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Original Article

Evaluation of Persian Shallot (*Allium hirtifolium*) Ecotypes for Phytochemical Components and Antioxidant Activity

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Abstract

Phytochemical constituents, such as total phenol, allicin and pyrovic acid as well as antioxidant activity of thirteen ecotypes of *Allium hirtifolium* Boiss. from different regions of Iran were evaluated. Average contents of total phenol, allicin and pyrovic acid of bulbs extracts per g fresh weight were 0.647 mg gallic acid equivalent (GAE), 2.127 mg and 84.412 µmol, respectively and antioxidant activity was 63.717%. The ecotypes that exhibited greater contents of studied phytochemicals consequently possessed higher antioxidant activities. Among the analyzed antioxidant enzymes, superoxide dismutase activity was present in the greatest quantity (61.501 U mg^{-1} protein), followed by ascorbate peroxidase (54.182 U mg^{-1} protein), polyphenol oxidase (46.219 U mg^{-1} protein), peroxidase (1.972 U mg^{-1} protein) and catalase (0.49 U mg^{-1} protein). Correlation analysis showed the accumulation of *A. hirtifolium* phytochemicals was associated with mean annual temperatures and precipitation. Cluster analysis on phytomedical characters arranged the ecotypes in five groups. The ecotypes of group D presented by Isfahan ecotypes showed the highest pharmaceutical potential which could be considered in future breeding programs. The ecotype groups were not strictly concordant with their bioclimatic or geographic location, so it can be concluded that the genetic factors as well as environmental factors affected the antioxidant capacity of *A. hirtifolium* ecotypes.

Key words: *Allium hirtifolium*, Allicin, Pyrovic acid, Total phenol, Antioxidant activity

Introduction

Oxygen free radicals produced by living organisms are extremely reactive and unstable molecules. They are capable of inducing irreversible damages to biological structures [1]. Natural antioxidants occurring in the cell inhibited the harmful activity of these free radicals. Antioxidants may be divided into enzymatic and non enzymatic groups. Fortunately, organisms utilize both enzymatic and non enzymatic endogenous antioxidant defenses to minimize cell injury [2]. When free radical generation is enhanced, amplification of endogenous antioxidant may be necessary. Due to the side effects of synthetic antioxidants in human health there is a growing tendency to use natural antioxidant compounds derived from different plant species [3]. The diets rich in fruits and vegetables provide a great amount of antioxidant phytochemicals that could protect cells from damages caused by oxidants via neutralizing or trapping reactive oxygen species [4].

Plants belonging to *Allium* genus due to are rich in organosulfur and phenolic compounds, show high antioxidant activities [5]. Persian shallot (*Allium hirtifolium* L.) commonly known as mooseer in Iran, is a perennial diploid (2n=2X=16) plant, belongs to Alliaceae family. It is native to Iran and grows as a wild plant throughout in the Zagross Mountains range, western and southwestern Iran [6]. It is a bulbous herb and usually consists of a single main bulb or rarely two bulbs. Each bulb has a weight of about 8-15 times of a garlic clove [7,8].

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The bulbs of mooseer has been widely used as a traditional herb and spice plant that added to a variety of foods such as salads, pickles, yogurt and different sauces [2]. In Iran for many years, fresh and dry bulbs of *A. hirtifolium* are recommended as a folk medicine for treatment of rheumatic, inflammatory, arthritis, diarrhea and stomach pain [7]. Many authors have examined the antimicrobial [9], antifungal [10], antioxidant [11], antiparasitic activities [12] and immunomodulatory [3] effects of *A. hirtifolium* extracts. These results supposed mooseer bulbs as a potential source of natural antioxidants. Majority of biological activities of mooseer are attributed to the organosulphur and phenolic compounds [3]. Phenolic compounds possess strong antioxidant activities due to scavenging reactive oxygen species [13]. The flavor of *A. hirtifolium* such as garlic releases only after tissue disruption by the rapid degradation of S-alk(en)yl-L-cysteine sulphoxide (alliin) by the alliinase enzyme that produces allicin, pyruvate, ammonia and a range of sulphur compounds [14]. Allicin is identified as the chief biologically active constituent of sulphur containing compounds of mooseer [7]. Despite the high nutraceutical values of *A. hirtifolium*, unfortunately the natural habitat of this plant is under increasing pressure as a result of excessive harvest that caused to damage the plant density in its habitates in Iran.

