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Full Length Research Paper

Antioxidant capacities and phytochemical composition of *Hippophae rhamnoides* L. leaves methanol and aqueous extracts from trans-Himalaya

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The aim of this study was to assess the antioxidant capacities, phytochemical constituents, total phenolic and flavonoid contents of methanol and aqueous extracts of *Hippophae rhamnoides* L. leaves form trans-Himalayan cold desert of Ladakh. The 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) scavenging capacity of the methanol and aqueous extracts increased in a dose dependent manner (up to 0.1 mg/ ml) and plant leaves extract concentrations required for 50% inhibition of DPPH radical scavenging effect (IC_{50}) were recorded as 0.014 and 0.015 mg/ml for methanol and aqueous extracts, respectively. The leaf extracts also scavenged the $ABTS^{\cdot+}$ radical generated by 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)/2.4 mM potassium persulfate (PPS) system and the IC_{50} values were found to be 0.017 and 0.018 mg/ml for methanol and aqueous extracts, respectively. The total antioxidant power of the extract was determined by ferric reducing antioxidant power (FRAP) assay. Qualitative analysis of the phytochemicals of aqueous extract revealed the presence of alkaloids, carbohydrates, saponins, proteins, phenols and flavonoids. The methanol extract was found to contain phenols and flavonoids as major phyto-components. Total polyphenol and phenolic acid contents in the methanol extract were found to be 269.85 ± 15.25 and 410.72 ± 9.48 mg gallic acid equivalent (GAE)/g dry weight, respectively. Total flavonoid and flavonol contents were estimated to be 82.28 ± 3.21 and 151.25 ± 8.75 mg quercetin equivalent (QE)/g dry weight, respectively. Total polyphenol and phenolic acid contents in the aqueous extract were found to be 157.05 ± 11.21 and 490.50 ± 10.20 mg GAE/g dry weight, respectively. Total flavonoid and flavonol contents in the aqueous extract were estimated to be 95.80 ± 3.12 and 162.72 ± 9.18 mg QE/g dry weight, respectively. The methanol and aqueous leaf extracts indicated presence of vital phytoconstituents of polyphenols, flavonoids, flavonols that contributed significantly to its antioxidant capacity. Results obtained from the present study signify that the methanol and aqueous leaf extracts of *H. rhamnoides* possess antioxidant properties and could possibly act as primary antioxidants by scavenging free radicals in disease conditions.

Key words: *Hippophae rhamnoides* L., leaf extract, antioxidant capacities, phyto-components, phenolics, flavonoids.

INTRODUCTION

Reactive oxygen species (ROS) are involved in a variety of intercellular and cellular processes and they are responsible the progression of many diseases (Bland, 1995). Intake of bioadsorbable dietary antioxidant could prevent a large number of diseases in many paradigms by inhibiting or delaying the oxidation of susceptible

cellular substrates (Surh, 2002). Herbs, fruits and vegetables are the widely available natural products that become well accepted in recent years due to civic alertness and ever-increasing interest amongst consumers and scientific communities (Thaipong et al., 2006).

The biological and pharmacological activities, including antioxidative, antiimflammatory and antiviral effects of natural products have been reported from epidemiological evidences and these activities result from the active phytochemical constituents present in these products (Pawlowska et al., 2008; Wang and Ballington, 2007).

Sea buckthorn *(H. rhamnoides* L., Eleagnaceae*)* is native to Eurasia, and is mainly known in North America as an attractive ornamental shrub. *H. rhamnoides*, *H. salicifolia* and *H. tibetana* are the predominant sea buckthorn species in India. Sea buckthorn is naturally distributed in six valleys namely Leh, Nubra, Changthang, Suru, Lahaul and Spiti in trans-Himalayan cold desert of India at an altitude of 3000 to 4500 m above msl. Sea buckthorn is generally termed as the 'wonder plant' or 'golden bush' because of its wide applications.

Different parts of *H. rhamnoides*, especially leaves, pulp, bark, and oil of seeds have been used for the treatment of several diseases in traditional medicine in various countries over the world. In Indian system of medicine *H. rhamnoides* is an important medicinal plant and its leaves have been used in various ailments and as health tonic. Sea buckthorn based products have been comprehensively exploited in the treatment of slow digestion, stomach malfunctioning, cardiovascular problems, liver injury, tendon and ligament injuries, skin diseases and ulcers. In the recent years, medicinal and pharmacological activities of sea buckthorn have been well investigated using various *in vitro* and *in vivo* models as well as limited clinical trials and diverse pharmacological activities like cytoprotective, anti-stress, immunomodulatory, hepatoprotective, radioprotective, anti-atherogenic, anti-tumor, anti-microbial and tissue regeneration have been reported (Suryakumar and Gupta, 2011).

