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A comparative study of barberry fruits in terms of its nutritive and medicinal contents from CKNP region, Gilgit-Baltistan, Pakistan

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Abstract

Wild berries have remained as an important part of human nutrition. They are rich in essential nutrients, (sugars, fibers, amino acids, vitamins, and minerals), health promoting phytochemicals (phenolics compounds, flavonoids, carotenoids and tannins) and minerals. Berberry spices are a valuable medicinal shrub grown in Asia and Europe. Berberry have a distinct position due to many traditional pharmacological uses. The physio-chemical and phytochemical analyses of berberry (*B. Calliobotrys B. orthobotrys B. psedumbellata*) fruit were carried out to determine its nutritional and phytochemical constituents. The different parameters which were observed during the studies were, pH (3.91, 3.52 and 3.33), TSS, (20.22, 18.18 and 15.56 ^{0} Brix) titrateable acidity (2.26, 2.18 and 1.36% citric acid), ash (0.79, 1.05 1.13%) , moisture (80.47, 74.96 and 80.13%), total sugars (14.98, 12.44 and 12.99 %) reducing sugars (9.00, 7.23and 7.68) non-reducing sugars 6.10, 5.45 and 5.95%) crude protein (1.51.0.96 and 1.10 %), fat (0.69, 0.67 and 0.83%), fiber (0.73, 0.78 and 0.94%) ascorbic acid (10.70, 14.92 and 13.59%), antioxidant activity (76.01, 71 and 80.65 %), total carotenoids (370.1, 345.80 and 381.69 µg/100g), total flavonid (385.52, 376.93 and 395.09), total phenolics (689.82, 675.68 and 702.94) and total anthocyanins (80.78, 77.52 and 83.55) mg/100ml fresh fruits of berberry respectively.

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Introduction

Berberidaceae family includes about 650 plants, is a wild shrub, having 2.5-3 meters height and contain radish brown to dark brown fruits. It is a temperate spiny shrub widely distributed throughout Asia and Europe (Akbulut *et al.,* 2007). It is found in Himalayan range of Jammu and Kashmir and Humachal Perdaish (Sood *et al.,* 2010), and also in the Karakoram, and Hindukush regions of Indo-Pak sub-continent. In Gilgit-Baltistan it grows in wide range of elevations from the plane low lands (4000ft to 8000ft from mean sea level) high alpine areas. The common species found in Gilgit are *Berberis Pseudumbellata, B. Orthobotrys, B. Barndisiana, and B. Calliobotrys* (Khan and Khatoon, 2007).

Recent concepts of nutraceutical or medical foods have attracted the attention of many researchers towards the unexploited natural sources. Literature reveals that berberry is a good juncture of nutritional and medicinal constituents. Studies have been conducted on nutritionally and medicinally important shrubs around the globe. Barberry has also been investigated owing to its significant health promoting roles. It contains higher amounts of antioxidant components and low sugar, protein, fat and fiber (Sood *et al.,* 2010; Hanachi, 2009; Akbulut *et al.,* 2007), and phyto-chemicals of medicinal significance (Gulfraz *et al.,* 2007). The members of family Berberidaceae has extensive uses in traditional medicine for curing of different ailments especially internal wounds, bone healing and hepatitis (Gulfraz *et al.,* 2008). It is also used as a tonic for heart diseases to prevent cardiovascular disease and it prevents chronic bleeding by purifying blood and reduces blood pressure (Ardestani *et al.,* 2013). It has positive response to neurological disorders, gall bladder treatment, gout, kidney stone, asthma, colon cancer and prostate inflammation (Fatehi *et al.,* 2005).

The berberry fruit and its bioactive compounds are used in medical and food industry (Fallahi *et al.*, 2010). In Persian foods sauces, jellies, marmalades, carbonated drinks, chocolates and fruit nectars are made from berberry fruit, while natural food colorants are also manufactured due to its attractive color (Ardestani *et al.,* 2013). Berberry fruit contains color pigments (Giusti and Wrolstad, 2001) that contain health promoting constituents such as phenolic compound, flavonoids, carotenoids and anthocyanins. These phytochemicals contribute total anti-inflammatory, anti-mutagenic (Shamsasa *et al.,* 2010), antioxidant activity, prevents hepatitis, inhibits certain enzymes and protect food from damage in stomach from free radicals which lead to chronic diseases (Motalleb *et al.*, 2005; Svarcova *et al.*, 2007). *B. Lycium* reducing blood cholesterol (Sood *et al.,* 2009), serum cholesterol in broiler chicken and functioning as growth promoter (Chand *et al.,* 2007).

