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Diversity in antioxidant properties and mineral contents of *Allium paradoxum* in the Hyrcanian forests, Northern Iran

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Abstract. *Khodadadi S, Nejadsattari T, Naqinezhad A, Ebrahimzadeh MA. 2015. Diversity in antioxidant properties and mineral contents of* Allium paradoxum *in the Hyrcanian forests, Northern Iran. Biodiversitas 16: 281-287.* The knowledge about variation of antioxidant properties from local medicinal plant can be achieved by investigating all natural habitats of it. To reach this goal, a comprehensive survey on eight populations of *Allium paradoxum* (M. B.) G. Don, was conducted in the Hyrcanian forests. *A. paradoxum* (Amaryllidaceae) is a perennial, local plant, native to the northern Iran. Different parts of it, is largely used in food preparation and traditional medicine. Plants were collected randomly from different altitudes and forest sites of Iranian northern provinces ranging from west to east (Guilan, Mazandaran and Golestan Provinces). Samples were divided into aerial and bulbous parts. The antioxidant activities of the extracts were investigated with 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and reducing power assays. Total flavonoid content was determined by a colorimetric aluminum chloride method. The highest antioxidant activities and flavonoid contents in *A. paradoxum* were related to Varaki and Zarinabad sites (Mazandaran province) with relatively higher humidity, in the central part of the Hyrcanian area and in altitudinal range between 462-860 m asl. The content of total phenolics in the extracts was determined according to the Folin-Ciocalteu reagent method, and calculated as gallic acid equivalents (GAE). With respect to total phenolic contents, aerial parts of plants of Jahannama, the lowest elevation site (Golestan province), had the highest amount. Elemental composition (Fe^{++}, Mn^{++}) and total sulphur were also determined using Atomic Absorption Spectroscopy and digestion method, respectively. Higher contents of two elements, particularly Fe, and total sulphur can be found in bulbous part of Zarinabad and Kiasar populations (Mazandaran province) compared to other sites. The results of the current study indicated that there is a remarkable and significant variation of antioxidant activities among different studied populations of *A. paradoxum* in the Hyrcanian forests.

Key words: *Allium paradoxum*, antioxidant activity, medicinal plant, mineral contents

INTRODUCTION

The natural antioxidants present in dietary plants have great importance in favoring health and resistance to oxidative stress in humans (Dimitrios 2006). The total antioxidant potential (TAP) in food products is related to the different molecules present in foods. Antioxidant capacities are considerably modulated by numerous factors including biological and environmental factors, as well as their interaction (Lisiewska et al. 2006; Moore et al. 2006). Plant materials contain various forms of antioxidants. Phenolic compounds are found in plants, have multiple biological effects. Flavonoids and other phenolics have been suggested to play a protective role against damages caused by disease (Kähkönen et al. 1999; Mammadov et al. 2011).

Nutrients play a significant role in improving productivity and quality of plants. Sulphur supply influences bulb yield, plant dry matter, bulb pungency and flavor intensity in *Allium* crops (Durenkamp and De Kok 2004). Sulphur insufficiency will result in the loss of plant health, the plant's resistance to environmental stress, and in decreased food quality and safety (De Kok et al. 2002; Durenkamp and De Kok 2004). Micronutrients are involved in numerous biochemical processes and adequate intake of certain micronutrients related to the prevention of deficiency diseases (Ebrahimzadeh et al. 2011).

The genus *Allium*, a member of Amaryllidaceae family, contains more than 900 species (APG III 2009; Li et al. 2010; Tojibaev et al. 2014). The most important diversity source of the genus is considered in the mountainous areas of southwest and central Asia including the territory of Iran (Fritsch and Friesen 2002). *Allium* comprises many economical, ornamental and medicinal species that have been used as a remedy for treatment of certain diseases (Fritsch and Friesen 2002; Putnoky et al. 2013). Among the different medicinal plants, some endemic species may be used for phytochemicals with significant antioxidant capacities and health benefits (Exarchou et al. 2002).

Allium paradoxum (M.B.) G. Don is a perennial species of shady woods in the Caucasus, northern Iran and neighboring parts of central Asia (Vvedenskii 1935; Wendelbo 1971; Stearn 1992). This plant is one of locally vegetables that was known as "Alezi", and is used as a healthy vegetable in raw or cooked form for people who are living in northern provinces of Iran. The populations of the plant are obvious during late February to early April in the Hyrcanian forests. The Hyrcanian forest is a temperate deciduous forest ecosystem in the southeastern part of the Caucasus biodiversity hotspot, that are located in three northern provinces of Iran, namely, Guilan, Mazandaran and Golestan with a total surface area of 1.84 million hectares (Bobek 1951; Takhtajan 1986; Naqinezhad et al. 2015).

