Antioxidant potential and total phenolic contents of seabuckthorn (*Hippophae rhamnoides*) pomace

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ABSTRACT

Introduction: Seabuckthorn (*Hippophae rhamnoides L., Elaeagnaceae*) is an important medicinal resource found in abundance in Indian subcontinent especially the North Western Himalayan regions and all parts of the plant are rich in bioactive substances. **Methods:** The antioxidative activity of three different extracts (100% Methanolic, 70% Methanolic, 100% Aqueous) of pomace were evaluated by various *in vitro* methods such as ABTS, DPPH, nitric oxide, reducing power, superoxide and hydroxyl radical. Total phenolic contents were determined spectrophometrically. **Results:** The 100% methanolic extract showed higher recovery (11%) and total phenolic contents (302.72 ± 4.17 mg of GAE/gm of extract), as compared to the other extracts. All the extracts were able to scavenge different *in vitro* radicals *i.e.* ABTS, DPPH, superoxide, hydroxyl and nitric oxide radicals, in a concentration dependent manner. The IC_{50} values, determined by using linear regression analysis, revealed that 100% methanolic extract was better scavenger of ABTS, DPPH and hydroxyl radicals. However, 70% methanolic extract was better scavenger of superoxide and nitric oxide radicals. The reducing power of the extracts was also found in a dose dependent manner and was higher in methanolic extracts (100% and 70%). **Conclusion:** On the basis of present study, it could be concluded that pomace of seabuckthorn has pronounced antioxidant properties and can be incorporated in the feed as a nutraceutical after conducting safety and toxicological studies.

Keywords: Antioxidant activity, *In Vitro* radicals, Pomace, Phenolic contents, Seabuckthorn.

INTRODUCTION

Variety of wild edible plant species in the Himalayan region have played a prominent role in providing food and medicine for human beings as well as animals. Seabuckthorn *(Hippophae rhamnoides)* is one of the most valuable plant of cold deserts regions of India because of vitamin, organic acids, fiber, pectic compounds, carotenes, polyunsaturated fatty acids and other components. The berries contain more than 100 different kinds of nutrients and bio-active substances and are rich source of vitamins, carotenoids, flavonoids, minerals, essential oils, and essential fatty acids. The presence of polyphenols in seabuckthorn fruits have shown antioxidant properties and can be used to protect human body against the damaging effect of oxidized radicals.[1] The presence of these bioactive substances in the fruit are mainly responsible for its immunomodulatory,[2] antioxidant,[3]

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anti-carcinogenic,[4] hepato-protective,[5] wound healing[6] properties.

The commercial production of seabuckthorn (*Hippophae rhamnoides*) juice results in a large amount of pomace, which is suggested to contain substantial amounts of valuable natural antioxidants. The pomace, one of the by product of seabuckthorn and end product of seabuckthorn berries, has been utilized in Russia in baking of bread and manufacturing of vitamin mixtures for farm animals and birds.[7] Similarly, seabuckthorn pomace is used in Estonia to increase the nutritive value of wheat bread.[8] But, the studies on the validation of the nutritive or antioxidant properties of pomace in India are still lacking. So, the present study was aimed to evaluate the *in vitro* antioxidant properties of various extracts of Seabuckthorn pomace.

MATERIALS AND METHODS

Chemicals and reagents used

Sodium carbonate, Folin Coicaltue Reagent (FCR), gallic acid (GA), 2,2-diphenyl-1-picryl hydrazyl (DPPH), Butylated hydroxytolune (BHT), Potassium persulfate, (ABTS), Nitroblue tetrazolium (NBT), Nicotineamide adenine dinucleotide hydrogen salt (NADH), Phenazine methosulphate (PMS), Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Ascorbic acid (AA), 2-deoxy-D-ribose, Potassium dihydrogen phosphate (KH_2PO_4), Potassium hydroxide (KOH), Ethylene diamine tetramine (EDTA), Ferric chloride ($FeCl_3$), Hydrogen peroxide (H_2O_2), Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Griess' reagent (sulphanilamide 1%, o-phosphoric acid 2% and naphthyl ethylene diamine dihydrochloride 0.1%), Sodium nitroprusside and Potassium ferricyanide.

