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ANTIOXIDANT CAPACITY AND RELATED PHYTOCHEMICALS ANALYSIS OF METHANOLIC EXTRACT OF TWO WILD EDIBLE FRUITS FROM NORTH WESTERN INDIAN HIMALAYA

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ABSTRACT

Fruits of *Berberis asiatica* and *Pyracantha crenulata* are traditionally consumed by local people of north western Himalayan states of India for the treatment of oxidative stress induced ailments**.** Methanolic extract from mature fruits were evaluated for their total phenolics, related antioxidant compounds and activities. Result showed that *Berberis asiatica* possesses the higher level of total polyphenols, catechins and ascorbic acid then *Pyracantha crenulata* while *Pyracantha crenulata* possesses the higher level of β-carotene and lycopene then *Berberis asiatica*. Antioxidant and free radical scavenging activities at four different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) were evaluated. *Berberis asiatica* showed higher antioxidant potential as compare to the *Pyracantha crenulata.* Results of the finding provides evidence that the crude methanolic extract of the studied wild fruits are valuable source of natural antioxidant, which can be applied in both healthy medicine and food industry

KEY WORDS:Total phenolics, Antioxidant activities, Wild edible fruits, North-western Himalaya

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INTRODUCTION

Several studies have shown that high fruit intake may be associated with reduced disease like cancer and cardiovascular disease; one possible mechanism for the reduction of the said disease is antioxidant properties of the phytochemicals present in the Fruits[1,2]. The antioxidant or radical scavenging property is important because of its potential to provide health protection against reactive oxygen species and free radicals, which are the main cause of many diseases[3]. The search for new antioxidative metabolite with considerable antioxidative properties is a very active domain of research. Science long time fruits have been used as food and source of vitamins, minerals and are the very important source of various antioxidants. Various Fruits were also found to be medically active in several therapies, such as antitumour, antibacterial, antiviral, hematological and immunomodulating treatments. The antioxidative and free radical scavenging properties of the phenolic content of methanolic extracts have been reported for various wild fruits[3], suggesting possible protective roles of these compounds, due to their ability to scavenge free radicals, metals chelation and inhibition of lipoxygenase activity[4]. The north western part of Indian Himalaya, due to its climatic conditions, is one of the Indian regions with a high diversity of wild edible Fruits, these underexploited fruits generally have less economic importance than the most popular ones, but they may have the potential to contribute to food security, nutrition, health, income generation and environmental services. Many works have emphasized on the diversity and traditional uses of wild edible fruits from different part of country but there is no systematic record found for the antioxidative properties of major wild edible fruits from north western Indian Himalaya. Therefore in present investigation was design to evaluate antioxidant activity of two wild edible fruits viz, *Berberis asiatica*, *Pyracantha crenulata*.*Berberis asiatica* family (Berberidaceae) is locally known as Kilmora and occurring in the North Western Himalayan region. The fresh roots are used for curing diabetes and jaundice[5]. The stems are recommended in rheumatism. The berries fruits are reported to possess anti-cancer activity and mildly laxative and are given to children[6, 7]. *Pyracantha crenulata* family (Rosaceae) found in the North Western Himalayan region. In Uttarakhand region it is locally known as Ghingaru. The leaves are used to make herbal tea. The pome fruit is orange-red and rich in sugar. The ripe fruit is eaten fresh. Thus the results from this preliminary study will provide a better understanding of the antioxidant properties of these fruits.

