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Research Article / Araştırma Makalesi

MORPHOLOGICAL AND ANATOMICAL INVESTIGATIONS ON SOME *HYPERICUM* L. SPECIES GROWING NATURALLY IN AND AROUND ESKISEHIR *

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Abstract: The purpose of the present study is to determine some morphological and anatomical characteristics of the species of *Hypericum montbretii* Spach., *H. origanifolium* Willd. and *H. perforatum* L. that show natural distribution in Eskişehir and its surrounding area. *H. perforatum* was determined to exist more abundantly in the research area in comparison with *H. montbretii* and *H. origanifolium*. While *H. montbretii* and *H. origanifolium* have black glands, *H. perforatum* lack such glands. Upon the examination of the root cross-rections, the pith was determined to be completely covered by xylem cells. As to stem cross-sections, it was observed that large parenchymatic cells were present in the pith of the young stems, while old stems were observed to have formed cavity in their pith. The species examined were determined to bear resemblance to one another in consideration of their leaf anatomy. Leaves are equifacial and amphistomatic and they have amaryllis type stomata. They are mesomorphic and there are schizo-lisigenous type secretion pockets in the leaves. Compared to adjacent cells,stomata are anisositic or diasitic. Stomata on the lower surface of the leaves show higher frequencies than those on the upper surface.

Key words: *H. montbretii*, *H. origanifolium*, *H. perforatum*, anatomy, morphology

Eskişehir ve çevresinde yetişen bazı *Hypericum* L. Türleri üzerinde morfolojik ve anatomik çalışmalar

Özet: Bu çalışmada Eskişehir ve yöresinde doğal yayılış gösteren *Hypericum montbretii* Spach., *H. origanifolium* Willd. ve *H. perforatum* L. türlerinin bazı morfolojik ve anatomik özellikleri saptanmaya çalışılmıştır. Araştırma bölgesinde *H. perforatum*'un *Hypericum montbretii* ve *H. origanifolium*. türlerine göre daha geniş yayılış gösterdiği görülmüştür. *H. montbretii* ve *H. origanifolium* türlerinin sepallerinde siyah glandlar bulunmasına karşın bu glandlar *H. perforatum* türünün sepallerinde yoktur. İncelenen türlerin kök enine kesitlerinde, öz bölgesi tamamen ksilem hücreleri tarafından örtülmüştür. Gövde enine kesitlerinde türlerin öz bölgesinde genç gövdelerde iri parankimatik hücreler vardır, yaşlı gövdelerde ise öz boşluğu oluşmaktadır.İncelenen türlerin yaprak anatomileri birbirlerine çok benzemektedir. Yapraklar ekvifasial ve amfistomatiktir ve amarillis tip stomalara sahiptirler. Mezomorfik olan yapraklarda şizo-lizigen tipte salgı cepleri vardır. Stomalar komşu hücrelerine göre de anizositik ve diasitik tiptedirler. Türlerin yapraklarının alt yüzeylerindeki stoma frekansları, üst yüzeylerine göre daha yüksektir.

Anahtar kelimeler: *H. montbretii*, *H. origanifolium*, *H. perforatum*, anatomi, morfoloji

Introduction

The genus *Hypericum* L., a member of the Guttiferae (Hypericaceae) family, is a genus growing abundantly also in Turkey. Scattered across tropic and subtropic regions, as well as across Africa, North America, Asia and Europe, this family is represented on the Earth by 46 genera and 1000 species. The sole member of the family present in Turkey is *Hypericum*, 80 species of which show natural distribution in Turkey.

* This study is a part of İsmühan Potoğlu Erkara's master thesis entitled as "A Taxonomical, Morphological and Cytotaxonomical Investigation on Some *Hypericum* Species Growing Naturally In and Around Eskişehir".

Boissier (1867), Robson (1967), Tutin (1968), Alptekin (1974), Çakırer (1981), Tokur (1987, 1988), Robson (1988), Arda (1989) and Dönmez (2000) carried out systematical research into the *Hypericum* species present in Turkey. However, the number of anatomical data concerning the *Hypericum* species growing in Turkey is fairly scare (Arda 1989, Tokur and Mısırdalı 1989).

