Fatty Acids, Tocols, and Carotenoids in Pulp Oil of Three Sea Buckthorn Species (*Hippophae rhamnoides, H. salicifolia,* and *H. tibetana*) Grown in the Indian Himalayas

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ABSTRACT: Sea buckthorn berries from *Hippophae rhamnoides, H. tibetana,* and *H. salicifolia* were collected from the cold deserts of the Himalayas (Lahaul, Ladakh, and Spiti; India) and characterized in terms of the FA, carotenoid, tocopherol, and tocotrienol composition in their pulp oil. These varied from species to species. Total carotenoids ranged from 692 to 3420 mg/kg in pulp oils of fresh berries, and total tocols, from 666 to 1788 mg/kg. *Hippophae salicifolia* berries contained substantially lower amounts of pulp oil, with lower levels of carotenoids and tocopherols. There was little difference in the proportion of individual tocols in pulp among the three species. α-Tocopherol alone constituted 40–60% of total pulp tocols in berries. Pulp oils had palmitoleic acid (32–53%) as the most abundant FA followed by palmitic (25–35%), oleic (8–26%), linoleic (5–16%), and linolenic (0.6–2.6%) acids, with the highest deviation observed in the proportion of palmitoleic acid in these berries. *Hippophae rhamnoides* and *H. tibetana* contained the highest amount of the lipophilic carotenoids and tocols. *Hippophae salicifolia* berries had higher amounts of lipophobic constituents such as vitamin C and flavonols.

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Sea buckthorn (SB), a member of the *Hippophae* genus, grows in regions of Asia, Europe, and North America. In summer, plentiful round yellow-orange berries cover the female plants. These fruits have a long history of applications in Tibetan and Mongolian medicine (1). SB berries consist of a fairly tough skin and juicy pulp enveloping a small, hard, oval seed. Ripe berries from *H. rhamnoides* are orange-red and have a diameter of 10–15 mm and a soft outer fleshy tissue and hard seed. Soft parts of the berries, or pulp, contain 3–5% oil and seed contains 6–15% oil (1). SB berries are an excellent source of bioactive phytochemicals such as carotenoids, tocopherols, vitamin C, organic acids, and polyphenols (2). A wide spectrum of physiological effects of SB berries and berry products has been reported, including inhibition of LDL cholesterol oxidation and platelet aggregation, reduction of atopic dermatitis, immunomodulation, cytoprotective effects, and protection from gastric ulcer (3–7).

Both the soft parts (pulp and peel) and seeds contain oil that has high levels of tocopherols, carotenoids, and plant sterols. Both the oils have a quite different FA composition and hence different nutritional properties. Whereas seed oil TAG are rich in linoleic (18:2), a-linolenic (18:3), oleic (18:1), and palmitic acids (16:0), the pulp oil is rich in palmitoleic acid (16:1) which is rare among oils from plant sources (1,8,9). The reported chemical composition of SB berries varies considerably. This may be because of different origins/subspecies, the climate and geographical conditions of growing areas, and agronomic practices (1). A systematic mapping of the chemical composition of SB berries of different varieties and origins is still lacking.

In India, SB is grown in the Trans-Himalayan cold deserts such as Ladakh, Lahaul, and Spiti at altitudes above 2500–4500 m. The Ladakh region extends between 32°15′–36°N latitude and 75°15′–80°15′E longitude; Spiti and Lahaul lie between 31°44′–32°59′N latitude and 76°46′–78°41′E longitude. *Hippophae rhamnoides, H. salicifolia*, and *H. tibetana* are the predominant SB species in India. Of these, *H. rhamnoides* is widely distributed in the Trans-Himalayan region, but *H. salicifolia* and *H. tibetana* are observed only in the Lahaul and Spiti regions, respectively (10–13). The SB-growing areas in the Trans-Himalaya host unique geoclimatic conditions of high altitude coupled with extreme temperature variations (–40 to 30°C), low precipitation, and low oxygen in air. In these cold deserts plants are under extreme climatic stress and are expected to have a distinct phytochemical profile. No systematic studies have carried out from this perspective on Himalayan SB berries, particularly on *H. salicifolia* and *H. tibetana*, which are exclusive to high altitudes of the Himalayas. This paper deals with the chemical composition of pulp oil of three major SB species from different locations in the Himalayas.

