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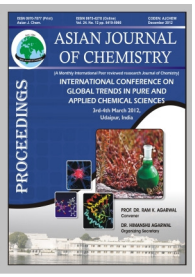

Health Promoting Phytochemicals Their Concentration and Antioxidant Activity of Wild Edible Fruits of Uttarakhand, India†

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Fragaria indica, Prunus armeniaca, Pyracantha crenulata and *Rubus ellipticus* belong family-Rosaceae, have great importance due to the presence of bioactive chemical constituents. By solvent extraction method fruit extracts were analyzed using HPLC and antioxidant activity was done by using DPPH, ABTS and FRAP method. Among all fruits, *Fragaria indica* had highest value of ascorbic acid (5.93 ± 0.40 mg /100g fw) and the same fruit species had lowest anthocyanin content (0.09 ± 0.02 mg/100 g fw) in the middle of all fruits. Total antioxidant activity, measured by the DPPH method ranged from 19.18 ± 0.14 to 29.22 ± 0.08 mM AAE/100 g fw, measured by the ABTS method, ranged from 0.47 ± 0.01 mM AAE/100 g to 4.41 ± 0.03 mM AAE/100 g fw and FRAP value was found within the range 0.09 ± 0.01-3.23 ± 0.03 mM AAE/100g fw.

Key Words: Wild Edible Fruits, Nutraceuticals, Antioxidants, Phytochemicals. Vitamin C.

INTRODUCTION

Phyto is a Greek word that means plant and phytochemicals are usually related to plant pigments. Fruits having bright colours-yellow, orange, red, green, blue and purple-generally contain the most phytochemicals and the most nutrients. These protective plant compounds are an emerging area of nutrition and health. Nutrients may help in slowing down the aging process and reduce the risk of many diseases, including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis and urinary tract infections[1-4]. Pronounced fight-o-chemicals, phytochemicals fight to protect the health. They can have complementary and overlapping mechanisms of action in the body, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of hormone metabolism and antibacterial and antiviral effect. Information about such foods is part of traditional knowledge, which is largely transmitted through participation of individuals of households. Further, such plants may serve as income source and may be marketed or traded locally, regionally, even internationally and the primary importance of edible wild species during periods of drought, social unrest or war is well documented. The most important nutrients present in plants are: carbohydrates, such as the starch and free sugars, oils, proteins, minerals, ascorbic acid and the antioxidant phenols, such as chlorogenic acid and its polymers[5,6]. Wild relatives of temperate fruits belonging to genera *viz.*, *Malus, Prunus, Pyrus, Vitis, Rubus, Fragaria* and others showed a wide range of diversity thereby possibility of utilizing large numbers of desirable genes/traits particularly the resistance to biotic and abiotic stresses which is generally lacking in their cultivated allies.

EXPERIMENTAL

Total phenolic content in the methanolic extract of fruit part of selected species were estimated calorimetrically using the Folin-Ciocalteu calorimetric method. Flavonoid content in the methanolic extract of plant was determined by aluminium chloride calorimetric method described by Chang *et al*. The absorbance of resulting reaction mixture was measured at 415 nm using UV-VIS spectrophotometer. Quantification of total flavonoid content was done on the basis of standard curve of quercetin prepared in 80 % (v/v) methanol. Results were expressed in mg quercetin equivalent to per gram of fresh weigh.

Quantification of phenolic compounds high performance liquid chromatography: Total anthocyanin contents of the hydrophilic extracts were measured by pH-differential method. $A = (A_{520\ nm}\text{-}A_{700\ nm})$ pH-1.0-$(A_{520\ nm}\text{-}A_{700\ nm})$ pH-4.5. The monomeric anthocyanin pigment concentration was calculated using following equation: Monomeric anthocyanin pigment (mg/L) $= (A \times MW \times DF \times 1000)/(\varepsilon \times l)$ where, MW (molecular weight)

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TABLE-1
HPLC CONDITIONS FOR QUANTIFICATION, SEPARATION AND IDENTIFICATION OF PHENOLIC COMPOUNDS, β-CAROTENE AND VITAMIN C

