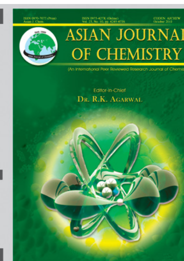
Asian Journal of Chemistry; Vol. 24, No. 8 (2012), 3335-3338

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Martynia annua: Comparative Antioxidant Potential of Its Stem and Leaves

AZIZ-UR-REHMAN[1,*], SHARGEEL AHMAD[1], TAUHEEDA RIAZ[1], ANSUR ABBAS[1], MUHAMMAD ATHAR ABBASI[1], SABAHAT ZAHRA SIDDIQUI[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. 264; E-mail: azizryk@hotmail.com

(*Received*: 9 May 2011; *Accepted*: 1 March 2012) AJC-11138

The present investigation was aimed to evaluate the comparative *in vitro* antioxidant and radical scavenging activity of various fractions of stem and leaves of *Martynia annua* L. The antioxidant components were initially extracted in methanol separately for stem and leaves using Soxhlet apparatus. These methanolic extracts of stem and leaves were subjected to the fractionation separately in solvents of different polarity. The comparative antioxidant potential and radical scavenging activities of these fractions were investigated using different antioxidant assays such as ferric reducing antioxidant power (FRAP), 2,2'-diphenyl-1-picrylhydrazil (DPPH) scavenging, total phenolic contents (TPC) and total antioxidant activity by phosphomolybdenum complex method. The results revealed that *n*-butanol soluble fraction of stem showed highest % scavenging of DPPH (83.62 ± 0.38 % at concentration of 250 μg/mL) as compared to other studied fractions. The ethyl acetate soluble fraction of leaves also displayed good activity (82.88 ± 0.34 %) nearly equal to it. However, the *n*-butanol soluble fraction of stem exhibited highest total antioxidant activity (0.187 ± 0.85), highest value of total phenolic contents (278.32 ± 0.73 mg/GAE) as well as highest FRAP value 149.00 ± 0.56 mg/TE. So it was concluded that *n*-butanol soluble fraction of stem contains many antioxidant compounds. Similarly, the ethyl acetate soluble fraction of leaves is also a valuable source of antioxidants.

Key Words: *Martynia annua* L., DPPH assay, Total antioxidant activity, FRAP value, Total phenolics.

INTRODUCTION

Free radicals and various other reactive oxygen species (ROS), such as hydroxyl radical ($OH^{\bullet}$), superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and whole class of highly reactive molecules are derived from the normal metabolism of oxygen or from exogenous agents and factors. Normally oxidative damage to crucial cellular molecules induced by reactive oxygen species has been implicated as a possible factor in the etiology of several human diseases, including cardiovascular disease, cancer and aging[1]. In recent years, there is a vibrant increasing interest in finding antioxidant phytochemicals because they can inhibit the propagation of free radical reactions and also protect the human body from lipid oxidative rancidity in foods and from various dangerous diseases. The most effective ones seem to be phenolic and flavonoids compounds of many plant raw materials, particularly in seeds, herbs and fruits. Their radical scavenging properties and metal-chelating capabilities have enabled these phenolic compounds to be thought of as most effective free radical scavengers and inhibitors of lipid peroxidation[2]. A large number of herbal drugs are rightly reputed to be of excellent medicinal value and are used for treatment of several ailments. In folk medicine, various indigenous drugs are used in single and/or in combined form to treat different types of inflammatory and arthritic conditions, with considerable success[3]. *Martynia annua* L. belonging to family Martyniaceae, is found in Pakistan. It is used as a remedy for scorpion-sting. Many useful compounds such as cynidin-3-galactoside, pelargonidin-3-5-diglucoside, hydroxyl benzoic acid, arachidic acid, gentisic acid, linolic acid, oleic acid, palmitic acid, stearic acid, apigenin, apigenin-7-O-glucuronide have been isolated from this species[4]. Its leaves are antiseptic and are used in epilepsy. Its fruit is used as anti-inflammatory and roots are used for the treatment of snake-bite. Also, entire plant is used to treat menstrual disorders. Dried entire plant has anticonvulsant activity and analgesic activity[5,6]. As a part of continues research on the chemical and biological investigations of the medicinal plants, we examined the comparative antioxidant potential of different fractions of leaves and stem of this plant. Although some primary studies on antioxidant potential of aqueous and methanolic extracts of the whole plant has been studied, but the comparative antioxidant potential of various fractions of leaves and stem in solvents of different polarity had not been studied yet. The objective of the present study was to evaluate comparative antioxidant potential of various fractions of stem and leaves

of this plant using four antioxidant methods *i.e.* ferric reducing antioxidant power (FRAP) assay, 2,2'- diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity, total phenolic contents and total antioxidant activity by phosphomolebdenum complex method.