MATERIALS AND METHODS

Chemicals Details

Sodium Carbonate (Na_2CO_3), Methanol, Hydrogen chloride (HCl), L-Ascorbic acid (Vitamin C), 2, 6-dichloroindophenol, Oxalic acid, Ferric chloride, Ferrous chloride, Ethylenediamine tetra acetic acid (EDTA), Trichloroacetic acid (TCA) and Folin-Ciocalteu's reagent were purchased from Merck Specialities Private Limited (Navi Mumbai, India). Tannic acid, Catechins, 1,1-diphenyl-2-picryl-hydrazl (DPPH), 2,2-azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2, 4, 6- tripyridyl-s-triazine (TPTZ), Ammonium persulfate (APS), Hydrogen peroxide (H_2O_2), ferrozine, L-3,4-dihydroxyphenylalanine (L-DOPA), Tyrosinase and other chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

Plant materials and extraction

Mature fruits of Kilmora (*Berberis asiatica*) and Ghingaru (*Pyracantha crenulata*), were collected by randomized sampling techniques from Uttarakhand regions of the north western Himalaya of India. After collection the plant samples were cut down into small pieces, air dried at 50^0C, powdered and stored at room

temperature in desiccator before analysis. Methanolic extract of fruits were taken to measure antioxidant metabolites and activities. Dried samples (5 g) were grinded in a super mill grinder 1500 series (Newport Scientific Pvt. Ltd.) and 1.5 gram grinded samples were extracted by semiautomatic soxlet apparatus (pelican, socsplus, 2AS, Chennai) in methanol at 100^0C at for 1 h and 90 % methanol was recovered during recovery phase at 130^0C for 30 min, The methanolic extract of each were then evaporated at 80^0C in to dryness, redissolved in methanol to a concentration of 10 mg/ml and stored at 4°C for further use. The all assays were carried out in triplicate and the results are expressed as mean values ± standard deviations.

Determination of total phenolic content (TPC)

Phenolic compounds content in the methanolic extracts was estimated by a colorimetric assay, based on procedures described by Singleton and Rossi[8] with some modifications. Briefly, 1 ml of extract (10 mg/ml) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Hitachi spectrophotometer). Gallic acid was used to calculate the standard curve (1-80 µg/ml). Estimation of the phenolic compounds was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg of gallic acid equivalents/g of extract (GAEs).

Determination of condensed tannins (CT)

Condensed tannins were estimated by the method of the Sun, Ricardo-da-Silva & Spranger[9] with some modification. A fine dried sample powder (100 mg) of each samples were dissolved in 20 ml of pre-boiled double distilled water, kept in an orbital shaker at 60^0C for 20 min and allowed to cool at ambient temperature and finally filtered through Whatman Grade No. 1 Filter Paper. To the freshly prepared aqueous extract (0.100 ml) was added 0.900 ml methanol, 2.500 ml 1% vanillin reagent and 2.500 ml 9 M HCl. The solution was mixed thoroughly with the vortex and absorbance at 500 nm was recorded after 20 minutes incubation at 30^oC. Condensed tannins content was calculated from the standard calibration curve based on catechins.

Determination of ascorbic acid (AA)

Ascorbic acid was determined according to the volumetric method[10]. Ten milliliter of 4% oxalic acid was added to standard solution of vitamin C (100µg/ml) and the resulting solution was titrated against 2, 6-dichloroindophenol dye until a pink colour end point was obtained and the titer value was noted as V_1. Again, dried methanolic extract of each samples (100 mg) were extracted with 4% oxalic acid and volume was made to 100 ml. The filtered extract (5 ml) was mixed with 10 ml of 4% oxalic acid and titrated against 2, 6-dichloroindophenol dye until a pink colour end point was obtained and the titer value was noted as V_2. Ascorbic acid content was calculated based on the following equation: Amount of ascorbic acid (mg/100 g extract) = $[(0.5 \text{ mg} \times V_2 \times 100 \text{ ml}) / (V_1 \times 15 \text{ ml} \times \text{Wt. of samples})] \times 100$, where V_1 is and V_2 were the volume of the dye used to titrate vitamin C and sample extract respectively. The result was expressed as mg ascorbic acid /g extract

Determination of β-carotene (β car) and lycopene (Ly)

β-carotene and lycopene were determined following the method of Nagata and Yamashita[11]. Dried methanolic extract of each sample (100 mg) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6) for 1 min., filtered with Whatman No. 1 filter paper and the absorbance was measured at 453, 505, 645 and 663 nm. Contents of β-carotene and lycopene were calculated by the equations: Lycopene (mg/100 ml) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$. β-Carotene (mg/100 ml) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$. The values were expressed as µg of β-carotene/g of extract and µg of carotenoid/g of extract.

