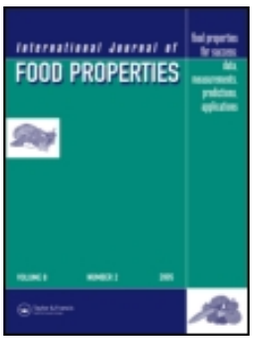

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IN VITRO ANTIOXIDANT AND ANTIHEMOLYTIC ACTIVITIES OF HYDROALCOHOLIC EXTRACTS OF *ALLIUM SCABRISCAPUM* BOISS. & KY. AERIAL PARTS AND BULBS

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This study is designed to evaluate the antioxidant and antihaemolytic activities of the hydroalcoholic extracts of* Allium scabriscapum *aerial parts and bulbs by employing eight* in vitro *assay systems. In 1,1-diphenyl-2-picryl hydrazyl radical scavenging assay, both the extracts show moderate scavenging activity. The reducing power ability of extracts increased with increasing in the samples concentrations. IC_{50} for metal chelating activity of aerial parts and bulbs extracts were 894.6 ± 31.29 and 746.2 ± 26.11 μg mL^{-1}, respectively. The aerial part extracts show better nitric oxide and hydrogen peroxide scavenging activities than the bulb. Extracts exhibited good antioxidant activity in linoleic acid emulsion system and were comparable to vitamin C ($p > 0.05$). Aerial parts extract showed better antihemolytic activity against cumene hydroperoxide and hydrogen peroxide-induced hemolysis. Among the extracts* A. scabriscapum*, aerial parts had higher phenolic and flavonoid contents.

Keywords: ***Antioxidant activity,* Allium scabriscapum*, Cumene hydroperoxyde.***

INTRODUCTION

The pathology of numerous chronic diseases, including heart diseases, inflammatory injuries, and neurodegenerative diseases, involves oxidative injury to cellular components. Reactive oxygen species (ROS), capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart failure, and many other health problems related to advancing age.[1–3] Minimizing oxidative injury can well be one of the most important approaches to the primary protection of the body from these aging-associated diseases and health problems, since antioxidants terminate direct ROS attacks and radical-mediated

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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 natural products, including cereal grains, vegetables, fruits, and plant extracts.[4,5] 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 biological effects. The genus *Allium* is a member of the *Alliaceae* family and contains 600 to 700 species, but only a few species have been domesticated so far as vegetables, spices, or ornamental plants. *Allium scabriscapum* Boiss. & Ky., which is locally called "piaz-e-golzard," was found to be a medicinal plant in northern Iran, especially in Mazandaran. The antibacterial activity of *Allium scabriscapum* was previously reported.[6] To the best of the authors' knowledge, there is negligible scientific information reported about antioxidant and antihemolytic activities of this species. In this study, the antioxidant and antihemolytic activities of aerial parts and bulbs extracts of *Allium scabriscapum* are examined employing various *in vitro* assay systems in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

EXPERIMENTAL PROCEDURES

Chemicals

Disodium salt of 3-(2-pyridil)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), linoleic acid, trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl (DPPH$^{\bullet}$), potassium ferricyanide, cumene hydroperoxide (CuOOH), and hydrogen peroxide were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Sodium nitropruside, gallic acid, quercetin, butylated hydroxyanisole (BHA), ascorbic acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, ethylenediaminetetraacetic acid (EDTA), and ferric chloride were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade or purer.

Plant Material and Preparation of Freeze-Dried Extracts

Allium scabriscapum aerial part and bulbs were collected in spring 2010 from the Veresk area, northern Iran, and identified by Dr. Bahman Eslami. A voucher (No. A4422) has been deposited in the Sari, School of Pharmacy herbarium. Materials were transported to the laboratory and kept at $<4^{\circ}C$ within 24 h prior to sample preparation.

Preparation of Extract

The materials were oven dried at 38°C, for 5 days. Dried materials were coarsely ground (2–3 mm) before extraction. Materials were extracted by percolation method using ethanol/distilled water (70/30) for 24 h at room temperature. Extracts were filtered and concentrated under reduced pressure at 40°C using a rotary evaporator.