Collection of byproducts of seabuckthorn plant

The pomace of seabuckthorn plant (*Hippophae rhamnoides L.*) was collected from Keylong region of Himachal Pradesh, India during the month of October, 2009 and was used for the present study for determining *in vitro* antioxidant potential.

Preparation of extract

After collection, the pomace was shade dried, powdered and stored at the room temperature (18–22ºC) till further process. The powder of pomace was soaked in different solvents [100% Methanol, 70% methanol (aqua-methanol) and 100% aqueous] for 24 hours and kept at room temperature with intermittent shaking. The mixture was then filtered and the extracts were prepared after drying the filtrate in rotatory vacuum evaporator at 40ºC. The percentage of recovery was calculated after determining the weight of the extracts. Finally, the dried extracts were lyophilized and stored at 4ºC till analysis of *in vitro* antioxidant parameters.

ANTIOXIDATIVE ACTIVITY ASSAYS

Determination of total phenolic content

The total phenolic content in the different extracts of seabuckthorn pomace was estimated by using Folin-Ciocalteu reagent (FCR) based assay of Gulcin.[9] The extract (50 µl of 1 mg/ml solution) was added to 950 µl of water. After proper mixing, FCR (500 µl) and 2.5 ml of the 20% sodium carbonate solution were added. The mixture was then kept for 40 min at room temperature and absorbance was recorded at 725 nm. Total phenolic contents in the extracts were calculated from the standard curve of Gallic acid. Through the standard curve of gallic acid following factor was used to determine the concentration of total phenols in terms of gallic acid equivalent per gram of extract (GAE/g of extract).

Total Phenolic contents (GAE/ g of extract) =
Optical Density × 860

Measurement of total antioxidant activity

The total antioxidant activity of different extracts were determined according to the method of Re[10] based on $ABTS^{\bullet+}$ scavenging assay. A 2 mM ABTS was taken in volumetric flask and 100ml double distilled water was added. ABTS radical cations ($ABTS^{\bullet+}$) were produced by mixing the stock solution with 400 µl of 70 mM potassium persulfate. To ensure complete oxidation of ABTS, the mixture was held at room temperature in the dark for 6 to 12 hr prior to analysis. The resulting $ABTS^{\bullet+}$ solution was diluted with phosphate buffer (about twelve fold) to give an absorbance reading of 0.750 at 734 nm. The antioxidant activity of different extracts of pomace with seeds was evaluated in concentration (mg/ml) ranging from 0.125 to 2.0.

The radical scavenging capacity was performed by mixing 30 µl of the extract (sample) solution (of different concentrations) into 3.0 ml of $ABTS^{\bullet+}$ solution. After

proper mixing, the absorbance was recorded at 734 nm after 3 minutes against distilled water. A control solution of 30 µl 70% methanol in 3.0 ml of $ABTS^{\bullet+}$ solution was also prepared and analyzed. The percentage of inhibition of $ABTS^{\bullet+}$ radicals at different concentations were determined by using the following formulae:

$$\% \text{ ABTS}^{\bullet+} \text{ inhibition} = [1 - (A_{734} \text{ nm Sample}/A_{734} \text{ nm Control})] \times 100$$

Free radical scavenging activity

The potential of extracts to scavenge DPPH radicals was determined according to the method of Hsu.[11] A 100 µM DPPH samples was taken in 50 ml volumetric flask and diluted with 70% methanol in water. The free radical scavenging activity of extracts was evaluated at different concentrations ranges from 10–500 µg/ml.