So far, most studies about *Allium* relating to cysteine sulphoxides and alliinase activity (Krest et al. 2000; Fritsch and Keusgen 2006), antioxidant activity of several *Allium* members (Yin and Cheng 1998) or have focused only on whole species such as *A. paradoxum* (Ebrahimzadeh et al. 2010; Nabavi et al. 2012; Elmi et al. 2014), *A. sativum* L. and *A. cepa* L. (Benkeblia 2005; Lawrence and Lawrence 2011), *A. ursinum* L. (Putnoky et al. 2013). Ghasemi et al. (2015) investigated properties of garlic under selenium and humic acid treatments. The results of their study show the positive effects of applied treatments. Ebrahimzadeh et al. (2010) reported trace elemental analysis in *A. paradoxum* and among them, higher contents related to Fe and Mn. There is no report about total sulphur content in *A. paradoxum*.

The purpose of this study is to provide the first report about variation in phytochemical properties of *A. paradoxum* and potential sources of natural habitats in Northern provinces of Iran. Moreover, we aim to estimate total sulphur and trace element (Fe, Mn) contents in the studied populations.

MATERIALS AND METHODS

Chemicals

Barium chloride, 1,1-diphenyl-2-picryl hydrazyl (DPPH), Magnesium nitrate, Perchloric acid and potassium ferricyanide were purchased from Sigma Chemicals Co. (USA). Gallic acid, Quercetin and Ferric chloride were purchased from Merck (Germany).

Study areas, plant material and preparation of extract

We selected eight forest sites (Zarinabad, Varaki, Kalaleh, Jahannama, Haftkhal, Asalem, Savadkooh and Kiasar) from northern provinces of Iran namely Guilan, Mazandaran and Golestan. These sites were located in the Hyrcanian area along an altitudinal gradient from 198 to 1980 m asl. (Figure 1, Table 1). Three Meteorological stations, i.e. Astara in Guilan province, Gharakhil in Mazandaran province and Hashemabad in Golestan province were the closest stations to the current studied sites. According to bioclimatic classification of Iran (Djamali et al. 2011), the climate of area varies from temperate oceanic in Astara (Guilan) to Mediterranean pluviseasonal oceanic in Hashemabad site (Golestan).

Samples were randomly collected from aforementioned sites during March to April 2014. The materials (aerial and bulbous parts) were dried at room temperature for two