Determination of Total Phenolic and Flavonoid Contents

Total phenolic contents of extracts were determined by the Folin-Ciocalteau method.[7] The extracts sample (0.5 mL) was mixed with 2.5 mL of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 mL of 75 g l^{-1} sodium carbonate were then added. The

absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature with a double beam spectrophotometer (UV–Visible EZ201, Perkin Elmer, LAMBDA, Well2582, Waltham, MA, USA). The result was expressed as gallic acid equivalents. Total flavonoid contents of extracts were estimated as previously described.[8] Briefly, 0.5 mL solution of extracts in methanol were 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. Total flavonoid content was calculated as quercetin from a calibration curve.

DPPH Radical-Scavenging Activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH$^{\bullet}$) was used for determination of free radical-scavenging activity of the samples.[9] Different concentrations of each sample were added, at an equal volume, to ethanolic solution of DPPH$^{\bullet}$ (100 μM). After 15 min at room temperature, the absorbances were recorded at 517 nm. The experiments were repeated three times. Vitamin C, BHA, and quercetin were used as standard controls. IC_{50} values denote the concentrations of each sample, which is required to scavenge 50% of DPPH$^{\bullet}$ free radicals.

Reducing Power Determination

Fe(III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action.[8] The reducing power of extracts was determined according to the authors' recently published paper.[8] Different amounts of each extract (25–800 μg mL^{-1}) 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 at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixtures to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as the positive control.

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.[9] The chelating of ferrous ions by extracts was estimated by the authors' recently published paper.[9] Briefly, extracts (0.2–3.2 mg mL^{-1}) were 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); the mixture was then shaken vigorously and left standing at room temperature for 10 min. Absorbances of the solutions were then measured spectrophotometrically 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 control, and A_s was the absorbance of the extract/standard. Na_2EDTA was used as the positive control.

Assay of Nitric Oxide Scavenging Activity

For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of each sample dissolved in water and incubated at room temperature for 150 min. The same reaction mixtures, without extracts, but with an equivalent amount of water, served as the control. 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 the positive control.[9]

Antioxidant Activity in a Hemoglobin-Induced Linoleic Acid System

The antioxidant activity of extracts was determined by a modified photometry assay.[10] Reaction mixtures (200 μl) containing 50 μl of each extract (125–1000 mg), 1 mmol L^{-1} of linoleic acid emulsion, 40 mmol L^{-1} 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^{-1} of $FeCl_2$ and 50 mL of ammonium thiocyanate (0.3 g mL^{-1}). Vitamin C was used as the positive control.

Scavenging of Hydrogen Peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to the authors' recently publisher paper.[11] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Samples (0.1–1 mg mL^{-1}) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbances of samples at 230 nm were determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard were calculated as follows: % 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 samples of extracts and standard.

Antihemolytic Activity

Preparation of rat erythrocytes. All the animal experiments were carried out with the approval of the institutional animal ethical committee. Male rats in the body weight range of 180–220 g were housed in individual polypropylene cages and had free access to food and water. The animals were fed a standard diet. The animals were sacrificed under anesthesia and blood was collected by heart puncture in heparinized tubes. Erythrocytes were isolated and stored according to the method described by Yuan et al.[10] Briefly, blood samples collected were centrifuged (1500× g, 10 min) at 4°C and erythrocytes were separated from the plasma and buffy coat and were washed three times by centrifugation (3000 rpm, 5 min) in 10 volumes of 10 mM phosphate buffered saline (pH 7.4). The supernatant and buffy coats of white cells were carefully removed with each wash. Washed erythrocytes were stored at 4°C and used within 6 h for further studies.

CuOOH-induced hemolysis. Red blood cells (RBC) were isolated from male wistar rats and suspended in balanced phosphate buffered saline to obtain a 1% RBC suspension.[11] Aliquots (3.5 mL) were incubated at 37°C for 210 min in the presence of 50

μM CuOOH (dissolved in ethanol) and the cellular integrity determined turbidimetrically at 710 nm at 30 min intervals.[11] The extracts (dissolved in EtOH; final concentrations 50 μg mL^{-1}) were preincubated for 30 min with RBC before the addition of CuOOH [blanks were RBC added with ethanol, at a final concentration always less than 0.1% (v/v)]. Percentages of hemolysis were determined setting as a 100% hemolysis the absorbance value determined in RBC suspensions sonicated for 5 s at 50% power (mean values of four determinations were used for the calculation).