A 0.5 ml sample solution (extract of different concentrations) was added to 2.0 ml of DPPH solution. A control solution was also prepared by adding 0.5 ml of 70% (v/v) methanol in water to 2 ml DPPH solution. Samples were vortexed for 10 to 15 seconds and kept at room temperature (22 ± 3°C) in the dark for 30 minutes. The absorbance of the samples and control solutions were determined at 517 nm against water and the % DPPH radical scavenging activity was calculated as follows:

$$\% \text{ DPPH radical scavenging activity} = [1 - (A_{517} \text{ nm sample}/A_{517} \text{ nm control})] \times 100$$

Superoxide anion radical scavenging assay

The superoxide anion radical-scavenging ability of extract was assessed by the method described by Nishikimi[12] followed by slight modification. The superoxide anion radical scavenging activity of extracts of pomace was evaluated in concentration ranging from 10–100 µg/ml. In this experiment, the reaction mixture contained 1 ml of NBT solution (156 µM prepared in phosphate buffer, pH-7.4), 1 ml of NADH solution (468 µM prepared in phosphate buffer, pH-7.4) and 0.5 ml diluted sample of different concentrations fraction. Finally, acceleration of the reaction was carried out by adding 100 µL PMS solution (60 µM prepared in phosphate buffer, pH -7.4) to the mixture. The reaction mixture and control sample (0.5 ml methanol instead of sample solution) were mixed properly and incubated at 25°C for 5 min and absorbance at 560 nm was measured against distilled water. Decreasing value of absorbance of reaction mixture indicated increasing superoxide anion scavenging activity. Percentage inhibition of the superoxide anion radicals was calculated using the following equation:

$$\% \text{ superoxide radical scavenging activity} = [1 - (A_{560} \text{ nm sample}/A_{560} \text{ nm control})] \times 100$$

Hydroxyl radical scavenging assay

The potential of different concentrations of pomace of Seabuckthorn to scavenge the hydroxyl radical generated by the Fenton reaction was measured according to the method of Elizabeth and Rao.[13] The hydroxyl radical attacks deoxyribose, which eventually results in thiobarbituric acid reacting substance (TBARS) formation. The reaction mixture contained in a final volume of 1.0 mL [100 µL of 2-deoxy-D-ribose (28 mM in KH_2PO_4-KOH buffer, 20 mM, pH 7.4), 100 µL of different concentrations of extacts (1.25–40 µg/mL) in KH_2PO_4- KOH buffer (800 µL 20 mM, pH 7.4)] was mixed with 200 µL of 1.04 mM EDTA and 200 µM of $FeCl_3$ (1:1 v/v), 100 µL of 1.0 mM of H_2O_2 and 100 µL of 1.0 mM ascorbic acid and incubated at 37ºC for 1 h. After incubation, thiobarbituric acid (1%) (1 mL) and trichloroacetic acid (2.8%) (1 mL) were added to the reaction mixture and incubated at 100ºC for 20 min. After cooling, absorbance was measured at 532 nm. The control sample was also prepared having no extract but instead of 800 µL have 900 µL KH_2PO_4- KOH buffer and rest of the procedure is same. From the absorbance the % scavenging activity was calculated using the following formula.

$$\% \text{ Hydroxyl radical scavenging activity} = [1 - (A_{532} \text{ nm sample}/A_{532} \text{ nm control})] \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured by the method of Green[14] with slight modification by using Griess' reagent. About 250 µl sample of different concentrations of the extracts (10–60 µg/ml) and control sample (250 µl distilled water instead of extract) were mixed with an equal volume (250 µl) of sodium nitroprusside solution (5 mM in standard phosphate buffer pH 7.4) and incubated at 25ºC for 1 h. After incubation, the incubation mixture was mixed with 0.5 ml of Griess' reagent (sulphanilamide 1%, o-phosphoric acid 2% and naphthyl ethylene diamine dihydrochloride 0.1%) and diluted with 2 ml of distilled water. Finally, the absorbance was measured at 546 nm. From the absorbance the % scavenging activity was calculated using the following formula.

$$\% \text{ Nitric oxide radical scavenging activity} = [1 - (A_{546} \text{ nm sample}/A_{546} \text{ nm control})] \times 100$$