The plants analysed in our study belong to the gene resources of our country and are also used for medical purposes. We set about doing our research upon conceiving the idea that biological characteristics of plants need to be supported by morphological and anatomical research in order to shed more light on evolutionary and systematic relationships.

Therefore, our purpose was to determine morphological and anatomical characteristics of the *Hypericum* species growing naturally in and around the city of Eskişehir.

Materials and Methods

The species of *Hypericum montbretii* Spach., *H. origanifolium* Willd. and *H. perforatum* L. examined in this study were collected in and around the city of Eskişehir, Turkey during the years 1994-1995 (Figure 1).

H. montbretii Eskişehir: Kalabak village and its surrounding area, 1300 m,. under the forest, 05.06.1994, İ. Potoğlu (OUFE: 10332). Eskişehir: Kirazlı village, Springs, 1500 m., alongside the road, 05.06.1994, İ. Potoğlu (OUFE: 10333).

H. origanifolium Eskişehir: Sivrihisar, 1100 m., Hub.-Mor., alongside the road to Tekören village, 19.06.1994, İ. Potoğlu (OUFE: 10334). Eskişehir: Yörükkırka village, 1000 m., rural area, 05.06.1994, İ.Potoğlu (OUFE: 10335).

H. perforatum Eskişehir: Türkmen Mountains, upper parts of Kalabak, 1300 m., 05.06.1994, İ.Potoğlu (OUFE: 10336). Eskişehir: the country road to Kızılinler, alongside the road to Kütahya, 900 m., 05.06.1994, İ.Potoğlu (OUFE: 10337). Eskişehir: Sarıcakaya, Sakarılıca, Mayıslar, Dağküplü, Muttalip, 1300 m., alongside the road, 12.06.1994, İ.Potoğlu (OUFE: 10338)

Morphological graphics of the plants were achieved using Stereo Zoom Microscope (Prior). For determination of anatomical characteristics of the plants, we obtained some cross-sections of roots, stems and leaves manually. Plant materials were directly fixed in alcohol of 70 % and then kept in same solution to the acquisition of cross-sections. We also obtained lower and upper cross- sections from the fresh materials. All the cross-sections were stained with Sartur (60 ml lactic acid, 45 ml Sudan III (Merck), 2 gr aniline, 0.2 gr I, 1 gr KI, 10 ml alcohol (95 %), 80 ml distilled water) and were thus made permanent preparates by means of glycerine-gelatine. The sections were photographed by the Nikon Optiphot Microphotography device in our Department.

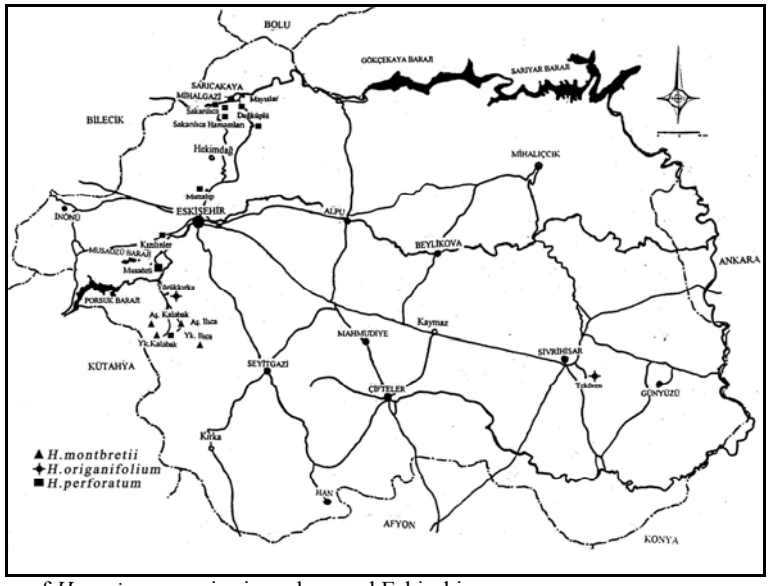

Figure 1. Distribution of *Hypericum* species in and around Eskişehir

Results

Morphology

The phenological characteristics of the species are illustrated in Table 1.

