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Original Article

Micro Morphological Study of Several Populations of *Zataria multiflora* Boiss.

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Abstract

Zataria multiflora Boiss. is one of the most important species of the Lamiaceae family, which has numerous medicinal properties in traditional medicine, and is related to the thyme species in terms of botanical features and composition of essential oil. In this research, the anatomical characteristics of vegetative organs including leaves, inflorescence, stems and petioles of 4 populations of shirazian thyme have been collected from different areas of Iran and were investigated and compared. Methods include making hand-cuts and observations with an optical microscope. The results of this study showed that there is a similarity to the characteristics of the inflorescence, stem, leaf and petiole in the populations of shirazian thyme. In terms of stem and leaf diameter, the thickness of the cork and xylem, frequency and type of non-glandular trichomes and glandular trichomes there are differences between the studied populations. The results indicate that the study of anatomical characters is an appropriate method for identification of populations of a species and the role of ecological factors on the development of the internal structures of plants could be studied too.

Keywords: Sh, Anatomy, Vegetativirazian thymee organs, Glandular trichome

Intruduction

Zataria multiflora Boiss. is one of the best-known medicinal herbs of the mint family (Lamiaceae). Native to southwestern Asia (Iran, Afghanistan, Pakistan, Kashmir). In Iran, the wild growth habitat is in the provinces of Isfahan, Yazd, Kohgiluyeh and Boyer Ahmad, Fars, Hormozgan, Khuzestan, Khorasan and Sistan and Baluchestan. The habitat of this species is the rocky slopes in Saharo-Sindian and Irano-Turanian regions [1].

It is a shrub which grows to a height of 40-80 cm, spicy, with twisted branches, branches with white skin, fluffy, heart-shaped or circular-shaped leaves, with an almost heart-shaped base with rounded head, a spike-shaped flower with a short calyx, it's harvest time is in from April to June when it is blossoming [1]. This plant has many medicinal properties and in food, pharmaceutical, sanitary and cosmetic industries it is used a lot. *Zataria multiflora* Boiss. (Shirazi thyme)is an ancient condiment with modern pharmaceutical uses [2]. Several studies on the anatomy of the vegetative organs, stem, leaf and petiole of the genera belonging to the Lamiaceae family, including species (*Mentha* spp) [3], Vitex [4], savory (*Satureja*) [5] and other genera *Ornithogalum* [6], *Louis* (*Typha*) [7], plantain (*Plantago*) [8], wormwood (*Artemisia*) [9]; *Scrophularia* [10], *oats* (*Avena*) [11], *Clematis* [12], *Astragalus* [13,14], a number of species of halophyte [15]; *Onosma* [16]; *Bolboschoenus* [17]of several genera of dark Alsinoideae [18]; Festuca [19], turpentine (*Pistacia atlantica*varkurdica) [20]; plain wormwood (*Artemisia sieberi*Besser) [21] have been made. The aim of this study was to investigate the anatomical structure of the vegetative organs and type of glandular and non-glandular trichome. Also

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comparing the relationship between population and habitat characteristics and ecological conditions on *Zataria multiflora* Boiss anatomical structure in Fars, Esfahan, Yazd and Hormozgan provinces.

Material and Methods

To evaluate anatomical characteristics of the inflorescence axis, the stem, leaf, petiole, and flowering shoot was isolated from herbarium specimens of 4 populations of *Zataria multiflora*.(Table 1). All samples were kept in fixative solution (FAA) (Formaldehyde Alcohol A**c**etic Acid, 5%:90%:5%) for 24 to 48 hours. In order to dehydrate, all samples were transferred to ethanol with solubility 70% and 90%, respectively. The time of keeping plant sample in ethanol soluble was between 24 to 48 hours. In the next step sectioning of the samples was done manually. Thin sections were prepared from the middle portion of all samples by using the hand–cut method and blade. Plant samples were placed on to graph paper, cutting was done vertically under a stereomicroscope using a scalpel. Cutting samples were then transferred to a filter and were kept in a petri dish containing distilled water. Bleaching of plant tissues was done using sodium hypochlorite soluble containing 1% active chlorine. The time required depends on the type of tissue, and lasted between 1 to 5 minutes. Then all samples were washed three times with distilled water. Double staining was done using carmine (5 minutes) and methyl blue (2 seconds). Micromorphological characters were studied using an OLYMPUS (CH30) stereo-microscope equipped with a Nikon camera. To evaluate the morphometry, a graded lens was used. All specimens used in this study were from the Research Institute of Forest and Rangelands of Iran (TARI) herbarium**.** The list of studied specimens and their voucher numbers are given in Table 1**.**

