

MORPHOLOGICAL AND ANATOMICAL CHARACTERIZATION AND TRACE ELEMENTS COMPOSITION OF *ONOSMA ARGENTATA* HUB.-MOR. (BORAGINACEAE) ENDEMIC TO TURKEY

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Abstract

Onosma argentata Hub.-Mor. is an endemic species growing in Turkey. It is under the threat like many other endemic species in Turkey and its IUCN category is evaluated as vulnerable. According to literature, this species was known only from the two collections, in this study the plants were collected from different localities mentioned in literature. Plant is perennial. The stem is 20-35cm, shortly hairy and rigid-setose, clothed at base with remains of leaves. The aim of this study is to provide information on the anatomical and morphological properties of *O. argentata*. Anatomical sections taken from the root, stem and leaves of the plants. The results have been compared with some species of Boraginaceae. In addition, the concentrations of trace elements *O. argentata* were determined by using atomic absorption spectrometry. Large amount of sodium (Na), aluminum (Al), potassium (K) and iron (Fe) were present in all flowers, leaf and stem of *O. argentata* plant.

Key words: *Onosma argentata*, Morphology, Anatomy, Trace elements, Vulnerable.

Introduction

In Turkey the genus *Onosma* L. (Boraginaceae) is represented by about 101 species of which 50 are endemic (Güner *et al.*, 2012). *Onosma argentata* Hub.-Mor. is endemic to Turkey. It is under threat as many endemic species in Turkey and its IUCN category is evaluated as vulnerable (Ekim *et al.*, 2000; Anon., 2001).

Some *Onosma* species are used as herbs, folk medicines and dyes. Fahad & Bano (2012) have reported, traditionally, *Onosma* plants are used as stimulant in rheumatism, bladder pain, kidney irritation and palpitation of heart. Dried flowers of some *Onosma* species are used in folk medicine to treat respiratory ailments and some of them are used in the treatment of body swellings and their flowers are eaten (Akçin & Engin, 2005). The roots of *Onosma argentata* are used traditionally in Turkey for wound healing and burns (Özgen *et al.*, 2004). It is stated that *O. argentata* extract is effective against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and the roots of *O. argentata* has antioxidant activity (Özgen *et al.*, 2003).

Studies on the anatomy of this genus are limited (Metcalf & Chalk, 1979; Binzet *et al.*, 2012). There are several reports on the anatomy of some *Onosma* species (Akçin *et al.*, 2011; Güven *et al.*, 2013; Akçin *et al.*, 2013; Binzet & Teke, 2014). In recent years, this species has been the subject of chemical studies (Özgen *et al.*, 2004; Çiftçi *et al.*, 2010). There is no detailed study on morphological and anatomical characteristics of *O. argentata*. Therefore the present study is undertaken to fill the gap.

Trace elements play a very important role in chemical, biological, biochemical and enzymatic reaction in the living cells of plants, animals and human bodies. The role of trace elements in the body metabolism is of prime importance (Yousufzai *et al.*, 2001). Little is known of the trace elements of *O. argentum*. The study is expected to deliver preliminary data on trace element composition of *O. argentum* grown in

Turkey and provide useful information for future studies which will be conducted on biochemistry, microbiology and physiology. Second the goal of this study was to present and investigate the composition of trace elements *O. argentata* by using atomic absorption spectrometry.

Material and Methods

Plant samples were collected from the following locations:

Erzincan: Sakaltutan, 2032m, 39 52 91 N, 39 10 23 E, 17.06.2006, Serpentine, (Kandemir 7241).

Sivas: Kızıldağ, 1700m, 12.07.2006, Serpentine, (Kandemir 7795).

Taxonomical description of the plant specimens was prepared according to Davis (1978). Fresh samples and herbarium material were used in each case for experimental analysis and morphological measurements. Analysis of morphological and anatomical studies were made using 20 plant specimens. Each value is the average of 20 measurements. Anatomical studies were carried out on fresh samples or samples kept in alcohol. Anatomical measurements were made with the help of micrometric ocular using sections from the which of plant parts. Results were presented by original drawings, photographs and tables. Photographs were taken with Leica DM LB microscopy.

Solutions and reagents: All solutions were prepared using ultra pure water (specific resistance 18 MΩ cm) from a Milli-Q purification system (Millipore Corporation, Massachusetts, USA). Standard solutions of analytes were prepared from their 1000 mg L⁻¹ stock solutions (Merck). Concentrated HNO₃ for digestion samples was used ultra pure grade (Merck).