The different phytochemical compositions of a species that are found in different origins could reflect the various environmental conditions of each particular location. Previous studies have shown that the amount of total phenol, organosulfur compounds, and antioxidant activities of different *Allium* species depend on biological factors, as well as edaphic, and environmental conditions [13,16]. The phenolic compounds of garlic cultivars had different responses to variability in environmental condition and *p*-hydroxybenzoic, and *p*-coumaric acids showed highly significant and negative correlations with both average temperature and total precipitation [13]. As well as Hirata *et al*. [16] showed the cysteine sulfoxide and phenolic contents of garlic bulbs influenced by temperature and humidity. Ghahremani-majd *et al*. [3] reported the occurrence of geographic variations of *A. hirtifolium* antioxidant capacity and according to results of Ghsemi Pirbalouti *et al*. [17] the extract of different *A. hirtifoluim* populations showed a variation of antioxidant activity. Since the same plant species growing in different geographic regions can exhibit different phytochemical compositions and occasionally some superior plants can be found in special sites. This study focused to assess antioxidant activity regards to some antioxidant compounds (total phenol, allicin and pyrovic acid) and antioxidant enzymes activity of *Allium hirtifolium* wild ecotypes were collected throughout the different parts of Iran. Variables such as temperature and annual rainfall factors examined for their effects on antioxidant capacity of different ecotypes of *A. hirtifolium.* This is the first report of allicin, pyrovic acid and antioxidant enzymes activity of *A. hirtifolium* species. The results of this study can aid to selection process of the ecotypes having high phytochemical contents and antioxidant capacity for use in domestication and breeding programs.

Material and Methods

Plant Materials

The bulbs of Persian shallot ecotypes for this study collected from thirteen regions of Iran with naturally grown plants (May-August 2014). Bulbs were harvested when the lower one-third to one-half of the leaves on the plants had dried. Climatic data of the locations were determined using data collected by the nearest meteorology stations (Table 1).

Extracts Preparation

After removing the skin, the bulb samples were cut into small pieces. Then, the samples were freeze dried, ground into fine powder and stored at -80°C for the analyses of contents of total phenol, allicin, antioxidant activity and some antioxidant enzymes. All determinations were carried out in triplicate. All chemicals and reagents were from Merck (Germany) with analytical grade.

DPPH Assay

Radical scavenging activity of plant extracts against stable 2, 2 diphenyl 2 picryl hydrazyl hydrate (DPPH) was determined by method of Brand-Williams *et al*. [18]. 0.5 g of mooseer powder was extracted with 5 ml methanol 85% at room temperature. 25 µl of the methanolic extract were rapidly mixed with 1975 µl of DPPH methanolic solution (40 mg L^{-1}, Sigma) in a cuvette placed in the spectrophotometer.

Table 1 *Allium hirtifolium* Boiss. ecotypes included in this study, their collection sites, climatic and geographical coordinates in Iran.

Ecotype No	Collecting place	Province	Altitude (m)	Latitude (N)	Longitude (E)	Av. annual temp (ºC)	Annual precipitation (mm)	Climate
1	Noor Abad	Lorestan	2200	33° 75′	48° 25′	12.71	458.16	Cold-Semi humid
2	Kohdasht	Lorestan	1195	33° 32′	47° 36′	16.73	369.97	Temperate-Semi arid
3	Khorramabad	Lorestan	1147	33° 29′	48° 21′	17.21	435.16	Cold-Semi humid
4	Khodabandeh	Zanjan	1756	36° 08′	48° 35′	11.10	407.18	Cold-Semi arid
5	Khoramdareh	Zanjan	1575	36° 11′	49° 12′	12.28	304.30	Cold-Semi arid
6	Isfahan 1	Isfahan	1570	32° 38′	51° 39′	17.20	154.46	Cold-Arid
7	Isfahan 2	Isfahan	1570	32° 38′	51° 39′	17.20	154.46	Cold-Arid
8	Sanjan	Markazi	1775	34° 38′	49° 42′	14.25	298.15	Cold-Arid
9	Hamadan	Hamadan	1765	34° 36′	48°20′	12.20	322.73	Cold-Semi arid
10	Saral	Kurdistan	1850	34° 32′	46° 01′	11.35	418.95	Cold-Semi arid
11	Divandareh	Kurdistan	1850	34° 32′	46° 01′	11.35	418.95	Cold-Semi arid
12	Yasuj	Kohgiluyeh and Boyer-Ahmad	1870	30° 40′	51° 36′	14.81	802.84	Cold-Humid
13	Sardasht	West Azerbaijan	1280	36° 09′	45°28′	13.60	840.19	Cold-Humid

After 50 min incubation in darkness at room temperature the absorbance at 515 nm was measured. The decline in radical concentration indicated the radical scavenging activity of the sample. DPPH radical scavenge rates were calculated as follows:

% Inhibition=$[(A_B-A_A)/A_B]\times100$, where A_B stands for absorbance at 517 nm before sample addition, and A_A stands for absorbance at 517 nm after sample incubation.

