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Antioxidant activity of sea buckthorn (*Hippophae rhamnoides*) seed oil extracted using various organic solvents

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Abstract−Sea buckthorn (SBT) combines very fascinating nutritional composition with vital vitamins (A, C, E, D, K, and B complexes). Flavonoids, sterols, α-carotene, linoleic acid, and many more unsaturated fatty acids are present in the sea buckthorn plant. The organic extract of SBT seeds is commonly utilized as an anti-aging ingredient in numerous cosmetics. SBT oil extracts are used in pharmaceuticals that treat diseases like diabetes, cancer, cardiovascular disease, and neurological disorders, in addition to cosmetology. In this investigation, various concentrations of organic solvents such as *n*-hexane, isopropyl alcohol, ethyl acetate, ethanol, methanol, and ascorbic acid (standard) were used for the extraction of oil from sea buckthorn seeds. The antioxidant activity of such extracts was checked by the iron chelating, commonly known as the ferric chloride ($FeCl_3$) method, which is based on the ferric reducing ability of plasma (FRAP) assay with the help of UV-Vis. Our results indicate that seed extract of *Hippophae rhamnoides*, should be considered as a non-toxic source and the ferric reducing ability of plasma (FRAP) assay is used to evaluate the antioxidant potential by various organic solvents. The highest (68%) of FRAP is scavenged by the ethyl acetate and least (53%) of isopropyl extracts.

Keywords: Antioxidant Activity, FRAP Assay, Sea Buckthorn Seeds, Solvents

INTRODUCTION

Hippophae rhamnoides is very hardy, cold-resistant, strong, deciduous, dioecious, plant that with increasing plantings of sea buckthorn will play crucial role in the future nutraceutical market as cultivated in Europe and Asian countries (Russia, Canada, and China) [1]. Sea buckthorn is the common name of *Hippophae rhamnoides* and it belongs to the Family: Elaeagnaceae, Genus: Hippophae, Major class is Dicotyledonae. But this whole plant is very essential owing to its medicinal, pharmaceutical applications; however, current study is carried out only on seeds of sea buckthorn instead of other parts of the plant. Seeds are dark brown, elliptical with 2.0-2.8 mm in size and containing 8-18% oil in normal [2].

Sea buckthorn (SBT) is a unique gift of nature for the cold desert of Pakistan, growing on rocky soils and is drought-tolerant. Its fruit is light orange and seeds are husky. Barriers of SBT have been used for many applications such as skin disorders, asthma, hepatitis, rheumatism, and gastrointestinal diseases. Sea buckthorn fruit is a very ironic that contains sugars, alcoholic compounds, fruit acids, and multivitamins such as C, E, and K. In addition, carotenoids, phenolic compounds, fiber, amino acids, some minerals are crucial parts of this fruit, due to which it is considered highly nutritious [3,4]. Recently, the antioxidant, anti-inflammatory, and anti-radiation activities were observed much better on burns and ulcer problems due to a variety of constituents such as nutrients, polysaccharides, vitamins, fatty acid, terpenoids, flavonoids, organic acids and volatile organic compounds [5-7]. The extracts of different parts of plants have been reported for usage in different applications [8-10]. Leaves, berries barks, husks, and other parts of the SBT plants have been used for the last century in various countries due to their significant medicinal and nutritional applications. Berries and leaves of SBT are a big producer of bioactive materials such as flavonoids (subclasses includes flavones, anthocyanins, flavanols, isoflavones, flavan-3-ols etc.) have positive effect on the human body, most importantly categories as an antioxidant, anticancer property, and antibacterial impacts. Moreover, the phenolic contents and antioxidant potential in leaves of sea buckthorn are superior as compared to berries [11-13]. The SBT fruit contains carbohydrates (hydrated sugars), fruity acids, water-soluble vitamins, [14,15] flavones, light-weight amino acids. 19 Amino Acids (AA) including eight essential and

