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FATTY ACID COMPOSITION OF FRUIT PULP AND SEED OILS OF HIMALAYAN SEABUCKTHORN (*HIPPOPHAE* L)

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ABSTRACT

Fatty acid composition of oils of fruit pulp and seeds was studied in 2 elite genotype of *Hippophae rhamnoides* ssp. *turkestanica* from "Madhagon" a low altitude (2625 m asl) place, and "Shego", a higher altitude place (3760 m asl), 1 ecotype of *H. salicifolia* from medium altitude place "Tinu" (3200 m asl) and an exotic "HI-I" (*H. rhamnoides* ssp. *mongolica*), all high yielding selections or cultivars, raised at High Land Agricultural Research and Extension Center of CSK Himachal Pradesh Agricultural University, located at Kukumseri (2730 m asl) in Lahaul valley of district Lahaul-Spiti, a dry temperate region of Himalayas. In pulp oils of all ecotypes, unsaturated fatty acids constituted the higher proportion (60.7-52.8%) than the saturated fatty acids (29.9-10.6%) of the total oil. In the seed oils, the unsaturated fatty acids also constitute higher proportion (86.5-51.7%) except H. salicifolia. In pulp oils, the major fatty acids are palmitoleic acid (46.4-37.1%), palmitic acid (28.1-9.1%), linoleic acid (15.0-6.4%) and oleic acid (6.9-4.0%). Pulp oil of H. salicifolia is richest source of Linoleic acid (15.0% of total oil) and α-linolenic acid (1.3%), also another very important unsaturated fatty acid. However, palmitoleic acid was a major dominating unsaturated fatty acid (46.4-37.1%), being maximum in the pulp oil of "Madhagon" ecotype (46.4%), whereas palmitic acid was a major saturated fatty acid, almost equal in the pulp oils of all ecotypes (28.1-26.4%), except "Shego" ecotype (9.1%). In seed oils, major FAs are linoleic acid (39.8-3.7%), palmitic acid (29.3-8.7%), Oleic acid (27.4-14.8%) and α-linolenic acid (25.4-3.6%), with exception in H. salicifolia. Seed oil of "Shego" ecotype from high altitude was richest source of linoleic acid (39.8%) and α-Linolenic acid (25.4%), which offers the best ratio of omega 6 and omega 3 followed by exotic "HI-1". The pulp oil of "Tinu" ecotype is a useful source for the Linoleic acid (15.0% of total oil) and α-linolenic acid (1.3%), whereas pulp oils of all ecotypes, specially "Madhagon" ecotype are rich source of Palmitoleic acid. The seed oils of *H. rhamnoides* ssp. *turkestanica* could be considered excellent sources of PUFAs due to their high contents of linoleic and α-linolenic acids, which in human body are precursors of other long-chain n-3 and n-6 fatty acids.

Keywords: *Hippophae rhamnoides ssp. turkestanica, ssp. mongolica, H. salicifolia,* cultivars, fruit pulp and seed oils' fatty acid composition and high altitude Himalayas.

INTRODUCTION

Seabuckthorn is a deciduous nitrogen fixing and thorny multipurpose plant widely distributed in dry temperate Himalayas (Singh et al. 1995, Singh and Singh 2004). Traditionally, it is being used in making health juice and jams and treating wounds, skin diseases, pulmonary and digestive disorders in the region (Brijlal et al. 2001). Fruit oil and leaves have been reported to be rich source of vitamin E, K, Carotenoids, polyphenols and sterols and other bioactive substances, which have been reported to have efficacy in a number of health problems (Yang and Kallio 2002; Suryakumar and Gupta 2011).

A number of factors, i.e. geographical environment of local habitat, soil properties, climate, genetic characteristics and developmental stage of seabuckthorn and many other factors affect the qualitative and quantitative composition of the seabuckthorn fruit (Yang and Kallio 2002). The quality and composition of seabuckthorn oil depends largely on the part of the plant and processing method used.

In literature, seabuckthorn oil has been reported to have several health protection functions, i.e. diminishing inflammations; relieving pain; improving the cardiovascular conditions; antibacterial properties; skin tissue regeneration after mechanical, chemical and burn injuries (especially the pulp oil); use in skin grafting; treatment of corneal wounds; anti-mutagen effects (Suryakumar and Gupta 2011). Some studies have also found anticancer and anticarcinogenic effects of seabuckthorn oil, as well as improving effects on immune function, peroxidation, mucosa and skin (In: Suryakumar and Gupta 2011). Seabuckthorn seed oil has significant anti-atherogenic and cardioprotective activity (Malik et al. 2012) Seabuckthorn extracts proved also anti-radiation le sion properties (Goel and Bala 2006). Clinical studies performed in Romania found efficiency of seabuckthorn oil used against skin physical and chemical burns, ocular damages and diabetes (Brad et al. 2002). Oils from both fruits and leaves are also used in cosmetic industry (Lu Rongsen 1992).