All parts of the plant are considered to be rich source of bioactive substances like flavonoids (isorhamnetin, quercetin, myricetin, kaempferol and their glycoside compounds), carotenoids (α, β, δ-carotene, lycopene), vitamins (C, E, K), tannins, triterpenes, glycerides of palmitic, stearic and oleic acids and some free amino acids (Zu et al., 2006). The leaves, in particular, reported to contain a wide number of bio-active constituents. The leaf metahnolic and ethyl acetate extracts were well characterized by polyamide and silica gel column chromatography (CC) and paper chromatography (PC). Quercetin, kaempferol, isorhamnetin, myricetin, and gallic acid were isolated from methanol extract of *H. rhamnoides* L. leaves (Guliyeva et al., 2004).

The volatile composition of leaves were analyzed by GC–MS and it was found to contain tetracosane (10 to 40%), hexadecanoic acid (<0.1 to 11%), octadecatrienol (5 to 27%), tetracosene (3 to 11%), eicosanol (<0.1 to 13%) and others (Tian et al., 2004). *H. rhamnoides* leaves extracts are traditionally been used to treat colitis and enterocolitis in Mongolia (Tsybikova et al., 1983). It is also used in the treatment of diarrhea (Vereshchagin et al., 1959), gastrointestinal complains, dermatologic disorders (Tsybikova et al., 1983) and rheumatoid arthritis in the Middle Asia. Extensive studies in the recent time revealed that the leaf alcoholic extracts of *H. rhamnoides* have adaptogenic activity, ani-oxidative action, immunomodulatory effect, anti-inflammatory activity and cytoprotective properties in stress conditions (Saggu and Kumar, 2007, 2008; Saggu et al., 2007; Padwad et al., 2006; Geetha et al., 2002a, b, 2003, 2005; Narayanan et al., 2005; Guliyeva et al., 2004).

The aim of the present investigation was to determine the antioxidant capacities, phytochemical constituents, total phenolic and flavonoid contents of methanol and aqueous extracts of *H. rhamnoides* L. leaves form trans-Himalayan cold desert of Ladakh.

MATERIALS AND METHODS

Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl radical (DPPH˙), 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid, $FeSO_4.7H_2O$, $AlCl_3$, sodium acetate, sodium bicarbonate (Na_2HCO_3), potassium persulphate ($K_2S_2O_8$), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ascorbic acid, quercetin, β-carotene, and $FeCl_3$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mercuric chloride, potassium iodide, iodine, copper sulphate, potassium sodium tartarate, ethanol, sodium hydroxide, potassium hydroxide, ferric chloride, sodium citrate, sodium carbonate, lead acetate, hydrochloric acid, Folin-Ciocalteus's phenol reagent, sulphuric acid, methanol, chloroform, ethanol and sodium carbonate were from Merck Chemical Supplies (Merck KGaA, Darmstadt, Germany). All the other chemicals used including solvents, were of analytical grade.

Plant materials and extraction

H. rhamnoides (sea buckthorn) leaves were collected from the Choglamsar village of Leh, Ladakh, India (altitude = 11500 ft. above mean sea level), in July, 2010. The plant samples were washed thoroughly in running tap water to clean the contaminants and debris, shade dried at room temperature for 15 days, finely powdered and used for extraction. Soxhlet extraction was carried out with 10 g of leaf powder with methanol and water at 40°C. The extracts were then filtered with a Buckner funnel and Whatman No 1 filter paper. The solvents were then removed by rotaevapour

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evaporation under reduced pressure. The material was then lyophilized to obtain the dry extract and stored at -80°C freezer.

DPPH radical scavenging assay

The effect of extract on DPPH radical was determined using the method of Liyana-Pathiranan and Shahidi (2005). A solution of 0.135 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of plant extract. Plant extract contained 0.02 to 0.10 mg of the dried extract. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Quercetin and BHT were used as standards. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging capacity (%) = ($Abs_{control}$ – Abs_{sample}) / ($Abs_{control}$) × 100

Where $Abs_{control}$ is the absorbance of DPPH radical + methanol or aqueous ; Abs_{sample} is the absorbance of DPPH radical with sample extract OR standard. The inhibitory concentration (IC_{50}), effective concentration (EC_{50}) and antiradical power (ARP) were estimated and calculated as described by Kroyer (2004).

