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Chemical and antioxidant properties of Sea buckthorn (*Hippophae rhamnoides*)

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Abstract

Sea buckthorn (*Hippophae rhamnoides*), an ancient crop with modern virtues has recently gained worldwide attention, mainly for its nutritional and medicinal value as the berries contain different kinds of nutrients and bioactive compounds including vitamins, fatty acids, free amino acids and elemental components. The present study was conducted to analyze the moisture, ash, crude protein, oil content, minerals (Fe, Mg, Ca, K and Zn), vitamin C and antioxidant activity of sea buckthorn (*Hippophae rhamnoides*) pulp from Ladakh. Percent moisture content, ash, crude protein and oil content were 85.76, 1.79, 1.37 and 2.12 respectively. The mineral analysis revealed that the mean values of Fe, Mg, Ca, K and Zn as 26.15 ppm, 19.04 ppm, 169.02 ppm, 247.14 ppm and 1.27 ppm respectively. Vitamin C content was 251 mg per 100g pulp. The free radical scavenging activity of sea buckthorn based on its ability to bleach the stable radical DPPH was 26%.

Keywords: Antioxidant activity, minerals, sea buckthorn, vitamins

1. Introduction

Herbal formulations have been in use for many years globally not only as therapeutic but also as prophylactic and health promotive agents. Sea buckthorn (*Hippophae rhamnoides* L.), a unique and valuable plant has gained worldwide attention, mainly for its medicinal and nutritional potential. Sea buckthorn (SBT) is a thorny nitrogen-fixing deciduous shrub of cold arid region native to Europe and Asia. It is currently domesticated in several parts of the world due to its nutritional and medicinal properties (Rousi, 1971; Li, 2003) ^[1]. It is a hardy plant, drought and cold resistant, useful for land reclamation and farmstead protection through its vigorous vegetative reproduction and strong, complex root system with nitrogen-fixing nodules (Rongsen, 1992) ^[2]. All parts of this plant are a good source of large number of bioactive substances like vitamins (A, C, E, K, riboflavin, folic acid), carotenoids, phytosterols, organic acids (malic acid, oxalic acid), polyunsaturated fatty acids and some essential amino acids (Beveridge *et al.*, 1999; Yang and Kallio, 2001; Pintea *et al.*, 2005) ^[3, 22, 5]. Sea buckthorn has been used in traditional Chinese medicine since the Tang Dynasty, going back more than 1000 years. In-depth survey and documentation of indigenous ethnobotanical knowledge of SBT reveal that this plant was traditionally utilized by local people of Asia, Nordic countries and the Baltic region in multidimensional aspects of food, fuel, medicine, veterinary, agricultural tools and bio-fencing (Yang *et al.*, 2000; Dhyani *et al.*, 2010) ^[24, 7]. This plant has been used extensively in oriental traditional system of medicine for treatment of asthma, skin diseases, gastric ulcers and lung disorders. Current research is now beginning to understand and support the traditional uses of SBT. A wide spectrum of pharmacological effects of SBT have been reported, including antioxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective and tissue repair (Suleyman *et al.*, 2001; Geetha *et al.*, 2002a, b; Goel *et al.*, 2002; Xing *et al.*, 2002; Gao *et al.*, 2003; Gupta *et al.*, 2005; Basu *et al.*, 2007; Chawla *et al.*, 2007; Saggi *et al.*, 2007; Upadhyay *et al.*, 2009, 2011) ^[8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19].

SBT berries have a unique composition, combining a cocktail of components usually only found separately. The bioactive components vary with fruit maturity, fruit size, species, geographic locations, climate and methods of extraction (Zeb, 2004; Leskinen *et al.*, 2010) ^[20, 21]. The berries are orange-yellow to red color fruits which are a rich source of valuable compounds such as multiple vitamins (C and E), carotenoids (carotene, lycopene, lutein and zeaxanthin), flavonoids (isorhamnetin, quercetin, isorhamnetin-3-beta-d-glucoside; isorhamnetin-3-beta-d-glucosaminide; kaempferol, etc.) organic acids, amino acids, micro and macronutrients (Yang and Kallio, 2001; Kallio *et al.*, 2002) ^[23].