Reducing power assay

Reducing power of extracts of pomace was determined by the method of Oyaizu[15] with slight modification. Different concentrations (10–200 μg/ml) of the sample (1 ml) and control sample (1 ml distilled water instead of sample solution) were mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml potassium ferricyanide (0.25%). The final mixture was properly mixed and incubated at 50ºC for 20 minutes. After incubation, the reaction mixture rapidly cooled, mixed with 2.5 ml of 10% TCA and centrifuged at 3000 rpm for 10 minutes. About 2.5 ml of the supernatant was taken and 2.5 ml distilled water and 0.5 ml of ferric chloride (0.025%) were added to it, mixed well and allowed to stand for 10 minutes. The absorbance was measured at 700 nm.

STATISTICAL ANALYSIS

All the experiments were repeated three times and the data were represented as mean ± SD. The linear regression analysis was used to calculate IC_{50} values.

RESULTS

The percentage recovery and total phenolic contents (mg of GAE/gm of extract) of different extracts are presented in Fig. 1 and 2. The percentage recovery was higher in methanolic extracts (100% and 70% methanolic) as compared to aqueous extract. The totals phenols were also more in methanolic extracts as compared to aqueous one. The inhibition of the different *in-vitro* free radicals i.e. ABTS (Fig. 3 and 4), DPPH (Fig. 5), superoxide (Fig. 6), hydroxyl (Fig. 7) and nitric oxide radicals (Fig. 8) by all the extracts was found in a concentration dependent manner. The activity of the extracts at different range of concentration for inhibiting these radicals was also compared with standard antioxidants i.e Trolox, BHT, and Ascorbic acid in the figures. Finally, the IC_{50} values of the different extracts for the total antioxidant activity, free radical scavenging assay, superoxide radical scavenging assay, hydroxyl and nitric oxide radicals scavenging assay are presented in Table 1.

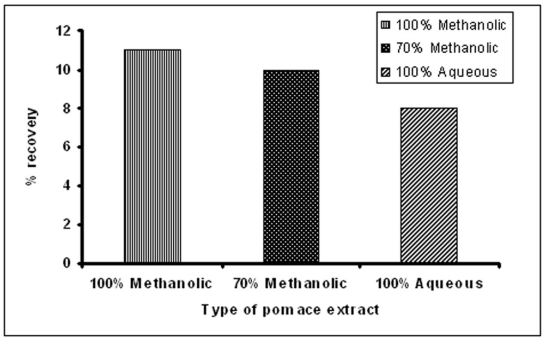

Figure 1. Percentage recovery of different extracts of pomace of Seabuckthorn.

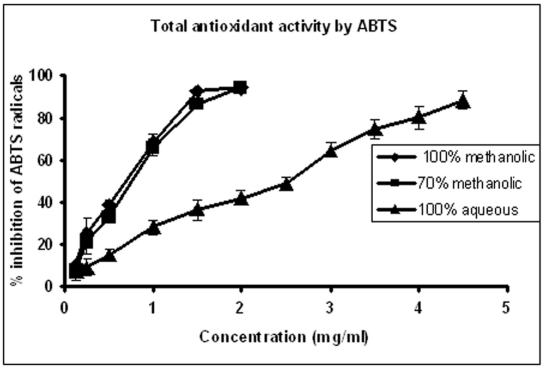

Figure 3. Total antioxidant activity of different concentrations of extracts of pomace by ABTS radical and results are expressed as mean ± SE. (n = 3).

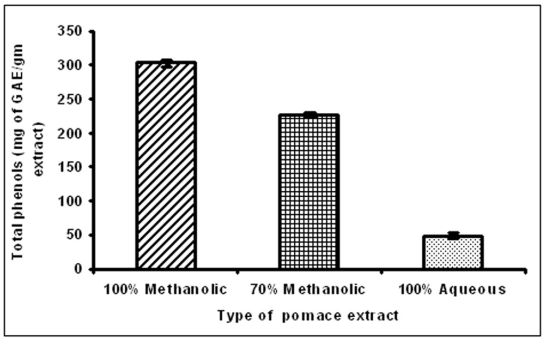

Figure 2. Total Phenols for different extracts of pomace of Seabuckthorn.

