International Journal of Anatomy and Physiology ISSN 2326-7275 Vol. 14 (4), pp. 001-005, April, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Exploring the Biochemical Properties of *Hippophae rhamnoides L. spp*. turkestanica Seeds

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Accepted 10 January, 2025

Sea buckthorn is a valuable medicinal plant, cultivated and naturally grown in northern Pakistan. The plant produces berry with small hard seed in the centre. The seed is the source of all nutrients and phytochemcials. However, there is lack of literature regarding the biochemical and physico-chemical quality of the seed. This study consists of evaluation of the physiochemical parameters; elemental components, seed storage protein profile and anatomical structure of sea buckthorn ssp turkestanica seed. Results showed that ash content was 2.05%, moisture content 5.5%, thousand seed mass value 10.20 g; sodium 47.65 ppm, potassium 88.0 ppm, phosphorus 0.43 ppm, magnesium 758.0 ppm, calcium 912.0 ppm, zinc 96.50 ppm, iron 290.25 ppm and silver 3.10 ppm. Seed storage protein electrophoresis reveals the presence of only low molecular weight proteins. SEM analysis shows that seed was 4.3 mm long and 2.4 mm wide with 0.1 - 0.2 nm thicken seed coat. The study provides a good source of information of sea buckthorn seed of Pakistani origin and its potential for cultivation in various parts of the world.

Key words: Hippophae, physiochemical parameters, elements, seed storage proteins, anatomy.

INTRODUCTION

Sea buckthorn (Hippophae) is a unique medicinal and aromatic plant and belongs to the family of Elaeagnaceae. It is a medium sized or small deciduous tree or large shrub with 2.5 – 6 m in height. The main trunk has a thick and rough bark. The young branches are smooth, grey and light ash colored with needle shaped thorns (Zeb and Khan, 2008a) . It is cultivated and naturally grown in the various parts of the world, including Pakistan. The distribution of sea buckthorn ranges from Himalayan regions including India, Nepal, Bhutan, Pakistan and Afghanistan, to China, Mongolia, Russia, Kazakhstan, Hungary, Romania, Switzerland, Germany, France and Britain, and northwards to Finland, Sweden and Norway (Rousi, 1971; Zeb, 2004a). The wide distribution of sea buckthorn is showing its habit-related variation in berry related characters such as fresh weight, chemical and sensory attributes (Yang and Kallio, 2001).

The plant yields various important products such as leaves, oil, juices etc with tremendous medicinal and pharmacological applications (Yang and Kallio, 2002; Zeb, 2004b). The wide chemical and phytochemical composition of sea buckthorn has been reviewed recently (Zeb and Khan, 2008b; Tiitinen et al., 2005) . It was found to vary with the origin, climate and method of extraction. In general, the major components of the seed are vitamin-C, large amount of carotenoids and vitamin-E, flavonoids, and kaempferol, fatty acids, triacylglycerol, phytosterols, sugar, organic acids, proanthocyanidins and phenolic compounds (Abid et al., 2007; Fan et al., 2007; Li et al., 2007).

In Pakistan *Hippophae rhamnoides*, that is, sub species turkestanica is distributed in northern areas like Kurram Agency, Chitral, upper Swat, Utror-Gabral, Gilgit, Astore, Skardu, Ganche, Baltistan, Ladak and nearly all over the northern areas from altitude of 1,219 to 4,266 m. The northern areas of Pakistan have tremendous potential of the wild sea buckthorn (*H. rhamnoides* subsp. turkestanica). The plant is spread over all five districts of the region. According to an estimate 3,000 ha of land in the northern areas is under natural sea buckthorn cover (Shah et al., 2007). Proper management and utilization can bring positive changes in the socio-economic condi-tions of the local communities dealing with sea buckthorn.

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However, literature describing the physicochemical and anatomical characterization of sea buckthorn seeds of Pakistani origin is limited (Sabir et al. 2005; Abid et al. 2007). The present study was therefore aimed to investigate the physiochemical, biochemical and anatomical characteristics of sea buckthorn seed, which is important for bringing awareness regarding nutritional, medicinal, and economic importance of the seed.

MATERIALS AND METHODS

Sample collection

Samples of fresh *H. rhamnoides* sp *turkestanica* berries were collected from different plants in Skardu Pakistan. The berries were dried in sun, and stored in cloth bags under room temperature. Seeds were then separated from the dried berries and processed for further analysis.

