In Vitro Assessment of Protection from Oxidative Stress by Various Fractions of *Mazus pumilus*

[1]SAMMIA SHAHID*, [2]TAUHEDA RIAZ, [2]MUHAMMAD ATHAR ABBASI, [2]FARAH KHALID AND [3]MUHAMMAD NADEEM ASGHAR

[1]*Department of Basic Sciences, School of Science and Technology, University of Management and Technology, Lahore-54770, Pakistan.*

[2]*Department of Chemistry, Government College University, Lahore, Pakistan.*

[3]*Department of Chemistry, FCC University, Lahore, Pakistan.*

*drsammiashahid@gmail.com**

(Received on 28th August 2012, accepted in revised form 1st March 2013)

Summary: Bioactive compounds in food have been shown to maintain human health. Therefore the aim of this investigation was to evaluate antioxidant potential of an edible herb *Mazus pumilus* (Burm. f.) Steenis by spectrophotometric methods. Methanolic extract of the herb was obtained on Soxhlet apparatus, and sequentially fractionized with organic solvents of increasing polarity. The antioxidant potential of all these fractions was evaluated by four methods: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, total antioxidant activity, Ferric reducing antioxidant power (FRAP) assay and ferric thiocyanate assay along with determination of their total phenolics. The results revealed that *n*-butanol fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 80.42±1.68 inhibition level, % of DPPH radical at a concentration of 60 µg ml^{-1}. The IC_{50} value of this fraction was 28.79±1.35 µg ml^{-1}, relative to ascorbic acid, having IC_{50} 58.84±0.89 µg ml^{-1}. It also showed highest total antioxidant activity (1.01±0.08) as well as highest FRAP value (626.83±0.39 TE / µM ml^{-1}), highest total phenolic contents (88.30±1.09 GAE / mg g^{-1}) and highest value of inhibition of lipid peroxidation (53.91±1.21 %) as compared to the other studied fractions. The results obtained from this study suggest that *Mazus pumilus* is a valuable herb, which due to the presence of larger quantities of antioxidant compounds inhibits the oxidative stress mechanism that lead to degenerative diseases.

Key Words: *Mazus pumilus*, DPPH assay, total antioxidant activity, reducing power, total phenolics, inhibition of lipid peroxidation.

Introduction

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins and isocatechins [1]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [2]. In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthene oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in long term complication of diabetes [3]. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ), have been added to foodstuffs. Although these synthetic antioxidants are efficient and relatively cheap, there are some disadvantages, because they are suspected of having some toxic properties. Therefore search for natural antioxidants has received much attention and efforts have been made to identify natural compounds that can act as suitable antioxidants to replace synthetic ones [4].

Currently, there is growing interest towards natural antioxidants of herbal resources. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [5]. Oxidative Stress *(OS)* is a general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ, caused by the Reactive Oxygen Species *(ROS)* and Reactive Nitrogen Species *(RNS)*.

***To whom all correspondence should be addressed.**

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage [6]. All forms of life maintain a reducing environment within their cells. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Antioxidants terminate the chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols which are abundantly present in natural products.

Mazus pumilus (Burm. f.) Steenis belongs to the family Scrophulariaceae, is an important medicinal plant [7]. Its flowering period is from May to October. It is an annual herb having height up to 15 cm. It grows in wet grassland, along streams, trail sides, waste fields, wet places and the edges of forests. The plant is aperient, emmenagogue, febrifuge and tonic. The juice of the plant is used in the treatment of typhoid [8]. According to our knowledge, no work has been done on the comparative antioxidant potential of various fractions of *Mazus pumilus* so, it was considered worthwhile to study the antioxidant potential of different fractions of *Mazus pumilus* whole plant for future investigations towards the finding of new, potent and safe antioxidant compounds.

