Research Article

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Ecological and phytochemical attributes of endemic *Ferula gummosa* Boiss. at vegetative and generative stages

Endemik *Ferula gummosa* Boiss’in Vejetatif ve Üretici Aşamalarında Ekolojik ve Fitokimyasal Özellikleri

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Abstract

**Objective:** This study was accomplished to find out the ecological as well as some biochemical and physiological properties of *Ferula gummosa* Boiss.

**Methods:** Soil samples were analysed. Different plant parts collected during different stages were analysed biochemically (catalase, peroxidase, ascorbate peroxidase and total protein) and physiologically (proline, sugars, phenolic components and photosynthetic pigments).

**Results:** Soil analysis showed that pH, EC and phosphatase activity were approximately 6.5–6.8, 200 μS/cm and 890 μmol/min, respectively. Among measured elements in soil, only P and Na had significantly higher concentrations at generative and vegetative stages, respectively. The biochemical and physiological analyses of *F. gummosa* Boiss. leaves and roots showed that the content of proline, sugar and phenolic components were significantly higher at generative stage than vegetative stage, while the content of photosynthetic pigments and activities of catalase, peroxidase and ascorbate peroxidase were significantly lower. Our qualitative analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that the total protein bands of generative stage were more intensive than vegetative stage.

**Conclusion:** The phytochemical results strongly supported the idea that the metabolic changes were developmental-dependent.

**Keywords:** *Ferula gummosa* Boiss; Antioxidant enzymes; Developmental stages; Photosynthetic pigments; Soil analysis.

Özet

**Amaç:** Bu çalışma, *Ferula gummosa* Boiss’in ekolojik ve bazı biyokimyasal ve fizyolojik özellikleri bulmak için yapılmıştır.

**Material ve Metod:** Toprak numuneleri analiz edildi. Farklı aşamalarda toplanan farklı bitki parçaları, biyokimyasal olarak (katalaz, peroksidaz, askorbat peroksidaz ve toplam protein) ve fizyolojik olarak (prolin, şekerler, fenolik bileşenler ve fotosentetik pigmentler) analiz edildi.

**Bulgular:** Toprak analizi, pH, EC ve fosfataz aktivitesinin sırasıyla yaklaşık 6.5–6.8, 200 μS/cm ve 890 μmol/dk olduğunu göstermiştir. Toprakta ölçülen elementler arasında sadece P ve Na konsantrasyonları önemlidi derecede yüksekti. *Ferula gummosa* Boiss’in yapraklarında ve köklerinde yapılan biyokimyasal ve fizyolojik analizlerde, prolin, şeker ve fenolik bileşenlerin içeriği vejetatif evrede daha yoğun olduğu görülmüştür. *Ferula gummosa* Boiss’in yapraklarında ve köklerinde yapılan biyokimyasal ve fizyolojik analizlerde, prolin, şeker ve fenolik bileşenlerin içeriği vejetatif evrede daha yoğun olduğu görülmüştür. Sodyum dodesil sülfat poliakrilamid jel elektroforezi ile nitel analiz sonucu, generatif evre toplam protein bantlarının vejetatif evrede göre daha yoğun olduğunu göstermiştir.
**Introduction**

Two major problems related with the sustainable use of medicinal plants, native to the certain countries, are the ecological requirements and biochemical properties that have not well understood [1]. *Ferula gummosa* Boiss., a member of Apiaceae family, is one of the most important medicinal plants with a large number of applications within both industry and traditional medicine [2]. Its main habitats are Turkmenistan, Pakistan, and a vast area in northeast Iran with an average annual precipitation of 250–500 mm [3]. Some of the 30 species of *Ferula* belonged to Iranian flora are endemic, including *Ferula tabasensis*, *F. gummosa* Boiss., *F. persica* and *F. gummosa* Boiss. lives about 6–8 years reaching nearly 0.8–3 m in height when growing voluntarily in Iran. The stem of this resinous and odoriferous plant contains typically several elliptical ducts dispersed in a phloem that can be exuded manually or naturally during the vegetative stage of its life cycle. In addition, the root of the plant is glandular and rich in oleogum-resin [3]. It has been reported that the root extracts of *F. gummosa* has inhibitory effects on growth of some bacterial pathogens. Moreover, long-term consumption of hydro-alcoholic extract of *F. gummosa* root increases the defence of the body against oxidative stress [4].