In the indigenous uses only roots are used as herbal medicine in Gilgit-Baltistan. Most of the investigations have been carried out on *B. Lycium* species in Iran, India and Turkey. Furthermore, climatic conditions, geography and varietal differences significantly affect the compositional characters. It is of great interest to investigate the chemical composition of plant parts, so that economical uses of the plant could be possible. No study has yet been conducted on the fruit of this region regarding its compositional characters. Therefore it is necessary to carry out a systematic study to explore the nutraceutical potential of this fruit.

Material and methods

Sample collection

Barberries fruit were collected from CKNP region (District Gilgit and Hunza-Nagar) and transported immediately to the Karakorum International University and the study was carried out in the Laboratory of department of Agriculture and Food Technology.

The following nutritional and phyto-chemical composition were analyzed during study.

Proximate composition

The Total soluble solids were measured by a digital hand rafrectometer (Atago, Japan). Crude ash, Crude fiber, Crude fat and Moisture content was estimated according to AOAC (1990). Reducing, non-reducing and total sugars were measured by Lane and Eynon method AOAC (1990). Crude protein was measured by micro Kjeldhal method (920.10) and converted into protein using conversion factor (N x 6.25)

Chemical and phytochemical composition

Titratable acidity was determined by the titration method of AOAC (1990). Ascorbic acid content was determined by direct colorimetric method. Total phenolics were measured by using the Folin-Ciocalteu assay as described by Sponas and Wrolstad, 1990. Total carotenoids were estimated by using the procedure reported by Rodriguez-Amaya, 1999. Flavonoid content was determined by calorimetrically through aluminum chloride method (Benherlal and Arumughan 2007). Total anthocyanins content was determined by the method reported by Giusti and Wrolstad (2001). Total anthocyanin amount was calculated as cyanidin-3-glucoside, by means of the pH differential method using 2 buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). Anthocyanin pigments change hue and intensity with pH. At pH 1.0, anthocyanins exist in the colored oxonium or flavylium form, where-as at pH 4.5 they are predominantly in the colorless carbinol form. An aliquot of the fruit extract anthocyanin solution was adjusted to pH 1.0 and another aliquot to pH 4.5. The difference in absorbance is proportional to the anthocyanin content. A first 1ml aliquot of the fruit extract was transferred to a 100ml volumetric flask, diluted to volume with pH 1.0 buffer, and mixed. A second 1ml aliquot of the fruit extract was placed in a 100ml volumetric flask, diluted to volume with pH 4.5 buffer, and mixed. The solutions were allowed to stand at room temperature for 30 minutes. Absorbance was measured using a spectrophotometer at 510 and 700 nm, using A = (A510 – A700) pH1.0 – (A510 – A700) pH4.5 with molar extinction coefficient of cyanidin-3-glucoside of 26900 and a molecular weight of 449.2. Results were expressed as mg of cyanidin-3-glucoside per 100 g of fresh weight.

Antioxidant activity was measured by using a modified version of DPPH free radical (2, 2-diphenyl-l-picrylhydrazyl) scavenging activity as described by Brand-Williams *et al.,* (1995).

Preparation of aqueous and methanol extracts

Fruit extracts were made as 1, 2.5 and 3.5% in methanol and distilled water and studied against bacterial species *E. coli*, *Pseudomonas* spp. and *Bacillus cereus*.

Data analysis

The triplicate data was analyzed by one-way analysis of variance (ANOVA). Significance of difference will be estimated at 5% level (Steel *et al.,* 1996).

Result and discussion

The ripe berberry fruits were harvested during, 2013 from Nagar, Bagrote and Heramosh valleys, of CKNP region and transported to the laboratory of Agriculture and Food Technology, Karakorum International University Gilgit.

1. B. Calliobotrys

2. B. *orthobotrys*

3.B. *psedumbellata*

There Berberies species from CKNP region

These fruits were washed with distilled water and sorted immature and damaged fruits. The following physiochemical attributes were investigated during study of Berberies fruit are presented in table 1.

Table 1. Proximate analysis of Berberies fruit from CKNP region.