Seabuckthorn oil has been found to treat skin and mucous membrane injuries (Yang et al. 1999). By application of seed oil, an increase in the level of α-linolenic acid in plasma lipids has been reported to improve the atopic dermatitis symptoms (Yang *et al*., 2000). Seabuckthorn oil stabilizes the cell membrane. It is because of the occurrence of polyunsaturated fatty acids (PUFA), particularly monounsaturated fatty acids (MUFA) in seabuckthorn oil, which are also component of membrane sphingolipids and glycerophospholipids, which carry out the epidermal barrier system function (Yang et al. 1999). PUFA and MUFA, being essential components of cell membrane, maintain the fluidity of the membrane (Yang et al. 2000). They also play important role in functioning of enzymes, receptors, ion channels and other substance transportation systems. Glycerophospholipids of cell membrane is a source of linoleic acid, necessary for the synthesis of acylceramides and 13-hydroxyoctadecadienoic acid (13-HODE). Acylceramides are the main components, which determine the epidermal barrier function, whereas, 13-hydroxyoctadecadienoic acid lowers the epidermal hyperproliferation and expected to decrease the inflammation (Yang et al. 1999). Seabuckthron pulp oil has been reported to decrease the risk of atherosclerosis (Yang et al. 1999), as application of seabuckthorn fruit pulp oil increased the HDL cholesterol from 1.38 to 1.53 mmol/l. Seabuckthorn oil contains high content of Palmitoleic acid, which may have cholesterol and triglyceride lowering and stroke reducing effects (Yamori et al. 1986; Colquhoun et al. 1996). Seabuckthorn oil is also rich in vitamin E, carotenoids and flavonoids, which have strong anti-anti-oxidant properties (Suryakumar and Gupta 2013).

Several health benefits, including antiatherogenic, cardioprotective, antiplatelet and antiulcer activities of the seed oil, as well as antioxidative activity of leaf extracts, have been demonstrated using cell culture and animal models (Johansson et al. 2000; Xing et al. 2002; Narayanan et al. 2005). The health protection properties of seabuckthorn largely depend on the composition of seabuckthorn fatty acids. Therefore, we analyzed the chemical composition of FA in fruit pulp and oils of most promising 4 ecotypes of seabuckthorn, including an exotic, growing in dry temperate Himalayas.

MATERIALS AND METHODS

Fatty acid composition of oils of fruit pulp and seeds was studied in 9 years old stands of *Hippophae rhamnoides* ssp. *turkestanica*, selected from Madhgaon village in Lahaul (Lahaul form), a semi-arid region (rainfall about 450 mm per year) and Shego village in Spiti (Spiti form), an arid region (rainfall about 125 mm), *H. salicifolia* ("Drilbu" cultivar) selected from "Tinu village" in Lahaul and "HI-1" form, an exotic (*H.rhamnoides ssp. mongolica*), from Altai, Russia, all raised at High Land Agricultural Research and Extension Center of CSK Himachal Pradesh Agricultural University, located at Kukumseri (2730 m asl) in Lahaul valley of district Lahaul-Spiti, a dry temperate region of Himachal Pradesh, a Himalayan region of India. Lahaul-Spiti is a cold desert area, quite rich in seabuckthorn resources (Fig. 1, Table 1).

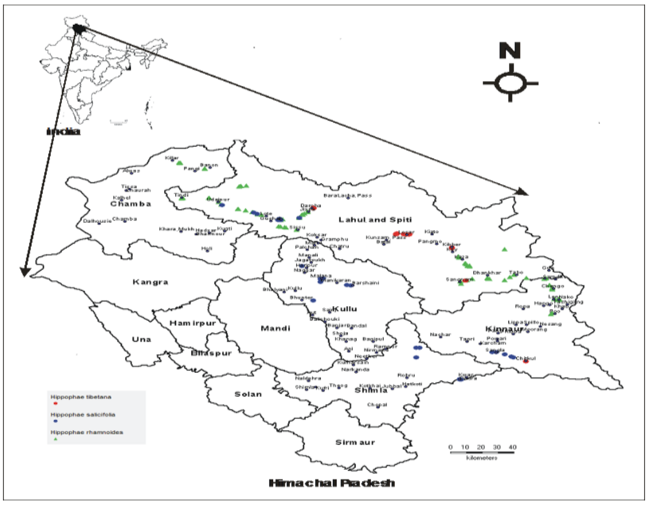

Fig. 1. Lahaul-Spiti and seabuckthorn growing areas in Himachal Himalayas

The place experiences a low rainfall (450 mm per) and heavy snow fall (200 cm per year) during winter (-20^{o}C). Fruits of seabuckthorn forms of *H. rhamnoides* ssp. *turkestanica* and *H. salicifolia* were collected during last week of September, whereas fruits of exotic "HI-1" were collected during second week of August, when they were fully ripe (Fig. 2).