The climatic conditions of the *Zataria multiflora* localities are as follows:

In the Isfahan, the average rainfall is 284 mm.. The minimum temperature is 2 °C and the maximum temperature is 38 °C. The climate was semi-arid,and dry. In Fars, the average rainfall is 346 mm.

The minimum temperature is 18 °C and the maximum temperature is 38 °C. and the climate was mostly semi-arid. In Yazd, the average precipitation is 110 mm. the minimum temperature is -1 and the maximum temperature is 35 °C. The climate was semi-arid areas. In Hormozgan, the average rainfall is 182 mm. the minimum temperature is 28 and the maximum temperature is 40 °C. The climate was, semi-arid, and semi-arid desert [22].

Table1. The list of the localities of *Zataria multiflora* Boiss. used for micro-morphological study

Locality and collection data	**Altitude(m)**
Esfahan: Qamishlou Protected Area**,** Golipour 103802 (TARI)	1900-2700
Fars: Southwest of Maharlu Lake in Shiraz, Golipour106261(TARI)	1580-1660
Yazd: Shirkooh Yazd, Golipour 106260 (TARI)	1700-2350
Hormozgan: Mountain Genu in Bandar Abbas, Golipour106262 (TARI)	700-1300

Table 2. Morphological characteristics of some populations of *Zataria multiflora* Boiss.

Habitat	**Altitude (m)**	**Xylem width** (stem mm)	**Cork width** (stem mm**)**	**Stem diameter** (mm)	**Inflorescence diameter** (mm)	**Hair frequency**	**Leaf width (**mm)
Isfahan	1900-2700	0.34-0.37	0.10-0.15	1.66-1.82	0.38-0.53	low	0.20.30
Fars	1560-1660	0.34-0.63	0.26-0.0.35	1.82-2.34	0.58-0.63	high	0.28-0.30
Yazd	1700-2350	0.14-0.26	0.05-0.20	0.88-1.17	0.56-0.61	medium	0.21-0.25
Hormozgan	700-1300	0.26-0.40	0.37-0.40	1.84-1.95	0.50.61	medium	0.35-0.37

Results

Leaf Anatomy

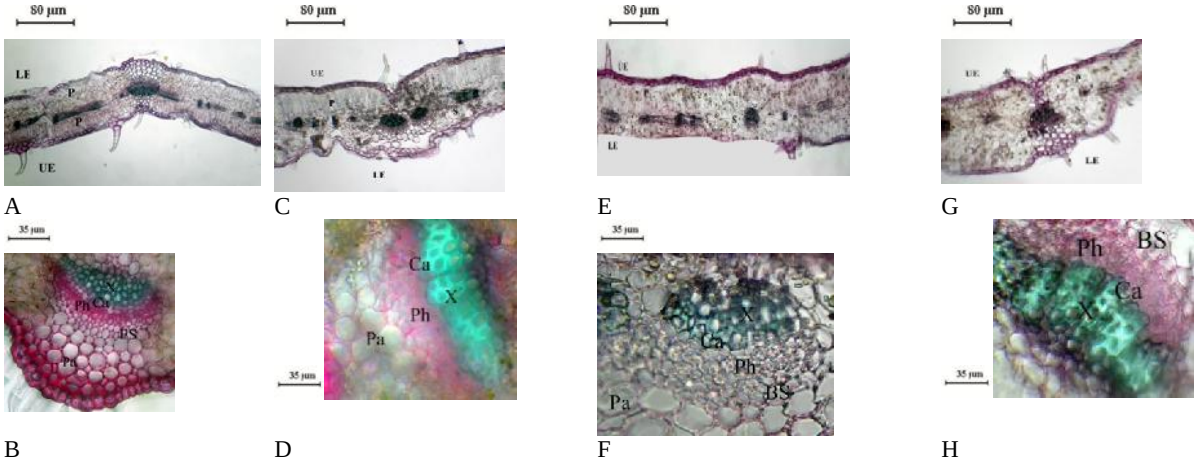
The leaves in all populations of *Zataria multiflora* Boiss. are elliptic. The epidermis on the adaxial and abaxial sides is represented by one layer, The leaves of the taxa show dorsiventral mesophyll. However a number of palisade layers and spongy layers differ among the taxa. (Fig. 1: A, C, E and G in Fars, Isfahan, Yazd and Hormozgan sample respectively). The anatomical features of midrib including one layer of vascular bundle, some layer of parenchyma cells, phloem and xylem layers (Fig. 1: B, D, F and H Fars, Isfahan, Yazd and Hormozgan sample respectively). The leaves' width size are as shown in Table 2.

