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In vitro evaluation of antidiabetic and antioxidant activity of Seabuckthorn *(Hippophae rhamnoides* L.) leaves

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Seabuckthorn (*Hippophae rhamnoides* L.) is thorny nitrogen fixing deciduous shrub native to Asia and Europe. The present study was undertaken to evaluate the antidiabetic and antioxidant effect of two different extracts, namely, methanolic and aqueous of seabuckthorn (*H. rhamnoides* L.) leaves by *in vitro* assays. Seabuckthorn leaves were procured from Lahul area of H.P. Leaves were dried and subjected to methanolic and aqueous extraction. Antidiabetic activity was measured by alpha-glucosidase inhibitor assay. Antioxidant activity was studied by measuring the total antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (DPPH) free radical scavenging assay and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method. IC_{50} values of both extracts were calculated for all the assays. The results showed that methanolic extract of seabuckthorn leaves have the potential to inhibit alpha glucosidase with IC_{50} value of 0.25 mg/ml. Similar to antidiabetic effect, methanolic extract also exhibited better antioxidant activity as compared to aqueous extract. Therefore, it is suggested that methanolic extract of seabuckthorn leaves is a potential source for natural antidiabetic and antioxidant compounds and could have potential use in the management of diabetes mellitus.

Key words: *Hippophae rhamnoides,* seabuckthorn, alpha glucosidase, antidiabetic, antioxidant.

INTRODUCTION

India continues to be the 'Diabetic Capital' of the world with 50.8 million diabetics (Sharma et al., 2011). Similar trends have also been found in the companion animals, that is, dogs and cats. Diabetes is a growing problem in dogs and cats and prevalence is increasing over time due to several reasons such as genetics, environmental, obesity, physical inactivity, etc. One therapeutic approach for reducing postprandial hyperglycemia in patients with diabetes mellitus is preventing the absorption of carbohydrates after food intake. Alpha glucosidase is the enzyme that catalyzes the cleavage of glycosidic bonds in oligosaccharides and thus compounds inhibiting this enzyme could help prevent postprandial hyperglycemia by decreasing the rate of carbohydrate degradation to

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glucose (Kim et al., 2004). The plant extracts have long been used for the ethnomedical treatment of diabetes in various systems of medicine and are currently accepted as an alternative for diabetic therapy. However, for many plant extracts, there is no clear understanding of the mechanism of action. Therefore, natural α-glucosidase inhibitors from plant sources offer an attractive strategy for the control of postprandial hyperglycemia.

As there are disturbances in antioxidant defense systems in diabetes mellitus (Strain, 1991), treatment with antioxidant may contribute to the prevention and delaying of diabetic complications (Armstrong et al., 1996). This is currently the basis of the "unifying hypothesis" that hyperglycemia-induced oxidative stress may account for the pathogenesis of all diabetic complications (Brownlee, 2001). Therefore, in addition to control of blood glucose levels, control of oxidative stress offers another avenue for the treatment of the disease. Seabuckthorn (*Hippophae rhamnoides L.,* Elaegnacea) is a thorny deciduous shrub found in Europe, Central Asia and temperate regions of South Asia, India and China (Rousi, 1971). In India the plant inhabits dry temperate regions and high altitude region of Himachal Pradesh, Jammu and Kashmir and Uttrakhand.

The leaves of the plant are rich in flavonoids, tannins and triterpenes, and they have been used in some countries to make extracts, tea, animal feed, pharmaceuticals and cosmetics (Beveridge et al., 1999; Kallio et al., 2002). Several studies have indicated that extracts from seabuckthorn leaves have antioxidant, anti-bacterial, anti-viral, anti-tumor and immunomodulatory properties (Tsybikova et al., 1983; Ganju et al., 2005). Further, it is reported to be well tolerated and without any untoward reaction or side effects in toxicological studies (Upadhyay et al., 2009).

However, there are no previous reports of any *in vitro* α-glucosidase inhibitory activity of seabuckthorn extract. In view of all the aforementioned reports, the present study was aimed to evaluate the antidiabetic (hypoglycemic) and antioxidant effects of aqueous (Aq) and methanolic (MeOH) extracts of seabuckthorn leaves by *in vitro* assays.

