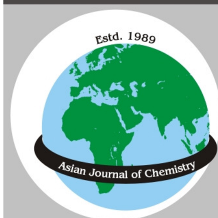

Asian Journal of Chemistry; Vol. 25, No. 15 (2013), 8569-8573

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14843

Rhamnus triquetra: A Valuable Source of Natural Antioxidants to Shield from Oxidative Stress

FARHANA MAZHAR[1], MUHAMMAD JAHANGIR[1], MUHAMMAD ATHAR ABBASI[1,*], AZIZ-UR-REHMAN[1], SYED ANAM ILYAS[1], FARAH KHALID[1], RAISA KHANUM[1], SABA JAMIL[1], SHAHEEN KAUSAR[1] and MUHAMMAD AJAIB[2]

[1]Department of Chemistry, Government College University, Lahore-54000, Pakistan
[2]Department of Botany, Government College University, Lahore-54000, Pakistan

*Corresponding author: Tel: +92 42 111000010; Ext. 266; E-mail: atrabbasi@yahoo.com

(*Received*: 20 November 2012; *Accepted*: 26 August 2013) AJC-13998

The aim of this study was to examine the *in vitro* antioxidant potential of different fractions of *Rhamnus triquetra*. The methanolic extract of this plant was dissolved in distilled water and successively partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Phytochemical screening revealed the presence of alkaloids, terpenoids, tannins, phenolics, sugars, saponins and flavonoids. The antioxidant potential of crude methanolic extract, all these fractions and remaining aqueous fraction was evaluated by different methods *i.e.*, DPPH free radical scavenging activity, total antioxidant activity, ferric reducing antioxidant power assay, ferric thiocyanate assay and total phenolic content assay. All the fractions exhibited significant antioxidant potential. The results revealed that ethyl acetate soluble fraction showed the highest % inhibition of DPPH radical (92.01 ± 0.21) having an IC_{50} value of 7.59 ± 0.65 μg/mL, total antioxidant activity value (1.840 ± 0.08), highest ferric reducing antioxidant power value of (2137.7 ± 0.58 μg/mL) and inhibition of lipid peroxidation (61.94 ± 1.17 %). The chloroform fraction showed the highest total phenolic content value (121.5 ± 1.10 μg/g) as compared to other fractions.

Key Words: *Rhamnus triquetra*, Antioxidant potential, Oxidative stress, Organic fractions, Lipid peroxidation.

INTRODUCTION

The scavenging of reactive oxygen species (ROS) is one of the possible mechanisms of action of antioxidant compounds. Chain breaking antioxidants prevent damage by interfering with the free radical propagation[1]. Reactive oxygen species may be the causative factor involved in many human degenerative diseases and antioxidants are known to have some degree of preventive and therapeutic effects on these disorders. Reactive oxygen species produced *in vivo* include superoxide radical, hydrogen peroxide (H_2O_2) and hypochlorous acid. Hydrogen peroxide and superoxide can interact in the presence of certain transition metal ions to yield a highly reactive oxidizing species, the hydroxyl radical[2]. Hydrogen peroxide, one of the main ROS, causes lipid peroxidation and DNA oxidative damage in cells[3]. Our group is working on evaluating the antioxidant and antimicrobial activity of medicinal plants. We are also extracting the pure natural metabolites responsible to the particular activity[4]. Recently, many synthetic antioxidant components have been shown undesirable side effects such as toxicity and carcinogenicity, so interest has been increased towards naturally occurring antioxidants. Vitamins, phenolic compounds, essential oils and extracts from the plants are naturally occurring antioxidants. At this time there is an increasing interest worldwide to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry[5]. Many plants produce significant amount of antioxidants to reduce the oxidative stress and are a potential source of new phytochemical with antioxidant activity[6]. Rhamnaceae, the Buckthorn family, is a large family of flowering plants, mainly trees, shrubs and some vines. About 50-60 genera and *ca.* 870-900 species are present in this family. The rhamnaceae is distributed throughout the world but most common in the sub-tropical and tropical areas. Economic uses of the rhamnaceae are essentially as ornamental plants and used as the material of various brilliant green and yellow dyes. *Rhamnus* belongs to family rhamnaceae. They are native throughout the temperate and subtropical Northern Hemisphere, and also more locally in the subtropical Southern Hemisphere parts of Africa and South America. The bark, leaves, and fruit of several species of *Rhamnus* have been used as laxatives, notably *R. cathartica* and *R. frangula* and some other species of *Rhamnus* have also been used as folk medicine for treating constipation, inflammation, tumors and asthma[7,8].

