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Original article

The Proximate, Mineral and Amino Acid Composition of Spring, Autumn Leaves and Roots of *Eryngium caeruleum* M.Bieb

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Abstract
Background and objectives: *Eryngium caeruleum* M.Bieb. (Syn. *Eryngium caucasicum* Trautv.) belongs to Apiaceae family. It is found abundantly in northern provinces of Iran as an edible plant. Hundreds of years ago, *Eryngium* genus was known as a medicinal herb in Persian medicine books which was named "Qaracaane" and the plant's roots were used in traditional medicine. The aim of this study was to evaluate the nutritional parameters in roots, spring and autumn leaves of *E. caucasicum* for the first time. **Methods:** The parameters including proximate composition (protein, carbohydrate, fat, fiber, ash, moisture and calorie) were measured by the standard methods of the AOAC, mineral contents (iron, zinc, copper and manganese) were measured by atomic absorption and amino acid contents was measured by RP-HPLC. **Results:** Regarding the results, it was found that the autumn leaves showed the highest amount of fiber, protein, moisture, zinc, copper and manganese. Also, spring leaves contained the highest levels of calorie, while the roots showed much more ash, carbohydrate and iron content. In terms of amino acids contents, threonine was the dominant among the rest of essential amino acids in all investigated parts of *E. caeruleum*. The results showed that both the aerial parts and the roots of *Eryngium caeruleum* could be good sources of nutritional ingredients. **Conclusion:** According to the obtained results it can be concluded that *E. caeruleum* has the capacity for prospective production of new natural medicinal supplements in order to improve body health and prevent or treat diseases.

Keywords: amino acids; *Eryngium*; medicinal; minerals; nutritive value; plants

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Introduction
The family of Apiaceae contains more than 300 genera [1]. These plants are used as medicinal plants, essential oil containing herbs, and vegetables. The plants of this family, and especially the *Eryngium* genus, have great potentials for producing herbal pharmaceutical products in the future [2]. *Eryngium* is the biggest and controversially the most taxonomically intricate genus that belongs to the family Apiaceae [3]. It contains more than 2500

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verified species, subspecies and varieties all around the world [4,5]. Among them only 23 species approximately have been studied phytochemically [6].
New research demonstrated that different species of *Eryngium* contain various phytochemical compounds and at least 127 compounds have been isolated and identified [6,7]. Latest studies revealed that the plants of this genus contain different types of nutrients including vitamins, minerals and proteins [8]. Most of these studies have focused on the aerial parts of *Eryngium* genus, while the roots have been rarely investigated.
Hundreds of years ago, the *Eryngium* genus was mentioned as a pharmaceutical herb in refernces of Persian medicine with the name of "Qaracaane", and widespread various descriptions and morphology have been recorded among different species due to the difference in location of growth [9].
In Persian medicine, foods and drugs are divided into different classification including absolute aliment, functional foods, pharconutrient, absolute medicament or drug, and poisons [10].
In Persian medicine references *Eryngium* is classified in pharconutrient group that is similar to nutraceutical foods in current concepts and the use of the roots has been confirmed. Also, various therapeutic effects have been referred in these references including diuretic, antidote (antitoxin), anti-inflammatory, aphrodisiac, emmenagogue, galactagogue, digestive, anti-flatulent and analgesic properties. Other indications attributed to this plant in traditional medicine are for snakebite and insect bites, cramps and gripes, pulmonary disease, halitosis and early stages of elephantiasis (lymphatic filariasis). Nowadays, some of Persian medicine practitioners recommend this medicinal plant as a nutritional herb for overcoming weakness and accelerating recovery [9,11].
Eryngium caeruleum M. Bieb. (Syn *Eryngium caucasicum* Trautv.) is one of the Iranian native medicinal plants, distributed especially in northern provinces of Iran especially in Mazandaran and Gilan. It is a perennial herb and its height can reach about hundred centimeters. Large quantities of young leaves are collected by native people and sold in local markets. It is used as a flavoring plant in different local foods and in food industries [2,12].
The aim of the present study was to evaluate the nutritional value of *Eryngium* as a nutraceutical herb. Considering the emphasis of Persian medicine references on the using of the plant root, nutritional value of both aerial parts and roots were investigated for the first time.

