Asian Journal of Current Research

Volume 9, Issue 4, Page 67-77, 2024; Article no.AJOCR.12416
ISSN: 2456-804X

Study on Biochemical Composition of Sea Buckthorn Fruit Cultivated from Leh, India and its Flavor Profile by Gas Chromatography Mass Spectrometry (GC-MS)

Selvamuthukumaran [a,b*]

[a] *Biochemistry and Nutrition Division, Defence Food Research Laboratory, Siddartha Nagar, Mysore-570 011, Karnataka, India.*
[b] *School of Agriculture Joy University, Vadakangulam, Tirunelveli District, Tamil Nadu, India.*

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: https://doi.org/10.56557/ajocr/2024/v9i48883

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12416

Original Research Article

Received: 22/07/2024
Accepted: 26/09/2024
Published: 08/10/2024

ABSTRACT

Sea buckthorn berries are among the most nutritious and vitamin rich fruit found in the plant kingdom. Biochemical composition of sea buckthorn berries cultivated from Leh, India was analyzed to study its suitability for making food products. Berry pulp was analyzed for sugars, acidity, TSS and pH. The results show that the sea buckthorn pulp exhibited higher acidity, lesser pH, total sugars and reducing sugars, when compared to fruits like pineapple and grapes. Calcium and potassium content of berry was found to be high when compared to other mineral content viz.

**Corresponding author: E-mail: kumarangitam@gmail.com;*

Cite as: *Selvamuthukumaran. 2024. "Study on Biochemical Composition of Sea Buckthorn Fruit Cultivated from Leh, India and Its Flavor Profile by Gas Chromatography Mass Spectrometry (GC-MS)". Asian Journal of Current Research 9 (4):67-77. https://doi.org/10.56557/ajocr/2024/v9i48883.*

sodium, iron and phosphorus. Antioxidant contents and antioxidant activity of pulp was determined and compared with other fruit pulps. Sea buckthorn fruit pulp exhibited significant antioxidant activity i.e. 88.72%, when compared to locally available fruits like grapes and pineapple. Totally 45 volatile flavour components were identified from sea buckthorn berry, which contains mainly esters, that contributes to significant berry aroma.

Keywords: Antioxidant activity; flavor profile; proximate composition; Sea buckthorn berries.

1. INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L.) is a high altitude temperate plant belonging to the family Elaegnaceae. Sea buckthorn is said to be a native of Himalayas, middle/ Mediterranean region, Asia and Siberia. In India, it is distributed wildly in various parts of the Himalayan region like Lahul and Spiti valley of Himachal Pradesh, in Uttaranchal Himalaya. It is found mainly in Bhagirathi Ghat, Sukki Ghat, Harsil Ghat, Gangotri Ghat, Mandakini Ghat, Alknanda Ghat, Jamuna Ghat etc. It also grows in several countries like China, Russia, Britain, Germany, Finland, Romania, France, Nepal, Bhutan and Pakistan at an altitude of 2500-4300 meters height [1,2].

Sea buckthorn berries are among the most nutritious and vitamin-rich fruit found in the plant kingdom. The vitamin C and carotenoids content of sea buckthorn fruit was found to be higher than in orange, papaya and apple [3]. Lu [4] reported that vitamin E content in sea buckthorn fruit is also higher than wheat embryo, maize, soya bean and safflower. Sea buckthorn berries contain up to 13% soluble sugars i.e. mainly glucose, fructose and xylose; 3.9% organic acids of mainly malic and succinic acids [5]. It is also rich in proteins and free amino acids. A total of 18 amino acids have been identified in sea buckthorn fruit [6]. There are at least 24 chemical elements present in sea buckthorn juice viz., nitrogen, phosphorus, iron, manganese, boron, calcium, aluminium, silicon etc. [6,7]. The oil content of the juice and the seeds ranges from 2-8%. Oil from the pulp is rich in palmitic and palmitoleic acids ($C_{16:0}$ and $C_{16:1}$), while the oil from the seed contains unsaturated fatty acids of C_{18} type (linoleic and linolenic acid). Oils from the seed and juice also contain vitamin E and carotenes [5]. In addition sea buckthorn berries, leaves and bark contain sitosterol, tocopherol and many other bioactive compounds [8].

