

Anatomical Investigations on Three Varieties of *Thalictrum minus* L. Growing in Turkey

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T. minus L. is a complex species which has been divided into three varieties in Turkey. These are var. *minus*, var. *majus* (Crantz) Crepin and var. *microphyllum* Boiss. In this study, stem and leaf anatomical features of the varieties were described, compared and some differences have been determined in between the varieties. In transverse sections of stems; the shape of stems, presence or absence of trichomes, the number of vascular bundles and in leaf sections; the structure of the mesophyll and the shape of the epidermal cells were identified as possible differential characters. We believe that our results were carried out to provide additional evidence for taxonomists and it can be help to separate of varieties.

Key words: Anatomy, Leaf, Ranunculaceae, Stem, *Thalictrum minus*, Variety

Türkiye’de Yetişen *Thalictrum minus* L.’un Üç Varyetesi Üzerinde Anatomik Araştırmalar

Thalictrum minus L. Türkiye’de üç varyeteye ayrılmış kompleks bir türdür. Bunlar; var. *minus*, var. *majus* (Crantz) Crepin ve var. *microphyllum* Boiss.’dur. Bu çalışmada varyetelerin gövde ve yaprak anatomik özellikleri tanımlanmış, karşılaştırılmış ve varyeteler arasında bazı farklılıklar belirlenmiştir. Gövdelerin enine kesitlerinde; gövdelerin şekli, tüy örtüsünün varlığı ya da yokluğu, iletim demetlerinin sayısı ve yaprak kesitlerinde; mezofil yapısı ve epiderma hücrelerinin şekli ayırıcı karakterler olarak tanımlanmıştır. Sonuçlarımızın taksonomistler için ilave kanıtlar sağlayacağına ve varyetelerin ayırımına yardımcı olabileceğine inanmaktayız.

Anahtar kelimeler: Anatomi, Yaprak, Ranunculaceae, Gövde, *Thalictrum minus*, Varyete

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INTRODUCTION

The Ranunculaceae is a large family containing of well-known wild flowers and garden ornamentals such as buttercups, anemones and hellebores and some very poisonous plants, such as *Aconitum* L. (1). *Thalictrum* L. is one of the large genera in Ranunculaceae with 150 species grown in the Northern Hemisphere, tropical S.America and S.Africa (2).

The first comprehensive taxonomic monograph concerning *Thalictrum* species was published by Lecoyer in 1885. It includes 69 species grown in the world (3). On the other hand, Flora Orientalis (4) is the first source of information for *Thalictrum* species growing in Turkey. Davis recorded 11 taxa, 9 species and 3 varieties of with Mediterranean, four Euro-Siberian and two Irano-Turanian elements in the Flora of Turkey (5). Comparative anatomical properties of three *Thalictrum* species were studied by Filipescu in 1969 (6). Hand (7) reported revision of subsectio *Thalictrum* (two subpecies out of Europa) in Europa. They are; ssp. *maxwellii* (Royle) Hand, ssp. *saxatile* DC. ex Ces., ssp. *caffrum* (Eckl. & Zeyh.) Hand, ssp. *minus*, ssp. *majus* (Crantz) Hook, ssp. *thunbergii* (DC.) Vorosch., ssp. *pratense* (F. W. Schultz) Hand and ssp. *elatum* (Jacq.) Stoj. & Stef. In the previous a study, Tatlıdil et al. (8) are reported pollen morphology of three varieties of *T. minus* growing in Turkey. According to their results, the pollen diameters, pore numbers, pore membrane features and distance between pores varies in varieties.

Members of the family have diverse chemical constituents. Numerous alkaloids have been identified from this genus, some with pharmacologic potential and ethnomedical usage. In the previous studies, the alkaloids (9–12) of three varieties of *T. minus* growing in Turkey are reported. And, some differences are found in their alkaloids. Numerous alkaloids such as isoquinoline, benzyloisoquinoline, bisbenzyloisoquinoline, aphyorphine, aphyorphine–bisbenzyloisoquinoline, phenanthrene and protoberberine were found in all three varieties. However, eight new compounds were isolated from the var. *microphyllum* (9), thalmineline, N-methylcanadine, northalibroline, thalivarminine and thalsivasine were found only in var. *minus* (10–11) and oxyacanthine was found only var. *majus* (12).

The varieties of *T. minus* grow on damp meadows, gorges and ditches in 500–2600 m. *T. minus* var. *microphyllum* is locally known as “Kahraman kaytaran” in East Anatolia where used as asthma, cardialgia and headache. Var. *minus* is also locally known as “Kara katranotu” in East Anatolia where used as eye diseases, diuretic, abscess (8).

