VERSITA

Cent. Eur. J. Biol. • 5(3) • 2010 • 338–345
DOI: 10.2478/s11535-010-0013-5

Central European Journal of **Biology**

Antihemolytic and antioxidant activities of *Allium paradoxum*

Research Article

Mohammad Ali Ebrahimzadeh[1]*, Seyed Fazel Nabavi[1,2], Seyed Mohammad Nabavi[1,3], Bahman Eslami[4]

[1]*Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, 48189 Sari, Iran*

[2]*Student Research Development Committee, Mazandaran University of Medical Sciences, 48189 Sari, Iran*

[3]*Department of Biology, University of Mazandaran, 48181-59376 Babolsar, Iran*

[4]*Department of Biology, Islamic Azad University, Ghaemshahr Branch, 32549 Ghaemshahr, Iran*

Received 28 August 2009; Accepted 22 January 2010

Abstract: Antioxidant activity of the aerial part and bulbs of *Allium paradoxum* was investigated by eight *in vitro* assay systems. Extracts showed good antioxidant activity. IC_{50} for 1,1-diphenyl-2-picryl hydrazyl radical-scavenging activity was 890.9±43.2 and 984.9±33.5 μg/ml for the aerial part and bulbs, respectively. The aerial parts have better reducing power than bulb extracts but not comparable with Vitamin C ($P<0.001$). Extracts showed weak Fe^{2+} chelating ability, the IC_{50} being 959±47 and 530±24 μg/ml for bulbs and aerial parts, respectively. Both tested extracts exhibited good hydrogen peroxide scavenging in a concentration dependent manner. They exhibited good antioxidant activity against the hemoglobin-induced linoleic acid system that was comparable with vitamin C ($P<0.01$). They showed good activity against cumene hydro peroxide induced hemolysis in RBCs. In addition, they possessed antihemolytic activity. The extract from aerial parts had significantly higher total phenol and flavonoid content than did bulbs. Amounts of eight elements (Cu, Mn, Zn, Fe, Ni, Pb, Cd and Cr) were also determined in the bulb and aerial part using atomic absorption spectroscopy. They contained higher Fe and Mn contents than other elements.

Keywords: *Allium paradoxum • Antihemolytic activity • Antioxidant activity • Cumene hydro peroxide • DPPH • Mineral contents • Phenolic contents*

1. Introduction

The pathology of numerous chronic diseases, including cancer and heart disease, involves oxidative damage to cellular components. Reactive oxygen species (ROS), capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age [1,2]. Minimizing oxidative damage may well be one of the most important approaches to the primary prevention of these aging-associated diseases and health problems, since antioxidants terminate direct ROS attacks and radical-mediated oxidative reactions, and appear to be of primary importance in the prevention of these diseases and health problems. Antioxidants have been detected in a large number of food and agricultural

* *E-mail: zadeh20@yahoo.com*

products, including cereal grains, vegetables, fruits, and plant extracts [3,4]. Also, many current human health problems relate to diets. Micronutrients are involved in numerous biochemical processes and an adequate intake of certain micronutrients relates to the prevention of deficiency diseases. Malnutrition is of major concern for many tropical developing countries, especially in the third world. Iron deficiency anaemia, for example, affects one third of the world population [5,6]. Fruits and vegetables are safe and valuable sources of minerals [7]. Among the various medicinal plants, some endemic and edible species are of particular interest because they may be used for producing raw materials or preparations containing phytochemicals with significant antioxidant capacities and high content of minerals with health benefits. The genus *Allium* is a member of *Liliaceae* family and contains 600 to 700 species, but only a few species have been domesticated so far as vegetables, spices or ornamental plants [8]. *Allium paradoxum* (M.B.) G. Don. is a cultivated vegetable and spice used in home gardens. In Iran it is locally called “Alezi”, and found as a cultivated vegetable and spice in the northern area of the country, especially in Mazandaran. The plant is used to prepare a variety of local and special foods. Cysteine sulfoxides and alliinase activity of *A. paradoxum* have been reported recently [9]. To the best of our knowledge there is no scientific reported about antioxidant and antihemolytic activities of this species. In this study, the antioxidant activity of aerial parts and bulbs of *A. paradoxum* at flowering stage examined by eight different *in vitro* assay systems in order to understand the usefulness of this plant as a foodstuff as well as in medicine. In addition, eight elements (Cu, Mn, Zn, Fe, Ni, Pb, Cd and Cr) were also determined in the bulb and aerial part.