During the last two decades, such medicinal plants had under increasing investigation. *Ferula gummosa* Boiss. as an Iranian traditional herbal drug has been employed individually or in mixtures for several purposes, e.g. wound-healing, memory enhancement, in gastrointestinal disorders, epilepsy and chorea [5]. Several biological activities, such as antibacterial, cardioprotective, spasmylic, anticonvulsant and antiepileptic effects [6], have been proved for extracts from different organs of *Ferula gummosa* Boiss.

Flower differentiation is an exciting event in plant morphogenesis with respect to the perception of external and/or internal signal(s) and switching the developmental process from vegetative to generative stage [7]. Carbohydrates, or sugars, regulate various aspects of plant growth through modulation of cell division and expansion. Besides playing essential roles as an energy sources for growth and as structural components of cells, carbohydrates also regulate the timing the expression of developmental programs. Recent studies suggested an important role for proline in reproduction development [8]. Interaction of phenolics with growth regulators could regulate developmental processes [9]. Also, considerable evidence supports the changes of antioxidant enzyme activities during the transition of plants to flowering stage [7]. Many genes and proteins were considerably upregulated during generative stage [10].

Recent research has mainly focused on chemical components of *F. gummosa* Boiss., whereas studies of physiological and biochemical characteristics of the plant remain scarce. Therefore, this study has been accomplished to evaluate the antioxidant enzymes, proline and sugar content, and phenolics and total protein in different parts of the plant (leaf and root) and various photosynthetic pigments in leaves of the plant during both vegetative and generative stages. Moreover, to the best of our knowledge, to date, no ecological research has been conducted on the plant growth habitats. Furthermore, nearly all essential and non-essential elements and toxic heavy metals in both soil and plant, soil electric conductivity (EC), pH, and phosphatase activity as well as geographical status of the plant habitats were determined. Our ecological data could be helpful not only for Geographical Information System (GIS), but also for medical plant growers to identify the areas appropriated for culturing this plant other than its natural habitats.

**Materials and methods**

**Plant material**

Plant materials for biochemical analysis were leaves and roots of non-flowering (vegetative stage) and flowering (generative stage) *F. gummosa* Boiss. collected from Taham Mountain (Zanjan Province, Iran) at an altitude of 2450 m around N: 36° 50’ 02.6”; E: 048° 37’ 50.1” in June 2014 and stored at 80°C. The taxonomic identification was determined by University of Zanjan, Department of biological sciences. The 18S rRNA sequence of the plant was also recorded in GenBank (Accession no. KM983398.1) by the authors.

**Proline assay**

Proline content was assayed according to the revised method of Bates et al. [8]. Samples were homogenized...
in 10 cm³ 3% (w/v) sulfosalicylic acid, and proline was assayed by the acid ninhydrin reagent. The absorbance was measured at 520 nm via using a spectrophotometer (Shimadzu Corporation UV-2450, Tokyo, Japan). Proline concentration was determined using a calibration curve and expressed as μmol/g fresh weight (FW).

### Total soluble sugar (TSS) and total phenolic content

In order to calculate TSS, fresh samples were extracted with 80% ethanol (1:10, w/v) and evaporated in 95°C hot water. Afterwards, they were made up to 2.5 cm³ with distilled-deionized water. The extract (0.2 cm³) was mixed with 5 cm³ of fresh 0.2% anthrone in sulfuric acid (w/v) and the mixture was then put in boiling water for 17 min. After cooling on ice, the absorbance was measured at 630 nm. Glucose was used as a standard for soluble sugars.

Total phenolic content was determined colorimetrically by using Folin-Ciocalteu reagent [11] modified as follows: each sample (1 g) was extracted with 6 cm³ of extraction buffer containing acetic acid and methanol (85:15, v/v). Afterwards, 10 μL of the methanic extract were mixed with 190 μL of distilled water in a test tube followed by addition of 1 cm³ of 10% Folin-Ciocalteu reagent and allowed to stand for 5 min. Then, 800 μL of 7.5% sodium carbonate solution was added. Each sample was allowed to stand for 90 min at room temperature in darkness and the absorbance was measured at 760 nm by using the spectrophotometer. Gallic acid equivalent (GAE) was used as a standard and the results were expressed as mg/g FW.