ABTS radical scavenging assay

The ABTS assay was performed as described by Re et al. (1999). The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate (PPS) solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml $ABTS^{\cdot+}$ solution with 60 ml of 80% ethanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using spectrophotometer (Spectramax $M2^e$, Molecular Devices, Germany).

Plant extracts (1 ml) were allowed to react with 1 ml of the $ABTS^{\cdot+}$ solution and the absorbance was taken at 734 nm after 7 min. The $ABTS^{\cdot+}$ scavenging capacity of the extract was compared with that of quercetin acid and BHT. The percentage inhibition was calculated as follows:

ABTS radical scavenging capacity (%) = ($Abs_{control}$ – Abs_{sample}) / ($Abs_{control}$) × 100

Where, $Abs_{control}$ is the absorbance of ABTS radical + methanol or aqueous; Abs_{sample} is the absorbance of ABTS radical with sample extract OR standard. The inhibitory concentration (IC_{50}), effective concentration (EC_{50}), and antiradical power (ARP) were estimated and calculated as described previously.

Total antioxidant capacity

A modified method of Benzie and Strain (1996) was adopted for the total antioxidant capacity assay. The stock solutions included 300 mM acetate buffer (3.1 g CH_3COONa and 16 ml CH_3OOH), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$.

Plant extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in dark. Observations of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. The calibration curve was prepared from the equation y = 0.058x + 0.048, R^2 = 0.913, where x was the absorbance and y was $FeSO_4$ concentration (µM). Results were expressed in µM Fe (II)/g dry mass and compared with that of ascorbic acid, BHA and BHT.

Reducing power assay

The reducing power of plant extract was estimated by a method described by Yen and Chen (1995). Extracts (100 to 1000 µg/ml) were mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50°C for 30 min. Afterwards 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm.

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was conducted using the method described by Wong et al. (2006). Two hundred microlitre of leaf extracts were added with 3 ml of FRAP reagent that was prepared with mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM FeCl3.6H2O at the ratio of 10:1:1. The reaction mixture was then incubated in a water bath at 37°C for 30 min. The antioxidant capacity based on the capability to reduce ferric ions of the extracts was calculated as percent of antioxidant. The percent of antioxidant was calculated using the formula, percent of antioxidant (%) = (A593 of sample- A593 of control)/A593 of sample] x 100

Phytochemical analysis

Phytochemical analysis of methanol and aqueous extracts of *H. rhamnoides* leaves were conducted by standard qualitative analytical methods for identification of diverse phytocompounds such as alkaloids (Evans, 1997; Wagner, 1993; Harborne, 1998), carbohydrates (Deb, 2001), saponins (Kokate, 1999), proteins (Gahan, 1984), phenolics (Mace, 1963; Raaman, 2006) and flavonoids (Harborne, 1983; Shinoda, 1928).

Total polyphenol assay

Total polyphenol content was measured using Folin-Ciacalteu colorimetric method as described previously by Gao et al. (2000). The extracts (100 µl) were mixed with 0.2 ml of Folin-Ciacalteu reagent and 2 ml of H_2O, and incubated at room temperature for 3 min. Following the addition of 1 ml of 20% sodium carbonate to the mixture, total polyphenol was determined after 1 h of incubation at room temperature. The absorbance of the resulting blue color was measured at 765 nm. Quantification was done with respect to the standard curve of gallic acid. The polyphenol content were expressed as mg/g gallic acid equivalent (GAE) using the following equation based on the calibration curve: y = 0.003x + 0.058, R^2 = 0.986, where x was the absorbance and y was the gallic acid equivalent (mg/g) at a final concentration of 0.1 mg/ml. The results were expressed as GAE, mg/g of dry weight.

Total phenolic acid assay

The total phenolic acid assay was carried out using Folin-

Table 1. Effect of *H. rhamnoides* leaf extracts on different radical-scavenging activities.