2. Experimental Procedures

2.1 Chemicals

Ferrozine, Linoleic acid, Trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl (DPPH), potassium ferricyanide, Cumene hydroperoxide (CuOOH), Hydrogen peroxide were purchased from Sigma Chemicals Co. (USA). Gallic acid, Quercetin, Butylated hydroxyanisole (BHA), Ascorbic acid, Sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, EDTA and Ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

2.2 Plant material and preparation of extract

A. paradoxum (M.B.) G. Don. aerial parts (at flowering stage) and bulbs were collected from Sari Forest (northern of Iran). Vouchers (No. A4411-A4412) have been deposited in the Sari School of Pharmacy herbarium. The materials were dried at room temperature for 2 weeks and then oven dried at 35°C, for 2 days. Dried materials were coarsely ground (2-3 mm) before extraction. Each part (100 g) was extracted by percolation using methanol/water (80/20 w/w) for 24 h at room temperature. The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper, repeated three times. The resultant extracts were concentrated in a rotary evaporator until a crude solid extracts were obtained which were then freeze-dried for complete solvent removal. The yields were 15.5 and 27% for bulbs and aerial parts, respectively.

2.3 Instrumentation and analytical procedures

The properly dried and ground plant samples were ash-dried overnight at 400-420°C in a Vitreosil crucible. Care was taken for the temperature not to exceed 450°C to avoid losses of zinc. The inorganic residue was kept in a desiccator until needed for analysis. Two grams of ash were dissolved in a 1:3 mixture of hydrochloric and nitric acids [10] diluted to 50 ml with distilled water and analysed with an atomic absorption spectrometer (Perkins Elmer AAS 100) (Wellesley, MA). Sixteen blank control solutions were used to estimate the detection limits of the investigated elements following the same analytical procedures. Three times the standard deviation was used as detection limit (Table 1).

2.4 Determination of total phenolic and flavonoid contents

Total phenolic compound contents were determined by the Folin-Ciocalteau reagent method [11,12]. The extract samples (0.5 ml) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g/l sodium carbonate then added. The absorbance was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Total flavonoid content was determined by a colorimetric method [13,14]. 0.5 ml solution of each plant extract in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid content was calculated as quercetin from a calibration curve.

2.5 DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts [15,16]. Different concentrations of each extract were added, at an equal volume, to a methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was measured at 517 nm. The experiment was repeated three times. Vitamin C, BHA and Quercetin were used as standard controls. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals.

2.6 Reducing power determination

Fe (III) reduction is often used as an indicator of electron- donating activity, an important mechanism for phenolic antioxidant action [17]. The reducing power of extracts was determined according to the method of Yen and Chen [18,19]. Different amounts of each extract (25-800 µg/ml) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixtures were incubated for 20 minutes at 50°C. 2.5 ml of trichloroacetic acid (10%) was added to the mixture to stop the reaction, then centrifuged at 3000 rpm for 10 min. The supernatant of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

2.7 Assay of nitric oxide-scavenging activity

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of each extract, dissolved in water and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control [20,21].

2.8 Metal chelating activity

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions *via* Fenton chemistry. The chelating of ferrous ions by extracts was estimated by the method of Dinis [22-24]. The extract (0.2-1.6 mg/ml) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured at 562 nm. The percentage inhibition of ferrozine- Fe^{2+} complex formation was calculated as $[(A_0 - A_s)/A_s] \times 100$, where A_0 was the absorbance of the blank, and A_s was the absorbance of the extract or positive control, EDTA.

2.9 Antioxidant activity in a hemoglobin-induced linoleic acid system

The antioxidant activity of extracts was determined by a modified photometry assay [25,26]. Reaction mixtures (200 ml) containing 10 ml extracts (10–400 mg), 1 mmol/l of linoleic acid emulsion, 40 mmol/l of phosphate buffer (pH 6.5) and 0.0016% hemoglobin, were incubated at 37°C for 45 min. After the incubation, 2.5 ml of 0.6% HCl in ethanol was added to stop the lipid peroxidation. The amount of peroxide value was measured in triplicate using the thiocyanate method by reading the absorbance at 480 nm after coloring with 100 ml of 0.02 mol/l of $FeCl_2$ and 50 ml of ammonium thiocyanate (30 g/100 ml). Vitamin C was used as positive control.