Determination of scavenging effects on DPPH• radicals

The DPPH assay was done by measuring the decrease in absorbance of methanolic DPPH solution at 515 nm in the presence of the extract[12]. The stock solution was prepared by dissolving 24 mg of DPPH with 100 mL methanol and stored at -20^{0}C and the working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to get an absorbance of 1.17 ± 0.02 units at 515 nm. methanolic extracts (150 µL) of different concentrations (0.5, 1.0 1.5 and 2 mg/ml) were allowed to react with 2850 µL of DPPH working solution for 24 h in the dark after which the absorbance was read at 515 nm. BHT was used as reference and the radical scavenging activity was calculated as a percentage of DPPH• discolouration by the equation: DPPH• radical scavenging (%) = $[(A_{control} - A_{sample})/ (A_{control})] \times 100$, where A_{sample} is the absorbance of the solution recorded during addition of extract/reference at a particular level, and $A_{control}$ is the absorbance of the DPPH solution without addition of extract.

Determination of scavenging effect on ABTS•+ radicals

The ABTS assay was done by measuring the decrease in absorbance of methanolic ABTS solution at 745 nm in the presence of the extract[13]. The stock solutions 7.0 mM ABTS and 2.3 mM ammonium persulfate were prepared and the working solution was prepared by mixing two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 3 mL methanol to obtain an absorbance of 0.9 ± 0.02 units at 745 nm. Methanolic extracts (200 µL) of different concentrations (0.5, 1.0 1.5 and 2 mg/ml) were allowed to react with 2000 µL of the freshly prepared ABTS solution for 30 min in dark condition and absorbance was taken at 745 nm. BHT was used as reference and the percentage inhibition was calculated by the equation: ABTS•+ radical scavenging (%) = $[(A_{control} - A_{sample})/ (A_{control})] \times 100$, where A_{sample} is the absorbance of the solution recorded during addition of extract/reference at a particular level and $A_{control}$ is the absorbance of the ABTS solution without addition of extract.

Determination of Metal chelating activity

The chelating of ferrous ions by extracts was determined by the modified method of Dinis, Madeira & Almeida[14]. Briefly, the samples (2.0 ml) of different concentration (0.5, 1.0 1.5 and 2.0 mg/ml) was added to a solution of 2 mM $FeCl_2$ (0.20 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.80 ml) and the mixture was shaken vigorously and kept at room temperature for 10 min. and the absorbance of the resulting solution was then measured at 562 nm. BHT was used as reference and the percent metal chelation activities were calculated according to the following equation: Metal chelating effect (%) = $[(A_{control} - A_{sample})/ (A_{control})] \times 100$, where A_{sample} is the absorbance of the solution recorded during addition of extract/reference at a particular level and $A_{control}$ is the absorbance of the $FeCl_2$ and ferrozine solution without addition of extract.

Reducing power assay

The reducing power was determined according to the method of Huda fujan *et al*.[15]. Various concentrations (0.5, 1.0, 1.5, and 2.0 mg/ml) of methanolic extracts (200 micro litre) were taken and volume made upto 1 ml by adding distilled water, in these added 2.5 ml of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 ^{0}C for 20 min. Afterward 2.5 ml of 10% trichloroacetic acid (w/v) were added; the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml demonized water and 0.5 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm: higher absorbance indicates higher reducing power. The extract concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. Butylated hydroxytoluene (BHT) was used as standards.