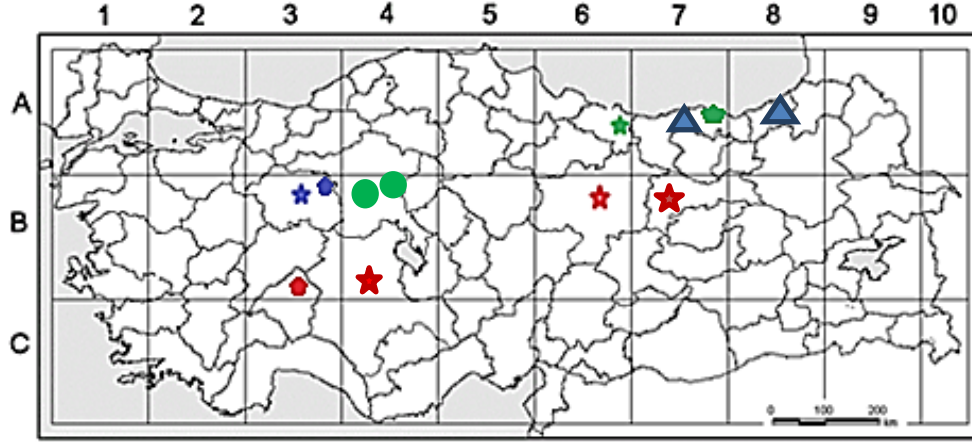
T. minus is usually glabrous. The stems are erect and up to 100 cm. Leaves are \pm triangular in outline, the two lateral divisions are almost as large as the middle division. Ultimate segments are very variable. It is a complex species which has been divided into many subordinate taxa. There are three taxa in Turkey; var. *minus*, var. *majus* and var. *microphyllum* which are often difficult to distinguish in Turkey and further work on the complex is necessary to Flora of Turkey (5). The largest ultimate leaf segments less than 9 mm in var. *microphyllum* as they are more than 9 mm in var. *minus* and var. *majus* according to Flora of Turkey. Inflorescence is a dense (var. *minus*) to lax (var. *majus*) panicle. Achenes are fusiform and ribbed.

In this paper, we report a comparative study on stem and leaf anatomical features of the plants in order to provide additional possibilities for the differentiation of the varieties.

EXPERIMENTAL

The plant materials were collected during the flowering period (in between May–July) from different localities of Turkey (Map and Table 1). Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy of Anadolu University, in Eskisehir, Turkey (ESSE). Living material was stored in 70 % alcohol for anatomical studies. Transverse sections and surface preparations of leaves were prepared manually. All sections were taken from middle section of leaves and stem. Many leaves and stem for each locality were used and prepared about

150 slides, that 6-9 sections in each slide, were investigated in total. All sections were embedded in glycerin-jelatine and mounted on microscope slides with Canada Balsam and photographs were taken through a light microscope (Olympus BX51T).



Map. The study area of varieties; var. *minus* ★ var. *majus* ● var. *microphyllum* ▲

Table 1. Collection data of studied *Thalicttrum minus* varieties

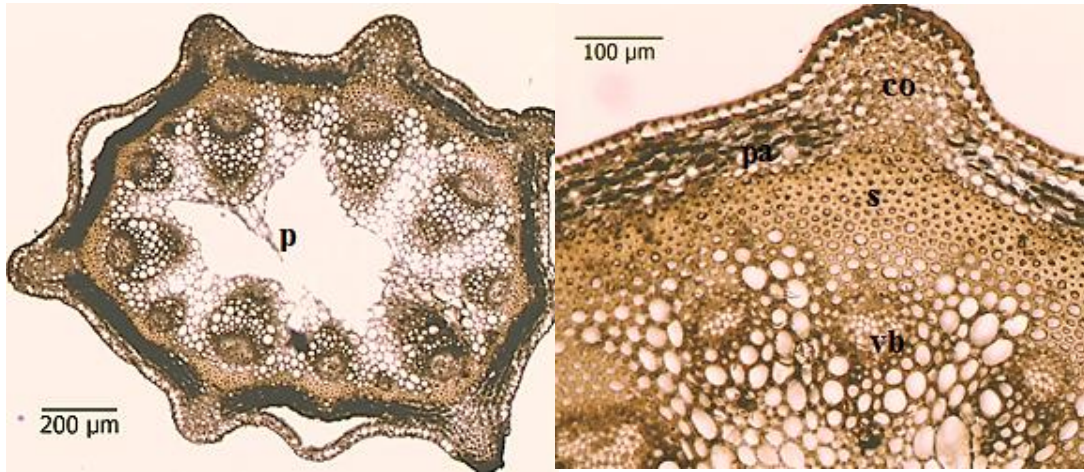
| Taxa | Collection data | ESSE |
|--------------------------|---|------|
| var. <i>minus</i> | B6: Sivas: between Şerefiye-Zara Kurbağalıbeli area | 2014 |
| | B3: Isparta: Gölcük, Karanlıkdere area | 7666 |
| var. <i>majus</i> | A6: Ordu: Ordu-Giresun road, 15. km, seaside | 1093 |
| | A8: Trabzon: Köprübaşı, Köprübaşı-Vizara plateau | 1107 |
| var. <i>microphyllum</i> | B3: Eskişehir: Mahmudiye, near hara | 1113 |
| | B3: Eskişehir: Çifteler meadow | 1114 |

