Physicochemical Characteristics and Fatty Acid Composition of Sea-Buckthorn (*Hippophae rhamnodes* L) Oil

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Summary: The study was carried out to evaluate the physico-chemical characteristics and fatty acid profiles of seed and pulp oils of Sea-buckthorn (*Hippophae rhamnodes* L) wildly grown in Northern Areas of Pakistan (Skardu). The yield of oils from seed and pulp were 10 % and 4.5 % respectively. Physical characteristics i.e. specific gravity, refractive index, acid value, iodine value, saponification value and unsaponified matters were determined according to standard procedures. The unsaturated fatty acid in seed oil is (85 %) and in pulp oil, is (63.4 %) while saturated fatty acid in seed oil is (14.1 %) and in pulp oil, is (36.0 %) respectively. The GLC analysis of methyl esters derived from the seed oil of Sea-buckthorn revealed the presence of Palmitic acid (11.1 %), Palmitoleic acid (9.8 %), Stearic acid (2.6 %), Oleic acid (Including Vaccenic acid) (22.1 %), Linoleic Acid (29.6 %) and α- and γ-Linolenic Acid (23.4 %). In pulp oil of Sea-buckthorn (*H. rhamnodes* L) we have Palmitic acid (34.5 %) Palmitoleic acid (33.4 %), Stearic acid (1.5 %), Oleic acid (Including Vaccenic acid) (15.3 %), Linoleic Acid (12.5 %) and α- and γ- Linolenic Acid (2.3 %).

Introduction

Sea-buckthorn (*Hippophae rhamnodes* L) is deciduous spiny shrub or small tree between two and four meter high, widely distributed throughout the temperate zones of Asia and Europe [1] and the sub-tropical zone of Asia at high altitude ranging from a few meters to 5200 meters [2]. The plant is hard, drought and usually cold tolerant, useful for the land reclamation and farmstead protection [3].

The *H. rhamnodes* sub species *Hippophae rhamnodes Turkistania* is found in Chitral and Northern Areas of Pakistan. Normally, it is spread throughout the Karakuram and Himalayan ranges at altitudes of 1500-3500 m. According to a Chinese *H. rhamnodes* expert, Professor Rongsen, there are about 3000 hectares of *H. rhamnodes* forests in Pakistan, annually producing 1200-2250 tons of Sea -buckthorn, and various industries producing jams, jellies, chocolates and capsules at small scale and exporting the berries abroad [4].

Sea-buckthorn (*H. rhamnodes)* is known to be one of the vitamin-rich berries in the plant kingdom, and has been credentialed as highly valued for healthy living, improving well-being, enhancing lifestyle, and preventing the disease [5]. Widely recognized in Northern regions of Europe and Asia, *H. rhamnodes* has been used medicinally for thousands of years [6]. Often referred to as a "miracle plant," *H. rhamnodes* has been used extensively in Eastern medicinal practices. Bright orange-yellow berries, the most popular harvestable commodity of the plant, have been used for many centuries in Europe and Asia as a nutritional food source. Examples of popular food items containing Sea-buckthorn berries include jams, jellies, juices, liquors, and wines. In addition to beverages, hundreds of other *H. rhamnodes* products have also been developed from the extracts of berries, leaves, and the bark [7]. Sea-buckthorn has been recognized in Canada as a specialty crop having economical viability in the functional foods and nutraceutical markets [8]. *H. rhamnodes* has been reported to contain more than 190 compounds in seed, pulp and juice. These compounds include fat soluble vitamins (A,E,K), 22 fatty acids, 42 lipids, carbohydrates, vitamins C, B_1, B_2, folic acid, α-tocopherol (generically referred to as vitamin E), phenolic compounds (flavonoids, carotenoids, sterol, terpene and tannin) [9].

Many substances found in *H. rhamnodes* have a beneficial effect on health and are expected to reduce the risk of heart diseases [10] and also have a role in the cancer prevention [11-12]. *H. rhamnodes* seeds and pulp have been targeted for their oil. Although the oil content of Sea-buckthorn is not as much as the amount found in most oil

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crops, its nutritional and medicinal values are much more than those of most crops, because it contains a lot of fat-soluble vitamins and plant sterols [13].