The sea buckthorn pulp, a source of sea buckthorn yellow contains mainly flavones and has a potential use as a food colouring material. The rich yellow pigment of sea buckthorn is widely used as food additive, in lieu of synthetic chemical pigments which have been proved to be harmful to the health of human beings. β-carotene is dominant amongst the carotenoid pigments in sea buckthorn, the concentration of which varies depending upon the place of origin and the part analyzed [9].

All parts of the plant viz., bark, leaves and fruits are rich in biomolecules with unique medicinal values. The array of biomolecules synthesized by sea buckthorn has imparted a central role to the plant not only as a health promoter but also for prevention and treatment of different diseases. The medicinal value of sea buckthorn is well documented in Asia and Europe. Investigations on modern medicinal uses were initiated in Russia during the 1950's [10]. In India it has been identified as Amalavetas, an ayurvedic plant. Amalavetas, the plant species which is real sea buckthorn in Himalayas is being used by the traditional doctors for curing various health problems [2]. The objective of this work was to explore the biochemical composition of sea buckthorn cultivated at Leh and studying the flavour profile of pulp extract using GC-MS (Gas Chromatography Mass Spectrometry).

2. METHODOLOGY

Berries were collected from one of the DRDO Lab i.e. Field Research Laboratory (FRL), located at Leh, Himachal Pradesh, India. All plants received similar water and fertilizer treatments. Samples were randomly collected, when optimally ripped from 30 plants in order to get a representative sample. Berries are harvested by hand, although investigations of mechanical harvesters are underway and hormonal treatment to decrease the force required to detach berries looks promising [11]. Samples were cleaned manually to remove surface dust with water; insect affected berries, stems, leaves or other debris collected during harvesting were also separated, as this debris would be expected to affect flavour by contributing off flavour from rotted berries or green flavor from leaves and other stem material. Sea buckthorn berries are well-known to carry a musky odour, detectable even in the sea buckthorn field itself. But washing berries using air–agitated tanks prior to processing may provide a method for reducing or adjusting this odour [12]. Water was completely drained and berries were frozen to – 20°C until its use. Chemicals used for experiments were procured from Sigma Aldrich, Bangalore and they were of both HPLC (High Performance Liquid Chromatography) and analytical grades. From seabuckthorn berries, the juice can be extracted by using different methods, the current methods includes juice extraction by using traditional cloth pressing method having sieve size of 0.8 mm. Bump [13] portrayed that this is the efficient method for extraction of liquid in the crushed berries. There are wide variety of press that can be utilized like screw press, serpentine belt press and cloth press. Among these various presses, serpentine belt press as well as rack and cloth press will provide juice content of 68%.

The berries were analyzed for its proximate constituents like fat, protein, moisture, carbohydrate, total ash and fibre contents according to the standard protocol described by AOAC [14]. Sample moisture can be calculated based on its weight difference from fresh as well as from dried samples. Kjeldhal method was used to quantify proteins, sample fat content by using Soxhlet method, sample carbohydrate content by variation. Sample was ignited and used for determining total ash content with the help of muffle furnace. Samples were both alkali and acid digested followed by subsequent washing cum drying in order to determine crude fibre content in the sample.

The mineral content in seabuckthorn berries was determined using atomic absorption spectrophotometer as per the method of AOAC [14]. A 20g sample was digested by heating in a kjeldahl flask with concentrated Nitric acid and Sulphuric acid mixture for oxidation of carbonaceous matter. For each sample, a blank was prepared simultaneously by taking same amounts of nitric and sulphuric acids mixture. Care was taken during heating so that no excessive foaming took place. Concentrated nitric acid in small amounts was added until all organic matter was oxidized. This point was reached when no further darkening of the solution occurred on continuous heating and a clear solution was obtained. It was cooled and transferred to 100 ml volumetric flask and the volume made up with distilled water. The concentration of various minerals was determined using atomic absorption spectrophotometer (Model AA 670, Shimadzu, Kyoto, Japan) by aspirating the solution into the oxygen–acetylene flame. The instrument was calibrated by using standard solutions (1-6 mg/l) of respective minerals.

