ANTIOXIDANT ACTIVITY AND NUTRACEUTICAL POTENTIAL OF SELECTED NEPALESE WILD EDIBLE FRUITS

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Abstract: Wild edible fruits play an important role in the nutrition of rural people especially in the hilly and mountainous region, where the wild fruits could be the only source to consume. Though wild edible fruits are widely utilized throughout the country, little works have been done in Nepal on their nutritional and phytochemical analysis. The main objective of this study was to evaluate the antioxidant activity and nutraceutical potential of the selected wild edible fruits. The fruit samples were extracted in appropriate solvents and all the analyses were done in triplicates using 96 well ELISA plate reader. Nutritionally, *Rubus acuminatus* was found to be rich in Vitamin C (0.78 ± 0.01 mg/g) over other fruits. Protein content was found to be high in *Berberis napaulensis* (2.26 ± 0.71 %) and *R. ellipticus* showed greater lipid (0.15 ± 0.01 %) and β-carotene content (1.08 ± 0.01 mg/100mg). *R. acuminatus* was found to have high flavonoid content (9.26 ± 0.40 mg QE/g) and exhibited higher antioxidant activity while *B. angulosa* (29.67 ± 2.28 mg GAE/g) had the highest phenolic content.

Keywords: Wild edible fruits; Radical scavenging activity; Phenolic content; Flavonoid content.

INTRODUCTION

Fruits, including the wild edibles are good source of carbohydrates, fibers, minerals, vitamins, essential dietary micronutrients, and more recently they have been recognized as important sources of phytochemicals that individually, or in combination, may benefit human health [1,2]. The nutritional value of fruits depend on their composition, which shows wide range of variation, depending on the species, cultivar, maturity and even cultivation conditions[3]. Interestingly, wild edible fruits (WEFs) are found to be nutritionally superior over the cultivated ones[4]. Several studies have shown that the fruits generally possess high levels of sugars and organic acids determining their sweetness and sourness[5,6]. Berries, which contain high amounts of bioactive phytochemicals, particularly polyphenolic antioxidants, are the focus of many studies as they are considered as valuable molecules which may reduce the risk of non-communicable diseases, such as cardiovascular disorders, cancers and others[7,8,9].

Antioxidants derived from plants including wild edible fruits can enhance the endogenous antioxidant system and reduce oxidative stress and the risk of chronic diseases[10,11,12]. The addition of WEFs with high antioxidant capacity can increase the nutritional value of the food/nutraceuticals and improve their organoleptic properties[13]. They have huge potential as sources of natural antioxidants, food supplements, nutrients and health promoting phytochemicals[11,13,14,15]. Antioxidant activity and nutritional composition of wild edible fruits have been reported from the various parts of the world. However, such information on Nepalese wild edible fruits is scarce [16,17,18]. We herein reported antioxidant activity and nutritional composition of selected wild edible fruits from Parbat district and Gaurishankar Conservation Area.

MATERIALS AND METHODS

Plant material

Rubus acuminatus was from Parbat district while rests of the sample were collected from Gaurishankar Conservation Area, which is situated in northern part of Central Nepal. Fruits commonly used by local people and that also have income generating opportunities were selected. Samples were collected for nutritional and phytochemical analysis. Fruits which were taken in this research work were: *Berberis angulosa, Berberis asiatica, Berberis thomsoniana, Berberis napaulensis, Eriobotrya dubia, Myrica esculenta, Rubus acuminatus* and *Rubus ellipticus.*

Collection and identification

Bunches of mature fruits were collected during their fruiting period during March-September. Selected samples were photographed in their natural habitat and GPS locations were recorded. Herbariums were prepared and were deposited at NAST and identified with the help of standard literature and herbarium specimens deposited at TUCH and KATH.

