

Original Article

## Antioxidant Activity, Phenolic and Flavonoid Contents of *Echium* Species from Different Geographical Locations of Iran

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### Abstract

Iranian *Echium* species (Boraginaceae), popularly known as "Gol-e-Gavzaban" are native plants that some of them have been used widely as food and traditional medicine since long times. In this work organs from different populations of two Iranian *Echium* species were collected from their natural locations in order to analyze their phenolic content and antioxidant activity. Hydroalcoholic extracts of organs were assessed for total phenolic content (TPC) and total flavonoid content (TFC). 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and ferric ion reducing antioxidant power (FRAP) assays were also used to evaluate the antioxidant properties of the extracts. Among the examined organs, leaves of *E. amoenum* (Hezar Jarib location) contained the highest ( $119.50 \pm 2.00$  mg GAE/g DW) TPC, followed by seeds of *E. italicum* L. (Alamute Qazvin location) ( $117.91 \pm 7.29$  mg GAE/g DW). Seeds of *E. amoenum* (Ramsar location) showed the highest ( $62.17 \pm 3.59$  mg QE/g DW) TFC value. All extracts also exerted antioxidant properties, the most active one was the seed extract of *E. amoenum* (Behshahr location) which contained the highest free radical scavenging effect ( $76.67 \pm 0.33\%$ ) and the highest FRAP value ( $20.88 \pm 0.72$  mg GAE/g DW). The study revealed that phenolic compounds may be the main contributors to the antioxidant activity of some organs of *Echium* plants and they could be explored as potent natural antioxidants in the future. Correlative effects of altitude and precipitation, as two important environmental factors on the content of phenolics were also investigated. Results showed that the patterns of phenolic and flavonoids contents in the different organs of *Echium* plants were differently affected by two environmental factors.

**Key words:** *Echium* species, Antioxidant activity, DPPH radical scavenging, Phenolic and flavonoid contents, FRAP

### Introduction

Reactive oxygen species (ROS) including radicals and non-radicals are involved in various physicochemical processes in the body. Excessive generation of ROS will result in oxidative damage, a harmful process that contributes to the pathogenesis of a large number of serious diseases in human including cardiovascular diseases, cancers, AIDS, inflammation and neurodegenerative diseases [1-3]. Indeed, ROS are highly reactive metabolites can simply react with biomolecules such as proteins, lipids, lipoproteins and DNA [4] or initiate the lipid free radical chains, leading to the lipid peroxidation

[5]. Antioxidants refer to the enzymes or other organic substances in foods that can delay or inhibit the oxidation process of lipid or other molecules by mechanisms such as prevention of ROS construction, free radicals scavenging, metal ions chelating and other mechanisms [1,2,6].

The health-promoting activities of plant-based foods are mainly due to the presence of different antioxidants including some vitamins, terpenoids and phenolic components, such as flavonoids and polyphenolic compounds [1,6]. The antioxidant activities of phenolic compounds are mainly due to their redox properties, which allow them to act as free radical quenchers, hydrogen donors, and reducing agents [1,3]. As synthetic antioxidants are

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suspected to be toxic and carcinogenic, therefore there is a considerable interest to replace them with naturally originated antioxidants, especially obtained from plant sources [2,3]. Therefore in the last years some medicinal plants have been extensively studied for their antioxidant and radical scavenging activities [1-3,7-13].

Recently, it has been shown that remarkable *in vitro* antioxidant and antiradical properties of seeds [14-16] and leaves [9] extracts of borage (*Borago officinalis* L.) from Boraginaceae family, are attributed to their phenolic constituents. Other reports have demonstrated that antioxidative stress potential of *Echium amoenum* (Boraginaceae) extracts could be due to the presence of bioactive antioxidant components, especially rosmarinic acid, flavonoids and tannin [7,11,17].

A total of 4 *Echium* species at the specific level were reported to be present in Iran [18]. *Echium amoenum* Fisch & C.A. Mey (Iranian Gol-e-Gavzaban or Iranian borage), is one of the most important medicinal plants in Iranian traditional medicine and its dried flower has been used as a demulcent, anti-inflammatory and analgesic, anxiolytic, and sedative [11,15,19]. The presence of bioactive compounds such as phenolics and flavonoids in these plants could be correlated with multiple biological effects including antioxidant activity. Therefore, the aims of the present study were to evaluate total phenol and flavonoid contents, and antioxidant potential of different organs of two frutescent *Echium* species which were collected from six natural localities in Iran. In addition the possible correlation between some environmental factors and the contents of phenolic compounds was also assessed. This study represents the first comprehensive report, where phenolic compounds and antioxidant activities of seeds, leaves and stems of some Iranian *Echium* species are systematically compared.