TSS (Total Soluble Solids) of various fruit pulps was carried out with the help of brixmeter (Erma, Japan) and the results were represented in °Brix. Sample acidity was carried out by titrimetric procedure by using 0.01N NaOH and phenolphthalein indicator as prescribed by AOAC [14], Fehling's titrimetric method was used to quantify total as well as reducing sugar content [14]. The total carotenoids and anthocyanin content in sample was determined colorimetrically at a wavelength of 450 and 535 nm [15]. Total phenol content in sample was quantitatively measured using standard i.e. gallic acid and detected at wavelength of 765 nm [16]. Vitamin like C and E was measured according to the protocol prescribed by Ranganna [15] and Desai [17]. The vitamin C was quantified by using 2,6 dicholorphenol indophenol and E with α- tocopherol by detecting at 536 nm wavelength [15]. DPPH assay was carried out to analyze the free radical scavenging activity as per the protocol of Singh et al. [18].

The volatile components from sea buckthorn pulp were obtained by mixing 200ml of pulp with 300ml of water in round bottom flask and distilled using simultaneous distillation and extraction apparatus (Likens Nickerson distillation apparatus) with diethyl ether as solvent for a period of 2 h 30 min. Traces of water molecules were removed using anhydrous sodium sulphite and ether extract was concentrated by flushing with 99.998% nitrogen gas and contents were made up to known volume of 0.5ml with diethyl ether. Sample was stored at -5ºC for further analysis of flavor components by GCMS.

Gas Chromatographic analysis of volatile oils was carried out using a Chemito GC 1000 HR (M/S Chemito, Chennai, India) system equipped with a flame ionization detector (FID) and BP-5 column (30 m X 0.25 mm I.D) with 0.25 µm film thickness. Injector and detector temperatures were maintained at 240ºC and 230ºC. Oven temperature was programmed from 35ºC to 210ºC with initial hold time of 20 min. Injection volume used was 0.5 µl with split ratio of 1:25. Helium gas (30cm/s) was used as mobile phase.

Electron impact mass spectrophotometric data were collected on Finnigan Mat GCQTM,UK. GC column conditions maintained as same as those used in the GC Flame ionisation detector. The Mass Spectrometer was operated at 70 eV, mass range 40-330 m/z and scan mode EI. Source temperature was 200 ºC and transfer line temperature was 250 ºC. Sample components were identified by comparing mass spectral data with the NIST library, as well as published kovatz's retention indices. Percentage of individual components detected is reported as a mean of three runs.

Profile of volatiles extracted from sea buckthorn was determined by comparison of individual components mass spectra with those present in the built-in NIST 98 library data base and also by comparison of kovatz's retention index calculated using standard formula as mentioned below for non-isothermal conditions with theoretical kovatz's retention indices [19,20]. Only those compounds, whose calculated kovat's retention index by injecting sample in GC which matches with NIST 98 MS library data were only considered.

$$I = 100 \, X \left[n + (N-n) \frac{t'_{r(unknown)} - t'_{r(n)}}{t'_{r(N)} - t'_{r(n)}} \right]$$

Where I = Kovatz retention index,

n = the number of carbon atoms in the smaller n-alkane,

N = the number of carbon atoms in the larger n-alkane,

t'_r = the retention time

The experiments were carried out in triplicate and the results were statistically analyzed using ANOVA (Analysis of Variance) with the help of Microsoft Excel 2010 (Microsoft Corporation, Washington, USA). The Duncan's test was used to compare the sample means prescribed by Steel and Torrie [21].

3. RESULTS AND DISCUSSION

The proximate composition of sea buckthorn fruit (*Hippophae rhamnoides* L.) is given in Table 1. The colour of the sea buckthorn fruit was orange-yellow, oval shaped, 5-7 mm in size and has sour taste. The results showed that the berries had good amount of carbohydrate, fat, crude fibre, protein and minerals. Proximate composition of sea buckthorn berry varies with origin, climate and nature of soil. Berries with highest moisture content 80-87 % [22] and lowest in the range of 20- 32% [23] were available. Sea buckthorn berries harvested during November 2015 from Leh, India has 53.03% moisture and ash content was 1.75% (Table 1). The crude fibre content of berries was 4.71%, protein content was 2.21 % and carbohydrate content was 29.08 %. This is in agreement with data reported by Chauhan et al*.* [3] and Katiyar et al*.* [24].