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Table 1. List of wild edible fruit samples with their common names and GPS locations

Scientific Name	Common Name	Altitude (m)	Latitude	Longitude
Berberis angulosa Wall. ex Hook. f. & Thoms.	Chutro	3747	N27°45.460'	E086°02.031'
Berberis asiatica Roxb. ex DC.	Chutro	2037	N27°52.567'	E086°14.114'
Berberis thomsoniana C.K. Schneid.	Chutro	3747	N27°45.460'	E086°02.031'
Berberis napaulensis (DC) Laferr	Jamane mandro	2506	N27°53.194'	E086°15.327'
Eriobotrya dubia (Lindl.) Decne.	Jure kafal	2216	N27°53.212'	E086°14.969'
Rubus acuminatus Sm.	Fusre aiselu	2579	N27°21.191'	E086°37.600'
Rubus ellipticus Sm.	Aiselu	2100	N27°52.147'	E086°14.500'
Myrica esculenta Buch.-Ham. ex D. Don	Kaphal	2200	N27°53.212'	E086°14.969'

Preparation of samples

Healthy fruits were carefully plucked from the bunch and air dried for about 20 days to remove moisture content. Then after, oven dried for a day at 37 °C. Seeds were separated from fruit pulps using forceps and needles. Then the samples were fine powered with the help of grinder and stored in dry, air-tight plastic containers with proper labeling.

Preparation of fruit extract

One gram of finely powered fruit pulp was poured into conical flask and 20 mL of methanol was added to it. Well labeled flasks were kept into shaking incubator (Innovative life science tool) at 37 °C for 24 hours with gentle shaking (100 rpm). After 24 hours, the samples were filtered through Whatman No. 1 filter paper (GE Healthcare UK Limited). The residue was subjected to extraction with additional 20 mL of methanol in the same manner and then filtered. Both the filtrates were mixed and the final volume was made up to 40 mL adding methanol in falcon tubes. All the methanolic extracts were stored in refrigerator at 4 °C until future use.

Similarly for the protein content analysis, 0.2 gram of sample was weighed and poured in conical flask and 20 mL of double distilled water was added in it. Then the conical flasks were kept into shaking incubator at 50 °C for 24 hours with 100 rpm. Filtration was done and final volume was made up to 20 mL.

Antioxidant activity

Antioxidant activity of methanolic extracts of all samples was assessed on the basis of radical scavenging effect of the stable 1, 1 –diphenyl -2 picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) free radical activity following the method of Blois[19] modified by Singh et.al.[20]. Ascorbic acid was used as standard. Plant extracts of various concentrations and the standard was prepared on clean and dry ependorf tubes. An aliquot of the sample (100 µL) was introduced into 96 well ELISA plate followed by 100 µL DPPH solution (0.2 mM). Control was prepared as above but without fruit extract and methanol was used as blank. Sample was introduced in triplicate form for the reproducible result. Well plate was incubated in dark for half an hour and absorbance was taken in an ELISA reader (Epoch 2 plate reader, Biotech) at 517nm.

Radical scavenging activity was calculated using the formula:

Radical scavenging activity (RSA) % = (Abs. control - Abs. sample/Abs. control)*100

Total phenolic content

Total phenolic content of all methanol extract samples was determined by using the Folin-Ciocalteau phenol reagent with slight modifications [21]. In brief, extract sample was diluted 10 times and an aliquot of the diluted samples (30 µL) was introduced into 96 well ELISA plate followed by 150 µL Folin-Ciocalteu reagent (Merck Specialities Private Limited) and 120 µL 0.7 M Na_2CO_3 (LOBA Chemie.). Positive control was prepared by replacing methanol in place of sample. Now the well plate was incubated in dark for half an hour and absorbance was measured at 765 nm against that of methanol (blank) using ELISA reader. Gallic acid was used as standard for the reference. Different concentration series of gallic acid (Fisher scientific) was made and used as reference standard to prepare calibration curve. Based on the calibration curve obtained from gallic acid, concentration of phenolic content of the plant sample was calculated as milligrams of the gallic acid equivalent per gram of the dry mass (mg GAE/g).