Determination of metals: The analysis was performed by ContrAA 300 Flame Atomic Absorption Spectrometer (HR-CS AAS) (GLE, Germany) equipped with a 50 mm burner head and an injection module (SFS-6). All absorption lines of an element in the spectral range of 185-900nm were analytically evaluated by using a Xe short-arc lamp as a continuum lamp source. Microwave-Assisted Acid Digestions have been made using a Premier microwave system.

Samples were digested using microwave system. 2.00 g portion of each sample dried at 80°C was accurately and 0.40 g directly weighted into PTFE (polytetrafluorethylen) bombs. For the samples decomposition concentrated 7 mL HNO₃ acid were added. In a tightly closed system, the following six-step microwave digestion program was applied according to Yousufzai *et al.* (2001). PTFE bomb was waited for an hour to cool and was carefully opened. Colorless solution was transferred into beaker and evaporated to dryness on hot plate. Afterwards final volume was diluted to 10 mL with 0,1 mol L⁻¹ HNO₃. The blank digests were carried out in the same way. Sample solutions were analyzed by HR-CS AAS.

Results

Morphological properties: Plant is perennial. Stems are numerous, up to 35cm, shortly hairy and rigid-setose, clothed at base with remains of leaves. Basal and lower cauline leaves 70-100 x 3-7mm, narrowly linear-lanceolate, margins revolute, subsesile, densely silvery adpressed-setose on both surfaces, subadpressed to patent setose on margins, middle and upper cauline leaves similar but shorter. Inflorescence of 1-3 terminal, many flowered cymes. Bracts lanceolate to linear. Pedicels to 11mm, long and short patent setose. Calyx 14-22 mm in flower, scarcely accrescent in fruit, densely patent-setose. Corolla whitish to cream, longer than calyx, cylindrical-inflated, sparsely hairy in upper. Anthers included. Nutlet 4 mm, ovoid-triangular, apex acuminate (Fig. 1A, B).

This species can be easily recognize from other *Onosma* species except *Onosma discedens* Hausskn. ex Bornm. with discolorous densely silvery adpressed-setose leaves. It is difficult to distinguish *O. argentata* from *O. discedens* due to similar these floral and leaves characteristic. *O. argentata* is restricted with small serpentine areas around Erzincan and Sivas, located East Anatolia. It has concolorous leaves with adpressed-setose on both surfaces, patentsetose on margins. But, *O. discedens* have discolorous leaves that silvery beneath with almost silky dense adpressed hairs, green above hairs sparse.

Anatomical Properties

Root: Periderm is 5-16 layered on the outer surface of root. Elements of phellem are dark coloured, crushed, break up and sometimes fell out. Cortex is multilayered and parenchymatic. Cell size is larger in primary cortex than secondary cortex. Phloem elements are located in small region of root. Cambium cells are 2-4 layered, flat and distinguishable. Xylem occupies a large area. It is of sclerenchymatic cells and trachea. Trachea different in size. The center of root are occupied by primary xylem tissue. The measurements of root are given in Table 1. (Fig. 2. I-II).

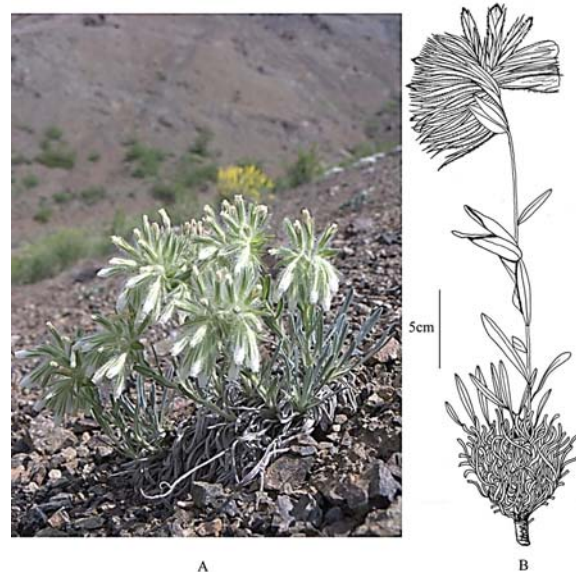


Fig. 1. General appearance of *O. argentata* (A. in field B. Drawing).