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MATERIALS AND METHODS

SB berries. Berries of three SB species from major SB growing areas in the Trans-Himalayan region of India were used in this study. Fresh berries of *H. rhamnoides* were collected from the Indus valley of Ladakh (3,500–4,000 m), Spiti (3,500–4,500 m), and Lahaul (2,500–3,000 m) regions. *Hippophae tibetana* and *H. salicifolia* berries were collected from Spiti and Lahaul regions, respectively. Berries were collected in triplicate from three different location (3 × 3) of the same geographic area and labeled as Lahaul 1, Lahaul 2, and Lahaul 3. Fruits were collected during June to November of 2003 at the stage of commercial maturity, as judged by juiciness and appearance. Berries from Ladakh, Lahaul, and Spiti (*H. rhamnoides* and *H. tibetana)* were collected and identified with the help of experts from the Field Research Laboratory, Leh and Himachal Pradesh Agricultural University, Kullu. Berries were stored in polyethylene bags and ferried to the Regional Research Laboratory Trivandrum by air under cold conditions and stored at −20°C until analysis.

Chemicals and reagents. Biochemical standards such as β-carotene, FA, and ascorbic acid were obtained from Sigma Chemical (St. Louis, MO). Tocopherols/tocotrienols were purchased from Calbiochem (Merck Ltd., Bombay, India). Hexane, isopropyl alcohol, methanol, and water of HPLC grade were obtained from Merck Ltd. Other chemicals and reagents were of analytical grade.

Composition of berries. Seeds were separated from the berries just before analysis at the laboratory. Frozen berries were taken from the freezer (−20°C), thawed at 25°C for 1 h, and crushed gently so that the seeds remained intact. The seeds were then separated manually from the pulp. Proximate composition of SB berries was determined in duplicate using AOAC Methods for moisture (No. 930.15), crude protein (No. 988.01), ash content (No. 942.2), and seed content (No. 949.08) (14). Analysis was carried out in triplicate, and mean values and SD were determined.

Vitamin C. For the extraction of vitamin C, 5 g of the pulp was ground in a mortar and extracted repeatedly with a 6% TCA solution (5 × 5 mL). Extracts were pooled and then filtered. The solution was kept under refrigeration until use. Vitamin C was analyzed by the dinitrophenylhydrazine method (15).

Flavonols. Flavonols in the berries were extracted using 95% ethanol and stored in amber bottles under refrigeration. The amount of total flavonols was estimated by the aluminum chloride method suggested by Chang *et al*. (16). Quercetin, which has a moderate absorbance at 415 nm, was used as standard, and the absorbance of mixtures was measured at this wavelength (16).

Extraction of lipids. The total lipids of the berries were extracted using a chloroform/methanol mixture. Pulp (1 g) of berries was homogenized in methanol (10 mL) for 2 min in a blender, then chloroform (20 mL) was added and homogenization continued for 5 min more. The mixture was centrifuged at 1500 × *g* and filtered through filter paper. Solid residue was resuspended in chloroform methanol (2:1 vol/vol, 30 mL) for 5 min. The mixture was filtered, and the residue was washed with a chloroform/methanol mixture (1:1, vol/vol, 30 mL). The filtrates and washings were combined, and one-fourth of the total volume of a 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 vol/vol). The purified lipid layer was filtered and dried over anhydrous sodium sulfate, the solvent was removed in a rotary film evaporator, and the amount of lipids (pulp oil) was noted. Lipids were stored in chloroform at −20°C for further analysis. This pulp oil was used for the analysis of FA, carotenoids, tocopherols, and tocotrienols (17).

FA. FAME of berry lipids were prepared according to the IUPAC method (18). FAME were quantified using a Hewlett-Packard 5890 series II model gas chromatograph equipped with an FID. The column used was an HP-FFA (cross-linked FFAP: 30 m × 0.5 mm × 1 μm; Hewlett-Packard, Avondale, PA). Injector and detector port temperatures were 250 and 300°C, respectively. The column temperature was maintained at 100°C for 1 min and then increased to 180°C at 5°C/min and maintained at that temperature for 15 min. The carrier gas used was nitrogen at 20 mL/min. By comparing with standard FAME run under the same conditions, component FAME were identified. FA composition was expressed as a weight percentage of the total FA.