EXPERIMENTAL

The plant *Martynia annua* L. was collected from P.O.K. in August 2010 and identified by Muhammad Ajaib (Taxonomist), Department of Botany, GC University, Lahore. A Voucher specimen (GC. Herb. Bot. 810) has been deposited in the Herbarium of the Botany Department of the GC University, Lahore, Pakistan.

Extraction and fractionation of antioxidants: The shade-dried ground stem (4.5 kg) and leaves (3.7 kg) were exhaustively extracted separately with methanol using Soxhlet apparatus. These extracts were evaporated to yield the residues 315 g and 198 g respectively. These extracts were dissolved in distilled water (1.5 L) separately and partitioned with *n*-hexane (1 L × 4), dichloromethane (1 L × 4), ethyl acetate (1 L × 4) and *n*-butanol (1 L × 4) respectively.

DPPH^ (1,1-Diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-tripyridyl-s-triazine), Trolox, gallic acid, Folin-Ciocalteu reagent and BHT (butylated hydroxytoluene) were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride and ferrous chloride from Merck (Pvt.) Ltd. (Germany). Spectrophotometric measurements were made on UV-2300 Shimadzu, Japan equipped with temperature control device. All the measurements were made in triplicate and the results thus obtained were averaged.

Antioxidant assays

DPPH Radical scavenging activity: The DPPH radical scavenging activities of various fractions of stem and leaves of this plant were examined by reported method[7]. Briefly, various concentrations of the samples (1000, 500, 250, 125, 60, 30 mg/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 1 h. Then absorbance was measured at 517 nm against methanol as a blank in the UV-Visible spectrophotometer (CECIL Instruments CE 7200 Cambridge England). Lower absorbance of spectrophotometer indicated higher free radical scavenging activity.

The per cent of DPPH decolouration of the samples was calculated according to the formula:

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

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

Total antioxidant activity by phosphomolybdenum complex method: The total antioxidant activities of the studied fractions of this plant were evaluated by phosphomolybdenum complex formation method[8]. Briefly, 500 µg/mL of 1each 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 1.5 h. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. All determinations were assayed in triplicate and mean values were calculated.

Total phenolic contents: Total phenolic contents of the fractions were determined by a reported method[9]. Stock solution of gallic acid was made by dissolving 0.5 g gallic acid in 10 mL of ethanol in a 100 mL volumetric flask and diluting to volume with double distilled water. Sodium carbonate solution was prepared by dissolving 200 g of anhydrous sodium carbonate in 800 mL of double distilled water. After boiling and subsequent cooling of the solution, a few crystals of sodium carbonate were added. The solution was stand for 24 h, filtered and volume was raised to 1 L with double distilled water. To prepare a calibration curve, 0, 1, 2, 3, 5 and 10 mL of stock solution of phenol were added into 100 mL volumetric flask separately and then diluted to volume with double distilled water. The resultant solutions contained concentrations of 0, 50, 100, 150, 250 and 500 mg/L gallic acid. From each calibration solution and sample or blank, 40 µL were pipetted into separate cuvettes and to each 3.16 mL of double distilled water was added. Folin- Ciocalteu's reagent (200 µL) was added and mixed well. After 8 min, 600 µL of sodium carbonate solution was mixed thoroughly in the solution. The solution was allowed to stand at 40 ºC for 0.5 h and absorbance of each solution was noted at 765 nm against the blank (without phenolic solution). A concentration versus absorbance linear plot was thus obtained. The concentration of total phenolic compounds of each plant extract in milligram of gallic acid equivalent (GAE), was determined by using the following standard equation.

$$\text{Absorbance} = 0.118\,x + 0.0824\ [\text{gallic acid (mg/mL)}]$$

Ferric reducing antioxidant power assay: The reducing capacity of plant fractions was measured according to the reported method[10]. Freshly prepared FRAP solution contained 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution in 40 mM HCl solution and 2.5 mL of 20 mM ferric chloride solution. The mixture was incubated at 37 ºC throughout the monitoring period. 3 mL of FRAP reagent was mixed with 100 µL of each sample and 300 µL of distilled water. Solutions were kept in dark for 40 min and the absorbance was determined at 593 nm. Results were compared with standard curve of Trolox.