## Materials and Methods

### Plant Materials

Mature seeds of two *Echium* species were collected from their natural habitats in different regions of Iran. Seeds of *E. amoenum* Fisch & C.A. Mey were obtained from three mountainous areas (Behshahr, Hezar Jarib and Ramsar (Jannat Roudbar), Mazandaran province) during August-September 2010. Leaves and stems were also harvested from the wild plants in June 2010. Seeds of *E. italicum* L. were gathered from three different localities (Boumehen in Tehran province, Kaleybar in East

Azerbaijan province and Alamute Qazvin in Qazvin province) during August-September 2010. Leaves and stems of *E. italicum* plants were harvested from the mentioned locations in June, 2010. The different botanical taxa studied in this work are shown in Table 1, together with some information about their date and location of collection. Voucher specimens were deposited at Tehran University Central Herbarium (TUH).

### Preparation of the Extracts

One gram of each freeze-dried sample was crushed mechanically into powder form using a mortar and pestle in liquid nitrogen, and extracted with 70% aqueous EtOH through maceration (48 h for three times) at room temperature. The supernatants were collected, and filtrated over glass wools. The resultant total extract was dried by rotary evaporation under pressure at 30 °C. The crude extract was then weighed and stored at -20 °C for further analysis.

### Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) of each extract was determined spectrophotometrically using Folin–Ciocalteu phenol reagent according to the method described by Singleton et al. [20]. In brief, an aliquot of 100 µL of dissolved extract (4 mg/400 µL DMSO) was transferred to a 10 ml volumetric flask, containing ca. 6.0 ml distilled water, to which 500 µL Folin–Ciocalteu phenol reagent was subsequently added. After 1 min, 1.5 ml of 200 g/l Na<sub>2</sub>CO<sub>3</sub> was added and the volume was made up to 10 ml with H<sub>2</sub>O. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm by a UV-visible spectrophotometer (Shimadzu UV-1601 PC).

Measurements were carried out in triplicate and calculations were based on a calibration curve obtained with gallic acid. TPC was expressed as mg gallic acid equivalents/gram dry weight (mg GAE/g DW). The calibration equation for gallic acid was  $y = 0.011x + 0.013$  ( $r^2 = 0.998$ ).

### Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) in extracts was determined with aluminium chloride (AlCl<sub>3</sub>) according to the method of Zhishen et al. [21]. Briefly, 0.25 ml of optimal diluted sample (4 mg/1 ml DMSO) was added into a tube containing 1 ml of double distilled water. Then, 0.75 ml of 5% NaNO<sub>2</sub>, 0.075 ml of 10% AlCl<sub>3</sub>, and 0.5 ml of 1 M NaOH were added at 0, 5 min and 6 min, sequentially. Finally, the volume of reacting solution was adjusted to 2.5 ml with double distilled water. The absorbance of the solution at a wavelength of 510 nm was

detected using a spectrophotometer. All determinations were made in triplicate and values were calculated from a calibration curve obtained with quercetin. Flavonoid contents were expressed as milligram of quercetin equivalents (QE) per gram of dried weight ( $y = 0.288x - 0.007$ ;  $r^2 = 0.9967$ ).

#### DPPH Radical Scavenging Activity Assay

The DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical scavenging of the corresponding extracts was estimated using the method of Liyana-Pathriana and Shahidi [22]. Fifty microlitres of extracts in methanol (4 mg/200  $\mu$ l) were added to 5 ml of a 0.004% methanol (w/v) solution of DPPH. After 30 min incubation period at room temperature, the absorbance of the mixture was measured against a control at 517 nm. Trolox (1 mM) (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The percentage of DPPH radical scavenging activity (I%) of extracts was calculated using the following equation:

$$I\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where  $A_{\text{control}}$  is the absorbance of the control reaction mixture (DPPH radical + methanol), and  $A_{\text{sample}}$  is the absorbance of the test compound (DPPH radical + sample extract/reference). Tests were carried out in triplicate.

#### Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing property of the extracts was determined using an assay described by Lim *et al.* [23]. One milliliter of extract in DMSO (4 mg/4 ml) was added to 2.5 ml of potassium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was incubated at 50 °C for 20 min, after which 2.5 ml of 10% (w/v) trichloroacetic acid was added. The mixture was then separated into aliquots of 2.5 ml and mixed with 2.5 ml of deionized water. Then, 0.5 ml of 0.1% (w/v)  $\text{FeCl}_3$  was added to each tube and allowed to stand for 30 min. Absorbance for each tube was measured at 700 nm. The FRAP was expressed as gallic acid equivalents (GAE) in mg/g of samples used. The calibration equation for gallic acid was  $y = 16.654x - 0.002$  ( $r^2 = 0.997$ ).

#### Statistical Analysis

All measurements were carried out in triplicate and the data were expressed as mean  $\pm$  SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Duncan's test and P values less than 0.05 were considered to be statistically significant. Regression and correlation (Pearson) analyses were carried out to determine the

possible relations between the contents of phenolic compounds in different organs of the studied plants and environmental factors.