Parameters	Conditions		
	Phenolic compounds	β-carotene	Vitamin C
Mobile phases	Water: methanol: glacial acetic acid (80:20:1 ratio)	Acetonitrile: methanol :Tetra hydrofuran (45:50:5 ratio)	95 % water with 0.01 % formic acid: Acetonitrile
Flow rate	0.8 mL/min	1.0 mL/min	0.8 mL/min
Column	Puro spher^{R}, RP-C-8 column	Puro spher^{R}, RP-C-8 column	Puro spher^{R}, RP-C-18 column
Detection	254 nm	450 nm	254 nm

TABLE-2
TOTAL PHENOL, TOTAL FLAVONOID, TOTAL ANTHOCYANIN, VITAMIN C AND β-CAROTENE CONTENT

Plant species	Total phenol (mg GAE/g fw)	Total flavonoid (mg/g fw)	Total anthocyanin (mg/100 g fw	Vitamin C (mg/100 g fw)	β-carotene (mg/100 g fw)
Fragaria indica	05.91 ± 0.31	5.12 ± 0.02	0.09 ± 0.02	5.93 ± 0.40	ND
Prunus armeniaca	0.92 ± 0.02	4.94 ± 0.14	0.27 ± 0.01	3.59 ± 0.72	ND
P.crenulata	05.59 ± 0.05	5.46 ± 0.04	0.44 ± 0.05	3.30 ± 0.34	ND
Rubus ellipticus	03.95 ± 0.05	4.99 ± 0.15	0.58 ± 0.02	4.46 ± 0.53	1.81 ± 0.02

*Data taken in immature stage of fruits, ND-not detected

TABLE-3
PHENOLIC COMPOUNDS (mg/100 g fw) OF WILD FRUITS OF GARHWAL HIMALAYA

Plant species	Gallic acid	Catechin	Caffeic acid	Totalphenol
Fragaria indica	07.26 ± 0.77	08.27 ± 0.79	ND	15.53
Prunus armeniaca	06.59 ± 0.02	ND	ND	06.59
Pyracanthacrenulata	10.15 ± 1.40	ND	ND	10.15
Rubus ellipticus	40.45 ± 0.67	ND	40.55 ± 0.70	81.00

= 449.2 and ε (molar absorptivity) = 26,900 of cyanidin-3-glucoside, which was used as a standard; DF - the dilution factor; l - the path length. The total monomeric anthocyanins were reported on the basis mg/g fw. For the extraction and quantification of ascorbic acid was extracted according to the modified method of Abdulnabi *et al*..The compounds spectra were monitored/recorded at 254 nm. The β-carotene in the sample was extracted according to the method described by Tee *et al*. The compounds spectra were monitored/recorded at 450 nm. Standard solution of β-carotene with concentrations from 0.5-10 μg/mL was used to obtain a standard curve[7-12].

Assay of antioxidant activity: The ABTS [2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging ability of fruit extract was carried out from a modified method as described by Re *et al*. and Cai *et al*. Ferric reducing antioxidant potential assay was measured calorimetrically according to the method developed by Benzie and Strain[13] with some modifications. Readings were recorded on the UV-VIS spectrophotometer at 593 nm. A blank sample was prepared by ascorbic acid and results were expressed in mM ascorbic acid equivalent (AAE) per 100 g fw of plant material. Traditional DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was carried out following the Brand-William *et al*.[14] with minor modification[13-17].

RESULTS AND DISCUSSION

The therapeutic benefits of fruits are often attributed to their antioxidant properties. The extracts of wild edible fruits have strong antioxidant activity, which aroused an increasing interest in the formulation of nutraceuticals. Results are summarized in the Tables 1-3. Highest level of total phenolic content was found in *Fragaria indica* (5.91 ± 0.31 mg GAE/g fw) followed by *Pyracantha crenulata* (5.59 ± 0.05 mg GAE/g fw) and *Rubus ellipticus* (3.95 ± 0.05 mg GAE/g fw) while the lowest was found in *Prunus armeniaca* (0.92 ± 0.02 mg GAE/g fw) (Table-2). The order of the species based on total phenolic content is as follows: *Fragaria indica* > *Pyracantha crenulata* > *Rubus ellipticus* > *Prunus armeniaca.*