Fruits were hand cleaned to remove dry leaves, branches and fruits damaged by harvesting and then wind screened. They were frozen at 0^{o}C before use. In order to extract high quality oil, only ripe fruits were used for analysis. They were cleared and washed in distilled water. The fruit pulp was separated from the seed and seed coat by mixer operated for short time intervals (about 3 seconds repeatedly). A choloroform/methanol mixture was used to estimate the total lipids of seabuckthorn fruit. 1 gm of pulp of fruit was homogenized in 10 ml methanol for 2 minutes in a blender followed by adding 20 ml choloroform and them homonizing for 5 minutes. The mixture was centrifuged at 1500 x g and then filtered through filter paper. The filtrates and washings were combined, and one-fourth of the total volume 0.88% aqueous potassium chloride solution was added, shaken well, and allowed to settle. The lower layer was separated off and washed with methanol/water (1:1 v/v). The purified lipid layer was filtered and dried over anhydrous Na2SO4, the solvent was removed in a rotary film evaporator, and the amount of lipids was noted. Lipids were stored in chloroform at −20°C for further analysis (Kallio *et al.*, 2002).

Table 1-Characteristics of four seabuckthorn ecotypes of growing at HAREC, Kukumseri (2730 m asl), dry temperate Himalayas

Source of genotype	**Madhgaon**	**Shego**	**Drilbu**	**HI-1**
Species	*H.rham*.ssp. *turkestanaica*	*H.rham*.ssp. *turkestanaica*	*H. salicifolia*	*H.rhamnoides* ssp. *mongolica*
Place of origin	Madhgaon, Lahaul	Shego, Spiti	Tinu, Lahaul	Altai, Russia
Altitude (m asl)	2625	3760	3200	NA
Climate	Semi-arid	Arid	Semi-arid	Semi-arid
Rainfall (mm yr^{-1})	450	75	200	NA
Fruit weight (g/100)	13.5	18.2	30.0	30.5
Fruit colour	Reddish orange	Reddish orange	Yellow	Yellowish-orange

Note: Rainfall and snowfall at Kukumseri are 450 mm and 200 cm/year, respectively (Average of last 5 years), NA= Not available.

a. b. c. d.

Fig. 2. Fruits of seabuckthorn genotypes of a. Madhgaon, b. Shego, c. Drilbu and d. HI-1

In order to measure the fatty acids, FAME of berry lipids was prepared according to the IUPAC method (Paquot and Hautefenne, 1987)). FAME was estimated by a Hewlett-packard 5890 series II model gas chromatograph equipped with an FID. The column used in the analysis was an HP-FFA (cross-linked FFAP: 30 m X 0.5 mm X µm; Hewlett-Packard, Avondale, PA). Injector port temperatures were 250 and 300^0 C, respectively. The column temperature was maintained at 100^0 C for 1 minute and then enhanced to 180^0 C at 5^{0}C/min and kept at that temperature for 15 minutes. The carrier gas used was nitrogen at 20 mL/min. By comparing with standard, FAME run under the same conditions, component FAME were identified. The separetd FA were identified using a standard mixture of known fatty acids (sigma Chemical Co, USA). The Quantities of various FA are presented in percentage (%) of their total quantity. The estimations were done based on the SPSS programme.

RESULTS

PULP OIL COMPOSITION

It was found that pulp of "Madhgaon" had highest oil content (25.3% on dry weight basis), followed by exotic HI-1 (21.5%), "Shego" (19.4%) and a minimum value of 16.1%) in "Drilbu" (Table 2).

Saturated Fatty Acids: In pulp oil, saturated fatty acids (SFAs) make 28.5-29.8% of total oil and much lesser of 10.6% in "Drilbu". Among the saturated fatty acids, palmitic acid is a major fatty acid, value being the almost equal (26.4-28.1%) in all three forms, except much lesser value in "Shego" (9.1%). Next SFA was stearic acid was maximum in "HI-1" (1.1%), which was higher than three forms (0.7-0.8%). Similar was the trend for myristic acid. Arachidic acid was highest and same in "HI-1" and "Drilbu" ecotype (0.2%). Tricosanoic Acid was absent in both local forms of ssp. *turkestanica* (Table 2). Others SFAs were present in negligible amounts.

Table 2- Total oil and fatty acids profile in fruit pulp of four ecotypes of seabuckthorn

	Madhgaon	**Shego**	**Drilbu**	**HI-1**
Total oil (% on dry weight basis)	25.25±1.27	19.44±0.9	16.11±0.7	21.54±1.3
Saturated Fatty Acids				
Butyric Acid (4:0)	0.004±0.0001	Abs	0.008±0.0001	Abs
Caproic Acid (6:0)	0.005±0.0001	0.006±0.0001	0.011±0.0002	0.008±0.0001
Caprylic Acid(8:0)	0.004±0.0001	0.003±0.0001	0.006±0.0002	0.009±0.0001