Inflorescence Axis Anatomy

Fars sample: Inflorescence axis cover with non-glandular and rarely glandular hairs. It include one layer of epidermis cell, collenchyma tissue in four corner of Inflorescence axis, parenchyma cells, endodermis, one layer of cork tissue, phloem tissue, xylem and fiber layer, the pith consists of parenchyma cells (Fig. 2: A, B,C and D in Fars, Isfahan, Yazd and Hormozgan sample respectively). Inflorescences axis diameter are in Table 2.

Stem Anatomy

Fars sample: Hairs were found in the young part of the stem and were not found in the older part of the stem. White periderm was observed in the stem. The stem is 1.82-2.34 mm in width (Table 2). The anatomical features include one layer of epidermis cells, parenchyma cells, a layer of cork tissue, endodermis, phloem tissue, primary xylem and secondary xylem with fiber cells, pith consisting of parenchyma cells. The cork layer is 0.26-0.35 mm in width. The xylem layer is 0.34-0.63 mm in width (Fig. 3-A) (Table 2).



A C E G

B D F H

Fig. 1 Anatomical characteristics of leaf and midrib in populations of *Z. multiflora*
A and B: Fars, C and D: Isfahan, E and F: Yazd, G and H: Hormozgan
P,Parenchyma. ph,Phloem. X,xylem. Ca, Cambium. Pi-pith, Mx Methaxylem. Ue,Upper epiderm., Le,lower epiderm. S, stomata. P, Parenchyma.

B C D

Fig. 2 Anatomical characteristics of inflorescence axis in populations of*Z. multiflora*
A: fars, B: Isfahan, C: Yazd, D: Hormozgan

Isfahan sample: Hairs were found in the young part of the stem and were not found in the older part of the stem. White periderm was observed in the stem. The stem is 1.66-1.82 mm in width (Table 2). Anatomical features include one layer of epidermis cells, parenchyma cells, a layer of cork tissue, endodermis, phloem tissue, primary xylem with fiber cells, pith consisting of parenchyma cells. The cork layer is 0.1-0.15 mm in width. The xylem layer is 0.34-0.37 mm in width (Fig. 3-B) (Table 2).

Yazd sample: Hairs were found in the young part of the stem and were not found in the older part of the stem. White periderm was observed in the stem. The stem is 0.88-1.17 mm in width (Table 2). Anatomical features include one layer of epidermis cells, parenchyma cells, a layer of cork tissue,endodermis, phloem tissue, primary xylem with fiber cells, pith consisting of parenchyma cells. The cork layer is 0.05-0.2 mm in width. The xylem layer is 0.14-0.26 mm in width (Fig. 3-C) (Table 2).

Hormozgan sample: Hairs were found in the young part of stem and were not found in the older part of the stem. White periderm was observed in the stem. Stem is 1.84-1.95 mm in width (Table 2). Anatomical features include layers of cork tissue, phloem tissue, primary xylem and secondary xylem with fiber cells, pith consisting of parenchyma cells. The cork layer is 0.37-0.40 mm in width. The xylem layer is 0.26-0.40 mm in width (Fig. 3-D) (Table 2).