### Pigment assays

Chlorophylls (Chl a and Chl b) and carotenoids were determined via the method developed by Kichtenthaler and Wellburn [12]. Fresh plant material (0.5 g of leaf) was homogenized in 20 cm³ of 80% acetone (v/v). The absorbance of the supernatant measured at 663 and 645 nm for chl a and b, respectively, and at 470 nm for carotenoids using the spectrophotometer. The amounts of the pigments were finally calculated and expressed as mg/g FW.

### Protein analysis

Fresh samples (leaves or roots) were frozen in liquid nitrogen immediately after harvesting and stored at −80°C. One gram of sample was homogenized in 3 cm³ of 0.05 M Na-phosphate buffer (pH 7.8) including 1 mM EDTA and 2% (w/v) PVPP (polyvinylpolypyrrolidone). The homogenates were centrifuged at 14,000 rpm for 30 min at 4°C. Supernatant was used for protein determination and enzyme assays. All assays were done at 4°C.

The protein content was determined according to the method of Bradford [13] using bovine serum albumin as a standard. For qualitative analysis of proteins, discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by using 12% acrylamide gel and protein bands were stained with silver. In addition, both quantity and quality parameters of total protein was assessed by TotalLab software version 1.10.

### Enzyme assay

Peroxidase (POD) activity was determined based on the method described by Trinder [14] using 4-aminoantipyrine as hydrogen donor, which measures the increase in absorbance at 510 nm resulting from the decomposition of hydrogen peroxide. One enzyme unit is defined as μmol cm⁻³ destroyed H₂O₂ per minute. Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada [15]. The assay depends on the decrease in absorbance at 290 nm as ascorbate was oxidized. One enzyme unit is defined as μmol cm⁻³ oxidized ascorbate per minute. Catalase (CAT) (EC 1.11.1.6) activity was determined according to the method of Aebi [16]. The reaction mixture contained enzyme extract, 100 mM potassium phosphate buffer (pH 7.0) and 10 cm³ of 30% H₂O₂. The reaction was initiated by adding H₂O₂ and the absorbance was measured at 240 nm. One unit of CAT was defined as the amount of enzyme catalyzing the decomposition of 1 mmol of H₂O₂ per minute.

### Soil material

The rhizosphere soil samples during vegetative and generative stages of the plant growth were collected separately from 0 to 30 cm depth, at least with five replicates, after removal of the litter layer. Five soil samples obtained from 5 cores (2.5 cm in diameter) were randomly selected and evenly combined to each other. After putting in sealed plastic bags, soil samples immediately taken to the laboratory. Each sample was passed through a 2 mm sieve after removing roots, stones and other impurities. Each sample was divided into two aliquots. One aliquot was air dried for analysis of related soil chemical properties. The other
one was kept without drying at 4°C until analysis of phosphatase activity was conducted.

**Soil analysis**

The air-dried and pulverized samples were analyzed for pH measurement in soil: deionized water suspension (1:2.5) using a glass electrode and for EC using a conductimetre (HQ40d Portable, Hach, USA) in extracts obtained from saturated soil. Potential soil phosphatase activity was measured within 72 h of sampling according to the method of Tabatabai and Bremner [17]. Briefly, 1 g soil was incubated in modified universal buffer solution (pH 11.0) with para-nitrophenyl phosphate (p–NP) substrate (Sigma-Aldrich, USA) at 37°C. After 1 h, reactions were stopped with 0.5 M NaOH, filtered with Whatman 42 paper and the formation of p-nitrophenol determined colorimetrically using a spectrophotometer at 420 nm. The phosphatase activity was expressed as μmol p-NP per gram dry soil and incubation time (μmol p-NP/g. h).

**Soil and plant elements**

In order to analyze elements in soil, air-dried and sieved soil samples were completely milled to 100 μm with an agate mill; 250 mg aliquot from each powdered and homogenized sample was mineralized with Aqua Regia (6 cm³ HCl 37% plus 3 cm³ HNO₃ 65% Suprapur, E. Merck, Germany) in a microwave oven (model Milestone 1200) in a Teflon vessel with specific soil digestion program. After cooling, solutions were made up to 20 cm³ with Milli-Q water and then filtered with Whatman 42 (Sigma-Aldrich, USA).