Total flavonoid content

Total flavonoid content was estimated using the aluminium chloride method[22] with slight modifications. First of all extract samples were diluted to 2.5 mg/mL and an aliquot (100 µL) of the diluted samples were introduced into 96 well ELISA plate followed by 100 µL 0.1M $AlCl_3$ (SD fine- Chem Ltd, Mumbai). Positive control was prepared by replacing the sample with methanol while methanol was taken as blank. The well plate was then incubated in dark for an hour and absorbance was measured at 510 nm using ELISA reader. Same experiment was also done with various concentration series of quercetin (Sigma-Aldrich). Based on the calibration curve obtained from series of quercetin concentration, total flavonoid content of the plant sample was calculated and expressed in terms of the milligrams

of the quercetin equivalent per gram of the dry mass (mg QE/g).

Total protein content

Total protein content of the aqueous extract of samples was determined following Fanglian, He with slight modifications[23]. Freshly prepared Bradford reagent was used to quantify the protein content. 100 µL sample was thoroughly mixed with one mL Bradford reagent in a two ml polypropylene tubes and then vortexes. Then 200 µL of the mixture was introduced into 96 well plate and incubated for 2- 10 minutes and absorbance was taken at 595 nm against blank in ELISA reader. Standard stock of bovine serum albumin (HIMEDIA Laboratories Private Ltd., Mumbai) mg/mL) was prepared and calibration curve was plotted with concentration ranging from 10 µg /mL - 1000 µg /mL.

Lipid content

Lipid content was determined by using semi-continuous method[24]. For this, one gram of the sample was extracted with 200 mL petroleum ether (HIMEDIA Laboratories Private Ltd., Mumbai) on a Soxhlet apparatus (S.M Scientific Instruments Pvt. ltd.) for 10 hours. After 10 hour, petroleum ether was removed by evaporation with the help of Rota-evaporator and the residue of the lipid was weighed. Residue was subtracted from the weight of sample taken which gave the content of lipid and the results were expressed in percentage.

Estimation of carotenoids

To determine the content of β- carotene and lycopene, the previous method was followed with slight modifications[25]. In brief, 0.1 gram samples were taken and shaken with 10 mL of acetone- hexane mixture in the ratio of 4:6 for one minute and filtered through Whatman No. 1 filter paper. Now, the samples were introduced into 96 well ELISA plate and absorbance was measured at 453, 505, 645 and 663 nm. Content of β-carotene and lycopene was calculated according to the following equations:

Lycopene (mg/100 mL) = $-0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$ and,

β- Carotene (mg/100ml) = $0.216A_{663} - 0.304A_{505} + 0.452A_{453}$.

Vitamin C content

Vitamin-C content was determined following previous method with slight modifications[26]. Firstly, the samples were extracted with 1% metaphosphoric acid (HIMEDIA Laboratories Private Ltd., Mumbai) for 10 hour at room temperature and the solutions was then filtered through Whatman No. 1 filter paper. To quantify the vitamin C content, an aliquot of the samples (20 µl) were introduced into 96 well ELISA plate followed by 180 µL 2, 6-dichloroindophenol (HIMEDIA Laboratories Private Ltd., Mumbai) and immediately, the absorbance was measured at 515 nm using ELISA reader against blank. Authentic L- ascorbic (CDH Laboratories reagents) acid was used as standard. Concentration series was prepared and calibration curve was plotted. Based on the calibration curve, content of vitamin C was calculated and result was expressed as milligrams per gram of the dry mass.

Statistical analysis

All the measurements were taken in triplicates and values were reported as mean ± SD. Statistical significance of differences in mean values of different parameters were determined by using one way analysis of variance (ANOVA). All the analyses were done using Microsoft Excel 2013.

RESULTS

Antioxidant activity

DPPH radical scavenging activity was used to quantify the free radical scavenging activity. In the present study, samples exhibited concentration dependent radical scavenging activity (Table 2). Among all samples, *R. ellipticus* showed the strongest activity (83.55±3.39 %) at lowest concentration tested while *B. napaulensis* was found to have weak scavenging activity. However, at 10 mg/g concentration all the samples showed similar scavenging activity except *B. napaulensis*. As the concentration increases, scavenging activity of samples gradually increases. Ascorbic acid which was used as a standard in this study exhibited 95% radical scavenging activity at 1mg/mL concentration.