Table 1. The phenological characteristics of the species

Species	*H. montbretii*	*H. origanifolium*	*H. perforatum*
Time of blossoming	April-July	May-August	April-July
Habitat	Damp or shady places among rocks	Dry grassy or rocky slopes or steppe	Dry habitats in mesophytic regions and near water elsewhere
Altitude	200-1750 m	50-2400 m	0-2500 m
Emergence of first leaves	March	April	March
Emergence of first flowers	April-May	May-June	April-July
Emergence of first fruits	June-July	July-August	May-July
Maturation of seeds	August	August	August -September

H. montbretii grows quite close to the surface of the soil and the roots protruding from the decumbent and short rhizome are not deeply seated. The stem of the plant, which is perennial, suffruticose and decumbent, has a height of 15-60 cm and is erect in appearance. With the exception of those with inflorescence, no other branches display any kind of branching out. The leaves are 15-55 mm in length, apart from being ovate and acute. Black glands are present on leaves (intramarginal) as well as over sepals and petals. Petals are 8-14 mm in length. Capsule is 7-10 mm in length and is covered with vesicles. Fruit is septicid capsule. Seeds have straight veins and lines on the surface (Figure 2).

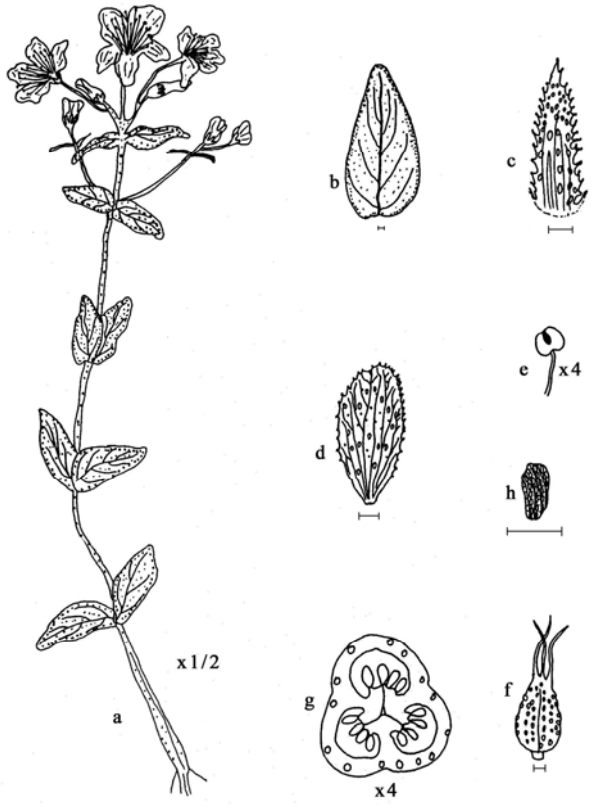

Figure 2. Morphological characteristics of *H. montbretii* a)Plant general appearance b)Leaf c)Sepal d)Petal e)Anther f)Capsule g)Cross-section of ovarium h)Seed (Scale 1 mm).

The stems of the rhizomes of *H. origanifolium* are 15-30 cm in length, with being perennial and suffruticose. There exist black and transparent glands over the surface of leaves, sepals and petals. Leaves have a lengthness of 5-30 mm, with being decussate and sessile. Petals are of 9-15 mm in length. Fruit is 7-12 mm in length in the form of septicid capsules with dorsal vittae and lateral vesicles. Seeds have a wrinkled appearance with rough lines (Figure 3).