## Results

### Total Phenolic and Flavonoid Contents

Table 2 reports the results of TPC and TFC analyses. The amounts of TPC varied widely in the different organ analyzed and ranged from  $8.55 \pm 0.69$  to  $119.50 \pm 2.00$  mg GAE/g dry weight (DW). Among seed extracts, the highest TPC was identified in seed extract of *E. italicum* from Alamute Qazvin (average:  $117.91 \pm 7.29$  mg GAE/g DW) followed by seed extract of *E. amoenum* from Behshahr ( $115.77 \pm 2.38$  mg GAE/g DW), whereas the lowest content value was found in seed extract of *E. amoenum* from Ramsar ( $65.37 \pm 0.22$  mg GAE/g DW). The TPC values of the leaf extracts ranged from  $17.42 \pm 0.47$  mg GAE/g DW in *E. italicum* (Boumehen) to  $119.50 \pm 2.00$  mg GAE/g DW in *E. amoenum* (Ramsar). The obtained results also showed that stems possessed lower TPC values in comparison to the seeds and leaves of the studied plants. Stem extracts of *E. amoenum* collected from Behshahr and Hezar Jarib locations had the highest and the lowest TPC with values of  $93.81 \pm 1.04$  and  $8.55 \pm 0.69$  mg GAE/g DW, respectively.

Similarly, data revealed that the amounts of TFC varied widely in different analyzed organ extracts. Seed extract of *E. amoenum* from Ramsar contained the highest TFC value ( $62.17 \pm 3.59$  mg QE/g DW), while the lowest level was found in seed extract of *E. amoenum* from Hezar Jarib ( $11.45 \pm 0.73$  mg QE/g DW) (Table 2). The TFC value of the leaf extracts varied from  $7.26 \pm 0.00$  mg QE/g DW in *E. amoenum* (Hezar Jarib location) to  $34.05 \pm 1.85$  mg QE/g DW in *E. italicum* (Alamute Qazvin). The highest TFC was identified in stem extract of *E. amoenum* (Ramsar) at  $19.93 \pm 1.60$  mg QE/g DW and stem extract of *E. italicum* (Alamute Qazvin) exhibited the lowest content at  $2.56 \pm 0.23$  mg QE/g DW. The TPC and TFC values were also determined for each sample as milligram GAE or QE per gram dry weight of the extracts (DE) in Table 2.

In this study, regression analyses demonstrated a strong positive linear relationship ( $r^2 = 0.91$ , Fig. 1) between TPC of the leaves with average annual precipitation in different localities, but TPC of the seeds and stems had no significant correlations with precipitation (Table 3). Also regression coefficients were less than 90% for equations of TFC of the seeds ( $r^2 = 0.54$ ), stems ( $r^2 = 0.71$ ) and leaves ( $r^2 = 0.31$ ) of

*Echium* plants with precipitation in different localities (Fig. 1). Except for TPC of the stems ( $r^2 = 0.81$ , Fig. 1), weak negative correlations were found between TPC and TFC of the organs of *Echium* plants with collection sites altitudes (Table 3). So with increasing in altitude, TPC and TFC of the different organs were decreased. The highest contents of phenolic compounds, as well as antioxidant activities were obtained for different organs of two populations of *E. amoenum* from Ramsar and Behshahr with the highest average annual precipitations (652.3 and 1300 mm) and lowest altitudes (1250 and 150 m).

#### DPPH Free Radical Scavenging Capacity

The DPPH free radical scavenging activities of the extracts from different organs of the studied *Echium* plants and of Trolox as a standard antioxidant have been shown in Fig. 2. Results indicated definite scavenging activity of the extracts towards DPPH radicals in comparison with Trolox. DPPH free

radical scavenging values for the three organs in all of the *Echium* plants were significantly different ( $P < 0.05$ ). According to our results, the seed extracts showed the highest power for scavenging of DPPH free radicals, and the lowest activities were obtained for the leaf extracts. The most powerful free radical scavenging activity ( $76.67\% \pm 0.33$ ) was exerted by seed extract of *E. amoenum* (Behshahr) and the weakest one ( $3.49\% \pm 0.25$ ) was exhibited by stem extract of *E. italicum* (Alamute Qazvin). The activities of the seed extracts of *E. amoenum* from Ramsar and Behshahr were equivalent. Among the leaf extracts, leaf extract of *E. amoenum* from Ramsar ( $29.15\% \pm 0.42$ ) with an inhibition value near to Trolox (28.3%) showed the highest activity for scavenging of DPPH free radicals. Compared to Trolox, most of the seed extracts showed much higher activity. It was noticeable that after seeds, the higher radical scavenging activities were obtained for stem samples, with the highest one ( $51.58\% \pm 0.39$ ) for stems of *E. amoenum* from Ramsar.