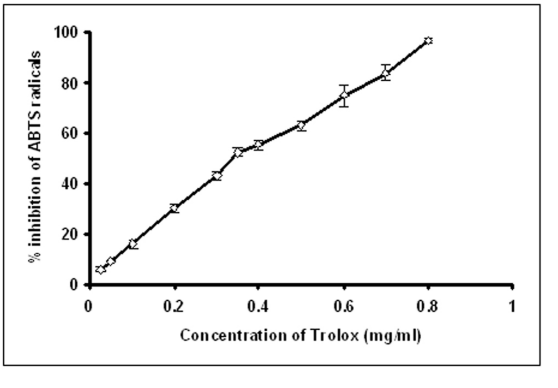

Figure 4. Total antioxidant activity of Standard Trolox by ABTS radical and results are expressed as mean ± SE. (n = 3).

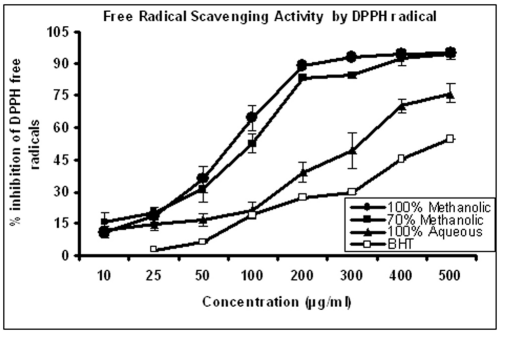

Figure 5. Free Radical Scavenging Activity of different concentrations of extracts of pomace by DPPH radical and results are expressed as mean ± SE. (n = 3).

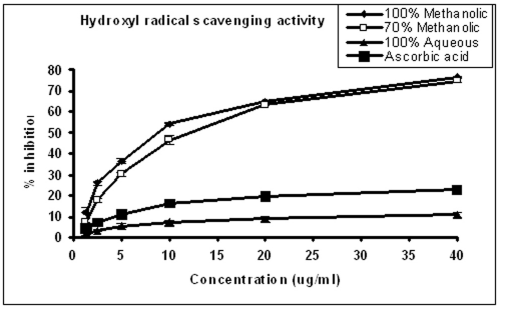

Figure 7. Percentage inhibition of Hydroxyl radical by different extracts of pomace and standard ascorbic acid at different concentrations. Results are expressed as mean ± SE. (n = 3).

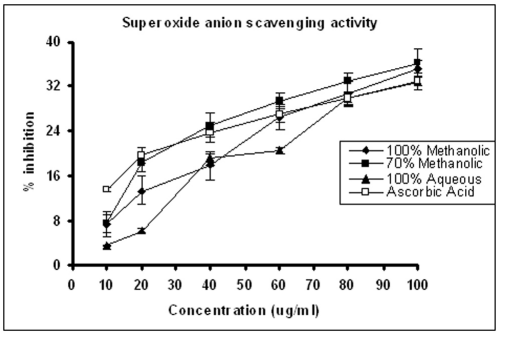

Figure 6. Superoxide anion scavenging activity of different extracts of pomace and standard ascorbic acid. Results are expressed as mean ± SE. (n = 3).

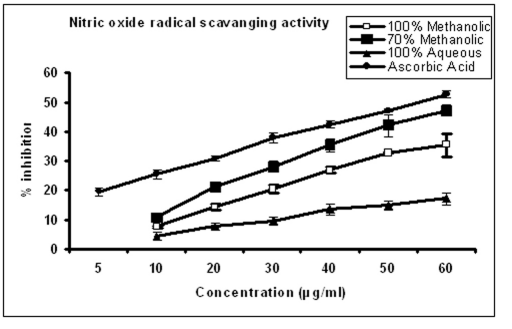

Figure 8. Percentage inhibition of Nitric oxide radical by different extracts of pomace and standard ascorbic acid at different concentrations. Results are expressed as mean ± SE. (n = 3).