Table 2. Antioxidant activity of samples and standard by DPPH assay

Plant name	DPPH radical scavenging activity (%) at different concentration				
	1 mg/g	1.25 mg/g	2.5 mg/g	5 mg/g	10 mg/g
B. angulosa	53.46±1.84	61.82±4.42	93.16±0.69	93.40±0.14	92.15±0.12
B. asiatica	76.24±3.66	88.43±0.24	94.61±0.41	94.76±0.44	94.02±0.23
B. thomsoniana	66.71±2.35	79.95±1.24	94.96±0.20	94.45±0.53	93.86±0.18
E. dubia	42.40±2.67	57.17±1.76	89.14±0.60	94.96±0.12	94.69±0.30
B. napaulensis	8.95±0.1	44.81±0.00	13.91±1.35	35.68±0.27	68.27±0.36
M. esculenta	23.52±0.27	27.39±1.26	42.48±4.75	73.54±0.54	94.49±0.42
R. acuminatus	82.53±1.75	92.50±0.65	94.45±0.44	93.01±0.18	89.68±0.12
R. ellipticus	83.55±3.39	90.70±0.75	95.35±0.18	93.35±0.36	94.61±0.51

DPPH Radical scavenging activity (%) of ascorbic acid						
	10(µg/mL)	20(µg/mL)	40(µg/mL)	60(µg/mL)	80(µg/mL)	100 (µg/mL)
%RSA	59.05±0.90	64.48±0.23	73.23±0.89	79.45±0.60	89.14±3.99	95.66±2.88

Total phenolic and flavonoid content

Total phenolic content of samples varied from 10.19 ± 0.69 to 29.67 ± 2.28 mg GAE/g dry weight of sample. The highest phenolic content was found in *B. angulosa* while the lowest was found in *B. napaulensis*. TPC of other fruit samples lies in between these two values (Table 3). Similarly, variation was obtained in total flavonoid content of selected samples. The highest amount of flavonoid content was found to be 9.26 ± 0.40 mg QE/g dry weight of sample for *R. acuminatus*, while the lowest was found to be 1.54 ± 0.03 mg QE/g dry weight of sample for *E. dubia* (Table 3). Total phenolic and flavonoid significantly varied among the fruit samples ($P < 0.001$).

Table 3. Total phenolic and flavonoid content of wild edible fruits

Sample	TPC(mg GAE/g)	TFC(mg QE/g)
B. angulosa	29.67 ± 2.28	7.55 ± 0.56
B. asiatica	23.59 ± 0.54	6.44 ± 0.79
B. thomsoniana	23.31 ± 1.59	6.17 ± 0.45
E. dubia	17.11 ± 1.35	1.54 ± 0.03
B. napaulensis	10.19 ± 0.69	2.11 ± 0.12
M. esculenta	13.42 ± 0.71	4.56 ± 0.17
R. acuminatus	25.24 ± 2.41	9.26 ± 0.40
R. ellipticus	19.72 ± 0.43	4.68 ± 0.66

Nutritional analysis

Protein, vitamin-C, lipid, β carotene and lycopene content were quantified for nutritional analysis (Table 4). Protein content of fruits varied from 0.79 ± 0.14 (*B. thomsoniana*) to 2.26 ± 0.71 percent (*B. napaulensis*) while vitamin C content varied from 0.19 ± 0.01 (*R. ellipticus*) to 0.78 ± 0.01 (*R. acuminatus*) mg AA/g dry weight of the sample. β- carotene, a precursor of vitamin A was detected in very negligible amount in almost all fruit samples. β-carotene content ranged from 0.04 ± 0.02 (*R. acuminatus*) to 0.11 ± 0.01 (*R. ellipticus*). Similarly, the values obtained for lipid content was also found in very negligible amount.