Concentration (mg/ml)	Inhibition (%)							
	DPPH radical-scavenging capacity				ABTS radical-scavenging capacity			
	Methanol extract	Aqueous extract	Quercetin	BHT	Methanol extract	Aqueous extract	Quercetin	BHT
0.02	70.15 ± 0.87	67.59 ± 0.57	65.04 ± 1.06	74.58 ± 0.56	59.26 ± 0.89	54.11 ± 0.77	57.39 ± 0.88	60.27 ± 1.14
0.04	74.71 ± 0.76	71.18 ± 1.02	75.95 ± 0.66	79.85 ± 0.98	64.77 ± 0.45	59.54 ± 0.54	63.58 ± 0.59	67.78 ± 0.35
0.06	79.80 ± 0.61	76.23 ± 0.55	78.77 ± 0.65	80.69 ± 0.86	69.15 ± 0.66	62.13 ± 0.90	68.25 ± 1.05	71.28 ± 0.45
0.08	83.34 ± 0.89	82.24 ± 0.75	80.45 ± 0.23	81.68 ± 0.78	74.58 ± 0.32	71.08 ± 0.86	75.29 ± 0.44	79.82 ± 0.66
0.1	88.28 ± 0.52	87.78 ± 0.79	86.61 ± 0.74	89.76 ± 1.32	80.16 ± 0.58	75.15 ± 0.72	79.21 ± 0.87	82.19 ± 0.84
IC_{50} (mg/ml)	0.014	0.015	0.016	0.013	0.017	0.018	0.017	0.016
EC_{50} (mg/ml)	0.61	0.66	0.65	0.57	0.74	0.79	0.72	0.70
ARP values	163.93	151.52	153.84	175.44	135.14	126.58	138.89	142.86

Values are given as mean ± SD of three replicates. BHT: Butylated hydroxytoluene.

Ciacalteu's reagent as depicted by Marinova et al. (2005). 1 ml of plant extract was added into a flask containing 9 ml of distilled water. Then 1 ml of Folin-Ciocalteu's phenol reagent was added and the mixture was thoroughly mixed. After 5 min of incubation, 10 ml of 7% Na_2CO_3 were added. Then the mixture was diluted to 25 ml with the addition of 4 ml of distilled water. Then the mixture was incubated at room temperature for 90 min. Finally, the absorbance was measured using spectrophotometer at 750 nm. The total phenolic acid content was expressed as mg GAE/g samples.

Total flavonoids assay

Estimation of the total flavonoids in the plant extracts were carried out using the method of Ordon et al. (2006). Briefly, to 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. The contents were incubated for 1 h at room temperature and the absorbance was measured at 420 nm. Total flavonoid content was calculated using the following equation based on the calibration curve: $y = 0.009x + 0.137$, $R^2 = 0.992$, where x was the absorbance and y was the quercetin equivalent (mg/g) at a final concentration of 0.1 mg/ml.

Total flavonols assay

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran (2007). To 2 ml plant extract, 2 ml of 2% $AlCl_3$ ethanol and 3 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was measured after 2.5 h at 20°C. Total flavonoid content was calculated using the following equation based on the calibration curve: $y = 0.009x + 0.137$, $R^2 = 0.992$, where x was the absorbance and y was the QE (mg/g) at a final concentration of 0.1 mg/ml.

Statistical analysis

All the experimental results are expressed as mean ± standard deviation (SD) using statistical analysis with Statistical Program for Social Sciences (SPSS), (SPSS Corporation, Chicago, IL) version. Analysis of variance (ANOVA) in a completely randomised design, Duncan's multiple range test and Pearson's correlation coefficients were performed to compare the data. Post hoc analysis was performed using Neumen Keuls Test, and values with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

DPPH radical scavenging capacity

The DPPH radical is regarded as a model for a lipophilic radical and used as a stable free radical to determine the antioxidant capacity of natural compounds (Ramnik et al., 2008; Shimada et al., 1992). The free radical scavenging capacity of methanol and aqueous extracts of *H. rhamnoides* leaf and the two positive controls, viz; ascorbic acid and BHT were compared. The IC_{50} values were found to be 0.014, 0.015, 0.016 and 0.013 mg/ml for methanol extract, aqueous extract, quercetin and BHT, respectively.

The DPPH scavenging capacities of the leaf methanol extract, aqueous extract, quercetin and BHT expressed as an EC_{50} value, were 0.61,

Table 2. Ferric reducing antioxidant power (FRAP) of *H. rhamnoides* leaf extracts.

