 11, 12, 13, 14
20	14.35	436	435	273, 167, 123	Phloridzin	2, 3, 4, 7, 8, 9, 12, 13, 14
21	15.52	164	163	119, 163	m-Coumaric cid	1, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14
22	16.30	356	355	193, 119, 217, 175	Ferulic acid-O-hexoside	1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14
23	16.91	194	193	149, 134, 178	Ferulic acid	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
24	17.21	492	491	315, 300, 271	Isorhamnetin-O-glucouronide	2, 3, 4, 5, 7, 8, 9, 10, 13
25	18.15	610	609	301, 255, 179	Rutin	1, 2, 3, 5, 6, 7, 9, 12
26	18.95	464	463	301, 343	Quercetin-3-O-glucoside	DIAV
27	19.25	448	447	285, 327	Luteolin-7-O-glucoside	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14
28	20.11	164	163	119, 163	o-Coumaric acid	1, 6, 7, 8, 9, 10, 11, 12, 13, 14
29	20.26	594	593	285	Luteolin-7-O-rutinoside	1, 2, 3, 4, 5, 6, 7, 12
30	21.45	432	431	311, 283, 341, 312	Vitixin	1, 4, 8, 10, 11, 13, 14
31	21.95	578	577	269, 431	Apigenin-7-O-rutinoside	1, 3, 8, 11, 12, 14
32	22.12	490	489	284, 255, 210, 285	Kaempferol-3-O-acetylhexoside	2, 3, 5, 6, 9, 12
33	22.21	594	593	285, 327, 258	Kaempferol-3-O-rutinoside	1, 2, 5, 6, 9, 12, 13, 14

34	22.36	434	433	271	Naringenin-7-O-glucoside	1, 4, 7, 8, 9, 10, 11, 13
35	22.42	448	447	285, 257	Kaempferol-3-O-glucoside	DIAV
36	22.56	448	447	301	Quercetin-3-O-rhamnoside	2, 3, 4, 5, 6, 7, 8, 10, 11, 12
37	22.71	154	153	109	β- resorcylic acid	2, 3, 9, 12, 14
38	23.06	464	463	301, 151, 179	Quercetin-3-O-hexoside	1, 2, 3, 4, 5, 8, 10, 11, 12, 14
39	23.32	432	431	269, 285	Apigenin-7-O-glucoside	2, 3, 5, 6, 7, 8, 9, 10, 11, 14
40	23.72	462	461	415, 269	Apigenin-7-O-rhamnoside	1, 3, 8, 9, 11, 14
41	24.21	480	479	317, 179, 151	Myricetin-3-O-glucoside	1, 2, 3, 4, 5, 6, 9, 11, 12, 13
42	25.58	154	153	109, 137	α- resorcylic acid	1, 2, 6, 7, 12, 13
43	29.44	196	195	151, 107	Homoveratic acid	2, 8, 12, 13
44	31.12	432	431	341, 311, 269	Isovitexin	1, 4, 8, 10, 11, 13, 14
45	33.10	302	301	151, 227, 179	Quercetin	DIAV
46	33.40	286	285	217, 199, 175, 241	Luteolin	1, 2, 3, 4, 5, 6, 7, 9, 10, 12
47	34.51	272	271	177, 151, 119	Naringenin	1, 4, 7, 8, 9, 10, 13
48	34.85	270	269	151	Apigenin	1, 3, 8, 11, 14
49	35.10	286	285	217, 151, 133	Kaempferol	1, 2, 5, 6, 9, 12
50	35.30	386	385	223, 247, 190, 205	Sinapic acid-o-hexoside	1, 2, 3, 4, 12, 13
51	37.07	224	223	208, 179, 149	Sinapic acid	1, 4, 6, 12, 13
52	37.25	318	317	151, 179	Myricetin	1, 2, 3, 4, 5, 6, 9, 11, 12, 13, 14

Note. The base peaks are identified in bold font. DIAV = detected in all varieties; 1 = Halman; 2 = Rakhchekarpo; 3 = Khante; 4 = Shakanda; 5 = Nugget; 6 = Venatchaa; 7 = Shakarpara; 8 = Rakausilk; 9 = Sterling; 10 = Vivagold; 11 = CITH1; 12 = CITH 2; 13 = Newcastle; 14 = Turkey.

