IJFANS
International Journal of Food And Nutritional Sciences

Volume 4, Issue 4, Jul-Sep 2015, www.ijfans.com e-ISSN: 2320-7876

# INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

## IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

IJFANS International Journal of Food And Nutritional Sciences

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

*e-ISSN* 2320 –7876 *www.ijfans.com*
Vol.4, Iss.4, Jul-sep, 2015


*Research Paper* *Open Access*

# ANTIOXIDANT PROPERTIES OF FRUIT PULP AND PEEL OF EIGHT APPLE CULTIVARS GROWN IN HIMACHAL PRADESH

**R K Sharma*, N Sharma, S Khatri and R. Kundra**
G. B. Pant Institute of Himalayan Environment and Development, Himachal Unit, Mohal-Kullu (H.P.), India

*Corresponding author: rajeshbhu78@gmail.com

**Received on: 21th May, 2015** **Accepted on: 14th September, 2015**

## ABSTRACT

Eight apple cultivars grown in Himachal Pradesh were tested for total phenolics and flavonoids contents and antixodant activities using standard methods. Total phenolics and flavonoids contents in peels (75.5–174 mg/g and 25.9–140.6 mg/g, respectively) of all test apple cultivars were higher than their pulp (52.23-95.20 mg/g and 12–27.6 mg/g, respectively). Extracts of both pulp and peels also showed significant variations among test cultivars and maximum activities of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) followed by 2, 2'–azino-bis-ethylbenzthiazoline-6-sulfonic acid (ABTS) and least by ferric reducing antioxidant power (FRAP). Relationships between concentrations of extracts of apples and DPPH inhibition was found positive and significant for all the tested cultivars ($p<0.05$). The present study concludes that test apple cultivars had significant amounts of total phenolics and flavonoids compounds as well as possesses significant antioxidant activities. Thus consumption of test apple cultivars grown in Himachal Pradesh is highly encouraged among mountain people for gaining more nutrititional and health benefits.

**Keywords:** Antioxidant activity, Total phenolics contents, Apple, Cultivars, Hiamchal Pradesh

## INTRODUCTION

Now a days, antioxidants have been determined in many food stuffs like fruits, vegetables, herbs, cereals, shrubs, sprouts and seeds, etc., as they are the most abundant sources of natural antioxidant (Velioglu et al. 1998; Sharma et al. 2012; Lila et al. 2014). Characters of fruits such as appearance, quality, taste, flavor and health promoting properties are influenced by contents of carbohydrates, organic acids and phenolics compounds, etc. (Hudina and Atampar , 2000; Treutter, 2001; Lattanzio, 2003). Some fruits like apple and orange have remarkable antioxidant potential (Scalzo et al. 2003; Gardner et al. 2000; Leong and Shui, 2002) due to high phenolics contents. Flavonoids contents, antioxidant contents, or potential of fruits varies with cultivars, tissue types, plant nutrition, climatic region, cold storage, species, parts of fruit and physiological stage (Wolfe et al. 2003; Drogoudi et al. 2008; McGhie et al. 2005; Goulding et al. 2001; Sharma et al. 2013).

Many studies showed that many diseases such as cancer, Alzheimer's disease, stroke, cardiovascular disease, iron overload and ageing might be caused by reactive oxygen species (Demirkol et al. 2004). Antioxidants may be natural, originated from food related materials e.g. carotenoids, flavonoids, phenolic acids, tocopherols, tocotrienols etc., or may be synthetic, manufactured from chemical molecules e.g. butylated hydroxyanisole, butylated hydroxytoluene, ethoxyquin, propyl gallate, tertiary butylhydroquinone, etc., helps to maintain the food quality and increase their shelf life.

Consumption of fruits and vegetables in routine are essential for healthy life as they decreases risk of occurance of diseases like cancer, related to cardiovascular diseases, etc, (Liu et al. 2002). Many epidemiological studies showed that the risk of some disease like cancer, thrombotic stroke, ischemic heart disease, asthma and type-2 diabetes could be reduced by consumption of apples (Boyer and Liu, 2004; Knekt et al. 2002). Studies have also shown that phytochemicals found in the fruits and vegetables increases their nutritional values and potential to prevent them from diseases might be due to higher contents of phytochemicals than other contents (Chu et al. 2002; Meyers et al. 2003)

As the wonderful fruit "apple" a rich source of phyto-nutrients, indispensable for optimal health has health promoting and disease prevention properties, thus truly justifying the sentence, "an apple a day keeps the doctor away". Studies also showed that peels contains more antioxidants than apple pulps (Wolfe et al. 2003; Taso et al. 2005; Łata and Tomala, 2007; Drogoudi et al. 2008; Khanzadeh et al. 2008; Vieria et al. 2009). Studies further showed that apples have higher amounts of free phenolics as compared to other fruits (Sun et al. 2002), and they have ranked second after blueberries for total phenolics contents and antioxidant activities (Jose et al. 2009).