Statistical analysis: All the measurements were done in triplicate and statistical analysis was performed by Microsoft excel 2003. Results are presented as average ± SEM.

RESULTS AND DISCUSSION

2,2′-Diphenyl-1-picrylhydrazyl (DPPH) radical is largely used in the model systems to investigate the scavenging activities of several natural compounds such as phenolics and anthocyanins. DPPH radical is a stable free radical at room temperature and accepts an electron or radical to form a most stable diamagnetic molecule. DPPH radical is scavenged by an antioxidant through the donation of proton by it forming the reduced DPPH. The colour changes from purple to yellow after reduction can be quantified by the decrease of absorbance at the 517 nm. Radical scavenging activity increases with

increasing percentage of the free radical inhibition[11]. DPPH radical scavenging activities of all the studied fractions *i.e.* *n*- hexane soluble fraction of stem, dichloromethane soluble fraction of stem, ethyl acetate soluble fraction of stem, *n*-butanol soluble fraction of stem, *n*- hexane soluble fraction of leaves, dichloromethane soluble fraction of leaves, ethyl acetate soluble fraction of leaves and *n*-butanol soluble fraction of leaves were determined and results have been shown in Table-1. As the concentration of sample increased, the per cent inhibition of DPPH free radical also increased. It has been found from the results that *n*-butanol soluble fraction of stem showed good % scavenging of DPPH *i.e.* 83.62 ± 0.38 % at concentration of 250 μg/mL as compared to *n*-butanol soluble fraction of leaves having 38.42 ± 0.14 % at the same concentration. It also exhibited good scavenging potential relative to all the remaining studied fractions. However, ethyl acetate soluble fraction of leaves displayed a nearly equal scavenging potential *i.e.* 82.88 ± 0.34 %. Butylated hydroxytoluene (BHT) was used as a standard antioxidant. The IC_{50} (concentration of sample required to scavenge 50 % free radical) values of all the studied fractions were also calculated and results have been shown in Table-2. The IC_{50} value is inversely related to the activity *i.e.* the lower the IC_{50} value the higher will be the antioxidant potential. It has been revealed from the results that *n*-butanol soluble fraction of stem possessed lowest IC_{50} value (72.71 ± 0.022 μg/mL) as compared to *n*-butanol soluble fraction of leaves (421.29 ± 0.42 μg/mL) as well as all other fractions. This signifies that it has highest DPPH radical scavenging activity. The ethyl acetate soluble fraction of leaves also demonstrated lower IC_{50} value of 83.57 ± 0.025 μg/mL relative to similar fraction from stem of this plant *i.e.* 295.49 ± 0.33 μg/mL. Similarly, the dichloromethane soluble fraction of leaves also revealed lower IC_{50} value, 268.09 ± 0.04 μg/mL, relative to similar fraction from stem of this species having IC_{50} value of 411.20 ± 0.32 μg/mL. However, the IC_{50} value of *n*- hexane soluble fraction of leaves was found to be higher *i.e.* 659.36 ± 0.065 μg/mL than that of *n*-hexane soluble fraction of stem *i.e.* 558.50 ± 0.52 μg/mL.

The total antioxidant capacity of all the studied fractions was measured spectrophotometrically by phosphomolybdenum complex method which is based on reduction of Mo(VI) to Mo(V) by sample analyte and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at 695 nm[12]. The phosphomolybedenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids. From the results (Table-2) it was revealed that *n*-butanol soluble fraction of stem exhibited highest total antioxidant activity *i.e.* 0.187 ± 0.85 while that of *n*-butanol soluble fraction of leaves was found to be 0.137 ± 0.55. The total antioxidant activity for *n*- hexane soluble fraction of stem was found to be 0.102 ± 0.61 which is almost equal to the similar fraction of leaves (0.109 ± 0.29). The total antioxidant activity value for dichloromethane soluble fraction of stem was 0.150 ± 0.34, which is slightly greater than that of leaves' fraction *i.e.* 0.122 ± 0.73. The ethyl acetate soluble fractions of stem and leaves showed almost equal total antioxidant activity *i.e.* 0.153 ± 0.25 and 0.160 ± 0.61, respectively.