Many bioactive compounds have been isolated from the berries of SBT such as hippophae cerebroside, oleanolic acid, ursolic acid, 19- α -hydroxyursolic acid, dulcic acid, 5-hydroxymethyl-2-furancarbox-aldehyde, cirsiomaldehyde, octacosanoic acid, palmitic acid and 1-Ohexadecanolenin (Zheng *et al.*, 2009) [24]. Isorhamnetin isolated from barc of SBT, has shown significant antioxidant activity in several antioxidant assays (Pengfei *et al.*, 2009) [25]. The berries are also rich in fatty acids (saturated 13.7% and 86.3% unsaturated) including palmitic acid, oleic acid (omega-9), palmitoleic acid (omega-7), linoleic acid (omega-6), and linolenic acid (omega-3); and phytosterols. The most recognized product of SBT is comprised of seed oil that is enriched in essential fatty acids (omega-3 and 6) and pulp oil that contains high levels of omega-7 (Yang and Kallio, 2005) [26].

2. Materials and methods

The sea buckthorn pulp was purchased from Ladakh and cold stored for further analysis.

2.1 Moisture content (%)

Moisture content was estimated as per the method described by Ranganna (1986) [27].

2.2 Protein (%)

Total nitrogen was determined by the Macro-Kjeldhal procedure as per AOAC (1995) [28]. Two grams of sample was digested in Kjeldhal flask with digestion mixture (copper sulphate and potassium sulphate in 1:9 ratio) and concentrated H₂SO₂ (20 ml) till light green colour appeared and finally cooled. Ammonia released by distillation of digested samples with saturated NaOH (80 ml) was captured in 0.1N HCl and per cent nitrogen was estimated. Total nitrogen determined was converted into protein using conversion factor N \times 6.25.

2.3 Oil content (%)

The Soxhlet extraction method AOAC (1995) [28] was used to determine fat content of the samples. Sample (2.0g) was weighed and put in the extraction thimble and plugged. It was then placed in the Soxhlet apparatus. Weighed flat bottom flask (B) was thereafter filled to about three quarters of its volume with petroleum ether. The apparatus was then set up and the experiment was carried out for a period of 4-8 hours for complete extraction. The petroleum ether was recovered by evaporation using water bath and the remaining sample in the flask was dried in the oven, cooled in a desiccator and finally weighed. The difference in the weight of the empty flask and the flask with oil gave the oil content, which was calculated as percent fat content as follows;

$$\text{Crude fat (\%)} = \frac{W_1 - W_2}{W} \times 100$$

Where,

W = Weight of sample

W₂ = Weight of empty flask

W₁ = Weight of flask + oil

2.4 Ash (%)

Ash content of material represents inorganic residue remaining after destruction of organic matter or the mineral content present in the sample. Total ash content was determined according to AOAC (1995) [28] procedures. 10 gm

of sample in triplicate was weighed accurately into pre-weighed crucibles, charred over a flame and kept in muffle furnace at 600 °C for 4-6 hours. The dish was cooled in desiccators & weighed. Ash content (%) was determined by formula as given:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.5 Total solids (%)

Total solids of the sample were determined by drying the samples in hot air oven at 70 °C as per the method described by Ranganna (1986) [27] using following formula.

Total solids (%) = 100 - moisture content (%)

2.6 Ascorbic Acid (mg/100g)

Ascorbic acid was estimated by the method as described by Rangana (1986) using 2, 6-dichlorophenol indophenol dye. Dye factor was calculated by titrating 5 ml standard ascorbic acid plus 5 ml (3%) metaphosphoric acid against 2, 6-dichlorophenol indophenol till pink colour appeared and volume used was noted.

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

Ascorbic acid was estimated by taking 5g of sample, volume made upto 100 ml with (3%) metaphosphoric acid and filtered. Then aliquot of 10 ml was taken in a titration flask and titrated against 2, 6-dichlorophenol indophenol till light pink colour appeared (which persisted for 15 seconds). Vitamin C was calculated using the following formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre value} \times \text{dye factor} \times \text{volume made up}}{\text{ml of filtrate taken for estimation} \times \text{weight of sample}} \times 100$$

2.7 Mineral analysis

The mineral contents of sea buckthorn were determined according to AOAC (1990) [29]. The samples (0.8–1 g) were ashed in a muffle furnace at a temperature of 550 \pm 10 °C for 6 h and the ash obtained was digested with 5ml 6M HCl on a water bath. After drying 7ml 0.1M HNO₃ was added and contents were diluted to 100 ml with double deionized water. Calcium (Ca), Iron (Fe), Zinc (Zn), Magnesium (Mg) were determined in an Atomic Absorption Spectrophotometer whereas Potassium by Flame Photometer.