Atomic absorption spectroscopy results showed that 24 chemical elements were present in sea buckthorn whole berry [6,7]. Potassium is the most abundant (675mg/100g) of all elements present in *Hippophae rhamnoides L.* [6,7,25]. Fe, Ca, Na, Mg and phosphorous content of berries were given under Table 1. Fruit maturity and natural contents of elements in the soil, as well as contamination in both soil and air affects mineral concentration in berries [25].

Table 1. Proximate composition* of sea buckthorn fruit

Composition	Content
Moisture (%)	53.05 ± 0.03
Ash (%)	1.75 ± 0.05
Protein (%)	2.21 ± 0.37
Crude fibre (%)	4.71 ± 0.25
Total carbohydrate (%)**	29.08 ± 0.46
Total fat (%)	9.20 ± 0.22
Sodium (mg/100g)	67.00 ± 0.47
Potassium (mg/100g)	675.00 ± 0.86
Calcium (mg/100g)	627.00 ± 0.39
Magnesium (mg/100g)	22.45 ± 0.29
Iron (mg/100g)	17.00 ± 0.54
Phosphorus (mg/100g)	72.00 ± 0.77

**Values are Mean ± SD of triplicate analyses; **Total carbohydrate by difference*

Sea buckthorn berry contains 9.2% crude fat. This includes tocopherols, tocotrienols, carotenoids as well as omega-3 and omega-6 fatty acid [26]. The fruit berry contains omega-3 and omega-6 fatty acids @ of 34 & 31%, respectively [26]. The composition of the sea buckthorn oils varies according to the subspecies, origins, cultivation activities, harvesting time and extraction method [27]. Chinese varieties contain 10.19 to 22.74% crude fat [6].

The Table 2 shows the physico-chemical characteristics of sea buckthorn fruit pulp in comparison with other fruit pulps. The results shows that the sea buckthorn pulp exhibited lesser TSS, total sugars, reducing sugars and pH when compared to other fruit pulp viz. pineapple and grapes. The acidity of the sea buckthorn pulp was found to be quiet high, when compared to other fruit pulps. The fruit is not readily liked due to its acidic and astringent taste and also whey like unpleasant flavor [28].

The results clearly depicts that sea buckthorn needs to be blended with other fruits during its processing so that the intensity of the acidity level will be minimized or toned down in the finished end product. On the other hand the judicious blending will reduce the sharp whey like flavor of sea buckthorn, which can be further minimized by incorporating pleasant exotic fruit flavor derived from use of other fruits viz. pineapple, grapes etc. Chauhan et al. [3] were also reported similar results of lesser sugars, lesser pH and higher acidity in sea buckthorn fruit while comparing with other fruits viz. papaya, apple and oranges. Significant difference ($p<0.05$) in physico-chemical characteristics of sea buckthorn fruit pulp were found, while comparing them with other fruit pulps viz. pineapple and grapes.

The Table 3 shows the antioxidant contents of sea buckthorn pulp in comparison with other fruit pulps viz. pineapple and grapes. Vitamin C, which is one of the important vitamins and it is required for the growth and maintenance of good health in human beings. The results show that the vitamin C content was found to be higher in seabuckthorn fruit pulp, when compared to other fruit pulps. Chauhan et al. [3] were also reported more amount of vitamin C in sea buckthorn fruit, while comparing its content with fruits like papaya, orange and apple. Vitamin E is an important dietary constituent, as it is essential in maintaining the stability and integrity of the cell membrane. This is an antioxidant and free radical scavengers and its presence has been linked to prevention of chronic diseases and premature ageing, cancer, cardiovascular diseases and strokes [29]. As like vitamin C, sea buckthorn pulp accorded more vitamin E content, when compared to other fruit pulps. Lu [4] were also reported higher concentration of vitamin E in sea buckthorn fruit, while comparing its content with other plant products viz. maize, wheat embryo, safflower and soybean.