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three major amino acids, such as glutamic acid (11.76-16.48%) aspartic acid (0.43-55.68% of total amino acids), serine (0.03-11.12% of in the sea buckthorn have been reported [16]. Specially, the fruit is thought to be extremely nourishing due to its rich nitridation. SBT contains oil both in soft components and inside the seed of the fruit. Maceration/Soxhlet extractor was used for extracting oil from the seeds of the test plant, which is very simple and reliable, and the extraction rate is high. The state-of-the-art of potential applications of SBT play a crucial role due to nature of oil such as solid lipid content, chain length of hydrocarbon and degree of saturation. The emulsion effect, which comprises size, viscosity, and stability with bioactive incorporated [17-19]. Besides, SBT has a positive impact on the nutrition and health of animals to improving their productivity. On the other hand, due to its excellent physicochemical, antioxidant properties, and shelf life of the wheat bread can be improved, which is a prolific sign to diversify the range of bakery products [20]. In addition, broad applications of SBT in the food including cheese, yoghurt, or beverages as well as in feed industries to improve the quality of final products such as meat, egg, and fish farming along with new opportunities for the development of food have been considered [21]. Green solvents are becoming sustainable products which are not limited to ethanol, ethyl acetate, or the combination of water and water-soluble and other low chain organic solvent [22-24]. Hexane, ethanol, diethyl ether and 2-methyltetrahydrofuran were used for the extraction of oil from sea buckthorn berries. Extractions with 2-MTHF had yielded oils with higher monounsaturated fatty acid as compared to hexane [25,26]. Ethanol is used because of its low cost; another fascinating green solvent that has been studied recently is 2-methyltetrahydrofuran (2-MTHF), which is produced out of carbohydrates from lignocellulose biomass [27].

The novelty of this work is how it emphasizes the seed by-products, complex and rational use of plant raw materials, to assess the potential of SBT seeds using five herbal extracts such as ethanol, methanol, ethyl acetate, *n*-hexane, and isopropyl alcohol by ferric reducing ability of plasma (FRAP) assay for evaluation of antioxidant activity to determine their practical significance. The findings outlined in the present research will provide theoretical support for the further utilization of sea buckthorn resources.

EXPERIMENTAL

1. Materials and Reagents

Ethanol (Sigma-Aldrich), methanol (Sigma-Aldrich), isopropyl alcohol (Sigma-Aldrich), ethyl acetate (Sigma-Aldrich), *n*-hexane, (Sigma-Aldrich), ascorbic acid (Sigma-Aldrich), ferric chloride, and 0.5% *O*-phenanthroline. All reagents were of analytic grade. Husky SBT (seeds were purchased from Namco's Ltd. Gilgit-Baltistan, Pakistan with 95% purity), and distilled water was used throughout the experimentation.

2. Plant Material

Sea buckthorn seeds were purchased from Namco's Ltd Gilgit-Baltistan, Pakistan with 95% purity. The samples collected were washed with distilled water and dried in an open atmosphere just to remove the water contents. Then, they were dried in incubator at 40 °C. Dried samples were ground by mortar and pestle to get granular form of seeds. Furthermore, 30 g of the sample was added in the thimble (a part of Soxhlet apparatus) and it related to 250 mL of round bottom flask with 300 mL of selected solvents (ethanol, methanol, ethyl acetate, *n*-hexane, and isopropyl alcohol) used for oil extraction from seeds at room temperature. The plant extracts were then stored at 4 °C.

3. Herbal Extract of SBT Seeds with Various Solvents

Five different solvents were taken separately into a round bottle flask, and 100 g of sea buckthorn seeds were put into each flask. Then, flasks were placed in dark for 24 hours. Afterwards, the flasks were set into an isomantle to extract the oil along with respective solvents. The crude herbal extracts were then distilled until the total volume of 50 mL remained for each herbal extract. These were regarded as stock solutions of the herbal extracts in the concerned solvents.

4. Preparation of Various Concentrations Solutions in Different Solvents of the Herbal Extracts

In this work, five herbal extracts of sea buckthorn seed oil were prepared in five different solvents, such as ethanol, methanol, *n*-hexane, ethyl acetate, and isopropyl alcohol with concentration 1-5 mL; then the prepared solutions were incubated for 24 hours. Moreover, 200 mM ferric chloride and *O*-phenanthroline solution were added and absorbance was observed by using a UV-Vis spectrophotometer at 510 nm. Solutions of different concentrations ranging from 1-5 mL were made by taking 1 mL of the herbal extract with a pipette and shifted to the 5 mL volumetric flask and filled with the concerned solvent, in which its herbal extract was prepared, up to the mark. In this way, a solution of 1 mL was prepared. For the preparation of 2 mL solution, 5 mL of herbal extract of each solvent was taken into the volumetric flask and made up to the mark with relevant solvent, in which its herbal extraction was done. Moreover, for the preparation of 3 mL, 4 mL and 5 mL solutions, 3 mL, 4 mL, and 5 mL of the herbal extract from each solvent was taken into the volumetric flask and made up to the mark, respectively.