Rhamnus triquetra belong to family rhamnaceae and are widely distributed in Pakistan as well as in Jammu and Kashmir. It is an evergreen shrub and it grows up to 7 m. The plant

needs light, substrate and heavy soils. The plant depends on acid, neutral and alkaline clay. Leaves and fruits are used mostly and their extracts are useful for hemorrhagic septicemia in cattle. The bark is astringent and deobstruent[9]. The juice of the bark is used in the treatment of diarrhea and dysentery[10]. Wood is hard, so it is used for agricultural implements and small carving[11].

To the best of our knowledge no studies on antioxidant activity has been reported yet on *Rhamnus triquetra* therefore it is important to report *in vitro* antioxidant potential of aqueous and organic fractions of this species by using ferric reducing antioxidant power, total antioxidant activity by using different assays. These antioxidant assays can give an idea about the chemistry of this plant.

EXPERIMENTAL

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, 2,4,6-tripyridyl-*s*-triazine (TPTZ), trolox, gallic acid, Folin-Ciocalteu phenol reagent and butylated hydroxytoluene (BHT) were obtained from the Sigma Chemical Company Ltd. (USA) and the organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride from Merck (Pvt.) Ltd. (Germany).

Collection and identification of plant material: The aerial part (stem) of *Rhamnus triquetra* was collected from the Azad Jammu and Kashmir in March 2010. The plant was identification by Mr. Muhammad Ajaib of the Department of Botany, GC University, Lahore and a voucher specimen has been deposited in the herbarium (voucher specimen numbers are GC.Herb.Bot.854) of the same university.

Extraction of plant material: Since certain compounds get denatured in sunlight, so plant material was dried under shade for 2 weeks after which it was grinded to a uniform powder. The methanolic extract was prepared by soaking 6 kg of dry powdered of plant material in methanol (10 L × 5) at room temperature for few days so that alkaloids, terpenoids and other organic constituents get extracted from plants. The methanolic extract was concentrated using a rotary evaporator to yield the residue (575 g), which was dissolved in distilled water (1 L) and partitioned with *n*-hexane (1.5 L × 3), chloroform (1.5 L × 3), ethyl acetate (1.5 L × 4) and *n*-butanol (2 L × 4), respectively. These organic fractions and remaining water fraction was concentrated separately on rotary evaporator and these residues thus obtained and initial crude methanolic extract were used to evaluate their *in vitro* antioxidant activities.

Phytochemical screening of the plant extract: Qualitative phytochemical screening were performed using standard procedures to identify the phytochemical constituents, *i.e.*, alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids and cardiac glycosides, using standard procedures[12,13].

Test for terpenoids (Salkowski test): 2 mL of chloroform was added to 0.5 g each extract followed by 3 mL of conc. H_2SO_4 that was added carefully to form a layer. Presence of terpenoids was indicated by preparation of a reddish brown layer at the interface of the organic and aqueous layer.

Test for flavonoids: We used two methods for flavonoids. In the first test added few drops of 1 % aluminium chloride solution to plant extracts, a persistent yellow colouration indicates the presence of flavonoids. While in second method, Benedict's reagent was sprayed on TLC card which have spots of samples. In UV light green fluorescence showed flavonoids presence.

Test for saponins: 0.5 g of plant extracts were added to 5 mL distilled water in a test tube. Stable persistent froth that is observed after vigorous shaking mixed with 3 drops of olive oil forms an emulsion indicates presence of saponins.