2.10 Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (0.1-1 mg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid and BHA were used as standards [19,27]. The percentage of hydrogen peroxide scavenging by the extracts and the standard compounds was calculated as follows:

$$\% \text{ Scavenged } (H_2O_2) = [(A_o - A_1)/A_o] \times 100$$

where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard.

2.11 CuOOH-induced hemolysis

Red blood cells (RBC) were isolated from male Wistar rats and suspended in balanced phosphate buffered saline (PBS) to obtain a 1% RBC suspension [28]. Aliquots (3.5 ml) were incubated at 37°C for 210 min in the presence of 50 µM Cumene hydroperoxide (CuOOH, dissolved in ethanol) and the cellular integrity determined turbidimetrically at 710 nm at 30 min intervals [28]. The extracts (dissolved in EtOH, final concentration 50 µg/ml) were pre-incubated for 30 min with RBC before the addition of CuOOH [blanks were

Elements	Detection wavelength (nm)	Drying temp. (°C)	Melting point (°C)	Detection limits (μg/g)
Cu	324.8	120	1085	0.033
Fe	248.3	120	1535	0.047
Zn	213.9	120	450	0.035
Mn	279.5	120	1246	0.009
Ni	232.0	120	1455	0.056
Cr	357.9	120	1900	0.018
Pb	283.4	120	327.5	0.020
Cd	228.8	120	321.1	0.007

Table 1. Operating Conditions of the Atomic Absorption Spectrometer[a] (AAS 100).

[a] *Slit width 0.2 nm. Air and acetylene flow rates 4.0 and 0.5 l/min, respectively.*

RBC with ethanol, at a final concentration always less than 0.1% (v/v)]. Percentage hemolysis was determined (100% hemolysis was determined as the absorbance value in RBC suspensions sonicated for 5 s at 50% power, mean values of 4 determinations were used for the calculation).

2.12 Antihemolytic activity

Antihemolytic activity of the extracts was assessed as described by Naim *et al.* [29,30] with a slight modification. Erythrocytes from male Wistar rat blood were separated by centrifugation and washed with phosphate buffer (pH 7.4), and diluted with phosphate buffered saline to give a 4% suspension. 1 g of extract/ml of saline buffer was added to 2 ml of the erythrocyte suspension and the volume made up to 5 ml with saline buffer. The mixture was incubated for 5 min at room temperature and then 0.5 ml of H_2O_2 solution in saline buffer added to induce oxidative degradation of the membrane lipids. The concentration of H_2O_2 in the reaction mixture was adjusted to bring about 90% hemolysis of the blood cells after 240 min. After incubation the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis determined by measuring the absorbance, corresponding to hemoglobin liberation, at 540 nm.

A. paradoxum	Zn	Mn	Fe	Cu	Cr	Cd	Pb	Ni
Bulb	3.1	7.7	12.0	5.78	0.2	0.5	ND	1.7
Aerial part	5.1	20.4	38.1	4.62	ND	0.6	ND	3.0

Table 2. Amounts of trace elements in plants of *A. paradoxum* by AAS Analysis (μg/g)

Values are averages of three independent measurements having a precision of approx. ±2.5%.
ND: Not detected

2.13 Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by analysis of variance ($P<0.05$) and the means separated by Duncan's multiple range test. The IC_{50} values were calculated from linear regression analysis.

3. Results and Discussion

3.1 Elemental composition

Results of elemental analysis in *A. paradoxum* ash by AAS are presented in Table 2. The concentration of various elements decreases in the order: *Bulbs*: Fe> Mn> Cu> Zn> Ni> Cd> Cr; aerial part: Fe> Mn> Zn> Cu> Ni> Cd. Aerial parts contained higher trace elements than bulbs. The daily elemental requirements of an adult male are as follows (mg/d): 10–15 Fe, 12–15 Zn and 2–3 Cu [7]. Foods consumed by third world country populations are poor in important elements, such as Fe, and an increased consumption of *A. paradoxum* could be useful in meeting the daily requirements. *A. paradoxum* is native to northern of Iran and it has the potential to be of considerable benefit to human nutrition. A knowledge of the chemical forms of important elements in plants of economic interest is crucial because actions can be taken to reduce or minimize the toxic effects of the environment pollutant heavy metals [31].