Antihemolytic activity. Briefly, erythrocytes from male rat blood were separated by centrifugation and washed with phosphate buffer (pH 7.4). Erythrocytes were then diluted with phosphate buffered saline to give 4% suspension. One gram of sample mL^{-1} of saline buffer was added to 2 ml of erythrocyte suspension and the volume was made up to 5 ml with saline buffer. The mixture was incubated for 5 min at room temperature and then 0.5 mL of the H_2O_2 solution in saline buffer was added to induce the oxidative degradation of the membrane lipids. The concentration of H_2O_2 in the reaction mixture was adjusted to bring about 90% hemolysis of blood cells after 240 min. After incubation, the reaction mixture was centrifuged at 1500 rpm for 10 min and the extend of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.[12]

Statistical Analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an 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.

RESULTS

Total phenol compounds reported as gallic acid equivalents were derived from a standard curve ($y = 0.0063x$, $r^2 = 0.987$). Total phenolic contents of aerial parts and bulbs extracts of *Allium scabriscapum* were 24.40 ± 0.71 and 6.64 ± 0.20 mg gallic acid equivalent/g of extract powder. The total flavonoid contents of aerial parts and bulbs extracts of *Allium scabriscapum* were 13.04 ± 0.35 and 10.52 ± 0.25 mg quercetin equivalent/g of extract powder, respectively, as derived from a standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$). IC_{50} for DPPH radical scavenging activity was 611.1 ± 21.99 μg mL^{-1} for aerial parts and was 1738.5 ± 52.14 μg mL^{-1} for bulbs extract. The IC_{50} values for vitamin C, quercetin, and BHA were 5.05 ± 0.10, 5.28 ± 0.21, and 53.96 ± 3.10 μg mL^{-1}, respectively. Figure 1 shows the dose-response curves for the reducing power of extracts. It was found that the reducing power of samples also increased with the increase of its concentration. Extracts showed good activity but it is not comparable with vitamin C ($p < 0.01$). Bulb extracts showed better metal chelating activity than aerial parts extracts. IC_{50} was 746.2 ± 26.11 μg mL^{-1} for aerial parts and was 894.6 ± 31.29 μg mL^{-1} for aerial part extract. EDTA showed better activity ($IC_{50} = 18$ μg mL^{-1}). The *Allium scabriscapum* aerial parts and bulbs extracts also showed good nitric oxide scavenging activity ($IC_{50} = 666 \pm 23.31$ μg mL^{-1} for aerial parts and was 1020.4 ± 38.77 μg mL^{-1} for bulbs). Although quercetin showed very potent nitric oxide radical scavenging ($IC_{50} = 20 \pm 0.01$ μg mL^{-1}), its carcinogenic activity has been reported.[13] Scavenging of hydrogen peroxide by *Allium scabriscapum* extracts may be attributed to their polyphenolic contents, and/or other active components, which can donate electrons

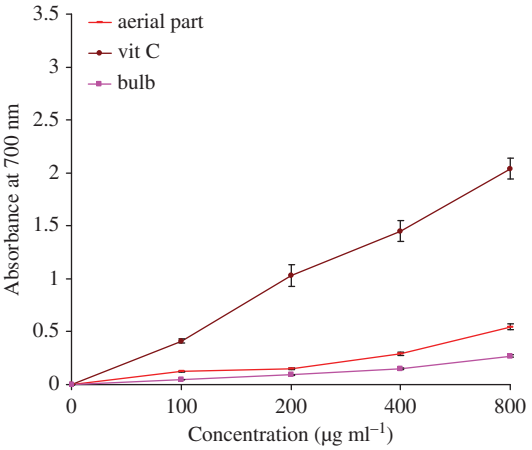

Figure 1 Reducing power of *Allium scabriscapum* extracts. (Color figure available online.)

