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Anatomical investigations on eleven *Hypericum* L. taxa

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Abstract

In this study, anatomical features of *Hypericum capitatum* Choisy. var. *luteum* N. Robson, *H. lydium* Boiss., *H. malatyanum* Peşmen, *H. microcalycinum* Boiss. & Heldr., *H. perforatum* L. *H. retusum* Aucher, *H. scabrum* L., *H. spectabile* Jaub. & Spach, *H. thymopsis* Boiss., *H. triquetrifolium* Turra and *H. uniglandulosum* Hausskn. ex Bornm. from Türkiye were examined. The cross-section of the root, stem and leaves were investigated with light microscopy. The leaf surfaces were examined with scanning electron microscopy. The roots have 2-3 or 4-5 layers of periderm. The stem wings were various in shape and number, the epidermis is single or double layered. The leaves are equifacial or bifacial. The micro-anatomy of leaf surface show that the epidermis cell walls are straight to slightly undulate, straight to undulate or straight to curved and the stomata are anisocytic. The characteristics obtain from this study were aimed to make contributions to the diagnostic features of genus *Hypericum*.

Key words: anatomy, leave, root, SEM, stem

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Onbir *Hypericum* L. taksonu üzerinde anatomik araştırmalar

Özet

Bu çalışmada Türkiye'den *Hypericum capitatum* Choisy. var. *luteum* N. Robson, *H. lydium* Boiss., *H. malatyanum* Peşmen, *H. microcalycinum* Boiss. & Heldr., *H. perforatum* L. *H. retusum* Aucher, *H. scabrum* L., *H. spectabile* Jaub. & Spach, *H. thymopsis* Boiss., *H. triquetrifolium* Turra and *H. uniglandulosum* Hausskn. ex Bornm. taksonlarının anatomik özellikleri çalışıldı. Kök, gövde ve yaprak enine kesitleri ışık mikroskobu ile, yaprak yüzeyleri taramalı elektron mikroskobu ile incelendi. Köklerde 2-3 veya 4-5 sıra periderm bulunur. Gövde kanatları farklı şekil ve sayıda, epidermis tek veya çift sıralıdır. Yapraklar ekvifasiyal yada bifasiyaldir. Yaprak yüzeyinin mikro anatomisi epidermis hücre duvarlarının düzden hafif dalgalıya, düzden dalgalıya veya düzden kıvrımlıya kadar olduğunu göstermektedir, stomalar anizositiktir. Bu çalışmadan elde edilen özelliklerin *Hypericum* cinsinin tanısal özelliklerine katkı sağlaması amaçlanmıştır.

Anahtar kelimeler: anatomi, gövde, kök, SEM, yaprak

1. Introduction

Hypericum L. (Hypericaceae) is consist of 469 species with the distribution all over the world, except Antarctica [1]. The genus represented by 80 species in Flora of Türkiye (including supplements) which are composed from herbs and shrubs [2-4]. The species number given as 96 in the checklist by Arslan (2012) [5]. With the four new species added recently, the number of species has become 100 in Türkiye [6-9].

The species of the genus *Hypericum* are traditionally used and *Hypericum perforatum* L. is an important medicinal herb of which extracts have activity against mild to moderate depression [1]. The characteristics used in

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identification are generally based on morphology that including glands configuration. The marginal, intramarginal, superficial glands and vittae (occurs on ovary and the capsule) are commonly used in classification [2].

There are several studies focused on anatomy of the *Hypericum* species. Perrone et al. (2013) were studied leaf and stem anatomy of eight *Hypericum* species [10]. In addition, distribution of secretory structures and leaf vascular patterns in some species of the genus were investigated [11]. The translucent glands and secretory canals of the floral and vegetative parts of *Hypericum perforatum* L. was given from morphological, anatomical and histochemical points of view [12]. Anatomical characteristics of the *Hypericum montbretii* Spach., *H. origanifolium* Willd., *H. perforatum* L. and *H. kotschyanum* Boiss. from Türkiye were given [13, 14]. Transverse sections of the roots, stem, leaves and surface sections of the leaves of *Hypericum sechmenii* Ocak & Koyuncu were investigated [15].

The genus *Hypericum* is taxonomically complex group. This study is aim to make contributions to its identification characters with the anatomical properties.