Physiochemical parameters

Ash content was determined using high temperature in a muffle furnace at 900^{O}C. Moisture contents, electrical conductivity (EC), thousand seeds mass (TSM), pH and titratable acidity were determined according to the methods described earlier (Chapman and Pratt, 1978). Vitamin C contents of the seed were measure according to AOAC method (AOAC, 1990). Oil content was measured according to standard methods described by AACC (2000).

Elemental composition

Elemental contents were determined by wet digestion procedure. Minerals like sodium and potassium were determined with the help of flame photometer (Jenway PFP7) (Khan et al., 2007). Heavy and alkaline metals like Ag, Zn, Fe, Mg, and Ca, were determined with help of atomic absorption spectrometer (Perkin Elmer, model AAnalyst 700) with air/acetylene flame at 2200 – 2400 K (photo multiplier tube detector), against the standard (Hanlon, 1992). The phosphorus content of seed was determined using UV-visible spectrophotometer (Shimadzu UV-1700) at 660 nm as described in our previous work (Khan et al., 2007).

SDS-PAGE electrophoresis

The seed storage-proteins were analyzed by SDS-PAGE (Shuaib et al., 2007). The grains were ground to fine powder and 10 mg was weighed in 1.5 ml microtube. 400 ml protein extraction buffer (Tris-HCl 0.05 M, pH 8), 0.02% SDS, 30.3% urea, 1% 2- mercapto-ethanol) was added to each micro tube, kept overnight at 40°C and centrifuged at 13000 rpm for 10 min. The supernatant containing dissolved protein was stored at 4°C. Gel preparation running and staining were standard procedures. The molecular weight of protein subunits was measured by comparing sample bands to the standard protein molecular weight marker bands in the electrophorogram.

Scanning electron microscopy

Single sea buckthorn seed was sectioned longitudinally with microtome. The section was then attached to aluminum stub with help of silver past and placed in stub holder under vacuum. The sample was examined under scanning electron microscope (JEOL JSM–5910, Japan) at magnification of 23X. The results are the mean of triplicate readings.

Table 1. Physiochemical characteristics of sea buckthorn seed.

Parameter	Quantity[a]
Ash (%)	2.05 ± 0.200
Moisture (%)	5.50 ± 0.051
pH	5.70 ± 0.084
Titratable acidity (%)	4.80 ± 0.210
Vitamin C (mg/100 g)	35.40 ± 0.042
Total seed mass (g)	10.20 ± 0.035

[a]Values are expressed as mean ± standard deviation (n = 3).

RESULTS AND DISCUSSION

Physiochemical characteristics

Physiochemical characteristics are important to study the quality of a species. The data regarding physiochemical characteristic such as ash, moister content, electrical conductivity, pH, titratable acidity, vitamin C and TSM are presented in Table 1. Sea buckthorn seed contains 2.05% ash and 5.5% moisture. The electrical conductivity and TSM were 5µS and 10.2 g, respectively. Ash content of seed was in agreement with earlier results (Khan et al., 2005), while moister content shows deviation, which may be due to stored sample used for analysis. TSM was reported for the first time with a value of 10.2 g. The small increase in the electrical conductivity (Figure 1) with passage of time reflects the hardness of the seed. Crude oil content in seeds of sea buckthorn was found to be 6.0%, comparable with 7.03 to 12.86% reported by Shah et al. (2007).

Elemental composition

Elemental components like Na^+, K^+ , P, Mg, Ca, Zn, Fe and Ag are important for nutritional purposes, cultivation and growth. Minerals composition revealed the high content of calcium (912.00 ppm), magnesium (758.00 ppm), iron (290.25 ppm), zinc (96.55 ppm), and potassium (88.00 ppm), while sodium concentration was 47.65 ppm and phosphorous (0.43 ppm) was the lowest in all elements (Figure 2). Investigated value of Na^+ is close to the value given by Sabir et al. (2005), while K^+ values are in close agreement with the reported value of 100 ppm by Tong et al. (1989). The phosphorous, mag-nesium, calcium, zinc, iron, and silver show high degree of variation from the reported literature (Zeb, 2004a). Variation may originate from the natural content of elements in the soil, geographical location, contami-nation of soil and air, and the different method and equipment used for analysis.