RESULTS

Stem

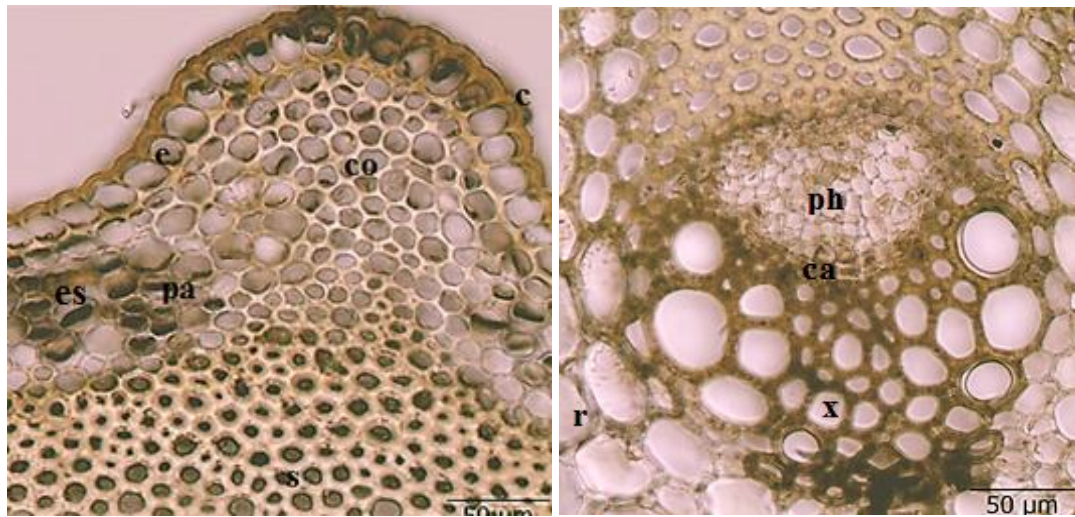
Transverse sections from the middle part of the stem revealed the following elements (Figs. 1-3): The stems are polygonal in var. *minus*, almost orbicular in var. *majus* and kidney shaped in var. *microphyllum* (Figs. 1a-3a). The epidermis is composed of a single layer of almost square or ovoid compactly arranged cells. The outer cell wall is covered with an almost a thick cuticle. The outer and inner walls of the epidermis are thick, the lateral walls of the epidermis cell are thin in var. *minus* and var. *microphyllum* while the outer walls of the epidermis cells are thick, the inner and the lateral walls are thin in var. *majus*. Covering trichomes are observed only in

var. *microphyllum* (Fig. 3). They consist of glandular (head and stalk unicellular) and non-glandular type (simple, uni-three-cellular). Covering trichomes occur more frequently. The collenchyma tissue is located under the epidermis of the stem ridges. The shape of collenchymatous cells is ovoid or polygonal. They are 7-9 layered in var. *minus*, 2-3 layered in var. *majus* and they form a projecting part towards the outside. However, in var. *microphyllum*, there are 5-6 layered parenchymatous cells instead of collenchyma cells and they are ovoid or orbicular shaped and they slightly form a projecting part towards the outside (Fig. 3b). The cortex tissue composed of parenchyma cells which 4-6 layered in var. *minus*, 3-4 layered in var. *majus* and 5-6 layered in var. *microphyllum*. The parenchyma tissue are contained some ergastic substances in var. *minus* and var. *majus* (Figs. 1c-2c). However, they are observed 1-2 seriate in parenchymatous cells of var. *microphyllum*. A ring of pericyclic sclerenchyma is 5-7 layered and consists of polygonal or oval forming. Endodermis is inconspicuous. The numerous (14-33) vascular bundles are arranged in one circle in var. *minus* and var. *majus* while one or two circles in var. *microphyllum*, (with V-shaped xylem) under the pericycle. The numbers of vascular bundle are 16-17 in var. *minus*, 20-33 in var. *majus* and 14-29 in var. *microphyllum* (Figs. 1a-3a). It is collateral, typically with the xylem concave on the side towards the phloem, so that the latter is often partly surrounded by xylem. The xylem is especially surrounded by sclerenchymatous cells in var. *microphyllum*. Cambium is usually squashed, indistinct or several layered in all varieties (Figs. 1d-2d). Medullary rays are multiseriate, usually up to 7 cells wide. They are 2-7 layered in var. *minus*, 3-5 layered in var. *majus* and 1-5 layered in var. *microphyllum*. The pith is composed of large orbicular or polyhedral parenchymatous cells, often with intercellular spaces. It is hollow in var. *minus* and in var. *majus* (Figs. 1a-2a).