Sample	FRAP (µM Fe (II)/g)
Methanol extract	557.24 ± 12.15
Aqueous extract	510.18 ± 11.77
AA	4421.44 ± 402.86
BHA	4118.10 ± 706.07
BHT	3918.10 ± 496.62

Values are given as mean ± SD of three replicates. AA: Ascorbic acid. BHA: Butylated hydroxyanisole. BHT: Butylated hydroxytoluene.

0.66, 0.65 and 0.57 mg/ml, respectively. The ARP values of the methanol and aqueous extracts were also calculated and were found to be 163.93 and 151.52, respectively.

The DPPH activity of leaf methanol and aqueous extracts were found to increase in a dose dependent manner. The leaf extracts at the used concentrations exhibited potential effect of DPPH activity as percentage of free radicals inhibition (Table 1).

A higher DPPH radical scavenging capacity is associated with a lower IC_{50} value. These results suggested that the leaf methanol and aqueous extracts contained strong free radical scavenging capacity.

ABTS radical scavenging capacity

The $ABTS^{\cdot+}$ radical scavenging capacity of the methanol and aqueous extracts of *H. rhamnoides* leaf compared to quercetin and BHT has been depicted in Table 1. The methanol and aqueous extracts of *H. rhamnoides* leaf at concentrations of 0.02 to 0.1 mg/ml scavenged the ABTS radical in a dose dependent manner. The positive controls viz; quercetin and BHT at a concentration of 0.02 to 0.1 mg/ml were also found to produce dose dependent inhibition of ABTS radical. The quantities of methanol and aqueous extracts of *H. rhamnoides* leaf required to produce 50% inhibition (IC_{50}) of ABTS radical were found to be 0.017 and 0.018 mg/ml, respectively. Analogous effects were produced by quercetin and BHT at concentration of 0.017 and 0.016 mg/ml, respectively. The EC_{50} and ARP values of leaf extracts were also comparable to the standards. EC_{50} of leaf methanol and water extracts were found to be 0.74 and 0.79 mg/ml. BHT and ascorbic acid were set up at EC_{50} of 0.01 and 0.02 mg/ml, respectively. The ARP value of the leaf methanol and aqueous extracts was 135.14 and 126.58, respectively (Table 1). The scavenging capacities of the extracts on $ABTS^{\cdot+}$ were similar to the results of the scavenging activities on DPPH radicals.

Total antioxidant power

The total antioxidant power assay (Benzie and Strain, 1996) was used in the present study to determine the ability of the leaf extracts to reduce ferric ions. An antioxidant capable of providing a single electron to the ferric-TPTZ Fe(III)-TPTZ complex would cause the reduction of this complex into the blue ferrous-TPTZ Fe(II)-TPTZ complex which absorbs strongly at 593 nm. The FRAP values of leaf methanol and aqueous extracts were found to be 557.24 and 510.18 µM Fe (II)/g (Table 2). The FRAP values for the leaf extracts were significantly lower than that of ascorbic acid, BHA and BHT (Table 2).

Reducing power

The reducing ability of a compound could serve as a potent indicator of its antioxidant capacity (Meir et al., 1995). We examined Fe^{3+}–Fe^{2+} transformation in the presence of crude plant extracts and ascorbic acid for measurements of reducing power. The reducing power increases with increasing concentrations of the extract that was similar to the antioxidant activity.

The reducing ability of leaf extracts increased with an increase in concentration when compared with the positive standard, ascorbic acid (Figure 1).

In general, the reducing ability of a compound depends on the presence of reductones (Duh, 1998), which exhibits antioxidative capacity, by breaking the free radical chain and donating a hydrogen atom (Gordon, 1990). The presence of reductants (that is, antioxidants) in the extract fractions causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing capacity of a compound may serve as a significant indicator of its antioxidant capacity (Thaipong et al., 2006). The methanol and aqueous extracts of *H. rhamnoides* leaves at all concentrations exhibited higher activities when compared with control, which was found to be statistically significant ($p < 0.05$).