TABLE-1
FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS FRACTIONS OF STEM AND LEAVES OF *Martynia annua* L. USING 1,1-DIPHENYL-2-PICRYLHYDRAZYL RADICAL (DPPH)

Samples	Concentration in Assay (μg/mL)	% Scavenging of DPPH ± SEM[a]
n-Hexane soluble fraction of stem	1000	79.06 ± 0.85
	500	50.55 ± 0.13
	250	26.84 ± 0.17
Dichloromethane soluble fraction of stem	1000	82.75 ± 0.39
	500	57.56 ± 0.74
	250	39.16 ± 0.15
Ethyl acetate soluble fraction of stem	1000	83.12 ± 0.95
	500	68.71 ± 0.13
	250	46.4 ± 0.66
n-Butanol soluble fraction of stem	250	83.62 ± 0.38
	125	64.20 ± 0.45
	60	49.33 ± 0.31
	30	37.86±0.36
n-Hexane soluble fraction of leaves	1000	79.10 ± 0.46
	500	45.19 ± 0.21
	250	4.31 ± 0.51
Dichloromethane soluble fraction of leaves	1000	80.17 ± 0.34
	500	62.55 ± 0.75
	250	47.55 ± 0.52
Ethyl acetate soluble fraction of leaves	250	82.88 ± 0.34
	125	63.25 ± 0.45
	60	42.06 ± 0.66
n-Butanol soluble fraction of leaves	1000	85.96 ± 0.75
	500	56.89 ± 0.65
	250	38.42 ± 0.14
BHT[b]	60	91.17 ± 1.20
	30	72.14 ± 1.14
	15	65.86 ± 2.32

[a]All results are presented as mean ± standard mean error of three assays; [b] Standard antioxidant; Note: IC_{50} should be calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested samples. So the concentrations of fractions were taken in a way that some concentrations show % inhibition above 50 % and some show below 50 %

Plant phenolics are commonly found in both edible and non-edible plants and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers[13]. Therefore, the antioxidant activities of plant extract are often explained by their total phenolics. This assay is an electron transfer based assay and gives reducing capacity, which has normally been used to estimate phenolic contents of biological materials[14]. Total phenolics assay was employed to measure the total phenolic contents of various fractions of this plant and results have been shown in Table-2. The *n*-butanol soluble fraction of stem exposed highest value of total phenolic contents (278.32 ± 0.73 mg/GAE) as compared to all other studied fractions while that of *n*-butanol soluble fraction of leaves exhibited 248.70 ± 0.21 mg/GAE. The *n*-hexane soluble fraction of stem showed more total phenolics (184.88 ± 0.81 mg/GAE) relative to similar fraction from leaves (144.20 ± 0.65 mg/GAE). The dichloromethane soluble fractions of both stem and leaves showed almost equal total phenolics *i.e.* 232.55 ± 0.29 mg/GAE and 244.20 ± 0.65 mg/GAE, respectively. However, ethyl acetate soluble fractions of leaves revealed slightly greater number of

TABLE-2
IC_{50}, TOTAL ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS AND FRAP VALUES OF VARIOUS FRACTIONS OF STEM AND LEAVES OF *Martynia annua* L.

Samples	IC_{50} value (µg/mL) ± SEM[a)]	Total phenolic contents (mg/GAE) ± SEM[a)]	Total antioxidant activity ± SEM[a)]	FRAP value (mg/TE) ± SEM[a)]
n-Hexane soluble fraction of stem	558.50 ± 0.52	184.88 ± 0.81	0.102 ± 0.61	85.00 ± 0.16
Dichloromethane soluble fraction of stem	411.20 ± 0.32	232.55 ± 0.29	0.150 ± 0.34	110.50 ± 0.54
Ethyl acetate soluble fraction of stem	295.49 ± 0.33	256.27 ± 0.19	0.153 ± 0.25	114.50 ± 0.21
n-Butanol soluble fraction of stem	72.71 ± 0.22	278.32 ± 0.73	0.187 ± 0.85	149.00 ± 0.56
n-Hexane soluble fraction of leaves	659.36 ± 0.65	144.20 ± 0.65	0.109 ± 0.29	35.50 ± 0.67
Dichloromethane soluble fraction of leaves	268.09 ± 0.45	298.49 ± 0.98	0.122 ± 0.73	58.00 ± 0.54
Ethyl acetate soluble fraction of leaves	83.57 ± 0.25	275.11 ± 0.52	0.160 ± 0.61	124.00 ± 0.55
n-Butanol soluble fraction of leaves	421.29 ± 0.42	248.70 ± 0.21	0.137 ± 0.55	92.00 ± 0.64
BHT[b)]	12.1 ± 0.92	-	1.218 ± 0.09	-

[a)] Standard mean error of three assays; [b)] Standard antioxidant

total phenolics (275.11± 0.52 mg/GAE) than that of similar fraction of stem (256.27 ± 0.19 mg/GAE).