Carotenoids. Pulp oil (1 mL) was dissolved in hexane (0.1 g/mL), vortexed 30 s with 0.5 mL of 0.5% NaCl, and centrifuged for 10 min at 1500 × *g*. Supernatant was diluted and measured at 460 nm (Shimadzu UV-2450 UV-vis spectrophotometer). The calibration curve was plotted with an authentic standard of β-carotene, and the amount of total carotenoids was expressed in terms of β-carotene (19).

Tocopherols/tocotrienols. Pulp oils were dissolved in hexane and analyzed in a Shimadzu HPLC binary system (LC-10A) with an LC-10AD pump, a model 7125 Rheodyne injector fitted with a 20 μL sample loop, a model SPD-10A UV-vis detector, and a CR 7Ae data processor for data acquisition, analysis, and display. A Shimadzu CLC-NH2 (M) column (4.6 mm i.d. × 25 cm) was used in normal phase with a solvent system of *n*-hexane/isopropanol (96:4 vol/vol) at a flow rate of 1 mL/min. The UV detector was set at 297 nm. The HPLC conditions were standardized using individual standards and their various mixtures (20).

RESULTS AND DISCUSSION

Composition of berries. The proximate composition of berries of the three different species is summarized in Table 1. The moisture content of fresh berries of *H. tibetana* and *H. rhamnoides* was in the range of 67–77%, and in *H. salicifolia* was 82–86%. Seeds constituted 7.4–10% of the berries in *H. tibetana* and *H. rhamnoides* and 3.4–4.1% in *H. salicifolia.* In these berry samples, the oil content of the pulp of *H. rhamnoides* contained higher amounts of oil (3.0–3.6% of fresh berries) than for *H. tibetana* (2.2–2.5%) and *H. salicifolia*

TABLE 1
Composition of Berries of Indian *Hippophae rhamnoides, H. salicifolia,* and *H. tibetana* (mean ± SD; *n* = 3)

Sea buckthorn species	Geographic location	Seed (%)	Moisture (%)	Pulp oil (%)	Total ash (%)	Total protein (%)	Vit. C (mg/kg)	Flavonols (mg/kg)
Hippophae rhamnoides	Lahaul 1	10.4	74.4 ± 0.5	3.08 ± 0.22	1.05 ± 0.02	2.25 ± 0.62	2924 ± 36	308 ± 12
	Lahaul 2	8.1	74.4 ± 0.4	2.99 ± 0.32	0.79 ± 0.14	1.86 ± 0.59	2305 ± 29	285 ± 21
	Lahaul 3	9.7	72.7 ± 0.2	3.04 ± 0.26	0.93 ± 0.19	2.11 ± 0.45	4877 ± 19	212 ± 49
Hippophae rhamnoides	Spiti 1	7.9	76.9 ± 0.7	3.38 ± 0.45	0.60 ± 0.29	2.17 ± 0.49	1866 ± 17	122 ± 20
	Spiti 2	7.4	74.6 ± 0.5	3.14 ± 0.23	0.73 ± 0.09	2.15 ± 0.34	1444 ± 36	156 ± 17
	Spiti 3	9.3	67.2 ± 1.3	3.38 ± 0.17	0.99 ± 0.14	2.44 ± 0.19	1666 ± 23	180 ± 9
Hippophae rhamnoides	Ladakh 1	9.8	72.3 ± 0.9	3.60 ± 0.15	0.89 ± 0.12	2.12 ± 0.25	2145 ± 53	251 ± 25
	Ladakh 2	8.5	72.1 ± 0.5	3.52 ± 0.31	0.79 ± 0.11	1.99 ± 0.23	2568 ± 42	291 ± 17
	Ladakh 3	8.6	73.4 ± 0.4	3.61 ± 0.25	0.92 ± 0.09	2.34 ± 0.39	2425 ± 36	261 ± 22
Hippophae salicifolia	Lahaul 1	3.4	85.8 ± 1.0	1.49 ± 0.20	0.26 ± 0.06	1.13 ± 0.29	27692 ± 91	428 ± 51
	Lahaul 2	3.7	82.3 ± 0.9	1.53 ± 0.15	0.40 ± 0.08	1.54 ± 0.38	29840 ± 185	353 ± 16
	Lahaul 3	4.1	83.5 ± 0.6	1.65 ± 0.28	0.39 ± 0.02	1.62 ± 0.32	22979 ± 78	398 ± 9
Hippophae tibetana	Spiti 1	9.0	74.5 ± 0.4	2.25 ± 0.56	1.02 ± 0.10	3.09 ± 0.13	9279 ± 79	392 ± 12
	Spiti 2	8.8	73.2 ± 1.1	2.45 ± 0.32	0.96 ± 0.09	2.98 ± 0.22	8789 ± 65	401 ± 15
	Spiti 3	8.7	72.9 ± 0.9	2.50 ± 0.19	0.87 ± 0.12	2.96 ± 0.29	8985 ± 45	342 ± 14