Total Phenol Assay

Total phenol content was measured with the Folin-Ciocalteu reagent [19]. The extract obtained for DPPH analysis was also used for the measurement of total phenol content. The extract was centrifuged at 12000 g for 10 min at 4°C. 200 µl of the supernatant was diluted with 300 µl of water, and 2.5 ml of freshly prepared 50% Folin-Ciocalteu reagent was then added. The solution was incubated in darkness at room temperature for 3 min then 2 ml of a 7.5% sodium carbonate solution was added. After 30 min incubation in darkness at room temperature, the absorbance was measured at 760 nm. Gallic acid standards of different concentrations were used for the calibration, and total phenol content was expressed as mg of gallic acid equivalent per gram of fresh weight.

Allicin Assay

A spectrophotometric method using 4-mercaptopyridine (4-MP) was used for quantification of allicin content according to Miron *et al*. [20]. 0.5 g of mooseer powder was extracted with 10 ml distillated water at room temperature. After 30 min incubation at room temperature, the extract was centrifuged at 6000 g for 30 min. 1 ml of the supernatant was added to 1 ml of 4-mercaptopyridine buffer (Na_3Po_4 50 mM, EDTA 2 mM and 4-MP 10^{-4} mM). Change in absorbance was noted for 60 seconds at intervals of 1 second in 324 nm. The amount of allicin in a sample determined from the initial rate of 4-MP reaction with allicin.

Pyruvic Acid Assay

The pyruvate levels of samples were assessed according to the colorimetric procedure proposed by Anthon and Barrett [21] with some modifications. A spectrophotometer based on the reaction of 2,4-dinitrophenylhydrazine (DNPH) with pyruvic acid was used for pyruvic acid assay. 1 ml of clarified mooseer juice was added to 1 ml distillated water and 1 ml of 0.125 g L^{-1} DNPH in 2 M HCl. The samples were placed in a 37 ◦C water bath, then removed after 10 min and 2.5 ml of 0.6 M NaOH was added. The pyruvate content was measured at 420 nm for both samples and standards. Standard solutions had been prepared by sodium pyruvate.

Antioxidant Enzymes Assay

1 g of sample powder was homogenized with a mortar and pestle in 10 ml of ice cold 50 mM potassium phosphate buffer (pH 6.8). The homogenates were centrifuged at 12000 g for 20

min at 4°C and the supernatant fraction used as the source of protein and enzymes. All the steps were carried out at 0-4 °C. The amount of soluble protein in the extracts was determined using the Bradford [22] protein assay, with bovine serum albumin (BSA) as the standard.

Catalase Assay: Catalase (CAT, EC 1.11.1.6) activity was estimated by the UV method of Aebi [23]. The reaction mixture contained 50 µL of enzyme extract, 500 µL of 10 mM H_2O_2 and 2450 µL of 50 mM potassium phosphate buffer. The decrease in absorbance was recorded at 240 nm for 60 seconds. Specific activity of enzyme expressed as mmoles H_2O_2 decomposed per mg protein per minute by using the H_2O_2 extinction coefficient 39.4 $mM^{-1}cm^{-1}$.

Peroxidase Assay: The activity of peroxidase (POX, EC 1.11.1.7) was determined by adding 200 µl of the enzyme extraction to 1000 µl of 100 mM potassium phosphate buffer, 500 µl of 20 mM guaiacol solution, and finally 300 µl of 10 mM H_2O_2 were added. Change in absorbance was noted for 90 seconds at intervals of 5 seconds in 470 nm [24]. By using the extinction coefficient of tetraguaiacol product (26.6 $mM^{-1}cm^{-1}$) the specificity of enzyme was expressed as mm produced tetraguaiacol per mg protein per minute.

Polyphenol Oxidase Assay: Polyphenol oxidase (PPO, EC 1.10.3.1) activity was determined by oxidation of catechol to benzoquinone. The reaction mixture included 1400 µl of 100 mM potassium phosphate buffer with 100 µl enzyme extraction and 500 µl of freshly prepared 100 mM 4-methyl catechol. Change in absorbance was noted for 120 seconds at intervals of 5 seconds in 410 nm and specific enzyme activity was calculated as mmol catechol oxidized per mg protein per min with 1.01 $mM^{-1}cm^{-1}$ as the extinction coefficient [25].