2. Materials and methods

The samples were collected from the field studies by the authors from Malatya province, in Türkiye. The localities including the habitat properties are given (Table 1). The specimens were identified according to the literature given by Robson (1967) [2]. The method given by Altınbaşak et al. (2020) was used with some modification for preparation of material [14]. Plant materials were stored in 70% ethanol-water solution and transverse section of root, stem and leaf were taken by hand using a blade. The sections were kept about 5 min in SARTUR reagent for dye. Leica DM 1000 light microscope and Leica DFC 290 digital camera were used for take photographs. The leaf surface scanning electron microscopy (SEM) photographs were taken with a Leo Evo 40 scanning electron microscope. Before scanning, the leaf pieces were mounted on stubs using double-sided adhesive tape and coated with gold using a BALTEC SCD-050 coater.

Table 1. Localities of examined *Hypericum* taxa

Taxa	Localities
Hypericum capitatum var. *luteum*	Malatya: Between Hekimhan and Darende 59. km, step, 1500 m, 07.06.2020, Arabacı & Gürhan (IG 1014)
H. lydium	Malatya: Between Malatya-Hekimhan Kocaözü village, 1000 m, 07.06.2020, Arabacı & Gürhan (IG 1011)
H. malatyanum	Malatya: Eskiköy, Melekbaşı, rocky areas, 1800 m, 14.06.2020, Arabacı & Gürhan (IG 1027)
H. microcalycinum	Malatya: Between Balaban and Malatya, 16. km, 1700 m, 07.06.2020, Arabacı & Gürhan (IG 1019)
H. perforatum	Malatya: Between Hekimhan and Darende, 1150 m, garden sides, 07.06.2020, Arabacı & Gürhan (IG 1022)
H. retusum	Malatya: Between Hekimhan and Saz, junction of Deveci 1600 m, 07.06.2020, Arabacı & Gürhan (IG 1020)
H. scabrum	Malatya: Above Eskiköy, slopes, 14.06.2020, Arabacı & Gürhan (IG 1026)
H. spectabile	Malatya: Akçadağ, 1 km West of Dümüklü, *Quercus* L. openings, 1500 m, 05.06.2021, Arabacı & Gürhan (IG 1031)
H. thymopsis	Malatya: Between Darende and Gürün, 11. km, 07.06.2020, Arabacı & Gürhan (IG 1024)
H. triquetrifolium	Malatya: İnönü University Campus, around the Fine Arts Faculty, 900 m, 16.07.2020, Gürhan (IG 1025)
H. uniglandulosum	Malatya: İnönü University Campus, 950 m, Arabacı (TA 3089)

3. Results

Anatomical properties of taxa and the photomicrographs of root, stem, leaf cross section and leaf blade epidermis scanning electron microscopy (SEM) images are given below (Figures 1-4).

Hypericum capitatum Choisy. var. *luteum* N. Robson

The root has 4-5 layers of periderm, canals are numerous with wide lumen. The stem has 4-5 wings, epidermis is biseriate, secretary canals are absent in phloem. The leaves are equifacial, phloem has no secretory canals, the epidermis has straight to slightly undulate cell walls, stomata are anisocytic and sunken.