(1.5–1.6%). The ash content of *H. rhamnoides* and *H. tibetana* (0.6–1.05% of berries) was also higher than in *H. salicifolia* (0.26–0.4%). Total protein content in the pulp of the berries was found to be higher for *H. tibetana* (2.96–3.09% of fresh berries) for than for *H. rhamnoides* (1.86–2.44%) and *H. salicifolia* (1.13–1.62% of berries). In general, proximate composition of SB berries varied from species to species. Vitamin C values for berries of *H. salicifolia* (22,979–29,840 mg/kg) and *H. tibetana* (8,789–9,279 mg/kg) were much higher than in *H. rhamnoides* (1,444–4,877 mg/kg). The amount of flavonols (122–428 mg/kg) in the berries of these three species was also found to be slightly higher than the reported amounts of flavonols in cranberry (100–263 mg/kg), bog whortleberry (184 mg/kg), lingonberry (74–146 mg/kg), black currant (115 mg/kg), and crawberry (102 mg/kg) (21). The results presented here indicate that the chemical profile of *H. salicifolia*, with a lower seed content, lower levels of fatty matter, and high levels of vitamin C and flavonoids, differed perceptibly from the other two. The chemical properties *of H. salicifolia* and *H. tibetana* have not been well documented until now..

FA. The FA compositions of pulp oil of the three species of berries are summarized in Table 2. In the oil from berry pulp, the dominating FA were palmitoleic, palmitic, oleic, linoleic, and linolenic acids. The highest variations among the three species were observed in the proportion of palmitoleic acid (32–50%). *Hippophae rhamnoides* contained the highest proportion of this rare FA (16:1) in the pulp oil (45–50%). Variation in the next highest FA, 16:0, was in the range of 25–36% in the pulp oil from the three species. The proportion of oleic acid (22–26%) in pulp oil of *H. tibetana* was higher than that of *H. salicifolia* (13-18%) and *H. rhamnoides* (10–17%). The proportion of 18:3 FA was found to be slightly higher in

TABLE 2
FA Composition of Pulp Oils of Berries from Indian *Hippophae rhamnoides, H. salicifolia,* and *H. tibetana* (mean ± SD; *n* = 3)