Test for tannins: For indicating presence of tannins in plant extracts, *ca.* 0.5 g of the extracts were boiled in 10 mL of water and then filtered, then added few drops of 0.1 % ferric chloride, brownish-green or blue- black colouration indicates presence of tannins in the extracts.

Test for alkaloids: Draggendroff's reagent was sprayed on TLC card on which sample spots are present. If orange colour has appeared then alkaloids confirmed.

Test for phenolics: Neutral solutions of ferric chloride was added to *ca.* 0.5 g of each extract. The bluish green colour indicates the presence of phenolics.

Test for reducing sugars (Fehling's test): 1 mL of water and 5-8 drops of Fehling's solution (A and B) was added to 0.5 g of each plant extract. It is heated and observed for brick red precipitate, indicating the presence of sugars.

Test for cardiac glycosides (Keller-Killiani test): In aqueous solution (0.5 g each sample added in 5 mL water) added glacial acetic acid (2 mL) having ferric chloride (1 drop). Conc. H_2SO_4 was added in this solution. At the interface brown ring shows the deoxysugar characteristic of cardenolides presence. Below brown ring violet ring may be formed. Just above the brown ring a greenish ring may be formed in the acetic acid layer and this ring spread gradually throughout this layer.

Determination of antioxidant activity

Sample preparation for antioxidant activity: The sample solutions of different extracts were prepared by dissolving 0.02 g of the extract in 20 mL of the methanol (1000 μg/mL).

DPPH radical scavenging activity: The DPPH radical scavenging activities of various fractions of plant were examined by comparison with that of a known antioxidant *i.e.*, butylated hydroxytoluene (BHT), using the method of Tauheeda *et al.*[14]. The following concentrations of the samples (1000, 500, 250, 120, 60, 30 and 15 μg/mL) were mixed with 3 mL of methanolic solution of DPPH (0.1 mM). The mixture was shaken vigorously and allowed to stand at room temperature for one an hour. IC_{50} values (concentration of sample required to scavenge 50 % of free radicals) were calculated from the regression equation. Synthetic antioxidant reagent, BHT was used as reference positive.

Inhibition free radical DPPH was calculated in following way:

$$I\,(\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Each sample was assayed in triplicate and the mean values were calculated.

Total antioxidant activity by the phosphomolybdenum method: The total antioxidant activities of various fractions of the plant were evaluated by the phosphomolybdenum complex

formation method[15]. Briefly, 500 µg/mL of each sample was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 4 mL of reagent solution. The vials were capped and incubated in water bath at 95 ºC for 90 min. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against the blank. The antioxidant activity was expressed relative to that of BHT. All determinations were assayed in triplicate and mean values were calculated.

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was performed according to Benzie and Strain[16] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $CH_3COONa{\cdot}3H_2O$ and 16 mL CH_3COOH, pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM hydrochloric acid, and 20 mM ferric chloride hexahydrate solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL ferric chloride hexahydrate solution, which was then warmed to 37 ºC before using. The solutions of plant fractions and that of trolox were prepared in methanol (500 µg/mL). 10 µL of each fractions and BHT solution were taken in separate test tubes and 2990 µL of FRAP solution was added to each to make a total volume upto 3 mL. The fractions were allowed to react with the FRAP solution in the dark for 0.5 h. The absorbance of the coloured product (ferrous tripyridyltriazine complex) was checked at 593 nm. The FRAP values are determined as micromoles of Trolox equivalents (TE) per mL of the sample using the standard curve constructed for different concentrations of Trolox. The results were expressed in Trolox equivalents µM/mL.