Determination of total antioxidant activity

The total antioxidant activity of the methanolic extract of both the sample was measured by spectrophotometrically using a phosphomolybdenum method[16], based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of specific green phosphate / Mo (V) compounds. A 0.3 ml of sample extract of different concentrations (0.5, 1.0, 1.5, and 2.0 mg/ml) were combined with 2.7 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample was capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm. The total antioxidant activity was expressed as equivalents of trolox (µM /g of extract).

Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was done according to Benzie and Strain[17] with some modifications. The stock solutions 300mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution were prepared. The fresh working FRAP solution was prepared by mixing 25mL acetate buffer, 2.5mL TPTZ solution and 2.5mL $FeCl_3.6H_2O$ solution and warmed at 37^0C before use. Methanolic extracts (150 µL) of different concentration were allowed to react with 2850 µL of the FRAP solution for 30 min in the dark condition and the readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The FRAP value was determined by plotting a standard curve obtained by addition of ferrous sulfate heptahydrate (20-200 µM) to the FRAP reagent and the results were expressed in µM equivalent to $FeSO_4.7H_2O$ as FRAP value.

STATISTICAL ANALYSIS

The statistical analyses were performed using the statistical package SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL). Analyses of variance were performed by ANOVA and significance of each group was verified with one-way analysis of variance followed by Duncan's multiple range test ($P < 0.05$). The 50% inhibitory concentration (IC50) was calculated according to Concentration-Effect regression line.

RESULTS

Total phenolic, condensed tannins, β-carotene, lycopene and ascorbic acid

The classic antioxidants are vitamin C, E and β-carotene. Other antioxidants include phenolic compounds which have been identified as important antioxidants found in fruits. Some of these compounds have been shown to have even more antioxidant activity than vitamin C and E in vitro and significant bioavailability has been demonstrated by animal and human studies[18-20].The amount of total phenolic content (TPC), condensed tannins (CT), β-carotene and lycopene and ascorbic acid (AA) were significantly different among both the fruits (Table 1). The TPC was determined from the regression equation of the calibration curve obtained from tannic acid ($y = 0.0210x$, $r > 0.99$). The TPC found higher (30.47±1.520 mg/g extract) in *Berberis asiatica* as compare to *Pyracantha crenulata* (7.43±0.371 mg/g extract). The total CT content of methanol extract from the crops extract was assayed by vanillin-HCl colorimetric assay from the regression equation of calibration curve ($y=0.0022x+0.013536$; $r = 0.997$) and expressed in catechin equivalents. The result showed that *Berberis asiatica* possesses the higher level of CT (7.93±0.381 mg /g extract), as compare to *Pyracantha crenulata* (2.26±0.108 mg/g extract).

Table 1
Total Polyphenols, Condensed tannins, Ascorbic acid, β-Carotene and Lycopene contents of Underutilized Horticultural Crops

Crop Name	Total Polyphenols (mg Tannic acid equivalents/g of extract)	Condensed tannins (mg Catechins/g of extract)	Ascorbic acid (mg ascorbic acid/g of extract)	β-Carotene (µg of β-carotene/g of extract)	Lycopene (µg of carotenoid/g of extract)
P.Crenulata	7.43±0.371	2.26±0.108	18.68±0.891	5.08±0.253	16.86±0.841
B. asiatica	30.47±1.520	7.93±0.381	31.96± 1.524	4.53± 0.216	10.62±0.484

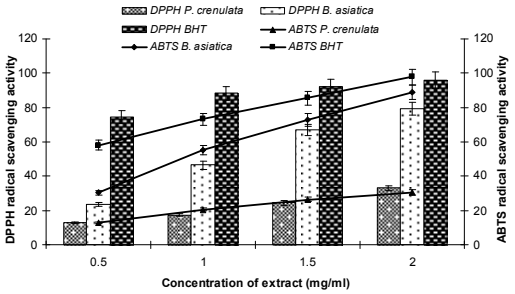
Values are expressed as means ± S.D. of triplicate measurements. Values with different letters indicate significant difference (P<0.05).