a

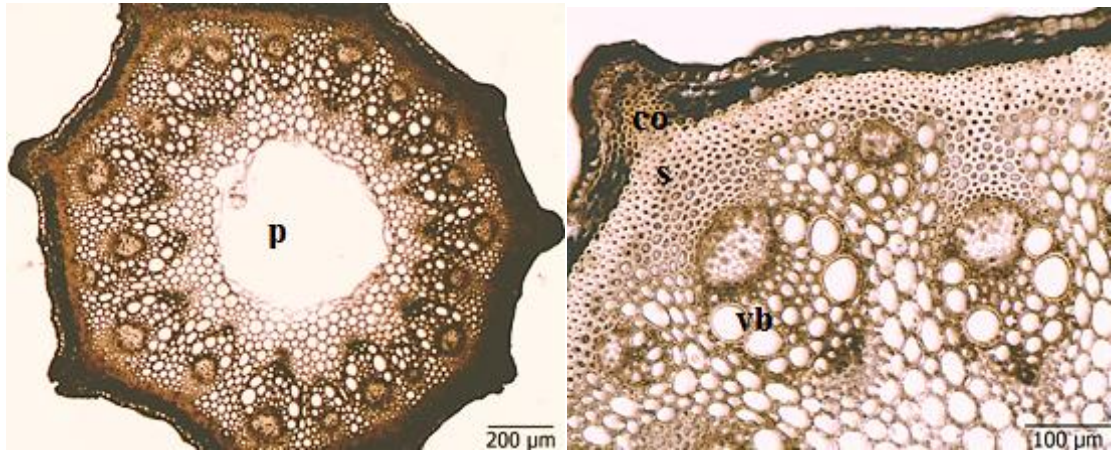
b



c

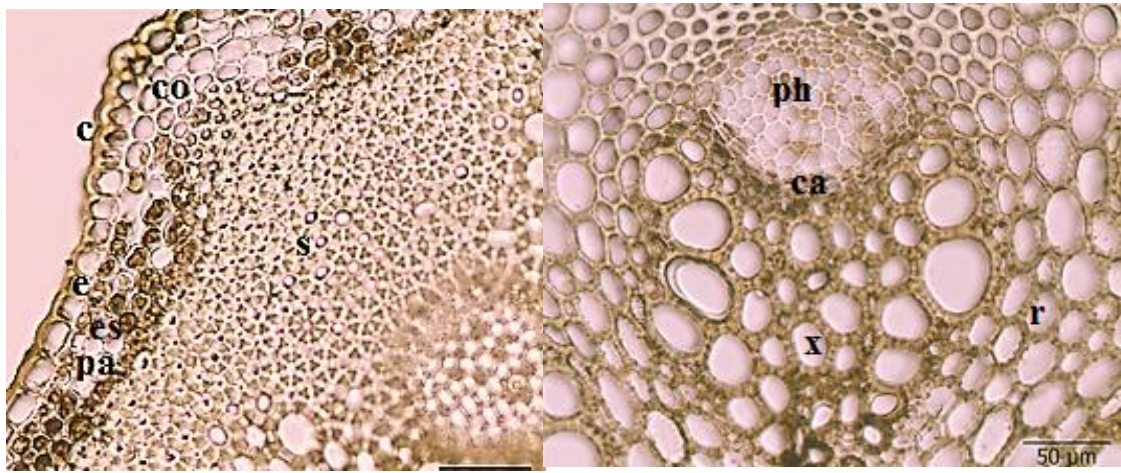
d

Fig. 1. *T. minus* var. *minus*: Stem transverse section (a-d): c-cuticle, ca-cambium, co-collenchyma, e-epidermis, es-ergastic substance, ph-phloem s-sclerenchyma x-xylem pa-parenchyma p-pith, r-ray, vb-vascular bundle



a

b



c

d

Fig. 2. *T. minus* var. *majus*: Stem transverse section (a-d): c-cuticle, ca-cambium, co-collenchyma, e-epidermis, es-ergastic substance, ph-phloem s-sclerenchyma x-xylem pa-parenchyma p-pith, r-ray, vb-vascular bundle