Being one of the important cash crops of Himachal Pradesh, north west Indian Himalaya apple (*Malus sp*.) plays an important role in horticulture. In Himachal Pradesh, large area (47 percent) has been engaged for the cultivation of apples as compared to other

fruits grown in Himachal Pradesh and has largest production (83 percent) among all fruits grown in Himachal Pradesh (Anonymous, 2007). The literature on the total phenolics and flavonoids contents as well as the antioxidant activities of different cultivars of apple commonly grown in north west Indian Himalaya and particularly Kullu district of Himachal Pradesh are rare or not available. Therefore, the present study was carried out to evaluate and compare the total phenolics and flavonoids contents and antioxidant activities of pulp and peel of different apples cultivars grown in Himachal Pradesh.

## MATERIALS AND METHODS

For the analysis of total phenolics and total flavonoids contents and antioxidant activities, 1kg fruits of eight cultivars of *Malus sp*. Namely Royal Delicious, Rich-a-Red, Commercial, Black-Vem-Devis, Red Gold, Golden Delicious, Granny Smith and Red delicious were collected in triplicates in a distilled water rinsed polyethylene bag from apple orchids located in Kullu, Himachal Pradesh (Lat: $32^0$12'; Long: $77^0$04' and Lat: 1500.m amsl). The samples were brought back to laboratory, washed with running tap water and separated in pulp and peel.

Total moisture content was obtained using an oven dry method. 100g of fresh peels and pulp of apples were taken separately, wrapped in brown paper and kept in oven at 70 °C till a constant weight of samples were acheived. The total moisture content was calculated using following formula as [(100 × (fresh weight-dry weight)/ dry weight]. Fresh pulp and peel of apple cultivars was used to prepare the extracts. 5g of pulps and peels of each cultivar were crushed separately in mortar and pestle using 80% ethanol (v/v) in five replicates and final volume was made to 20 ml using ethanol. The extracts of the samples were filtered through Whatman no 1 filter paper and all the five replicates of each sample were combined to achieve the concentrations 250 mg/ml and stored at 4 $^0$C in a refrigerator till further analysis.

Total flavonoids contents in the extracts of each apple cultivar was estimated using an aluminium chloride reagent and quercetin as standard (Ordon-Ez et al. 2006). One milliliter of extract solution containing 250 mg extract was taken in a test tube, 1ml of 2% $AlCl_3$ was added and test tube was shaken thoroughly. After one hour, absorbance of the golden color reaction mixture was measured at 420 nm using an UV-VIS spectrophotometer (Ultrospec 2100, ProHealth Care Bioscience AB, Uppsala, Sweden). The same procedure was repeated for each standard solutions of quercetin ranging between 0-100 mg/ml and standard curve was obtained using equation: absorbance = m× quercetin (mg).

Method of Wolfe et al. (2003) was used for the estimation of total phenolics contents in the etahnolic extracts of different apple cultivars involving Folin-Ciocalteu Phenol Reagent and tannic acid as standard. One ml of extract solution (250mg) was taken in a test tube. 1 ml of Folin Ciocalteu [diluted with double distilled water, 1:1 (v/v)] Phenol Reagent was added and the whole reaction mixture was gently shaken. After five minutes, 2ml of 20% sodium carbonate ($Na_2CO_3$; w/v) and 6 ml of double distilled water were further added. Then, the mixture was shaken gently and heated for one hour at waterbath at 80 $^0$C or till blue colour developed. Absorbance of reaction mixture was measured at 650 nm using an UV-VIS spectrophotometer (Ultrospec 2100, Pro Health Care Bioscience AB, Uppsala, Sweden). The above method was also repeated with each concentration of tannic acid (0.01-1 mg/ml) and standard curve was obtained using equation: absorbance = m × gallic acid (mg).

2,2-diphenylpicrylhydrazyl (DPPH) assay was done according to the method of Liyana-Pathirama and Shahidi (2005) after some modifications (w/v). A stock of DPPH solution was prepared by dissolving 10 mg DPPH in 25 ml ethanol and stored in refrigerator at 4°C. Working solution of DPPH was further prepared by mixing 10 ml of stock DPPH solution in a volumetric flask and final volume was maintained using ethanol. One ml of extract solution (250mg) was taken and mixed with 4 ml of DPPH (0.004%). Then the mixture was gently shaken and kept in dark for 30 minutes. Absorbance of reaction mixture was read using an UV-VIS Spectrophotometer (Ultrospec 2100, Pro Health Care Bioscience AB, Uppsala, Sweden) at 517 nm. The above procedure was also repeated with different concentrations of ascorbic acid (0.1-1 mg/ml) for the preparation of standard curve (DPPH inhibition vs ascorbic acid concentration). The standard curve was obtained using an equation i.e. DPPH inhibition = m ×ascorbic acid (mM)

2,2-Azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay for ethanol extracts of different apple cultivars was achieved using a literature method (Re et al. 1999). The stock solution of 7 mM 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 2.4 mM potassium persulphate was prepared in methanol. The working solution was prepared by mixing equivalent of 7 mM ABTS and 2.4 mM potassium persulphate and allowing them to react for 12-16 hours in dark at room temperature. The solution was then diluted using methanol to obtain an absorbance of 0.706 ± 0.001 at 734 nm. One ml of extract solution (250 mg) was mixed with 1 ml of reaction mixture shaken.. Absorbance of the reaction mixture was measured at 420 nm using UV-VIS Spectrophotometer (Ultrospec 2100, Pro Health Care Bioscience AB, Uppsala, Sweden). The standard curve was prepared using ascorbic acid solution at concentrations 10 to 100 µM using the above procedure. The standard curve was obtained using an equation i.e. ABTS inhibition = m × Ascorbic acid (mM)