Table 1 The IC_{50} values of different extracts of pomace of Seabuckthorn for different parameters. The values are expressed as Mean ± SE. (n = 3)

Parameters	Type of Pomace Extracts			Standard		
	100% Methanolic	70% Methanolic	100% Aqueous	Trolox	BHT	Ascorbic Acid
Total Antioxidant activity (ABTS) (mg/ml)	0.717 ± 0.049	0.802 ± 0.032	2.050 ± 0.099	0.38 ± 0.01	–	–
Free Radical Scavenging activity (DPPH) (µg/ml)	105.62 ± 21.35	120.61 ± 21.89	199.82 ± 17.44	–	559.37 ± 4.97	–
Superoxide Radical scavenging activity (µg/ml)	149.85 ± 16.25	142.15 ± 14.43	207.88 ± 13.53	–	–	182.10 ± 9.35
Hydroxyl (OH.) radical scavenging activity (µg/ml)	16.67 ± 0.31	19.34 ± 0.45	229.25 ± 22.85	–	–	96.38 ± 6.003
Nitric Oxide (NO.) reducing power (µg/ml)	82.61 ± 5.06	61.99 ± 4.25	192.58 ± 19.40	–	–	55.46 ± 1.44

From the IC_{50} values of the extracts for the different radicals, it was observed that 100% methanolic extract was better scavenger of ABTS, DPPH and hydroxyl radicals, whereas, 70% methanolic extract was better scavenger of superoxide and nitric oxide radicals. The reducing power of the extracts was found in a dose dependent manner (Table 2). It was observed that reducing power ability was more in methanolic extracts (100% and 70%) as compared to aqueous extract.

DISCUSSION

The potentially derivative of oxygen known as Reactive Oxygen Species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) are continuously generated inside the human body as a result of exposure to exogenous chemicals and/or various endogenous metabolic processes which involved redox enzymes and bioenergetic electron transport.[16]

Table 2 Reducing power capability for different extracts of pomace of seabuckthorn and values are expressed as absorbance as Mean ± SE. (n = 3)

Conc. of extract (µg/ml)	Type of Pomace Extracts			Standard
	100% M	70% M	100% A	BHT
10	0.21 ± 0.01	0.20 ± 0.01	0.19 ± 0.02	0.32 ± 0.01
20	0.23 ± 0.01	0.22 ± 0.01	0.21 ± 0.02	0.42 ± 0.01
40	0.26 ± 0.02	0.26 ± 0.02	0.22 ± 0.02	0.62 ± 0.01
60	0.29 ± 0.02	0.28 ± 0.02	0.23 ± 0.01	0.78 ± 0.01
80	0.32 ± 0.02	0.32 ± 0.02	0.25 ± 0.01	0.83 ± 0.01
100	0.37 ± 0.03	0.34 ± 0.03	0.26 ± 0.01	0.88 ± 0.01
200	0.46 ± 0.04	0.44 ± 0.03	0.30 ± 0.03	0.92 ± 0.01

The inadequate antioxidant defense and or overproduction of ROS leads to upsurge and results in oxidative stress. Some plants have good antioxidant availability and are safer than the synthetic antioxidants.[16] The plant/any substance shows its antioxidant activity by various mechanisms like prevention of chain initiation, chelation of transition metal, ion/catalyst, decomposition of peroxides, reductive capacity, and radical scavenging activity.[16]

Total phenols

The high levels of phenolic compounds in the plant extract are indicator of the radical scavenging activity.[17] More total phenolic contents in methanolic extracts revealing that methanolic extract possesses better antioxidant potential than aqueous extract. Phenols are very important plant constituent because of their scavenging ability due to their hydroxyl group[18] and have important role in stabilizing lipid oxidation and are associated with antioxidant activity.[19]