Stem: There is a cuticular layer on epidermis. Epidermis is 1-2 layered and consist of ovoidal cell. There are glandular and aglandular hairs on epidermis. Collenchyma is 1-4 layered. Cortex is 8-20 layered cells ovoidal, sometimes flatted ovoidal and different in size. Diameter of cortex cells on the endodermis are to large. Endodermis is located between cortex and vascular tissue. Cambium cells are distinguishable and 2-3 layered. Phloem covers the major part and xylem below it reaches up to the pith. There are pith rays extending up to the pith from cambium in the xylem tissue. Xylem has trachea cells different in size. The pith occupies a large space and consists of parenchymatic cells. Thicknesses of tissues in the stem were measured with the help of micrometer and results are given Table 1 (Fig. 3A, Table 1).

Leaf: Leaves are covered by a thick cuticular layer on both upper and lower surfaces. There is a single layered epidermis on both upper and lower surface of the leaf. Mesophyll consists of 1-2 layer of palisade parenchyma cells and 5-7 layers of spongy parenchyma cells. Palisade cells are rich in chloroplasts. Spongy parenchyma cells occupy a wide area in the mesophyll. Stomata are present on both upper and lower epidermis. Vascular bundles are surrounded by a parenchymatic bundle sheath (Fig. 3B, Table 1).

Trace elements: In this study, the analysis was performed by ContrAA 300 Flame Atomic Absorption Spectrometer (HR-CS AAS) (GLE, Germany). The levels of metals were calculated on dry weight ($\mu\text{g g}^{-1}$). Ten elements (Fe, K, Mn, Na, Cd, Cu, Pb, Cr, Al and Be) were detected in the in all flowers, leaf and stem of *O. argentata*. As it is known metals are unique because of their important role in metabolism (Çiftçi *et al.*, 2009). They are essential part of many important enzymes and they also play roles as catalysts and antioxidants (Hambidge *et al.*, 1987). The concentrations of the trace elements in the samples are presented in Table 2. According to our results, Na, Al, K and Fe contents were high in all studied plant's sections. Mn, Cd, Cu, Pb, Cr and Be contents of same samples were low.