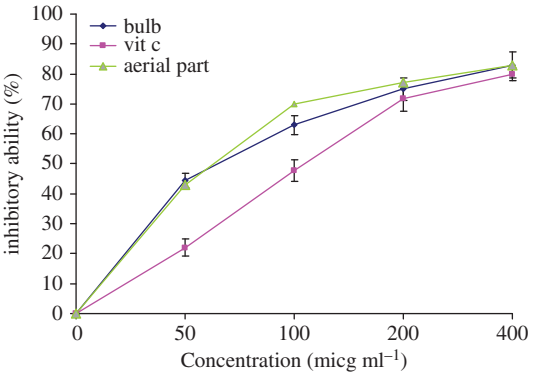

Figure 2 Antioxidant activities of *Allium scabriscapum* against linoleic acid peroxidation induced by hemoglobin. Each value is expressed as mean 3 standard deviation positive control (vitamin C). (Color figure available online.)

to hydrogen peroxide, thus neutralizing it to water.[14] The extracts showed good hydrogen peroxide scavenging in a concentration dependent manner. Both of the extracts showed good hydrogen peroxide scavenging activity (IC_{50} was 724.63 ± 21.01 μg mL^{-1} for aerial parts and was 862.06 ± 28.44 μg mL^{-1} for bulbs). The IC_{50} values for vitamin C and BHA were 21.4 ± 1.12 and 52 ± 2.61 μg mL^{-1}, respectively. Tested extracts show good activity in the hemoglobin-induced linoleic acid system. There were no significant differences between extracts ($p > 0.05$). Vitamin C showed a higher inhibition (Fig. 2). Effects of extracts were tested and found that they did not show any harmful effects on erythrocytes. Both of the extracts showed moderate antihemolytic activity (IC_{50} was 548.7 ± 17.01 μg mL^{-1} for aerial parts and was 733.4 ± 19.06 μg mL^{-1} for bulbs and 235 ± 9.10 μg mL^{-1} for vitamin C). In RBC exposed to cumene hydroperoxyde (Fig. 3), hemolysis started after 30 min incubation, extract dose-dependently delays the onset of the cumene hydroperoxyde induced hemolysis at 120 min, and hemolysis was inhibited by 22 ± 1% already at 2 μg mL^{-1} of aerial parts, by 19.9 ± 1.07% at 0.2 μg mL^{-1} of bulbs, respectively.

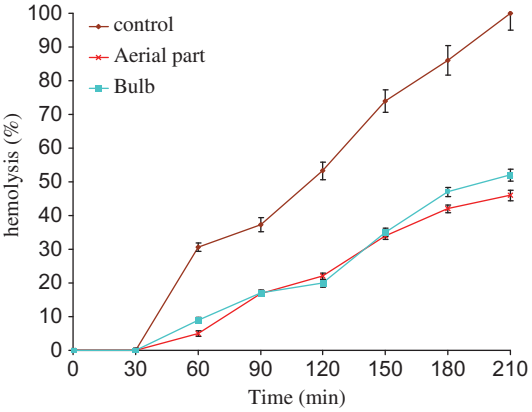

Figure 3 Protective effect of *Allium scabriscapum* on red blood cell hemolysis induced by CuOOH (50 μM). Both the extracts at 0.2 μg ml^{-1}. Values are the mean ± S.D. of three independent experiments. (Color figure available online.)

DISCUSSION

Polyphenolic compounds were widely found in naturally originated natural products, and they have been shown to possess significant biological activities.[15] $DPPH^{\bullet}$ is a stable nitrogen-centered free radical. Any substances that can donate hydrogen or electron, can change its color of which changes from violet to yellow, so can be considered as antioxidants and, therefore, radical scavengers.[9] Phytochemical contents of this plant seem to have direct roles for its moderate DPPH scavenging activity.[11]

Another assay that is mechanism related to electron donating ability of this sample is reducing power assay. In this assay, phenols or other electron donor compounds in the sample can reduce Iron (III) to Iron (II). An amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm.[8] Increasing absorbance at 700 nm indicates an increase in reductive ability.

Figure 1 shows the dose-response curves for the reducing power of extracts. It was found that the reducing power of samples also increased with the increase of their concentrations. The extract showed good activity but it is not comparable with vitamin C ($p < 0.01$). 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.[16,17] For example, brain iron dysregulation and its association with amyloid precursor protein plaque formation are implicated in Alzheimer's disease pathophysiology and so iron chelation could be considered a rational therapeutic strategy for Alzheimer's disease.[7]