Ferric thiocyanate (FTC) assay: The antioxidant activities of the various fractions of the plant on the inhibition of linoleic acid peroxidation were assayed by the thiocyanate method[17]. 0.1 mL of each of sample solution (0.5 mg/mL) was mixed with 2.5 mL of linoleic acid emulsion (0.02 M, pH 7) and 2 mL of phosphate buffer (0.02 M, pH 7). The linoleic emulsion was prepared by mixing 0.28 g of linoleic acid, 0.28 g of Tween-20 as emulsifier and 50.0 mL of phosphate buffer. The reaction mixture was incubated for 5 days at 40 ºC. The mixture without extract was used as the control. The 0.1 mL aliquot of the mixture was taken and mixed with 5 mL of 75 % ethanol, 0.1 mL of 30 % ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5 % hydrochloric acid and allowed to stand at room temperature. Precisely after 3 min the addition of ferrous chloride to the reaction mixture, the absorbance was recorded at 500 nm. The antioxidant activity was expressed as percentage inhibition of peroxidation (IP %):

$$\text{Inhibition of lipid peroxidation (\%)} = \left[\frac{(A_{sample})}{(A_{control})}\right] \times 100$$

The antioxidant activity of BHT as reference standard was assayed for comparison.

Total content of phenolics: The total phenolics of the various fractions of plant were determined with the Folin Ciocalteu reagent by a reported method[18]. The 0.1 mL (0.5 mg/mL) of sample was combined with 2.8 mL of 10 % sodium carbonate and 0.1 mL of 2 N Folin-Ciocalteu reagent. After 40 min, the absorbance at 725 nm was measured using a UV-visible spectrophotometer. The total phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample using a standard calibration curve constructed for different concentrations of gallic acid. The curve was linear between 50 and 400 µg/mL of gallic acid. Results were expressed in GAE µg/mL.

Statistical analysis: All the measurements were performed in triplicate and statistical analysis was realized by statistical software. Results are presented as average ± SEM.

RESULTS AND DISCUSSION

The phytochemical analysis conducted on *Rhamnus triquetra* extracts revealed the presence of tannins, flavonoids, sugars, alkaloids, terpenoids, phenolics and saponins in methanolic, *n*-hexane, chloroform, ethyl acetate and *n*-butanol soluble fractions.

The amount of common bioactive components like terpenoids, alkaloids, phenolics, flavonoids and saponins were concentrated in medium polar and polar fractions (*i.e.*, chloroform, ethyl acetate and butanol) as expected, while the quantity of sugars was good in remaining aqueous fraction. The results were tabulated in Table-1.

Antioxidant potential of the plant extracts were summarized in Table-2. The DPPH radical is widely used in the model system to evaluate antioxidant activities in a relatively short time. DPPH scavenging was due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom or transfer electron to DPPH thus neutralize the free radical character and then this gives rise to the reduced form 1,1-diphenyl-2-picryl hydrazine (non radical) with the loss of the violet colour. Radical scavenging activity increased with increasing percentage of the free radical inhibition[19]. The

TABLE-1
PHYTOCHEMICAL CONSTITUENTS OF VARIOUS FRACTIONS OF *Rhamnus triquetra*.
("+" REPRESENTS PRESENCE AND "–" REPRESENTS ABSENCE)

Test	*n*-Hexane soluble fraction	Chloroform soluble fraction	Ethyl acetate soluble fraction	*n*-Butanol soluble fraction	Remaining aqueous fraction
Terpenoids	+	++	+++	++	+
Flavonoids	+	++	+++	+++	+
Tannins	–	–	+	+	–
Alkaloids	–	+	+	++	–
Phenolics	+	++	+++	++	+
Sugar	–	–	+	+	++
Cardiac glycosides	–	–	+	–	–
Saponins	–	–	+	+	–