Ferric reducing antioxidant power assay (FRAP)

The antioxidant capacity of leaf extracts was evaluated by FRAP assay because it also showed high reproducibility (Thaipong et al., 2006). When the sample solution reacted with FRAP solution, dark blue coloration will appear owing to the formation of ferrous tripyridyltriazine complex that was detected at 593 nm. The amount of Fe^{3+} reduced to Fe^{2+} is directly proportional total antioxidant. As exhibited in Figure 2, methanol extract had higher total antioxidant compared to aqueous extract. The percentage of antioxidant for Ferrous tripri-

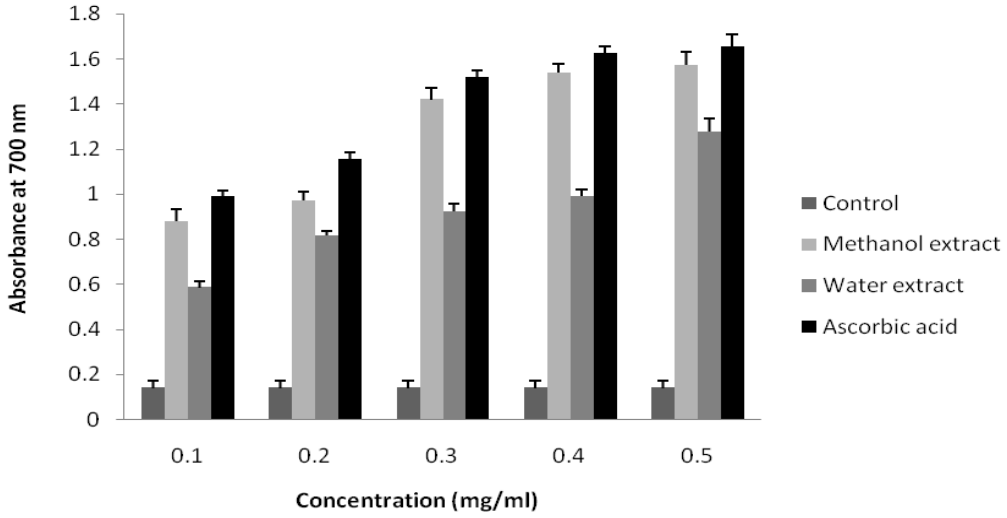

Figure 1. Reducing power activity of *H. rhamnoides* leaf extracts.

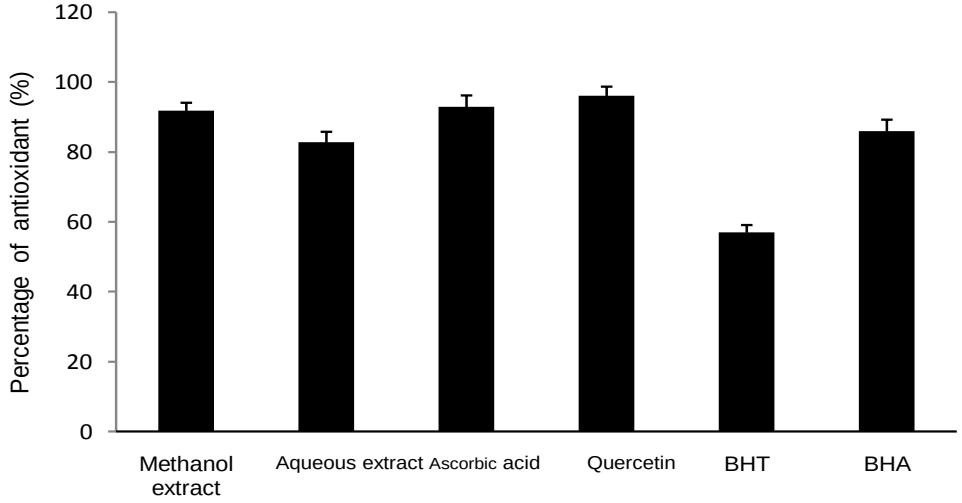

Figure 2. Ferric reducing antioxidant power of *H. rhamnoides* leaf extracts.

dyltriazine complexes were formed as a product from the reaction where the samples had the capacity to reduce Fe^{3+} to Fe^{2+}. methanol and aqueous extracts were found to be 91.82 and 82.72%, respectively. Ascorbic acid, quercetin, BHT and BHA were used as positive controls and the antioxidant percentage were determined to be 92.92, 96.10, 56.94 and 85.89%, respectively.

Qualitative phytochemical analysis

The phytochemical screening revealed the presence of alkaloids, carbohydrtates, saponins, proteins, phenols and flavonoids in the aqueous leaf extract (Table 3). Alkaloids, carbohydrates, proteins and saponins were found to be absent in the methanol extract. The methanol extract was found to contain phenols and flavonoids as major phyto-components (Table 3).