For analysis of elements in F. gummosa Boiss. leaf and root, according to the method recommended by Unterbrunner et al. [18], 0.5 g of milled sample was digested in an acid mixture containing 5 cm³ 65% HNO₃ and 3 cm³ 30% H₂O₂ in open vessels on a hot plate, followed by filtration with filter cellulose Whatman 42. Contents of 14 elements (As and P, Mg, K, Na, Mn, Fe, Ni, Cu, Zn, Cd, Co, Cr, Pb) were determined by Inductive Coupled Plasma Optical Emission Spectrometry (ICP-OES, Spectro Ametek, Arcos) for both soil and plant samples.

**Statistical analyses**

Plant and soil samples were collected at least with five replicates. All experiments were repeated at least three times for each sample. The data were expressed as mean ± S.E. Analysis of variance was conducted using one-way ANOVA test using SAS version 9.1. p-Values <0.05 were regarded as statistically significant. Mean comparisons to identify significant differences among treatments were performed using Dunnett’s test.

**Results and discussion**

**Proline, TSS and total phenol contents**

The proline accumulation in organs of flowering plants observed in the leaves and roots at generative stage had the proline content 50.46% and 52.96%, respectively, higher than those in vegetative one (Figure 1A). In our results, proline accumulation has been shown to occur in generative stage. It may back to the ability of plant to remain more sustainable under low precipitation condition at generative stage. By increasing proline as a smolite, plant cells miss less water, thereby metabolic activity will be nearly protected from low water conditions. Proline accumulation was largely viewed as a specific reaction to overcome stress conditions, while recent studies suggested important functions of proline metabolism also in generative development. Also, it has been reported that proline content is related to plant age and leaf age [19].

The amount of TSS in roots of F. gummosa Boiss. significantly changed during developmental transition, while it remained unchanged in leaves. As shown in (Figure 1B), TSS content of roots increased to 7.03396 mg/g FW in generative one, whereas it was only 3.10688 mg/g FW in vegetative one. These results indicated either the transmission or the consumption of root-stored sugars during generative stage. It has been reported that plants store energetic substances during their vegetative stage and use them in generative stage [20]. The pivotal role of sugars as signalling molecules has been illustrated by the variety of sugar-sensing and signalling mechanisms discovered in plants (Arabidopsis) and microorganisms (yeast and bacteria) [21]. Sugar signals have been reported to be generated either by carbohydrate concentration or by relative ratios to other metabolites [21]. In the last decade, trehalose-6-phosphate (T6P), an intermediate in trehalose metabolism, has been shown to regulate embryonic and vegetative development, flowering time, meristem determinacy and cell fate specification in plants. The accumulation of sugar in F. gummosa Boiss. during generative stage could be associated with not only energy supply, but also signalling for flowering. Moreover, a rapid breakdown of...
The total phenolic content in leaves and roots of flowering plants were higher than those of vegetative plants (Figure 1C). It might reveal that the accumulation of total phenolics in *F. gummosa* Boiss. was greatly dependent on the plant developmental stage. Increased levels of phenolic compounds similar to our results during flower development has been reported by Ebrahimzadeh and Abrishamchi [6]. It has been proposed that phenolics play a considerable role in enhancing plant growth and development. The preferential oxidation of phenolics by POD prevents peroxidase-catalyzed oxidation of auxin. Through their interaction with growth regulators, phenolics intervene in the regulation of developmental processes [9]. In addition, the defensive role of them was reported against a variety of biotic and abiotic stresses [14].

**Photosynthetic pigments contents**

Chlorophyll and carotenoid contents changed remarkably during flower development. A 58.78% and 75.01% reduction was observed in total chlorophyll and carotenoid contents, respectively, during generative stage as compared with vegetative one (Figure 2). The reduction in chlorophyll content under generative stage was also reported in some plants like barley [23]. Generative development may trigger leaf senescence in many plant species, especially in monocarpic plants that have single generative growth in their life history. Obvious visual symptom of leaf senescence is loss of chlorophyll. Based on our results, chlorophyll and carotenoid degradation massively occurred during generative stage followed by fruit ripening and leaf senescence.
Protein content

Protein content in different parts of *F. gummosa* Boiss. was analyzed as both quantity and quality. The results of quantitative analysis showed the higher accumulation of proteins in leaves and roots of flowering plants (Figure 3A).