Results and Discussion

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron donating anti-oxidants can be described as reductants and inactivation of oxidants by reductants can be described as redox reactions. This assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of tripyridyltriazine [TPTZ] forming an intense blue Fe^{2+}–TPTZ complex with an absorbance maximum at 593 nm [9]. Increasing absorbance indicates an increase in reductive ability. The FRAP values of the studied fractions were calculated and it was found that among all the fractions the *n*-butanol fraction showed highest FRAP value (626.83±0.39 TE μM ml^{-1}). FRAP values exhibited by chloroform soluble fraction and ethyl acetate soluble fraction were 116.76±0.34 TE μM mL^{-1} and 225.58±1.30 TE μM ml^{-1} respectively while that of aqueous fraction and *n*-hexane fraction were found to be poor (Table-1). High FRAP values obtained for more polar fractions may be ascribed partially to the presence of phenolic and flavonoid contents. The FRAP values of chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and aqueous fraction were found to be non significant ($p > 0.05$) when compared with blank.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity is widely used to evaluate antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability [10]. It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compound is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to yellow [11]. DPPH is a preformed stable radical used to measure radical scavenging activity of antioxidant samples. This method is based on the reaction of DPPH radical that is characterized as a stable free radical with deep violet colour and any substance that can donate hydrogen atom to DPPH thus reduces it to become stable diamagnetic molecule [12]. Reduction of DPPH radical was observed by the decrease in absorbance at 517 nm where as colour changes from purple to yellow. The various fractions of *Mazus pumilus* significantly reduced DPPH radicals. It was found (Table-2) that activity increases by increasing the concentration of the fractions in the assay. The various concentrations of *n*-butanol soluble fraction exhibited highest percent of inhibition of DPPH radical as compared to other fractions. It showed 80.42±1.6 % inhibition of DPPH radical at a concentration of 60 μg ml^{-1}. The various concentrations of the fractions which showed percent inhibition greater than 50% were found to be significant ($p < 0.05$) when compared with negative control i.e. blank. IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds. IC_{50} is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process. A lower value would reflect greater antioxidant activity of the fraction [13]. The IC_{50} values of the studied fractions were calculated (Table-1). *n*-Butanol soluble fraction exhibited lowest IC_{50} value i.e. 28.79±1.35μg ml^{-1} as compared to other

studied fractions, relative to ascorbic acid, a standard reference antioxidant, having IC_{50} value 58.84±0.89. Ethyl acetate fraction also showed good IC_{50} value (64.76±0.98 µg ml^{-1}), while chloroform soluble fraction showed moderate value (272.35±1.16 µg/ml). Very poor IC_{50} values were found for *n*-hexane soluble fraction and remaining aqueous fraction. The results for chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and remaining aqueous fraction were found to be non significant ($p > 0.05$) when compared with ascorbic acid, a reference standard.

Ferric Thiocyanate (FTC) Assay

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Especially, linoleic and arachidonic acids are targets of lipid peroxidation. The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities [14]. The FTC assay measures the amount of peroxide value in the beginning of the lipid peroxidation, where ferric ion was formed upon reaction of peroxide with ferrous chloride. The ferric ion will then unite with ammonium thiocyanate producing ferric thiocyanate, a red-coloured substance. The darker the colour, the higher will be the absorbance [15]. The inhibition of lipid peroxidation was checked for all the fractions. The results (Table-1) showed that *n*-butanol soluble fraction showed highest percent inhibition of lipid peroxidation i.e. 53.91±1.21 %. Ethyl acetate soluble fraction also exhibited good value (46.81±1.03 %) while *n*-hexane soluble fraction (5.31±0.38 %), chloroform soluble fraction (18.09±0.56 %) and remaining aqueous fraction (8.24±0.65 %) didn't show good results. The results were compared with BHT having percent inhibition 62.35±1.52 %. The values of lipid peroxidation inhibition shown by ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction, chloroform soluble fraction and remaining aqueous fraction were found to be non significant ($p > 0.05$) when compared with BHT