Capric Acid (10:0)	0.004±0.0001	0.007±0.0001	0.006±0.0001	0.009±0.001
Lauric Acid (12:0)	0.017±0.0003	0.019±0.0002	0.022±0.0005	0.047±0.0003
Myristic Acid (14:0)	0.230±0.0015	0.292±0.008	0.258±0.006	0.448±0.012
Pentadecanoic Acid (15:0)	0.008±0.0002	0.048±0.002	0.060±0.002	0.068±0.003
Palmitic Acid (16:0)	27.602±1.937	9.137±0.17	28.138±1.140	26.376±1.605
Heptadecanoic Acid (17:0)	0.037±1.700	0.029±0.0002	0.047±0.002	0.061±0.003
Stearic Acid (18:0)	0.704±0.023	0.750±0.025	0.829±0.031	1.077±0.028
Arachidic Acid (20:0)	0.154±0.009	0.166±0.004	0.196±0.008	0.200±0.005
Behenic Acid (22:0)	0.055±0.003	0.059±0.002	0.071±0.002	0.072±0.002
Tricosanoic Acid (23: 0)	Abs	Abs	0.170±0.006	0.088±0.006
Lignoceric Acid (24:0)	0.054±0.002	0.080±0.003	0.068±0.002	0.083±0.003
Total saturated fatty acids (%)	28.878±2.17	10.596±0.605	29.86±1.400	28.546±0.965
Unsaturated Fatty Acids				
Palmitoleic Acid (16:1)	46.423±3.173	38.439±4.065	37.121±2.648	37.674±2.260
Cis-10 Heptadecenoic Acid (17:1)	0.069±0.004	0.052±0.002	Abs	Abs
Oleic Acid (18:1) n9c	4.038±0.35	6.891±0.325	4.576±0.233	4.068±0.238
Linoleic Acid (18:2) n6c	9.317±0.57	6.384±0.437	15.042±0.635	10.134±0.760
γ-Linolenic Acid (18:3) n6	0.042±0.003	0.046±0.003	0.043±0.002	0.022±0.001
α-Linolenic Acid (18:3) n3	0.632±0.040	0.820±0.028	1.307±0.106	0.794±0.045
Cis-11-Eicosenoic Acid (20:1) n9	0.071±0.005	0.087±0.005	0.095±0.007	0.037±0.002
Cis-11, 14-Eicosadienoic Acid (20:2) n9	0.021±0.001	0.015±0.001	0.035±0.002	0.016±0.001
Arachidonic Acid (20:4) n6	0.017±0.001	0.028±0.003	0.033±0.002	0.034±0.002
Erucic Acid (20:1) n9	0.017±0.001	0.026±0.002	0.070±0.005	0.023±0.001
Nervonic Acid (24:1) n9	0.033±0.002	0.035±0.002	Abs	Abs
Total unsaturated fatty acids (%)	60.68±3.45	52.823±4.835	58.934±3.618	52.802±4.684

Note: Values are percentage of the total, Lahaul= Semiarid form of *H.rhamnoides* ssp. *turkestanica*, Spiti= Arid form of *H.rhamnoides* ssp. *turkestanica*, *H.sal.*= *H.salicifolia*, "HI-1"= exotic form of *H.rhamnoides*. All values are presented as means, n=5. Abs-Absence.

UNSATURATED FATTY ACIDS

In pulp, unsaturated fatty acids constitute most of the oil, varying from a maximum of 60.7% in "Shego", followed by "Tinu" (58.9%) and lesser and same value (52.8%) in other two forms. Palmitoleic acid, a very useful; UFA, was the major unsaturated fatty acid, being maximum in "Shego" (46.4% of the total), which was much higher than other three forms, having almost the same values (37.1-38.4). Linoleic acid, another important fatty acid varied from a maximum of 15.0% in "Tinu", followed by "HI-1" (10.1%), "Madhgaon" (9.3%) and a minimum of 6.4% in "Shego". α-Linolenic acid, also very important unsaturated fatty acid, being also maximum in "Tinu" (1.3%), however is quite low in pulp oils of all three forms (0.8-0.6%). Oleic acid varied from a maximum of 6.9% in "Shego" to a minimum of 4.0% being same both in "Madhgaon" and "HI-1", which was lesser than "Tinu" (4.6%). Nervonic Acid was absent in "Tinu" and "HI-1", whereas present in small amounts in other two forms (Table 2, Fig. 3)).

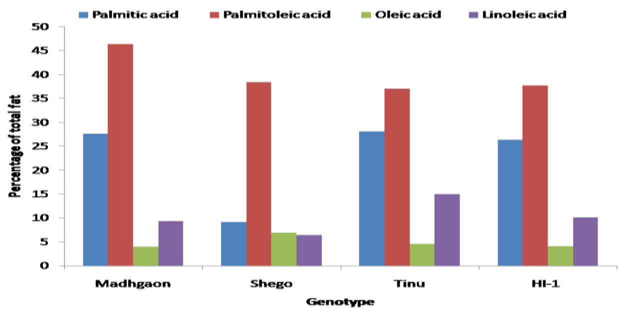

Fig. 3. Proportion of major fatty acids in the pulp oils of 4 genotypes of seabuckthorn

SEED OIL COMPOSITION

The oil content of seabuckthorn seeds was found to be maximum in "Shego" (26.3%) followed by "HI-1" (13.0%), "Madhgaon" 10.2%) and a minimum value in "Tinu" (3.5%) (Table 3).