Ascorbate Peroxidase Assay: Ascorbate peroxidase activity (APX, EC 1.11.1.11) was measured by the method of Nakano and Asada [26]. 20 µl of enzyme extract was added to reaction mixture containing 680 µl of 100 mM potassium phosphate buffer, 100 µl of 10 mM ascorbic acid and 100 µl of 10 mM EDTA. The reaction started with addition of 100 µL of 100 mM H_2O_2. The decrease of absorbance at 290 nm was monitored in 60 seconds. APX specific activity expressed as mmol ascorbate oxidized per mg protein per min with 2.8 $mM^{-1}cm^{-1}$ as the extinction coefficient.

Superoxide Dismutase Assay: The activity of superoxide dismutase (SOD, EC 1.15.1.1) was estimated by the method of Giannopolitis and Ries [27]. The reaction mixture contained 300 µl of enzyme extract, 200 µl of 50 mM potassium phosphate buffer, 300 µl of 12 mM methionine, 300 µl of 75 µM nitro blue tetrazolium (NBT) and 1 mL of 50 mM Na_2CO_3. The end, 30 µl of 1 µM riboflavin was added. The reaction and control mixture were placed 30 cm from light source consisting of a 40 W fluorescent lamp for 15 min. The absorbance was recorded at 560 nm. A non-irradiated reaction mixture which did not develop a color, served as the blank. The SOD activity was measured by its ability to inhibit the photochemical reduction of NBT. One unit of SOD activity was expressed as the quantity of SOD required to produce a 50% inhibition of NBT photochemical reduction. The specific enzyme activity was said as units per mg protein.

Statistical Analysis

The experiment was arranged in a randomized complete block design. Data were analyzed by SPSS software and means were compared according to the Duncan's multiple ranges test at $P \leq 0.05$ (SPSS ver.16.0). Correlation studies were conducted by SPSS program. The content of phenolic compound and allicin samples were used to determine the relationship among the different ecotypes of *A. hirtifolium* by cluster analysis using the SPSS software (Ver. 16). Euclidean distance was selected as a measure of similarity, and the unweighed pair-group method with arithmetic average (UPGMA) was used for cluster dentition.

Results and Discussion

The thirteen ecotypes of studied *Allium hirtifolium* were collected from Northwest, West, Central and Southwest of Iran, in an altitude ranges from 1147 m (ecotype 3) to 2200 m (ecotype 1), as indicated in Table 1. The present study explores the variations of the total phenol, allicin and pyruvic acid contents as well as antioxidant activity of wild *A. hirtifolium* ecotypes of Iran.

Antioxidant Activity

Antioxidant activity of mooseer extract was measured by the DPPH radical scavenging method. The DPPH radical is one of the most commonly used subtract for the fast evaluation of antioxidant

activity because of its stability, reproducibility and simplicity [28]. The antioxidant capacity of different ecotypes was found to be significantly variable. Differences in antioxidant capacity reflect the variability of ecotypes. Variation of antioxidant activity previously was reported for different populations of *A. hirtifolium* [3,17], garlic [29] and onion [28]. Average antioxidant activity was 63.717% with the range of 74.133 to 55.7% (Fig. 1). Ecotype 6 followed by ecotypes 10 and 7 exhibited statistically higher antioxidant activities than the other studied ecotypes, which were probably due to the high values of antioxidant compounds and enzymes in these ecotypes [30]. According to the obtained data in *A. hirtifolium* plants, with decreasing precipitation and increasing temperature, antioxidant activity increased, as well as correlation results showed significant negative correlation between antioxidant activity and precipitation rate (Table 3). Previous studies have shown that environmental factors largely affected antioxidant activity in mooseer plants [3][17].Various factors, such as genotype, temperature and precipitation affected the contents of phytochemicals, so these parameters are responsible for the variation of antioxidant activity of different plant populations [29].

Total Phenol Content

Total phenolic compounds, expressed as gallic acid equivalents (GAE), are well known antioxidant constituents of plants and possess chemical and biological activities in vegetables and fruits [4,13]. In this study total phenol content ranged from 1.001 (ecotype 10) to 0.385 (ecotype 12) mg GAE g^{-1} FW with an average of 0.647 mg GAE g^{-1} FW. The highest phenolic content was obtained from Kordistan ecotypes followed by Noor Abad and Isfahan ecotypes (Table 2). Similar to the reports of Ghahremani-majd *et al*. [3] and Ghasemi Pirbalouti *et al*. [17], the variation of total phenol content among different ecotypes was statistically significant. Ghasemi Pirbalouti *et al*. [17] reported the total phenolic content of *A. hirtifolium* populations from Southwest of Iran was between 44.28–34.50 mg GAE per g of bulbs methanolic extract. Also Ghahremani-majd *et al*. [3] indicated that the total phenol content ranged from 8.4 to 0.5 mg GAE per gram sample of different *A. hirtifolium* populations. The differences among our results and literatures might be due to the differences in genotypes, environmental conditions and extraction methods [13]. The correlation among total phenol content, temperature and annual precipitation of different ecotypes was assayed and the results were negative and non significant (Table 3).