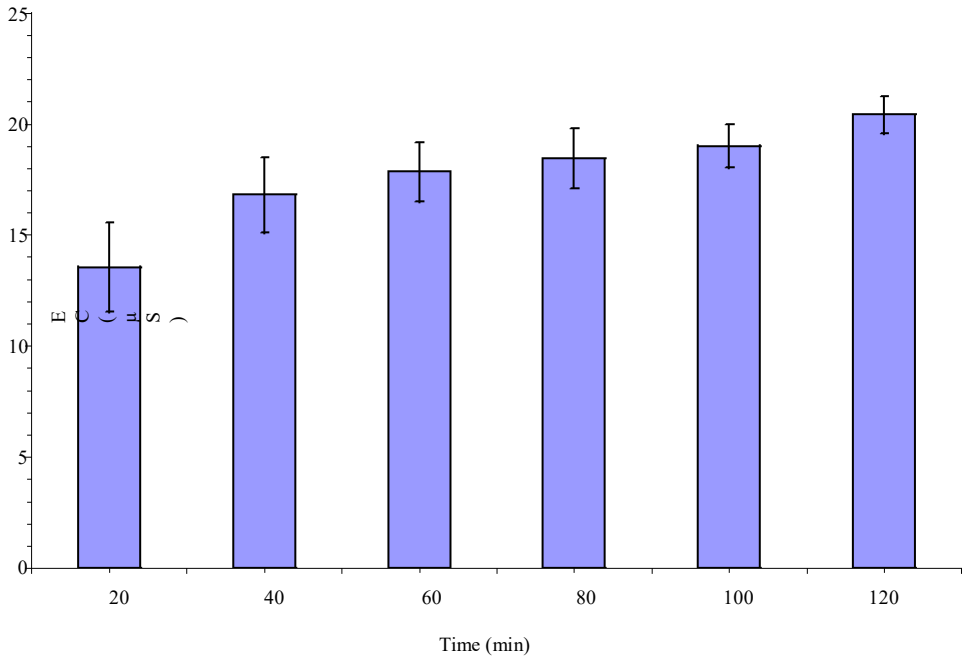

Figure 1. Variation of electrical conductivity of sea buckthorn seed with respect to time.

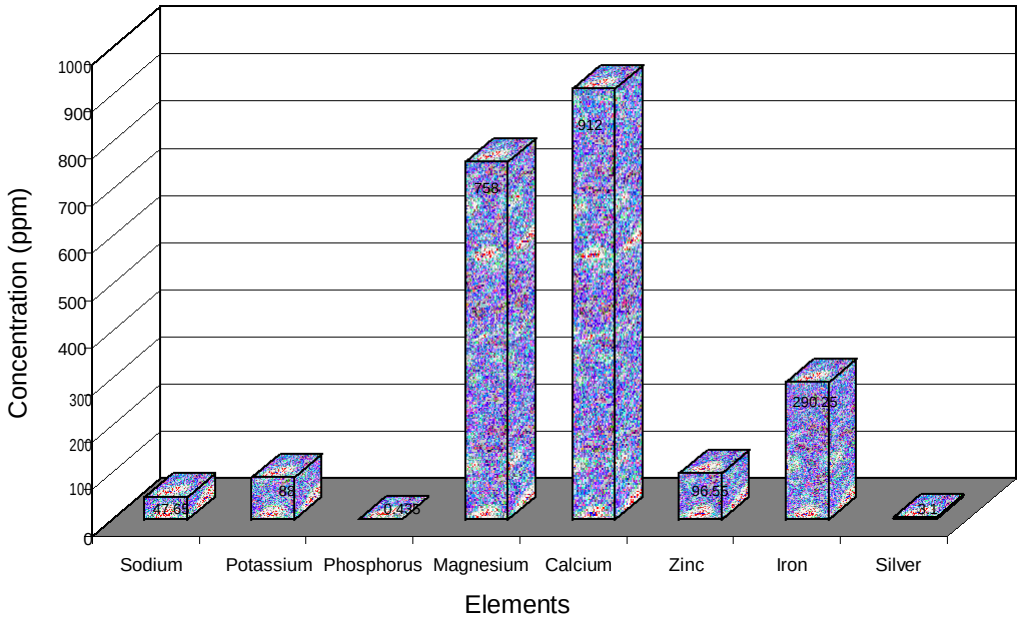

Figure 2. Elemental composition of sea buckthorn seeds.