Sea buckthorn species	Geographic location	FA						
		14:0	16:0	16:1	18:0	18:1	18:2	18:3
Hippophae rhamnoides	Lahaul 1	0.5 ± 0.1	28.4 ± 1.1	50.3 ± 1.2	0.6 ± 0.0	11.3 ± 1.7	8.1 ± 0.3	1.3 ± 0.9
	Lahaul 2	0.6 ± 0.0	29.4 ± 0.7	50 ± 1.1	0.7 ± 0.1	9.7 ± 0.6	7.9 ± 0.2	1.6 ± 0.7
	Lahaul 3	0.5 ± 0.0	28.3 ± 1.1	49.7 ± 0.9	0.5 ± 0.0	11.5 ± 1.6	8.5 ± 0.4	1.7 ± 0.8
Hippophae rhamnoides	Spiti 1	0.6 ± 0.2	35.8 ± 1.2	45.6 ± 2.3	0.5 ± 0.1	9.8 ± 1.5	8.2 ± 0.2	0.8 ± 0.1
	Spiti 2	0.3 ± 0.1	35.8 ± 1.1	45.6 ± 1.9	Trace[a]	9.8 ± 1.3	8.2 ± 0.8	0.8 ± 0.1
	Spiti 3	0.6 ± 0.0	30.7 ± 2.6	51.8 ± 0.9	Trace	12.3 ± 0.6	5.9 ± 1.0	0.4 ± 0.0
Hippophae rhamnoides	Ladakh 1	0.4 ± 0.0	33.8 ± 1.5	46.4 ± 2.0	1.0 ± 0.1	13.4 ± 0.94	5.4 ± 1.3	1.3 ± 0.4
	Ladakh 2	0.9 ± 0.1	31.2 ± 1.4	49.1 ± 3.1	0.8 ± 0.0	13.9 ± 3.49	4.6 ± 0.6	Trace
	Ladakh 3	0.8 ± 0.1	30.9 ± 1.3	45.6 ± 2.6	0.9 ± 0.0	16.8 ± 1.73	5.8 ± 0.4	0.8 ± 0.4
Hippophae salicifolia	Lahaul 1	0.3 ± 0.1	29 ± 3.1	32.9 ± 0.5	0.1 ± 0.0	17.6 ± 0.5	16.1 ± 0.9	0.6 ± 0.1
	Lahaul 2	0.1 ± 0.0	28.9 ± 0.8	41 ± 1.2	0.7 ± 0.1	12.6 ± 1.1	14.3 ± 1.8	1.0 ± 0.0
	Lahaul 3	0.2 ± 0.1	28 ± 1.9	35 ± 3.0	0.6 ± 0.2	13 ± 1.3	15.0 ± 1.3	0.8 ± 0.2
Hippophae tibetana	Spiti 1	1.1 ± 0.2	25.7 ± 0.5	32.1 ± 4.2	0.5 ± 0.1	26.0 ± 1.9	9.3 ± 1.1	5.2 ± 1.0
	Spiti 2	0.9 ± 0.3	28.5 ± 2.4	34.1 ± 2.3	0.4 ± 0.0	22 ± 2.8	9.2 ± 0.5	3.5 ± 0.6
	Spiti 3	0.8 ± 0.1	26 ± 1.8	36.0 ± 1.4	0.4 ± 0.2	23.5 ± 2.4	8.3 ± 0.8	5.6 ± 0.9

[a]Trace, ≤ 0.1%.

TABLE 3
Carotenoid, Tocopherol, and Tocotrienol Composition of Pulp Oils of Berries of Indian *Hippophae rhamnoides, H. salicifolia,* and *H. tibetana* (mg/kg of pulp oil) (mean ± SD; *n* = 3)

Sea buckthorn species	Geographic location	Carotenoids	Tocopherols/tocotrienols[a]						Total
			α T1	α T3	β T1	γ T1	γ T3 + δ T1	δ T3	
Hippophae rhamnoides	Lahaul 1	2660 ± 82	859 ± 40	293 ± 24	16 ± 5	152 ± 21	362 ± 45	12 ± 2	1694 ± 61
	Lahaul 2	3420 ± 102	1068 ± 49	227 ± 16	12 ± 2	165 ± 13	288 ± 21	17 ± 2	1788 ± 41
	Lahaul 3	2940 ± 42	773 ± 28	148 ± 28	19 ± 4	149 ± 28	344 ± 21	16 ± 4	1564 ± 67
Hippophae rhamnoides	Spiti 1	2576 ± 108	1006 ± 26	183 ± 22	18 ± 3	170 ± 35	228 ± 24	10 ± 3	1615 ± 76
	Spiti 2	2820 ± 62	1046 ± 71	164 ± 34	9 ± 4	120 ± 22	117 ± 23	16 ± 3	1494 ± 76
	Spiti 3	2706 ± 35	679 ± 54	247 ± 28	11 ± 4	121 ± 20	241 ± 14	17 ± 1	1318 ± 59
Hippophae rhamnoides	Ladakh 1	2400 ± 60	954 ± 73	95 ± 15	27 ± 6	126 ± 5	187 ± 11	14 ± 2	1394 ± 87
	Ladakh 2	2350 ± 22	858 ± 73	120 ± 27	22 ± 4	82 ± 10	201 ± 22	15 ± 1	1301 ± 80
	Ladakh 3	2650 ± 61	903 ± 50	109 ± 27	21 ± 5	90 ± 5	208 ± 35	17 ± 1	1348 ± 93
Hippophae salicifolia	Lahaul 1	801 ± 25	354 ± 15	46 ± 7	5 ± 2	94 ± 9	291 ± 18	8 ± 1	799 ± 23
	Lahaul 2	840 ± 42	517 ± 30	124 ± 23	4 ± 1	57 ± 18	183 ± 12	18 ± 6	902 ± 35
	Lahaul 3	692 ± 52	390 ± 22	89 ± 12	9 ± 2	33 ± 6	128 ± 35	18 ± 1	666 ± 62
Hippophae tibetana	Spiti 1	3166 ± 32	867 ± 46	127 ± 17	15 ± 3	86 ± 16	431 ± 46	19 ± 4	1546 ± 47
	Spiti 2	2693 ± 41	895 ± 67	95 ± 7	18 ± 3	62 ± 18	363 ± 30	17 ± 5	1448 ± 109
	Spiti 3	2840 ± 18	864 ± 13	85 ± 11	19 ± 2	64 ± 16	336 ± 33	12 ± 4	1368 ± 25