Ferric-reducing antioxidant power (FRAP) assay was performed according to the method described by Benzine and Strain, (1996). The stock solutions of 300mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ were prepared. The fresh working solution was prepared by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ and 2.5 ml of $FeCl_3.6H_2O$ and then the temperature of working solution was raised to 37 °C before use. One ml of extract solution (250 mg) was mixed with 1 ml of working reagent. Then, the mixture was kept at room temperature for 30 minutes. The absorbance of reaction mixture was measured at 593 nm using UV-VIS Spectrophotometer (Ultrospec 2100, Pro Health Care Bioscience AB, Uppsala,

Sweden). The standard curve was prepared by using ascorbic acid solution at concentrations ranging between 0.1 to 1 mM using above procedure. Standard curve was obtained using equation : absorbance = m× ascorbic acid (mM)

All the measurements were done in triplicates and obtained data are expressed as a mean ± SE. Data were analyzed by analysis of variance ($P<0.05$). Means were separated by Duncan Multiple Range Test and results was considered significant at $p < 0.05$. All the data were processed by computer programs SPSS (version 16), Sigma (version 10) and Microsoft office (2007).

## RESULTS AND DISCUSSION

The markets price and moisture contents of pulps and peels of fruits of different cultivars of apples commonly grown and sold in Himachal Pradesh, India are given Table 1. Market price was found maximum for Royal and Red delicious followed by Grammny Smith, Richa-a-Red, Commercial and Golden Delcicous and minimum price was observed for Red Gold (Table 1). The moisture contents in pulps and peels ranged between 81.6% - 90% and 72.2% - 85.3%, repectively. The results further showed that moisture content in pulps was found higher than those of peels of tested cultivars of apples as pulp peel ratio of moisture content was found more than a unit for all the tested apple cultivars (Table 1). Moisture contents in pulps and peels of apple varied significantly due to cultivars ($p<0.05\%$). Total moisture contents in pulps and peels of locally produce apple cultivars was found maximum in Grammny Smith and minimum in commercial cultivars of apples (Table 1).

Antioxidant activities of a plant mainly depends on contents of their secondary metabolites such as flavonoids, phenolic acids, etc. The release of secondary metabolites from plants varied from plant to plant, tissues to tissues, species to species and environmental conditions of plants growing medium. At present, more than 8000 phenolic compounds from plants having different chemical structures have been identified. These phenolic compounds are known to act as an effective free radical scavengers and antioxidants (Li et al. 2006; Kuti 2004). The antioxidant potentials of a plant can be accurately revealed by expressing antioxidant activities in terms of total phenolics and flavonoid contents (Kuti 2004; Pan et al. 2010).

The amounts of total phenolic and flavonoids compounds present in ethanolic extracts from pulps and peels of different apple cultivars are given in Tables 2 and 3, respectively. Total phenolic compounds in pulps, peels and pulps+peels expressed as mg /g fresh weight varied significantly due to variations in cultivars ($p<0.05$) with a range of minimum to maximum as 51.5 -99.5, 74.5 – 174.3 and 126.8 – 174.34, respectively (Table 2). Total phenolic compounds was found higher in peels than those of pulps of all the tested apple cultivars (Table 2). Maximum and minimum contents of mean total phenolics compounds was found in pulps extracts of Grammny Smith and Red Gold and in peels extracts of Black-vem-Devis and Red Gold, respectively (Table 2). The total phenolics compounds in pulp + Peels of differen apple cultivars were found maximum in Black-vem-Devis followed by Rich-a-Red, Royal Delicious, Golden Delcious, Gramny Smith, Red Delicious, Commercial and minimum was found in Red Gold (Table 2). From the present results it can be suggested that consumption of pulp of Gramny Smith and peels of Black-Vem-Devis of tested apple cultivars, produced locally provides more total phenolic compounds as compared to other tested apple cultivars.

The amounts of total flavonoid compounds were found lower than those of total phenolic compounds in both pulps and peels of tested apple cultivars. The amounts of total flavonoid compounds quantified as mg/g fresh weight of tested varieties of apples varied from minimum of 11.8 to a maximum of 28.0, 25.8 to 141.7 and 46.8 to 164.5 in pulps, peels and pulps+peels, respectively (Table 3). The order of total flavonoid compounds from maximum to minimum in extracts of pulps+peels of different tested apple varieties was found as Royal Delicious > Red Delicious > Black-Vem-Devis > Golden Delicious > Grammny Smith > Commercial > Red Gold > Richa-a-Red (Table 3). Variation in cultivars of apples significantly influences the amounts of total flavonoid compounds in pulp, peels and pulp+peel at $p<0.05$. The results indicates that significant amounts of total flavonoid compounds can be obtained from locally cultivated apple varieties in Himachal Pradesh. In the present study Royal delicious was found as a rich source of total flavonoids compounds.