Petiol anatomy:

In all populations, the epidermis on the adaxial and abaxial sides is represented by one layer, collenchyma and parenchyma tissues. There is one midrib including phloem tissue, cambium and xylem layers (Fig. 4 A-H).

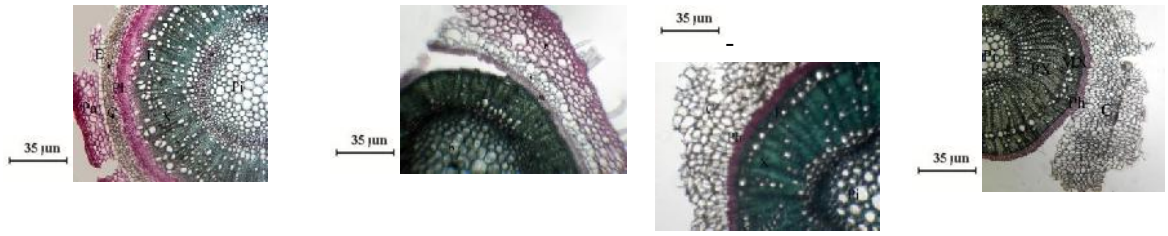


Fig. 3 Anatomical characteristics of stem in populations of*Z. multiflora*
A fars, B: Isfahan, C: Yazd, D: Hormozgan
P,Parenchyma. Ph,Phloem. X,xylem. Ca, cambium. Pi-pith, Mx Methaxylem.

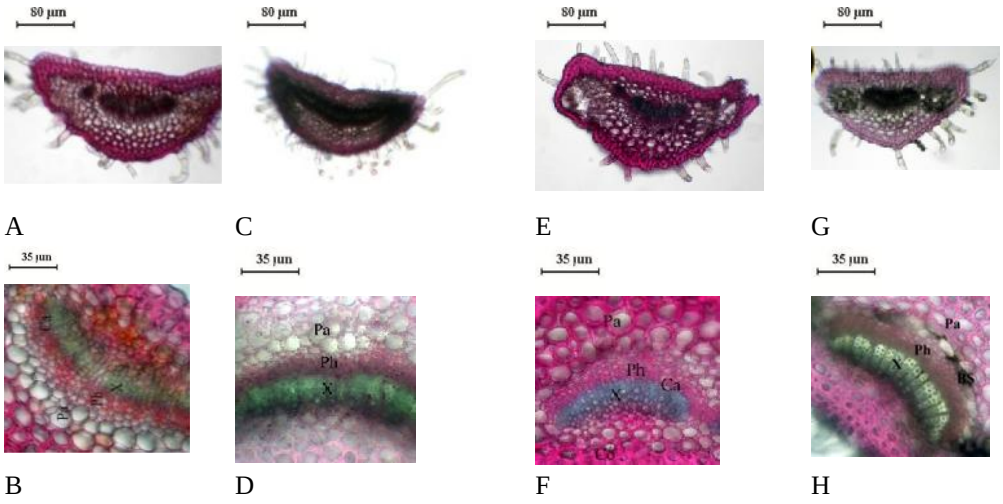


Fig. 4 Anatomical characteristics of petiol and midrib in populations of *Z. multiflora*
A and B: fars, C and D: Isfahan, E and F: Yazd, G and H: Hormozgan.
P,Parenchyma. ph,Phloem. X,xylem. Ca, Cambium. Pi-pith, Mx Methaxylem.

Glandular and Non-glandular Hairs

Fars sample: One, two and three cells of non-glandular trichomes are scattered on the inflorescence axis, young stems, leaves and petioles (Fig. 5:A,B and C). The density of trichomes on the leaf surface is high. Secretory trichomesincluding peltate and capitate are observed on the surface of leaves, petioles and inflorescences (Fig. 5-D and Fig. 5-E). The frequency of trichomes on the abaxial surface are between 2 to 5, and on the adaxial surface between 3 to 7.