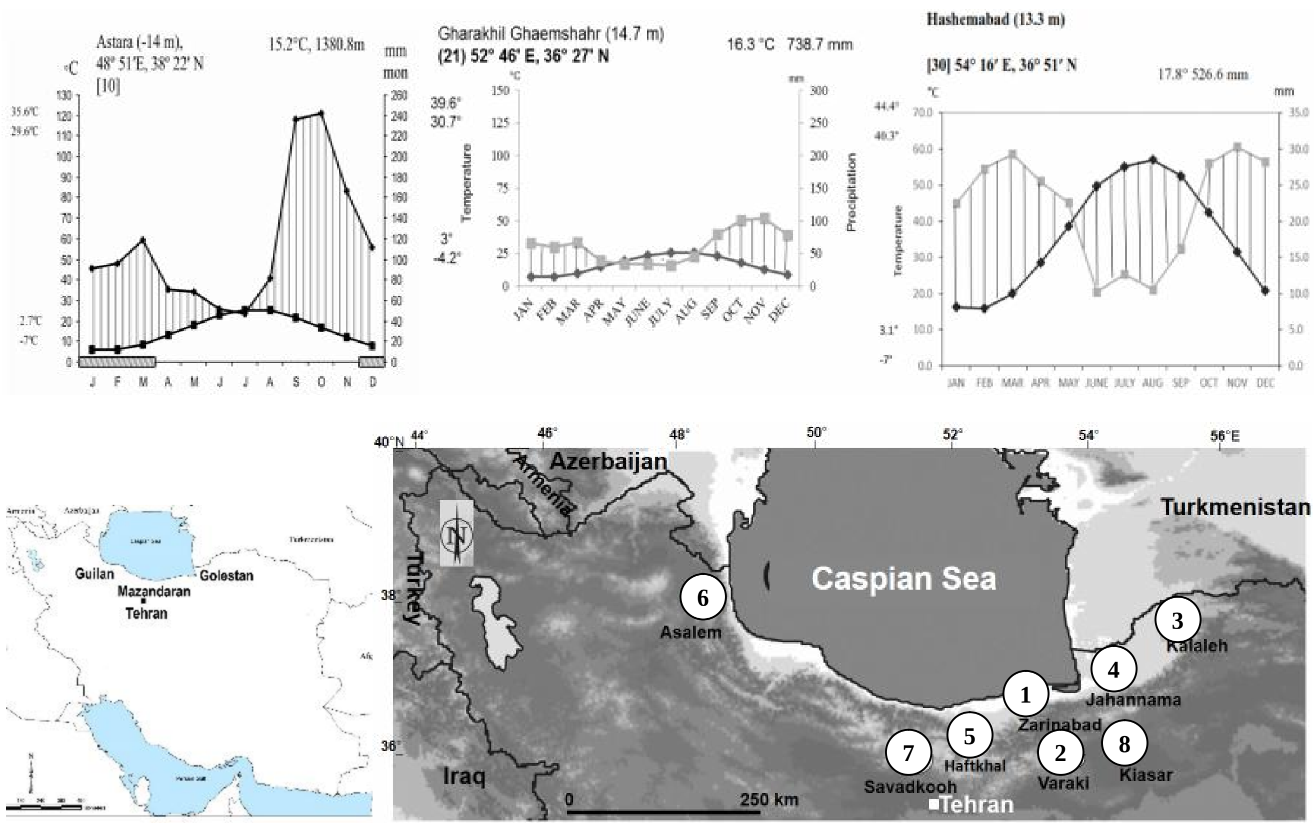

Figure 1. Studied sites of *Allium paradoxum* populations in the Hyrcanian forest. Climatic diagrams are shown from the nearest stations in each of three provinces (from left: Guilan, Mazandaran and Golestan, respectively).

Table 1. The characteristics of eight sites for *Allium paradoxum* sampling.

Forest site	Province	Longitude	Latitude	Altitude (m a.s.l)
Zarinabad	Mazandaran	E 053°12′ 19.2″	N 36°29′ 44.4″	462
Varaki	Mazandaran	E 53°07′ 29.6″	N 36°17′ 13.9″	860
Kalaleh	Golestan	E 055°36′ 52.1″	N 37°24′ 35.2″	698
Jahannama	Golestan	E 54°07′ 23.5″	N 36°44′ 37.8″	198
Haftkhal	Mazandaran	E 53°23′ 59.2″	N 36°17′ 22.1″	897
Asalem	Guilan	E 048°49′ 07″	N 37°39′ 32.8″	1200
Savadkooh	Mazandaran	E 52°48′ 29.9″	N 36°06′ 27.8″	1980
Kiasar	Mazandaran	E 053°36′ 39.5″	N 36°06′ 27.8″	1825

weeks (bulbs were 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 extracts were then separated from the sample residues by filtration through Whatman No.1 filter paper. The extractions were repeated three times. The resultant extracts were concentrated in a rotary evaporator until crude solid extracts were obtained which were then freeze-dried for complete solvent removal (Ebrahimzadeh et al. 2010). The yields of each sample were presented in Tables 2 and 3.

DPPH radical-scavenging activity

The stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Yamaguchi et al. 1998; Nabavi et al. 2009). Different concentrations of each extract (100, 200, 400, 800 and 1600 µg mL^{-1}) 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. Methanol with DPPH was used as control. The experiment was repeated three times. Vitamin C and butylated hydroxyanisole (BHA) were used as standard controls. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation: Scavenging effect (%) = $[(A_0 - A_s)/A_0] \times 100$, where A_0 is the absorbance without extract and A_s is the absorbance of extracts or standards.

Reducing power determination

The reducing power of extracts was determined according to the method of Yen and Chen (1995) (Nabavi et al. 2008). Different amounts of each extract (50-800 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 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. Vitamin C was used as positive control.

Total phenol and flavonoid contents

Total phenolic contents were determined by the Folin-Ciocalteu reagent method (Ebrahimzadeh et al. 2008; Singleton et al. 1999). The extract samples (0.5 mL) were mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent for 5 min and 2.0 mL of 75 g l^{-1} sodium carbonate then added. The absorbance was measured at 760 nm after 2 h of incubation at room temperature. The standard curve was prepared by 0, 50, 100, 150, 200 and 250 mg mL^{-1} solutions of gallic acid in methanol: water (50: 50, v/v). Results were expressed as mg gallic acid equivalents (GAE) g^{-1} of extract. Total flavonoid content was determined by a colorimetric method (Chang et al. 2002; Ghasemi et al. 2009). 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 (Perkins Elmer AAS 100).The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg mL^{-1} in methanol. The total flavonoids were expressed as mg quercetin equivalent (QE) g^{-1} of extract powder.