The present study highlights the antioxidant contents and their activities in ethanolic extracts of different varieties of locally grown/cultivated apple crops of Himachal Pradesh. From the present study, it is clear that total phenolics and flavonoids compounds as well as antioxidant activities of extracts of apples depends on types of varieties. Total phenolics and flavonoids contents in four varieties of apples from New York were also found higher in peels as compared to that of pulps (Wolfe et al. 2003) and five cultivars from Pakistan (Manzoor et al. 2012). The results of the present study are found similar to those earlier reported in literatures (Vieira et al. 2009; Abrosca et al. 2007; Drogoudi et al. 2008). The content of total phenolics and flavonoids in pulp extracts were also found in between 0.4 -0.8 and 0.2 – 1 times lower than those of peel extracts of different apple cultivars (Tables 2 and 3). This results were found inconsistent with those reported by Vieira et al. (2009), Drogoudi et al. ( 2008), Leontowicz et al. (2003) and Lata & Tomala (2007). Total phenolics contents in pulps and peels of Golden and Red Delicious cultivars of apple were found 4.5-7.5 folds higher in the present study than those reported by Manzoor et al. (2012) i.e. 12.98 and 21.02 in Golden Delicious and 14.76 and 25.88 in Red Delicious. Similarly total flavonoid contents in pulps and peels of Golden and Red Delicious cultivars of apple were found 2.8-5.3 folds higher in the present study than those reported by Manzoor et al. (2012) for these cultivars from Pakistan.

The antioxidant potentials of a plant depends on the amounts of total phenolic compounds present in them. Antioxidant potential of etahnolic extracts of pulps and peels of different varieties of apples were evaluated using in-vitro DPPH, ABTS and FRAP assay and expressed as mMAAE/g fw. The results were presented in Table 4 and Figure 1. Figure 1 shows that antioxidant activities in

ethanol extracts from pulps+peels possess maximum DPPH activities followed by ABTS and minimum by FRAP assays in all the tested apple cultivars. The results further showed that DPPH, ABTS and FRAP activities in ethanolic extracts of pulps, peels and pulps+peels of different cultivars varied significantly ($p<0.05$) due to variations in cultivars. DPPH, ABTS and FRAP activities were found maximum in pulp extract of Golden Delicious, Black-Vem-Devis and Black-Vem-Devis (4.48, 3.70 and 2.13 mMAAE/g fw, respectively), in peel extracts of Golden delicious, Black-vem-Devis and Royal Delicious (4.50, 4.03 and 2.13, mM AAE/g fw, respectively) and pulps+peel extracts of Golden and Royal delicious (8.98, 7.37 and 4.95, mM AAE/g fw, respectively). Among the extracts of the tested apple cultivars, DPPH, ABTS and FRAP activities ranged between 4.2 to 4.5, 0.2 to 3.8 and 0.8 to 2.1 in pulps, 4.4 to 4.6, 3.6 to 4.04 and 2.1 to 2.9 in peel and 8.6 to 9, 4 to 7.5 and 3.3 to 5 in pulp+peel, respectively (Table 4 and Fig.1). From the study, it is also clear that peels of tested apple varieties possess high antioxidant activities as compared those of pulps. The present study further indicate that Golden delicious possess more DPPH activities, whereas Royal delicious possess more ABTS and FRAP activities. Thus consumption of these fruits cultivars may be helpful in reducing the load of free radicals in Human bodies. The results of present study further showed that ratio of DPPH, ABTS and FRAP activities in pulps to pulp of different tested apple cultivars were less than a unit which further indicates that peels possess more free radical scavenging potential as compared to the pulps extracts.

**Table 1-Cultivars, sample ID, market price (Rs/kg) and total moisture content (%) of pulp and peels of fruits of different apple cultivars**

| Cultivars | ID | Market price[a] | Moisture content | | | Pulp Peel Ratio |
|---|---|---|---|---|---|---|
| | | | Pulp | Peel | Pulp+Peel | |
| Royal Delicious | ROD | 150.00 | $82.99^{cd} \pm 0.31$ | $76.44^{cd} \pm 0.89$ | $159.43^{cd} \pm 0.83$ | $1.09^{b} \pm 0.01$ |
| Richa-a-Red | RAR | 70.00 | $84.67^{bc} \pm 0.35$ | $77.65^{bc} \pm 0.39$ | $162.32^{c} \pm 0.60$ | $1.09^{b} \pm 0.01$ |
| Commercial | COM | 70.00 | $81.94^{d} \pm 0.22$ | $75.75^{d} \pm 0.83$ | $157.69^{d} \pm 0.89$ | $1.08^{b} \pm 0.01$ |
| Black-Vem-Devis | BVD | - | $83.57^{cd} \pm 0.40$ | $76.92^{bcd} \pm 0.79$ | $160.49^{cd} \pm 0.53$ | $1.09^{b} \pm 0.02$ |
| Red Gold | REG | 60.00 | $86.60^{ab} \pm 0.40$ | $72.30^{e} \pm 0.15$ | $158.90^{d} \pm 0.53$ | $1.20^{a} \pm 0.00$ |
| Golden Delicious | GOD | 70.00 | $83.74^{cd} \pm 0.42$ | $78.56^{b} \pm 0.22$ | $162.30^{c} \pm 0.22$ | $1.07^{bc} \pm 0.01$ |
| Granny Smith | GRS | 80.00 | $87.24^{a} \pm 1.16$ | $84.37^{a} \pm 0.47$ | $171.61^{a} \pm 1.38$ | $1.03^{cd} \pm 0.01$ |
| Red Delicious | RED | 150.00 | $84.98^{abc} \pm 1.69$ | $83.49^{a} \pm 0.13$ | $168.48^{b} \pm 1.72$ | $1.02^{d} \pm 0.02$ |
| | | | | | | |
| Mean | | | 84.19 | 78.19 | 162.65 | 1.08 |
| Std Dev | | | 2.05 | 3.19 | 4.88 | 0.05 |
| Min | | | 81.59 | 72.15 | 155.98 | 0.98 |
| Max | | | 88.99 | 85.29 | 173.00 | 1.20 |
| F-Values | | | $5.20^{**}$ | $49.79^{***}$ | $26.28^{***}$ | $16.08^{***}$ |