Total Antioxidant Activity

The total antioxidant activity of the studied fractions was measured 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 phosphate / Mo (V) compounds with a maximum absorption at 695 nm. Hydroxyl group of the 6-hydroxychroman ring, which is shared by all flavan-3-ols (condensed tannins) and flavonoids, is considered to be, the base of the phosphomolybdenum complex method [15, 16]. It is a quick, cheap and reproducible test, gives the measurement of the antioxidant potential of an analyte. In the presence of a reducing agent, reduction of phosphormolybdate acid to phosphomolybdate blue (Mo^{+6} - Mo^{+5}) took place. This method has been used successfully to determine the antioxidant potentials of plant extracts and isolated compounds. The reaction takes place at acidic pH. The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids [17]. From results (Table-1), it was observed that *n*-butanol soluble fraction showed highest total antioxidant activity i.e. 1.01±0.08 as compared to other fractions. Ethyl acetate fraction also showed good total antioxidant activity (0.92±0.04). Chloroform soluble fraction showed moderate activity (0.53±0.04) while *n*-hexane soluble fraction (0.25±0.02) and remaining aqueous fraction (0.38±0.05) didn't show good activity. The results were compared with butylated hydroxytoluene (BHT), a reference standard whose total antioxidant activity was found to be 1.15±0.07. The results for chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and remaining aqueous fraction were found to be non significant ($p > 0.05$) when compared with BHT.

Total Phenolic Contents

Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals [18]. In this assay Folin-Ciocalteu reagent, a mixture of phosphor-tungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids, is reduced to blue oxides of tungstene (W_8O_{23}) and molybdene (Mo_8O_{23}) during phenol oxidation. This reaction occurs under alkaline condition provided by sodium carbonate. The intensity of blue colour reflects the quantity of phenolic compounds, which can be measured using spectrophotometer [19]. Table-1 shows the phenolic concentration in the different fractions, expressed as milligram of gallic acid equivalents (GAEs) per gram of fraction. Among these five fractions *n*-butanol soluble fraction possessed the highest amount of total phenolics compounds i.e. 88.30±1.09 GAE / mg g^{-1}) followed by the ethyl acetate soluble fraction (66.76±0.98 GAE / mg g^{-1}), chloroform soluble fraction (38.50±1.35 GAE mg g^{-1}), remaining aqueous fraction (7.81±1.10 GAE / mg g^{-1}), while *n*-hexane soluble fraction exhibited the lowest total phenolic content (4.42±1.55 GAE / mg g^{-1}) respectively.

Table-1: IC_{50}, total antioxidant activity, FRAP values, total phenolics and lipid peroxidation of different fractions of *Mazus pumilus*.

Sample	IC_{50} (µg ml^{-1})	Total antioxidant activity	FRAP value TE (µM / ml^{-1})	Total phenolics (*GAE* / mg g^{-1})	Inhibition of Lipid Peroxidation (%)
***n*-Hexane soluble fraction**	878.61±1.74	0.25±0.02	22.51±1.27	4.42±1.55	5.31±0.38
Chloroform soluble fraction	272.35±1.16**	0.53±0.04**	116.76±0.34*	38.50±1.35*	18.09±0.56
Ethyl acetate soluble fraction	64.76±0.98**	0.92±0.04**	225.58±1.30*	66.76±0.98*	46.81±1.03**
***n*-Butanol soluble fraction**	28.79±1.35**	1.01±0.08**	626.83±0.39*	88.30±1.09*	53.91±1.21**
Remaining aqueous fraction	911.16±1.23	0.38±0.05	42.36±1.01	7.81±1.10	8.24±0.65
Ascorbic acid[a)]	58.84± 0.89	-	-	-	-
BHT[a)]	-	0.91±0.07	-	-	62.35±1.52
Blank	-	-	15.34	3.57	-

All results are presented as mean ± standard mean error of three assays.
[a)] Standard antioxidant, *$p < 0.05$ when compared with negative control i.e. blank/solvent ($p < 0.05$ is taken as significant), **$p < 0.05$ when compared with reference standards (BHT, Ascorbic acid)

Table-2:DPPH radical scavenging activity of *Mazus pumilus* (Burm. f.) Steenis.