Malaria is one of the most important health problems that affect about 40% of the world's population in 100 countries. An urgent need for the development of new antimalarial agents faces the scientific community. Iron chelators have been shown to have antimalarial activity through the mechanism of withholding iron from vital metabolic pathways of the intra-erythrocytic parasite. Evidence is now available that iron chelation therapy with desferrioxamine has clinical activity in malaria in humans.[18] On the other hand, many studies have demonstrated that the chelator, such as deferoxamine, has antiproliferative effects against both leukemia and neuroblastoma. Its anticancer mechanism is

severely limited due to its poor ability to permeate cell membranes and chelate intracellular iron pools.[19] Deferoxamine is an iron chelator that is being evaluated as a treatment for iron overload. Use of deferoxamine in excessive dosages may result in some adverse effects.[20] Deferiprone is an orally absorbed bidentate iron chelator that can induce urinary iron excretion, promote negative iron balance, and reduce hepatic iron levels in some transfusion-dependent patients. A number of adverse effects may occur in 0.6 to 4% of patients, including arthritis, nausea, and agranulocytosis, and require cessation of therapy in up to 30% of patients. There remains an urgent need to identify an orally active chelator regimen that is as effective as deferoxamine and has an acceptable degree of tolerability.[20] Thus, many researches focused on some natural product, especially flavonoids, that possess direct influence on Iron (III) ions level within tissues; 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 the extracts and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The nitric oxide assay 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. The % inhibition increased by increasing concentration of the extracts. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cáncer, and other pathological conditions.[21] A number of disease states, including sepsis and hepatic failure, are characterized by abnormally high nitric oxide production and removing the excess nitric oxide could have salutary effects.[22] The plant/plant products may have the property to counteract the effect of nitric oxide formation and, in turn, may be of considerable interest in preventing the ill effects of excessive nitric oxide generation in the human body. Furthermore, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of nitric oxide that are detrimental to human health. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing hydrogen peroxide is very important throughout food systems.

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Linoleic acid and arachidonic acid are especially targets of lipid peroxidation.[23] Erythrocytes are considered as prime targets for free radical attack owing to the presence of both high membrane concentration of polyunsaturated fatty acids and the oxygen (O_2) transport associated with redox active hemoglobin molecules, which are potent promoters of reactive oxygen species. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation.[23] The inhibition of lipid peroxidation by antioxidants may be due to their free radical scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical.[24–27] Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. Antihemolytic activity of quercetin and other flavonoids have previously reported and good activity of extracts may be the result of high flavonoid content especially quercetin.[28] The antioxidant activity of the samples were confirmed in rat erythrocytes (RBC) exposed to cumene hydroperoxyde, by measuring the erythrocyte membrane resistance to free radical-induced hemolysis. When control RBC were incubated with a sample (50 $\mu g\ mL^{-1}$), no significant hemolysis was observed within 3 h, thus to exclude any membrane-perturbing effect of the compounds.

CONCLUSION

The present study shows that phytochemical constituents, biological effects of aerial parts and bulbs extracts for the first time, might be considered to be a therapeutic effect in clinical situations. Results demonstrate that both extracts have good DPPH, nitric oxide radical, hydrogen peroxide scavenging, reducing power and metal chelating ability, as well as antihemolytic activity due to the presence of polyphenolic compounds. Thus, it can be concluded that the antioxidant properties of *A. scabriscapum* maybe could explain some of the traditional uses attributed to this plant since the excessive production of free radicals are involved in several pathologies. These results can be useful as a starting point of view for further applications of this plant or its constituents in pharmaceutical preparations after performing clinical researches.