ABTS (2, 2, azonobis 3, ethylene benzothiazoline-6-sulphonic acid) radical scavenging assay

ABTS on reacting with potassium persulphate produces ABTS radical cation ($ABTS^{\cdot+}$), a blue green chromogen with absorption maximum at 734 nm. The extent of decolorization is significant indicator of antioxidant activity of the sample. The effects of antioxidants, reductants on ABTS radical cation is due to its hydrogen donating availability which is visually observed by a change in color radical cation ($ABTS^{\cdot+}$) to colorless ABTS. In the present study, the lower IC_{50} values of methanolic extracts (100% methanolic, 70% methanolic) as compared to aqueous extracts for total antioxidant activity indicates that methanolic extracts are better scavenger of ABTS ($ABTS^{\cdot+}$) radical.

DPPH (1, 1 diphenyl – 2-picrylhydrazyl) scavenging activity

The stable DPPH radical formed is widely used, relatively quick method for the evaluation of free radical scavenging assay.[20] DPPH is a stable free radical that accepts free electron or hydrogen radical to become a stable diamagnetic molecule.[21] The effect of antioxidant on DPPH radical scavenging is thought to be due to their hydrogen donating availability,[20] visually noticeable as a change in color from purple to yellow. DPPH free radical scavenging is an acceptable mechanism by which antioxidant act to inhibit lipid peroxidation, so scavenging of DPPH was used in the present study.[22] In the present study the comparison of scavenging effect of methanolic, aqua methanolic and aqueous extracts on DPPH radical showed that methanolic (100% methanolic, 70% methanolic) extracts had high radical scavenging activity as compared to aqueous extract. The findings of the present study revealed that the extracts were free radical inhibitors and acted as primary antioxidants. The results are in accordance with the findings of Negi et al,[23] who observed free radical scavenging activity of various seabuckthorn seed extracts.

Superoxide anion radical ($O_2^{\cdot-}$) scavenging activity

Superoxide anion radicals ($O_2^{\cdot-}$) are formed by activated phagocytes such as monocytes, macrophages, eoisinophills and neutophills and the production of superoxide anion is an important factor in the killing of bacteria by phagocytes.[20] Superoxide anions are the most common free radicals *in vivo* whose concentration increases under the conditions of oxidative stress. Their generation occurs in variety of biological system by auto-oxidation processes or enzymatic reactions and also produces other cell damaging free radicals and oxidizing agents.[24] Superoxide anion ($O_2^{\cdot-}$) is a reduced form of molecular oxygen has been implicated in the initiating oxidation reactions associated with aging.[25] They are also involved in several pathophysiological processes due to their transformation into more reactive species such as hydroxyl radical ($OH^{\cdot}$) that initiate lipid peroxidation.[22] The decrease in absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion ($O_2^{\cdot-}$) in the reaction mixture. In the

present study, the IC_{50} value of different extracts for the scavenging of superoxide anion radical was in the following descending order: 70% methanolic > 100% methanolic >100% aqueous. This order indicates that 70% methanolic extract is the best scavenger of superoxide anion radical.

Hydroxyl radical ($OH^{\cdot}$) scavenging activity

Hydroxyl radical is the most reactive free radical and can be formed from the superoxide anion (O_2^{-}) and hydrogen peroxide (H_2O_2) in the presence of metal ions such as copper and iron.[26] Among the oxygen radicals especially the hydroxyl radical is the most reactive and severely damages adjacent biomolecules such as proteins, DNA, PUFA, Nucleic acids and almost every biomolecule it touches. This damage causes aging, cancer and several diseases.[27] Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of living body against various diseases. In the present study, the scavenging of hydroxyl radical was determined using the ascorbic acid-iron-EDTA model of hydroxyl radical generated system. Generated hydroxyl radicals in the solution were detected by their ability to degrade 2 deoxy-2-ribose into fragments that on heating with TBA and at low pH form a pink chromogen.[28] Generally, the molecules that inhibit deoxyribose degradation are those that can chelate the iron ions and there by prevent them from complexing with deoxyribose and render them inactive in a Fenton reaction.[29] In the present study, the IC_{50} value of aqueous extracts of seabuckthorn byproducts was much higher as compared to methanolic seabuckthorn byproducts, which indicates that methanolic extracts are more potent scavenger of hydroxyl radical.