However; Hirata *et al*. [16] reported that high latitude areas tended to have low phenolic content. Manuel Beato *et al*. [13] found the correlation between different types of phenolic compounds of garlic bulbs with climatic condition was varied.

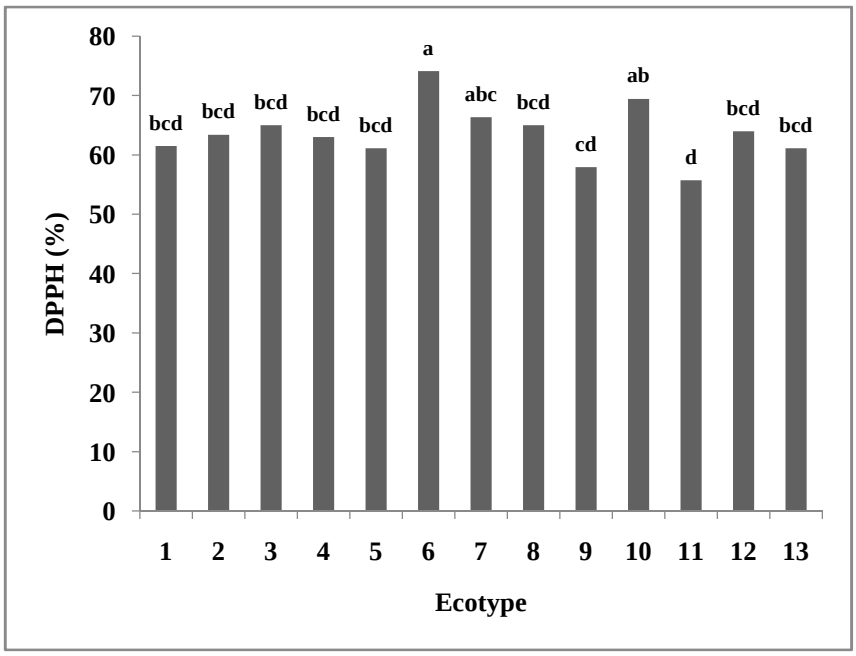

Fig. 1 Antioxidant activity of different ecotypes of *Allium hirtifolium* Boiss.

According to their results caffeic acid showed a highly significant and positive correlation with the average temperature but did not correlate with total precipitation; however vanillic, *p*-hydroxybenzoic, and *p*-coumaric acids showed high significant and negative correlations with both average temperature and precipitation. Climatic conditions influenced the activity of key enzyme in phenolic biosynthesis means phenylalanine ammonia lyase (PAL), which could explain the changes in phenolic content in various environmental conditions [31].

Allicin Content

Allicin content ranged from 3.664 (ecotype 7) to 0.861 (ecotypes 11, 12) mg g^{-1} FW with an average of 2.127 mg g^{-1} FW (Table 2). The values observed in this study was higher than those reported by Asili *et al*. [7] who reported a variation of allicin content from 0.62 to 3.26 mg per gram among *A. hirtifolium* ecotypes collected from the Lorestan province in west of Iran. The content of allicin in *A. hirtifolium* is comparable with the allicin content of garlic that reported 3.3 mg g^{-1} FW by Khar *et al*. [32]. The collected bulbs of two regions of Isfahan exhibited statically higher allicin content as compared to other ecotypes. These results suggest the high health beneficial value of these ecotypes because allicin is one of the most important bioactive compounds possessing anti-fungal, anti-bacterial, anti-viral, anti-tumor and anti-hypertensive effects [33].

According to the obtained data, the bulbs of regions with high temperature and low rate of precipitation showed high allicin content. Results from Table 3 revealed the correlations among allicin, temperature and precipitation rate of plant growing site were significantly positive and negative, respectively. This suggestion also supported by previous results that demonstrated by Hassan *et al*. [34] who reported that high allicin content of garlic might be closely associated with the climatic conditions of high temperature and low precipitation. Coolong and Randle [35] reported that the alliin content increased linearly in response to temperature. In contrast with these results, Hirata *et al*. [16] reported high temperature and humidity affects garlic quality by decreasing the cysteine sulfoxide content. Generally in present study plants from ecotypes 6, 7 and 3 have good potential for produce the high allicin content and bulbs of these sites could be used for domestication programs in access to populations with high contents of allicin.