Material and Methods

Plant material

Spring leaves, autumn leaves and roots of *Eryngium caeruleum* M.Bieb. were collected from Sesar village, Gilan province (the north of Iran) in October 2016 and April 2017. The identity of the plant was confirmed at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The voucher specimen (TEH-6610) has been deposited. The plant materials were dried in shade and room temperature, and were separately ground coarsely for next analyses.

Chemicals

Amino acid standard solution (L- Alanine, L-Arginine, L-Aspartic Acid, L-Cystine, L-Glutamic Acid, Glycin, L-Histidine, L-Hydroxylysine, Hydroxy-L-proline, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L- Serine, L-Threonine, L-Tyrosine, L-Valine) the derivatizing reagent PITC (phenyl isothiocyanate), and triethylamine (TEA) were purchased from Sigma (Spain). All other reagents and solvents were purchased from Merck (Germany) and Sigma -Aldrich (USA). The chemicals used in the experiments were of analytical grade.

Proximate compositions

The contents of ash, moisture, protein, fat, fiber and carbohydrate in the samples were determined for proximate compositions by standard methods described in the AOAC (Official Methods of Analysis of the Association of Official Analytical Chemists) and Pearson [13,14].
Briefly, weight difference method was used for determination of the ash and moisture contents. The protein content was estimated using Kjeldahl method. In this method, the nitrogen value was determined and then was multiplied in conversion factor of 6.25 in order to calculate the protein amount. Fiber content was also evaluated with measurement of the loss in the crucible weight on ignition. The percentage of moisture, ash, crude protein and crude fiber were added together and were subtracted from 100 for

determination of carbohydrate contents. Fat content was determined by Soxhlet method.
The energy values of the samples were determined as: Energy value=4× (carbohydrate %+ protein %) + 9× fat % [15]. All the proximate values were presented as percentage.

Mineral contents
The microwave digestion method was used to determine the amount of four elements (Zn, Mn, Cu and Fe) in dried roots, spring and autumn leaves of *E. caeruleum*. One gram of dried plant samples was transferred to TFM vessels; 6 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%) were added and digested for 15 min at 180 °C at 500 watt power.
The residue was diluted to 25 mL by ultrapure water and then filtrated by 0.25 µm filter and injected to atomic absorbance spectrometry (AAS, Spau GBC Model Savant AA, AUS) [16].

Amino acid composition
The amino acid contents of the samples were determined by RP-HPLC method with pre-column PITC derivation according to Tayade et al. [17] with minor modifications.
The amino acids were extracted from the samples by acid hydrolysis using HCl solution (6N) containing phenol 0.1%. Then, plant extracts were concentrated and dried under vacuum (37 °C, 20 mmHg). Ten µL of the following solution (ethanol: water: TEA, 2:2:1, v/v) was added and dried immediately under vacuum. Afterwards, 20 µL of the derivative solution (ethanol: water: TEA, PITC, 7:1:1:1, v/v) was added and dried under vacuum passing 20 min to remove the extra derivative solution. For sample analysis by HPLC, derivative samples were dissolved in solution of ethanol: water: TEA, 2:2:1, v/v and then 20 µL of them were injected to HPLC system. Chromatographic analysis was carried out on a Knauer HPLC system (Germany) coupled to a UV Detector K-2500 monitored at the 269 nm, equipped with a degasser, an injection valve (20-µL sample loop), a quatry pump, an oven Knauer, a reverse phase C_{18} column (5 µm, 4.6 ×250 mm) as the stationary phase and the Chem32 software. The mobile phase consisted of two solvents A: sodium acetate buffer (11.48 g sodium acetate dissolved in 1000 mL distilled water, pH=6.1): acetonitrile (940:60, v/v) and B: acetonitrile: water (60:40, v/v). In order to achieve a suitable resolution, gradient elution programs were tested. The gradient started with 100% solvent A, decreasing to 0% in 12 min, left at 0% till 5 min, and increasing to 100% in 8 min. The flow rate was kept constant at 1 mL/min and the analysis was performed at 38°C.

Statistical analysis
Proximate and mineral compositions were evaluated in triplicate for each sample and mean values with standard deviation were presented. The obtained data were analyzed with the SPSS statistical package, version 21 (SPSS Inc. Chicago, IL, USA). Analysis of variance (ANOVA) was used to evaluate the differences of distribution between different parts of *Eryngium caeruleum*. For multiple comparisons TUKEY test was used. Statistical significance was set at ($p<0.05$).