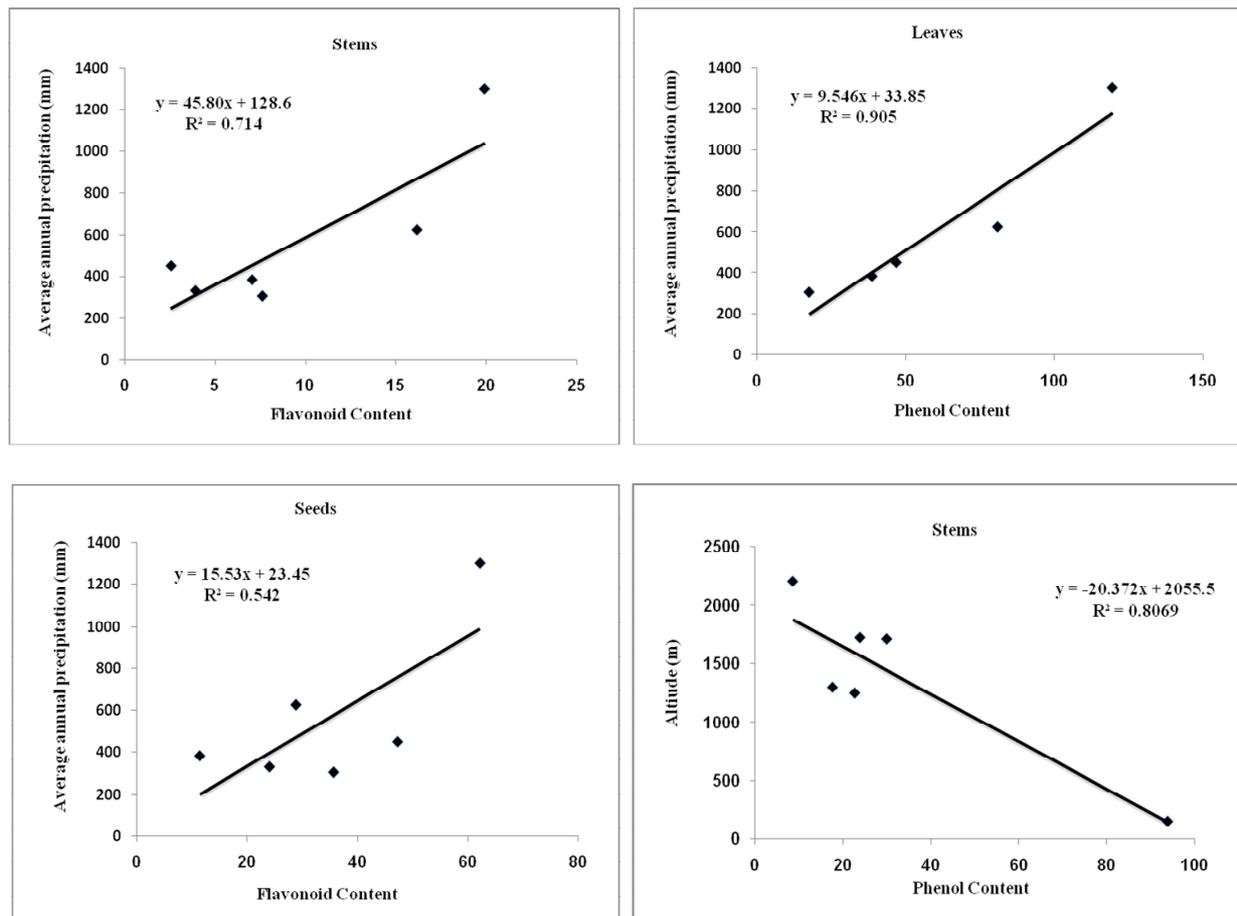
**Table 1** The collected plant materials, localities and date of collection. TUH= Tehran University Central Herbarium, AAP: average annual precipitation.

Species	Locality	Climate	Latitude [N]	Longitude [E]	Altitude (m)	AAP (mm)	Date	TUH No.
<i>E. italicum</i>	Alamute Qazvin	Cold and semi-humid	36° 26'	50° 23'	1300	450.4	June and September 2010	38893
<i>E. italicum</i>	Boumehen	Semi-cold and semi-humid	35° 44'	51° 52'	1722	305.5	June and September 2010	38891
<i>E. italicum</i>	Kaleybar	Mountain-temperate and semi-humid	38° 51'	47° 00'	1710	330.7	June and August 2010	38892
<i>E. amoenum</i>	Hezar Jarib	Mountain-temperate and semi-humid	36° 28'	53° 58'	2200	383.2	June and August 2010	38894
<i>E. amoenum</i>	Behshahr	Temperate and humid	36° 41'	53° 32'	150	652.3	June and August 2010	38895
<i>E. amoenum</i>	Ramsar	Mountain-temperate and humid	36° 43'	50° 34'	1250	1300	June and August 2010	38896