Table 2 Comparison of phytochemicals and antioxidant enzymes activity of different *Allium Hirtifolium* Boiss. ecotypes.

Eco type	Total phenol (mg GAE g^{-1} FW)	Allicin (mg g^{-1} FW)	Pyruvic acid (µmol g^{-1} FW)	CAT (U mg^{-1} protein)	POX (U mg^{-1} protein)	APX (U mg^{-1} protein)	PPO (U mg^{-1} protein)	SOD (U mg^{-1} protein)
1	0.773abc	1.492g	81.817f	0.582a	1.274c	43.537bc	46.095bcd	54.757ab
2	0.571bcd	2.439c	88.582c	0.381a	0.797c	55.289ab	46.289bcd	46.769ab
3	0.546bcd	2.994b	92.616b	0.607a	0.944c	51.289ab	50.264b	61.337ab
4	0.631bcd	2.105e	82.413f	0.430a	2.814b	53.956ab	31.439cde	65.086a
5	0.586bcd	2.165de	84.868d	0.294a	1.246c	66.791a	29.420de	64.195a
6	0.799abc	3.581a	99.007a	0.618a	3.176b	66.316a	79.286a	66.265a
7	0.761abc	3.664a	98.953a	0.638[a]	5.369a	63.791a	40.378bcde	66.323a
8	0.511cd	1.491g	87.682c	0.629a	1.325c	53.622ab	73.129a	59.790ab
9	0.478cd	2.109e	85.713d	0.252a	0.740c	30.304cd	27.568e	58.295ab
10	1.001a	2.21d	85.669d	0.755a	5.121a	66.225a	66.379a	62.793ab
11	0.858ab	0.861h	84.585de	0.432a	0.731c	60.136ab	27.332e	66.176a
12	0.385d	0.875h	79.729g	0.377a	1.269c	34.304cd	34.374bcde	48.770b
13	0.507cd	1.659f	813.286ef	0.368a	0.823c	58.803ab	48.417bc	66.325a

* Values followed by the same letter within a column indicate they are not significantly different ($p < 0.05$).

Pyrovic Acid Content

Pyruvic acid content is an index to evaluate pungency and flavor of *Allium* crops [36]. In this study pyruvic acid content among studied ecotypes showed significant differences that ranged between 98.98 to 54.64 µmol g^{-1} FW with average 84.412 µmol g^{-1} FW (Table 2). Abedi *et al*. [37] reported 60-84 µmol pyruvate per gram in garlic ecotypes of Hamadan province in west of Iran. Natale *et al*. [38] characterized Argentine garlic cultivars by their pungency; the pyrovic acid mean value of these cultivars was 80.467 µmol per gram fresh weight.

In this study, results showed a progressive increase in pyrovat content with increasing the temperature and decreasing precipitation rate. In agreement with these findings the correlation results (Table 3) showed a significant positive and negative correlation among pyrovic acid content with temperature and precipitation parameters, respectively. As a result, pyrovic acid content reached to greatest value in the sites with highest temperature and lowest precipitation rate (Isfahan and Sanjan ecotypes). Several studies have been reported that climatic conditions such as temperature and precipitation rate affected the pungency and flavor of alliums [35]. Temperature determined the type of sulfur accumulation in plants so affects the rate of pungency. With low temperature, sulfur accumulates mainly as SO_4^{2-} which does not enter to flavor biosynthetic pathway [35] so in this condition the pyrovate content decreases. Garlic pungency and flavor vary according to variety and environmental conditions. Drought may affect garlic pungency and high temperature prior to harvest may increase the pungency [39].

Antioxidant Enzymes Activity

The literature survey showed that different *Allium* species possess well defined activities of antioxidant enzymes [40]. The enzymatic antioxidant systems inhibit or alleviate the damage of free oxygen species. Hydrogen peroxide is destroyed by catalase, peroxidase and ascorbate peroxidase, also superoxide radicals are detoxified by superoxide dismutase [41]. In presence of atmospheric oxygen, polyphenol oxidase enzyme oxidized diphenol to quinones which then undergo polymerization to yield dark brown polymers [42]. This reaction affects the plant resistance to stress conditions.