Sample	Conc. (µg ml^{-1})	Scavenging of DPPH radical(%)±S.E.M[a)]
***n*-Hexane soluble fraction**	1000	55.07 ± 1.31*
	500	34.16 ± 1.53
	250	18.80 ± 1.05
Chloroform soluble fraction	500	76.31 ± 0.34*
	250	48.80 ± 1.44
	120	31.59 ± 0.35
Ethyl acetate soluble fraction	120	76.23 ± 1.30*
	60	52.16 ± 1.27*
	30	33.34 ± 1.16
	15	21.18 ± 1.04
***n*-Butanol soluble fraction**	60	80.42 ± 1.68*
	30	57.12 ± 0.33*
	15	31.84 ± 0.23
Remaining aqueous fraction	1000	53.37 ± 0.91*
	500	36.43 ± 1.74
	250	25.47 ± 0.33
	120	23.29 ± 1.42
Ascorbic acid[b)]	125	79.14 ± 0.93
	60	58.09 ± 0.86
	30	29.97 ± 0.55

[a)] standard mean error of three assays.
[b)] a reference standard antioxidant
*$p < 0.05$ when compared with negative control i.e. blank/solvent ($p < 0.05$ is taken as significant)

The values of total phenolic contents of chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and aqueous fraction were found to be non significant ($p > 0.05$) when compared with blank.

Experimental

Chemicals

Folin Ciocalteu reagent and BHT (butylated hydroxytoluene), TPTZ (2,4,6-Tripyridyl-s-triazine), $DPPH^{\cdot}$ (1,1-Diphenyl-2-picrylhydrazyl radical), Trolox, Gallic acid, were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (*n*-hexane, *n*-butanol, chloroform, ethyl acetate), sulphuric acid, sodium phosphate, ferric chloride, ferrous chloride ammonium molybdate, linoleic acid and tween-20 from Merck (Pvt.) Ltd. (Germany).

Plant Materials

The herb *Mazus pumilus* (Burm. f.) Steenis was collected from Jallo Park, Lahore, Pakistan in November 2011, and identified by Mr. Muhammad Ajaib (Taxonomist), Department of Botany, GC University, Lahore. A voucher specimen (GC-Herb-Bot-1177) has been deposited in the Herbarium of the Botany Department of the same university.

Extraction and Fractionation of Antioxidants

The shade-dried ground whole plant (0.4 kg) was exhaustively extracted with methanol (6 L × 4) on the Soxhlet apparatus. The extract was evaporated to yield the residue (94 g), which was dissolved in distilled water (1 L) and partitioned with *n*-hexane (1 L × 4), chloroform (1 L × 4), ethyl acetate (1 L × 4) and *n*-butanol (1 L × 4) respectively. These organic fractions and remaining water fraction was concentrated separately on rotary evaporator. The

residues thus obtained were used to evaluate their *in vitro* antioxidant potential.

Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing capacity of herbal extracts was calculated according to the method of Benzie and Strain [9] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $CH_3COONa.3H_2O$ and 16 ml CH_3COOH), pH 3.6, 10 mM TPTZ (2, 4, 6-Tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3.6H_2O$ solution and then incubated at 37°C. The solutions of plant samples and that of Trolox were prepared in methanol (500µg ml^{-1}). 10 µl of each of sample solution and BHT solution were taken in separate test tubes and 2990 µl of FRAP solution was added in each to make total volume up to 3 ml. The herb samples were allowed to react with FRAP solution in the dark for 30 minutes. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The FRAP values were determined as micromoles of Trolox equivalents per ml of sample by computing with standard calibration curve constructed for different concentrations of Trolox. Results were expressed in TE µM m^{-1}.

