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Original Article

Anatomical Structure Study of Aerial Organs in Four Populations of *Urtica dioica* L.

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Abstract

Urtica dioica L. is an Iranian native pharmacological plant for which little attention has been paid to the anatomical structure. Medical applications of this plant include diabetes therapy, digestive improvement, anemia and kidney disease therapy. Because climatic conditions can affect the anatomical structure which in turn affects pharmacological compositions, research on the anatomy of this plant is needed. In this research, plant samples were collected from populations near the cities of Brojerd, Mashad, Ghazvin, and Ramsar. Cross sections, were made from stem, leaf, and petiole at the second internodes, and stained using double staining methods. Differences between stem, leaf, and petiole tissues confirmed that climatic factors produced differences among the populations in anatomical structure of aerial organs. Noted differences included: 1- Number and diameter of vascular bundles with five vascular bundles in Ghazvin population, five to seven vascular bundles in the Brojerd population, and four vascular bundles in populations in Mashad and Ramsar. The Mashad and Ramsar populations differed in diameter. 2- Protective tissue and thickness of the cuticle of plants from Ghazvin had more tissues because of lower thermal mean and mountain region. 3- Differences in petiole diameter with the largest petiole diameter in the Mashad population and the smallest petiole diameter in the Ramsar population. These observed differences in anatomy confirmed the effect of climate on differentiation in anatomical structure in *Urtica dioica* L.

Key words: *Urtica dioica* L., Anatomical structure, Climatic stem, Leaf, Petiole.

Introduction

Urtica dioica L. (Urticaceae), stinging nettle, is a long-lived perennial plant that is common in nutrient-rich habitats [1]. This plant is one of the Iranian native pharmacological plants and little attention has been paid to the anatomical structure of the plant.

Nettle is dioecious (- obligatory out-crossing) and wind-pollinated [2]. In addition to sexual reproduction by seed, the plant spreads asexually by overwintering underground rhizomes and by aboveground stolons that can root. Stinging trichomes are on the stem and leaves and suggests that they function as a defense against mammalian herbivores [3]. No evidence that stinging trichomes have any function in against parasitic plants exists [4].

The blood sugar lowering effect of nettle as a medicinal plant has a long history in old script, such as that written by Avicenna.

Other reports have indicated the benefits of using an infusion or extract of the leaves or other plant parts for diabetes [5-6] as well as other disorders like prostatic hyperplasia [7-10], inflammation [11], rheumatoid arthritis, hypertension, and allergic rhinitis [12-13]. The aim of this research was to show how climatic conditions could affect the anatomical structure which directly affects the pharmacological compositions. So, considering the important effects of different climatic conditions on anatomical structure as well as pharmacological compositions of this plant a research on the anatomy of this plant is needed.

The soft green leaves were 3 to 15 cm long and borne opposite on an erect, wiry green stem. All the leaves

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have a strongly serrated margin, a cordate base and an acuminate tip with a terminal leaf tooth longer than adjacent laterals.

The plant bears small greenish or brownish tetramer flowers in dense axillary inflorescences [14]. Both leaves and stems are pubescent with non-stinging and stinging hairs (trichomes), whose tips come off when touched, cross forming the hair into a needle that will inject several chemicals: Acetylcholine, histamine, serotonin, and possibly formic acid [15].

Materials and Methods

In this research, four populations were selected and ten samples were analyzed in each population. The plant population samples were collected from Brojerd, Mashad, Ghazvin, and Ramsar cities in summer, 2009. Climate map of the geographical area was introduced in which the method of De Martonne Aridity Index provided that most of the areas in the province of Ghazvin are semi-arid and cold climate. It is a mountainous region. Ramsar has a very wet climate with hot summers and relatively cold winters. The weather of Brojerd is cold being a semi-arid region as depicted in the category of De Martonne Aridity Index. In Mashad, the climate is variable and tends to be more moderate, cold and dry[16-17].

The samples were fixed in 70% alcohol and transverse sections of leaf were prepared by hand cutting. Cross sections were made from stem, leaf and petiole of the second internode. Staining followed the methylene blue-carmine procedure as described by Sharifnia and Alboyeh [18]. The specimens were studied with an Olympus (3H-Z, made in Japan) light microscope equipped with a camera.