**Table 2** TPC and TFC values of seed, leaf and stem extracts of *Echium* species from different localities.

Species	Location	Organ	TPC (mg GAE/g DW)	TPC (mg GAE/g DE)	TFC (mg QE/g DW)	TFC (mg QE/g DE)
<i>E. italicum</i>	Alamute Qazvin	Seed	117.91 ± 7.29 <sup>b</sup>	782.22 ± 49.24 <sup>b</sup>	47.26 ± 1.18 <sup>b</sup>	319.36 ± 7.98 <sup>b</sup>
<i>E. italicum</i>	Boumehen		105.27 ± 0.96 <sup>d</sup>	680.11 ± 3.50 <sup>d</sup>	35.67 ± 1.71 <sup>c</sup>	240.32 ± 6.22 <sup>c</sup>
<i>E. italicum</i>	Kaleybar		66.11 ± 1.37 <sup>e</sup>	423.81 ± 8.79 <sup>e</sup>	24.07 ± 1.89 <sup>f</sup>	154.36 ± 12.11 <sup>ef</sup>
<i>E. amoenum</i>	Hezar Jarib		67.98 ± 0.47 <sup>e</sup>	424.86 ± 2.96 <sup>e</sup>	11.45 ± 0.73 <sup>i</sup>	71.53 ± 4.57 <sup>hi</sup>
<i>E. amoenum</i>	Behshahr		115.77 ± 2.38 <sup>c</sup>	727.85 ± 14.66 <sup>c</sup>	28.87 ± 3.65 <sup>de</sup>	178.24 ± 22.55 <sup>def</sup>
<i>E. amoenum</i>	Ramsar		65.37 ± 0.22 <sup>e</sup>	422.52 ± 1.13 <sup>e</sup>	62.17 ± 3.59 <sup>a</sup>	422.67 ± 25.65 <sup>a</sup>
<i>E. italicum</i>	Alamute Qazvin	Leaf	46.81 ± 1.79 <sup>h</sup>	350.00 ± 8.00 <sup>h</sup>	34.05 ± 1.85 <sup>cd</sup>	191.35 ± 8.65 <sup>d</sup>
<i>E. italicum</i>	Boumehen		17.42 ± 0.47 <sup>jk</sup>	125.66 ± 2.49 <sup>jk</sup>	19.35 ± 0.35 <sup>e</sup>	103.79 ± 1.88 <sup>e</sup>
<i>E. amoenum</i>	Hezar Jarib		38.62 ± 1.32 <sup>i</sup>	274.00 ± 7.02 <sup>i</sup>	7.26 ± 0.00 <sup>ji</sup>	40.74 ± 0.00 <sup>ji</sup>
<i>E. amoenum</i>	Behshahr		80.91 ± 1.21 <sup>e</sup>	629.33 ± 12.67 <sup>e</sup>	25.90 ± 2.23 <sup>f</sup>	146.40 ± 5.50 <sup>f</sup>
<i>E. amoenum</i>	Ramsar		119.50 ± 2.00 <sup>a</sup>	906.67 ± 3.55 <sup>a</sup>	32.98 ± 0.10 <sup>de</sup>	183.75 ± 0.31 <sup>de</sup>
<i>E. italicum</i>	Alamute Qazvin	Stem	17.62 ± 0.62 <sup>k</sup>	104.63 ± 3.44 <sup>k</sup>	2.56 ± 0.23 <sup>t</sup>	13.08 ± 1.25 <sup>j</sup>
<i>E. italicum</i>	Boumehen		23.83 ± 0.50 <sup>jk</sup>	130.91 ± 2.78 <sup>jk</sup>	7.62 ± 0.92 <sup>ji</sup>	41.87 ± 5.05 <sup>ji</sup>
<i>E. italicum</i>	Kaleybar		29.93 ± 1.95 <sup>j</sup>	155.84 ± 10.15 <sup>j</sup>	3.91 ± 0.35 <sup>l</sup>	19.98 ± 2.00 <sup>j</sup>
<i>E. amoenum</i>	Hezar Jarib		8.55 ± 0.69 <sup>l</sup>	43.02 ± 3.49 <sup>l</sup>	7.05 ± 0.53 <sup>ji</sup>	39.19 ± 2.69 <sup>ji</sup>
<i>E. amoenum</i>	Behshahr		93.81 ± 1.04 <sup>f</sup>	519.10 ± 5.15 <sup>f</sup>	16.19 ± 3.10 <sup>h</sup>	82.96 ± 15.49 <sup>h</sup>
<i>E. amoenum</i>	Ramsar		22.66 ± 0.36 <sup>jk</sup>	127.30 ± 2.01 <sup>jk</sup>	19.93 ± 1.60 <sup>e</sup>	109.38 ± 8.96 <sup>e</sup>

Results are mean ± SD (n=3). For each column, values followed by the same letter (a-l) are not statistically different at  $P < 0.05$ , DW: dry weight; DE: dry extract.