DPPH Radical Scavenging Activity

The DPPH radical scavenging effect of various fractions of herb was determined by comparison with that of known antioxidant, butylated hydroxytoluene (BHT) using the method of Lee and Shibamoto [12]. Briefly, various amounts of the samples (1000 µg ml^{-1}, 500 µg ml^{-1}, 250 µg ml^{-1}, 125 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1}, 8 µg ml^{-1}) were mixed with 3 ml of methanolic solution of DPPH (0.1mM). The mixture was shaken vigorously and allowed to stand at room temperature for an hour. Absorbance was measured at 517 nm against methanol as a blank in the spectrophotometer. Lower absorbance of spectrophotometer indicated higher free radical scavenging activity.

The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = A_{control} - A_{sample} / A_{control} \times 100$$

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

Ferric Thiocyanate (FTC) Assay

The antioxidant activities of various fractions of herb on inhibition of linoleic acid peroxidation were assayed by ferric thiocyanate method [20]. The 0.1 ml of each of sample solution (0.5 mg ml^{-1}) was mixed with 2.5 ml of linoleic acid emulsion (0.02 M, pH 7.0) and 2.0 ml of phosphate buffer (0.02 M, pH 7.0). 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°C. The mixture without extract was used as control. The 0.1 ml of the mixture was taken and mixed with 5.0 ml of 75 % ethanol, 0.1 ml of 30 % ammonium thiocyanate and 0.1 ml of 20 mM ferrous chloride in 3.5 % HCl and allowed to stand at room temperature. Precisely 3 minutes after addition of ferrous chloride to the reaction mixture, absorbance was recorded at 500 nm. The antioxidant activity was expressed as percentage inhibition of peroxidation (IP %)

$$[IP\% = \{1 - (\text{abs. of sample})/(\text{abs. of control})\} \times 100].$$

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

Total Antioxidant Activity

The total antioxidant activities of various fractions of plant were evaluated by phospho-molybdenum complex formation method [17]. Briefly, 500 µg ml^{-1} 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°C for 90 minutes. The absorbance of mixture was measured at 695 nm against blank after the samples had been cooled to room temperature. The antioxidant activity was expressed relative to that of butylated hydroxytoluene (BHT). All determinations were assayed in triplicate and mean values were calculated.

Total Phenolic Contents

Total phenolics of various fractions of plant were determined by the method of Makkar *et al* [21]. The 0.1 ml (0.5 mg ml^{-1}) of sample was combined with 2.8 ml of 10 % Na_2CO_3 and 0.1 ml of 2N Folin-Ciocalteu's reagent. Absorbance at 725 nm was measured by UV-visible spectrophotometer after 40 minutes of incubation at 40 °C. Total phenolics were determined as milligrams of gallic acid equivalents per gram of sample by computing with standard calibration curve constructed for different

concentrations of gallic acid. Results were expressed in GAE / mg g^{-1}.

Statistical Analysis

Statistical analysis were performed using one way analysis of variance (ANNOVA) followed by post-hoc Tukey's test. All the measurements were done in triplicate and statistical analysis was performed by Statistical software. All the data were expressed as ± S.E.M. Differences at P , 0.05 were considered statistically significant.

Conclusions

Our results of antioxidant assays justified and supported the popular usage of Mazus species as traditional remedies for some infections and edible vegetables. It was observed from the results that ethyl acetate soluble fraction and *n*-butanol soluble fraction showed good antioxidant activity. Chloroform soluble fraction showed moderate activity while *n*-hexane soluble fraction and remaining aqueous fraction showed no activity. *n*-butanol fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 80.42±1.68 % inhibition of DPPH radical at a concentration of 60 µg ml^{-1}. The IC_{50} of this fraction was 28.79±1.35 µg ml^{-1}, relative to ascorbic acid, having IC_{50} 58.84±0.89 g ml^{-1}. It also showed highest total antioxidant activity (1.01±0.08) as well as highest FRAP value (626.83±0.39 TE / µM ml^{-1}), highest total phenolic contents (88.30±1.09 GAE / mg g^{-1}) and highest value of inhibition of lipid peroxidation (53.91±1.21%) as compared to the other studied fractions. Ethyl acetate soluble fraction also showed good results. So it was concluded from the present study that ethyl acetate and *n*-butanol fractions are rich in strong antioxidants, these fractions are potentially valuable sources of natural antioxidants and bioactive materials and further phytochemical investigations on this plant may bring new natural antioxidants into the food industry that might provide good protection against the oxidative damage which occurs both in the body and our daily foods.