Determination of total sulphur content and mineral analysis

Total sulphur of aerial parts and bulbs were measured in the digest as described by Quin and Wood (1976). Samples (0.1 g) were analyzed for sulphur after magnesium nitrate and perchloric digestion. Barium chlorate was added to the mixture and it was left overnight, following which the absorbance of the final reaction mixture was measured at 420 nm (Ghasemi et al. 2015). Results are expressed as µg in g dry matter. Iron and manganese were analyzed in the samples. Briefly, the properly dried and ground plant samples were ash-dried overnight at 400-420°C in a Vitreosil crucible. The inorganic residue was kept in a desiccator until needed for analysis. Two-tenths of a gram of ash was dissolved in a 1: 3 mixture of hydrochloric and nitric acids (Han et al. 2008) diluted to 50 mL with distilled water and analyzed with an atomic absorption spectrometer (Perkins Elmer AAS 100) (Wellesley, MA). The results were expressed in mg g^{-1} of sample.

Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data was analyzed by one-way ANOVA and the means separated by Tukey's post-hoc test ($P < 0.05$).

RESULTS AND DISCUSSION

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 (Ebrahimzadeh et al. 2009). Results of our study show that scavenging effect of samples is increased by increasing their concentrations. Among these samples, the aerial parts generally had the strongest radical scavenging activity than bulbous parts (Tables 2 and 3). The lowest IC_{50} (the highest activity) for DPPH radical-scavenging activity was 0.96 µg mL^{-1} for Varaki aerial parts (Mazandaran). The IC_{50} values for Vitamin C and BHA were 3.7 ± 0.1 and 29.3 ± 5.9 µg mL^{-1}, respectively.

Reducing power of extracts

The reducing power capacity of compounds may serve as a significant indicator of its potential antioxidant activity (Meir et al. 1995). This reducing power capacity was determined using a Fe^{3+} to Fe^{2+} reduction system. 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. Figures 2 and 3 show the dose-response curves for the reducing power of extracts (at 50-800 µg mL^{-1} concentration). The reducing power of extracts also increased with increasing their concentrations. There were significant differences between extracts and vitamin C ($P < 0.01$). The highest reducing power activity was observed in aerial parts of Varaki site (1.32, at 800 µg mL^{-1}). The aerial part extracts showed higher reducing power than bulb extracts.

Extraction yield, total phenol and flavonoid contents

The extraction yield of different fractions of *A. paradoxum* was varied from 4.7 to 26.1% (Tables 2 and 3). The extraction of aerial parts resulted in the higher amount of total extractable compounds than bulb extracts. The amount of total phenolics varied in different populations and ranged from 5.7±0.5 to 112.4±7.5 mg GAE g^{-1} of extract. The highest total phenolic contents were 112.4±7.5 and 111.2±5.4 mg GAE g^{-1} of extract, bulbs and aerial parts of "Jahannama" and "Varaki" samples, respectively. The content of flavonoid varied from 1.3 to 294.33±11.3 mg QE g^{-1} of extract powder (Tables 2 and 3). Aerial part extracts had significantly higher flavonoid contents than bulb extracts. Plants of "Zarinabad" site (Mazandaran province) showed the highest amount of flavonoid contents followed by Varaki (294.33±11.3 and 173±4.4, respectively).

Total sulphur and mineral analysis

The results of the current study showed that bulbous parts had higher total sulphur than aerial parts. According to Tables 2 and 3, total sulphur content in *A. paradoxum* ranged from 69-210 µg in g dry matter. The highest sulphur content was found in bulbs of Kiasar (Mazandaran province). Results of elemental analysis were presented in Tables 2 and 3. The comparative study showed that the bulbous part of Zarinabad sample had the maximum value of Fe and Mn contents.

Discussion

Investigation on antioxidant properties with particular concern of geographical distribution of plant populations has become of interest of current relevant studies (Pisoschi and Negulescu 2011). This kind of research can be also very important particularly on endemic and other restricted-range plants.