**Fig. 1** Correlation between phenol and flavonoid contents of some organs of *Echium* plants and average annual precipitation and altitude of the collection sites.

**Table 3** Pearson correlation coefficients ( $r$ ) between TPC and TFC in the different organs of *Echium* plants with average annual precipitation and altitude of the localities.

	Average annual precipitation ( $r$ )	Altitude ( $r$ )
Seeds		
TPC	0.29	-0.57
TFC	0.73*	-0.31
Leaves		
TPC	0.95*	-0.53
TFC	0.55	-0.59
Stems		
TPC	0.09	-0.90*
TFC	0.85*	-0.56

\*Significant coefficient ( $P < 0.05$ ).

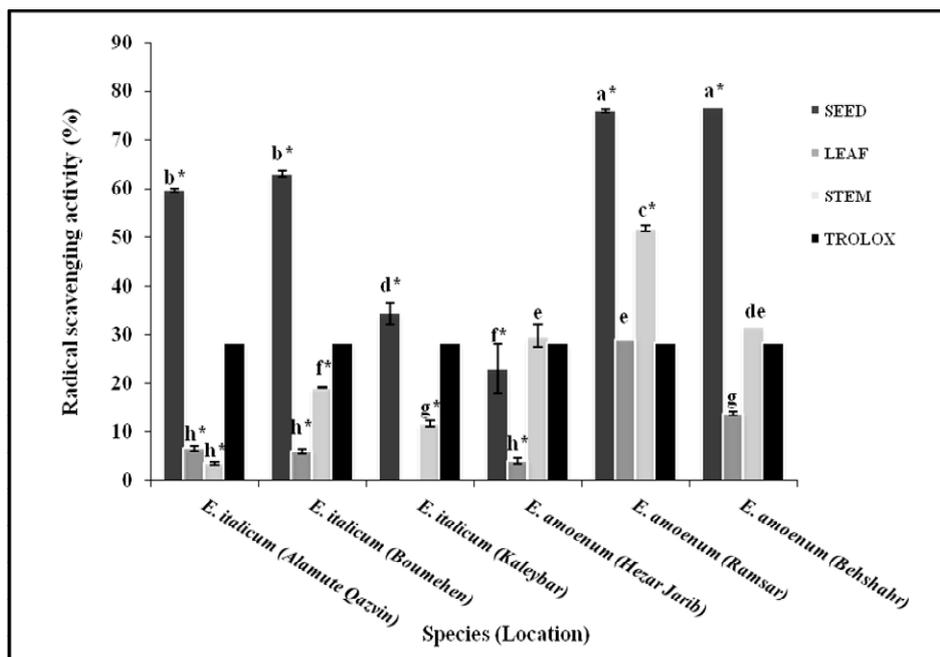
#### Ferric Reducing Antioxidant Power (FRAP)

The ability of the studied plant extracts to reduce ferric ions was determined by FRAP assay (Fig. 3). As expected, FRAP values of the seeds were significantly higher than those of stems and leaves; also they were significantly ( $P < 0.05$ ) different from each other. Among the seeds, the highest FRAP

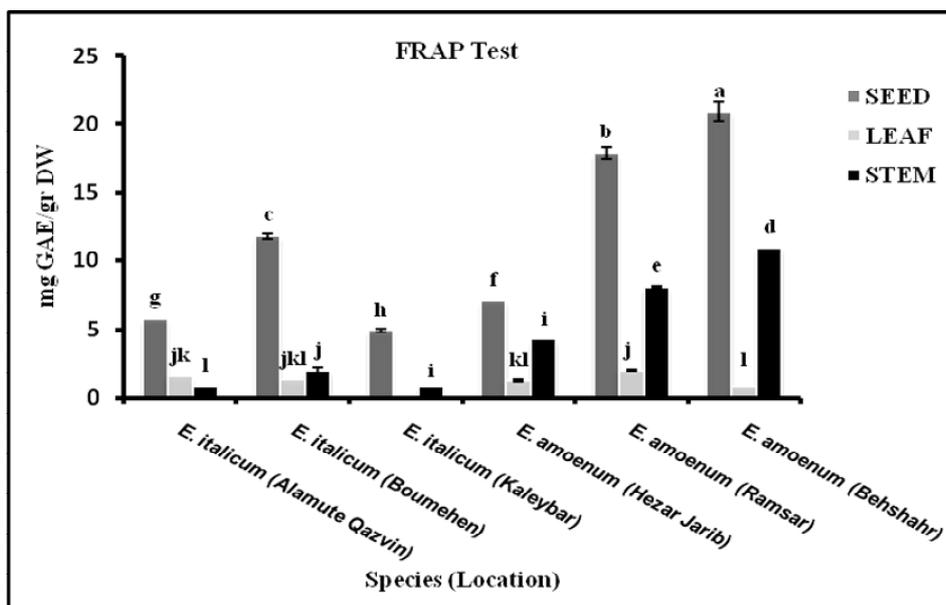
values were obtained for seed extracts of *E. amoenum* from Behshahr ( $20.88 \pm 0.72$  mg GAE/g DW) and Ramsar ( $20.66 \pm 0.47$  mg GAE/g DW) locations. Seed extract of *E. italicum* (Kaleybar) showed the lowest FRAP value of  $4.90 \pm 0.13$  mg GAE/g DW. The FRAP values of leaf extracts varied from  $0.78 \pm 0.00$  to  $1.95 \pm 0.09$  mg GAE/g DW for *E. amoenum* that have been collected from Behshahr and Ramsar, respectively. Among the stem extracts, that of *E. amoenum* from Behshahr had the highest FRAP value of  $10.92 \pm 0.00$  mg GAE/g DW; while stem extracts of *E. italicum* from Kaleybar and Alamute Qazvin with  $0.82 \pm 0.00$  mg GAE/g DW had the lowest one.