MATERIALS AND METHODS

Plant collection

Seabuckthorn leaves were procured from Agricultural Research Extension Centre, Kukumseri, Lahual, Himachal Pradesh (India).

Sample processing

Leaves were dried and powdered. The powder was extracted in water and methanol. The extracts were filtered and filtrate were concentrated *in vacuo* using rotary evaporator at 45°C. The concentrated extracts were subjected to freeze drying to obtain dry powdered extracts. The lyophilized samples were used for further studies.

Antidiabetic activity

Antidiabetic activity was measured by alpha-glucosidase inhibitor assay (Srianta et al., 2013) at different concentrations of the sample ranging from 0.0625 to 0.50 mg/ml. Sample (0.1 ml) was added to a test tube containing 0.1 ml of 20 mM p-Nitrophenyl α-D-glucopyranoside (pNPG) and 2.2 ml of 100 mM phosphate buffer at pH 7.0, and then incubated for 5 min at 37°C. The reaction was initiated by addition of 0.1 ml of enzyme solution (1 mg/0.1 ml) followed by 15 min incubation at 37°C. The reaction was stopped by addition of 2.5 ml of 200 mM Na_2CO_3. The absorbance of p-nitrophenol released from pNPG was measured at 400 nm. Percentage inhibition of α-glucosidase activity was determined by the following equation:

$$\text{Alpha-glucosidase inhibition (\%)} = [1-(A_{400nm}\ \text{test}/A_{400nm}\ \text{control})] \times 100$$

IC_{50} value was calculated, that is, the amount of sample required to inhibit 50% α-glucosidase activity.

Antioxidant activity

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

The ability of extracts to scavenge DPPH radical was determined according to the method of Hsu et al. (2006) at different concentrations ranging from 0.0625 to 0.50 mg/ml. Butylated hydroxytoluene (BHT) was taken as reference standard. The absorbance of the test and control solutions was determined at 517 nm. The percent DPPH radical scavenging was determined by using the following formulae:

DPPH radical scavenging activity

$$(\%) = [1-(A_{517nm}\ \text{test}/A_{517nm}\ \text{control})] \times 100$$

The amount of the sample needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity.

Measurement of total antioxidant activity by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method

The total antioxidant activity was determined according to the method of Re et al. (1999) at different concentrations of the sample ranging from 0.0625 to 0.50 mg/ml. Trolox was taken as reference standard. ABTS reacts with potassium persulphate to produce ABTS radical cation (ABTS•+), a blue green chromogen with absorption maxima at 734 nm. The extent of decolorization is a significant indicator of antioxidant activity of the sample. The effects of antioxidants on ABTS radical cation is due to its hydrogen donating availability which is observed by a change in color radical cation (ABTS•+) to colorless ABTS. The absorbance of the test and control solutions was determined at 734 nm. The percentage of inhibition of ABTS•+ radicals at different concentrations were determined using the following formulae and further IC_{50} value was calculated:

$$\text{ABTS•+ inhibition (\%)} = [1-(A_{734nm}\ \text{test}/A_{734nm}\ \text{control})] \times 100$$

IC_{50} value denotes the concentration of sample required to scavenge

Table 1. Alpha glucosidase inhibitory activity of seabuckthorn extracts.

S/N	Concentration (mg/ml)	Inhibition (%)	
		S (Aq)	S (MeOH)
1	0.0625	14.5±0.047	8.57±0.029
2	0.125	40.24±0.041	39.39±0.009
3	0.250	40.67±0.038	64.08±0.004
4	0.500	43.09±0.652	73.67±0.008
	IC_{50} value mg/ml	n.d	0.25

n.d: not determined. Values are mean ± SEM; Means with different superscripts vary significantly ($p<0.001$) with each other.

Table 2. Antioxidant activity of seabuckthorn extracts.

S/N	Seabuckthorn Extract	$IC_{50\ Value}$ (mg/ml)	
		DPPH Method	ABTS Method
1	S (Aq)	$0.38±0.012^a$	n.d
2	S (MeOH)	$0.07±0.005^b$	$0.15±0.002^a$
3	Standard	$0.04±0.002^b$	$0.12±0.017^a$

n.d: Not determined. Values are mean ± SEM; Means with different superscripts vary significantly ($p<0.001$) with each other within columns.

scavenge 50% ABTS•+ radicals.