5. Preparation of Ferric Chloride Solution

200 mM ferric chloride ($FeCl_3$) solution was prepared in 1 dm^3 of distilled water by adding 0.27 g of ferric chloride (Hexa-hydrated form), i.e., $FeCl_3 \cdot 6H_2O$ in a 1,000 mL volumetric flask making the volume up to the mark. A dark yellowish transparent solution was obtained. To synthesize the 0.5% *O*-phenanthroline solution, 0.5 g of *O*-phenanthroline was taken in the 100 mL volumetric flask and a small amount of absolute ethanol was added and shaken with stirring continuously. Then, adding more ethanol filled the flask up to the mark. 0.05 M solution of ascorbic acid was also synthesized by the dissolution of 0.88 g ascorbic acid in a 100 mL volumetric flask along with distilled water.

6. Ferric Chloride Method

In this method, the sample mixture had 2 mL of 0.5% *O*-Phenanthroline, 200 mM Ferric chloride solution, and same quantity of extracts at various concentrations ranging from 1-5 mL. Then, sample mixture was incubated for 30 minutes at ambient temperature. Ascorbic acid was added as standard, and absorbance was obtained 100% having a reduction of all ferric ions. In term of chemistry, all ferric ions in the form of ferric chloride get reduced to ferrous ions, the organic reagent that is itself a locating agent, that is, *O*-Phenanthroline forms an abruptly bright red colored complex which is fer-

rous ion. Blank absorbance was observed without extract by UV-Vis (CECIL CE 7400s, Cambridge UK) at 510 nm. From each extract, five solutions of concentration ranging from 1-5 mL were prepared in the respective solvents as which solvents were utilized for extraction. 2, 2 mL of ferric chloride (200 mM) and 1, 1 mL of 0.5% *O*-phenanthroline (in ethanol) were added and incubated continuously for around 30 minutes at ambient temperature.

RESULTS AND DISCUSSION

1. Antioxidant Activity

An antioxidant is a molecule that prevents the other molecules from being oxidized and plays a significant role as a preservative agent. Oxidation is a chemical reaction that may propagate through chain reactions and cause the deterioration of living healthy cells and stop the free radical chain reaction, for instance, phenols and ascorbic acid (vitamin C) [28-31]. Vitamin C is widely known as a redox catalyst, that has the ability for reduction, and thus neutralization will occur resulting in the formation of reactive oxygen species (ROS) including H_2O_2. Antioxidant potential of various parts of the plants are reported as: seed extract>root extract>leaf extract> stem extract [32]. Some diets containing antioxidant dietary supplements are not useful from a health point of view or they do not show any positive behavior toward preventing diseases. Polyphenols, regularly have cancer prevention agent characteristics *in vitro*, and are not cell reinforcements *in vivo* because of broad digestion [33]. This vitamin (ascorbic acid) plays a significant role in the transformation from procollagen to the collagen, which then oxidizes proline constituent to hydroxyproline.

2. Ferric Reducing Ability of Plasma (FRAP) Assay Quantification

FRAP assay quantifies the antioxidant activity by reducing the ferric ions (Fe^{3+}) to the ferrous ones (Fe^{2+}) present in the sample [34]. A biological antioxidant is a constituent that exists at lower concentrations than the oxidizable substratum, which on the other hand suggestively suspends or stops the substrate oxidation. The only alteration is that the oxidizing species reacts with the antioxidant, not to that of the substrate. For example, the antioxidant property of vitamin C reduces oxidizing substances like hydrogen peroxide (H_2O_2) along with reduction of metal ions involves generating the free radicals through the Fenton reaction mentioned below.

i. 2 Ferric ions+Ascorbate
→2 Ferrous ions+Dehydro-ascorbate
ii. 2 Ferrous ions+2 hydrogen peroxide
→2 Ferric ions+2 hydroxyl radicals+2 hydroxyl ions