Carotenoids are important food constituents, owing to their color and nutritive value as provitamin A apart from being strong antioxidants. The sea buckthorn pulp recorded more total carotenoids content, when compared to other fruit pulps viz. grapes and pineapple. Chauhan et al*.* [3] were also reported more carotenoids in sea buckthorn fruit, while comparing its content with fruits like apple, papaya and oranges.

Anthocyanins are responsible for the attractive colour of the product and they have been recognized as important antioxidants [30]. The phenolic compounds contribute immensely towards better human health due to their multiple biological effects such as antioxidant capacity, antimutagenic and anticarcinogenic activity and anti-inflammatory action [31]. As like other antioxidants, the sea buckthorn pulp showed higher total anthocyanin and total phenol contents, when compared to other fruits pulps viz. pineapple and grapes. Sea buckthorn fruit pulp exhibited significant antioxidant contents ($p<0.05$) when compared to other fruit pulps.

Sea buckthorn fruit pulp accorded significant antioxidant activity, when it is being compared with pulps obtained from fruits like grapes and pineapple (Table 4). Antioxidant activity enhancement may be ascribed to antioxidant like carotenoids, anthocyanins, phenols and vitamins. Therefore, sea buckthorn fruit is an appropriate raw material source for development of health enriched antioxidant rich functional food products.

The profile of the flavor compounds of sea buckthorn pulp was very typical and distinctive from that of other common berries. The pulp is immensely flavored with exotic flavor and considered to be a good appetizer, which has health imparting attributes. The volatile flavor components of the sea buckthorn pulp are given in the Table 5. A total of 45 volatile flavor components were identified in sea buckthorn pulp by GC MS, which represents 80.89% of the total volatile flavor content. The volatile compounds of the sea buckthorn fruit pulp were predominantly esters [32] followed by phenols, carbonyl compounds, terpene and alkanes. It was found that 23.69% of ether extract contains esters which is responsible for aroma of the berry, among them the major ester compounds detected from sea buckthorn fruit pulp were ethyl dodecenoate followed by 3-methyl-1-butyl benzoate, 3-methylbutyl octanoate, ethyl tetra decanoate, benzyl benzoate, ethyl 2- methyl butanoate, ethyl cinnamate, cis-2-hexenyl acetate, ethyl butanoate and ethyl 3-methyl-butanoate. Among 5.75% of carboxylic acid, phenylacetic acid, tetradecanoic acid and hexadecanoic acid were the major acids. Sea buckthorn ether extract was found to contain 26.12% of alkanes, among the alkanes n-pentadecane, n-pentacosane, n-heneicosane were major compounds. In addition to the above one, the other flavour compounds present in pulp were 1.631% of linalool, 1.638% linalool oxide, n-hexadecanol 2.771% and the major terpene hydrocarbon detected from the sea buckthorn fruit pulp was sabinene. Chinese berries contained higher proportions of ethyl 3-methyl butanoate, butyl pentanoate, 2-mthyl propyl 3-methylbutanoate and pentyl 3-methyl butanoate [25]. Finnish berries contained higher proportion of ethyl 2-methylbutanoate, ethyl 3-methyl butanoate and ethyl hexanoate.

Table 2. Physico–chemical characteristics* of sea buckthorn fruit pulp in comparison with other fruit pulps

Parameters	Sea buckthorn	Pineapple	Grapes
Total soluble solids (ºbrix)	12.00 ± 0.28 ^{a}	14.06±0.11^{b}	13.83±0.28^{b}
Acidity (%)	1.84 ± 0.03 ^{a}	0.96±0.02 ^{b}	1.09±0.04 ^{c}
Total sugars (%)	6.45 ± 0.59 ^{a}	10.37±0.32^{b}	13.97±0.40^{c}
Reducing sugar (%)	**0.85 ± 0.40 ^{a}**	**4.29±0.23 ^{b}**	**9.00±0.37 ^{c}**
pH	**2.90 ± 0.01^{a}**	**4.21±0.03 ^{b}**	**3.92±0.04 ^{c}**

**Values are Mean ± SD of triplicate analyses; ^{a,b,c} Mean values in the same row bearing the common superscript do not differ significantly (p>0.05)*