SATURATED FATTY ACIDS

In seeds, saturated fatty acids (SFAs) make about 40.2% of total oil in "Tinu", whereas it is 27.1%, 22.7% and 12.4% in "HI-1", "Madhgaon" and "Shego", respectively. Among the saturated fatty acids, like pulp oil, in seed oil also, palmitic acid is most abundant, being maximum in "Tinu" (29.3%) followed by "HI-1" (17.4%), "Madhgaon" (14.8%) and a minimum in "Shego" (8.8%). The next SFA is stearic acid, being almost same in all forms (5.8-4.6%), except in "Shego form" (2.8%). Pentadecanoic acid was present in trace amount in "Shego", whereas tricosanoic acid was absent in "Tinu". Interestingly, all other SFAs, though constitute small amounts, are again highest in "Tinu" and lowest in "Shego" (Table 3).

Table -3 Total oil and fatty acids profile in seeds of four ecotype of seabuckthorn

	Madhgaon	Shego	Drilbu	HI-1
Total oil (on dry weight basis)	10.18± 0.715	26.29	3.47	13.01
Saturated fatty acids				
Butyric Acid (4:0)	0.013±0.001	0.005±0.001	0.051±0.003	0.027±0.002
Caproic Acid (6:0)	0.281±0.027	0.019±0.002	0.682±0.028	0.536±0.049
Caprylic Acid(8:0)	0.352±0.024	Abs	0.732±0.031	0.714±0.035
Capric Acid (10:0)	0.021±0.001	0.003±0.001	0.080±0.003	0.037±0.002
Lauric Acid (12:0)	0.032±0.002	0.013±0.001	0.096±0.006	0.045±0.002
Myristic Acid (14:0)	0.281±0.023	0.143±0.017	0.698±0.045	0.470±0.025
Pentadecanoic Acid (15:0)	0.291±0.025	Tr	0.658±0.032	0.329±0.017
Palmitic Acid (16:0)	14.800±2.178	8.772±0.364	29.259±2.504	17.408±1.266
Heptadecanoic Acid (17:0)	0.081±0.003	0.052±0.003	0.167±0.008	0.100±0.007
Stearic Acid (18:0)	4.641±0.360	2.813±0.205	5.499±0.246	5.767±0.360
Arachidic Acid (20:0)	0.848±0.052	0.432±0.029	1.178±0.097	0.940±0.055
Behenic Acid (22:0)	0.476±0.034	0.112±0.007	0.813±0.407	0.309±0.021
Tricosanoic Acid (23: 0)	0.325±0.028	0.055±0.002	Abs	0.280±0.015
Lignoceric Acid (24:0)	0.313±0.019	0.052±0.003	0.342±0.029	0.210±0.013
Total saturated fatty acids (%)	22.755±2.48	12.471±0.809	40.255±2.864	27.172±1.570
Unsaturated Fatty Acids				
Palmitoleic Acid (16:1)	4.738±0.261	2.588±0.173	8.088±0.503	4.682±0.307
Oleic Acid (18:1) n9c	23.255±1.980	16.333±0.961	14.759±0.983	27.413±2.066
Elaidic Acid (18:1) n9t	3.215±0.203	1.781±0.114	4.026 ±0.221	2.971±0.219
Linoleic Acid (18:2) n6c	23.792±1.750	39.833±2.173	3.737±0.281	11.756±0.960
γ-Linolenic Acid (18:3) n6	Tr	0.012±0.001	0.056±0.003	0.025±0.001
α-Linolenic Acid (18:3) n3	8.685±0.517	25.435±2.410	0.339±0.017	3.597±0.263
Cis-11-Eicosenoic Acid (20:1) n9	0.497±0.021	0.241±0.017	1.255±0.090	0.786±0.401
Cis-11,14-Eicosadienoic Acid (20:2) n9	0.061±0.003	0.050±0.002	0.035±0.002	Abs
Arachidonic Acid (20:4) n6	0.577±0.041	0.051±0.004	0.274±0.028	0.224±0.019
Erucic Acid (20:1) n9	0.136±0.009	0.018±0.001	0.127±0.009	0.041±0.001
Nervonic Acid (24:1) n9	0.161±0.008	0.160±0.012	Abs	0.154±0.008
Total unsaturated fatty acids (%)	65.117±3.825	86.502±6.760	32.891±2.510	51.649±4.471

Note: Values are percentage of the total, Lahaul= Semiarid form of *H.rhamnoides* ssp. *turkestanica*, Spiti= Arid form of *H.rhamnoides* ssp. *turkestanica*, *H. sal.*= *H. salicifolia*, "HI-1"= exotic form of *H. rhamnoides*. All values are presented as means, n=5. Abs-Absence. Tr-Trace

UNSATURATED FATTY ACIDS

In seeds, unsaturated fatty acids constitute a major part of total oil (86.5-51.7%), except in "Tinu" (32.9%). The amount of unsaturated fatty acids was maximum in "Shego" (86.5%) from an arid region, followed by "Madhgaon" (65.1%) and "HI-1" (51.7%) and a minimum amount in "Tinu" (32.9%) (Table 3, Fig. 4).