Total polyphenol, phenolic acid, flavonoids and flavonols content

It had been well documented that the phenolic content of plant materials is strongly associated with their antioxidant capacity (Skerget et al., 2005). Phenolics are the principal plant compounds with antioxidant capacity attributable to their redox properties, which exhibit an important function in neutralizing free radicals (Zheng and Wang, 2001). Plant derived phenolics are well-known natural antioxidants and can contribute directly to the antioxidative action (Duh et al., 1999). Applications of

Table 3. Qualitative analysis of the phytochemicals of methanol and aqueous extracts of *H. rhamnoides* leaves.

Phytochemicals	Methanol extract	Aqueous extract
Alkaloids	-	+
Carbohydrates	-	+
Saponins	-	+
Proteins	-	+
Phenolics	+	+
Flavonoids	+	+

+Compound present. - Compound absent.

Table 4. Polyphenol, phenolic acid, flavonoid and flavonol contents of *H. rhamnoides* leaf extracts.

Phyto-component	Methanol extract	Aqueous extract
Total polyphenol[a]	269.85 ± 15.25	157.05 ± 11.21
Total phenolic acid[b]	410.72 ± 9.48	490.50 ± 10.20
Total flavonoids[c]	82.28 ± 3.21	95.80 ± 3.12
Total flavonols[d]	151.25 ± 8.75	162.72 ± 9.18

Values are given as mean ± SD of three replicates. [a]Expressed as mg GAE/g of dry plant material. [b]Expressed as mg GAE/g of dry plant material. [c]Expressed as mg QE/g of dry plant material. [d]Expressed as mg QE/g of dry plant material.

In the present study, the content of phenolic compounds in methanol and aqueous extracts of *H. rhamnoides* leaf was found to be 269.85 and 157.05 mg GAE/g of dry plant material respectively Table 4. Total phenolic acid content in methanol and aqueous leaf extracts were 410.72 and 490.50 mg GAE/g of dry plant material respectively (Table 4). Results obtained from the current investigation strongly recommend that phenolics are vital components of the leaf extracts of *H. rhamnoides* and a number of their pharmacological antioxidant properties may possibly due to the these constituents.

Flavonoids are natural phenolic compounds and recognized antioxidants. Several studies revealed that antioxidant capacity of plant extracts was moderately high which are rich in flavonoids (Cakir et al., 2003). The concentration of flavonoids in the leaf metahanol and aqueous extracts of *H. rhamnoides* were found to contain 82.28 and 95.80 mg QE of dry plant material, respectively (Table 4).

Flavonols are the active constituents in variety of fruits and vegetables and possess high antioxidant and antiradical capacity with various therapeutic applications (Peterson and Dwyer, 1998).

In the present study, the content of flavonol in *H. rhamnoides* leaf metahanol and aqueous extracts has been depicted in Table 4 and it was found to be 151.25 and 162.72 mg QE/g of dry plant material respectively.

Plant leaves are one of the most important resources of natural antioxidants responsible for inhibiting the harmful consequences of oxidative stress. A number of scientific reports signify that certain plant compounds viz. terpenoids, steroids and phenolic compounds such as tannins, coumarins and flavonoids have protective effects owing to their antioxidant capacities (Chandrasekhar et al., 2006).

The most wide spread secondary metabolite in plant kingdom reported so far are the phenolics and they have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. Several studies have shown that the higher antioxidant capacity associated with medicinal plants is attributed to the total phenolic compounds (Hong et al., 2008). Thus, the outcome of the present study highlights the antioxidant capacities rendered by leaf metahanol and aqueous extracts of *H. rhamnoides* under oxidative stress conditions.

Conclusion

The antioxidant capacities of the methanol and aqueous extracts from the leaves of *H. rhamnoides* were assessed in an effort to validate the medicinal potential of the subterranean leaf part. The methanol and aqueous leaf extracts indicated presence of vital phytoconstituents of polyphenols, flavonoids, flavonols that contributed significantly to its antioxidant capacity. A significant correlation existed between concentrations of the extract and percentage inhibition of free radicals and reducing power. Hence, from the results of our study, it can be concluded that the methanol and aqueous extracts of *H. rhamnoides* may have health benefits and can be used as a source of

natural antioxidants for pharmacological preparations. Further studies should be conducted in *in vivo* systems for the toxicity and safety assessment of the leaf extracts for novel product development and human consumption.

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