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Analysis of minerals and vitamins in sea buckthorn (*Hippophae rhamnoids*) pulp collected from Ghizer and Skardu districts of Gilgit-Baltistan

Maqsood Hussain[1], Shamsher Ali[1*], Saeed Awan[2], Manzoor Hussain[3], Ishtiaq Hussain[3]

[1]*Integrated Mountain Area Research Center, Karakorum International University, Gilgit-Baltistan, Pakistan*

[2]*Department of Food Science and Technology, Karakorum International University, Gilgit-Baltistan, Pakistan*

[3]*Department of Chemistry, Karakorum International University, Gilgit-Baltistan, Pakistan*

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Abstract

Sea buckthorn (*Hippophae rhamnoides* ssp. *turkestanica*) is a very important medicinal plant wildly grown in Gilgit-Baltistan of Pakistan. Recently this plant gained more attraction of scientists and researcher due to its nutriental, medicinal and economical significance. The present study was conducted to analyze the minerals (Cu, Cr, Ca, Fe and Zn), vitamin C and Phytosterol content in sea buckthorn (*Hippophae rhamnoids*) pulp collected from different localities of Ghizer and Skardu Districts of Gilgit-Baltistan. The mineral analysis revealed that the mean values of were 0.099 and 0.675ppm, 138.88 and 84.38 ppm, 33.97 and 57.20 ppm respectively in yellow and red varieties. The concentrations of Zn, ca, appreciably higher in red variety than yellow variety while the concentrations of Fe, Cu, and Cr were higher in yellow variety than red. Statistical analysis showed that the mineral contents were significantly influenced ($p<0.05$) by variation in varieties and localities. The interaction between varieties and localities was also found significantly different. Results showed that sea buckthorn pulp containing vitamin C and Phytosterol in yellow and red verities were 371.05 and 283.70 mg/100g, 3.60g/100g and 4.11g/100g respectively. Results showed that vitamin C content in yellow variety is higher than red variety whereas the phytosterol contents were slightly higher in red variety as compare to yellow variety. Statistical analysis showed that vitamin C and phytosterol content were significantly influenced ($p<0.05$) by variation in varieties and localities. The interaction between varieties and localities was also found significantly different.

* **Corresponding Author:** Shamsher Ali ✉ shamsher_pcsir@yahoo.com;

Introduction

Sea buckthorn is a general term given to the shrub or small tree of genus *Hippophae*. This genus belongs to the family *Elaeagnaceae* that consists of six species and ten sub species, among which the most economically important one is *Hippophae rhamnoides* L., commonly know as sea buckthorn. Because it is mainly this species that has been used for economic and biological purpose. The term sea buckthorn has been associated with *Hippophea rhamnoides* (Rongsen, 1992). The only subspecies *H. rhamnoides* ssp.*turkestanica*, found in the Gilgit-Baltistan of Pakistan and also grown in the Central Asia and West Asia, which includes Afghanistan, Tajikistan, Turkmenistan, Uzbekistan, Kirghisistan, the Xinjiangprovince of China, and Northern India. This subspecies that can withstand the harsh biophysical conditions, characterized by arid, hot summers and cold winters (Rongsen, 1992). Sea buckthorn is popular due to its high nutritional contents in the berries. The fruit contains 60-80% juice rich in sugar, organic acids, amino acids and vitamins. Vitamins C contents is 200 to 1 500 mg 100 g^{-1} which is five to 100 times higher than any other fruit or vegetable known (Ahmad and Kamal, 2002). The carotene content ranges from 30-40 mg $100g^{-1}$ of berries (Bernath and Foldsi, 1992). Sea buckthorn berries contain up to 13% soluble sugars, mainly glucose, fructose, xylose and 3.9% organic acids, mainly malic and succinic acid (Ma and Cui, 1989). There are at least 24 chemical elements present in Sea buckthorn juice, e.g. nitrogen, phosphorus, iron, manganese, boron, calcium. Aluminum, silicon and others (Zhang *et al.,* 1989, Tong *et al.,* 1989). The oil content ranges from 1.5-3.5% in fruit pulp and 9.9-19.5% in seeds (Rongsen, 1992). The oil contains significant amounts of β-carotene and Vitamin E, making the oil of Sea buckthorn and effective medicine for many diseases (Ahamd and Kamal, 2002). Yang *et al.* (2001) described that Phytosterols are the major constituents of the unsaponifiable fraction of sea buckthorn oils. The major phytosterols in sea buckthorn oil is sitosterol (β-sitosterol), with 5-avensterol second in quantitative importance. Other phytosterols are present in relatively minor quantities. The total quantity of phytosterols is quite high in sea buckthorn and may exceed soybean oil by 4-20 times. It was reported that the total sterol content, varied between subspecies and collection sites, in the seeds, fresh pulp/peel, and the whole berries were 1200-1800, 240-400, and 340-520 mg/kg, respectively.