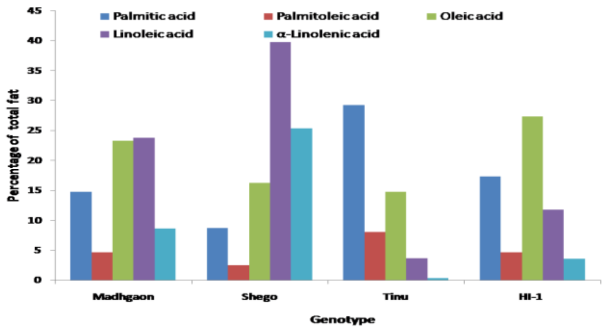

Fig. 4 Proportion of major fatty acids in the seed oils of 4 genotypes of seabuckthorn

Among the unsaturated fatty acids, unlike pulp, linoleic acid is the most abundant in the seed oils and varied from a maximum of 39.8% in "Shego" followed by "Madhgaon" (23.8%), "HI-1" (11.8%) and a minimum of 3.7% in "Tinu". Oleic acid was also present in high amount, being maximum in "HI-1" (27.4%), followed by "Madhgaon" (23.3%), "Shego" (16.3%) and a minimum of 14.8% in "Tinu". Similarly, another omega AFA α-linolenic acid value was highest in "Shego" (25.4%) considerably higher than "Madhgaon" (8.7%), "HI-1" (3.6%) and a minimum and insignificant amount in "Tinu" (0.3%) (Fig. 4). "Tinu" was, however, was most rich in Palmitoleic acid (8.1%), value being lesser and almost same in "Madhgaon" and "HI-1" and lowest in "Shego". Cis-11-eicosaneoic acid found in very low content, was also maximum in "Tinu" cultivar (1.3%) and a minimum in "Shego" (0.2%). Other UFAs were present in insignificant amounts, except elaidic acid (1.7-4.2%). γ-Linolenic Acid was present in very small amount in all forms and in trace amount in "Madhgaon", whereas, nervonic Acid was absent in "Tinu" (Table 3).

COMPARISON BETWEEN PULP AND SEED OILS

While comparing the oils of pulp and seeds of various forms of seabuckthorn, the proportion of UFAs was generally higher in seed oils (86.5-51.6%) than pulp oils (60.7-52.8%) with the exception of "Tinu". However, for palmitoleic acid, an omega UFA, pulp was richer (46.4-37.1%) than the seeds (8.1-2.6 %). However, in case of linoleic acid, another omega UFA, the seeds were richer (39.8-3.7%) than the pulp (15.0-6.4%). Oils of seeds were also richer in α-linolenic acid, an Omega UFA (25.4-0.3%) than the pulp oils (1.3-0.6%). Similarly seeds have higher amount of oleic acid (27.4-14.8%) than the pulp (6.9-4.0%).

Among the saturated fatty acids, palmitic acid had a similar content both in pulp (28.1-9.1%) and seeds (29.3-8.8%). Stearic acid was more in seeds (5.8-2.8%) than the pulp (1.1-0.7%). Myristic acid was present in very low and same content in both pulp and seeds. Seeds were richer in arachidic acid (1.2-0.4%) than the pulp (0.2%).

Palmitic acid and palmitoleic acid were the predominant fatty acids in the pulp oils of four forms of seabuckthorn, followed by linoleic acid and oleic acid and other FAs in insignificant amounts. In seed oils, linoleic acid, oleic acid, and palmitic acid and, are predominant FAs, followed by palmitoleic acid and α-linolenic Acid is a predominant fatty acid in "Shego" ecotype.

DISCUSSION

In present study, in seed oil as well as pulp oil, unsaturated fatty acids (UFAs) constitute majority of the oil in all the forms of seabuckthorn in the present study, except for the seeds of "Tinu" (*H. salicifolia).* The UFAs particularly, palmitoleic acid, linoleic acid and linolenic acid are considered very important for health functions in human beings.

In the present study, seabuckthorn seed oil is rich in the two essential fatty acids, linoleic (18:2 n-6) and α--linolenic (18:3 n-3) acids. The proportion of linoleic acid of total oil generally is 39.8-11.8%, whereas α-linolenic acid makes (25.4-8.7%), with low values in *H. salicifolia* and exotic ssp. *mongolica.* The proportion of palmitoleic acid, a rare essential UFA was also 8.1-2.6%. The other major fatty acids in seeds are UFA oleic acid (27.4-14.8%) and SFA palmitic acid (29.3-8.7%). The other FAs are stearic acid (5.8-2.8%) and elaidic Acid (4.2-18%), which are similar as reported in ssp. *sinensis* (from China), *mongolica* (from Russia) and *rhamnoides* (from Finland) have almost identical fatty acid composition in seeds (Franke and Mu¨ ller 1983; Kallio et al. 1999, Kallio et al. 2002; Yang and Kallio 2001; Yang and Kallio 2002).