P. crenulata possessed the comparatively higher levels of β-carotene and lycopene (5.08±0.253 µg/g extract and 16.86±0.841 µg/g extract) then the *B. asiatica* (4.53± 0.216µg/g and 10.62±0.484 µg/g), respectively and got results are comparable to those of barberry (*Berberis vulgaris*) and *Pyracantha fortuneana* fruit extracts[21,22]. Ascorbic acid (ASA) content was recorded significantly higher in *B. asiatica* (31.96± 1.524 mg/g extract), while in the *P. crenulata* ASA was obtained 18.68±0.891mg/g extract.

Radical scavenging activity

The abilities for each sample extract at four different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) to scavenge DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals are shown in Figure1. The results showed that these extracts significantly inhibited the activities of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals in dose-dependent manner ($P<0.05$). The percent scavenging activities of methanolic extracts of *P. crenulata* and *B. asiatica* on DPPH$^{\bullet}$ radical ranged from 13.00 to 32.98 and 23.52 to 79.45, respectively. However, at BHT showed excellent scavenging activities ranged from 74.28 to 95.89 percent inhibition. ABTS$^{\bullet+}$ radicals scavenging effect of the methanolic extracts increased with increase in concentration in a dose-dependent manner and almost complete inhibition of ABTS$^{\bullet+}$ radicals (88.91%) was observed for 2.0 mg/ml of fruit extract of *B. asiatica.* The scavenging activities of methanolic extract of *P. crenulata* and *B. asiatica* on ABTS$^{\bullet+}$ radical ranged from 12.95 to 30.75 % and 30.38 to 88.90%, respectively. At similar concentration standard BHT showed 58.03, 73.32, 85.49 and 97.86 percentage inhibition. The results are comparable with as proposed by Saklani, *et al*.[23] in *Pyracantha crenulata* methanolic extract.

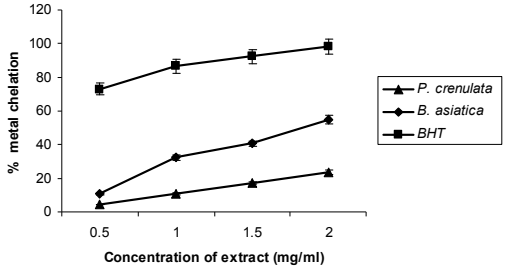
Graph 1
Radical scavenging activity as measured by DPPH and ABTS assay



[legend: DPPH- DPPH$^{\bullet}$ radical scavenging activity (0.5, 1.0, 1.5 and 2.0 mg/ml), ABTS - ABTS$^{\bullet+}$ radical scavenging activity (0.5, 1, 1.5 and 2 mg/ml)]. Values are the mean of triplicate determinations ± SD Metal chelating activity

The relative metal chelating activities of methanolic extract from both the fruits are depicted in the Figure 2. In the present study, the chelating activity at four different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) toward ferrous ions was investigated and found that chelating activity of the extracts increased with increase in concentration. BHT was used as reference standards on ferrous ions.

Graph 2
Metal chelating activity of the methanolic extracts, values are the mean of triplicate determinations ± SD

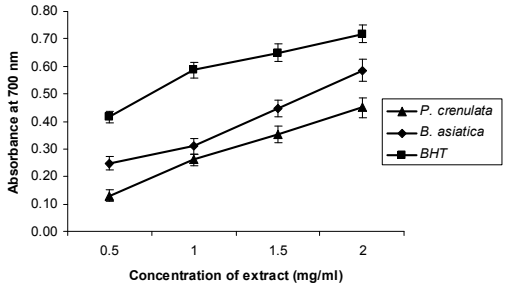


Metal chelating activities of methanolic extracts of P. crenulata and B. asiatica ranged from 4.35 to 23.80% and 10.87 to 54.89%, respectively. At similar concentrations standard BHT showed 72.93, 86.63, 92.28 and 98.26 percentage chelation. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion[19].