Upon fragmentation, vanillic acid and 4-hydroxybenzoic acid produced the ions at m/z 123 and m/z 93 due to the loss of CO_2 from their respective precursor ions. Hence peak Nos. 7 and 14 were identified as 4-hydroxybenzoic acid and vanillic acid. The precursor ion of p-coumaric acid and gentisic acid at m/z 163 and m/z 153 upon fragmentation also generated a fragment ion at m/z 119 and m/z 109 which is a characteristic of CO_2 loss; hence peak Nos. 17 and 19 were identified as p-coumaric acid gentisic acid. The MS/MS fragmentation of the precursor m/z 353 ion identified as chlorogenic acid gave dominant product ions at m/z 191, m/z 179 and m/z 173. The product ions m/z 191 for quinic acid and m/z 179 for caffeic acid revealed the constituent of chlorogenic acid prior to condensation. On the basis of this, the peak 2 was identified as quinic acid. In the present study we were able to characterize three phenolic acid derivatives which showed the neutral loss of a hexose moiety. Based on the MS/MS fragmentation pattern, these compounds were identified as p-coumaric acid hexoside (peak 6), ferulic acid hexoside (peak 22) and sinapic acid hexoside (peak 50) respectively. The precursor ion at m/z 479 produced a fragment ion at m/z 359 which upon subsequent fragmentation produced another fragment ion at m/z 167 due to the loss of hexose moiety and is a characteristic of vanillic acid. Based on this the peak 13 was assigned as vanillic acid derivative (Ornelas-Paz et al., 2013). Peak 1 had an $[M-H]^-$ ion at m/z 301 which yielded a fragment ion at m/z 257 with the loss of CO_2 group from carboxylic acid function; a characteristic of ellagic acid fragmentation. Hence peak 1 was assigned as ellagic acid. Cinnamic acid has an $[M-H]^-$ ion at m/z 147 and a fragment ion at m/z 62. On the basis of this peak 10 was identified as cinnamic acid. Further based on the literature, peak Nos. 37, 42 and 43 have been assigned to beta resorcylic acid, alpha resorcylic acid and homoveratic acid respectively as their precursor ions at m/z 153 and 195 produced the respective fragment ions at m/z 109 and 151 due to the loss of CO_2 group from their carboxylic acid function (Sun et al., 2007; Ibrahim, El-Halawany, Saleh, El-Naggar, El-Shabrawy, & El-Hawary, 2015).

Flavonoids generally exist as glycosides, hence mass spectra shows both the deprotonated molecule $[M-H]^-$ of the glycoside and the ion corresponding to the deprotonated aglycone $[A-H]^-$. During MS analysis, the glycosidic linkage of the phenolic glycosides gets cleaved; hence mass fragments at m/z 162 amu (hexose, glucose, galactose), 146 amu (deoxyhexose, rhamnose), 132 amu (pentose, xylose, aribinose) and 176 amu (glucouronic acid) or loss of these fragments is observed. Catechin ($[M-H]^-$ m/z 289) yields fragment ions at m/z 245, m/z 179 and m/z 205. The isomer epicatechin gives the same fragment ions, as the stereoisomers could not be distinguished by mass spectrometry. The $[M-H-44]^-$ fragment ion at m/z 245 in catechin or epicatechin is formed by the loss of a $(CH)_2OH$ group (Stoggl, Huck, & Bonn, 2004). The mechanism of production of fragment ions at m/z 179 and m/z 205 has already been explained (Bravo, Silva, Coelho, Vilas-Boas, & Bronze, 2006). Gallocatechin ($[M-H]^-$ m/z 305) yields the fragment ions at m/z 125 and 179, while as epicatechin gallate