In Table 2, some antioxidant enzymes activity of different *A. hirtifolium* ecotypes are presented. All samples exhibited SOD, APX, PPO, POX and CAT activity. CAT activity ranged from 0.252 (ecotype 9) to 0.755 (ecotype 10) U mg^{-1} protein and POX activity from 0.731 (ecotype 11) to 5.369 (ecotype 7) U mg^{-1} protein and APX activity varied between 30.304 (ecotype 9) and 66.791 (ecotype 5) U mg^{-1} protein. PPO activity differed from 27.332 (ecotype 11) to 79.286 (ecotype 6) U mg^{-1} protein. The SOD activity was between 48.770 U mg^{-1} protein in ecotype 12 and 66.325 U mg^{-1} protein in ecotype 13. According to these results the examined mooseer ecotypes had different susceptibility to the action of toxic oxygen species in respect to different activity of antioxidant enzymes. Ecotype 10 exhibited high activity of all enzymes and may possess a strong resistance to the action of toxic oxygen species so could be used in breeding oxygen stress resistant. The bulb samples which collected from Hamadan (ecotype 9) exhibited poor activity of all investigated antioxidant enzymes probably due to unpleasant conditions during the growth and maturation. The variation found among the ecotypes is probably due to the metabolic behavior of each ecotype in relation to the genotype and different climatic and environmental conditions [29].

Correlations between Phytochemicals

In order to determine the contribution of various phytochemicals for the antioxidant activity, the correlation among the above phytochemicals and antioxidant activity were evaluated (Table 4).

Table 3 Correlation coefficients among antioxidant activity, total phenol, pyrovic acid, ascorbic acid contents and environmental parameters.

Factors	DPPH	Total phenol	Allicin	Pyruvic Acid
Temperature	0.138^{ns}	-0.210^{ns}	0.630^{*}	0.723^{**}
Precipitation	-0.629^{*}	-0.419^{ns}	-0.647^{*}	-0.712^{**}

** Significant with P <0.01; * significant with P<0.05; ^{ns}: non-significant (n=5).

There are several reports regarding to the correlation between antioxidant activity, total phytochemicals and antioxidant enzyme activity in various fruits and vegetables [29][30][33]. The overall results revealed that, total phenol, allicin, pyrovic acid contents and different antioxidant enzymes activity had significant positive relationship with antioxidant activity. Antioxidant activity showed the highest correlation with antioxidant enzymes activity (r=0.668), which was followed by pyrovic acid (r=0.637), total phenol (r=0.618) and allicin (r=0.576) contents. There are several reports regarding the correlation between antioxidant activity, total phenol and organosulfur compounds in various fruits and vegetables [30,43]. Ghahremani-majd *et al*. [3] Ghsemi Pirbalouti *et al*. [17] reported the positive correlation between phenolic content and antioxidant activity of *A. hirtifolium* extract. Phenolic compounds due to having hydroxyl groups that increase the stability of the free radical show antioxidant potential. In agreement with our results Bhandari *et al*. [29] and Abedi *et al*. [37] reported a positive and significant relationship between the organosulfur, pyrovic acid contents and the radical scavenging capacity. All studied antioxidant enzymes showed a significant correlation with antioxidant activity. Escobar *et al*. [44] reported the antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase constitute a system that keeps O_2^-, $OH^\cdot$ and H_2O_2 at low steady state concentrations in plants cells and tissues so increase the antioxidant capacity and prevent oxidative stress situations.

Also our result showed significant relationship between allicin and pyrovic acid contents. Coolong and Randle [35] reported the flavor and pungency of different *Allium* species attributed to some organosulfur compounds and pyrovic acid content. Kim *et al*. [45] reported that that the allicin content affected the pungent of garlic, and relationship between the pungency score and allicin content demonstrated a highly positive correlation.

Cluster Analysis

Prospecting the distribution area of *A. hirtifolium* in Iran allowed the collection of thirteen ecotypes. They were located in different geographical regions belonging to cold-arid, cold-semi arid, cold-humid, cold-semi humid and temperate-semi arid bioclimatic zones. The average of annual rainfall of the collection sites varied between 154.46 (ecotypes 6, 7) and 840.19 (ecotype 13) mm, and the altitudes ranged from 1147 (ecotype 3) to 2200 (ecotype 1) m. A cluster analysis using total phenol and allicin contents of thirteen ecotypes of *A.hirtifolium*, grouped these ecotypes in five groups (Fig. 2). Ecotypes 2, 4, 5 and 9 were grouped into group A and ecotypes 1, 8, 10 and 13 were clustered into group B. Group C represented by ecotype 11 and 12. Ecotypes 6 and 7 were clustered as group D. At least ecotype 3 was distinguished from the others as group E. The highest phenol (0.780 mg GAE g^{-1} FW) and allicin (3.623 mg g^{-1} FW) contents were included in group D represented by Isfahan ecotypes. This potential should be noted for the domestication and breeding programs. The ecotypes of this group originated from same area in the center of Iran. Some of ecotype groups were not strictly concordant with their bioclimatic condition such as ecotypes 1 and 13 with cold-humid climatic were grouped with ecotypes 8 and 10 from cold-arid area. So according to these results we can conclude that genetic factors in addition to environmental factors affected the phytochemical content of *A. hirtifolium*. Asili *et al*. [7] and Ebrahimi *et al*. [8] revealed a high level of genetic diversity in mooseer ecotypes of Iran. Manuel Beato *et al*. [13] reported that phenolic compounds of plants mainly influenced by genetic factors and environmental conditions as well as Kaur *et al*. [15] observed the genotypic and environmental factors largely affect the antioxidant compounds in onions.