Table 3. Antioxidant contents* of sea buckthorn pulp in comparison with other fruit pulps

Name of the antioxidant	Sea buckthorn	Pineapple	Grapes
Vitamin C (mg/100g)	504.00± 0.38^{a}	11.98 ± 0.51^{b}	16.86 ± 0.69^{c}
Vitamin E (mg/100g)	190.54± 0.86 ^{a}	0.00± 0.00 ^{b}	78.98 ± 0.48^{c}
Total carotenoids (mg/100g)	6.85 ± 0.34 ^{a}	0.31± 0.14^{b}	0.24 ± 0.11^{b}
Total anthocyanin (mg/100g)	1.48 ± 0.21^{a}	0.10± 0.03^{b}	0.18 ± 0.06^{b}
Total phenols (mg/100g)	560.00± 0.71^{a}	41.03 ± 0.85^{b}	72.66 ± 0.63^{c}

** Values are Mean ± SD of triplicate analyses; ^{a,b,c} Mean values in the same row bearing the common superscript do not differ significantly (p>0.05)*

Table 4. Antioxidant activity* of sea buckthorn pulp in comparison with other fruit pulps

Name of the fruit pulp	Antioxidant activity (%)
Sea buckthorn	88.72 ± 0.58^{a}
Pineapple	37.54 ± 0.86^{b}
Grapes	55.85 ± 0.44^{c}

**Values are Mean ± SD of triplicate analyses; ^{a,b,c} Mean values in the same column bearing the common superscript do not differ significantly (p>0.05)*

Table 5. Flavour profile* of sea buckthorn fruit pulp

Name of the compound	Kovatz RI	% Peak area
Acetic acid	707	0.430±0.029
Ethyl butanoate	803	0.162±0.012
Furfural	841	0.790±0.035
Ethyl 2-methylbutanoate	850	0.555±0.021
Ethyl 3-methyl-butanoate	852	0.154±0.009
2-Acetylfuran	922	0.436±0.027
Hexanoic acid	972	0.514±0.034
Sabinene	995	1.223±0.051
Ethyl hexanoate	1002	1.730±0.064
Cis-2-Hexenyl acetate	1018	0.207±0.013
Cis- bita-Ocimene	1044	1.607±0.074
Linalool oxide	1077	1.638±0.083
Terpinolene	1083	0.229±0.015
Propyl hexanoate	1099	0.428±0.021
Linalool	1102	1.631±0.094
2-Phenylethanol	1122	2.91±0.097
Isobutyl hexanoate	1131	1.162±0.071
Ethyl benzoate	1175	5.256±0.180
alpha-Terpineol	1195	2.259±0.115
Methyl salicylate	1198	0.538±0.034
Ethyl octanoate	1201	4.461±0.102
Phenylacetic acid	1255	1.505±0.052
n-Tridecane	1299	0.603±0.037
Butyl benzoate	1304	1.256±0.086
3-methyl butyloctanoate	1329	1.213±0.075
3-methyl-1-butyl benzoate	1444	1.731±0.071
Ethyl cinnamate	1472	1.301±0.063
n-Pentadecane	1498	5.141±0.098
Ethyl dodecenoate	1575	0.356±0.0142
Ethyl dodecanoate	1599	1.467±0.082
n-Heptadecane	1700	0.690±0.037
Benzyl benzoate	1773	0.904±0.068

Name of the compound	Kovatz RI	% Peak area
Tetra decanoic acid	1777	1.773±0.085
Ethyl tetradecanoate	1788	1.133±0.0768
1,2-benzenedicarboxylic acid, bis-(2-methylpropyl ester)	1873	0.916±0.066
Tetramethyl pentadecanone	1894	2.105±0.137
n-Hexadecanol	1960	2.771±0.091
Hexadecanoic acid	1970	1.515±0.067
Ethyl hexadecanoate	1973	1.525±0.085
n-Eicosane	1997	0.740±0.098
n-Heneicosane	2099	6.198±0.214
n-Tetracosane	2398	4.393±0.202
n-Pentacosane	2500	5.501±0.121
n-Hexacosane	2599	2.824±0.092
n-Heptacosane	2698	3.883±0.054