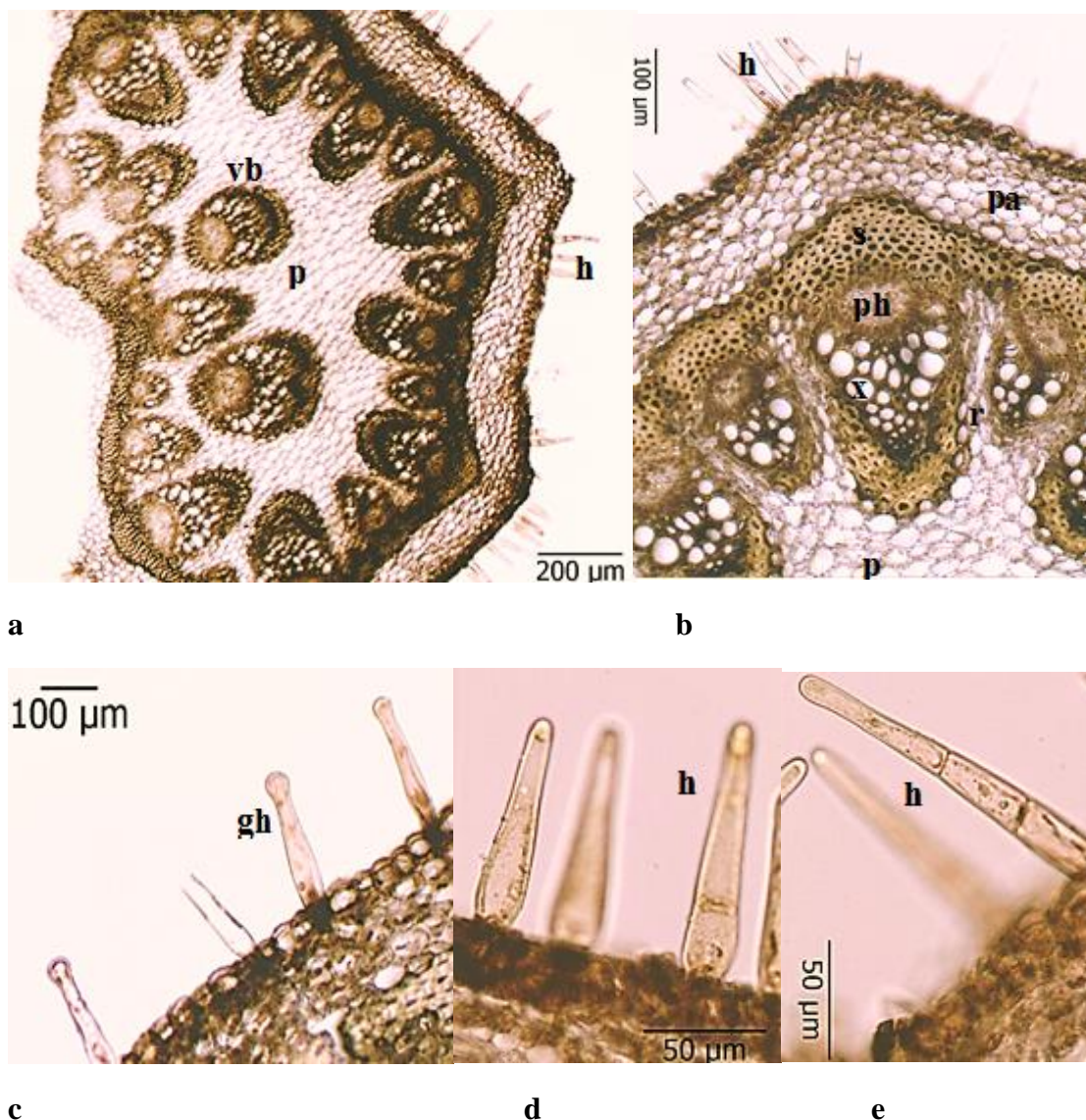


Fig. 3. *T. minus* var. *microphyllum*: Stem transverse section (a-e): gh-glandular hair, h-hair, ph-phloem, s-sclerenchyma, x-xylem, pa-parenchyma, p-pith, r-ray, vb-vascular bundle

Leaf

Transverse sections of the lamina midrib and surface preparations of both abaxial and adaxial epidermis revealed the following elements (Figs. 4-6):

The upper and lower epidermis consist of a single cell layer and the shape of cells is ovoid, square or rectangular. Cells of the upper epidermis are larger than cells of the lower epidermis (Figs. 4-5c, 6b). The walls of upper and lower epidermal cells are sinuous in var. *minus* and var. *microphyllum* while they are slightly sinuous in var. *majus*. The outer and inner walls of the epidermis cells are thicker than the anticlinal walls. Both epidermis are covered with a thin cuticle. Covering trichomes are absent in all varieties. Anomocytic stomata type (Figs. 4d-5d, 6c) occurs on the lower surfaces of the leaf only (hypostomatic) (Figs. 4a-6a). They are located on the same level with epidermal cells (mesomorphic type). In transverse section the structure of the leaf is of the bifacial type. The mesophyll is differentiated into a palisade tissue

consisting of 2-3 layers and 2-seriate spongy tissue (arm-palisade) in var. *minus*, 2 seriate palisade tissue and 2-seriate spongy tissue in var. *majus* and 2-seriate palisade tissue and 1-2-seriate spongy tissue (arm-palisade) in var. *microphyllum*. The shape of the cells of spongy tissue is almost elongated, elongated-ovoid in the last layer of var. *minus*. The mesophyll is arranged densely in var. *minus* and var. *majus* while it is arranged in a more loose way in var. *microphyllum* (Figs. 4a-6a). Type of vascular bundles is collateral and occur in a small area. The midrib is less developed. In this regions, vascular bundles are accompanied by collenchymatous tissue. The midrib region forms a projecting part in all varieties (Figs. 4b-6b).