TABLE-2 FREE RADICAL SCAVENGING ACTIVITY OF THE VARIOUS FRACTIONS OF *Rhamnus triquetra* USING 1, 1-DIPHENYL-2-PICRYL HYDRAZYL RADICAL (DPPH)		
Sample	Concentration in assay (µg/ml)	Scavenging (%) of DPPH radical ± SEM[a]
Crude methanolic extract	250	89.01 ± 0.89
	120	62.89 ± 0.21
	60	52.1 ± 0.86
	30	40.47 ± 0.61
n-Hexane soluble fraction	1000	71.43 ± 0.49
	500	60.28 ± 0.58
	250	42.36 ± 0.71
	120	33.29 ± 0.62
Chloroform soluble fraction	250	87.49 ± 0.31
	120	71.24 ± 0.29
	60	54.72 ± 0.10
	30	41.09 ± 0.37
Ethyl acetate soluble fraction	60	92.01 ± 0.21
	30	89.72 ± 0.68
	15	51.2 ± 0.89
n-Butanol soluble fraction	60	80.43 ± 0.73
	30	62.69 ± 0.91
	15	48.71 ± 0.68
Remaining aqueous fraction	500	79.93 ± 0.34
	250	60.47 ± 0.90
	120	45.21 ± 0.39
BHT[b)]	60	91.49 ± 0.13
	30	75.54 ± 0.07
	15	42.62 ± 0.04

[a]Standard error mean of three assays. [b]Standard reference antioxidant.

colour change from violet to yellow and fall in absorbance of the stable radical DPPH was measured for different concentrations and results are shown in Table-2. In our results, the ethyl acetate soluble fraction showed the highest DPPH scavenging activity of 92.01 ± 0.21 at a concentration of 60 µg/mL, while *n*-hexane soluble fraction revealed the lowest scavenging value of 71.43 ± 0.49 at a concentration of 1000 µg/mL. In the present study, in some cases, methanolic extract and polar fraction (ethyl acetate) showed higher or similar antioxidant activities as compared to the standard BHT. This is due to most bioactive compounds such as polyphenols including tannins, flavonoids existed in higher polar fraction[20].

For each fraction, the IC_{50} value was calculated from the plotted curves. Lower IC_{50} value indicates better DPPH radical scavenging activity. The polar fraction (ethyl acetate) of methanolic extract of *Rhamnus triquetra* provided the IC_{50} value of 7.59 ± 0.65 µg/mL than the non polar fraction (*n*-hexane) with IC_{50} of 182.99 ± 1.48 (Table-3). Total antioxidant capacity/activities of all fractions was evaluated by spectrophotometrically by phosphomolybdenum method which is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate Mo(V) complex at acidic pH. Electron transfer occurs in this assay depends upon the structure of the antioxidant[21]. The antioxidant activities of the fractions were compared with the reference standard antioxidant BHT. The ethyl acetate fraction displayed the highest total antioxidant activity value (1.840 ± 0.08), followed by the slightly less potent methanol, *n*-butanol, chloroform and remaining aqueous soluble fraction, while the *n*-hexane soluble fraction had the lowest total antioxidant activity (Table-3).

The FRAP assay is a simple and inexpensive procedure that measures the total antioxidant levels in a sample by taking into account of their oxidation-reduction potential. The method described measures the ferric reducing ability of plasma (FRAP). It utilizes the reducing potential of the antioxidants to react with the ferric tripyridyltriazine (Fe(III)-TPTZ) complex and produce the intense blue colour ferrous tripyridyltriazine (Fe(II)-TPTZ) complex[22]. The reaction conditions favour reduction of the complex and thereby colour development provided that a reductant (antioxidant) is present. Among all the *Rhamnus triquetra* fractions, the ethyl acetate fraction showed the highest FRAP value (2137.2 ± 0.58 µg/mL) and then the other fractions which followed the order: methanolic extract (2126.7 ± 0.91 µg/mL) > *n*-butanol (1709.3 ± 0.19 µg/mL) > chloroform (1565.2 ± 0.94 µg/mL) > aqueous fraction (357.5 ± 0.57µg/mL) while the *n*-hexane soluble fraction revealed the lowest FRAP value (266.5 ± 0.46 µg/mL) (Table-3).