Due to lake of awareness regarding the importances of this precious plant. The people of the study area are still used this plant as fencing and fuel. This may lead drastic reduction in future. In the light of above fact the present studied was undertaken to identify the minerals, vitamin C, and phytosterol content in the berries of sea buckthorn wildly grown in Ghizer and Skardu districts of Gilgit Baltistan. The outcome of this work will hopefully bring awareness regarding nutritional, medicinal and economical importance of the berries of this fruit.

Materials and methods

Fully mature and healthy sea buckthorn (sub.species *Turkistanica)* berries of yellow and red varieties were collected from six different localities (Hussain Abad, Rundo, Siksa, Yaseen, Hamochal and Ponial) of District Skardu and Ghizer of Northern Areas of Pakistan. Berries were thoroughly washed and crushed with blender for the analysis of minerals, vitamin C in pulp and phytosterol from pulp oil.

Analysis of Minerals

Mineral content of sea buckthorn pulp samples were determined by atomic absorption spectroscopy, according to the method of A.O.A.C (2000).

Digestion of Samples

For the digestion of samples, exactly 1gm of sample was taken in digestion tubes 10ml of HNO_3 (Nitric acid)was added to the samples and the mixture was kept for over night .4ml of $HClO_4$ (Perchloric acid) was added to that mixture and was kept in fumes block for digestion. The temperature was increased gradually starting from 50˚C and increased up to 250˚C-300˚C. The digestion was completed in about 70-85 minutes as indicated by the appearance of

white fumes the mixture was left to cool down and then was made into100 ml solution by adding distilled water in a 100 ml volumetric flask.

Determination of minerals

The diluted samples were introduced to Atomic Absorption Spectrophotometer (Model parkin Elmer 2380) for the determination of minerals i.e. Zn, Ca Cu, Cr, Fe,

Analysis Ascorbic acid (Vitamin C)

Vitamin C was determined by the titrametric method as reported in (AOAC1984). 5 ml of was taken from each sample in 100 ml volumetric flask and volume was made up to the mark by adding 0 .4% meta phosphoric acid solution.5 ml of this diluted sample was titrated against standardized dye until light pink color which was end point. Three replications were taken for each determination.

Analysis of phytosterol in pulp oil

Phytosterol estimation was carried out by Liberman-Burchard method (Sabir *et al.* 2003). 1 gm of oil was taken. Chloroform was used as solvent to make the volume 10 ml. samples were stirred to dissolve completely and diluted to 10 times. 3 ml of diluted samples solutions were taken and their absorbance was determined on spectrophotometer after adding the reagent, containing 0.5ml sulphuric acid in 10ml of acetic anhydrate. Liberman-Burchard reagent reacts with the sterol to produce a characteristics green color. Whose absorbance is determined on Spectronic 20-D (Milton Roy Company) spectrophotometer at 640 nm.10mg of standard cholestrol was dissolved in 10ml chloroform. Choles-5-en-3-β-ol was used as standard cholestol whose minimum purity was 95% the result was drawn by plotting the calibration curve using he different concentrations of sterol against absorbance.