References

1. F. Aqil, I. Ahmed and Z. Mehmood, *Turkish Journal of Biology*, **30,** 177 (2006)
2. T. P. A. Devasagayam, J. C. Tilak and K. K. Boloor, *Journal of the Association of Physicians of India*, **52,** 794 (2004)
3. N. Ara and H. Nur, *Research Journal of Medicine and Medical Sciences,* **4,** 107 (2009).
4. T. Riaz, M. A. Abbasi, T. Shahzadi, A. Rehman, S. Z. Siddiqui, H. Khalid and M. Ajaib, *Journal of Medicinal Plants Research,* **5,** 6597 (2011)
5. E. Souri, G. Amin, H. Farsam, H. Jalalizadeh and S. Barezi, *Iranian Journal of Pharmaceutical Research*, **7,** 149 (2008)
6. B. Halliwell, *Free Radical Research*, **9**, 1 (1990)
7. R. K. Manhas, M. K. Gautam and D. Kumari, *The Journal of American Science,* **5,** 1 (2009)
8. G. Kunkel, Plants for human consumption, Koeltz Scientific Books, Box 1360, Koenigatein, West Germany, p. 393, (1984).
9. E. F. Benzie and J. J. Strain, *Analytical Biochemistry,* **239,** 70 (1996).
10. M. N. Siddaraju and S. M. Dharmesh, *Journal of Agricultural and Food Chemistry,* **55**, 7377 (2007).
11. S. J. Meir, B. Kanner and S. P. H. Akiri, *Journal of Agricultural and Food Chemistry*, **43**, 1813 (1995).
12. K. G. Lee and T. Shibamoto, *Food Chemistry*, **74,** 443 (2001).
13. T. Riaz, M. A. Abbasi, A. Rehman, T. Shahzadi and M. Ajaib, *Journal of Pharmaceutical and Scientific Innovation,* **1**, 16 (2012)
14. M. A. Ebrahimzadeh, F. Pourmorad and S. Hafezi, *Turkish Journal of Biology,* **32**, 43 (2008).
15. N. Huda-Faujan, A. Noriham, A. S. Norrakiah and A. S. Babji, *African Journal of Biotechnology,* **8**, 484 (2009).
16. P. Prietto, M. Pineda and M. Aguilar, *Analytical Biochemistry*, **269**, 337 (1999).
17. M. A. Abbasi, K. Rubab, A. Rehman, T. Riaz, T. Shahzadi, M. Khalid and M. Ajaib, *Pakistan Journal of Pharmaceutical Sciences*, **25**, 357 (2012).
18. M. R. Saha, M. S. R. Hasan, R. Akter, M. M. Hossain, M. S. Alam, M. A. Alam and M. E. H. Mazumder, *Bangladesh Journal of Veterinary Medicine,* **6**, 197 (2008).
19. K. K. Rahim A. Almey, A. J. Khan, S. Zahir, K. Suleiman and M. R. Aisyah, *International Food Research Journal,* **17**, 1077 (2010)
20. P. Valentao, E. Fernandes, F. Carvalho, P. B. Andrade, R. M. Seabra and M. L. Bastos, *Journal of Agricultural and Food Chemistry,* **50,** (2002)
21. H. P. S. Makkar, M. Bluemmel, N. K. Borowy and K. Becker, *Journal of the Science of Food and Agriculture,* **61**, 161 (1993).