Results and Discussion

The amounts of the proximate composition (moisture, ash, fiber, carbohydrate, protein, fat and calorie) measured in dried roots, spring and autumn leaves of *E. caeruleum* have been shown in table 1.

Table 1. Proximate composition of roots, spring and autumn leaves of *Eryngium caeruleum* (%dry weight ± SD).

Parameters	Spring leaves	Autumn leaves	Roots
Fiber	16.69 ± 0.11	22.63 ± 0.24	18.73 ± 0.18
Protein	16.88± 0.21	17.9± 0.32	7.7 ± 0.14
Fat	1.61 ± 0.11	1.54 ± 0.15	1.06 ± 0.08
Carbohydrate	49.75 ± 0.21	39.56 ± 0.13	55.86 ± 0.33
Ash	7.58 ± 0.11	9.45 ± 0.24	10.12 ± 0.18
Moisture	7.49 ± 0.26	8.6 ± 0.31	6.53 ± 0.12
Calorie (Kcal)	281.01± 1.52	236.78 ± 1.31	267.58 ± 1.27

According to One-way ANOVA analysis, there was a significance differences between the fiber, protein, carbohydrate, ash, moisture and calorie amounts in various studied parts of *E. caeruleum* ($p<0.05$). In contrast, fat did not show significant difference in various studied parts.
As observed, autumn leaves showed the highest amount of fiber, protein and moisture contents. Spring leaves contained the highest levels of calorie, while roots ranked first in ash and carbohydrate contents.
The mineral contents (iron, zinc, copper and manganese) measured in dried roots, spring and autumn leaves of *E. caeruleum* have been demonstrated in table 2.

Table 2. Mineral composition of roots, spring and autumn leaves of *Eryngium caeruleum* (mg/kg dry weight ± SD)

Elements	Spring leaves	Autumn leaves	Roots
Iron (Fe)	994.76 ± 38.69	2632.78 ± 60.36	2723.79 ± 73.98
Copper(Cu)	11.75 ± 0.10	12.22 ± 0.37	6.20 ± 0.29
Zinc (Zn)	62.56 ± 5.28	136.21 ± 6.68	129.53 ± 12.47
Manganese (Mn)	158.99 ± 1.15	218.71 ± 4.26	203.31 ± 5.86

Table 3. Amino acid composition of spring leaves, autumn leaves, roots of *Eryngium caeruleum* (g/100 g protein ± SD) and the FAO/WHO (2007) recommended amounts a of the essential amino acid

Amino acid (Abbreviation)	Spring leaves	autumn leaves	roots	FAO/WHO/UNU	
				Child	Adult
EAA					
Histidine(His)	2.9	2.8	3.0	1.90	1.60
Threonine(Thr)	9.5	10.7	5.5	3.40	0.90
Valine(Val)	5.2	5.3	3.2	3.50	1.50
Methionine(Met)	1.5	1.3	3.9	2.70	1.70
Isoleucine(Ile)	2.7	3.0	1.9	2.80	1.30
Leucine(Leu)	2.9	4.5	2.2	6.60	1.90
Phenylalanine(Phe)	3.7	3.9	1.2	6.30	1.90
Lysine(Lys)	2.2	1.2	2.4	5.80	1.60
TEAA	38.6	40.5	38.5		
NEAA					
Aspartic Acid (Asp)	9.4	7.1	15.2	-	-
Glutamic Acid (Glu)	13.8	15.2	14.7	-	-
H-Proline (H-Pro)	1.9	1.2	4.4	-	-
Serine (Ser)	8.1	7.1	6.1	-	-
Glycine (Gly)	10.3	10.0	5.7	-	-
Arginine (Arg)	8.0	7.8	15.2	-	-
Alanine (Ala)	4.6	6.3	3.3	-	-
Proline (Pro)	9.5	8.4	10.3	-	-
Tyrosine (Tyr)	3.6	4.2	1.8	-	-
TNEAA	61.4	59.5	61.5		

TEAA: total essential amino acids; EAA: essential amino acids; NEAA: nonessential amino acids; TNEAA: total nonessential amino acids

FAO/WHO (2007). Protein and amino acid requirements in human nutrition. Report of a Joint WHO/FAO/UNU expert consultation, WHO technical report series no. 935. Geneva, Switzerland: World Health Organization

One-way ANOVA analysis approved that mineral contents showed significant differences in various investigated parts of *E. caeruleum* ($p<0.05$).Tukey analysis showed that Fe amount in spring leaves had significant differences in comparison with other studied parts. Additionally, Cu content in roots showed significant differences compared to rest of the investigated parts.