**Values are Mean ± SD of triplicate analyses*

Waxes, which have a higher molecular weight, i.e. longer retention times were completely separated from the fragrance compounds (terpenes and phenols) with shorter time. Waxes were mainly formed by n- heneicosane, n-pentacosane, n-tetracosane and n-heptacosane. The lower concentration of esters in distillate may be due to the hydrolysis and subsequent production of acetic acid as by product. This was confirmed by the presence of acetic acid. Della-porta et al. [33] reported that the maximum yield of clove bud volatile oil was measured at the end of steam distillation process and they found that the resultant yield was 0.74% by weight of the material charged in the extractor. Steam distillation and supercritical extraction cannot be differentiated in terms of yield. The composition of steam distillate and supercritical extract of natural products differs significantly, that is different extraction methods will have different flavor profile [34].

4. CONCLUSION

Biochemical analysis of fruit revealed that sea buckthorn is one of the richest fruit in terms of nutritional value. These fruits possess immense health benefits due to their rich content of vitamins C and E, which apart from their role as vitamins also function as efficient antioxidants. Berries are a good source of carotenoids, anthocyanins and phenols, which once again add to the antioxidant properties of the fruit, apart from carotenoids being a source of vitamin A. However, the fruit is highly acidic and cannot be consumed as such, all the nutritive and medicinal value of the fruit could be used ~~only~~, when it is converted to edible form i.e. in the form of food products after judicious blending with other fruits. Flavour profile of the fruit pulp steam extract was rich in ethyl octanoate (4.46%), ethyl benzoate (5.25%), 2-phenyl ethanol (2.91%) and 2-phenyl ethanol (2.91%). Both library mass spectra and calculated kovatz retention index were used to confirm volatile compounds present in sea buckthorn. Therefore, the fruit can be effectively utilized as one of the major ingredient for manufacturing several functional and nutraceutical based food products.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENT

The authors are thankful to Defence Research and Development Organization for providing grant from project DIP-233 to carry out this research work.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Singh V. Sea buckthorn: A wonder plant of dry temperate Himalayas. IndianJournal of Horticulture. 1998;43:6-9.
2. Uniyal MR, Uniyal RC. Sea buckthorn- A source of ayurvedic drugs. Proceedings of International Workshop on Sea buckthorn, Palampur, India. 2001;243-244.
3. Chauhan AS, Rekha MN, Ramteke RS, Eipeson WE. Preparation and quality evaluation of processed products from sea buckthorn (*Hippophae rhamnoides* L.) berries. Beverage and Food World. 2001; 27:31-34.
4. Lu R. Sea buckthorn: A multipurpose plant species for fragile mountains. International Centre for Integrated Mountain Development, Kathmandu, Nepal. 1992;1-62.
5. Ma Z, Cui Y. Studies on the fruit character and biochemical composition of some forms within the Chinese sea buckthorn (*Hippophae rhamnoides* Sinensis) in Shanxi, China. Proceedings of International Symposium on Sea buckthorn (*H.rhamnoides* L.), Xian, China. 1989;106-112.
6. Zhang W, Yan J, Duo J, Ren B, Guo J. Preliminary study of biochemical constituents of sea buckthorn berries growing in Shanxi Province and their changing trend. Proceedings of International Symposium on Sea buckthorn (*H.rhamnoides* L.), Xian, China. 1989;96-105.
7. Tong J, Zhang C, Zhao Z, Yang Y, Tian K. The determination of the physical–chemical constants and sixteen mineral elements in raw sea buckthorn juice. Proceedings of International Symposium on Sea buckthorn (*H.rhamnoides* L, Xian, China. 1989;132-137.
8. Mironov VA. Chemical composition of *Hippophae rhamnoides* of different