In this framework, antioxidant power may be denoted as reducing ability using the reductants analyzed by spectrophotometry. Free radical formation such as superoxide ($O_2^{\cdot-}$) is produced from O_2 via hydroperoxyl radicals in the basic media as well as NADPH oxides give rise H_2O_2. Superoxide ($O_2^{\cdot-}$) then can be converted into H_2O_2 by Heber Weiss reaction. Furthermore, ($OH^{\cdot}$), (OH^-) and H_2O can be obtained through a chain-like reaction mechanism. In the same way, when Fe^{2+} reacts with H_2O_2 in Fenton reaction to give Fe^{3+}, ($OH^{\cdot}$), (OH^-) and finally H_2O [35]. The free radical formation can interact with other very active species and cause damage to the system.

Table 1. Absorbance of three different solvents at standard concentration and 510 nm

Sample name	Concentration (mL)	Absorbance
n-Hexane	1	0.133
	2	0.166
	3	0.233
	4	0.366
	5	0.600
Isopropyl extract	1	0.760
	2	0.866
	3	0.960
	4	1.033
	5	1.073
Ethyl acetate extract	1	0.090
	2	0.155
	3	0.255
	4	1.320
	5	1.455

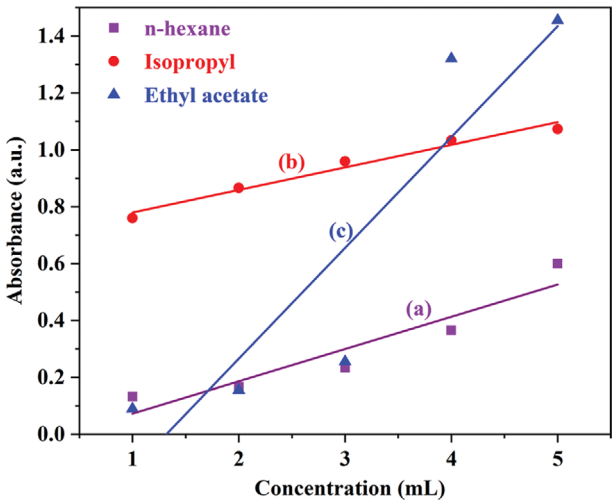

Fig. 1. SBT seeds extract of vitamin C at standard concentration of different solvents: (a) *n*-hexane extract, (b) isopropyl extract, and (c) ethyl acetate extract.

The FRAP assay was performed according to the method described by Szydłowska-Czerniak [36].

Antioxidant activity is largely affected by polarity index of the solvents in which herbal extracts of sea buckthorn seeds have been prepared. Absorbance was detected by UV-Vis, such as *n*-hexane extract showed absorbance at 510 nm for 1-5 mL with relative concentrations 0.133, 0.166, 0.233, 0.366 and 0.600 consistently as shown in Table 1, which also can be clearly shown in Fig. 1(a). Isopropyl alcohol extract for concentration 1-5 mL at 510 nm was observed as 0.760, 0.866, 0.960, 1.033 and 1.073 shown in Fig. 1(b) and Table 1. Moreover, ethyl acetate extracts showed absorbance 0.090, 0.155, 0.255, 1.320 and 1.455 respectively for concentration 1-5 mL at 510 nm, shown in Fig. 1(c).

Similarly, ethanol showed absorbance at 510 nm for the concentration of 1-5 mL, which was 0.049, 0.102, 0.202, 0.256 and 0.345

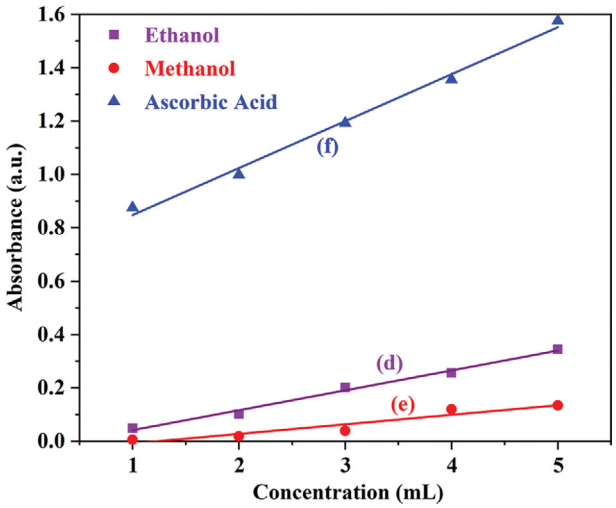

Fig. 2. SBT seeds extract of vitamin C at standard concentration of different solvents (d) ethanolic extract, (e) methanolic extract, (f) ascorbic acid (standard).