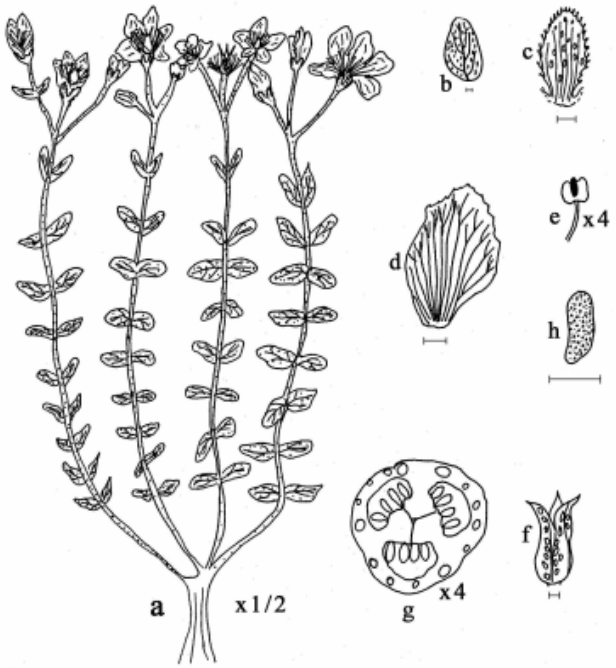

Figure 3. Morphological characteristics of *H. origanifolium* a) Plant general appearance b) Leaf c) Sepal d) Petal e) Anther f) Capsule g) Cross-section of ovarium h) Seed (Scale 1 mm).

Stems emerging from *H. perforatum* rhizome reach quite a depth under the soil. Branches, which are perennial, suffruticos and erect, branch out of the stem with a length of 10-110 cm. They grow alternately and densely. Leaves, which are lanceolate are 5-35 mm in length. No black glands are present on sepals. Petals are (5-) 8-15 mm in length and they have glands. Their styles are twice as long as ovary. The reddish brown fruit capsule is (4-) 5-9 mm in length, with also being septicid. Seeds have a reticular surface (Figure 4).

Anatomy

Root cross-sections

The root anatomy of *H. montbretii*, *H. origanifolium* and *H. perforatum* shows a remarkable similarity to one another. There is a thick cuticle in the outermost part, under which there lies a peridermis of 4-5 layers. There is also a parenchymatic cortex with a few layers. There lies a single-layered flat pericycle beneath the single layer of endodermis. There follow a narrow phloem with 2-3 layers, a cambium of 1-2 layers, apart from a widespread xylem. The pith of the root is entirely covered with xylem elements (Figure 5-7).

Stem cross-sections

The stem cross-sections *of H. montbretii, H. origanifolium* and *H. perforatum* bear a remarkable resemblance to one another. There is a cuticle in the outermost part and under this very layer lies a single-layered epidermis. Also, there lies a plate collenchyma with a few layers as well as a parenchymatic cortex under the epidermis. The single-layered endodermis cells are long and flattened, under which lies a single-layered pericycle. Phloem in rather narrow region with 2-3 layers precedes a cambium with a few layers and a rather widespread xylem. There is a parenchymatic pith located in the centre in addition to a pith cavity in the old stems (Figure 8-10).

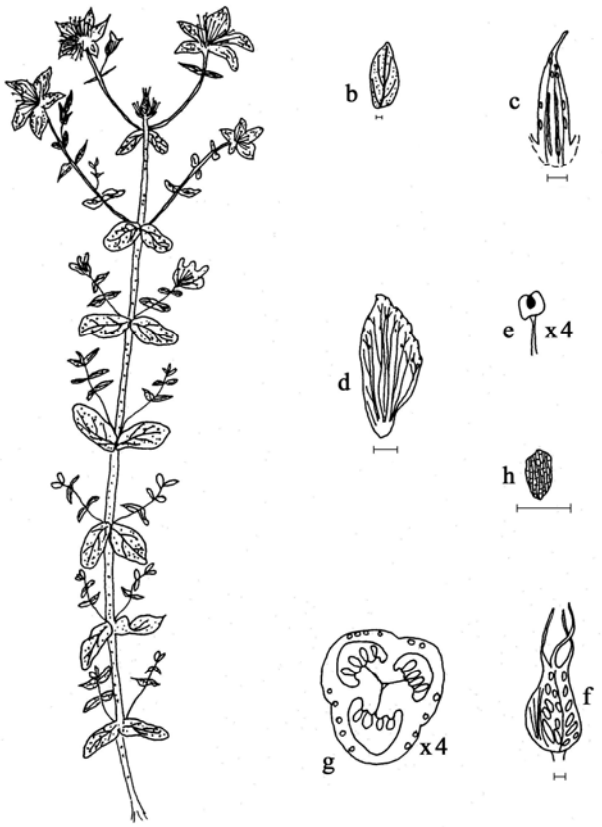

Figure 4. Morphological characteristics of *H. perforatum* a) Plant general appearance b) Leaf c) Sepal d) Petal e) Anther f) Capsule g) Cross-section of ovarium h) Seed (Scale 1 mm).