Nitric oxide ($NO^{\cdot}$) scavenging activity

Instead of possible benefit of nitric oxide radical ($NO^{\cdot}$), its contribution to oxidative damage is increasingly becoming evident. This is due to the fact that $NO^{\cdot}$ can react with superoxide to produce reactive peroxynitrite anions ($ONOO^{-}$), which is a potential strong oxidant that can decompose to produce hydroxyl ($OH^{\cdot}$) and nitric oxide (NO).[30] Increased concentration of $NO^{\cdot}$ has harmful effects in the body, so the regulation of the production of nitric oxide is necessary.[31] When nitric oxide is produced by macrophages, nitric oxide radical can be converted to $ONOO^{-}$, which will cause various deleterious chemical reactions in the biological system including triggering of lipid peroxidation, nitration of tyrosine residue of proteins, inactivation of aconites, inhibition of mitochondrial electron transport, and oxidation of biological thiol compounds.[32] Nitric oxide ($NO^{\cdot}$) is also implicated in inflammation, cancer and other pathological conditions in addition to ROS.[33] The plant extract may have the property to counteract the formation of nitric oxide (NO) radical and in turn may be of considerable interest in preventing the harmful effect of excessive nitric oxide generation in the body. The present study revealed that extracts exhibited strong nitric oxide radical scavenging activity which leads to the reduction of nitric concentration in the assay medium, a possible protective effect against oxidative damage. The IC_{50} value for nitric oxide scavenging activity of the aqueous extract was higher as compared to methanolic extracts indicating that the methanolic extracts are better scavenger of nitric oxide radicals.

Reducing power capability

The measurement of reductive ability can be investigated by the ferric (Fe^{3+}) to ferrous (Fe^{2+}) transformation in the presence of extract. The presences of reductants such as antioxidant substances in the samples (extracts) cause the reduction of Fe^{3+}/ferricyanide complex to the Fe^{2+} form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.[34] In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of the antioxidant samples. Antioxidants can be explained as reductants and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of the other.[35] The reducing capacity of a compound may serve as a significant indicator of its antioxidant potential. Similar to the antioxidant activity, the reducing power of the extract increased with increasing concentration of sample i.e. antioxidant activity may have a mutual correlation with reducing effect. In the present study, methanolic extracts had higher activity as compared to aqueous extract. Negi *et al.*[23] also reported the highest reducing power of methanolic extracts in seabuckthorn seed extracts. The results of reducing power demonstrate the electron donor properties of extracts of seabuckthorn byproducts thereby neutralizing free radicals by forming stable products. The result of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging. A recent study by Kruczek *et al*[36] has showed the antioxidant capacity of crude extracts containing carotenoids from the berries of various cultivars of Seabuckthorn (*Hippophae rhamnoides* L.). Strong correlation was also observed between DPPH scavenging activity and total phenolic content from all

the extracts of two species of sea-buckthorn; namely, *Hippophae salicifolia* D. Don and *Hippophae rhamnoides* L.[37] In review of Patel *et al*,[38] it has been described that seabuckthorn reduced recurrence of angina, ischemic electrocardiogram which might be due to decreased myocardial oxygen consumption and inhibition of platelet aggregation induced by collagen. So, in addition of the antioxidant properties of these extract, they can be validated for the prevention of these complications.

CONCLUSION

On the basis of present study, it could be concluded that pomace of seabuckthorn has pronounced antioxidant properties and can be incorporated in the feed as a nutraceutical after conducting safety and toxicological studies. They can also be tried to cure the various diseases particularly associated with oxidative stress.

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