Properties	*B. calliobotrys*	*B. orthobotrys*	*B. psedumbellata*
TSS (^{0} Brix)	20.22a	18.18b	15.56c
Moisture (%)	80.47a	74.96b	80.13a
Ash (%)	0.79c	1.05b	1.13a
Total sugars (%)	14.98a	12.44c	12.99b
None reducing sugars (%)	6.10a	5.45b	5.95a
R. sugars (%)	9.00a	7.23c	7.68b
Crude protein (% N @ 6.25)	1.51a	0.96b	1.10b
Crude fat (%)	0.69b	0.67b	0.83a
Crude fiber (%)	0.73b	0.78b	0.94a

Table 2. Physicochemical analysis of Berberies fruit from CKNP region.

Properties	*B. calliobotrys*	*B. orthobotrys*	*B. psedumbellata*
pH	3.91a	3.52b	3.33c
Titrateabl acidity (% citric acid)	2.26a	2.18b	1.36c
Ascorbic acid (% mg/100ml juice)	10.70c	14.92a	13.59b
Antioxidant activity (%)	76.01ab	71.15b	80.65a
Total Carotenoids (µg/100g)	370.15b	345.80c	381.69a
Total Flavonid (mg/100g)	385.52b	376.93c	395.09a
Total Phenolics (mg/100g)	689.82b	675.68c	702.94a
Total Anthocyanins (mg/100g)	80.78b	77.52c	83.55a

Table 3. Antibacterial efficacy of Berberies fruits aqueous and methanol (Zone of inhibition in mm) extracts against bacterial species.

Concentration	*E.coli*		*Pseudomonas spp*		*Bacillus cereus*	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
1 %	15.0c	18.33c	15.33b	19.00c	13.00b	17.33b
2.5 %	19.66b	22.00b	17.33b	23.33b	15.00b	22.00a
3.5 %	23.0a	25.66a	24.00a	26.00a	22.00a	24.00a

Proximate composition

The proximate properties of berberry fruits are shown in table 1. TSS, moisture, ash, total, reducing and non-reducing sugars, crude fat, crude protein and fiber content ware determined.

After harvest TSS was measured by digital rafrectometer. The Total Soluble Solids in *B. Calliobotrys B. orthobotrys B. psedumbellata* fruit were (20.22 , 18.18 and 15.56^0 Brix) is shown in table .1. The TSS in berberry fruit has been reported by Sood *et al.,* is (18.28) TSS ^{0} Brix of *B.* vulgaris from Palampur Himachal Pradesh and 17.33 ^{0} Brix from Iranian *B.* vulgaris by Ardestani *et al.,* 2013.

The moisture content of the berberry fruit were (80.47, 74.96 and 80.13%), this result agrees the findings of Ardestani *et al.,* (2013) who estimated moisture content (75.01%) in *B. vulgaris* from Iran and Sood *et al.* (2010) has estimated (83.29%) from Palampur Himachal Pradesh India. A little bit differences were reported 71. 42% by Akbulut *et al.* (2007) in berberry fruit from Turkey.

The ash content of a sample is the inorganic residue, which is left after the combustion of organic matter (Egan *et al.,* 1981). The important factor which affects the ash evaluation is the combustion temperature, particle size and organic materials also affects the ash content. Higher temperature leads to decomposition of organic matters, temperature low than 600^0 C is

suitable for smooth combustion of the sample (Babayemi *et al.,* 2010). During analysis ash content of the three varieties were (0.79, 1.05 1.13%) while 0.73, 0.82 and 1.12% were presented by Ardestani *et al.,* (2013); Sood *et al.,* (2010) and Akbulut *et al.,* 2007, respectively. These changes may be due to different species, agro-climatic changes and environmental conditions of the fruit sample (Zolfighari *et al.,* 2010).

Sugars (total, reducing and non-reducing) were estimated total sugars (14.98, 12.44 and 12.99 %) reducing sugars (9.00, 7.23 and 7.68) non-reducing sugars 6.10, 5.45 and 5.95 %) during study respectively. Sugars in this range 15.45, 9.61 and 5.53 have been reported by Sood *et al.*, (2010). While Ardestani *et al.* (2013) have reported 13.85% total and 8.84% reducing sugars in *B. Integerrima* and 9.48% total and 6.66 reducing sugars in *B. vulgaris* from Iran. These results agrees the findings of Akbulut *et al.* (2007) who reported 6.52% reducing sugars in berberry fruit from Turkey.