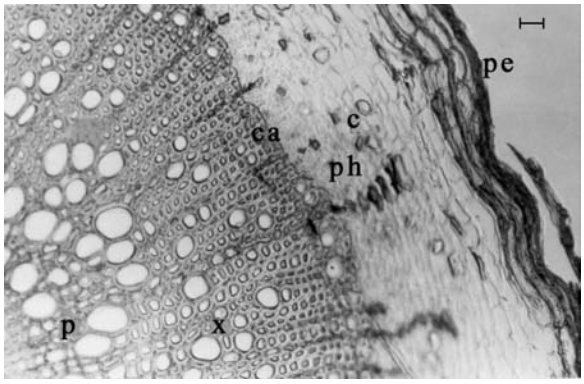

Figure 5. Cross-section of *H. montbretii* root (pe: Peridermis, co: Cortex, , ph: Phloem, ca: Cambium, x: Xylem, p: Pith; Scale 50 μm).

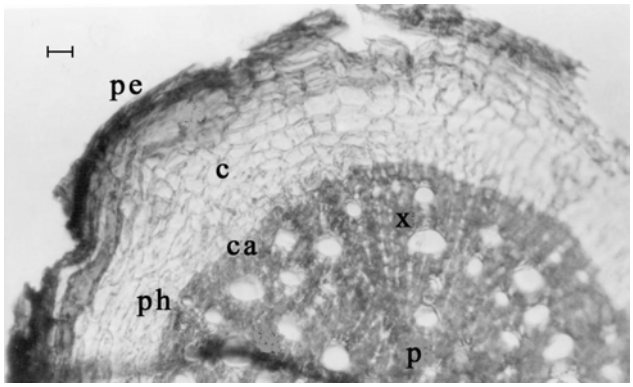

Figure 6. Cross-section of *H. origanifolium* root pe: Peridermis, co: Cortex, , ph: Phloem, ca: Cambium, x: Xylem, p: Pith; Scale 50 μm).

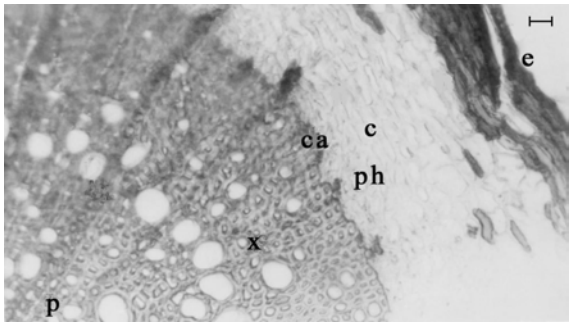

Figure 7. Cross-section of *H. perforatum* root pe: Peridermis, co: Cortex, , ph: Phloem, ca: Cambium, x: Xylem, p: Pith; Scale 50 μm).

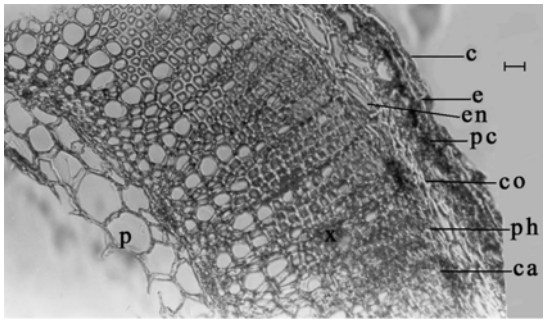

Figure 8. Cross-section of *H. montbretii* stem (c: Cuticle, e: Epidermis, pc: Plate collenchyma, co: Cortex, , ph: Phloem, ca: Cambium, x: Xylem, p: Pith; Scale 50 μm).