[a]Values obtained form local fruit venders. Values are mean ± S.E. of three replicates.
Values in each column followed by different letter are significantly different at $p \leq 0.05$ (Ducan's Multiple Range Test).
Level of significance; $^{***}$= $p<0.001$, $^{**}$= $p<0.01$.

**Table 2-Amounts of total phenolics compounds in ethanolic extracts of pulp and peel of different cultivars of apple fruits**

| Cultivars/ Extracts | Total Phenolic Compounds (mg TAE/g FW) | | | Pulp Peel Ratio |
|---|---|---|---|---|
| | Pulp | Peel | Pulp+Peel | |
| Royal Delicious | $86.82^{b} \pm 0.14$ | $154.18^{e} \pm 0.95$ | $241.01^{a} \pm 1.00$ | $0.56^{c} \pm 0.00$ |
| Richa-a-Red | $75.32^{c} \pm 0.67$ | $166.42^{c} \pm 0.78$ | $241.73^{a} \pm 1.44$ | $0.45^{d} \pm 0.00$ |
| Commercial | $52.90^{f} \pm 0.41$ | $109.20^{g} \pm 0.21$ | $162.11^{c} \pm 0.61$ | $0.49^{d} \pm 0.00$ |
| Black-Vem-Devis | $69.90^{d} \pm 0.26$ | $174.01^{a} \pm 0.23$ | $243.91^{a} \pm 0.15$ | $0.40^{e} \pm 0.00$ |
| Red Gold | $52.23^{f} \pm 0.36$ | $75.45^{i} \pm 0.32$ | $127.67^{d} \pm 0.51$ | $0.69^{b} \pm 0.01$ |
| Golden Delicious | $72.99^{c} \pm 0.27$ | $154.58^{e} \pm 0.58$ | $227.57^{b} \pm 0.34$ | $0.47^{d} \pm 0.00$ |
| Granny Smith | $95.20^{a} \pm 3.50$ | $130.94^{d} \pm 0.19$ | $226.13^{b} \pm 3.31$ | $0.73^{a} \pm 0.03$ |
| Red Delicious | $69.05^{dc} \pm 0.25$ | $95.34^{h} \pm 0.49$ | $164.40^{c} \pm 0.60$ | $0.73^{a} \pm 0.00$ |
| | | | | |
| Mean | 71.80 | 132.52 | 204.32 | 0.57 |
| Std Dev | 14.29 | 34.29 | 43.65 | 0.13 |
| Min | 51.50 | 74.95 | 126.84 | 0.40 |
| Max | 99.51 | 174.34 | 244.55 | 0.76 |
| F-values | $133.04^{***}$ | $4388^{***}$ | $1099^{***}$ | $34.01^{***}$ |

Values are mean ± S.E. of three replicates.
Values in each column followed by different letter are significantly different at $p \leq 0.05$ (Ducan's Multiple Range Test).
Level of significance; $^{***}$= $p<0.001$.

**Table 3-Amounts of total flavonoids compounds in ethanolic extracts of pulp and peel of different cultivars of apple fruits**