Statistical Analysis

All the data regarding different parameters were statistically analyzed by CRD and means were separated by applying LSD test as recommended by Steel and Torrie, 1980.

Results and discussion

When the yellow and red varieties of sea buckthorn grown in six different localities were compared on the basis of minerals i.e. Zn, Ca Cu, Cr, Fe, vitamin C and phytosterol a wide range of variation was observed among the varieties and localities.

Table 1. Zn content in sea buckthorn pulp (ppm).

Locations	Varieties		Mean
	Yellow	Red	
Hussain Abad	0.029 c ±0.04	0.054 c ±0.05	0.041 c ± 0.17
Rundo	0.051 c ± 0.03	1.127 a ±0.11	0.589 a ± 0.76
Siksa	0.038 c ± 0.06	0.625 b ± 0.46	0.332 b ± 0.41
Yaseen	0.346 bc ± 0.10	1.007 a ± 0.10	0.676 a ± 0.46
Hamochal	0.068 c ± 0.03	1.153 a ± 0.06	0.611 a ± 0.76
Ponial	0.063 c ± 0.04	0.083 c ± 0.01	0.073 c ± 0.01
Mean	0.099 b ± 0.24	0.675 a ± 0.50	

Means sharing same small letters are not significantly different at ($p<0.05$).

Data represents in table is mean of three replications. ± Standard deviation.

The mean values of Zn content in pulp of these samples ranged from 0.029 to1.153 ppm (Table 1). The highest concentration (1.153ppm/) of Zn was found in red variety of Hamochal location of Ghizer

district, where as the lowest concentration (0.029ppm) was observed in Hussain abad location of skardu location. The mean values of Zn of these localities, Hussain Abad, Rundo, Siksa, Yaseen, Hamochal and Ponial were in the range of 0.041, 0.589, 0.332, 0.676, 0.611, 0.073ppm respectively. While the mean values of yellow and red varieties were 0.099 and 0.675ppm respectively. The findings of this study are in conformity with Zhang *et al.*, (1989) they had reported that Zn content in Chinese origin were 0.431-1.25 ppm.

Table 2. Calcium content in sea buckthorn pulp (ppm).

Locations	Varieties		Mean
	Yellow	Red	
Hussain Abad	26.28 hi ± 4.79	49.03 d ± 4.24	37.66 c ± 16.08
Rundo	35.65 fg ±3.91	58.95 b ± 2.43	47.30 b ± 16.47
Siksa	21.47 i ±2.08	51.54 cd ± 2.78	36.50 c ± 21.26
Yaseen	42.21ef ±3.30	67.50 a ± 3.50	54.85 a ± 17.88
Hamochal	48.25 de ± 5.68	59.82 b ± 5.76	54.03 a ± 8.18
Ponial	29.96 gh ± 2.36	56.37 bc ± 3.42	43.17 b ± 18.67
Mean	33.97 b ± 10.86	57.20 a ± 6.56	

Means sharing same small letters are not significantly different at ($p<0.05$)

Data represents in table is mean of three replications. ± Standard deviation.

Table 3. Chromium content in sea buckthorn pulp (ppm).

Locations	Varieties		Mean
	Yellow	Red	
Hussain Abad	0.568 f ± 0.02	0.826 b ± 0.04	0.698 ± 0.18
Rundo	0.466 g ± 0.01	0.722cd ±0.02	0.594 ± 0.18
Siksa	0.973 a ± 0.03	0.587 ef ±0.01	0.780 ± 0.27
Yaseen	0.881 ab ± 0.06	0.638def ±0.02	0.759 ± 0.17
Hamochal	0.693 d ± 0.09	0.671 de ±0.05	0.682 ± 0.02
Ponial	0.877 b ± 0.02	0.794 bc ± 0.03	0.836 ± 0.05
Mean	0.7430 a ± 0.19	0.707 b ± 0.09	

Means sharing same small letters are not significantly different at ($p<0.05$).

Data represents in table is mean of three replications. ± Standard deviation.