According to SDS-PAGE and TotalLab software analysis (Figure 4), protein pattern of leaves and roots of *F. gummosa* Boiss. in both vegetative and generative stages were different. The protein bands of generative stage were more intensive than vegetative stage. Also, the number of protein bands in leaf was more than that in root. These qualitative data confirmed our protein quantitative results. So it seems, because of their metabolic activity, proteins are concentrated in the leaf of the *F. gummosa* Boiss. Also, TotalLab software are shown the results of protein densitometry (Figure 4). It shows that both the number and the concentration of protein at generative stage were more than that at vegetative one in roots and leaves. So that, in root and leaf, 34 and 44 protein bands during generative stage was found respectively, while only 15 and 22 at vegetative stage. The areas surrounded by each peak in generative stage are greater than those in vegetative stage. It means that the concentration of total protein in generative stage are also higher than that in vegetative stage. These results support quantitative analysis of total protein.

Digits represent the number of protein, and the area surrounded by each peak shows the concentration of protein. A large number of genes and proteins were upregulated during generative stage. Some proteins are necessary for plant responses to environmental conditions [24]. Proteins are involved in processes such as catalyzing chemical reactions (enzymes), facilitating membrane transport, intracellular structure and energy generating reactions involving electron transport. Also, Zhang et al. [10] proposed during flowering transition, the genes and proteins linked to carbohydrate metabolism, fatty acid biosynthesis and redox reaction were mainly upregulated at the induced stage, as well as the proteins related to starch synthesis, lipid metabolism, cytoskeleton proteins, IAA and ethylene signalling were continuously upregulated. Moreover, high content of total protein in generative
stage could be indicated the synthesis of new proteins by the plant required for flowering [25]. It is thus likely that the presence of proteins as indicators of developmental changes over the plant life.

**Antioxidant enzyme activities**

Investigation for the presence of antioxidant enzymes in *F. gummosa* Boiss. showed the activity of POD, APX and CAT at both vegetative and generative stages (Figure 3B, C and D). The activities of these three enzymes at vegetative stage were significantly (p ≤ 0.05) greater than those at generative one. Plant cells possess well-developed systems to regulate the level of reactive oxygen species (ROS), and the concentration of ROS can be changed or affected by several antioxidant enzymes such as superoxide dismutase (SOD), CAT and POD [14–16].

It was reported that leaf cells express POD gene in order to diminish the adverse effects of environmental conditions [26]. It has been demonstrated that POD plays a key role in cell wall stiffening through formation of cross-links between polymers of wall. In addition, high POD activity during plant growth may be attributed to its involvement in auxin metabolism, lignin biosynthesis, and wound healing [26]. It is possible that these enzymes participate in indole 3-acetic acid (IAA) catabolism, so that the IAA levels become favourable for the initiation and differentiation of floral organs [7]. Moreover, it has been shown that the level of antioxidant enzymes may be reduced during plant generative stages followed by increase in ROS, and the latter resulted in programmed cell death. Ye et al. [27] established a model for the coupling of floral initiation and senescence suggested that down-regulation of APX and SOD would cause an accumulation of H$_2$O$_2$ to activate senescence-associated genes expression in leaves. APX and CAT have been reported to play key roles in detoxification of ROS, particularly in H$_2$O$_2$ removal from cells. It has been shown that the cells containing enough amount of ascorbic acid could express APX during plant growth and development. The decline in APX activity in leaves of *F. gummosa* Boiss. during floral transition is parallel with the findings of Bañuelos et al. [28] in *Arabidopsis*. They suggested that floral transition depended on APX decline, and subsequent H$_2$O$_2$ elevation was involved in activating plastid 13- lipoxygenase
nutrients is particularly important during generative stage when seeds, fruits and storage organs are formed. At this growth stage, root activity and nutrient uptake generally decrease, mainly because of decreasing carbohydrate supply to the roots. The extent of remobilization is also important for diagnosis of the nutritional status of plants. During the generative stage, the degree of remobilization of micronutrients is often surprisingly high compared with that of during vegetative growth [29]. According to our results, more Fe, Cu and Mn remobilization to flowers of *F. gummosa* Boiss. resulted in decrease of them in leaves. It seems that Fe, Cu and Mn were more effective in flower formation. Also, the extent of remobilization of the micronutrients such as Cu, Fe and Zn, but not Mn, is closely related to leaf senescence [29].