3.2 Total phenol and flavonoid contents

Total phenol compounds, as determined by the Folin Ciocalteu method, are reported as gallic acid equivalents by reference to a standard curve ($y=0.0054x+0.0628$, $r^2=0.987$). The total phenolic content of aerial parts and bulbs was 62.7±3.5 and 7.4±0.2 mg gallic acid equivalent/g of extract, respectively. The total flavonoid

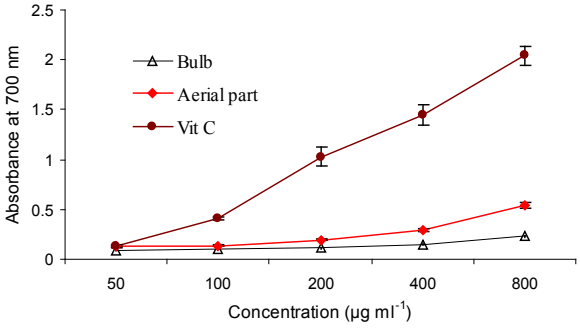

Figure 1. Reducing power of *A. paradoxum*.

contents of aerial parts and bulbs was 47.9±2.6 and 23.61±1.1 mg quercetin equivalent/g of extract powder, respectively, determined by reference to a standard curve (y=0.0063x, r^2=0.999). Aerial part extracts had significantly higher total phenol and flavonoid content than did bulb extract. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [32,33]. Increasing the levels of flavonoids in the daily diet may decrease the impact or occurrence of certain human diseases [34].

3.3 DPPH radical-scavenging activity

Scavenging of the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [21]. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [27]. IC_{50} for DPPH radical-scavenging activity was 890.9±43.2 and 984.9±33.5 µg/ml for aerial parts and bulbs, respectively. Despite the significantly higher total phenol and flavonoid content in aerial parts, both extracts showed about the same activity. The IC_{50} values for Ascorbic acid, quercetin and BHA were 1.26±0.11, 1.32±0.07 and 13.49±1.04 µg/ml, respectively.

3.4 Reducing power of extracts

In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donation of an electron. The amount of Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1 shows the dose– response curves for the reducing power of *A. paradoxum* extracts. The reducing power of extracts also increased with an increase in their concentration. There were significant differences between extracts and vitamin C (P<0.001). The aerial part extracts showed better reducing power than bulb extracts (P<0.05).

3.5 Assay of nitric oxide-scavenging activity

The extracts also showed very weak nitric oxide-scavenging activity between 0.1 and 1.6 mg/ml. The % inhibition increased with increasing concentration of the extract. Aerial part extracts showed better (33.3% scavenging activity in 800 µg/ml) scavenging activity than bulb extracts (17% scavenging activity in 800 µg/ml). Quercetin was a better scavenger with IC_{50}=200±13 µg/ml. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions [35,36]. The plant products may have the ability to counter the effect of NO formation and in turn may be of considerable interest in preventing the harmful effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by the detrimental excess generation of NO.

3.6 Fe^{2+} chelating activity of *A. paradoxum* extracts

The transition metal iron is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease [36,37]. Because Fe^{2+} also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe^{2+} concentration in Fenton reactions affords protection against oxidative damage. Iron chelators mobilize tissue iron by forming soluble stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications in humans and thereby improves quality of life and overall survival in some diseases such as Thalassemia major, cancer, HIV or Wilson's disease [38,39]. Chelation of ferrous ions by the extract was estimated according to our recently published papers [23,24]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. In this assay, both extract and EDTA interfered with the formation of ferrous and ferrozine complexes, suggesting that it has chelating activity and captures ferrous ions before ferrozine. The absorbance of the Fe^{2+}-ferrozine complex decreased dose-dependently, *i.e.* the activity was increased on increasing concentration from 0.1 to 1.6 mg/ml. Metal chelating capacity was significant since the extract reduced the concentration of the catalyzing transition metal in lipid per oxidation.

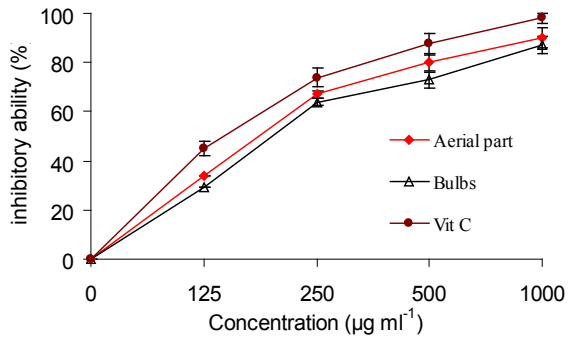

Figure 2. Antioxidant activities of *A. paradoxum* extract against linoleic acid peroxidation induced by hemoglobin. Each value is expressed as mean ±3 standard deviations positive control (Vitamin C).

Chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [40]. *A. paradoxum* extract showed weak Fe^{2+} chelating ability. IC_{50} was 959± 47 and 530±24 µg/ml for bulbs and aerial parts, respectively. EDTA showed very strong activity (IC_{50}=18 µg/ml).

3.7 Antioxidant activity in a hemoglobin-induced linoleic acid system

The antioxidant activity of *A. paradoxum* was determined by the method of Kuo *et al.*, [25] using the hemoglobin-induced linoleic acid system. This method gives results with only 1 h for oxidation time. Generally, antioxidant assays with linoleic acid need auto-oxidation for 5-6 days. The extracts showed good inhibitory ability on lipid oxidation, 67 and 64% for aerial parts and bulbs respectively, at 250 µg/ml and higher inhibitory ability, 90 and 87% respectively, at 1 mg/ml (Figure 2). There were no significant differences (P>0.05) among extracts in the hemoglobin-induced linoleic acid system that were comparable with vitamin C (P>0.05). The extracts show a rapid and concentration-dependent increase of antioxidant activity. The high reducing power indicates some compounds in the extract were electron donors and could react with free radicals to convert them into more stable products and to terminate radical chain reactions.

3.8 Scavenging H_2O_2

Scavenging of H_2O_2 by *A. paradoxum* extracts may be attributed to their phenolic compounds which can donate electrons to H_2O_2, thus neutralizing it to water [26,41]. The differences in H_2O_2 scavenging capacities between the extracts may be attributed to the structural features of their active components which determine their electron donating abilities. Extract was capable of scavenging hydrogen peroxide in a concentration dependent manner. IC_{50} for H_2O_2 scavenging activities were 412.2±18.5 and 529.8±24.9 µg/ml for aerial parts and bulbs respectively. The IC_{50} values for ascorbic acid and BHA were 21.4 and 52.0 µg/ml, respectively. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems [41,42].

3.9 CuOOH-induced hemolysis

The antioxidant activity of the extracts was confirmed using rat erythrocytes (RBC) exposed to CuOOH, by measuring the erythrocyte membrane resistance to free radical-induced hemolysis. When control RBC were incubated with extracts (50 µg/ml), no significant hemolysis was observed within 3 hours. This excludes any membrane-perturbing effect of the extracts. In RBC exposed to CuOOH (Figure 3), hemolysis was deetected after 30 minutes incubation. Extracts delayed the onset of the CuOOH-induced hemolysis. By the 150th minute hemolysis was inhibited for aerial part and bulbs by 14.6 and 41.9% respectively, compared to the control group.

3.10 Antihemolytic activity

Hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidants, but few studies have been performed with erythrocytes in whole blood. In this study, we used a biological test based on free radical-induced erythrocyte lyses in rat blood. This assay is useful for screening studies on various molecules and their metabolites, especially those having an oxidizing or antioxidizing activity, and molecules having a long-term action [43]. Lipid oxidation of rat RBC membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. The

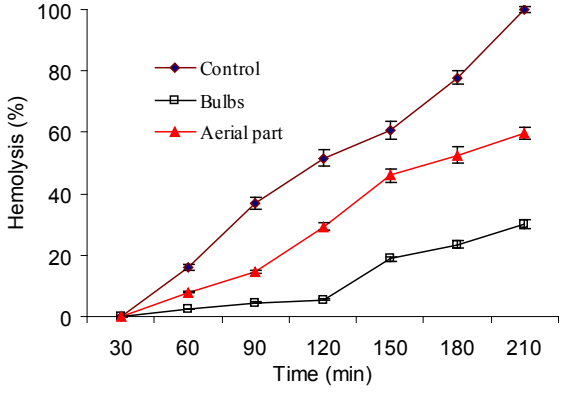

Figure 3. Protective effect of *A. paradoxum* extract on rat red blood cell hemolysis induced by CuOOH (50 µM). Values are the mean ±S.D. of 3 independent experiments.

extracts showed weak inhibiting activity, with bulbs showing slightly better activity than aerial parts (22 *vs*. 18% at 1 g/ml).