An ingredient of the oil, Palmitoleic acid, is a component of skin. It is considered a valuable topical agent in treating burns and healing wounds. This fatty acid can also nourish the skin when taken orally. If adequate quantities of *H. rhamnodes* or its oil are consumed, this is a useful method for treating systemic skin diseases, such as atopic dermatitis. *H. rhamnodes* oil is already widely used topically alone or in various preparations for burns, scalds, ulcerations and infections. It is an ingredient in sun blocks. *H. rhamnodes* oil has UV-blocking activity as well as emollient properties and it is an aid in promoting regeneration of tissues [14 - 15]. Gastric ulcers are growing fast in human beings, especially in the developing countries like Pakistan, due to unfavourable and non-assessed diet, ignorance, and carelessness. *H. rhamnodes* is traditionally used in the treatment of gastric ulcers and laboratory studies confirm the efficacy of the seed oil in this application. *H. rhamnodes* was found to be active in preventing gastric injury [16-19].

For this reason, *H. rhamnodes* oils have attracted considerable attention from researchers mainly for their nutraceutical value and are currently being incorporated into several products having economic potential in cosmetic, nutraceutical, and pharmaceutical markets [20]. *H. rhamnodes* oil treatments are vast and research on their medicinal uses is growing. Primary areas of treatment include, but are not limited to, antioxidation, skin and mucosa repair, cardiovascular disease prevention, immune system restoration, and anticancer applications [13].

The purpose of present study was to analyze the physicochemical and fatty acids composition of *H. rhamnodes* wildly grown in Northern Areas of Pakistan. The out come of this work will hopefully bring awareness regarding the nutritional and economical importance of oil of this fruit.

Results and Discussion

The oil was analyzed for its suitability for nutritional purpose and was subject to physico-chemical characterization and determination of chemical composition. It was observed that physical and chemical properties of *H. rhamnodes* pulp oil and seed oil are different from each other. Table- 1 shows that specific gravity of seed and pulp oil was (0.9124) and (0.8967) respectively. The acidic value of oil or fat is the extent to which hydrolysis results in the liberation of the fatty acid from their ester linkage with parent triglyceride molecules. The acidic value of *H. rhamnodes* seed oil was (6.3 mg/ g) while that of pulp oil was (9.5 mg/ g).

Table- 1: Physicochemical properties of sea buckthorn seed and pulp oils

Physicochemical properties of oils	Seed oil	Pulp oil
Yield (%)	10 ±0.20	4.5 ±0.12
Specific gravity	0.9124 ±0.03	0.8967 ±0.02
Refractive Index	1.4762 ±0.05	1.4668 ±0.04
Acid value (mg/g)	6.3 ±0.10	9.5 ±0.15
Iodine value (g of I/ 100g of oil)	125 ±1.50	63 ±2.0
Saponification-value(mg KOH/g)	181 ±3.20	196 ±3.58
Unsaponifiable matters (%)	0.9 ±0.02	2.06 ±0.15

Values are means ± SD (triplicate)

Iodine value is measure of degree of the unsaturation or number of double bonds present in oil or fat. The fat was deteriorated due to oxidation and hence its iodine value was reduced. The iodine value of *H. rhamnodes* seed oil is (125) which was greater than the pulp oil (63). It shows that seed oil is more unsaturated then pulp oil.

Saponification value is the index of mean molecular weight of the triglycerides comprising the fat. The saponification value of *H. rhamnodes* seed and pulp oils were 181 and 196 mg KOH/ g respectively. The unsaponifiable matters in oils mainly contain vitamins (vitamin E and K), pigments (carotene and lycopene) etc; 2.06 % for pulp oil which is more than that of seed oil (0.9 %). From the high value of unsaponifiable matter it is observed that the pulp oil contains more bioactive compounds as compared to seed oil. Table-2 indicates that unsaturated fatty acids in the seed oil (85.0 %) and in the pulp oil is (63.4 %) are higher than saturated fatty acids of seed oil (14.1 %) and pulp oil (36.0 %) respectively.