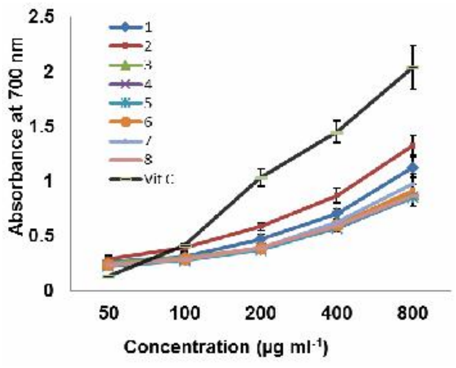

Figure 2. Reducing power of aerial parts extracts of *Allium paradoxum* populations. Vitamin C used as control. Numbers show studied sites. For names of 1-8 see Figure 1.

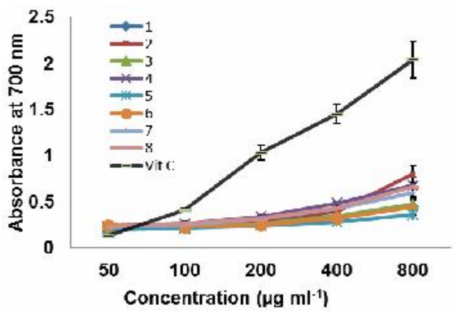

Figure 3. Reducing power of bulbous parts extracts of *Allium paradoxum* populations. Vitamin C used as control. Numbers show studied sites. For names of 1-8 see Figure 1.

Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities. Because of this, the antioxidant activities of *A. paradoxum* populations were determined by two spectrophotometric methods. The DPPH method rely on the reaction of 1, 1-diphenyl-2-picrylhydrazyl radical with an antioxidant molecule. It decolorizes the DPPH solution and the degree of color change is proportional to amount of the antioxidants (Saeed et al. 2012). In reducing power test, the presence of the reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+}can be monitored by absorbance measurement at 700 nm. In the present study, the results of two antioxidant tests showed that the aerial part extract of Varaki population in Mazandaran province had higher antioxidant action than other populations. Based on altitudinal range of current survey (198- 1980 m a.s.l) (Table 1), this site is located in the middle of the cited range.

Although phenolic acids and flavonoids are not essential for survival, may provide protection against a number of chronic diseases over the long term consumption (Bravo 1998). Variation of total phenol and flavonoids in our study area showed that the plant extracts of all populations had the potential for accumulating phenol and flavonoids. Bulbous part of Jahannama population (eastern part of our study area with low rainfall) possessed the

Table 2. Yield, total phenolic and flavonoid contents of methanolic extract, total sulphur and trace elements from aerial part of different populations of *Allium paradoxum* in Iran.

Parameters	Zarinabad	Varaki	Kalaleh	Jahannama	Haftkhal	Asalem	Savadkooh	Kiasar	F
Total phenol content (mg GAE g^{-1})	64.4 ^{a}± 3.2	111.2 ^{b}± 5.4	54.4 ^{c}± 3.2	62.6 ^{af}± 2.6	47.4 ^{d}± 3.6	56.4 ^{c}± 2.8	5.7 ^{e}± 0.5	60.6 ^{f}± 2.2	2325.84*
Flavonoid content (mg QE g^{-1})	294.3 ^{a}± 1.3	173 ^{b}± 4.4	80.3 ^{c} ± 3.5	51.0 ^{d}± 1.3	31.7 ^{e}± 2.2	77.8 ^{c}± 3.4	61.3 ^{f}± 1.1	103.7 ^{g}± 5.1	29300.50*
DPPH radical cavenging, IC_{50} (µg mL^{-1})	1.50 ^{af}± 0.01	0.96 ^{b}± 0.00	1.43 ^{acf}± 0.01	1.56 ^{adf}±0.01	1.38 ^{c}± 0.01	1.46 ^{acf}± 0.01	1.66 ^{df}± 0.01	1.57 ^{adf}± 0.00	101.83*
Total sulphur (µg in g dry matter)	81 ^{a}± 3.2	99 ^{b}± 3.1	110 ^{c}± 5.2	120 ^{d}± 3.7	110 ^{c}± 5.1	69^{e} ± 1.9	88 ^{f} ± 2.3	120 ^{d}± 5.8	1056.80*
Fe (mg g^{-1})	0.93 ^{a}± 0.03	0.82 ^{a}± 0.03	3.35 ^{bc}± 0.11	3.19 ^{bc}± 0.17	0.54 ^{a}± 0.01	1.41 ^{ab}± 0.10	2.40 ^{b}± 0.16	0.77 ^{a}± 0.02	25.04*
Mn (mg g^{-1})	0.05 ^{a}± 0.01	0.05 ^{a}± 0.01	0.07 ^{ab}± 0.01	0.08 ^{b}± 0.01	0.03 ^{ac}± 0.00	0.05 ^{a}± 0.01	0.05 ^{a}± 0.00	0.04 ^{ac}± 0.00	7.50*
Extraction yield (%)	18	21.2	19.8	18.2	21.2	20.1	26.1	23.2	