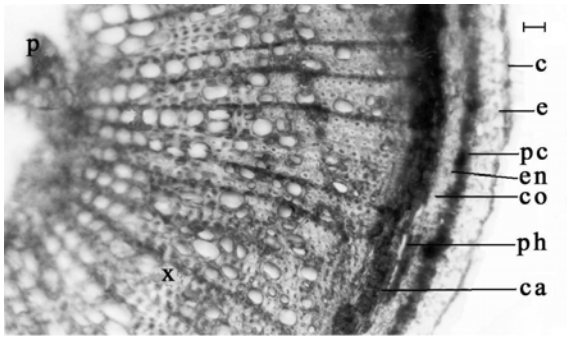

Figure 9. Cross-section of *H. origanifolium* stem (c: Cuticle, e: Epidermis, pc: Plate collenchyma, co: Cortex, , ph: Phloem, ca: Cambium, x: Xylem, p: Pith; Scale 50 μm).

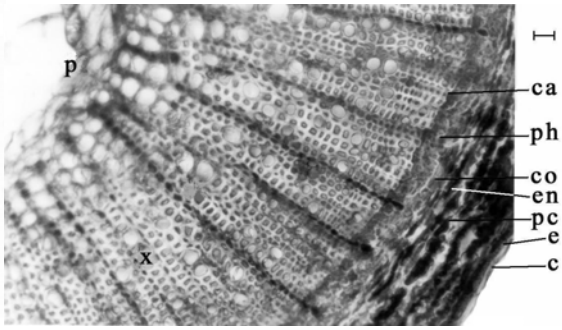

Figure 10. Cross-section of *H. perforatum* stem (c: Cuticle, e: Epidermis, pc: Plate collenchyma, co: Cortex, , ph: Phloem, ca: Cambium, x: Xylem, p: Pith; Scale 50 μm).

Leaf sections

Upon the examination of leaf anatomy, it was determined that all the three species of *Hypericum* have equifacial, amphistomatic and mesomorphic leaves that have amaryllis type stomata. There were schizo-lysigenous type secretion pockets on the leaves. The leaf surface sections revealed that stomata were anisositic and diasitic (Figure 11-19). The highest stomata frequency among the upper and lower surface sections was in *H. montbretii*. Its stomata frequency was 17 % in upper surface and 19 % in lower surface of leaves (Table 2).

Table 2. Stoma frequencies of the species (%)

Species	*H. montbretii*	*H. origanifolium*	*H. perforatum*
Stoma frequency of lower surface	19	17	16
Stoma frequency of upper surface	17	12	10

Discussion

Findings of the morphological characteristics of species complied with that of researches while some results were different. The sepal and petal lengths of the plants examined in our study were determined to comply with the findings of Robson (1967, 1988), Alptekin (1974), Tokur (1987-1988), Arda (1989) and Dönmez

(2000) while they were also determined to differ in some of their characteristics such as stem length and leave size.

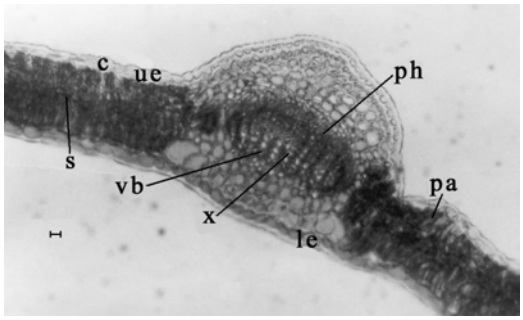

Figure 11. Cross-section of *H. montbretii* leaf (c: Cuticle, ue: Upper epidermis, le: Lower epidermis, pa :Palisade parenchyma, s: Spongy parenchyma, vb: Vascular bundle, ph: Phloem, x: Xylem; Scale 50 μm).