The most important factor defining the nutritional value of oils is the ratio of unsaturated

Table- 2: Saturated and Unsaturated fatty acids composition (g / 100g) in oil of seed and pulp of *Hippophae rhamnodes Subs. Turkistania.*

Parts of fruit	Saturated fatty acids	Unsaturated fatty acids
Seed oil	14.1 ± 0.21	85.0 ± 3.20
Pulp oil	36.0 ± 0.65	63.4 ± 2.75

Values are means ± SD (triplicate)

fatty acids present in oil. The fatty acid composition given in Table- 3 shows that seed oil of *H. rhamnodes* contains Palmitic acid (11.1 %), Palmitoleic acid (9.8 %), Stearic acid (2.6 %), Oleic acid (including Vaccenic acid) (22.1 %), Linoleic acid (29.6 %) and ∝- and γ-Linolenic acid (23.4 %). Pulp oil of *H. rhamnodes* has Palmitic acid (34.5 %), Palmitoleic acid (33.4 %), Stearic acid (1.5 %), Oleic acid (Including Vaccenic acid) (15.3 %), Linoleic acid (12.5 %) and ∝- and γ-Linolenic acid (2.3 %). The palmitic acid and palmitoleic acid ratio in pulp oil was more than in seed oil. While steric acid, Oleic acid (including Vaccenic acid), Linoleic acid (29.6 %), and ∝- and γ-Linolenic Acid (18.6) in seed oil have a higher ratio than that of pulp oil. It shows that unsaturated fatty acids in seed oil are much higher than in pulp oil. It has already been established that unsaturated fatty acids are beneficial to the human health, especially linoleic and linolenic acids, which are often used for curing disease relating to cardiovascular system and high blood cholesterol [14]. It can thus be concluded that seed oil is better than pulp oil because the former contains 23.4 % linolenic acid whereas the latter contains only 2.3 %.

Experimental

Sample Collection, Preparation and Extraction of Oil

The Sea-buckthorn (*Hippophae rhamnodes* L) berries were procured from PCSIR Laboratory Skardu, Northern Areas. The pulp and seeds were dried in oven at 105 ± 1°C and crushed into fine powder. The oil was extracted with hexane by Soxlet solvent extraction method [21]. The hexane extracts were dried over anhydrous sodium sulphate. The percentage yield of seeds and pulp oils was 9.5 % and 4.5 % respectively.

Physicochemical Values of the Oil

The physico-chemical properties, like specific gravity, refractive index, iodine value and, acid value, were determined according to standard procedure [22-23].

Preparation of Methyl Ester of Fatty Acids

Weighed amounts of oil (1.0 g) were transferred to a Teflon test tube. Methanolic potassium hydroxide (0.5N 10 ml) was then added to the oil samples. The mixture was refluxed until the globules of oil got into solution (90 minutes). Sulphuric acid (2N) was then added to the cooled mixture to liberate fatty acids. Esterification of the liberated fatty acids was carried out in the presence of catalytic amount of methanol BF_3 (10 ml) followed by boiling for about 20 minutes. The esterfied mixture was cooled and extracted with hexane. Separate hexane layers were washed with water and dried over anhydrous sodium sulphate [24].

Determination of Fatty Acids Methyl Esters

The fatty acids composition of oils was determined by Gas chromatography (Perkin Elmer 8410 Series) using a column (1.5 m x 4 mm i.d.) packed with Celite coated with 10 % DEGS. The GLC operating conditions were: column temperature 200 °C, FID temperature 250 °C, injector temperature 220 °C and carrier gas nitrogen with flow rate of 40 ml/ min. The determined percentage fatty acid composition is given in Table- 2.

Table- 3: Fatty acid composition (g /100g of fatty acids) of seed and pulp oil of Sea-buckthorn

Part of fruits analysis	Palmitic acid C 16:0	Palmitoleic acid C 16:1	Stearic acid C 18:0	Oleic acid (Including Vaccenic acid) C 18:1	Linoleic Acid C 18:2	∝- and γ-Linolenic Acid C 18:3
Seed oil	11.1 ± 0.36	9.8 ± 0.30	2.6 ± 0.04	22.1 ± 0.62	29.6 ± 1.60	23.4 ± 0.49
Pulp oil	34.5 ± 1.55	33.4 ± 1.30	1.5 ± 0.05	15.3 ± 0.53	12.5 ± 0.85	2.3 ± 0.05

Values are means ± SD (triplicate)

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