Different species of Indian seabuckthorn showed difference in abundance of various FAs in the seed oil. Among the omega fatty acids, linoleic acid (39.8%) and α-linolenic acid (25.4%) were highest in "Shego" cultivar of ssp. *turkestanica* a genotype naturally growing at higher altitude in arid region of Spiti, a trans-Himalayan region, bordering with Tibet, followed by another form "Madhgaon" cultivar of ssp. *turkestanica* a genotype naturally growing at lower altitude in arid region of Lahaul, a semi-arid region and other two forms. Does it have something to do with adaptation to extreme conditions of Shego at higher altitude? However, interestingly, all other FAs of the seed oil are higher in "Madhgaon" than "Shego". Linoleic acid proportion was also quite adequate in exotic "HI-1" exotic (11.8%). Pamitoleic acid was, however, maximum (8.1%) in the seed oil of *H. salicifolia*. Oleic acid was highest in "HI-1". Among the SFAs, palmitic acid is highest in seed oil of "Tinu" followed by "HI-1" and others.

Seed oil of "Shego" cultivar of *H. rhamnoides* ssp. *turkestanica*, is rich in the occurrence of essential UFAs, linoleic acid (39.8%) as well as α-linolenic acid (25.4%), values being also quite high (23.8% and 8.7%, respectively) in "Madhgaon" cultivar of this subspecies, a selection from the lower altitude of semi-arid region of Lahaul. Seed oil of another local form "Tinu" cultivar is high in palmitoleic acid (8.1%). The values of UFAs for exotic "HI-1" cultivar has intermediate values in UFAs and meets the requirements of quality seed oil. Presence of high proportion of essential UFAs along with oleic acid in the seed oils, the local forms of *H. rhamnoides* ssp. *turkestanica* in the present study, make them a useful source of quality seed oil for pharmaceutical industries. This differentiates seabuckthorn from other vegetable oils and makes it unique for health function (Ursin 2003).

While in pulp oils, predominant essential unsaturated fatty acid are palmitoleic acid (16:1 n-7, 46.4-37.6%), linoleic acid (18:2 n-6, 15.0-6.4%) and UFA oleic acid (6.9-4.1%). In the present study too, the amount of palmitoleic acid correlates negatively with that of oleic acid, as reported by others (Kallio et al. 2002; Yang and Kallio 2001). The maximum level of palmitoleic acid (up to 46.4%), and the lowest level of oleic acid (as low as 4.0%) were found in subsp. *turkesatnica* from Madhagon, from semi-arid region. Others are present in insignificant amounts. Therefore, palmitoleic acid is a predominant UFA in pulp oils in the present study, as also reported for Chinese and European seabuckthorn (Yang and Kallio, 2002). Palmitic acid was the predominant among the saturated fatty acids both in pulp and seed oils, as reported for Chinese and European seabuckthorn Like findings of other studies on Chinese and European (Yang and Kallio 2002), Russian and German seabuckthorn (Jamyansan and Badgaa,2005; Mörsel et al. 2005), in the present study, palmitoleic acid (46.4-37.1%) is a predominate essential UFA in the pulp oil. Fatima *et al.* (2012) have also found palmitoleic acid as a dominant SFA (32-42%) followed by palmitic acid (41-34%) in pulp oil in Canadian seabuckthorn, which are introduction from Russia. Since palmitoleic acid is a major constituent of skin fat, the pulp oil is used for cosmetic and healing purposes (Gupta et al. 2008). Pulp oils of all the forms in the present study are very rich in palmitoleic acid, being in "Madhgaon" cultivar of ssp. *tukestanica*. However, "Tinu" form of *H. salicifolia* having maximum values in the occurrence of essential UFAs, linoleic acid (15.0%) as well as α-linolenic acid (1.3). Therefore ,we can say that the pulp oil of "Tinu" (Drilbu cultivar) is most suitable for all essential UFAs. The exotic "HI form" along with other forms also meet this requirement of quality pulp oil.

Presence of high levels of omega 6 and 3 fatty acids in oil for human consumption is beneficial, but it is the low ratio of omega 6 and 3 fatty acids, which is important, particularly in the range of 1:1-3:1 (Simopoulos 2002), which is important for prevention of various diseases. Global studies (Yang and Kallio 2002) have shown that the unsaturated fatty acid composition of seabuckthorn seed oil is particularly interesting due to occurrence of appropriate ratio of v-3 fatty acids as compared to v-6 fatty acids. A high amount of v-3 fatty acid is not common in seed oils, and is usually accompanied by a high v-6: v-3 ratio, with the exception of flaxseed oil, having more than 50% α-linolenic acid. As the health food industry is looking for the production of v-3 fatty acid-enriched products, both for the human consumption and as animal feed. In the present study, the ratio of v-3 fatty acids to v-6 fatty acids varied from a maximum of 11:1 in "Tinu" followed by 3:1 in Madgaon", growing at lower altitude (2700 m asl) and exotic ssp. *mongolica* and a minimum ratio of 1.5:1 in "Shego", growing at higher altitude of arid region of Spiti. That shows that growing seabuckthorn at high altitude is beneficial than low altitude. Furthermore, seed oils of sp. *rhamnoides* are better source of omega fatty acids than ssp. *salicifolia* and shows a very promising source of UFAs for human health and nutrition. Presence of high level of oleic acid (omega 9), particularly in seed oils of all 4 forms make them useful for human consumption.