[a]α T1, α-tocopherol; α T3, α-tocotrienol; β T1, β-tocopherol; γ T1, γ-tocopherol; γ T3, γ-tocotrienol; δ T1, δ-tocopherol; δ T3, δ-tocotrienol.

H. tibetana (3.5–5.6%) than in the other two species. Traces (>1%) of myristic and stearic acids were present in all three species. Trace amounts of arachidic acid (20:0) were observed in pulp oil of *H. salicifolia* (data not shown).

Palmitoleic acid (16:1) is reported to facilitate cell membrane fluidity in a manner similar to that of PUFA but to have low susceptibility to oxidation. An increase in the level of dietary palmitoleic acid has been suggested to improve the metabolism of vascular smooth muscle cells (22). Hypocholesterolemic and hypoglyceridemic activities comparable to that of linoleic and linolenic acids also have been reported for palmitoleic acid (23). The occurrence of 16:1 FA in large proportion as shown in this study is unique among oils of plant origin. The presence of 16:1 in various SB species was reported earlier. Vereshchagin *et al*. (24) investigated the regioisomers and FA composition of TAG in the pulp/peel of wild SB berries of three geographic (Central Asian, Baltic, and Caucasian) regions, i.e., Tadzhikistan, the Baltic seashore, and Georgia, respectively. The Central Asian and Baltic forms contained higher proportion of palmitoleic acid in the TAG of berry pulp (55 and 42%, respectively) than the Caucasian form (16%). The FA composition of the pulp oil of a Siberian form reported by Ozerinina *et al*. (25) was similar to that of the Central Asian and the Baltic form. Cakir (26) reported palmitoleic (47.9 %) and palmitic (25%) acids were major FA in mesocarp oil of *H. rhamnoides* from Turkey. The results of the present study also showed extreme variations of palmitoleic acid, from 32 to 50% in the pulp oil from fresh berries. In considering the therapeutic value of this rare FA, these results indicate the potential of breeding and industrial application to evolve 16:1-rich varieties.

Carotenoids. The amounts of total carotenoids in the pulp oils of berries were quantified and expressed in terms of β-carotene (Table 3). The amount of carotenoids in pulp oils of these species varied from 700 to 3500 mg/kg. The carotenoid content of pulp oils of *H. rhamnoides* (2350 ± 22–3420 ± 102 mg/kg) and *H. tibetana* (2693 ± 41–3166 ± 32 mg/kg) were similar, and *H. salicifolia* had lowest amount (692 ± 52–840 ± 42 mg/kg). Carotene content in the pulp oil of *H. rhamnoides* berries from Ladakh (2350 ± 22–2650 ± 61 mg/kg) was slightly lower than that from the Lahaul (2660 ± 82–3420 ± 102 mg/kg) and Spiti (2576 ± 108–2820 ± 62 mg/kg) regions. The carotenoid content in *H. rhamnoides* was comparable with the values reported for the berries of Chinese origin. However, authentic reports on the carotenoid content in *H. tibetana* and *H. salicifolia* from other geographical areas are not available. β-Carotene as the major component in SB pulp oils as well as α-carotene, γ-carotene, dihydroxy carotene, lycopene, and xeaxanthin has been reported (27).