| Cultivars/ Extracts | Quercetin Equivalents (mg/g FW) | | | Pulp Peel Ratio |
|---|---|---|---|---|
| | Pulp | Peel | Pulp+Peel | |
| Royal Delicious | $22.70^{b} \pm 0.14$ | $140.55^{a} \pm 1.11$ | $163.25^{a} \pm 1.22$ | $0.16^{f} \pm 0.00$ |
| Richa-a-Red | $23.81^{b} \pm 1.44$ | $25.93^{h} \pm 0.11$ | $49.74^{g} \pm 1.47$ | $0.92^{a} \pm 0.05$ |
| Commercial | $11.98^{d} \pm 0.13$ | $58.35^{f} \pm 0.32$ | $70.33^{e} \pm 0.25$ | $0.20^{e} \pm 0.00$ |
| Black-Vem-Devis | $20.69^{c} \pm 2.00$ | $86.66^{c} \pm 0.45$ | $107.35^{c} \pm 2.43$ | $0.24^{d} \pm 0.02$ |
| Red Gold | $18.23^{c} \pm 0.15$ | $46.34^{g} \pm 0.45$ | $64.57^{f} \pm 0.33$ | $0.40^{b} \pm 0.01$ |
| Golden Delicious | $26.80^{a} \pm 0.19$ | $78.85^{d} \pm 0.59$ | $105.65^{c} \pm 0.72$ | $0.34^{b} \pm 0.00$ |
| Granny Smith | $27.58^{a} \pm 0.25$ | $71.66^{e} \pm 1.72$ | $99.24^{d} \pm 1.55$ | $0.38^{b} \pm 0.01$ |
| Red Delicious | $25.71^{a} \pm 0.18$ | $92.80^{b} \pm 0.09$ | $118.51^{b} \pm 0.18$ | $0.28^{c} \pm 0.00$ |
| Mean | 22.19 | 75.14 | 97.33 | 0.36 |
| Std Dev | 5.11 | 32.89 | 34.38 | 0.23 |
| Min | 11.79 | 25.77 | 46.81 | 0.16 |
| Max | 28.01 | 141.73 | 164.54 | 0.99 |
| F-values | $34.27^{***}$ | $1864^{***}$ | $816.31^{***}$ | $120.98^{***}$ |

Values are mean ± S.E. of three replicates.
Values in a column followed by different letter are significantly different at p≤0.05 (Ducan's Multiple Range Test).
Level of significance; $^{***}$= p<0.001.

**Table 4-Antioxidant activities in ethanolic extracts of pulp and peel of different cultivars of apple fruits**

| Cultivars/ Extracts | DPPH (mM AAE/g FW) | | FRAP (mM AAE/g FW) | | ABTS (mM AAE/g FW) | |
|---|---|---|---|---|---|---|
| | Pulp | Peel | Pulp | Peel | Pulp | Peel |
| Royal Delicious | $4.42^{bc} \pm 0.01$ | $4.44^{cd} \pm 0.01$ | $2.10^{a} \pm 0.01$ | $2.85^{a} \pm 0.02$ | $3.34^{b} \pm 0.06$ | $4.03^{a} \pm 0.00$ |
| Richa-a-Red | $4.42^{bc} \pm 0.01$ | $4.48^{ab} \pm 0.01$ | $1.54^{e} \pm 0.03$ | $2.16^{d} \pm 0.01$ | $3.13^{c} \pm 0.08$ | $3.89^{b} \pm 0.05$ |
| Commercial | $4.20^{e} \pm 0.02$ | $4.50^{a} \pm 0.02$ | $2.01^{b} \pm 0.02$ | $2.12^{d} \pm 0.03$ | $3.70^{a} \pm 0.03$ | $3.79^{c} \pm 0.01$ |
| Black-Vem-Devis | $4.40^{cd} \pm 0.01$ | $4.45^{bcd} \pm 0.01$ | $2.13^{a} \pm 0.00$ | $2.24^{c} \pm 0.02$ | $3.38^{b} \pm 0.04$ | $3.63^{d} \pm 0.02$ |
| Red Gold | $4.20^{e} \pm 0.01$ | $4.48^{ab} \pm 0.00$ | $1.75^{d} \pm 0.01$ | $2.25^{c} \pm 0.01$ | $0.25^{d} \pm 0.05$ | $4.02^{a} \pm 0.01$ |
| Golden Delicious | $4.48^{a} \pm 0.01$ | $4.50^{a} \pm 0.03$ | $1.90^{c} \pm 0.01$ | $2.29^{c} \pm 0.00$ | $0.26^{d} \pm 0.06$ | $3.83^{bc} \pm 0.03$ |
| Granny Smith | $4.44^{b} \pm 0.01$ | $4.46^{abc} \pm 0.01$ | $2.03^{b} \pm 0.01$ | $2.52^{b} \pm 0.03$ | $2.99^{c} \pm 0.05$ | $3.89^{b} \pm 0.05$ |
| Red Delicious | $4.37^{d} \pm 0.02$ | $4.41^{d} \pm 0.01$ | $0.85^{f} \pm 0.01$ | $2.55^{b} \pm 0.02$ | $3.09^{c} \pm 0.05$ | $4.01^{a} \pm 0.01$ |
| | | | | | | |
| Mean | 4.37 | 4.47 | 1.79 | 2.37 | 2.52 | 3.89 |
| Std Dev | 0.10 | 0.04 | 0.41 | 0.24 | 1.35 | 0.14 |
| Min | 4.16 | 4.40 | 0.83 | 2.09 | 0.17 | 3.60 |
| Max | 4.49 | 4.55 | 2.14 | 2.89 | 3.74 | 4.04 |
| F-values | $82.23^{***}$ | $5.42^{**}$ | $10.30^{***}$ | $138.28^{***}$ | $650.75^{***}$ | $27.01^{***}$ |

Values are mean ± S.E. of three replicates.
Values in each column followed by different letter are significantly different at p≤0.05 (Ducan's Multiple Range Test).
Level of significance; $^{***}$= p<0.001.