The ferric thiocyanate method measures the amount of peroxide generated at the initial stage of linoleic acid emulsion during incubation. Here, peroxide reacts with ferrous chloride to form ferric chloride, which in turn reacts with ammonium thiocyanate to produce ferric thiocyanate, a reddish pigment. Low absorbance values measured *via* the ferric thiocyanate method indicate high antioxidant activity[23]. Our results revealed that ethyl acetate soluble fraction rendered the maximum inhibition of lipid peroxidation (61.94 ± 1.17 %) and *n*-hexane displayed minimum value (46.07 ± 0.51). The inhibition of lipid peroxidation by BHT (standard) was 62.91 ± 0.16. The antioxidant activity of phenolic compounds is mainly due to their redox properties and chemical structure, which allow them to act as reducing agent, hydrogen donators

TABLE-3
IC_{50}, TOTAL ANTIOXIDANT ACTIVITY, FRAP VALUES, LIPID PEROXIDATION INHIBITION AND TOTAL PHENOLICS VALUES OF DIFFERENT FRACTIONS OF *Rhamnus triquetra*

S. No	Sample	IC_{50} of DPPH assay (µg/mL) ± SEM[a]	Total antioxidant activity ± SEM[a]	FRAP value TE (µM/mL) ± SEM[a]	Inhibition of lipid peroxidation (%) ± SEM[a]	Total phenolics (GAE mg/g of extract) ± SEM[a]
1	Crude methanolic extract	70.26 ± 0.27	1.740 ± 0.01	2156.7 ± 0.91	57.12 ± 0.73	114.17 ± 1.63
2	*n*-Hexane soluble fraction	182.99 ± 1.48	0.100 ± 0.026	266.5 ± 0.46	46.07 ± 0.51	9.3 ± 0.51
3	Chloroform soluble fraction	60.09 ± 0.54	1.359 ± 0.43	1565.2 ± 0.94	53.39 ± 0.56	121.5 ± 1.10
4	Ethyl acetate soluble fraction	7.59 ± 0.65	1.840 ± 0.08	2137.2 ± 0.58	61.94 ± 1.17	80.74 ± 1.29
5	*n*-Butanol soluble fraction	37.98 ± 1.35	1.537 ± 0.002	1709.3 ± 0.19	49.68 ± 0.92	98.17 ± 1.54
6	Remaining aqueous fraction	94.73 ± 0.74	1.183 ± 0.016	357.5 ± 0.57	54.21 ± 0.88	28.83 ± 1.2
7	BHT[b]	12.10 ± 0.29	1.2186 ± 0.015	–	62.91 ± 0.16	–
Blank	–	–	–	2.30	–	1.15

[a]Standard error mean of three assays. [b]Standard reference antioxidant.

and singlet Q- quenchers[24] and chelating transitional metals, inhibiting lipoxygenase and scavenging free radical[25]. Folin-Ciocalteu (FC) reagent was used to determine total polyphenols in samples. Folin-Ciocalteu reagent consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. These reagents oxidizes phenolates resulted in the production of complex molybdenum- tungsten blue which can be detected spectrophotometrically at 725 nm and calculated as gallic acid equivalents. Gallic acid is a water soluble polyhydroxy phenolic compound which can be found in various natural plants[26]. The standard curve equation is y (absorbance) = 0.006x + 0.139. The absorbance value was inserted in the above equation and the total amount of phenolic compound was calculated. In present result chloroform soluble fraction possessed the highest amount of total phenolic compounds, having value (121.5 ± 1.10 μg/g) followed by crude methanolic fraction (114.17 ± 1.63), *n*-butanol fraction (98.17 ± 1.54 μg/g), ethyl acetate fraction (80.74 ± 1.29) and aqueous fraction (28.83 ± 1.2 μg/g), while the *n*-hexane fraction exhibited the lowest total phenolic contents (9.3 ± 0.51 μg/g) (Table-3).

Conclusion

According to the results of this study, the methanolic extract of *Rhamnus triquetra* may be suggested as a potential source of natural antioxidant. It was concluded that as ethyl acetate soluble fraction of this plant exhibit the highest percentage inhibition of DPPH radical (92.01 ± 0.21) as compared to other fraction and also lower IC_{50} value (7.59 ± 0.65 μg/mL). This fraction also showed total antioxidant activity FRAP value and inhibition of lipid peroxidation while chloroform showed highest total phenolic contents when assayed while *n*-hexane soluble fraction showed the minimum value. Thus further phytochemical investigations may bring new natural antioxidants into the food industry that might provide good protection against the oxidative damage which occur in the body and our daily foods.