As shown in table 2, iron (Fe) showed the highest amount in all parts of E. caeruleum. Autumn leaves contained the highest amount of zinc (Zn), copper (Cu) and manganese (Mn), whereas roots had the highest level of iron.

The amino acid composition investigated in dried roots, spring and autumn leaves of *E. caeruleum* have been presented in table 3. In the present study, 17 amino acids were measured including eight essential amino acids and nine non-essential amino acids.

For reference purpose, the FAO/WHO (2007) recommended amounts of the essential amino acids [18] has been also presented in table 3.

Our results showed that threonine was found to be the dominant essential amino acid in all parts of *E. caeruleum*, whereas the major non-essential amino acids in spring leaves and autumn leaves was glutamic acid and in roots arginine and aspartic acid.

Several studies have been carried out on the nutritional value of several plants previously but the nutrient composition of different parts of *Eryngium caeruleum* M.Bieb. has been measured for the first time in the current study.

In a study on 14 medicinal plants growing in Pakistan by Hussain et al. (2009), the contents of moisture, ash, fiber, carbohydrate, protein, fat and calorie were in the range of 3.71-19.20%, 2.45-18.56%, 1.20-32.62%, 55.81-84.80%, 1.81-13.32%, 0.41-5.76% and 316.87-377.40%, respectively. Also the mineral contents including Fe, Zn and Cu were in the range of 7.6-4225, 3.4-51.4 and 0.2-47.4 ppm, respectively [19]. Adnan et al. investigated the proximate composition of five medicinal herbs collected from Pakistan. The amounts of moisture, ash, carbohydrate, protein, fat and mineral contents (Fe, Zn, Cu and Mn) were in the range of 1.32-7.42%, 7.49-24.38%, 45.66-80.48%, 5.26-8.59% and 1.89-14.35%, 5-2787 ppm, 18-39 ppm, 5-10 ppm and 25-269 ppm, respectively [20]. Hussain et al. carried out an investigation on four species including *Sonchus eruca*, *Melia azadirchta*, *Withania coagulans* and *Fagonia indica* in Pakistan to find the proximate composition (moisture, ash, fiber, carbohydrate, protein, fat and calorie contents) to be 6.69-12.72%, 10.62-15.68%, 15.10-45.44%, 18.28-65.43%, 6.48-42.72%, 2.29-2.59% and 267.56-332.01%, respectively and the amounts of Fe, Cu and Mn were reported in the range of 1.69-3.92, 0.31-7.21 and 0.19-0.38 ppm, respectively [21].

Another research was carried out on ten traditional plants including *Eryngium foetidum* in India by Singh et al. The results showed that the ash, fiber, protein, fat, Fe, Zn, Cu and Mn contents in dried sample were in the range of 1.2-13.50%, 1.75-9.72%, 2.63-6.75%, 0.26-4.17%, 61.30-273.30 ppm, 1.60-45.30 ppm, 5.20-768.70 ppm and 10.50-54.50 ppm, respectively [22].

The ash, total fat and protein contents in leaves and stems of *Eryngium creticum* reported by Dammous et al. were in the range of 18.07-22.1%, 0.84-4.10% and 4.30-13.30%, respectively. Also the amounts of Fe, Cu and Mn measured as 42.94, 2.35 and 18.38 ppm in the leaves and 30.21, 0.83 and 6.04ppm in the stems, respectively [23]. According to Chen et al., a research carried out on nutritional composition of maca (*Lepidium meyenii* Walp.) which cultivated in various regions of China. Nutritional compositions of maca were investigated across cultivation areas and between color types. The reported amounts for moisture, fiber, protein, fat, Fe, Zn, Cu and Mn contents of maca were in the range of 4.63-9.55%, 18.25-25.55%, 9.56-20.85%, 0.60-0.93%, 58.10-129.80 ppm, 23.3-39.7 ppm, 4.3-7.8 ppm and 9.8-17.1 ppm, respectively [24].