4. Conclusion

The aerial and bulb extracts of *A. paradoxum* exhibited good but different levels of antioxidant activity in all the models studied, both cell-free and in cell systems. They both contained high iron and manganese content. Further investigations of individual compounds as cost effective food/feed additives for human health, their *in vivo* antioxidant activities or other effects, are needed.

Acknowledgements

This research was supported by a grant from the research council of Medical Sciences University of Mazandaran, Iran.

References

[1] Cadenas E., Davies K.J.A., Mitochondrial free radical generation, oxidative stress, and aging, Free Radic. Biol. Med., 2000, 29, 222-230
[2] Marnett L., Oxyradicals and DNA damage, Carcinogenesis, 2000, 21, 361-370
[3] Burits M., Bucar F., Antioxidant activity of Nigella sativa essential oil, Phytother. Res., 2000, 14, 323-328
[4] Kalt W., Forney C., Martin A., Prior R.L., Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits, J. Agric. Food Chem., 1999, 47, 4638-4644
[5] Kumari M., Gupta S., Lakshmi A., Prakash J., Iron bioavailability in green leafy vegetables cooked in different utensils, Food Chem., 2004, 86, 217-222
[6] Leterme P., Buldgen A., Estrada F., Londono A.M., Mineral content of tropical fruits and unconventional foods of the Andes and the rain forest of Colombia, Food Chem., 2006, 95, 644-652
[7] Milton K., Micronutrient intakes of wild primates: are humans different?, Comp. Biochem. Phys. A, 2003, 136, 47-59
[8] Mozaffarian V., A dictionary of Iranian plant names, Tehran, Farhang Moaser, 2006
[9] Krest I., Glodek J., Keusgen M., Cysteine sulfoxides and alliinase activity of some allium species, J. Agric. Food Chem., 2000, 48, 3753-3760
[10] Han C., Li J., Hui Q., Determination of trace elements in Jinqi, a traditional chinese medicine, Biol. Trace Elem. Res., 2008, 122, 122-126
[11] Ebrahimzadeh M.A., Pourmorad F., Hafezi S., Antioxidant activities of Iranian Corn Silk, Turk. J. Biol., 2008, 32, 43-49
[12] Singleton V.L., Orthofer R., Lamuela-Raventos R.M., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent, Meth. Enzymol., 1999, 299, 152-178
[13] Ghasemi K., Ghasemi Y., Ebrahimzadeh M.A., Antioxidant activity, phenol and flavonoid contents of 13 Citrus species peels and tissues, Pak. J. Pharm. Sci., 2009, 22, 277-281
[14] Chang C., Yang M., Wen H., Chern J., Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food Drug Anal., 2002, 10, 178-182
[15] Nabavi S.M., Ebrahimzadeh M.A., Nabavi S.F., Fazelian M., Eslami B., In vitro antioxidant and free radical scavenging activity of Diospyros lotus and Pyrus boissieriana growing in Iran, Pharmacognosy Magazine, 2009, 4, 123-127
[16] Yamaguchi T., Takamura H., Matoba T., Terao Y., HPLC method for evaluation of the free radical-scavenging activity of food by using 1,1-diphenil,-2-picrylhydrazil, Biosci. Biotechnol. Biochem., 1998, 62, 1201-1204
[17] Nabavi S.M., Ebrahimzadeh M.A., Nabavi S.F., Hamidinia A., Bekhradnia A.R., Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica Mey, Pharmacologyonline, 2008, 2, 560-567
[18] Yen G.C., Chen H.Y., Antioxidant activity of various tea extracts in relation to their antimutagenicity, J. Agr. Food Chem., 1995, 43, 27-32
[19] Nabavi S.M., Ebrahimzadeh M.A., Nabavi S.F., Jafari M., Free radical scavenging activity and antioxidant capacity of Eryngium caucasicum Trautv and Froriepia subpinnata, Pharmacologyonline, 2008, 3, 19-25
[20] Sreejayan N., Rao M.N.A., Nitric oxide scavenging by curcuminoids, J. Pharm. Pharmacol., 1997, 49, 105-107