Table 2. Absorbance of three various solvents at standard concentration and 510 nm

Sample name	Concentration (mL)	Absorbance
Ethanol extract	1	0.049
	2	0.102
	3	0.202
	4	0.256
	5	0.345
Methanol extract	1	0.006
	2	0.018
	3	0.039
	4	0.120
	5	0.134
Ascorbic Acid (standard)	1	0.875
	2	0.998
	3	1.192
	4	1.356
	5	1.576

correspondingly as shown in Fig. 2(d) and Table 2. Methanol extract showed absorbance at 510 nm for same concentration as mentioned above and 0.006, 0.018, 0.039, 0.120, and 0.134 as shown in Fig. 2(e). In addition, absorbance observed for ascorbic acid (standard) for various concentration 1-5 mL at 510 nm was claimed 0.875, 0.998, 1.192, 1.356 and 1.576 denoted in [37,38] Fig. 2(f).

Absorbance values of various organic solvents at given different concentrations as shown in Table S1. Maximum ferric reducing ability (antioxidant activity) for *n*-hexane, isopropyl alcohol, and ethyl acetate extract for highest concentration of 5 mL was observed 55%, 53% and 68% 56%, 62%, and 99%, respectively, as shown in Table 3. However, highest ferric-reducing ability of ethanol, methanol extracts for the same concentration 1-5 mL was 56% and 60% correspondingly. However, the highest FRAP ability of ascorbic acid (vitamin C) for the maximum concentration of 5 mL was perceived 99%.

The antioxidant characteristic of the emulsions was observed as greater oil content and a higher scavenging activity for the DPPH radicals [39]. Polyphenol, flavonoids, vitamins like vitamin C and E are higher in leaves than barriers of sea buckthorn [40]. However, *Hippophae rhamnoides* (Sea buckthorn) seeds were successfully extracted by using different organic solvents such as chloroform ($CHCl_3$), acetone (C_3H_6O), ethyl acetate ($C_4H_8O_2$), and methanol (CH_3OH) using a Soxhlet extractor for eight hour each studied. It has been reported that methanol showed maximum antibacterial activity as compared to other solvents [41], which proved experimentally that DPPH scavenging activity of sea buckthorn seed extract was maximum for that particular solvent, which had maximum polarity index such as water reported the efficiency of reducing power of sea buckthorn seed oil extract in different solvents and suggested that study that ascorbic acid had maximum value in this regard [42]. Comparative studies with other organic solvents like ethanol, methanol, *n*-hexane, di-ethyl ether, and 2-MTHF have been reported [43-45].

The temperature effect was studied in SBT and total phenolic compounds were slightly higher at maximum temperature 20 °C than 0.5 and 10 °C. No positive effect in the concentrations of ascorbic acid in fruit even stored for two days. Later, a significant low was observed in the fruit maintained at 0.5 °C and 20 °C but, on the other hand, remained unchanged at 10 °C. However, at 10 °C, the antioxidant capability of the stored fruit was improved than that of 0.5 °C and 20 °C on the third day [46]. However, percent ferric reducing ability of ferric ions to ferrous ions with different organic solvents (*n*-hexane, isopropyl alcohol, ethyl acetate, ethanol, methanol, and ascorbic acid) was observed for the concentration of 1-5 mL with 68% in case of ethyl acetate as shown in Fig. 3.