Reducing power assay

Reducing power serves as a significant indicator of potential as antioxidant. Four different concentrations of methanolic extract (0.5, 1.0, 1.5 and 2.0 mg/ml) were used to evaluate the reducing power of *P. crenulata* and *B. asiatica* fruit samples. Figure 3 shows that reducing power of the samples is in increasing order with the increase in concentration, more precisely 0.130, 0.268, 0.353, 0.453 for *P. crenulata* 0.255, 0.310, 0.445, 0.586 for *B. asiatica* respectively. At similar concentration standard BHT showed 0.416, 0.586, 0.650 and 0.718 reducing power. Similar observations of antioxidant properties in terms of reducing power activity have been reported for methanolic extract of *B. aristata* stem bark by Gupta *et al*[24].

Graph 3
Reducing power of the methanolic extracts



Values are the mean of triplicate determinations ± SD

The IC_{50} values were calculated and tabulated in Table 2 and it was used for the comparison of the free radical scavenging activities (FRSA) of different samples. The IC_{50} value is the concentration of the samples required to scavenge 50% of the free radicals present in the system. It was calculated using the Concentration-Scavenging activity curve ($r > 0.99$). Lower IC_{50} implies a higher scavenging activity, the IC_{50} of *Berberis asiatica* extracts was lower than those of *Pyracantha crenulata*, suggesting that radical scavenging by extracts from *Berberis asiatica* was more effective than *Pyracantha crenulata*. The BHT reference displayed excellent FRSA and its IC_{50} values were computed (Table 2).

Table 2
Comparison of the IC_{50} values for radical scavenging assays of Methanolic extracts of the wild fruits as well as Standard BHT

Crop Name	IC_{50} values of each free radical scavenging assay (mg/ml)			
	DPPH[a]	ABTS[b]	RPA[c]	MCA[d]
B. asiatica	1.18	1.05	1.67	1.79
P. crenulata	2.96	2.93	2.12	4.33
BHT	0.33	0.47	0.82	0.32

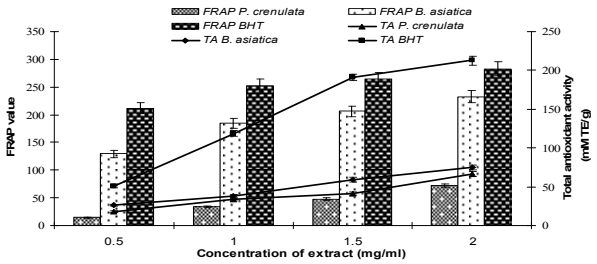
[a] *IC_{50} (mg/ml): effective concentration at which 50% of DPPH$^{\cdot}$ radicals are scavenged.*
[b] *IC_{50} (mg/ml): effective concentration at which 50% of $ABTS^{\cdot+}$ radicals are scavenged.*
[c] *IC_{50} (mg/ml): effective concentration at which 0.50 absorbance got (Reducing power)*
[d] *IC_{50} (mg/ml): effective concentration at which 50% of metal chelation observed*

In the report by Adnan *et al*[25] it was shown that synthetic polyphenols BHT exhibited a particular high radical scavenging activity compared with plant extracts.

Total antioxidant activity

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids[12]. The antioxidant activity of the methanolic extract of the samples and reference antioxidants BHT at four different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) measured by phosphomolybdenum method is presented in Figure 4. Total antioxidant activity expressed as µM Trolox equivalents and at four different concentrations the values were found to be 18.05, 33.52, 41.26, 66.68 for *P. crenulata* and 26.15, 37.94, 58.94, 74.78 for the *B. asiatica* respectively. At similar concentration standard BHT showed 51.03, 118.44, 191.01 and 212.64 µM Trolox equivalents antioxidant activity. The results are comparable with *Berberis vulgaris* antioxidant activity of absolute methanol extraction[26]. Antioxidant activity is highly correlated with total polyphenol content and with anthocyanin concentration Antonieta *et al*.[27].