It is notable that mineral nutrients have specific and essential function in plant metabolism. The pivotal role of Fe has been previously proved not only in chlorophyll biosynthesis, but also in ferredoxin, a component of electron transfer chain in photosynthesis apparatus. Moreover, Cu is a component of plastocyanin structure. In photosynthesis, plastocyanin functions as an electron transfer agent between photosystem II and photosystem I. Apart from Fe and Cu, Mn has an essential role in photosynthesis process by affecting the Hill reaction. It seems that *F. gummosa* Boiss. has an efficient capability of absorbing the necessary elements from soil during active growth period, vegetative stage, and saving mineral nutrients required for flowering.

### Soil analysis

The investigation concerning soil medium of voluntarily grown *F. gummosa* Boiss. showed pH values between 6.5 and 6.8, and EC 200 μS/cm. This was the first report exhibited that *F. gummosa* Boiss. grows appropriately in nearly neutral soils with moderate EC. No previous studies were reported to reveal such soil characteristics for this plant. The changes of soil elements concentrations are shown in Table 1 Among soil measured elements, the concentration of P and Na significantly changed as plant developmental stage changed. So that, P concentration was higher at generative stage in soil surrounded the roots than that at vegetative stage. Inversely, soil Na concentration was higher at vegetative stage. It has been previously reported that Na could stimulate growth through increase in cell expansion in some plants. Our results suggested the probable requirement of *F. gummosa* Boiss. for Na during vegetative growth period, since it has been accumulated in rhizosphere during that period. The

### Plant elements

Among plant measured elements, only Fe, Mn and Cu were significantly higher in leaves and roots of non-flowering plants (Figure 5A–C). Also, only Mn significantly increased in roots during flowering. Remobilization of

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**Figure 5:** Mn (A), Fe (B) and Cu (C) contents in the different organs of *F. gummosa* Boiss. at both vegetative and generative stages. Vertical bars represent ±S.E. and different letters are significantly different at p < 0.05.
accumulation of P in the surrounding of roots might be attributed to the more requirement of *F. gummosa* Boiss. for P at generative stage followed by P movement toward rhizosphere from bulk soil. The crucial role of P was extensively explained in energy-containing molecules, that is, nucleic acids, and phosphorylated sugars, lipids as well as in proteins controlling the flowering and fruiting of plants [7]. Moreover, the soil pH range with greatest phosphorous availability is 6.0–7.0, which measured soil pH showed the value between the ranges. Based on our results, soil phosphatase activity in the region was 890 μmol/min. The enzyme in soils originates not only from microbial sources but also from plants. It has been reported that phosphatase activity in soil is negatively correlated with increasing pH and available phosphorus level in the nutrient medium. The mineralization of organic P is affected by the action of phosphatases. In our study, phosphorous was accumulated just when the plant came into generative stage. It may suggest the involvement of *F. gummosa* Boiss. root in phosphorous availability probably through phosphatase secretion. Šarapatka et al. [30] have demonstrated that root-secreted phosphatase activity, called extracellular, is related to plant ability to make soil P available for absorption. They also have reported that phosphatase activity is influenced by crop plants, soil properties and farming systems. According to our result, the effect of plant developmental stage on activity of soil phosphatase could be focused by more researches.

Finally, *F. gummosa* Boiss. grows appropriately in its habitat, Zanjan Mountains, in a neutral soil, pH values between 6.5 and 6.8, with no salinity problem (EC: 200 μS/cm). Overall, the plant contains more amounts of etabllites including total phenol, proteins and sugars in generative stage in comparison with vegetative growth period. Moreover, *F. gummosa* Boiss. have a high ability to absorb the required elements for growth and development. The beneficial use of the plant organs could be recommended preferably during generative stage. Also, results of soil analysis could help to the medical plant growers for identifying the areas appropriated for culturing this plant other than its habitats. However, future studies on tissue culture are thus required in order to obtain pharmaceutical compounds in *F. gummosa* Boiss.

**Table 1:** Element content (mg g⁻¹ soil) in natural habitat of *Ferula gummosa* Boiss.

| K   | Na  | Mg  | P   | Mn  | Fe  | Ni  | Cu  | Zn  | Cd  | Co  | Cr  | Pb  | As  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 0.011 | 0.052 | 0.233 | 2.137 | 0.411 | 14.129 | 0.008 | 0.173 | 0.006 | 0.004 | 0.005 | 0.005 | 0.019 | ND  |

Values are mean of three replications. ND, not determined.

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**Conflict of interest statement:** The authors have no conflict of interest.

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