The reduction capability of the ascorbic acid, as well as the SBT

Table 3. Comparison of % ferric reducing ability of ferric ions to ferrous ions for concentration of 1-5 mL

Concentration (mL)	% Reducing Ability (*n*-hexane)	% Reducing Ability (2-propanol)	% Reducing Ability (Ethyl acetate)	% Reducing Ability (Ethanol)	% Reducing Ability (Methanol)	% Reducing Ability (Vita. C)
1	15%	33%	30%	31%	12%	89%
2	23%	40%	39%	44%	22%	92%
3	31%	45%	48%	49%	35%	95%
4	42%	52%	54%	53%	52%	97%
5	55%	53%	68%	56%	62%	99%

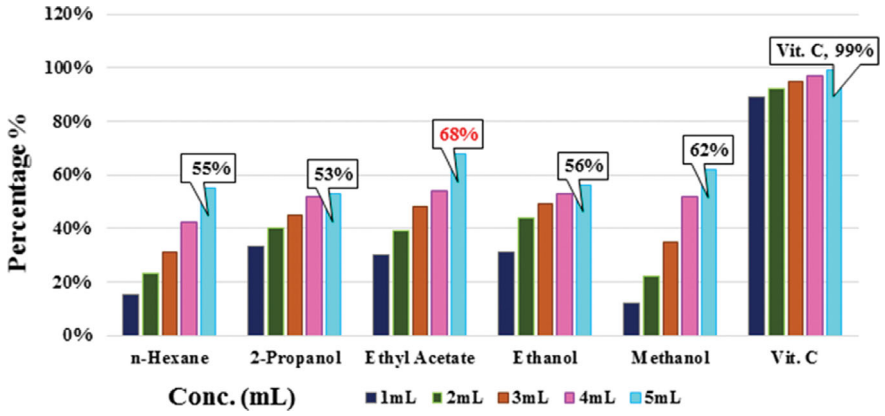

Fig. 3. Optimization of ferric reducing ability of ferric ions to ferrous ions under various concentration of solvents.

seeds extract, tracked the succeeding trend, i.e., Maceration<Soxhlet< SWE 150<ascorbic acid. This was observed that 30 v/w% dried sea buckthorn seed presented a yield of carotenes and tocopherols of nearly 69.6% and 91.1%, respectively, in the presence of 2-propanol as an entrainer, at optimized conditions [47].

SBT seeds were successively extracted by using the following solvents--ethyl acetate, acetone, chloroform, and methanol--by using a Soxhlet extractor for eight hours [47]. However, the activity performance showing the likelihood of employing sea buckthorn seeds for food preservation and for the medicinal uses [41] indicated that tocotrienols and tocopherols contents in the fresh berries had two to three times greater than the other two respective subspecies (120 mg/kg vs 40 mg/kg in *rhamnoides* and 50 mg/kg in *mongolica*). The proportions of linoleic acid (47.7 vs 42.7%, $p<0.05$, in seeds) and palmitic acid (17.0 vs 14.1%, $p<0.05$, in seeds) [48].

The antioxidant, antibacterial effects of aqueous and hydroalcoholic extracts of sea buckthorn leaves by employing in the *vitro* systems and examination were made by the RP-HPLC [49]. SBT leaves *in vitro* systems and their examination for the marker complexes with total phenol and flavonoids contents were measured by RP-HPLC. The contents of saturated, monounsaturated, and polyunsaturated fatty acids were reported as 13.70-42.68%, 40.73-60.37% and 3.70-24.62%, of total fatty acids [50]. Moreover, it can be used as a contender for the functional food with superior antioxidative characteristics studied in the SBT seedcake extract to assess the antioxidant potential [51-53]. It was investigated that pure methanolic extract presented a maximum recovery of up to 14%, and the phenolic compounds in the range of around 236.50±2.60 mg of GAE/gram extract), in comparison with the associated extracts. The outcomes obtained by the IC_{50} values showed that the pure methanolic extract was a superior scavenger of DPPH, ABTS, hydroxyl, superoxide, and nitric oxide. Furthermore, the sea buckthorn seeds were dipped for 24 hours in herbal extractions. This might have caused a big difference in their antioxidant values by using DPPH method, which had already been discussed in [17,18,54]. IC_{50} values for DPPH scavenging activity ranged from 1.6 (gallic acid) to 10.0 μg mL^{-1} (rutin); IC_{50} values for the hydroxyl radical scavenging were 18.0 (quercetin) 89.0 μg mL^{-1} (rutin); quercetin had the highest Fe^{2+} chelation capacity (83%) [55]. Moreover, good antioxidative properties of sea buckthorn seedcake were observed *in vitro* than *in vivo* study [40,52]. The crude ethanolic extracts of SBT root, stem, leaf, and seed along with other segments were investigated by LLE by employing the ethyl acetate, hexane, water, and their antimicrobial as well as antioxidant activities. Roots extracts were better radical scavenger observed by HPLTC than that of leaves and stems [32].