Fig. 5 Glandular and non-glandular trichome of *Zatarai multiflora*
Non glandular trichome (A, B and C) and glandular trichome (Dand E) in Fars population
Non glandular trichome (F and G) and glandular trichome (H and J) in Isfahan population
Non glandular trichome (K, L and N) and glandular trichome (M) in Yazd population
Non glandular trichome (O) and glandular trichome (P and Q) in Hormozgan population

Isfahan sample: Non-glandular trichomes are one and two cells, scattered on the inflorescence axis, young stems, leaves and petioles (Fig. 5: F and G). The density of trichomes on the leaf surface is medium. Secretory trichomesincluding peltate and capitates are observed on the surface of leaves, petioles and inflorescences (Fig. 5: H and I) The frequency of trichomes on the abaxial surface are between 2 to 3, and on the adaxial surface between 3 to 5.

Yazd sample: One, two and four cells of non-glandular trichomes are scattered on the inflorescence axis, young stems, leaves and petioles (Fig. 5:J, K and L). The density of trichomes on the leaf surface is medium. Secretory trichomeincluding peltate are observed on the surface of leaves, petioles and inflorescences (Fig. 5-M). The frequency of trichomes on the abaxial surface are between 3 to 6, and on the adaxial surface between 3 to 6.

Hormozgan sample: One and two cells of non-glandular trichomes are scattered on the inflorescence axis, young stems, leaves and petioles (Fig. 5:N and O). The density of trichomes on the leaf surface is medium. Secretory trichomeincluding peltate and capitate (Fig. 5:P and Q) are observed on the surface of leaves, petioles and inflorescences axis. The frequency of trichomes on the abaxial surface are between 3 to 6, and on the adaxial surface between 1 to 4.

Discussion

Anatomical and morphological traits were used as appropriate tools for the identification, classification and phylogenetic relationship of plant taxa. In this study, the anatomical features of stems, inflorescences axis, leaves and petioles in populations collected from four areas were investigated and compared. Some differences were observed in relation to inflorescence axis diameter, stem diameter, cork thickness and also leaf thickness in populations. In total, the maximum thickness of the leaves was observed in the Hormozgan sample and the minimum was seen in the Fars sample. Leaf thickness, number of layers of palisade and spongy parenchyma, cuticle thickness and stomatal index, are important features of leaf anatomy and reflecting the ecological condition of the plants' habitat. [23,24]. When the thickness of the leaves is high, indicating that this plant was grown under drought conditions.[23] The minimum diameter of inflorescence was seen in he Isfahan sample and the maximum diameter of inflorescence axis was observed in the Yazd sample. The minimum and maximum diameter of stem was seen in the Fars and Yazd samples, respectively. The highest cork thickness was observed in the Isfahan sample and the minimum cork thickness was seen in the Hormozgan sample. The maximum diameter of xylem thickness was seen in the Fars sample and minimum diameter of inflorescence axis was observed in the Yazd sample. Therefore, altitude effects on anatomy characteristics of *Zataria multiflora*. altitude increased the leaf lamina thickness and shoot diameter in Plantago species [8]. In a previous study, the amount of calcium in the growth zone of this plant in Maharlou region was high [22]. In another study, increasing the amount of calcium increased the diameter of the flowers and stems [25]. It could be concluded that according to Table 2, increasing in diameter of the flowers and stems is related to the amount Calcium in soil, although this conclusion requires more research

Glandular trichomes consisting of peltate and capitate were observed in populations of this species. These trichomes are as secretory vesicles, for oil accumulation. Peltate trichomes were observed in all populations, and capitates trichomes were seen in populations of Fars, Isfahan, and Hormozgan. Glandular and non-glandular trichomes are scattered on the inflorescence axis, young stems, leaves, petioles and flowers. Satil and Kaya (2007) were examined. The anatomy of leaves and leaf trichomes of species grown in Turkey. They reported that all *Satureja* species has non-glandular and glandular trichomes including peltate and capitates. Glandular trichomes of the Lamiaceae family can be divided into two categories, peltate and capitate [26]. Peltate glandular trichomes are formed from one basal cell, stem cells and some secretory cells in the head. Peltate glandular trichomes are observed in all populations of this species. Peltate glandular trichome are reported in *Thyme*, *Satureja*, *Salvia* Spp [27,]. Capitate glandular trichome are different in relation of their size and structure. Usually, they include one basal cell, stem cells and one secretory cell. This structure was observed in *Satureja* [5] and Salvia [28]. Density differences of secretory trichome were observed among populations of this species. Since glandular trichome are the site of