There were significant effects of cultivars on DPPH, ABTS and FRAP activities of ethanolic extracts of pulps, peels and pulps+peels. The higher antioxidant activities of peels extracts of all the tested apple cultivars might be due to their exposure to environmental stresses such as temperature or the fact that reductants/phenolic compounds are present in higher concentrations in the outer tissues i.e. epidermal and sub-epidermal tissues than those of inner mesocarp and pulp tissues of fruits (Abrosca et al. 2007; Vieira et al. 2009; Wolfe et al. 2003). The higher antioxidant activities of plant derived extracts is largly due to the ability of intrinsic phenolics to donate hydrogen atoms or electron to capture the free readicals (Stoilova and Krastanov 2008).

The correlation coefficients ($R^2$) calculated for each variety of tested apple cultivars between concentrations of pulp/peel extracts and their DPPH radical inhibition potential revealed a positive and significant relationships except red delicious and pulps of Gramny Smith and peels of Golden Delicious (Fig. 2). The $R^2$ values ranged between 0.4 – 0.95 for pulp vs DPPH and 0.4 – 0.94 for peels vs DPPH among the tested apple cultivars. The strongest correlations between concentration of extracts and their DPPH radical inhibition was observed for Red Gold and Rich-a-Rich apple cultivars, respectively for pulps and peels (Fig. 2).

## CONCLUSION

As a conclusion, the results obtained from the present study clearly showed that both pulp and peels of all the tested varieties of apple produced locally in Himachal Pradesh have higher amounts of total phenolics and flavonoids compounds. The results further showed that these varieties also possess antioxidant activities evaluated in terms of FRAP, DPPH and ABTS in-vitro assays. The

positive correlations between the extracts of pulp and peels of tested varieties of apples and their DPPH radical inhibition potential were also found. Thus on the basis of results, it can be strongly suggested that locally produced apple varieties could be an important source of natural antioxidants for local population. Study on altitudtional trends and individual chemical compound of apple fruits should be carried out to strengthen the present findlings.

## ACKNOWLEDGEMENTS

Dr. P. P. Dhyani, Director, G.B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora and MOEF & CC are gratefully acknowledged for providing the necessary research facilities and financial support, respectively to present work.

## REFERENCES


- Abrosca, B.D.; Pacifico, S.; Cefarelli, G.; Mastellone, C.; and Fiorentino, A. (2007). Limoncella apple, an Italian apple cultivar: phenolic and flavonoid contents and antioxidant activity. Food Chem., 104: 1333–1337.
- Anonymous (2007). Horticulture – Area under fruits/Production of fruits. Statistical Outline of H.P. Economics and Statistics Department, Shimla, H.P. :88-9.
- Benzine, I. F. F.; and Strain, J. J. (1996). Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem., 239:70-76.
- Boyer, J.; and Liu R.H. (2004). Apple phytochemicals and their health benefits. Nutr J., 3: 5.
- Chu. Y. F.; Sun, J.; Wu, X.; and Liu, R. H. (2002). Antioxidant and antiproliferative activities of common vegetables. J. Agric. Food Chem., 50: 6910-6916.
- Demirkol, O.; Adams, C.; and Ercal, N. (2004). Biologically important Thiols in various vegetables and fruits. J. Agric. Food Chem., 52, 8151-8154.
- Drogoudi, P.D.; Michailidis, Z.; and Pantelidis, G. (2008). Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivar. J. Sci Hortic., 115, 149-153.
- Gardner, P.T.; White, T.A.C.; McPhal, D.B.; and Duthie, G.C. (2000). The relative contribution of vitamin C, carotenoids and phenolics to antioxidants potential of fruit juices. Food Chem., 68: 471-474.
- Goulding, E.G.; McGlasson, J. B.; Wyllie S.; and Leach, D. (2001). Fate of apple phenolics during cold strage. J. Agric. Food Chem., 49: 2283-2289.
- Hudina, M.; and Atampar, F. (2000). Sugars and organic acids contents of European (*Pyrus communis* L.) and Asian (*Pyrus serotina* Rehd.) pear cultivar. Acta Aliment., 29: 217-230.
- Jose, A.; Yuri.; Amalia, N.; Alvaro, Q.; Yoshie, M.; and Ivan, P. (2009). Antioxidant activity and total phenolics concentration in apple peel and flesh is determined by cultivar and agroclimatic growing regions in Chile. J. Food, Agric. Environ., 7 (3 & 4): 513-517.
- Khanzadeh, S.; Tsao, D.; Rekika, R.; Yand, M.T.C.; and Rupasinghe, H.P. (2008). Polyphenol composition and total antioxidant capacity of selected apple genotype for processing. J. Food Comp. Anal., 21: 396-401.
- Knekt, P.; Kumpulainem, J.; Jarveinen, R.; Rissanen, H.; Heliovaara, M.; Reunanem, A.; Hakulinen, T.; and Aromaa, A. (2002). Flavonoid intake and risk of chronic disease. Am. J. Clin. Nutr., 76: 560-568.
- Kuti, J.O. (2004). Antioxidant compounds from four *Opuntia cactus* pear fruit varieties. Food Chem., 85: 527–533.
- Łata, B.; andTomala, K. (2007). Apple peel as a contributor to whole fruit quantity of potentially healthful bioactive compounds cultivar and year implication. J. Agric. Food Chem., 55: 10795-10802.
- Lattanizo, V. (2003). Bioactive polyphenols: the role in quality and storability of fruit and vegetables. J. Appl. Bot., 77: 128-146.
- Leong, L.P.; and Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. J. Food Chem., 76, 69-75.
- Leontowicz, M.; Gorinstein, S.; Leontowicz, H.; Krzeminski, R.; Lojek, A.; Katrich, E.; Ciz, M.; Martin-Belloso, O.; Soliva-Fortuny, R.; Haruenkit, R.; and et al. (2003). Apple and pear peel and pulp and their influence on plasma lipids and antioxidant potentials in rats fed cholesterol-containing diets. J. Agric. Food Chem., *51:* 5780–5785.
- Li, Y.; Guo, C.; Yang, J.; Wei, J.; Xu, J.; and Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem., *96:* 254–260.
- Lilia, A.; Hernandez, C.; Jose, A.; and Beltran, G. (2014). Total phenolics and antioxidant activity of Piper auritum and Porophyllum ruderale. Food Chem., 142: 455-460.
- Liu, M.; Li, X.Q.; Weber, C. Y.; Brown, J.; and Liu, R. H. (2002). Antioxidant and antiproliferative activities of raspberries. J. Agric. Food Chem., 50: 2926-2930.
- Liyana-Pathirama, C.M.; and Shahidi, F. (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH condition. J. Agric. Food Chem., 53: 2433-2440.
- Manzoor, M.; Anwar, F.; Saari, N.; and Ashraf, M. (2012). Variations of antioxidant characteristics and mineral contents in pulp and peels of different apple