([M-H]$^-$ m/z 441) gives fragment ions at m/z 289 which results from the cleavage of the ester bond and the loss of a gallic acid moiety, and those at m/z 169 from the cleavage of the ester bond and the loss of epicatechin units (Miketova, Schram, Whitney, Li, Huang, & Klohr, 2000). Based on this, the peak Nos. 9, 11, 16 and 18 were identified as gallocatechin, catechin, epicatechin and epicatechin gallate respectively. The flavonoids namely apigenin, quercetin, luteolin, naringenin, kaempferol and myricetin were identified on the basis of comparison of their mass fragmentation data with authentic standards and available literature. Quercetin, myricetin, kaempferol, apigenin and naringenin produced fragment ions at m/z 151 and 179, which result from the cleavage of the heterocyclic C-ring. Standard luteolin produced fragment ions at m/z 217 and 241. Hence the peak Nos. 45, 46, 47, 48, 49 and 52 were assigned to quercetin, luteolin, naringenin, apigenin, kaempferol and myricrtin respectively. Peak 30 and 44 exhibited a [M-H]$^-$ ion at m/z 431 and its characteristic fragment ions [M-H-90] and [M-H-120] at m/z 341 and m/z 311 and are characterized as vitexin and isovitexin based on the literature data (Zucolotto et al., 2012).

For flavonol-O-glycosides, the mass spectra showed both the deprotonated molecule [M-H]$^-$ of the glycoside and the ion corresponding to the deprotonated aglycone [A-H]$^-$. The latter ion is formed by loss of the rhamanose, glucose or galactose moiety from the glycoside. The same behavior was observed for flavone O-glycosides such as luteolin-7-O-glycoside (m/z 447 to m/z 285), luteolin-7-O-rutinoside (m/z 593 to m/z 285), apigenin-7-O-glucoside (m/z 431 to m/z 269), apigenin-7-O-rutinoside (m/z 577 to m/z 269) and for the flavanone-O-glucosides namely naringenin-7-O-glucoside (m/z 433 to m/z 271) with the loss of glucose and rutinose residue. Therefore the peak Nos. 27, 29, 31, 34 and 39 were assigned to luteolin-7-O-glucoside, luteolin-7-O-rutinoside, apigenin-7-O-rutinoside, naringenin-7-O-glucoside and apigenin-7-O-glucoside respectively (Spinola, Pinto, & Castilho, 2015). The mass spectrum of the peak 26 (m/z 463 to m/z 301), peak 36 (m/z 447 to m/z 301) and peak 38 (m/z 463 to m/z 301) showed identical fragmentation pattern as that of standard quercetin (m/z 301) with the loss of glucose, rhamanose and hexose unit, hence the peaks were identified as quercetin-3-O-glucoside, quercetin-3-O-rhamanoside and quercetin-3-O-hexoside respectively (Abu-Reidah, Ali-Shtayeh, Jamous, Arraez-Roman, & Segura-Carretero, 2015). Similarly the mass spectrum of peak 41 (m/z 479 to m/z 317, m/z 151) showed identical fragmentation pattern as that of standard myricetin (m/z 317) with the loss of glucose unit, hence the peaks was identified as myricetin-3-O-glucoside. Peak 20 with molecular ion at m/z 435 was characterized as phloridzin based on the data available in literature (Roowi & Crozier, 2011). Peak 24 showed [M-H]$^-$ ion at m/z 491 and loss of glycoside moiety resulted in the formation of isorhamnetin aglycone (m/z 315). Hence, the peak was characterized as isorhamnetin-o-glucouronide (Rivera-Pastrana, Yahia, & Gonzalez-Aquilar, 2010). Peak 25 with [M-H]$^-$ ion at m/z 609 gave rise to quercetin aglycone (m/z 301) by the loss of rutinose moiety, hence was characterized as quercetin-3-O-rutinoside/rutin (Aaby, Skrede, & Wrolstad, 2005). Peak Nos. 32, 33 and 35 showed loss of different glycosides resulting in a common characteristic aglycone fragment at m/z 285 attributed to kaempferol. Therefore the compounds were characterized as kaempferol-3-O-acetylhexoside, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside respectively (Ornelas-Paz et al., 2013).