Ferric reducing antioxidant power (FRAP) is a simple direct test for measuring antioxidant capacity. This method was initially developed to assay plasma antioxidant capacity, but can be used with plant extract too. Ferric reducing antioxidant power assay measures the reducing ability of antioxidant against oxidative effect of reactive oxygen species. Electron donation antioxidant can be described as reductants and inactivation of oxidants by reductants can be described as redox reaction. This antioxidant activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from ferric reducing antioxidant power reagents containing TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) and $FeCl_3.6H_2O$. The absorbance is measured spectrophotometrically at 595 nm[10]. The ferric reducing antioxidant power values of all the studied fractions were determined and results have been shown in Table-2. The *n*-butanol soluble fraction of stem possessed highest ferric reducing antioxidant power value, 149.00 ± 0.56 mg/TE, among all the fractions while that of similar fraction of leaves had 92.00 ± 0.64 mg/TE. The ferric reducing antioxidant power value for the *n*-hexane soluble fraction of stem was found to be 85.00 ± 0.16 mg/TE, which is higher than that of similar fraction of leaves, 35.50 ± 0.67 mg/TE. Similarly, dichloromethane soluble fractions of stem also showed greater ferric reducing antioxidant power value, 110.50 ± 0.54 mg/TE than that similar fraction of leaves, 58.00 ± 0.54 mg/TE, but the ethyl acetate soluble fractions of stem revealed lower ferric reducing antioxidant power value, 114.50 ± 0.21 mg/TE, than the ethyl acetate soluble fractions of leaves, which was found to be 124.00 ± 0.55 mg/TE.

Conclusion

From the results it was evident that the *n*-butanol soluble fraction of stem exhibited highest % scavenging of DPPH, 83.62 ± 0.38 % at concentration of 250 µg/mL as compared to all the remaining studied fractions and ethyl acetate soluble fraction of leaves also exhibited scavenging activity nearly equal to it *i.e.* 82.88 ± 0.34 %. Moreover, the *n*-butanol soluble fraction of stem also showed highest total antioxidant activity *i.e.* 0.187 ± 0.85, highest value of total phenolic contents (278.32 ± 0.73 mg/GAE) as well as highest ferric reducing antioxidant power value *i.e.* 149.00 ± 0.56 mg/TE. So it was concluded that both *n*-butanol soluble fraction of stem and ethyl acetate soluble fraction of leaves were the rich sources of antioxidants in this plant which would further be expected to increase shelf life of foods and fortify against peroxidative damage in living systems in relation to aging and carcinogenesis.

REFERENCES

1. B. Halliwel and J.M.C. Gutteridge, Oxford University Press (2006).
2. Y.C. Chung, C.T. Chein, T.K. Yun and S.T. Chou, *Food Chem.*, **97**, 418 (2006).
3. A.C.B. Paula, L.S.S. Hayashi and J.C. Freitas, *Braz. J. Med. Bio. Res.*, **36**, 105 (2006).
4. R.P. Rastogi and B.N. Mehtra, Compandium of Indian Medicinal Plants, CDRI, Lukhnow, vol. **2**, p. 446 (1993).
5. S.A. Nirmal, *Ind. Drug*, **44**, 772 (2006).
6. P.C. Mali, *J. Ethnopharmacol.*, **82**, 61 (2002).
7. K. Lee and T. Shibamoto, *Food Chem*., **74**, 443 (2001).
8. P. Prieto, M. Pineda and M. Aguilar, *Anal. Biochem.*, **269**, 337 (1999).
9. K. Slinkard and V.L. Singleton, *Am. J. Enol. Viticult.*, **28**, 49 (1977).
10. I.F.F. Benzie and J.J. Strain, *Method. Enzymol.*, **299**, 5 (1999).
11. D.J. Huang, H.L. Chen, C.D. Lin and Y.H. Lin, *Bot. Bull. Acad. Sin.*, **46**, 99 (2005).
12. S. Miladi and M. Damak, *J. Soc. Chim. Tunisie*, **10**, 101 (2009).
13. N. Huda-Faujan, A. Noriham, A.S. Norrakiah and A.S. Babji, *Afr. J. Biotech.*, **8**, 484 (2009).
14. R.G. Marwah, M.O. Fatope, R. Mahrooqi, G.B. Varma, H. Al-Abadi and S.S. Al-Burtamani, *Food Chem*., **101**, 465 (2007).