populations of the USSR. Proceedings of International Symposium on Sea buckthorn (*H.rhamnoides* L.), Xian, China. 1989;132-136.
9. Dwivedi SK, Ranjit Singh, Zakwan Ahmed. The sea buckthorn. Field Research Laboratory Publication, Leh, India. 2006; 1-57.
10. Gurevich SK. The application of sea buckthorn oil in ophthalmology. Vesttn Oftalmologii. 1956;2:30-33.
11. Li TSC, Schroeder WR. Sea buckthorn (*Hippophae rhamnoides* L.): A multipurpose plant. Hort Technology. 1996; 6:370-380.
12. Beveridge T, Li TS, Oomah BD, Smith A. Sea buckthorn products: Manufacture and composition. Journal of Agriculture and Food Chemistry. 1999;47:3480-3488.
13. Bump VL. Apple pressing and juice extraction. In: Processed apple products, Browning DL (Eds), Van Nostrand Reinhold Publishing Co, New York. 1989; 53.
14. AOAC. Official Methods of Analysis. 14th Eds. AOAC, Washington, DC; 1984.
15. Ranganna S. Hand book of analysis of quality control for fruit and vegetable products. Tata McGraw Hill, New Delhi; 1986.
16. Jayaprakasha GK, Tamil Selvi, Sakariah KK. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Research International. 2003;36:117 – 122.
17. Desai ID. Improved spectrophotometric assay for vitamin E: Oxygen radicals in systems. In: Methods in Enzymology, Packer L (Eds), Academic Press Inc, Orlando. 1984;105:142-144.
18. Singh RN, Singh BS, Saini, Rao H. In vitro antioxidant activity of pet ether extract of black pepper. Indian Journal of Pharmacology. 2008;40:147-151.
19. Jennings W, Shibamoto T. Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. Academic Press, New York. 1980;472.
20. Acree TE, Arn H. Flavornet: Gas chromatography – olfactometry of natural products.
Available:http://www.flavornet.com
Accessed 10 October 2001, 2001.
21. Steel RGD, Torrie JH. Principles and procedures of statistics: A biometrical approach. Tata McGraw Hill, New York; 1980.
22. Lougas T, Laos K, Vokk R. Physical parameters of sea buckthorn berries.Journal of Food Physics. 2006;19: 36−44.
23. Sabir SM, Maqsood H, Ahmed SD, Shah AH, Khan MQ. Chemical and nutritional constituents of sea buckthorn (*Hipophae rhamnoides* ssp. turkestanica) berries from Pakistan. Italian Journal of Food Science. 2005;17:455−462.
24. Katiyar SK, Sharma K, Kumar N, Bhatia AK. Composition of some unconventional Himalayan wild fruits. Journal of Food Science and Technology. 1990;27: 309−310.
25. Kallio K, Yang BR, Tahvonen R, Hakala M. Composition of sea buckthorn berries of various origins. Proceeding of International Symposium on Sea Buckthorn (*Hippophae rhamnoides* L.), Beijing, China; 1999.
26. Bounous G, Zanini E. The variability of some components and biometric characteristics of fruits of six tree and shrub species. Horticulture Abstract.1988; 60:4153.
27. Yang B, Kallio H. Composition and physiological effects of sea buckthorn (Hippophae) lipids. Trends in Food Science and Technology. 2002;13: 160−167
28. Lange E, Klein G, Gerber J, Bauer F, Fetkenhauer W, Sievert B. A procedure for manufacturing semi-finished sea buckthorn products. Food Science and Technology Abstract. 1991;25:5T30.
29. Packer L, Fuchs J. Vitamin E in health and diseases. Marcel Dekker, New York. 1993; 131-139.
30. Rice-Evans AC, Miller NJ, Paganga G. Structure – antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine. 1996;20:933- 956.
31. Parr AJ, Bolwell GP. Phenols in the plant and in man: The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of Science of Food and Agriculture. 2000;80:985 – 1012.
32. Wu D, Xia Q, Cheng H, Zhang Q, Wang Y, Ye X. Changes of volatile flavor compounds in sea buckthorn juice during fermentation based on gas chromatography-ion mobility spectrometry. Foods. 2022;11(21): 3471.

33. Della-porta G, Taddeo R, D'Urso E, Reverchon E. Isolation of clove bud and star anise essential oil by supercritical Co_2 extraction. LWT- Food Science and Technology. 1998;31:454-460.

34. Pourmortazavi SM, Sefidkon F, Hosseini SG. Supercritical carbon dioxide extraction of essential oils from *Perovskia atriplicifolia.* Journal of Agriculture and Food Chemistry. 2003;51:5414-5419.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/12416