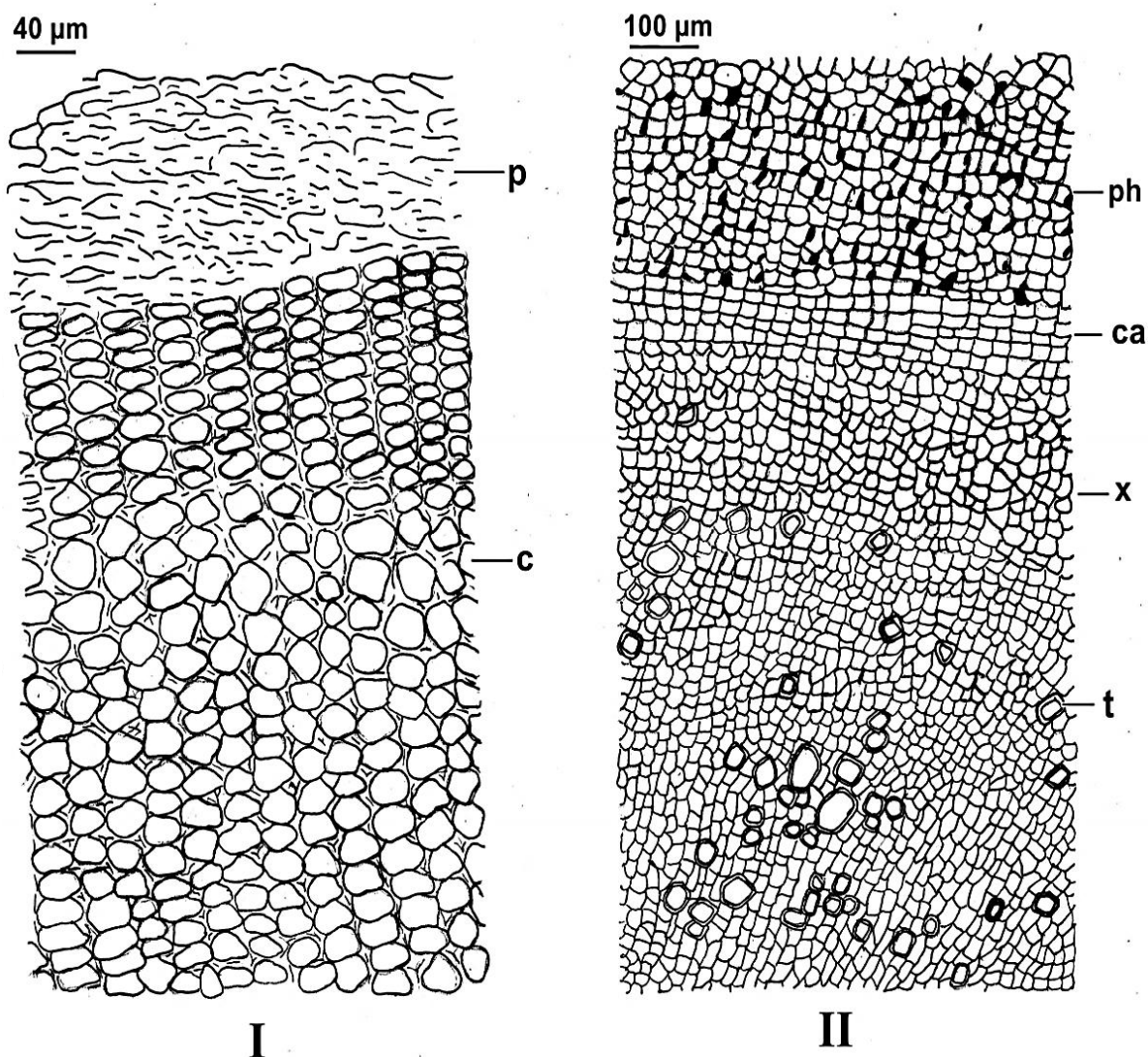


Fig. 2. The cross-sections of root of *O. argentata* (I-II)
 p. periderm, c.cortex parenchyma, ca. cambium, ph. phloem, t. trachea, x. xylem

Table 1. Measurements of various tissue of *O. argentata*.

	Width (μm)		Height (μm)	
	Min. - Max.	Mean \pm SD	Min. - Max.	Mean \pm SD
Root				
Peridermis cell	10.48 - 41.92	24.06 \pm 11.25	7.86 - 36.68	20.60 \pm 9.62
Cortex parenchyma cell	15.72 - 41.92	29.50 \pm 9.16	10.48 - 36.68	23.30 \pm 8.24
Pith ray diameter	15.72 - 104.8	71.90 \pm 29.24		
Trachea diameter	13.10 - 68.12	32.40 \pm 18.76		
Stem				
Epidermis cell	13.10 - 39.30	25.40 \pm 9.25	15.72 - 36.68	26.70 \pm 6.85
Cortex parenchyma cell	15.72 - 94.32	55.30 \pm 33.47	10.48 - 83.84	39.80 \pm 25.69
Trachea diameter	15.72 - 52.40	33.80 \pm 13.89		
Pith cell diameter	31.44 - 157.20	89.10 \pm 51.22		
Leaf				
Upper epidermis cell	20.96 - 73.36	39.80 \pm 19.82	13.10 - 52.40	30.30 \pm 10.73
Lower epidermis cell	18.34 - 52.40	30.00 \pm 11.94	15.72 - 41.92	30.30 \pm 10.73
Trachea diameter	3.75 - 12.50	8.80 \pm 2.65		
Palisade cell	28.82 - 83.84	54.00 \pm 21.19	18.34 - 36.68	26.50 \pm 6.33
Spongy cell diameter	15.72 - 36.68	26.20 \pm 8.29		