essential oil accumulation, the frequency of these glandular trichome affects the essential oil content. Satil and Kaya (2007) observed that there are some differences among population of *Satureja* species in relation of Peltate glandular trichome species. Furthermore, non-glandular trichomes on the leaf surface are considered as a diagnostic trait among species of genera such as *Onosma* [16], Satureja [5], Mentha Spp [3]. In this study, one and two cells of non-glandular trichomes were seen in all populations, and three-and four-cell non-glandular trichomes were observed in the Fars and Yazd population.

Conclusion

As the results indicate, the leaf thickness of the Hormozgan populations has increased and the diameter of the leaf is higher than other populations. On the other hand, stem thickness has increased in the Fars population and the frequency of trichome in the Fars population is more than the other populations studied. In the population of Yazd, the diameter of the stem, the thickness of the leaves, the diameter of the xylem all declined, indicating a decrease in the biomass due to the climate of the region. Therefore, various populations of *Zataria multiflora* have different strategies in terms of morphological and anatomical characteristics for coping and survival with ecological and climatic conditions of their habitats. As shown in Table 2, Stem diameter , Inflorescence diameter in the Fars localities is higher than the other habitats. Also, the prevalence of secretory trichome in the Fars populations is more than others. The abundance of corks can affect the content and accumulation of essential oils. Climate factors are affected on plant growth. The results of this study indicate that in Fars (Maharlo) region, the highest vegetative characteristics are present. Therefore, Maharloo region has provided more favorable vegetative conditions than the other areas. The high average rainfall in Fars area has led to better vegetative growth. Due to the severe degradation of the habitats of this plant, it seems that this species is at high risk and This plant needs protection.

References

1. Jamzad Z, Flora of Iran Lamiaceae pub Research Institute of Forest and Rangelands. 2012;76.
2. Sajed H, Sahebkar A, Iranshahi M. *Zataria multiflora* Boiss. (Shirazi thyme)- an ancient condiment with modern pharmaceutical uses. J Ethnopharmacol. 2013;145:686-698.
3. Albouyeh RM, Mirzaie-Nadoushan H, Majd A, Rezaie MB, Investigation of several anatomic characteristics in menthe species. Iranian Rangelands and Forest Plant Breeding and Genetic Research. 2002;8:81-92.
4. Abbas Azimi R, Jamzad Z, Sefidkon F, Bakhshi-Khaniki S, The potential value of phytochemical and micromorphological characters in taxonomic treatment of genus Vitex L. Lamiaceae. Iranian J Botany. 2006;12:15-35.
5. Satil F, Kaya A. Leaf anatomy and hairs of Turkish *Satureja* L. (Lamiaceae). Actabiologica Cracoviensia series Botanica. 2007;49:67-76.
6. Nejadsattari T, Hamdi S. M. M, Heidarian F, Assadi M. Anatomical studies of leaf and stem on Ornithogalum (Hyacinthaceae) in Iran.Taxonomy and Biosystematics. 2013;15:49-66.
7. Güven S, Beyazoğlu O, Makbul S, Türkmen Z, Kandemir A. Anatomica features of six Onosma L. (Boraginaceae) species From Turkey. Iranian J Botany. 2013;19:94-103. Tehran.
8. Zarinkamar F, Marzban A. Effect of altitude on anatomy of *Plantago major* and *Plantago lanceolata*. Iranian J Biology. 2009;23:532-540.
9. Azarnivand H, Jafari M , Zarechahoki M. A, Rabiee M. Anatomical characteristics of *Artemisia sieberi* (Case study: Vard-Avard, Garmsar, Semnan). Pajouhesh Sazandgi. 2011;80:100-108.
10. Makbul S, Beyazoğlu O. Morphological and anatomical studies on some Scrophularia L. (Scrophulariaceae) taxa from NE Anatolia, Turkey. Iranian J Botany. 2010;30:186-195.
11. Seifi Z, Zarinkamar F. Anatomical studies of some Avena species in Iran. Rostaniha. 2006;8:186-203.
12. Hamdi SMM, Assadi M, Iranbakhsh A, Malekloo M, Ebadi M. Anatomical studies on leaf on Typhaceae in Iran. Taxonomy & Biosystem. 2012;11:13-22.
13. Mehrabian AR, Azizian D, Zarre Sh, Podlech D. Petiol anatomy in Astragalus Sect. Incani DC. (Fabaceae) in Iran. Iranian J Botany. 2007;13:138-145.
14. Zarrinkamar F, Jalili A, Hamzehee B, Asri Y, Hodgson J G, Thompson K, Shaw S. Foliar anatomy of Carex in Astragalus, NW. Iran. The Iranian J Botany. 2002;9:261-266.
15. Ramazannejad Ghadi R, Azizian D, Assadi, M. Comparative anatomical analysis of stem in four genera of the tribe Salsoleaa, Chenopodiaceae. Iranian J Botany. 2006;12:169-182.
16. Habibi M, Azizian D, Sheidai M, Khatamsaz M. Comparative anatomical studies of some Clematis L. (Ranunculaceae) species in Iran. J Sci. 2008;2:20-29
17. Amini Rad M, Hroudová Z. Leaf and stem anatomy in Iranian Bolboschoenusspecies(Cyperaceae), as related to their taxonomy and ecology. Iranian J Botany. 2013;19:221-234. Tehran.