TABLE-4
ANTIOXIDANT ACTIVITIES (mM AAE/100 g fw) OF WILD FRUITS OF GARHWAL HIMALAYA

Plant species	ABTS assay	FRAP assay	DPPH assay
Fragaria indica	2.46 ± 0.08	3.23 ± 0.03	28.51 ± 0.30
Prunus armeniaca	0.47 ± 0.01	0.09 ± 0.01	28.02 ± 0.05
Pyracantha crenulata	4.41 ± 0.03	2.34 ± 0.01	19.18 ± 0.14
Rubus ellipticus	4.25 ± 0.12	3.10 ± 0.09	29.22 ± 0.08

The highest level of flavonoid was found in *Pyracantha crenulata* (5.46 ± 0.04 mg/g fw) and lowest in *Prunus armeniaca* (4.94 ± 0.14 mg/g fw). Based on *f*-test, these results were significantly varied ($P < 0.01$) among the species. Total anthocyanin content varied among the species. The values were ranged from 0.58 ± 0.02 to 0.09 ± 0.02 (mg/100g fw). The highest concentrations of anthocyanin was found in the fruits of *Rubus ellipticus* (0.58 ± 0.02 mg/100 g fw) followed by *Pyracantha crenulata* (0.44 ± 0.05 mg/100g fw) and *Prunus armeniaca* (0.27 ± 0.01 mg/100g fw) while lowest in *Fragaria indica* (0.09 ± 0.02 mg/100 g fw). Results showed a significant variation ($P < 0.01$) in total anthocyanin content among all the species. Significant ($p < 0.01$) levels were observed in vitamin C content among species. The highest ascorbic acid content was found in the fruits of *Fragaria indica* (5.93 ±

0.40 mg/100 g fw) followed by *Rubus ellipticus* (4.46 ± 0.53 mg/100 g fw) and *Prunus armeniaca* (3.59 ± 0.72 mg/100 g fw), while the lowest was found in *Pyracantha crenulata* (3.30 ± 0.34 mg/100 g fw). The β-carotene content was not detected in all selected fruits species except, *Rubus ellipticus* (1.81 ± 0.02 mg/100 g fw) showed the significant level of β-carotene. Only three phenolic compounds (gallic acid, catechin and caffic acid) were detected, when tested for HPLC analysis. A significant variation ($p < 0.01$) was observed in phenolic compounds among the species. Rubus ellipticus fruits (81.00 mg/100 g fw) showed the highest phenolic acids followed by *Fragaria indica* (15.53 mg/100 g fw) and *Pyracantha crenulata* (10.15 mg/100 g fw). Gallic acid was found maximum; however, caffeic acid and catechin were occurred in small quantities. The lowest amount of phenolic acids was found in *Prunus armeniaca* (6.59 mg/100 g). Total antioxidant activity, measured by the ABTS method, ranged from 0.47 ± 0.01 mM AAE/100 g to 4.41 ± 0.03 mM AAE/100 g fw. Pyracantha crenulata showed the highest antioxidant activity (4.41 ± 0.03 mM AAE/100 g fw) while *Prunus armeniaca* exhibited the lowest (0.47 ± 0.01 mM AAE/100 g fw). Results revealed significant variation ($p < 0.01$) in total antioxidant capacity measured by the Ferric reducing antioxidant potential method. The ferric reducing antioxidant potential value was found within the range 0.09 ± 0.01-3.23 ± 0.03 mM AAE/100 g fw. *Fragaria indica* (3.23 ± 0.03 mM AAE/100 g fw) exhibited the highest antioxidant activity while *Prunus armeniaca* exhibited lowest (0.09 ± 0.01 mM AAE/100 g fw). Similarly, total antioxidant activity, measured by the DPPH method ranged from 19.18 ± 0.14 to 29.22 ± 0.08 mM AAE/100 g fw. *Rubus ellipticus* exhibited the highest antioxidant activity (29.22 ± 0.08 mM AAE/100 g fw) while other species showed antioxidant activity between 19 and 30 mM AAE/100 g fw.

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