Tocopherols/tocotrienols. Tocols in pulp oil were quantified by HPLC, and the results are given Table 3. The amount of tocols in pulp oil varied from 600 to 1700 mg/kg among the three species studied. *Hippophae salicifolia* contained the least amount of tocols (666 ± 62–902 ± 35 mg/kg), as compared with *H. rhamnoides* (1301 ± 80–1788 ± 41 mg/kg) and *H. tibetana* (1368 ± 25–1546 ± 47 mg/kg). *Hippophae rhamnoides* berries from the Lahaul region showed a slightly higher content of tocols than those from Spiti and Ladakh. The relative proportion of individual tocols was almost identical in the three species studied here with α-tocopherol predominating (40–60%). Tocopherols constituted about 70 to 80% of total tocols, whereas tocotrienols accounted for 20 to 30%. The proportion of the other major isomers, α-tocotrienol and γ-tocopherol, was 5–25 and 4–16%, respectively. γ-Tocotrienol and δ-tocopherol together represented 10–25% of total tocols. Other isomers of tocols were present in trace amounts. The total amount of tocopherols in these berries, except for *H. salicifolia*, was in accordance with the values reported from China and Poland (17,28). A tocopherol profile of 75–89% of α-tocopherol, 4–11% of γ-tocopherol, 2.4–12.2% of β-tocopherol, 0.3–2.4%

of δ-tocopherol, 0.4–4.8% of β-tocotrienol, 0.4–3.2% of α-tocotrienol, and 0.6–2.5% of δ-tocotrienol was reported for the pulp of berries of the *sinesis* and *mongolica* subspecies of *H. rhamnoides* from China (17). Zadernowski *et al.* (28) reported 101–128 mg/100 g of tocopherols in pulp oil of *H. rhamnoides* berries of Polish origin with a profile of 62.5–67.9% of α-tocotrienol, traces of γ-tocotrienol, and 30–40% of δ-tocopherol (28). The tocopherol profile of Indian berries differed from the values reported for the Poland varieties in the proportion of δ-tocopherol. More resemblance in tocopherol profile of *H. rhamnoides* cultivars between Indian and Chinese origin may be due to the geoclimatic closeness in SB growing areas. Comparison of the tocopherol profile of berries of *H. salicifolia* and *H. tibetana* species with those of other geographic areas is not attempted here due to the lack of authentic reports. SB berries from the Indian region of the Himalayas were therefore a very rich source of tocols and were comparable with that of berries from other regions in the case of *H. rhamnoides*. It is well known that, as antioxidants, tocols, particularly tocotrienols, modulate diseases such as atherosclerosis, diabetes, cancer, aging, and the like (29).

The results of the present study showed that the pulp oil of two Indian SB berries (*H. rhamnoides* and *H. tibetana*) were rich sources of bioactive lipophilic compounds, i.e., carotenoids and tocopherols, and had a distinct FA profile, with palmitoleic acid as the major FA. The presence of 16:1 as the predominant FA is unique among oils of plant origin, and this observation verified previous reports based on Chinese varieties (1,8,17). For the first time the distinct nature of the berries of *H. salicifolia*, which is only found in the Himalayan region, was revealed, including a high content of hydrophilic components such as flavonols and vitamin C and a very low content of oil and lipophilic components such as carotenoids and tocopherols. The high nutritional quality of berries of *H. tibetana*, which has a limited distribution in India, is reported here for the first time. A high content of tocopherols and carotenoids, with high amount of 16:1 FA and other bioactive molecules, enhances the value of SB pulp oil as a health supplement. Reports on the lipophilic constituents of SB berries from China, Canada, Poland, and Russia are available. However, no authentic data on the SB berries grown in the Indian region of the Himalayas have been reported. The cold desert conditions in which Indian SB is grown may have an influence on the phytochemical profile, as shown in this preliminary study.

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