(malus domestica Borkh.) cultivars from Pakistan. Molecules, 17: 390-407.

- McGhie, T. K.; Hunt, M.; and Barnett, L. E. (2005). Cultivar and growingregion determine the antioxidant polyphenolic concentration and composition of apples grown in New Zealand. J. Agric. Food Chem., 53: 3065-3070.
- Meyers, K. J.; Watkins, C. B.; Pritts, P. M.; and Liu, R.H. (2003). Antioxidant and antiproliferative activities of common strawberries. J. Agric. Food Chem., 51: 6887-6892.
- Ordon-Ez, A.A.L.; Gomez, J.D.; Vattuone, M.A.; and Isla, M.I. (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. Food Chem., 97: 452-458.
- Pan, Y.; He, C.; Wang, H.; Ji, X.; Wang, K.; and Liu, P. (2010). Antioxidant activity of microwave-assisted extract of *Buddleia officinalis* and its major active component. Food Chem., 121: 497–502.
- Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; and Rice, E.C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med., 26: 1231-1237.
- Scalzo, J.; Polithi, A.L.; Pellegrini, N.; Mezzetti, B.R.; and Battino, M. (2003). Plant genotype affect total antioxidant capacity and phenolic content in fruit. Nutr., 21: 207-213.
- Sharma, R.K.; Samant, S.S.; Sharma, P.; and Devi, S. (2012). Evaluation of antioxidant activities of *Withania somnifera* growing in natural habitats of north-west Himalaya. J. Med. Plant Res., 6 (5): 657-661.
- Sharma, R.K.; Sharma, N.; Samant, S.S.; Nandi, S.K.; and Palni, L.M.S. (2013). Antioxidant activities in methanolic extracts of *Olea ferruginea* Royle fruits. Int. J. Biosci. Biochem.Bioinform., 3(2): 154-156.
- Stoilova, I.; Krastanov, A.; and Bui, H. (2008). Biodegradation of mixed phenolic compounds by a microbial association of *Aspergillus awamori* and *Thermoascus aurantiacus*. Elec. J. Environ. Agric. Food Chem., 7: 2625–2633.
- Sun, J.; Chu.; Wu, X.; and Liu, R. H. (2002). Antioxidant and antiproliferaive activities of common fruits. J. Agric. Food Chem., 50: 7449-7454.
- Taso, R.; Yang, R.; Xie, S.H.; Sockovie, E.; and Khanizadeh, S.H. (2005). Which polyphenolic compounds contribute to the total antioxidant activities of apple. J. Agric. Food Chem., 53: 4989-4995.
- Treutter, D. (2001). Biosynthesis of phenolic compounds and its regulation in apple. Plant Growth Regul., 34: 71-89.
- Velioglu, Y. S.; Mazza, G.; Goal., and Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem., 46: 4113-4117.
- Vieria, F.G.K.; Borges, G.D.S.C.; Copetti, C.; Ambani, R.D.D.M.C.; Denardi, F.; and Fett, R. (2009). Physico-chemical and antioxidant properties of six apple cultivars (*Malus domestica* Borkh) grown in Southern Brazil. J. Sci. Horticult., 122: 421-425.
- Wolfe, K.; Wu, X.; and Liu, R.H. (2003). Antioxidant activity of apple peels. J. Agric. Food Chem., 51: 609-61.