However, the values of ratio of v-6: v-3 ratio in pulp oil were 16:1 for "Madhgaon" ecotype, 8:1 for "Shego" ecotype, 12:1 for ssp. *salicifolia* and 13:1 for exotic HI-1. Therefore, ratio of v-6: v-3 ratio for all the pulp oils are very high, being a minimum in case of "Shego" ecotype in the present study.

A very high level of palmitoleic acid (Omega 7 FA) has also been reported in only few plants, like seabuckthorn pulp oil and macadamia nut (*Macadamia integrifolia)* oil (Cavaletto 1983). The pulp oil of all the species and subspecies of the present study are useful source of palmitoleic acid. Palmitoleic acid is known for its lower susceptibility to oxidation as compared to other polyunsaturated fatty acids, which may give it a utilization advantages such as its stability during frying and baking.

While its utilization in cosmetic industry is common, however, information is only limited on the effects of palmitoleic acid on cardiovascular disorders. Palmitoleic acid has been reported to prevent beta-cell apoptosis induced by glucose or saturated fatty acids (Morgan and Dhayal, 2010; Morgan et al. 2008). The diets, rich in palmitoleic acid, improved the circulating lipid profile in both animal model (Matthan et al. 2009) and human beings (Griel et al., 2008; Garg et al., 2003). A recent study has also found that palmitoleic acid functions as an adipose tissue-derived lipid hormone that stimulates muscle insulin action and suppresses hepatosteatosis in mice deficient in fatty acid binding protein (Cao et al. 2008). Recently, it has also been observed that palmitoleic acid improves hyperglycemia and hypertriglyceridemia by enhancing the insulin sensitivity, in part owing to suppressing proinflammatory gene expressions and improving hepatic lipid metabolism in diabetic mice (Zhi-Hong et al. 2011).

Matthan et al. (2009) observed the effects of dietary palmitoleic acid in hamsters, without any adverse effects on the plasma lipoprotein profiles or aortic cholesterol accumulation. Nestle et al. (1994) and Parameshwari and Nazni (2012) found palmitoleic acid to have effects similar to palmitic acid (saturated fatty acid) on plasma total and low density lipoprotein-cholesterol concentrations. Therefore, besides cosmetic industry, there is a lot of potential of palmitoleic acid as sustainable feedstock for producing industrially important octane, which is used as a comonomer in the expanding production of linear low-density polyethylene (Nguyen et al. 2010). Nguyen et al. (2010) showed the evidence in support of the theory that v-7 fatty acid amount can be increased to high levels in plants. Arabidopsis has been metabolically engineered to produce v-7 fatty acid to as high as 71%. As seabuckthorn is low-input, high yielding plant that can also grow on marginal lands, which are available in plenty in Himalayas, therefore, the pulp oil of seabuckthorn could be used as a sustainable source of plant feedstock for industrially important chemicals due to its unusually high level of palmitoleic acid. Then, in case, seabuckthorn pulp D9 desaturase enzyme is found to have higher specificity for conversion of 16:0 to 16:1D9 than its orthologs from other plants, the concerned gene can be used for increasing the monounsaturated fatty acid levels in plants and other living beings.

CONCLUSION

The present study provides valuable information about the fatty acid composition of the in the fruit oil and seed oils extracted from four different cultivars of seabuckthorn. Comparing with the other Asiatic and European subspecies, all berry parts of the analyzed cultivars, except *H. salicifolia*, exhibited higher oil content.

The seed oil of *H. rhamnoides ssp. turkestanica*, particularly from high altitude presented the highest ratio of v-6: v-3 ratio values, which were close to the recommended PUFA/SFA intake. The seed oils of *H. rhamnoides ssp. turkestanica* particularly from higher altitude area could be considered excellent sources of PUFAs due to their high contents of linoleic and α-linolenic acids, which in human body are precursors of other long-chain n-3 and n-6 fatty acids. Furthermore, the pulp oils of all the forms of seabuckthorn are the important source of palmitoleic acid, being considered useful for cosmetic industry and cardiovascular diseases. Pulp oil of *H. salicifolia* is also a useful source of palmitoleic acid and linoleic acid. The exotic form *H. rhamnoides* ssp. *mongolica*, an introduction from Altai region of Russia, is also moderate in the composition of UFAs and has potential for introduction in Himalayas. The results obtained in the present work are useful to identify suitable seabuckthorn cultivars for planning the berry breeding programme and also provides important information for food and pharmaceutical industry.

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