Proteins are building blocks of a body made from amino acids and provide energy (Shahnawaz *et al.,* 2009). On the other hand fats are the main source of energy provides twice amount of energy as compare to proteins and carbohydrate and provides essential fatty acids in the body. The true triglycerides omega 3 and 6 are essential for neurological development. Fruit commodity is the poor source of protein and fat content it possess 0.2-1.3% protein (Egan *et al.,* 1981). Crude protein (1.51, .0.96 and 1.10 %), fat (0.69, 0.67 and 0.83%), fiber (0.73, 0.78 and 0.94%) were analyzed during studies respectively. *B. vulgaris* and *B. Integerrima* contains fat fiber and protein in the ratio of 0.61, 2.62 and 0.12% and 2.96, 12.10 and 0.50% respectively (Ardestani *et al.,* 2013). According to Sood *et al.,* (2010) *B. vulgaris* contains (0.63%) crude fat and (0.81%) and crude protein. Akbulut *et al.,* (2007) reported B. *vulgaris* contains crude protein (10.32), crude oil (0.84) and (9.42%) crude cellulose. Dietary fiber has beneficial effects for human body it reduces constipation, blood cholesterol and sugar level (Goreinstein *et al.,* 2001).

Physiochemical attributes

During physico-chemical and nutraceutical analysis vitmin C, pH, acidity, total anthocyanins carotenoids, phenolic favonids, carotenoides and antioxidant activity of berberry fruits were analyzed. The values of pH and acidity of the Berberies fruit were (3.91, 3.52 and 3.33), and (2.26, 2.18 and 1.36%) at the stage of harvesting. It is a natural phenomenon that delayed in harvesting leads to more sweetness, increases total soluble solids (TSS) and pH values while declines titratable acidity. There are negative correlation between pH and acidity of a food commodity. (Fallahi *et al.,* 2010). This finding agrees the findings of Ardestani *et al.* (2013), who reported 3.06 pH and 2.62% acidity in terms of malic acid. While Akbulut *et al.* (2007) reported 3.35 pH valyes and 3.10% titrateable acidity in B *vulgaris* fruit from Turkey. A little bit difference in range of pH 3.08 and 1.32% in terms of citric acid has been estimated from this fruit (Sood *et al.,* 2010). These differences in values may be due to the changes in geographical and environmental conditions.

During ripening in fruits, the total antioxidant activity increases due to lipophilic antioxidant activity (Cano *et al.,* 2003). There is a positive correlation between antioxidant activity and phenolic content (Wang and Lin, 2000). The Total antioxidant activities of the fruit were (76.01, 71.15 and 80.65 %). The higher amount of antioxidant activity may be due to presence of ascorbic acid (10.70, 14.92 and 13.59% mg/100ml in terms of citric acid), phenolics (689.82, 675.68 and 702.94mg/100ml), flavonoids (385.52, 376.93 and 395.09), /100ml), anthocyanins (80.78, 77.52 and 83.55 mg/100ml) and carotenoids (370.1, 345.80 and 381.69 µg/100g µg/100ml).

Previous studies have been reported that *B. vulgaris* contains 10.83% mg/100gm of ascorbic acid, (343µg/100g) B-carotene and (82.470mg/100ml) total anthocyanins (Sood *et al.,* 2010). According to

Akbulut *et al.* (2007) *B. vulgaris* contain ascorbic acid (256.48g/kg), total phenolics (789mg/100g) and (931.01mg/kg) total anthocaynins content. *B. vulgaris* contain 27.99g/100g total phenolics and total anthocyanin 69.06mg/100g in ethanolic extract (Ardestani *et al.,* 2013).

The main antioxidants in fruit and vegetables are carotenoids, ascorbic acid, and phenolics compounds (Giovanelli *et al.,* 1999), phenolics compounds including flavonoids are good source of antioxidant (Shahidi and Naczk, 1995).

Anthocynins are color pigments and responsible for dark brown to red color of fruits and vegetables. Color and anthocyanins correlate during ripening (Andrew, 1994). It contains hydroxyl group in 3 carbon ring, having chelating affects act as a good antioxidant (Giusti and Worlasted 2001). Ripening is characterized by the conversion of green color in to red color and anthocyanins have the direct relationship with antioxidant potential in the fruit (Wang *et al.,* 1996).