Results

Anatomical structure of stem:

In this research, we demonstrated the parts from outer to inner and the studies are:

The cortex contains layers of epidermis, cortex (collenchyma and cortical parenchyma), and endodermises. The epidermis consists of a layer of epidermis cells with cellulosic wall and stinging hairs (trichomes), the cortex located at the corners of stem consist of layers of collenchymas that are thinner (4 rows) in the younger section as compared with older sections (11 rows) and because of these collenchymatic cells the stem seems outstanding.

The cortical parenchyma demonstrated many pith cells and pectocellulosic walls are the fundamental tissue in the cortex. The endoderm just occurs in the younger sections. In the center of the stem is the stele with outspread 4 to 5 vascular bundles and procambium that show in primary growth. In the inner the metaxylem and protoxylem could be distinguished. In the center of the stem, the pith contained parenchyma cells (Figs. 1 and 2).

Anatomical structure of leaf:

In the general anatomical account of Urticaceae, [19-20] reported the occurrence of laticiferous elements, glandular, non glandular and stinging hair types, hypostomata, presence of hydathodes, fluid-loaded epidermis, silicified and calcified cell walls which occur as cystoliths of different forms. The mesophyll has been with compact palisade parenchyma just under the upper epidermis. Spongy parenchyma cells under the palisade cells formed the pith. Under the upper epidermis in the hypodermis, the lithocyst cells with cystolith (the cystoliths contain calcium carbonate [21]) or crystals could be seen (Figs. 1-A). A thickened vascular bundle was present in the main midrib, and lower the midrib was collenchymas cells. Large stinging hairs (trichomes) were clearly visible (Figs 1 and 2).

Anatomical structure of petiole:

Both the adaxial and abaxial sides of the petiole are covered by a layer of epidermal cells. The adaxial side of the petiole has two swelling and is shaped in cross section similar to a horseshoe due to the arrangement and size of the vascular bundle. A protective layer of cells, such as collenchyma, was abundant (Fig 1 and 2).

The following was noted: The Ghazvin population had the largest number of vascular bundles viz. five, while Brojerd population had the thickest petiole diameter at 5-7mm, although Mashad and Ramsar each had four vascular bundles, they differed in diameter. The thinnest vascular bundles, collenchymas were observed in the Ramsar population, of the four tested populations, Ghazvin, because of growth at a lower thermal mean temperature and mountainous region, contained plants with more protective tissues and thicker cuticle layer, the Mashad population had the largest petiole and the Ramsar population had the smallest. The maximal lithocysts were observed in the Ramsar population.