4.6 Antioxidant Activity

DPPH radical scavenging activity of apricot varieties is shown in Figure 3 (A). Data analysis revealed that DPPH radical scavenging activity (expressed as inhibition percentage, IP) was significantly ($p \leq 0.05$) influenced by variety. The DPPH radical scavenging activity of apricot varieties was in the range of (30.2±1.6)-(71.2±2.3)%. Maximum IP of the order of 71.2±2.3% was observed in Rakausilk variety followed by Viva Gold (68.3%) and Rakhchekarpo (65.4%). CITH-1 variety showed lowest IP (30.2±1.6%) in DPPH radical activity. Close observation of the data indicated about a twofold variation in DPPH radical scavenging activity among the apricot varieties. Statistical analysis of the data indicated that there was no significant ($p \geq 0.05$) difference in DPPH radical scavenging activity between apricot varieties of Nugget and Shakarpara. Ferric reducing ability power of the apricot varieties is shown in Figure 3 (B). The data analysis revealed that among apricot varieties, reducing power was also significantly ($p \leq 0.05$) higher for Rakausilk variety (0.76) followed by Viva Gold (0.68) and Rakhchekarpo variety (0.65) respectively. Lowest reducing power was recorded in CITH-1 variety. Statistical analysis of the data revealed no significant ($p \geq 0.05$) difference in reducing power among apricot varieties of Sterling, Turkey, Newcastle, CITH-2 and CITH-1.

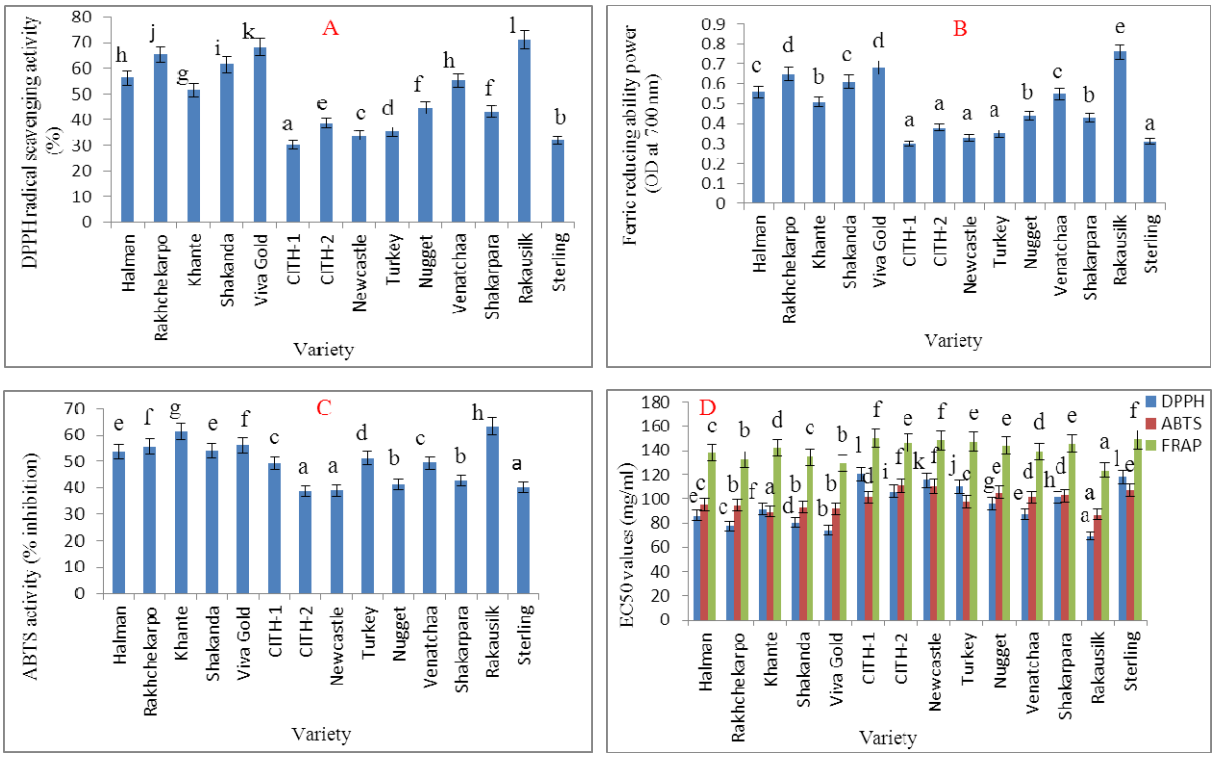

Figure 3. In-vitro antioxidant activities of apricot varieties of north India. (A) DPPH radical scavenging activity; (B) Ferric reducing ability power (FRAP); (C) ABTS assay; (D) EC_{50} values of apricot varieties for DPPH, FRAP and ABTS assays