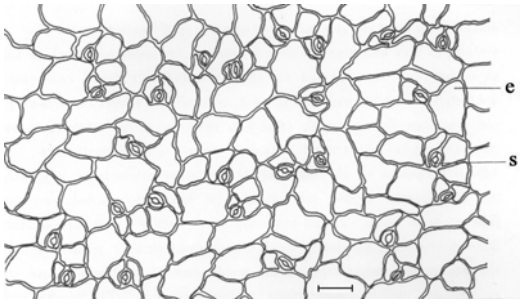

Figure 12. Upper surface section of *H. montbretii* leaf (e: Epidermis, s: Stoma; Scale 50 μm).

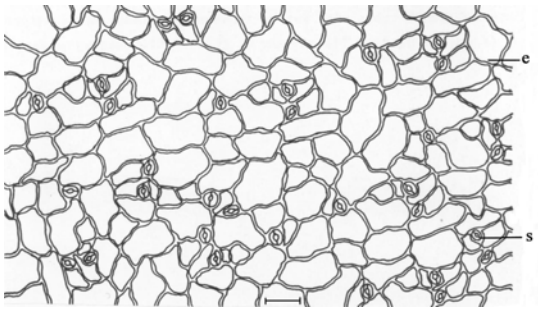

Figure 13. Lower surface section of *H. montbretii* leaf (e: Epidermis, s: Stoma; Scale 50 μm).

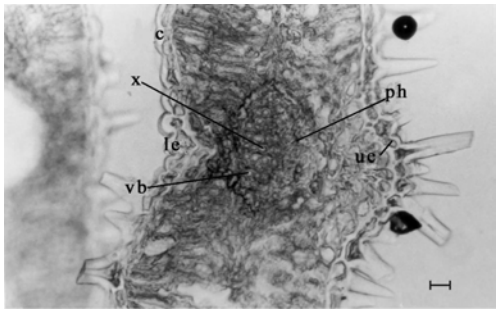

Figure 14. Cross-section of *H. origanifolium* leaf (c: Cuticle, ue: Upper epidermis, le: Lower epidermis, pa :Palisade parenchyma, s: Spongy parenchyma, vb: Vascular bundle, ph: Phloem, x: Xylem; Scale 50 μm).

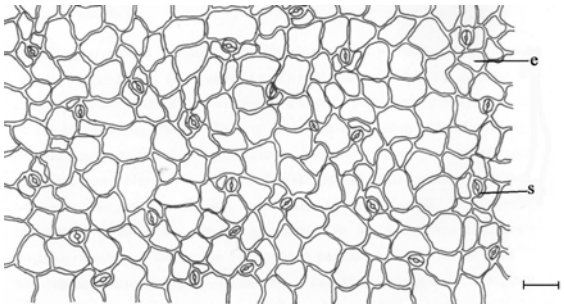

Figure 15. Upper surface section of *H. origanifolium* leaf (e: Epidermis, s: Stoma; Scale 50 μm).

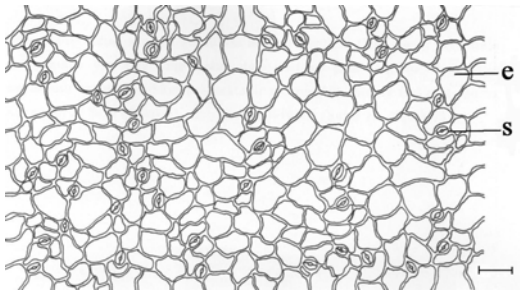

Figure 16. Lower surface section of *H. origanifolium* leaf (e: Epidermis, s: Stoma; Scale 50 μm).

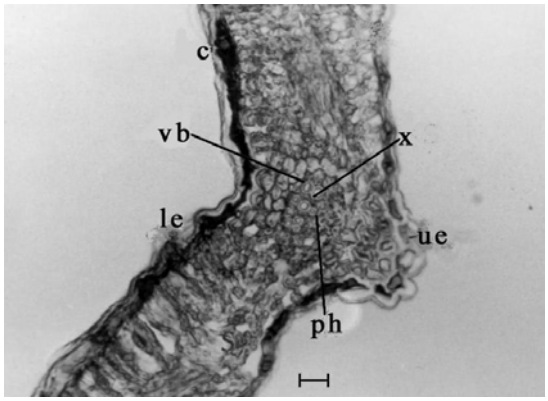

Figure 17. Cross-section of *H. perforatum* leaf (c: Cuticle, ue: Upper epidermis, le: Lower epidermis, pa :Palisade parenchyma, s: Spongy parenchyma, vb: Vascular bundle, ph: Phloem, x: Xylem; Scale 50 μm).