As mentioned before, the amounts of moisture, ash and carbohydrate in our study were approximately similar to amounts reported in the above mentioned papers. Additionally, the fiber contents in our investigation were in the range reported by Hussain et al. studies in 2009 and 2013 [19,21], but they were higher than those reported by Singh et al. (2011) [22].

The results we obtained from protein analysis were relatively higher than the amounts estimated in above mentioned papers. In contrast, fat and calorie amounts in our study were almost lower than those reported for the mentioned medicinal plants. Plants are not expected to have high amounts of fat and calorie. So the result could be considered as acceptable. Considering the high amounts of protein, fiber and carbohydrate, it can be concluded that the studied plant showed remarkable nutritional value.

The results of Fe measurement in our study were remarkably higher than majority of the plants in the above mentioned studies. Furthermore, the amounts of Zn and Mn were considerably higher than mentioned values in above articles.

Cu analysis results were higher than reported Cu contents by Hussain et al., Dammous et al. and Chen et al. [21,23,24], while they were lower than reported amounts reported by Singh et al. [22] and were approximately similar to the reported results by Hussain et al. [19] and Adnan et al. [20].

Considering the high iron contents in *E. caeruleum*, this plant can be suggested as a rich iron herbal source especially for women and children.

It has been demonstrated that many enzymes require small amounts of minerals for perfect activity [15]. Additionally, iron, zinc and manganese strengthen the immune system because of their antioxidant properties [25,26]. As a result, *E. caeruleum* can be used for perspective development of medical supplement due to its remarkable mineral contents.

In comparison with FAO/WHO (2007) recommended essential amino acids amounts [14], our results showed higher amounts of threonine and histidine in all studied parts of *E. caeruleum*. Furthermore, phenylalanine and valine amounts in spring and autumn leaves and methionine in roots were higher than recommended amounts by FAO/WHO (2007).

Qin et al. carried out a research on amino acid composition of several non-transgenic and beta-carotene-enhanced transgenic soybeans varieties [27]. The results of our study demonstrated that the amounts of most measured essential amino acids were higher than reported amino acids amounts in non-transgenic commercial soybeans by Qin et al. According to Chen et al., investigation on maca (*Lepidium meyenii* Walp.) the reported amounts for total essential amino acids (TEAA) in different types of maca were in the range of 18.91-31.29 g/100g protein [24]. The amounts of TEAA in all studied parts of *E. caeruleum* were higher than reported those amounts.

Essential amino acids should be supplied through dietary intake because human body cannot synthesize all required amino acids [15]. Therefore, it can be concluded that *E. caeruleum* can be a good source of rich protein and essential amino acids for daily intake. Both aerial parts and roots of *E. caeruleum* are rich sources of nutritional contents while autumn leaves were found to be richer than other studied parts of this plant in protein, essential amino acids and mineral contents.

These results of the study show a starting view point for further utilization of various parts of *E. caeruleum* in food preparations and medical supplements; however, it is recommended that *E. caeruleum* be assessed from the aspect of toxic effects. After checking of plant's safety for human intake, it can be recommended as a nutrient-rich plant in daily diet to achieve higher level of health and enhance the body's immune system.

Author contributions

Masoumeh G. Sepanlou: acquisition of data, analysis and interpretation of data; Fatemeh Salami: Drafting of the manuscript, statistical analysis; Mehran Mirabzadeh Ardakani: analysis and interpretation of data, critical revision of the manuscript for important intellectual content; Seyedeh Nargess Sadati Lamardi: administrative, technical and material support, statistical analysis; Sima Sadrai: study concept and design, critical revision of the manuscript for important intellectual content; Gholam-Reza Amin: drafting of the manuscript, study supervision; Naficeh Sadeghi: analysis and interpretation of data, administrative, technical and material support; Mannan Hajimahmoodi: study concept and design, study supervision, administrative, technical and material support

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

AOAC: official methods of analysis of the association of official analytical chemists; Cu: copper; Fe: iron; Mn: manganese; PITC: phenyl isothiocyanate; TEA: triethylamine; TEAA: total essential amino acids; Zn: zinc