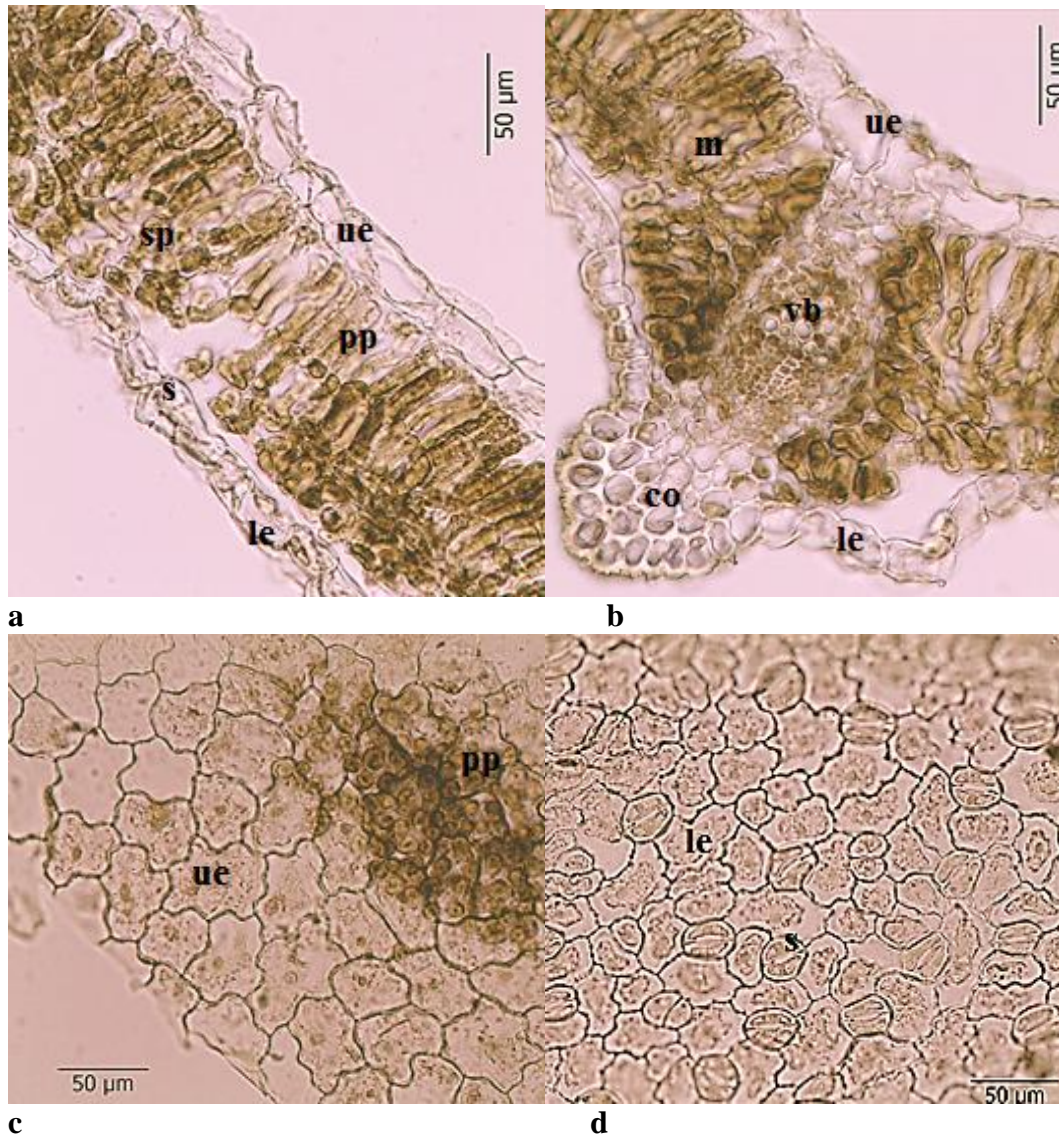


Fig. 4. *T. minus* var. *minus*: Leaf transverse section (a, b), surface preparation of upper epidermis (c), surface preparation of lower epidermis (d): co-collenchyma, m-mesophyll, pp-palisade parenchyma, sp-spongy parenchyma, s-stomata, ue-upper epidermis, le-lower epidermis vb-vascular bundle