Table 4. Protein, Vitamin C, Lipid, β -carotene and Lycopene content of wild edible fruits

Plant name	Protein content (%)	Vitamin C (mg/g)	Lipid (%)	β-Carotene (mg/g)	Lycopene (mg/g)
B. angulosa	1.01 ± 0.41	0.58 ± 0.01	0.025 ± 0.01	0.05 ± 0.02	0.03 ± 0.01
B. asiatica	1.43 ± 0.58	0.31 ± 0.01	0.035 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
B. thomsoniana	0.79 ± 0.14	0.39 ± 0.01	0.020 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
E. dubia	1.28 ± 0.29	0.27 ± 0.01	0.030 ± 0.01	0.07 ± 0.01	0.04 ± 0.01
B. napaulensis	2.26 ± 0.71	0.22 ± 0.01	0.035 ± 0.01	0.06 ± 0.03	0.02 ± 0.01
M. esculenta	0.90 ± 0.67	0.21 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
R. acuminatus	1.34 ± 0.79	0.78 ± 0.01	0.013 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
R. ellipticus	1.40 ± 0.84	0.19 ± 0.01	0.15 ± 0.01	0.11 ± 0.01	0.03 ± 0.01

DISCUSSION

Antioxidant activity including phenol and flavonoid content and neutraceutical potential of selected eight wild edible fruits were evaluated and considerable amount of variation was found. Among all tested fruit samples, stronger DPPH radical scavenging activity was found in *R. ellipticus* over the other fruits selected. High radical scavenging activity was shown by wild edible fruits (*Berberis*, *Myrica*, *Rubus*) from West Himalayan Regions of India[27,28,29,30]. The comparable results for different berries from other species conclude that berries are better source of antioxidants. Total phenolic content vary among the fruits while some researchers reported comparable

results[31] and in fruits of *B. angulosa* and *B. asiatica*, higher phenolic content was reported.

Anthocyanins are reported as common flavonoids in fruits and account for various coloration like blue, red, violet, etc. in berries[32]. Total flavonoid content obtained from the previous study of *M. esculenta* and various species of *Rubus* are comparable to the present study[33]. Studies done for some fruits are comparatively higher than the value obtained in the present study[18,27]. It has been reported that flavonoid content depends upon biological and environmental factors[34]. The findings of the present study are also consistent with that of wild fruits of Indian Himalayan Region[35].

Vitamin C content of *B. asiatica* in the present study was found similar to previous report[36]. Dramatic variations were found in berry fruits, with levels of ascorbic acid ranging from 0.14 to 1.03 mg/g among cultivars of raspberry, blackberry, red currant, gooseberry and cornelian cherry. The reported range of total ascorbic acid in present investigation also lies in the range reported previously[37]. Ascorbic acid is now known as an antioxidant and its deficiency may cause Scurvy[38]. Comparable amount of ascorbic acid in these fruits can be promoted as a source of natural ascorbic acid which helps to overcome many health problems.

Protein content of some fruit samples analyzed in the present study is comparable and higher than the previous studies[18,36,37,38]. β carotene, precursor of vitamin A is highly known for vision, reproduction, proper immune functions, including the deactivation of reactive oxygen species[39]. However, some papers had reported similar β carotene content for *R. ellipticus*[40]. It has been known that the level of carotenoids and lycopene in different fruits vary with cultivar, environmental conditions including solar radiations, growing seasons and maturity stages[41,42].

Present study has focused only on eight wild edible fruits and deals mainly with quantification of nutritional and phytochemical attributes. The data obtained from this study indicates that selected wild fruits are rich in nutritive and phytochemical constituents. Additionally, higher amount of phenolics and the stronger antioxidant activity shown by selected plants can be useful in combating free radical damage.

CONCLUSION

The wild edible fruits evaluated in this study showed a range of antioxidant activity and nutritional composition. Methanolic extracts of *R. acuminatus* and *R. ellipticus* showed stronger antioxidant ability among the tested samples. Fruits are comparatively rich in protein and vitamin C, however, contained low amount of lipid and negligible amount of carotenoids and lycopene. The present study only focused on the analysis of macronutrients. Hence, further study should be carried out on micronutrients and characterization of bioactive compounds present in the fruit samples.

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