Similar observation was recorded among Nugget, Shakarpara and Khante apricot varieties. $ABTS^{+}$ inhibition power of the apricot varieties is shown in Figure 3 (C). The data analysis revealed that among apricot varieties, $ABTS^{+}$ inhibition was also significantly ($p \leq 0.05$) higher for Rakausilk variety (63.2%) followed by Khante (61.3%) and Viva Gold variety (56.1%) respectively. Lowest $ABTS^{+}$ inhibition was recorded in CITH-2 variety. Statistical analysis of the data revealed no significant ($p \geq 0.05$) difference in $ABTS^{+}$ inhibition among apricot varieties of Sterling and Turkey; Newcastle and CITH-2; Venatchaa and CITH-1; Viva Gold and Rakhchekarpo and Shakanda and Halman apricots respectively.

4.6.1 EC_{50} Values in Antioxidant Assays

For better comparison of antioxidant activity of the apricot varieties, the results obtained from the DPPH radical scavenging, ferric reducing ability power and $ABTS^{+}$ inhibition were expressed as EC_{50} values. A low EC_{50} value is the indication of strong antioxidant activity. The EC_{50} (DPPH, FRAP and $ABTS^{+}$ inhibition) values for apricot varieties are plotted in Figure 3 (D) Data indicated that for DPPH and FRAP assay, EC_{50} values among apricot varieties were significantly ($p \leq 0.05$) lower in Rakausilk (69.2, 123.6 mg/ml) followed by Viva Gold (74.3, 129.5 mg/ml) and Rakhchekarpo (77.4, 132.4 mg/ml) varieties respectively. For $ABTS^{+}$ inhibition the EC_{50} values among apricot varieties were in the order of $EC_{50(Rakausilk)} < EC_{50(Khante)} < EC_{50(Viva\ Gold)}$. Comparison of the EC50 values for the antioxidant assays revealed that apricot varieties of north India showed significantly ($p \leq 0.05$) good scavenging activity towards DPPH and ABTS radical compared to ferric reducing power. Statistical analysis further indicated that for DPPH assays there was no significant ($p \leq 0.05$) difference in EC_{50} values among apricot varieties of Halman, Venatchae, Sterling and CITH-1 respectively. Similar observation was recorded for FRAP assay among apricot varieties of Sterling, Newcastle, and CITH-1, Shakarpar, Nugget and CITH-2. Positive correlations have been found between antioxidant activity and phenolics, indicating that antioxidant activity is directly related to phenolic profiles (Hussain, Wani, Meena, & Dar, 2010). Also the relative values of antioxidant activity can vary depending on the assay used. Our results revealed that a strong correlation existed between total phenolics and antioxidant activity for DPPH assay ($r = 0.91$) and ABTS radical inhibition ($r = 0.86$) while as moderate correlation existed for FRAP ($r = 0.76$).

5. Conclusion

The results of the present study demonstrate that content of bioactive compounds and antioxidant activity was significantly ($p \leq 0.05$) influenced by variety. Among the apricot varieties studied, cultivars of Rakausilk, Viva-gold and Rakhchekarpo possessed highest content of bioactive compounds. Comparison of the EC_{50} values for the antioxidant assays revealed that apricot varieties of north India showed significantly ($p \leq 0.05$) good scavenging activity towards DPPH and ABTS radical compared to ferric reducing power. Our results indicate a strong correlation existed between total phenolics and antioxidant activity for DPPH assay ($r = 0.91$) and ABTS radical inhibition ($r = 0.86$) while as moderate correlation existed for FRAP ($r = 0.76$). The present research offers an opportunity in the selection of apricot fruits to be incorporated in the daily diet schedule and other infant formulations so as to help in the prevention of countless diseases. The information from this research could also be useful in future breeding programs to identify new cultivars with enhanced health promoting compounds in apricot fruit.

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