Note: IC_{50} of BHA was 29.3 ± 5.9 µg mL^{-1}. Each value in Table 2 is represented as mean SD (n=3) and F-ratio (*) based on one-way ANOVA of studied variables. Values in the same row followed by a different letter are significantly different ($p<0.05$).

Table 3. Yield, total phenolic and flavonoid contents of methanolic extract, total sulphur and trace elements from bulbous part of different populations of *Allium paradoxum* in Iran.

Parameters	Zarinabad	Varaki	Kalaleh	Jahannama	Haftkhal	Asalem	Savadkooh	Kiasar	F
Total phenol content (mg GAE g^{-1})	47.2 ^{a}± 2.1	46.8 ^{a}± 1.7	49.2 ^{a}± 2.2	112.4 ^{b}± 7.5	30.2 ^{c}± 1.5	36.4 ^{d}± 1.9	59 ^{e}± 1.6	80.8 ^{f}± 4.1	2088.90*
Flavonoid content (mg QE g^{-1})	4.3 ^{a}± 0.2	1.3 ^{ac}± 0.0	1.7 ^{ac}± 0.0	7.7 ^{b}± 1.2	4.0 ^{a}± 0.1	5.3 ^{ab}± 1.2	3.3 ^{a}± 0.0	6.0 ^{ab}± 0.2	11.93*
DPPH radical cavenging, IC_{50} (µg mL^{-1})	2.32 ^{acd}± 0.01	3.19 ^{abcdf}± 0.02	2.34 ^{acd}± 0.02	1.78 ^{acde}± 0.01	4.21 ^{bcf}± 0.02	2.89 ^{abcd}± 0.02	3.51 ^{b}± 0.02	1.72 ^{acde}± 0.01	14.67*
Total sulphur (µg in g dry matter)	120 ^{a}± 4.4	81 ^{b}± 1.2	120 ^{a}± 4.9	150 ^{c}± 6.6	180 ^{d}± 6.4	110 ^{e}± 5.7	112 ^{e}± 4.1	210 ^{f}± 6.4	5311.66*
Fe (mg g^{-1})	17.30 ^{a}± 0.24	2.50 ^{b}± 0.09	2.25 ^{bcd}± 0.13	2.80 ^{bd}± 0.18	0.92 ^{c}± 0.04	1.05 ^{bc}± 0.15	1.95 ^{bcde}± 0.18	1.43 ^{bcde}± 0.1	28.21*
Mn (mg g^{-1})	0.1 ^{a} ± 0.01	0.05 ^{a} ± 0.00	0.07 ^{a}± 0.00	0.06 ^{a}± 0.01	0.02 ^{a}± 0.00	0.02 ^{a}± 0.00	0.01 ^{a}± 0.00	0.05 ^{a}± 0.01	2.20
Extraction yield (%)	9.8	8.1	6.4	4.7	6.1	11.6	11.0	12.3	

Note: IC_{50} of BHA was 29.3 ± 5.9 µg mL^{-1}. Each value in Table 3 is represented as mean SD (n=3) and F-ratio (*) based on one-way ANOVA of studied variables. Values in the same row followed by a different letter are significantly different ($p<0.05$).

highest amount of total phenol. In most measurements of the current experimental analysis, aerial parts had higher contents than bulbs that are compatible with the results of Ebrahimzadeh et al. (2010).

Minerals are usually divided in two groups macro- and micro- minerals (or trace elements), and also classified as either essential or nonessential, depending on whether or not they are required for human nutrition and have metabolic roles in the body (Reilly 2002). Sulphur content of plant varies from 0.03-2 mmol g^{-1} dry weight (DW) in different species (Durenkamp and De Kok 2004). In the current study, total sulphur content of bulbs showed higher amounts than in aerial parts. Study of Ghasemi et al. (2015) showed that total sulphur of *A. sativum* is in higher amount than in *A. paradoxum*. Moreover, the results of our study revealed that iron concentration is higher than manganese. However, this result can be varied in aerial and bulbous parts. Fe and Mn play vital roles in biochemical processes (Ebrahimzadeh et al. 2010, 2011). Element composition differs strongly between plant organs (Minden and Kleyer 2013). Variability may also come from differences in size, age, ontogenetic state and reproductive status between plant individuals (Agren 2008).