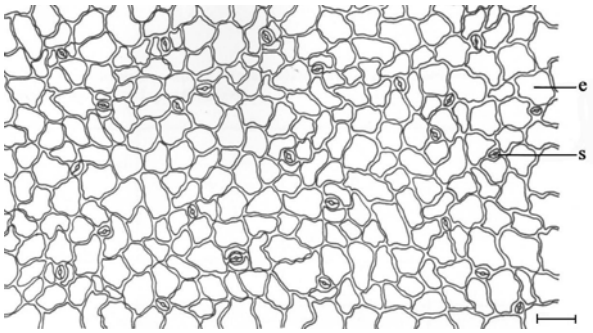

Figure 18. Upper surface section of *H. perforatum* leaf (e: Epidermis, s: Stoma; Scale 50 μm).

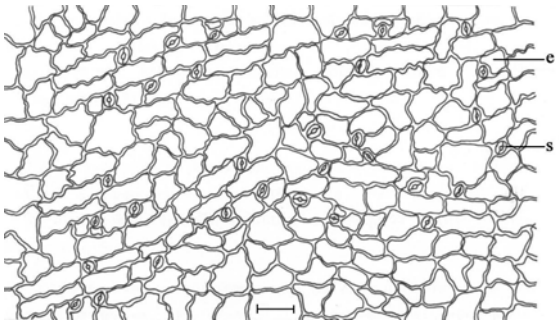

Figure 19. Lower surface section of *H. perforatum* leaf (e: Epidermis, s: Stoma; Scale 50 μm).

Based on the data obtained, we concluded that no remarkable morphological difference could be determined for various populations of all the three species analysed.

All three species prefer steppe habitats for their growth, which could be seen obviously by the widespread presence of xylem elements in the root anatomy of these species. This indicates that roots of the plants adjust themselves to the very habitat where they grow (Noack, 1939; Metcalfe and Chalk, 1957; Esau, 1977; Fahn, 1977; Cutter, 1978; Yentür, 1984; Özörgücü at al, 1991; Özörgücü, 1993 and Vardar and Seçmen, 1993).

The stem of *H. montbretii* was erect and decumbent, while the stem of *H. origanifolium* was suberect and ascending and that of *H. perforatum* was erect and sometimes ascending. These our data seem to be in conformity with those already existing in the literature (Robson, 1967,1988; Dönmez 2000). Secretion hairs on the epidermis and secretion cells present in the cortex of all the three species upon their stem analyses, confirm the findings of Noack (1939), Metcalfe and Chalk (1957), Cutter (1978) and Arda (1989).

Arda (1989) and Tokur and Mısırdalı (1989) reported that vascular bundles of the stems formed a circle, which was also observed for all the species analysed in our study.

The leaves of *H. montbretii* and *H. perforatum* were determined to be glabrous, while *H. origanifolium* leaves were determined to be covered with single-cellular pubescent hair apart from small tubercules. As far as *H. perforatum* is concerned, apart from the data obtained from the cross-sections, determination of the fact that its stomata frequencies were rather low on the upper surface, in particular seem to be an indication of the ability of this species to adapt itself to dry habitats with ease. Our results showed that stomata were anizositic in the surface sections of leaves and that secretion pockets are of a schizo-lysigenous type. These results are in agreement with the results of another authors. In addition, Ciccharelli et all. (2001) characterized the translu-

cent glands and secretory canals of *H. perforatum* in both the floral and vegetative parts, from morphological, anatomical and histochemical points of view.

Therefore, we suggest that future systematical studies into *H. montbretii, H. perforatum* and *H. origanifolium*, which is found abundantly in and around the city of Eskişehir, might shed further light on both morphological and anatomical characteristics of these species.

Acknowledgements

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