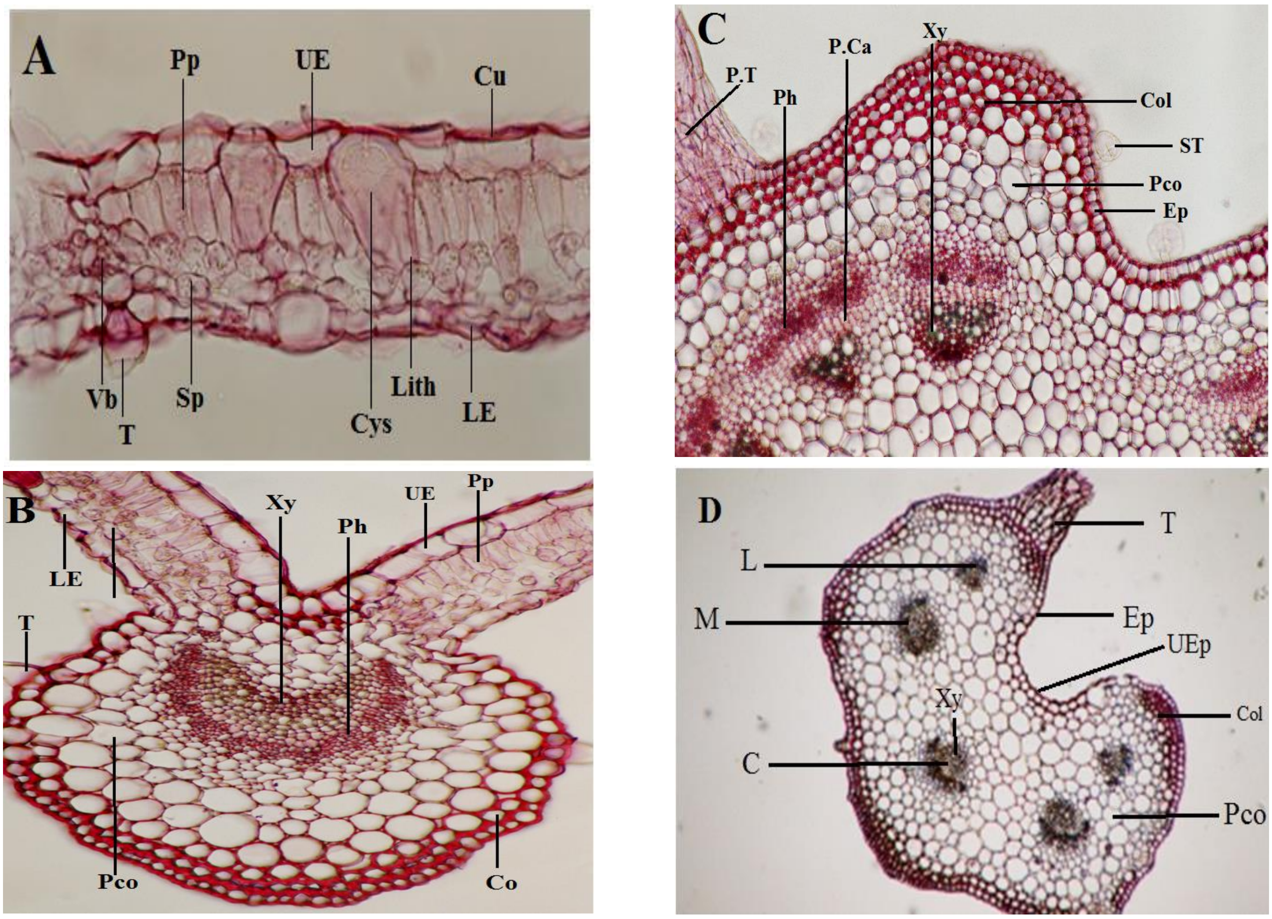

Fig 1 Cross section of leaf, stem and petiole in *Urtica dioica* L. from ramsar population ,A: leaf (scale = × 20), B: Midrib of leaf (scale = × 10), C: Stem(scale = × 10), D: petiole(scale = × 4).Cu: Cuticle, UE: Upper Epidermis, LE: Lower Epidermis, PT: Parenchymatous Trichome, Pp: Palisade parenchyma, Sp: Spongy parenchyma, ST: Secretory Trichome, Vb: Vascular bundle(central (C) and marginal (M) and the two minor [lateral (L)]) , Xy: Xylem, Pco: cortical Parenchyma, Ph: Phloem, Ep: Epidermis , P(co): Parenchyma (cortex), Col: Collenchymas, P.Ca: Pro. Cambium, Lith: Lithocyst , Cys: Cystolith, T: Trichome.

Discussion and Conclusions

This study confirmed an effect of climate on differentiation in anatomical structures of *U. dioica* L. Under climate stress conditions, the plants and rhizomes were shorter with fewer branches and rhizomes. Less biomass was produced as the water table approached the soil surface (Figs 1 and 2). Changes in stem, leaf, and petiole tissues confirmed that climatic factors have been many effects on populations of nettle expressed by differences in anatomical structure of aerial organs. By these results we confirmed that the effect of climate conditions on the differentiation anatomical structure in *U. dioica* L. The thickest and thinnest vascular bundles and collenchymas cells were observed in our study for Ghazvin and Ramsar populations, respectively. These results agree with observations by Miroslav [22] that showed: the plants were shorter with fewer branches, smaller rhizomes and less biomass under stress. The Ghazvin population had the most collenchymas, fiber, xylem bundles with larger diameters and trichomes, result that agree with accounts by Dibah [23] and Irimo website [17]. Apparently these differences are due to higher levels of ultraviolet light in the mountainous region of Ghazvin population. The plant has more protective tissue. Lithocyst abundance reports were similar to work of Dibah [23] and Han [24] that showed the abundances of secondary metabolites (cystoliths in lithocyst) dependent upon climatic conditions.

Finally, we can conclude that, the best condition was for Ramsar population. With the obtained results the effect of climatic conditions on the differentiation anatomical structure in *Urtica dioica* L. can be confirmed. The results showed that much diffraction in its organs has been changed corresponding to different clime by considering the aspects of the effect of secondary metabolite and medical component of different clime. It has been suggested with respect to the medical effect in this plant on different clime that by the results of this research, the comparison of secondary metabolite that related to medical effect in this plant on different clime will be studied in the next research.