Table 4 Correlation coefficient among phytochemical compounds, antioxidant enzymes activity and antioxidant activity in *Allium hirtifolium* Boiss. ecotypes.

Antioxidant	Total phenol	Allicin	Pyrovic acid	Catalase	Proxidase	Ascorbate peroxidase	Polyphenol oxidase	Superoxide dismutase
Antioxidant activity	0.618^{*}	0.576^{*}	0.560^{*}	0.577^{*}	0.554^{*}	0.860^{**}	0.629^{*}	0.624^{*}
Total phenol	-	0.237^{ns}	0.293^{ns}	0.625^{*}	0.639^{*}	0.623^{*}	0.287^{ns}	0.466^{ns}
Allicine	-	-	0.889^{**}	0.057^{ns}	0.534^{ns}	0.465^{ns}	0.060^{ns}	0.473^{ns}
Pyrovic acid	-	-		0.480^{ns}	0.479^{ns}	0.477^{ns}	0.467^{ns}	0.506^{ns}

** Significant with P <0.01; * significant with P<0.05; ^{ns}: non-significant (n=5).

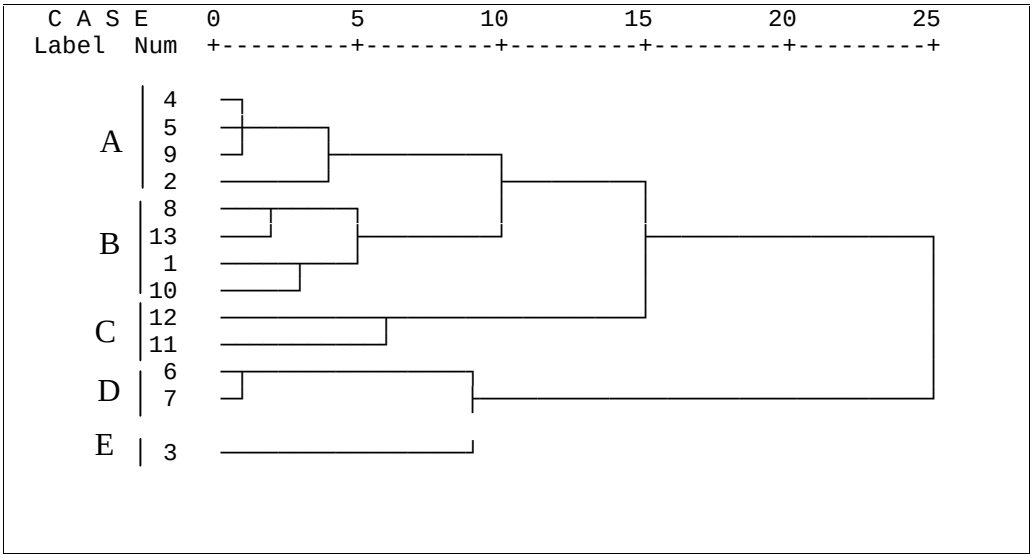

Fig. 2 Dendrogram of the thirteen ecotypes of *Allium hirtifolium* Boiss. resulting from the cluster analysis of the phytochemichal components based on Euclidean distances.

Conclusion

Allium hirtifoilum is a native plant to Iran with high nutraceutical values as well as the present results confirmed. The results of this study showed the presence of significant differences of phytochemicals content (total phenol, allicin and pyrovic cid) and antioxidant activity in different *A. hirtifolium* ecotypes. Also high antioxidant enzymes activity observed in these studied ecotypes. The presence of high phytochemical contents and antioxidant activity in Isfahan ecotypes suggests their higher nutritional values. These ecotypes should be considered for the domestication, breeding program and preservation of *A. hirtifoilum* that is under increasing pressure as a result of excessive harvest.

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