2.8 Antioxidant activity

The antioxidant activity of the extract was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) method described by Ravichandran *et al.* (2013) [30]. 0.2 ml of the methanol extract was mixed for 30 s with 3.8 ml of DPPH solution (6 \times 10⁻⁵ M), and left to react for 30 minutes, after which the absorbance of the mixture was measured at 517 nm. The DPPH solution without extract was analyzed as a control. The antioxidant activity was calculated as follows:

$$\text{DPPH radical-scavenging activity (\%)} = \frac{[A_{\text{control}} - A_{\text{sample}}]}{[A_{\text{control}}]} \times 100$$

Where A is the absorbance at 517 nm.

3. Result and discussion

The results for moisture, fat, ash, total solids, protein, vitamin C, mineral content and antioxidant activity are given in Table. 1. The moisture content (%) of pulp was 85.76. Moisture content of sea buckthorn pulp varies due to the variation in origin and climate. The results are in alignment with those of Dhyani *et al.*, 2007^[31] who reported moisture content of pulp from berries to be in the range of 84.9–97.6% for the Indian sea buckthorn. The sea buckthorn pulp had oil content of 2.12%. The most valuable components of the sea buckthorn berries are their oils. Generally, the oil from the pulp/peel fraction is combined due to the difficulty involved with separation. Berry pulp has high total lipid content, including tocopherols, tocotrienols, carotenoids, as well as omega-3 and omega-6 fatty acid families (Yang and Kallio, 2002)^[6]. Oil from the juice and pulp is rich in palmitic (16:0) and palmitoleic acids (16:1), while the oil from the seeds contains unsaturated fatty acids of C18 type oils, linoleic (18:2) and linolenic acid (18:3). The oil from the juice also contain vitamin E and carotene (Bernath and Foldesi, 1992; Ma and Cui, 1989)^[32]. The European sea buckthorn pulp oil had palmitoleic (16:1n-7), palmitic (16:0) and oleic acids as the major fatty acids. The pulp oil is rich in α -tocopherol. Ash content of material represents inorganic residue remaining after destruction of organic matter or the mineral content present in the sample. Ash content of pulp was 1.79%. These results are in alignment with those of Chauhan *et al.* (2001)^[34]; Katiyar *et al.* (1990)^[35] who reported ash content of

barriers varies from 1.76–1.8%. Protein content of pulp was 1.37%. A total of 18 out of 22 known amino acids have been found in sea buckthorn fruit (Mironov, 1989; Zhang *et al.*, 1989)^[36, 37], half of which are essential since they play a critical role in various processes within our bodies such as energy production, building cells and muscles, fat loss, and mood and brain functions. Sea buckthorn juice is rich in various free amino acids. Sea buckthorn is rich in antioxidant vitamins including vitamin C. The sea buckthorn pulp had vitamin C content of 251 mg per 100g pulp. The results for vitamin C are in alignment with those of Arimboor *et al.* (2006)^[38]. In comparison to common fruits, sea buckthorn is a rich source of vitamin C. Vitamin C is a natural water-soluble antioxidant which inhibits peroxidation of membrane phospholipids and acts as scavenger of free radicals. It also plays a major role in regeneration of vitamin E. Sea buckthorn is rich source of minerals. Potassium (247.14 ppm) was the most abundant of all the minerals. Mineral element composition revealed a high content of calcium (169.02 ppm), iron (26.15 ppm), magnesium (19.04 ppm) and zinc (1.27 ppm). The free radical scavenging activity of SBT was studied by its ability to bleach the stable radical DPPH. This assay provides information on the reactivity of compounds with a stable free radical. The bleaching of DPPH represents the capacity of SBT to scavenge free radical's independent of enzymatic activity. The present investigation shows that SBT is sufficiently effective in scavenging DPPH radicals. These results are in alignment with those of Badami *et al.*, 2003^[39].

Table 1: Chemical composition of sea buckthorn

Parameter	Sea buckthorn
Moisture (%)	85.76
Oil content (%)	2.12
Ash (%)	1.79
Total solids (%)	14.24
Protein (%)	1.37
Vitamin C (mg/100g)	251
Mineral content (ppm)	
Fe	26.15
Mg	19.04
Ca	169.02
K	247.14
Zn	1.27
Antioxidant activity (%)	26

4. Conclusion

Based on present study, it could be concluded that pulp of sea buckthorn has pronounced antioxidant properties. The mineral content of sea buckthorn is at par with other fruits. Sea buckthorn is one of the richest source of vitamin C. Thus, it can be incorporated into food products for development of functional foods.

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