FUTURE PERSPECTIVE AND CHALLENGES OF SBT FOODSTUFFS

Sea buckthorn berries are rich in health-supporting phytochemicals. SBT leaves, berries, roots, and all other parts are very crucial components from the nutritional point of view. Though, it would be more fascinating to develop a variety of food and other healthcare products from different parts of the SBT plant to boost its large-scale development. Moreover, advanced technologies and the selection of raw ingredients will strongly impact the final product. Besides that, qualitative and quantitative parameters, physical properties for instance acidity, bitterness, and excess of phenolic compounds are challenging steps for developers. In addition, various other parameters can be studied in SBT to observe the antimicrobial and drug delivery applications. However, some challenging properties have been already reported as malic acid and sourness of the SBT juice could be reduced by the addition of malolactic fermentation, which maintains the health-promoting phenolic compounds and leave extracts of composites play a crucial role for industrial applications and negative emission technologies [56-58]. The consumption of SBT berries as fresh fruit or as raw material for various food processing has great potential from the health point of view, but some sensory properties may have a negative impact or condense its use. It must be under consideration to make it more useful. Above and beyond, the major parts of the SBT plant have been under the consideration to study antioxidant, antimicrobial antibacterial, and other activities as well. Moreover, secondary constituents for instance bark, branches, stems can be converted into valuable products and useful for a variety of applications in the near future.

CONCLUSION

Sea buckthorn oil has strong potential to release the level of tox-

icity of oxidized lipids and maintains the stability of the immune system, which is protective to the liver against oxidative stress. Interestingly, the presence of phenolic in the SBT seeds and their herbal extracts showed good antioxidant activity checked by the iron chelating method, commonly known as the Ferric chloride ($FeCl_3$) method, which is based on the ferric reducing ability of plasma (FRAP) assay with the help of UV-Vis spectrophotometry. The maximum antioxidant potential was obtained in the case of ethyl acetate 68%, which can further reduce the free radical formation in the body because of its chemical and biological functions as less cell toxicity. Medium polarity and minimum cell toxicity of ethyl acetate increases the percentage antioxidant activity. Whereas, on the other hand, isopropyl showed minimum 53% antioxidant activity. Consequently, in-depth scientific research is in progress to see the effect of temperature, selection of other series of organic solvent to evaluate the comparative study for antimicrobial, antibacterial, antifungal activities and their exploitation are imperative for conversion of secondary constituents to valuable products.

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SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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Supporting Information

Antioxidant activity of sea buckthorn (*Hippophae rhamnoides*) seed oil extracted using various organic solvents

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Table S1. Taxonomical degree classification of sea buckthorn [51]

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Order	Rosales
Family	Elaeagnaceae
Genus	Hippophae
Species	Rhamnoides (Hippophae rhamnoides)

Table S2. Absorbance of various solvents at different concentrations

Concentration (μL)	Absorbance (n-hexane)	Absorbance (IPA)	Absorbance (Ethyl Acetate)	Absorbance (Ethanol)	Absorbance (Methanol)	Absorbance (Vitamin C)
1,000	0.133	0.760	0.090	0.049	0.006	0.875
2,000	0.166	0.866	0.155	0.102	0.018	0.998
3,000	0.233	0.960	0.255	0.202	0.039	1.192
4,000	0.366	1.033	1.320	0.256	0.120	1.356
5,000	0.600	1.073	1.455	0.345	0.134	1.576

Table S3. Comparison of % ferric reducing ability of ferric ions to ferrous ions for concentration of 1,000-5,000 μL

Concentration (μL)	% Reducing Ability (n-hexane)	% Reducing Ability (2-propanol)	% Reducing Ability (Ethyl acetate)	% Reducing Ability (Ethanol)	% Reducing Ability (Methanol)	% Reducing Ability (Vitamin C)
1,000	15%	33%	30%	31%	12%	89%
2,000	23%	40%	39%	44%	22%	92%
3,000	31%	45%	48%	49%	35%	95%
4,000	42%	52%	54%	53%	52%	97%
5,000	55%	53%	68%	56%	62%	99%