#### Discussion

During recent years, great attention has been paid by the public and the medical professionals to the use of indigenous drugs in the treatment of diseases [2]. Plant foods (fruits, grains and vegetables) are of great interest because they provide nutrients and bioactive components (phytochemicals).



**Fig. 2** Antioxidant activities of the different organs of *Echium* plants based on their abilities to scavenge DPPH free radicals, in comparison with that of Trolox as the reference. Data are expressed as mean  $\pm$  SD (n=3). Means not sharing a common letter (a-h) are significantly different ( $P<0.05$ ) among organs and \* compared with the Trolox.



**Fig. 3** Antioxidant activities of the different organs of *Echium* species based on their abilities to reduce ferric ions. Data are expressed as mean  $\pm$  SD (n=3). Means not sharing a common letter (a-l) were significantly different ( $P<0.05$ ).

Among them, polyphenols as abundant micronutrients in our diet are active substances which are found in many medicinal plants [24]. The antioxidative effect of phenolics in functional foods is due to a direct free radical scavenging activity, reducing activity and an indirect effect arising from chelation of metal ions [1,25,26]. Therefore, the putative therapeutic properties of many traditional

medicinal plants can ascribe mainly to the phenolic compounds in particular, the flavonoids present in them [1]. Some authors [2,3,12] have demonstrated a positive correlation between the content of phenolic compounds and their antioxidant activity, while others [8,9,13,15] reported poor or no relationship. Hence, it appears that differences in antioxidant activity could be related to the nature of the phenolic

compounds, from phenolic acids to flavonoids and not necessarily to their contents [15,27,28]. For instance, the radical scavenging activities of phenolic acids and their derivatives, such as esters as well as flavonoids in the plants depend on the number of hydroxyl groups in the molecules [2,29].

In agreement with some findings, our results showed that in some cases no clear correlation could be observed between antioxidant activities and phenolic content of the analyzed extracts. Some extracts had high phenol/flavonoid contents but low antioxidant activities and conversely, others possessed low phenol/flavonoid contents and high antioxidant activities. For instance, seed extracts of *E. italicum* plants (Alamute Qazvin and Boumehen) with the highest TPC and relatively high TFC showed lower antioxidant activities. As another example, seed extract of *E. amoenum* plants collected from Ramsar and stem extract of *E. amoenum* from Hezar Jarib showed significantly higher antioxidant activities, while they had the lowest TPC between the same organs examined. Although, the seed extract of *E. amoenum* from Ramsar had the highest value of TFC. In other words, extracts with higher radical scavenging and antioxidant activities did not show a high TPC or FTC. Apart from the above mentioned cases, a significant relationship was found among TPC/TFC and antioxidant activities of other extracts. Since Folin-Ciocalteu reagent is not specific just for polyphenols and will react with any other reducing substances, it has been shown that the Folin-Ciocalteu assay is used to obtain a crude estimate of the amount of phenolic compounds present in an extract. In addition, phenolic compounds, depending on the number of their phenolic hydroxyl groups, react differently to the Folin-Ciocalteu reagent [13,20]. Therefore, this may explain some observations about some organ extracts of *Echium* plants, where their high TPC values did not correspond to a high antioxidant activity.