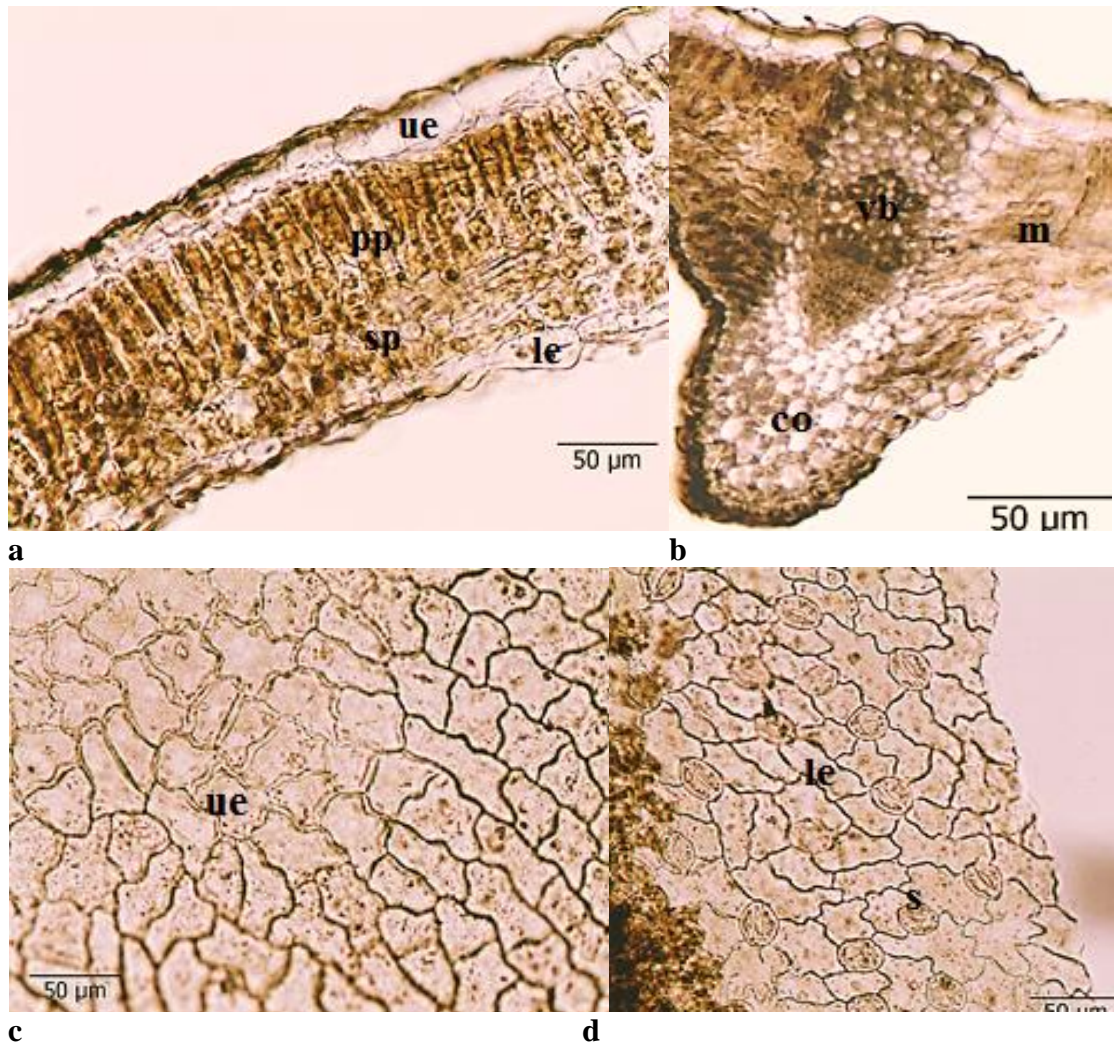


Fig. 5. *T. minus* var. *majus*: Leaf transverse section (a, b), surface preparation of upper epidermis (c), surface preparation of lower epidermis (d): co-collenchyma, m-mesophyll, pp-palisade parenchyma, sp-spongy parenchyma, s-stomata, ue-upper epidermis, le-lower epidermis, vb-vascular bundle

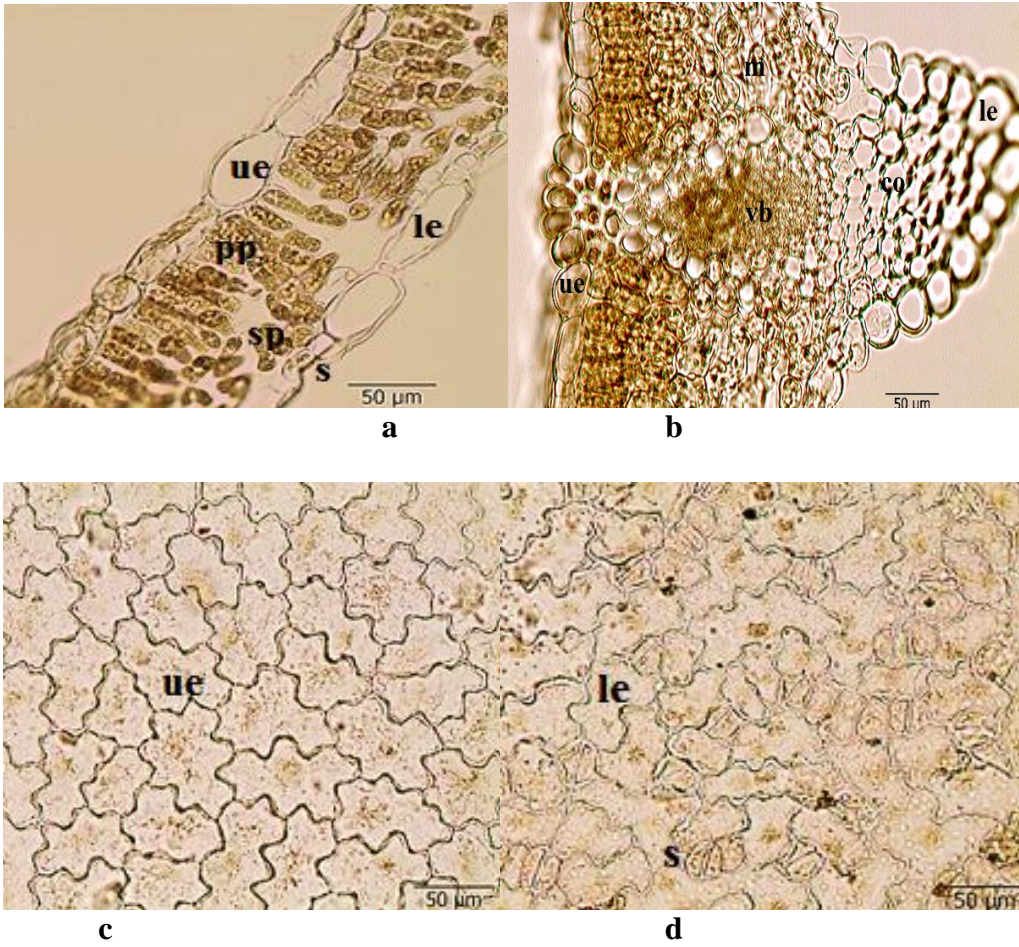


Fig. 6. *T. minus* var. *microphyllum*: Leaf transverse section (a,b), surface preparation of upper epidermis (c), surface preparation of lower epidermis (d): co-collenchyma, m-mesophyll, pp-palisade parenchyma, sp-spongy parenchyma, s-stomata, ue-upper epidermis, le-lower epidermis, vb-vascular bundle