The Ca content in pulp of these samples ranged from 21.47 to 67.50 ppm (Table 2). The highest concentration of Ca was found in red variety of Yaseen location of Ghizer district, where as the lowest concentration of Ca was found in Siksa red variety of skardu location. These results are nearly similar with Tong *et al.*, (1989) they reported that Ca content ranged from 64 to 256mg/L in sea buckthorn of Chinese origin which is slightly higher than our studies.

Similarly (Table 3) the mean values of Cr in pulp of these samples ranged from 0.466 to 0.973ppm. The highest content (0.973ppm) of Cr was found in yellow variety of Siksa location of Skardu district. On the other hand the lowest content (0.466 ppm) of Cr was observed in red variety of Rundo locality district skardu. These results are in agreement with Tong *et al.*, (1989) they found that Cr content ranged from 0.47 to 1.00 ppm in Chinese origin.

Table 4. Copper content in sea buckthorn pulp (ppm).

Locations	Varieties		Mean
	Yellow	Red	
Hussain Abad	0.386 a ± 0.04	0.286 b ± 0.01	0.336 a ± 0.07
Rundo	0.292 b ± 0.01	0.220 c ± 0.02	0.256 b ± 0.05
Siksa	0.292 b ± 0.08	0.208 cd ±0.01	0.250 b ± 0.04
Yaseen	0.189 de ±0.01	0.136 fgh ± 0.09	0.163 c ± 0.03
Hamochal	0.155 fg ± 0.07	0.127 gh ± 0.05	0.141 d ± 0.01
Ponial	0.164 ef ± 0.08	0.112 h ± 0.01	0.138 d ± 0.03
Mean	0.246 a ± 0.09	0.182 b ± 0.60	

Means sharing same small letters are not significantly different at ($p<0.05$).
Data represents in table is mean of three replications. ± Standard deviation.

Cu content range from (0.112 to 0.386ppm) was observed in seabuck thorn pulp (Table 4) . The highest concentration (0.386ppm) of Cu was found in yellow variety of Hussain Abad location of Skardu district, while the lowest concentration (0.112) of Cu was found in red variety of Punial location district Ghizer. Similar results i.e 0.158 to 0.653 ppm Cu was found by Zhang *et al.*, (1989) in Chinese origin.

Table 5. Iron content in sea buckthorn pulp (ppm).

Locations	Varieties		Mean
	Yellow	Red	
Hussain Abad	157.01ab ±15.84	64.00 g ±8.15	110.50 a ± 65.70
Rundo	125.73 cd ± 7.03	84.89 fg ± 10.01	105.31b ± 28.87
Siksa	150.77 b ± 6.85	106.18 de ± 14.21	128.47 a ± 31.50
Yaseen	136.65 bc ± 13.38	72.62fg ± 6.38	104.63 b ±42.27
Hamochal	82.46 fg ± 5.48	92.83 ef ± 10.28	87.65 c ± 7.33
Ponial	175.89 a ±8.63	85.76 ef ±8.70	130.82 a ± 63.73
Mean	138.88 a ± 32.24	84.38 b ±14.84	

Means sharing same small letters are not significantly different at ($p<0.05$).
Data represents in table is mean of three replications. ± Standard deviation.

Table-5 shows that the mean values of Fe in pulp of these samples ranged from 64.00 to175.89 ppm. The highest concentration (175.89ppm) of Fe was found in yellow variety of ponial location of Ghizer district. While the lowest concentration (64.00) of Cu was found in red variety of variety of Hussain Abad location of Skardu district. Results shown in table -5 are slightly different with Tong *et al.*, (1989) they

reported that Fe content ranged from 5.93 to 161ppm in sea buckthorn of Chinese origin.

Seabuck thorn is good source of vitamin C. In our study (Table 6) the mean values of vitamin C in pulp of these samples ranged from 209.01 to 514.00mg/100g, the highest concentration (514.00mg/100g) of vitamin C was found in yellow variety of Hussain Abad location of skardu district. Similarly, mean values of yellow and red varieties were 371.21 and 283.72mg/100g respectively. The results are in conformity with Zheng and song (1992) they reported that vitamin C concentration ranged from 200-780 mg /100 g in sea buckthorn Ssp. Sinensis of Chinese origin.