The obtained data in this study showed that almost all extracts evaluated from the different organs of the *Echium* plants possess antioxidant and free radical scavenging activities. It was also shown that among organs, seed extracts had significantly higher antioxidant activities in the scavenging of DPPH free radical than Trolox. This may be due to the high amounts of flavonoid and phenolic compounds in their extracts. The seed extracts of *E. amoenum* (Ramsar and Behshahr) remarkably reduced the concentration of DPPH free radicals, with a value higher than that of Trolox and slightly higher than that of *Thymus x -porlock* essential oil (69.29% inhibition) [10]. Also, in this study a strong

correlation was observed between values obtained in particularly for seed extracts, it was indicated that compounds in the extracts were capable of scavenging the DPPH free radical and reducing ferric ions. But the absence of a satisfactory correlation between TPC and TFC values with antioxidant activities of some extracts suggested that phenolic compounds are partly and not essentially responsible for antioxidant activities of the extracts [8,9,13,15]. Some studies have demonstrated that the phytosterols such as  $\beta$ -sitosterol strongly prevent the development of diseases due to ROS through modulation of antioxidant enzymes [30]. Moreover, the antioxidant effects of the phytosterols  $\beta$ -sitosterol, stigmasterol, and campesterol, against lipid peroxidation have been shown [31]. Furthermore, our results from the recent experiments have also revealed that all organs, especially seeds of the reported *Echium* plants possess phytosterols [32], so the antioxidant activity of them could be due to the presence of these compounds. On the other hand, in accordance with some reports the relatively high antioxidant and free radical scavenging activity of extracts containing low phenolic content could be attributed to the type of phenolics rather than their amounts [9,27,28].

Similar to our results many researchers have reported that pattern, production and composition of secondary metabolites in plants are influenced by different environmental factors. Sárosi et al. [33] showed that total phenolic content and total antioxidant activity of *Prunella vulgaris* L. from two different locations was affected by the weather condition. Giorgi et al. [34] have studied the effects of environmental growth conditions on the antioxidant capacity, total phenolic content and composition of *Achillea collina* Becker ex Rechb. They reported that climate (as influenced by altitude) was the main factor influencing the composition of phenolic antioxidants and their properties. Oloumi and Hassibi [35] investigated the correlative effect of some climatic factors on the content of some metabolites in the roots of *Glycyrrhiza glabra* L. gathered from different localities. They found that the pattern of phenolic contents differs based on climatic conditions. Based on their results total phenolic contents had correlation with longitude and average annual precipitation. Ghasemi et al. [36] have measured antioxidant activities, phenol and flavonoids contents of *Juglans regia* L. from 11 regions of Iran with different geographical and climatic conditions. They found a good correlation coefficient between the phenolic and flavonoid contents with collection altitudes. Jovancevic and co-workers [37] have shown the effect of altitude and

sun shining on the content of phenolic compounds of *Vaccinium myrtillus* L. Asadian et al. [38] reported that the highest values of hypericin, total phenols and flavonoids were obtained in *Hypericum perforatum* L. plants which grown wild in areas with high altitude. Ferreira and Domingos [39] showed that the seasonal antioxidant profile in *Ipomoea nil* (L.) Roth cv. Scarlet O'Hara was associated to variations in temperature, relative humidity and global radiation.

It is well known that the synthesis of secondary metabolites in different plant species is mainly controlled by genetics and could be stimulated by geographical and climatic factors such as temperature, precipitation, UV lights, light intensity, altitude, latitude and longitude, etc. [35-37]. Anyway, ecotype genetic analysis and further climate data on the locations would be necessary to establish a stronger correlation and a closer relationship between secondary metabolites, environmental factors and their interactions in the studied *Echium* plants.

## Conclusion

In the present study, we carried out a systematic record of the relative antioxidant activity in extracts of selected organs of some Iranian *Echium* plants. The results of this work revealed that extracts from different organs demonstrated a various range of phenolic compounds and different extent of antioxidant activity.

It is encouraging to note that seed extracts exhibited increased FRAP values and significantly higher antioxidant activities in the scavenging of DPPH free radical than Trolox. This may be due to the high amounts of flavonoid and phenolic compounds in seed extracts. However, we could not establish a strong correlation between antioxidant activity and phenolic contents in all of the extracts. It is worth mentioning that TFC more than TPC seems to determine antioxidant activity of some extracts. The results suggest that phenolic compounds, in particular the flavonoids, are the major contributors to the antioxidant capability of these extracts.

The seed and stem extracts of *E. amoenum* from two locations of Behshahr and Ramsar possessed the highest antioxidant activity followed by seed extracts of *E. italicum* from Boumehen and Alamute Qazvin. Consequently, some of Iranian *Echium* plants have great potential to be used as interesting sources of antioxidant principles in different industries (food, cosmetic and pharmaceutical).

In our case some studied species or populations were characterized by higher contents of phenolic contents

and consequently antioxidant activities; however we do not have enough data whether they are genetically based differences or are metabolic changes for adaptation of the plants to their environmental conditions.

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