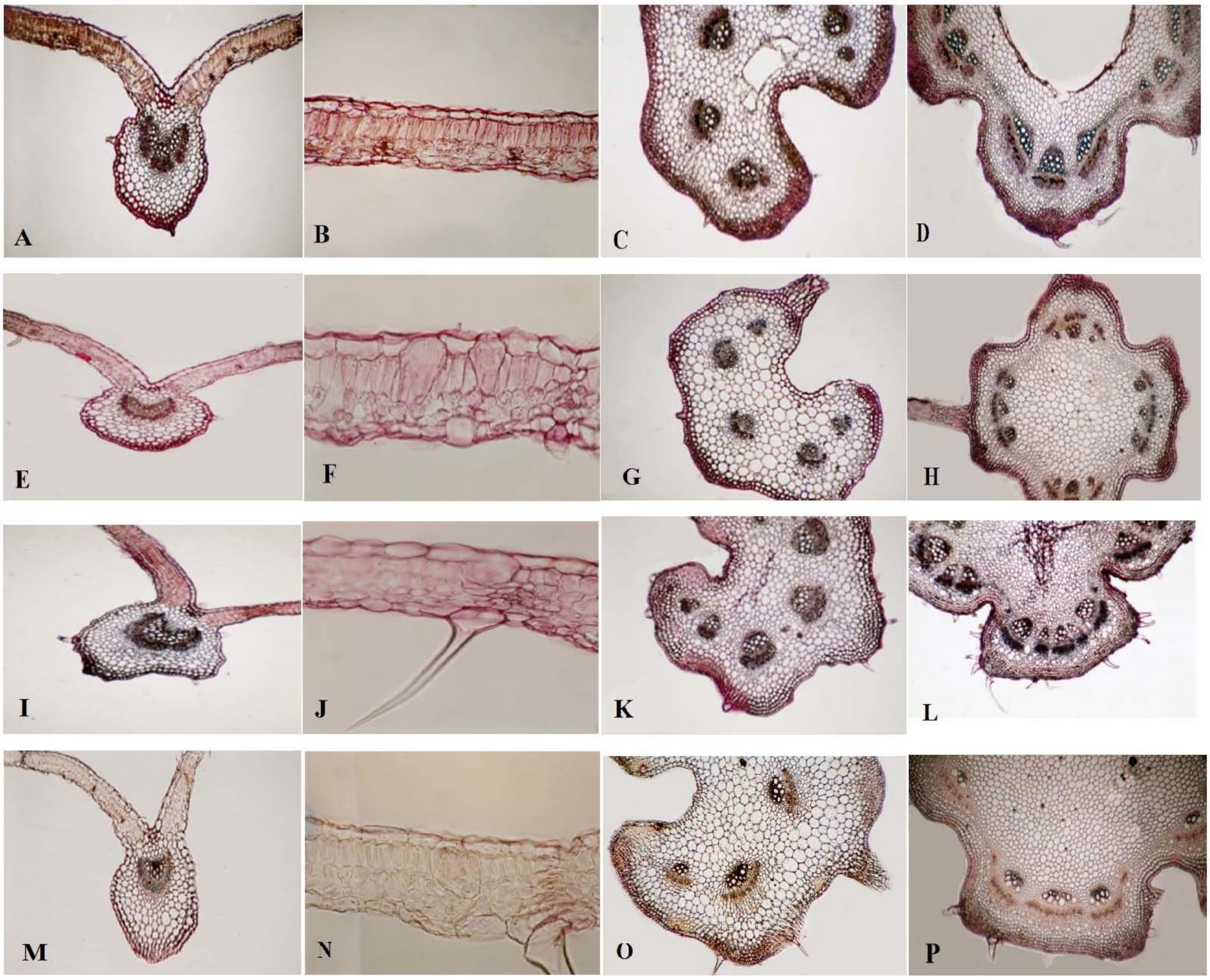

Fig 2 Comparative cross section of leaf, stem and petiole in *Urtica dioica* L. (A-D: Brojerd, E-H: Ramsar, I-L: Ghazvin, M-P: Mashad), A,E,I,M: Midrib of leaf (scale = × 4), B,F,J,N: leaf (scale = × 20), C,G,K,O: petiole (scale = × 4), D,H,L,P: Stem (scale = × 4).

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