ACKNOWLEDGEMENTS

The authors are thankful to Higher Education Commission of Pakistan for financial support.

REFERENCES

1. A. Matkowski, *Biotechnol. Advan.*, **26**, 548 (2008).
2. B. Halliwell, *Am. J. Med.*, **91**, 1422 (1991).
3. J. Nordberg and E.S.J. Arner, *Free Radic. Biol. Med.*, **31**, 1287 (2001).
4. S. Tayyaba, M.A. Abbasi, A.U. Rehman, R. Tauheeda, K.M. Khan, V.U. Ahmad, M. Ashraf, B. Hussain and M. Ajaib, *J. Chem. Soc. Pak.*, **34**, 442 (2012).
5. J. Javanmardi, C. Stushnoff, E. Locke and J.M. Vivanco, *Food Chem.*, **83**, 547 (2003).
6. B.N. Singh, B.R. Singh, R.L. Singh, D. Prakash, B.K. Sarma and H.B. Singh, *Food Chem. Toxicol.*, **47**, 1161 (2009).
7. S. Walter and G. Richard, *Am. J. Bot.*, **91**, 1627 (2004).
8. W.C. Ko, M.C. Chen, S.H. Wang, Y.H. Lai, J.H. Chen and C.N. Lin, *Planta Med.*, **69**, 310 (2003).
9. M. Ajaib, Z. Khan, N. Khan and M. Wahab, *Pak. J. Bot.*, **42**, 1407 (2010).
10. N.P. Manandhar and S. Manandhar, Plants and People of Nepal Timber Press Incorporated Portland, Oregon, p. 393 (2002).
11. D. Frohne and H.J. Pfander, A Colour Atlas of Poisonous Plant, Wolfe Publishing Ltd., p. 291 (1984).
12. G.E. Trease and W.C. Evans, Pharmacognosy, Bailliere Tindall, London, edn. 13, pp. 176-180 (1989).
13. B.O. Obadoni and P.O. Ochuko, *Global J. Pure Appl. Sci.*, **8**, 203 (2001).
14. R. Tauheeda, M.A. Abbasi, A.U. Rehman, S. Tayyaba, M. Ajaib and K.M. Khan, *Pak. J. Pharm. Sci.*, **25**, 679 (2012).
15. P. Prieto, M. Pineda and M. Aguilar, *Anal. Biochem.*, **269**, 337 (1999).
16. I.E.F. Benzie and J.J. Strain, *Anal. Biochem.*, **239**, 70 (1996).
17. H.P.S. Makkar, M. Bluemmel, N.K. Borowy and K. Becker, *J. Sci. Food Agric.*, **61**, 161 (1993).
18. P. Valentao, E. Fernandes, F. Carvalho, P.B. Andrade, R.M. Seabra and M.L. Bastos, *J. Agric. Food Chem.*, **50**, 4989 (2002).
19. D.J. Huang, H.J. Chen, C.D. Lin and Y.H. Lin, *Bot. Bull. Acad. Sin.*, **46**, 99 (2008).
20. B. Archana, N. Dasgupta and B. De, *Food Chem.*, **90**, 727 (2005).
21. S. Miladi and M. Damak, *J. Soc. Chim. Tunisie*, **10**, 101 (2008).
22. I.F.F. Benzie, *Clin. Biochem.*, **29**, 111 (1996).
23. C.A. Rice-Evans, N.J. Miller, P.M. Bramley and J.B. Pridham, *Free Radic. Res.*, **22**, 375 (1995).
24. E.A. Decker, *Nutr. Rev.*, **55**, 396 (1997).
25. J. Sun, Y.F. Chu, X. Wu and R.H. Liu, *J. Agric. Food Chem.*, **50**, 449 (2002).
26. J.S.J. Kim and M.J. Kim, *J. Med. Plants Res.*, **4**, 674 (2010).