Table 2. A comparison of stem and leaf anatomic characteristics in *Thalictrum minus* varieties

| Stem | var. <i>minus</i> | var. <i>majus</i> | var. <i>microphyllum</i> |
|----------------------------|--------------------------|--------------------------|---------------------------------|
| Shape (transverse section) | polygonal | almost orbicular | kidney |
| Trichome | absent | absent | present |
| Collenchyma | 7-9 layered | 2-3 layered | 5-6 layered parenchyma |
| Parenchyma | 4-6 layered | 3-4 layered | absent |
| Ergastic substance | more | more | less |
| Vascular bundle | 16-17 in one circle | 20-33 in one circle | 14-29 one or two circles |
| Medullary rays | 2-7 layered | 3-5 layered | 1-5 layered |
| Pith | hollow | hollow | not hollow |
| Leaf | | | |
| Pallisade parenchyma | 2-3 layers | 2 layers | 2 layers |
| Spongy parenchyma | 2 spongy pa. (arm-pa.) | 2 spongy pa. | 1-2 spongy pa. (arm-pa.) |
| | dense | dense | loose |
| Both epidermal walls | sinuous | slightly sinuous | sinuous |

DISCUSSION

Results of the study show that there are some anatomical variations in between varieties of *T. minus* growing in Turkey. However, many characters are same in all of them.

The shape of stems in transverse sections is different in all varieties. Especially, *T. minus* var. *microphyllum* can be easily separated than the others by the shape of stem and the presence of covering trichomes. The collenchymatous cells are located on under the epidermis in var. *minus* and var. *majus*. However, the parenchymatous cells are located on under the epidermis in var. *microphyllum*. It has been reported that, in stem transverse sections, vascular bundles are collateral and arranged in several circles in the genus *Thalictrum* to Metcalfe and Chalk (14) and Filipescu recorded the occurrence of two circles in *T. minus* (6). However, they consist of one circle in our var. *minus* and var. *majus* samples and one or two circles in var. *microphyllum* samples. The numbers of vascular bundles are observed 16-17 in var. *minus*, 20-33 in var. *majus* and 14-29 in var. *microphyllum*. They are recorded as 21-22 and 33-34 for *T. minus* in study of Filipescu. The xylem tissue in var. *microphyllum* is surrounded by prominent sclerenchymatous cells. The pith is hollow in var. *minus* and in var. *majus*. The hollow pith are recorded in many herbs of Ranunculaceae (14).

All the studied varieties were found to have hypostomatic leaves with anomocytic stomata. The hypostomatic leaves type is also recorded by Filipescu for *T. minus* (6). In surface preparations, sinuous lower anticlinal epidermal walls are observed in all varieties. This structure of lower epidermis are common in Ranunculaceae (14). However, the walls of upper and lower epidermis are sinuous in var. *minus* and var. *majus* as they are slightly sinuous in var. *microphyllum*. According to Metcalfe and Chalk (14), hairs are simple, unicellular in leaf of *Thalictrum*. However, covering trichomes are absent in the leaves of *T. minus* varieties. Filipescu reported that *T. minus* var. *flexuosum* (Bernh.) S. et K. and *T. lucidum* var. *stenophyllum* (Wimm. Et Grab.) f. *hirtostenophyllum* Nyar. have isobilateral type of leaves and *T. aquilegifolium* L has dorsiventral (6). *T. orientale* has also dorsiventral type of leaves to Kaya et al. (15). The leaves of *T. minus* varieties have bifacial type. The mesophyll is differentiated in palisade and spongy parenchyma and some differences are observed in the numbers of mesophyll cells. The spongy tissue has arm-palisade cells in var. *minus* and var. *microphyllum*. Arm-palisade cells are also common in leaf of Ranunculaceae (14) and they are reported in *T. orientale* (15). The vascular bundle is collateral type. The midrib region is less developed and forms a projecting part towards the outside in all varieties.

T. minus is a complex species which has been divided into three varieties in Turkey. The varieties must be necessary further work according to Flora of Turkey (5). We believe that our results were carried out to provide additional evidence for taxonomists and it can be help to separate of varieties.

Key to the taxa

1. Stems are kidney shaped, trichome present, without hollow pith and mesophyll tissue sparse.....var. ***microphyllum***
1. Stems are polygonal or orbicular shaped, trichome absent, with hollow pith and mesophyll tissue dense.....2
2. Stem polygonal, collenchyma 7-9 layered, vascular bundle numbers 16-17 and leaves with palisade parenchyma in 2-3 layers, anticlinal walls of both epidermal cells sinuous.....var. ***minus***
2. Stem orbicular, collenchyma 2-3 layered, vascular bundle numbers 20-33 and leaves with palisade parenchyma in 2 layers, anticlinal walls of both epidermal cells slightly sinuous.....var. ***majus***

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