Statistics

The percent activity was plotted against the sample concentration and a linear regression test was done using Graph Pad INSTAT and IC_{50} value was interpolated from standard curve. Lower IC_{50} values indicate higher antidiabetic/antioxidant activity. All the tests were done in triplicates and the results were expressed in mean ± standard error of mean (SEM). The data was analyzed statistically using Tukey-Kramer test.

RESULTS

Antidiabetic activity

The results of antidiabetic activity using alpha glucosidase inhibitory assay of the aqueous and MeOH extracts of seabuckthorn leaves are shown in Table 1. Aqueous leaf extract showed low alpha glucosidase inhibition activity, that is, less than 50% even at the highest concentration (0.50 mg/ml) taken in the experiment and thus IC_{50} value could not be determined. MeOH leaf extract exhibited better antidiabetic activity with 73.67% enzyme inhibition at 0.50 mg/ml concentration and IC_{50} value was found to be 0.25 mg/ml. This reflected that methanolic extract of seabuckthorn leaves contains certain compound(s) with alpha glucosidase inhibitory activity.

Antioxidant activity

The results of antioxidant activity of aqueous and MeOH extracts of seabuckthorn leaves using DPPH free radical scavenger method and ABTS total radical scavenging activity are shown in Table 2. IC_{50} value of aqueous extract was found to be significantly lower than BHT (standard) used in DPPH method, whereas IC_{50} value by ABTS method could not be calculated as extract exhibited very low antioxidant activity. IC_{50} values of MeOH extract obtained by DPPH and ABTS method were found to be significantly equal to the IC_{50} values of the respective reference standards (BHT & Trolox). This suggested that MeOH leaf extract possess antioxidant activity and is more active than aqueous extract.

DISCUSSION

In general, the control of blood glucose concentrations near the normal range is mainly based on the use of oral hypoglycaemic/antihyperglycemic agents and insulin. As all of these treatments have limited efficacy and are associated with undesirable side effects (Harrower, 1994; Reuser and Wisselaar, 1994; Campbell et al., 1996), there is a renewed interest in plant based medicines modulating physiological effects in the prevention and cure of diabetes. The inhibition of alpha glucosidase enzyme by MeOH extract of seabuckthorn leaves in the present study, provides a strong biochemical basis for the management of diabetes via the control of glucose absorption. Seabuckthorn leaves have also been found to possess antidiabetic activity when used in STZ-induced diabetic rats (Kim et al., 2010). Cao et al. (2003) have also reported that flavonoids from the seed and fruit

residue of *Hippophae rhamnoides* L. exhibited hypoglycaemia and hypolipidemic effects.

Although there are reports of antidiabetic and antioxidant activity of seabuckthorn based on free radical scavenging activity, inhibition of lipid peroxidation and protection of β-cells resulting in decreased blood glucose levels (Cao et al., 2003; Zhang et al., 2010; Pandurangan et al., 2011; Sharma et al., 2011), there is perhaps no previous study correlative on *in vitro* α-glucosidase inhibitory activity. Thus, the results obtained from the present study are useful in determining the mechanism of action of plant extracts. The *in vitro* α-glucosidase inhibitory activity may not always correlate with the *in vivo* one (Ye et al., 2002). So, it is necessary to confirm the *in vivo* action after oral administration to live animals, which is an important step in screening plant extracts for physiological and pharmacological effects. However, *in vitro* data is useful in testing different extracts/fractions to rule out inactive compounds and hence save considerable time and money.

Conclusions

The present investigation concluded that seabuckthorn leaves possess anti-diabetic and antioxidant activity and act by inhibiting alpha-glucosidase enzyme and scavenging free radicals. Moreover, methanolic extract of seabuckthorn leaves was found to be more effective than aqueous extract and thus could have potential for application in the management of diabetes mellitus. Further studies on *in vivo* action and isolation of the principal bioactive constituent(s) are also needed.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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