SDS-PAGE electrophoresis

The SDS-PAGE profile is helpful in measuring the genetic diversity in Sea buckthorn genotypes. It has been shown earlier (Ahmad et al., 2003), that there was high variation in protein mobility in the gel. The inter-relationship of sharing of at least one common band among all was established. However, they did not show

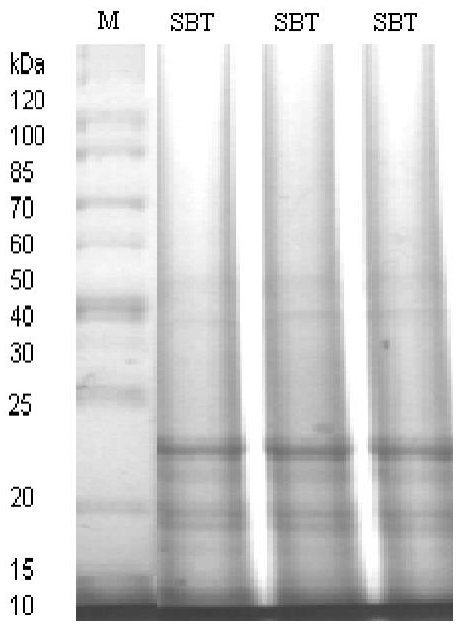

Figure 3. Seed protein electrophoresis of sea buckthorn seed.

Table 2. Molecular weight banding range of sea buckthorn seed.

S/N	Molecular weight range (kDa)	Number of protein bands
1	40–50	1
2	30–40	1
3	20–25	2
4	15–20	2

the exact molecular weight of each band. The results of SDS–PAGE analysis of total seed protein of sea buckthorn are shown in Figure 3. There was total of six bands in range of 15 - 50 kDa. The exact 50 kDa protein band was present in sea buckthorn SDS-PAGE profile. The molecular weight range of protein bands is presented in Table 2, which indicates that there are 4 protein bands in range of 15 – 25 kDa, one band in the range of 30 – 40 kDa while only one band is in the range of 40 – 50 kDa.

Scanning electron microscopic analysis

The scanning electron microscope creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electrons with the sample's surface. These signals come not only from the primary beam impinging upon the sample, but also from other interactions within the sample near the surface. SEM is capable of producing high-resolution images of a plant sample surface in its primary use mode, secondary electron imaging (Heywood, 1969). Each sea buckthorn

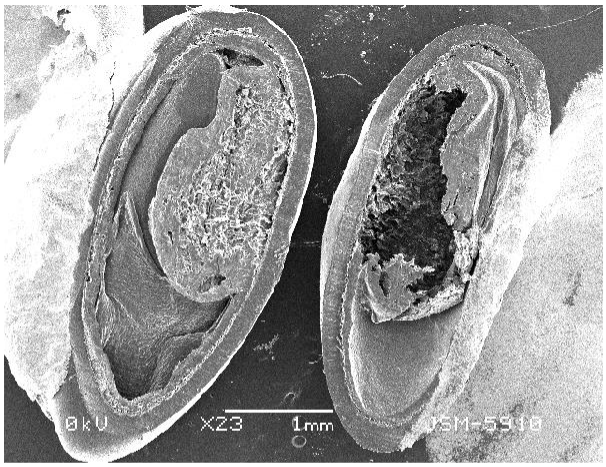

Figure 4. Scanning Electron Micrograph (SEM) of cross sectioned sea buckthorn seed.

fruit contains one seed. Sea buckthorn has dark brown and shiny seed coat. The scanning electron micrograph (SEM) of dissected sea buckthorn seed is shown in Figure 4. The seed was approximately 4.3 ± 0.01 mm (mean ± SD) long and 2.4 ± 0.01mm wide. The seed was slightly pointed at both ends. The seed has a furrow all around its body; one side of the seed is slightly smaller than the other. A cut section shows two distinct layers of the testa derived from the outer and inner integument of the ovule. The outer layer was thick and very hard, while the inner layer was thin and fibrous in appearance. The total thickness of the seed coat was 0.1 - 0.2 nm. The seed coat encloses the massive embryo, in which the axis was not visible. The SEM results were in close agreement with the seed length of 4.5 mm and seed width 2.33 mm of Canadian sea buckthorn variety reported by Harrison and Beveridge (2002).

Conclusion

Sea buckthorn is a kind of important crop with wide popularity across the world due to its medicinal and pharmacological importance and wide occurrence. Sea buckthorn seed of Pakistani origin have the potential for vigorous vegetative reproduction and because of its strong complex root system with nitrogen-fixing nodules, it should be considered as a best possible plant for soil and water conservation and reforestation in eroded areas. The present study investigated the physiochemical and biochemical features of sea buckthorn seed for cultivation in Pakistan and across the globe where sea buckthorn can grow. These features are also important for further analysis of sea buckthorn.

ACKNOWLEDGMENT

We re grateful for financial assistance of Higher Educa-

tion Commission (HEC) Islamabad Pakistan under Access to Scientific Instrumentation program for heavy metals and SEM analysis (No. HEC/FD/2007/670).

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