The results of this study demonstrated variation of antioxidant properties and mineral contents in plant organs of *A. paradoxum* populations along the Hyrcanian forests. The results generally indicated that populations of *A. paradoxum* in Mazandaran province had good capacity to utilize in medicine and food industry. Further field studies are needed to explain precise correlation between variation of mentioned properties in *A. paradoxum* and the environmental factors.

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REFERENCES

Agren G. 2008. Stoichiometry and nutrition of plant growth in natural communities. Annu Rev Ecol Evol Syst 39: 153- 170.

APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot J Linn Soc 161: 105-121.

Benkeblia N. 2005. Free-radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. Braz Arch Biol Techn 48 (5): 753- 759.

Bobek H. 1951. Die naturliechen Walder und Geholzfluren Iran. Bonner Geogr Abh 8: 1-62.

Bravo L.1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56 (11): 317-333.

Chang C, Yang M, Wen H, Chern J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10: 178-182.

De Kok LJ, Westerman S, Stuiver CEE, Weidner W, Stulen I, Grill D. 2002. Interaction between atmospheric hydrogen sulphide deposition and pedospheric sulphate nutrition in *Brassica oleracea* L. Phyton 42: 35-44.

Dimitrios B. 2006. Sources of natural phenolic antioxidants. Trends Food Sci Tech 17: 505-512.

Djamali M, Akhani H, Khoshravesh R, Andrieu-Ponel V, Brewer S. 2011. Application of the global bioclimatic classification of Iran: implications for understanding the modern vegetation and biogeography. Ecologia Mediterranea 37: 91-114

Durenkamp M, De Kok LJ. 2004. Impact of pedospheric and atmospheric sulphur nutrition on sulphur metabolism of *Allium cepa* L., a species with a potential sink capacity for secondary sulphur compounds. J Exp Bot 55 (404): 1821-1830.

Ebrahimzadeh MA, Pourmorad, F, Hafezi S. 2008. Antioxidant activities of Iranian Corn Silk. Turk J Biol 32: 43-49

Ebrahimzadeh MA, Nabavi SF, Nabavi SM. 2009. Essential oil composition and antioxidant activity of *Pterocarya fraxinifolia*. Pak J Biol Sci 12: 957-963.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B. 2010. Antihemolytic and antioxidant activities of *Allium paradoxum*. Cent Eur J Biol 5: 338-345.

Ebrahimzadeh MA, Eslami SH, Nabavi SM, Nabavi SF, Hajizadeh Moghaddam A, Bekhradnia AR. 2011. Estimation of essential and toxic mineral elements in *Mentha* species. Asian J Chem 23 (4): 1648-1650.

Elmi T, Gholami SH, Azadbakht M, Rahimi-Esboei B, Geraili Z. 2014. The effects of hydroalcoholic extract of leaves and onion of *Allium paradoxum* on *Giardia lamblia* in mice. J Shahrekord Univ Med Sci 16 (5): 13-22 (In Persian).

Exarchou V, Nenadis N, Tsimidou M, Gerothanassis IP, Troganis A, Boskou D. 2002. Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage and summer savory. J Agric Food Chem 50 (19): 5294-5299.

Fritsch RM, Friesen N. 2002. Evolution, domestication and taxonomy. In: Rabinowitch HD, Currah L (eds). *Allium* crop science: recent advances. CABI Publishing, Wallingford, UK.

Fritsch RM, Keusgen M. 2006. Occurrence and taxonomic significance of cysteine sulphoxides in the genus *Allium* L. (Alliaceae). Phytochem (67): 1127-1135

Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. 2009. Antioxidant activity, phenol and flavonoid contents of 13 *Citrus* species peels and tissues. Pak J Pharm Sci 22: 277-281.

Ghasemi K, Bolandnazar S, Tabatabaei SJ, Pirdashti H, Arzanlou M, Ebrahimzadeh MA, Fathi H. 2015. Antioxidant properties of garlic as affected by selenium and humic acid treatments. NZ J Crop Hort Sci 43 (3): 173-181.

Han C, Li J, Hui Q. 2008. Determination of trace elements in Jinqi, a traditional Chinese medicine. Biol Trace Elem Res 122: 122-126.