Table 6. Vitamin C content in sea buckthorn pulp (mg/100g).

Locations	Varieties		Mean
	Yellow	Red	
Hussain Abad	514.00 a ± 23.64	325.70 d ± 24.82	419.80 a ± 133.14
Rundo	456.30 b ± 38.88	379.71c ± 23.02	418.20 a ± 54.15
Siksa	389.13 c ± 17.50	286.07 def ± 20.59	338.30 b ± 79.23
Yaseen	297.20 de ± 21.54	209.01 h ± 23.81	253.12 d ± 62.35
Hamochal	259.12 efg ± 20.66	246.71 gh ± 15.55	252.83 d ± 8.77
Ponial	310.23 d ± 13.80	254.32 fg ± 22.59	282.55 c ± 39.53
Mean	371.21 a ± 99.70	283.72 b ± 61.31	

Means sharing same small letters are not significantly different at ($p<0.05$).

Data represents in table is mean of three replications. ± Standard deviation.

Table 7. Phytosterol content in sea buckthorn pulp (g/100g).

Locations	Varieties		Mean
	Yellow	Red	
Hussain Abad	3.56d ± 0.30	5.33 b ± 0.26	4.4 b ±1.25
Rundo	4.46 c ± 0.30	5.90 a ± 0.15	5.18a ±1.01
Siksa	2.66 f ± 0.15	4.30 c ± 0.40	3.4 c ±1.15
Yaseen	2.83 ef ± 0.20	3.36 d ± 0.15	3.10d ±0.37
Hamochal	3.40 d ± 0.30	3.26 de ±0.15	3.33cd ± 0.09
Ponial	4.70 c ±0.20	2.50 f ± 0.40	3.60 c ± 1.55
Mean	3.60 b ±0.83	4.11 a ±1.26	

Means sharing same small letters are not significantly different at ($p<0.05$).

Data represents in table is mean of three replications. ± Standard deviation.

Phytosterols are plant sterols which are similar in structure to Cholesterol, that on consumption are capable of lowering blood Cholesterol in humans. Elevated blood cholesterol is one of the well-established risk factor for coronary heart diseases, and lowering this indicator can impact on the incidence of heart diseases (Thurnham1999). Phytosterols are the major constituents of the un saponifiable fraction of sea buckthorn oils. The major phytosterols in sea buckthorn oil is sitosterol (ß-sitosterol), with 5-avenasterol second in quantitative importance. Other phytosterols are present in relatively minor quantities .The total quantity of sterol is quite high in sea buckthorn and may exceed Soya been oil by 4-20times Yang *et al.,* (2001). In Table-7 showed that the mean values of sterol in pulp of these samples ranged from 2.50 to 5.90g/100g, the highest concentration (5.90g/100g) of Phytosterol was found in red variety of Rundo location of skardu district. The mean values of Phytosterol of these localities, Hussain Abad, Rundo, Siksa, Yaseen, Hamochal and Ponial were in the range of 4.45, 5.18, , 3.48, 3.10, 3.33, 3.60g/100g respectively. While the mean values of yellow and red varieties were 3.60g/100g and 4.11g/100g respectively.

Conclusions

Research study was mainstay focused on minerals, vitamins and phytosterol in sea buckthorn a multi purpose plant from northern areas. There is high vitamin C content in sea buckthorn pulp which can be used for the production of blended juices and other beverages. Large amount of minerals in sea buckthorn especially Ca and Fe that are essential for human body have great importance. Sea buckthorn oil is rich source of phytosterols that are similar in structure of cholesterol are capable of lowering plasma cholesterol on consumption by humans. On the basis of above bio chemical composition of Sea buckthorn it is concluded that Sea buckthorn is best nutritional source for human body. One can not deny about the commercial importance because it provides cheapest raw material for Food and Pharmaceutical industries. On the basis of above facts it can also be consider as good source of income generating and income saving for local community.

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