[21] Ebrahimzadeh M.A., Nabavi S.F., Nabavi S.M., Essential oil composition and antioxidant activity of Pterocarya fraxinifolia, Pak. J. Biol. Sci., 2009, 12, 957-963
[22] Dinis T.C.P., Madeira V.M.C., Almeida L.M., Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Arch. Biochem. Biophys., 1994, 315, 161-169
[23] Ebrahimzadeh M.A., Pourmorad F., Bekhradnia A.R., Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran, Afr. J. Biotech., 2008, 7, 3188-3192
[24] Ebrahimzadeh M.A., Nabavi S.M., Nabavi S.F., Correlation between the in vitro iron chelating activity and poly phenol and flavonoid contents of some medicinal plants, Pak. J. Biol. Sci., 2009, 12, 934-938
[25] Kuo J.M., Yeh D.B., Pan B.S., Rapid photometric assay evaluating antioxidative activity in edible plant material, J. Agric. Food Chem., 1999, 47, 3206-3209
[26] Song-Hwan B., Hyung-Joo S., Antioxidant activities of five different mulberry cultivars in Korea, Lebenson. Wiss. Technol. Food Sci. Technol., 2007, 40, 955-962
[27] Dehpour A.A., Ebrahimzadeh M.A., Nabavi S.F., Nabavi S.M., Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition, Grasas Aceites, 2009, 60, 405-412
[28] Aldini G., Piccoli A., Beretta G., Morazzoni P., Riva A., Marinello C., et al., Antioxidant activity of polyphenols from solid olive residues of c.v. Coratina, Fitoterapia, 2006, 77, 121-128
[29] Naim M., Gestener B., Bondi A., Birk Y., Antioxidant and antihemolytic activities of soyabean isoflavones, J. Agric. Food Chem., 1976, 24, 1174-1177
[30] Yuan X., Wang J., Yao H., Chen F., Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber, Lebenson. Wiss. Technol. Food Sci. Technol., 2005, 38, 877-883
[31] Narendhirakannan R.T., Subramanian S., Kandaswamy M., Mineral content of some medicinal plants used in the treatment of diabetes mellitus, Biol. Trace Elem. Res., 2005, 103, 109-115
[32] Van Acker S.A.B.E., van Den Berg D.J., Tromp M.N.J.L., Griffioen D.H., Van Bennekom W.P., van der Vijgh W.J.F., et al., Structural aspects of antioxidant activity of flavanoids, Free Radic. Biol. Med., 1996, 20, 331-342
[33] Ebrahimzadeh M.A., Bahramian F., Antioxidant activity of Crataegus pentagina subsp. elbursis fruits extracts used in traditional medicine in Iran, Pak. J. Biol. Sci., 2009, 12, 413-419
[34] Nabavi S.M., Ebrahimzadeh M.A., Nabavi S.F., Bahramian F., In Vitro antioxidant activity of Phytolacca Americana Berries, Pharmacologyonline, 2009, 1, 81-88
[35] Moncada A., Palmer R.M.J., Higgs E.A., Nitric oxide: physiology, pathophysiology and pharmacology, Pharmacol. Rev., 1991, 43, 109-142
[36] Ebrahimzadeh M.A., Nabavi S.M., Nabavi S.F., Eslami B., Antioxidant activity of aqueous extract of Pyrus boissieriana fruit, Pharmacologyonline, 2009, 1, 1318-1323
[37] Halliwell B., Gutteridge J.M.C., Role of free radicals and catalytic metal ions in human disease: an overview, Meth. Enzymol., 1990, 186, 81-85
[38] Hebbel RP, Leung A, Mohandas N. 1990. Oxidation-induced changes in microheological properties of the red cell membrane, Blood 76, 1015-1022.
[39] Grazul M., Budzisz E., Biological activity of metal ions complexes of chromones, coumarins and flavones, Coord. Chem. Rev., 2009, 253, 2588-2598
[40] Ebrahimzadeh M.A., Nabavi S.F., Nabavi S.M., Eslami B., Antihypoxic and antioxidant activity of Hibiscus esculentus seeds, Grasas Aceites, 2010, 61, 30-36
[41] Ebrahimzadeh M.A., Nabavi S.F., Nabavi S.M., Antioxidant activities of methanol extract of sambucus ebulus L. flower, Pak. J. Biol. Sci., 2009, 12, 447-450
[42] Ebrahimzadeh M.A., Ehsanifar S., Eslami B., Sambucus ebulus elburensis fruits: A good source for antioxidants, Pharmacognosy Magazine, 2009, 4, 213-218
[43] Djeridane A., Yousfi M., Nadjemi B., Vidal N., Lesgards J.F., Stocker P., Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity, Eur. Food Res. Tech., 2006, 224, 801-809