Jafari SM, Zarre SH, Alavipanah SK, Ghahremaninejad F. 2015. Functional turnover from lowland to montane forests: evidence from the Hyrcanian forest in northern Iran. iForest 8: 359-367.

Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 47 (10): 3954-3962.

Krest I, Glodek J, Keusgen M. 2000. Cysteine sulfoxides and alliinase activity of some allium species. J Agric Food Chem 48 (8): 3753-3760.

Lawrence R, Lawrence R. 2011. Antioxidant activity of garlic essential oil (*Allium sativum*) grown in north Indian plains. Asian Pac J Trop Biomed 1 (1): S51-S54.

Li QQ, Zhou ZD, He XJ, Yu Y, Zhang YC, Wei XQ. 2010. Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast rps16 sequences, focusing on the inclusion of species endemic to China. Ann Bot 106: 709-733.

Lisiewska Z, Kmiecik W, Korus A. 2006. Content of Vitamin C, carotenoids, chlorophylls and polyphenols in green parts of dill (*Anethum graveolens* L.) depending on plant height. J Food Comp Anal 19: 134-140.

Mammadov R, Ili P, Ertem Vaizogullar H. 2011. Antioxidant Activity and Total Phenolic Content of *Gagea fibrosa* and *Romulea ramiflora*. Iran J Chem Chem Eng 30 (3): 57-62.

Meir S, Kanner J, Akiri B, Hadas SP, Hadas J. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agric Food Chem 43: 1813-1817.

Minden V, Kleyer M. 2014. Internal and external regulation of plant organ stoichiometry. Plant Biology 16 (5): 897- 907.

Moore J, Liu JG, Zhou K, Liangli Y. 2006. Effects of genotype and environment on the antioxidant properties of hard wheat bran. J Agric Food Chem 54: 5313-5322.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. 2008. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. Pharmacologyonline 2: 560-567.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. 2009. In vitro antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Phacog Mag 4: 123-127.

Nabavi SF, Nabavi SM, Hajizadeh Moghaddam A, Naqinezhad A, Bigdellou R, Mohammadzadeh S. 2012. Protective effects of *Allium paradoxum* against gentamicin-induced nephrotoxicity in mice. Food Funct 3: 28-29.

Naqinezhad AR, Zare-Maivan H, Gholizadeh H. 2015. A floristic survey of the Hyrcanian forests in Northern Iran, using two lowland-mountain transects. J For Res 6 (1): 187-199.

Pisoschi MA, Negulescu GP. 2011. Methods for total antioxidant activity determination: a review. Biochem Anal Biochem 1: 1: 106. doi: 10.4172/2161-1009.1000106

Putnoky S, Caunii A, Butnariu M. 2013. Study on the stability and antioxidant effect of the *Allium ursinum* watery extract. Chem Cent J 7: 21.

Quin BF, Wood PH. 1976. Rapid manual determination of sulfur and phosphorous in plant material. Comm Soil Sci Plant Anal 7: 415-426.

Reilly C. 2002. Minerals. In: Henry CJK, Chapman C (eds.). The nutrition Handbook for Food Processors. CRC Press, Boca Raton, CA.

Saeed N, Khan MR, Shabbir M. 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Compl Altern Med 12: 221.

Singleton VL, Orthofer R, Lamuela-Raventos RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth Enzymol 299: 152-178.

Stearn WT. 1992. How many species of *Allium* are known? Kew Mag 9: 180-182.

Takhtajan A. 1986. Floristic regions of the World. University of California Press, Berkeley, CA.

Tojibaev KSH, Turginov O, Karimov F. 2014. A new species and new records of *Allium* (Amaryllidaceae) for Uzbekistan (Central Asia). Phytoyaxa 177 (5): 291-297.

Vvedenskii AI. 1935. *Allium.* In: Komarov VL (ed). Flora U.S.S.R. 4: 254-255. Leningrad, USSR.

Wendelbo P. 1971. Alliaceae. In: Rechinger KH (ed). Flora Iranica 76: 66-67. Acad Druck-u Verlasanstalt.Graz, Austria.

Yamaguchi T, Takamura H, Matoba T, Terao Y. 1998. HPLC method for evaluation of the free radical scavenging activity of food by using 1, 1-diphenil,-2- picrylhydrazil. Biosci Biotechnol Biochem 62: 1201-1204.

Yen GC, Chen HY. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agr Food Chem 43: 27-32.

Yin MC, Cheng WS. 1998. Antioxidant activity of several *Allium* members. J Agric Food Chem 46 (10): 4097- 4101.