Inoculation of test Bacterial

100µl bacterial suspensions were aseptically introduced and spread using pre-sterilized cotton swabs on surface of MHA plates. The antimicrobial activity was determined by Agar well diffusion techniques as described by Adeniyi *et al.,* (1996). Wells of 6mm diameter with sterile cork borer were aseptically punched in the 90mm MHA agar plates. With the help of sterile micropipette tips aqueous and ethanol extract (100µl) were poured into the wells. The plates were incubated at 32 ± 2°C for 24 hours. After incubation, zone of inhibition was calculated with the help of Digital Vernier Caliper (Mitutoyo). The results of zone of inhibition of aqueous and menthol fruit extract of Berberies was present in Table 1. The result showed that both extract having antimicrobial efficacy against tested organisms. With the increase of extract concentration decreased the zone of inhibition. The mean value of zone of inhibition of aqueous and methanol extract against bacterial strain were recorded as *E.coli* (19.22 and 21.99mm), *Pseudomonas* spp (18.88 and 22.77mm) and *B.cereus* (16.66 and 21.11mm). Highest antimicrobial activity was found in methanolic extract compare to aqueous (Fig 2). The remote region of Gilgit-Baltistan possesses rich medicinal herbal resources, but has not been evaluated scientifically. Different medicinal plants or its parts are generally used to treat different ailments of animals as well as human being. In the present study fruit extract of Berberis were used against human pathogenic bacteria. Result indicated that fruit extract of Berberis has high potential against bacterial strains.

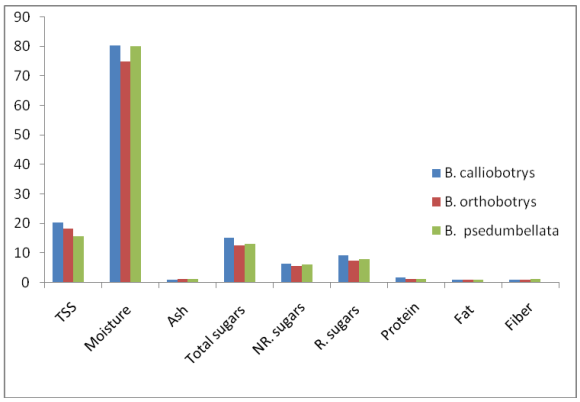

Fig. 1. Proximate analysis of berberry fruit (*Berberies vulgaris* L.).

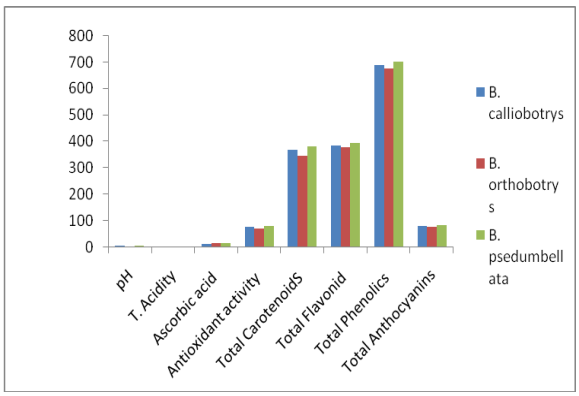

Fig. 2. Physicochemical analysis of berberry fruit (*Berberies vulgaris* L.).

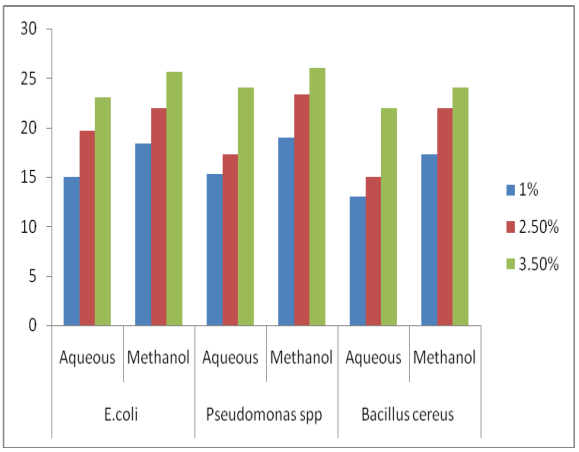

Fig. 3. Antibacterial efficacy of Berberies fruits aqueous and methanol (Zone of inhibition in mm) extracts against bacterial species.

Conclusion

The chemical and compositional properties revealed that berberry fruit is rich in physico-chemical and nutraceutical composition, such as ascorbic acid, carotenoid, flavonoids, anthocyanin and phenolic compounds, total, reducing and non-reducing sugar, protein, fiber, fat and ash. In addition, further studies are required to determine the phytochemicals and nutraceuticals in the root of this fruit. The roots of this fruit are being used in the folk medicine.

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