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REFERENCES

1. Cadenas, E.; Davies, K.J.A. Mitochondrial free radical generation, oxidative stress, and aging. Free Radical Biology and Medicine **2000**, *29*, 222–230.
2. Marnett, L. Oxyradicals and DNA damage. Carcinogenesis **2000**, *21*, 361–370.
3. Tepe, B.; Sokmen, A. Screening of the antioxidative properties and total phenolic contents of three endemic Tanacetum subspecies from Turkish flora. BioresourceTechnology **2007**, *98*, 3076–3079.
4. Burits, M.; Bucar, F. Antioxidant activity of Nigella sativa essential oil. Phytotherapy Research **2000**, *14*, 323–328.
5. Kalt, W.; Forney, C.; Martin, A.; Prior, R.L. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. Journal of Agricultural and Food Chemistry **1999**, *47*, 4638–4644.
6. Chehregani, A.; Azimishad, F.; and Haj Alizade, H. Study on antibacterial effect of some *Allium* species from Hamedan-Iran. International Journal of Agriculture and Biology **2007**, *9* (6), 873–876.
7. Nabavi, S.F.; Ebrahimzadeh, M.A.; Nabavi, S.M.; Eslami, B. Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss. Grasas Y Aceites **2010**, *61* (3), 244–250.
8. 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 Y Aceites **2009**, *60*, 405–412.
9. Mahmoudi, M.; Ebrahimzadeh, M.A.; Nabavi, S.F.; Hafezi, S.; Nabavi, S.M.; Eslami Sh. Antiinflammatory and antioxidant activities of gum mastic. European Review for Medical and Pharmacological Sciences **2010**, *14* (9), 765–769.
10. 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. LWT—Food Science and Technology **2005**, *38*, 877–883.
11. Ebrahimzadeh, M.A.; Nabavi, S.F.; Nabavi, S.M.; Eslami, B. Antihemolytic and antioxidant activities of *Allium paradoxum*. Central European Journal Biology **2010**, *5* (3), 338–345.
12. Ajila, C.M.; Prasada Rao, U.J.S. Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* L. peel extract. Food and Chemical Toxicology **2008**, *46*, 303–309.

13. Dunnik, J.K.; Hailey, J.R. Toxicity and carcinogenicity studies of quercetin, a natural component of foods. Fundamental and Applied Toxicology **1992**, *19* (3), 423–431.
14. Halliwell, B.; Gutteridge, J.M.C. Role of free radicals and catalytic metal ions in human disease: An overview. Methods in Enzymology **1990**, *186*, 1–85.
15. 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 Radical Biology and Medicine **1996**, *20* (3), 331–342.
16. Hebbel, R.P.; Leung, A.; Mohandas, N. Oxidation-induced changes in microheological properties of the red cell membrane. Blood **1990**, *76*, 1015–1022.
17. Grazul, M.; Budzisz, E. Biological activity of metal ions complexes of chromones, coumarins and flavones. Coordination Chemistry Reviews **2009**, *253*, 2588–2598.
18. Mabeza, G.F.; Loyevsky, M.; Gordeuk, V.R.; Weiss, G. Iron chelation therapy for malaria: A review. Pharmacology & Therapeutics **1999**, *81* (1), 53–75.
19. Richardson, D.R. Iron chelators as therapeutic agents for the treatment of cancer. Critical Reviews in Oncology and Hematology **2002**, *42*, 267–281.
20. Porter, J.B. A risk-benefit assessment of iron-chelation therapy. Drug Safety **1997**, *17* (6), 407–421.
21. Moncada, A.; Palmer, R.M.J.; Higgs, E.A. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacological Reviews **1991**, *43*, 109–142.
22. Shah, V.; Lyford, G.; Gores, G.; Farrugia, G. Nitric oxide in gastrointestinal health and disease. Gastroenterology **2004**, *126*, 903–913.
23. Yu, L.L. Free radical scavenging properties of conjugated linoleic acids. Journal of Agricultural and Food Chemistry **2001**, *49* (7), 3452–3456.
24. Ebrahimzadeh, M.A.; Nabavi, S.F.; Nabavi, S.M.; Eslami, B. Antihypoxic and antioxidant activity of *Hibiscus esculentus* seeds. Grasas Y Aceites **2010**, *61* (1), 30–36.
25. Ebrahimzadeh, M.A.; Nabavi, S.M.; Nabavi, S.F.; Eslami, Sh. Antioxidant and free radical scavenging activities of culinary-medicinal mushrooms, Golden Chanterelle *Cantharellus cibarius* and Angel's Wings *Pleurotus porrigens*. International Journal of Medicinal Mushroom **2010**, *12* (3), 265–272.
26. Gursoy, N.; Tepe, B.; Sokmen, M. Evaluation of the chemical composition and antioxidant activity of the peel oil of *Citrus nobilis*. International Journal of Food Properties **2010**, *13* (5), 983–991.
27. Tohma, H.S.; Gulcin, I. Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice (*Glycyrrhiza Glabra* L.). International Journal of Food Properties **2010**, *13* (4), 657–671.
28. Chaudhuri, S.; Banerjee, A.; Basu, K.; Sengupta, B.; Sengupta, P.K. Interaction of flavonoids with red blood cell membrane lipids and proteins: Antioxidant and antihemolytic effects. International Journal of Biological Macromolecules **2007**, *41*, 42–48.