Graph 4
FRAP value and Total antioxidant activity



[legend: FRAP- FRAP value (0.5, 1.0, 1.5 and 2.0 mg/ml), TA- Total antioxidant activity (0.5, 1, 1.5 and 2 mg/ml)].
Values are the mean of triplicate determinations ± SD Ferric reducing antioxidant power (FRAP)

FRAP assay is a colorimetric method based on the reduction of a ferrictripyridyltriazine (TPTZ) complex to its ferrous form. This reduction originates an intense blue complex with an absorption maximum at 593 nm[17]. The antioxidant capacity of all samples were expressed as FRAP value [FRAP (µM) equivalent to $FeSO_4.7H_20$] are shown in Figure 4. *B. asiatica* showed the higher FRAP value (129.42, 184.94, 206.26, and 232.37 µM) then by *P. crenulata* (14.71, 33.46, 47.43 and 72.06 µM) at all the four different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) respectively. At similar concentrations standard BHT showed 211.78, 252.59, 263.62 and 282.74 µM FRAP value. FRAP assay depends upon the ferric

tripyridyltriazine [Fe (III)-TPTZ] complex to the ferrous tripyridyltriazine [Fe (II)-TPTZ] by a reductant at low pH. FRAP method is sensitive in the measurement of total antioxidant power of the fresh biological fluids, such as plant homogenates[16].

DISCUSSION

The reactive oxygen species (ROS) and free radicals in the body are generated through exogenous (radiation, atmospheric pollutants, toxic chemicals, etc.) and endogenous (various cytokines) sources[28]. The free radicals, in excess are reported to result in oxidative stress and cause various degenerative diseases[29]. In this context, antioxidants play an important role in prevention the formation of ROS, radical scavenging and repairing the enzymes involved in the process of cellular development. Antioxidative metabolites like polyphenols and flavonoids of plant origin are important for maintenance of health as well as prevention from various degenerative diseases[18]. Considering the target species, study showed that total polyphenols, condensed tannins, ascorbic acid, β-carotene and lycopene contents of *Berberis asiatica* and *Pyracantha crenulata* fruits were considerably high. Overall *Berberis asiatica* contained higher polyphenols, condensed tannins and ascorbic acid then *Pyracantha crenulata*. The extracts showed different trends of antioxidant activity when determined using different methods. *Berberis asiatica* methanolic extracts showed potent antioxidant activity using the various free radical scavenging assay methods. Fruits are diverse in antioxidant composition and those with high antioxidant activity generally contain more antioxidants[30]. The phenolics and flavanoids (catechins) contents in relatively higher amount in *B. asiatica* fruits would justify its comparative advantage over *P. crenulata*. Polyphenols and flavanoids are act as primary antioxidants and are known to react with free redicals to protect DNA from oxidative damage, inhibitory against tumor cell[31]. Gallic acid and catechin are effective in preventing oxidative injuries in human epithelial cells under in vitro[32]. Significant scavenging and reducing capacity of the fruits extract was revealing in different methods.While considering relationship of phenolic content and antioxidant activity, the established scavenging and reducing capacity of *B. asiatica* fruits are indicative of their strength as an antioxidant. The remaining antioxidant activity may be attributed to other phytochemicals like vitamins, carotenoids, and so forth. The compounds present in the methanolic extract of the both the fruits are not only capable for scavenging of $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical but also to reduce the ferric ions. These results support the basic concept that antioxidants are reducing agents. Antioxidant compounds with a good reducing power functions as good electron and hydrogen-atom donors and therefore be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products[33].

CONCLUSION

Antioxidant activity of these wild fruits has not been done so far, the present study provides scientific basis of the use of these plant extracts in traditional health care system. *Berberis asiatica* and *Pyracantha crenulata* methanolic extracts showed potent antioxidant activity which can be applied in both healthy medicine and food industry. Detail work by using different methods for analysis of health promoting properties will be the aim of further investigation

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