18. Keshavarzi M, Esfandani Bozchaloyi S.Leaf and stem comparative anatomical analysis of three genera of Alsinoideae (Caryophyllaceae). Iranian J Botany. 2014;20:71-79.
19. Hosseini SZ, Rahiminejad MR, Saeidi H. Leaf anatomical structure of Iranian narrow-leaved species of the genus Festuca L. (Poaceae, Poeae). Iranian J Botany. 2013;10:86-93. Tehran
20. Seyedi N, Jalali S. Gh, Moghaddam M. Leaf anatomical investigation of Pistacia atlanticavarkurdica. Journal of Plant Biology. 2013;16:71-80.
21. Rabie M, Asri Y, Abbas Azimi R, Dehgan M. The effects of climatical parameters on anatomical structure of *Artemisia sieberi*Besser populations. Iranian J plant biol. 2012;4:57-70.
22. Najafpour Navaei M, shariat A. A study on ionomic indices of *Zataria multiflora* Boiss. an Iranian native medicinal plant. Iranian J Medicinal & Aromatic Plants. 2017;33:812-819.
23. Makbul S, Türkmen Z, Kandemir A, Beyazoglu O. Morphological anatomical and palynological properties of endemic *Onosma sintenisii*Hausskn. & Bornm. (Boraginaceae). Herb J Systematic Botany. 2008;15:125-136.
24. Makbul S, Coskuncelebi K, Türkmen Z, Beyazoglu O. Comparison of foliar anatomy of Scorzonera L. (Asteraceae) taxa from northeast Anatolia. Pakistan J Botany. 2011;43:135-155.
25. Ismaili S, Roohi V, Shiran B, Mohammadkhani AB,Effects of Calcium Chloride, Gibberellin and Benzyladenine on Qualitative and Quantitative Characteristics and Flower Longevity of Zinnia (Zinnia elegans). J Hortic Sci. 2014;4:444-452.
26. Fahn A, Structure and function of secretory cells In Hallahon DL, and Gray JC [eds.]. Advances in botanical research. Incorporating advances in plant pathology. Plant trichomes. Academic Press, London. 2000;31:37-75.
27. Bisio A, Corallo A, GastaldoP, Romussi G, Ciarallo G, Fontana N, Tommasi ND, and Profumo P. Glandular hairs and secreted material in Salvia blepharophyllaBrandegee ex Epling Grown in Italy. Annals Botany. 1999;83:441-452.
28. Kaya A, Demirci B, Baser K.H.C. Glandular trichomes and essential oils of *Salvia glutinosa* L. South African J Botany. 2003;69:422-427.