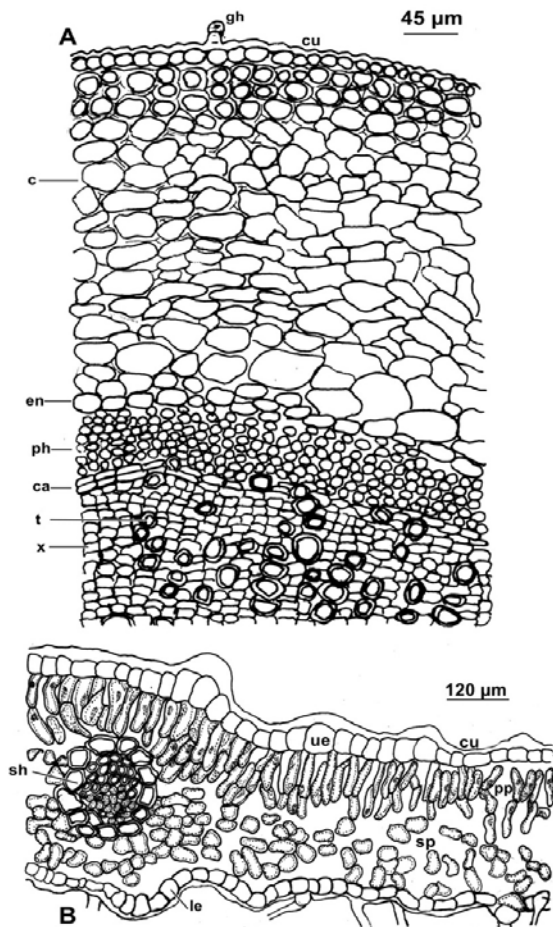


Fig. 3. The cross-sections of stem (A) and leaf (B) of *O. argentata* e. epidermis, gh. glandular hair, en. endodermis, c. cortex parenchyma, ca. cambium, cu. cuticle, le. lower epidermis, ph. phloem, pp. palisade parenchyma, ue. upper epidermis, sh. sheath, sp. spongy parenchyma, x. xylem.

Discussion

No morphological and anatomical information on *O. argentata* is available in literature except general taxonomic properties. In the results presented here, the morphological properties of *O. argentata* showed some similarities and differences compared to other findings in the Flora of Turkey. In the first description of *O. argentata*; stem is to 35cm, basal and lower cauline leaves 70-100 x 3-7 mm, pedicels to 11mm and calyx 14-22 mm. The present findings showed above mentioned measured; 30 cm, 70-90 x 3-5.5 mm, 10 mm, 15-20 mm respectively.

In our anatomical studies, it was determined that the species had a typical dicotyledonous root and stem. The

cambium is distinguishable and cortex is rather tight in cross-section. Metcalfe & Chalk (1979) gave information about general anatomical characteristics of Boraginaceae, pointing out that the family to which the species belongs has deeply and superficial periderm. Researchers have stated that *Onosma bracteosum* Hausskn. & Bornm. had secondary root structure, the periderm was multi-layered, the cortex was 10-15 layered and parenchymatic. The xylem was composed of scleranchymatic cells and trachea (Binzet & Akçin, 2012). In this study, it observed that investigated species had those properties. It has not been seen investigated species and *Onosma bracteosum* the stem. The same feature has not also been on the stem of *Symhytum asperum* Lepechin, *Symhytum ibericum* Steven and *Symhytum sylvaticum* Boiss. are belong to Boraginaceae family (Akçin *et al.*, 2007). The Palisade and spongy parenchyma cells are distinguishable in a cross-section of *O. argentata*. Metcalfe pointed out that in many plants, especially among the dicots, parenchyma can be distinguished in the mesophyll palisade and spongy parenchyma.

Akçin & Engin (2005) observed that in *O. bracteosum* mesophyll had a palisade parenchyma close to the upper epidermis, four to six layers of spongy and two to three layers of palisade parenchyma. In *Symhytum asperum*, *S. ibericum* and *S. sylvaticum* the mesophyll had palisade parenchyma close to upper epidermis, single layer of palisade and three to four layers of spongy parenchyma (Akçin & Engin, 2005).

As shown in Table 2, among the metals analysed; the Na, K, Al and Fe contents of flowers, leaf and stem found to be higher than other metals. Due to the high quantity of K, Mg and Ca together with the quantity of Na plus the content of the essential elements Fe, Mn, Zn and Cu allow the seeds are considered as excellent sources of bioelements (Calixto & Canellas, 1982). The analysis of data presented in Table 2 revealed that 4 elements (Na, Al, K and Fe) exist in significant quantities. The contents of iron was found range of 34.40 and 116.58 µg.g⁻¹ in all the samples studied (Table 2). The reason of consistence of iron in leaves more than flowers and bodies is due to the fact that iron exists in chlorophyll structure of plants which has a role for photosynthesis. Existing less quantity of Beryllium in each part of the plant (flowers, leaf, body) shows that Beryllium element doesn't play an important role in plant structure. The reason of trace element deposition can be explained by the implication of soil, water, air and various chemicals or related to the structure of genetical factors. Therefore, the studies to determine the level of trace elements in plants have contributed to basic biochemical, physiological, taxonomical, morphological and anatomical researches.

Table 2. The concentrations of the elements in the plant parts are presented.

Plant parts	Metal levels in <i>Onosma argentata</i> plant (µg/g)									
	Fe	K	Mn	Na	Cd	Cu	Pb	Cr	Al	Be
Flower	91,53	2133,06	5,04	409,10	0,17	6,10	0,80	13,27	62,88	<0,05
Leaf	116,58	1422,19	13,01	279,08	0,21	3,06	0,10	25,26	123,21	<0,05
Stem	34,40	1363,30	4,45	451,47	0,25	3,98	2,57	9,60	48,45	<0,05

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