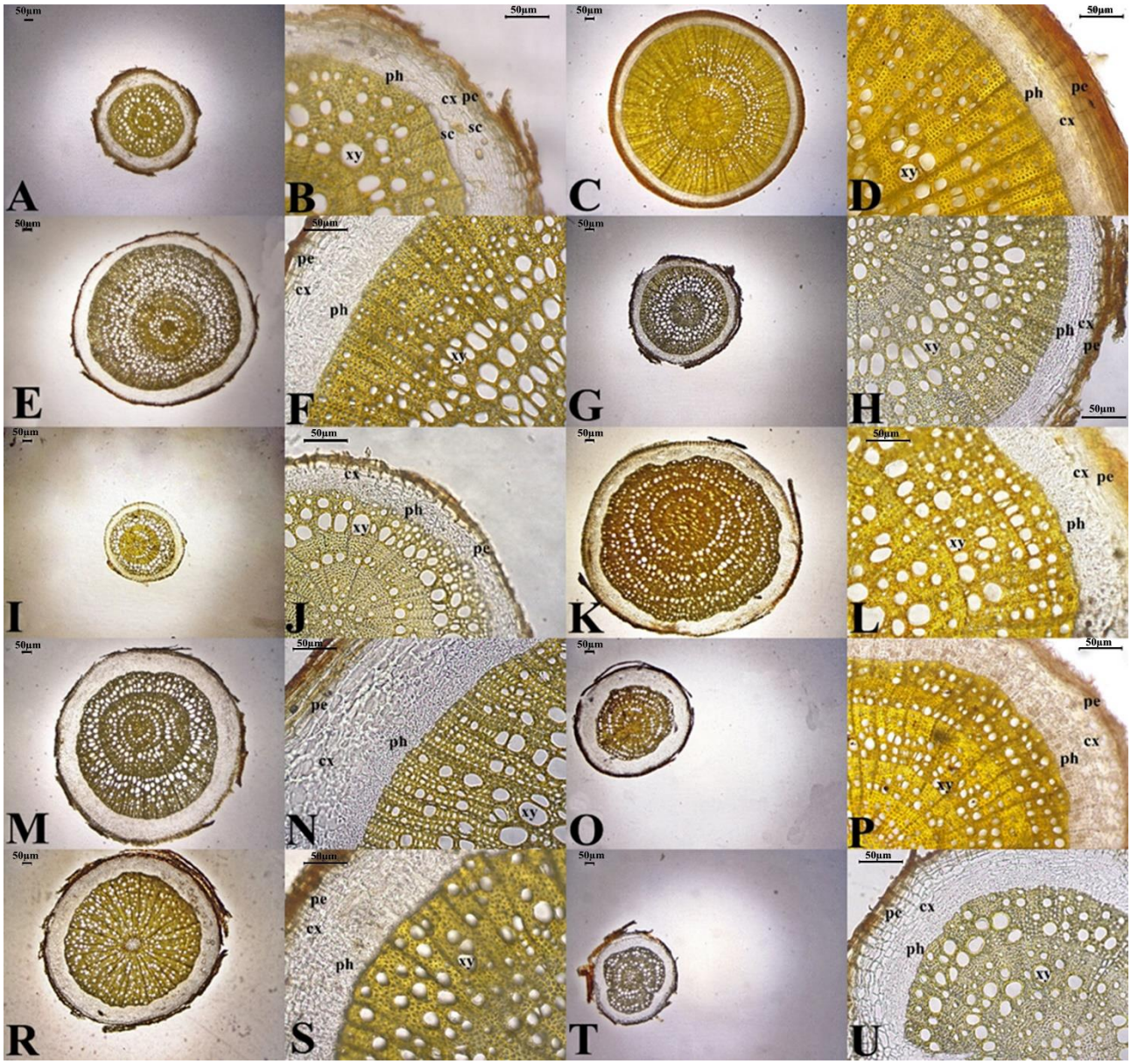

Figure 1. Photomicrographs of root cross section. A-B *Hypericum capitatum* var. *luteum*, C-D *Hypericum lydium*, E-F *Hypericum microcalycinum*. G-H *Hypericum perforatum*, I-J *Hypericum retusum*, K-L *Hypericum scabrum*, M-N *Hypericum spectabile*, O-P *Hypericum thymopsis*, R-S *Hypericum triquetrifolium*, T-U *Hypericum uniglandulosum*. (cx: cortex, pe: periderm, ph: phloem, sc: secretory canal, xy: xylem) (Scale bar 50 µm).

Hypericum lydium Boiss.

The root has 4-5 layers of periderm, canals are observed. The stem has a wing, epidermis is biseriate, the phloem has secretory canals. The leaves are equifacial, phloem has numerous (10-12) secretory canals, the epidermis has straight to curved cell walls, stomata are anisocytic and slightly sunken.

Hypericum malatyanum Peşmen

The stem is unwinged, epidermis is uniseriate, the phloem has secretory canals. The leaves are bifacial, phloem has 4-6 secretory canals, the epidermis has straight to slightly undulate cell walls, stomata are anisocytic and sunken.

Hypericum microcalycinum Boiss. & Heldr.

The root has 4-5 layers of periderm, canals are not observed. The stem has two wings, epidermis is uniseriate, secretary canals are absent in phloem. The leaves are bifacial, phloem has no secretory canals, the epidermis has straight to curved cell walls, stomata are anisocytic and slightly sunken.

Hypericum perforatum L.

The root has 2-3 layers of periderm, canals are observed. The stem has two wings, epidermis is uniseriate, the phloem has secretory canals. The leaves are bifacial, phloem has 4-6 secretory canals, the epidermis has undulate cell walls, stomata are anisocytic and sunken.

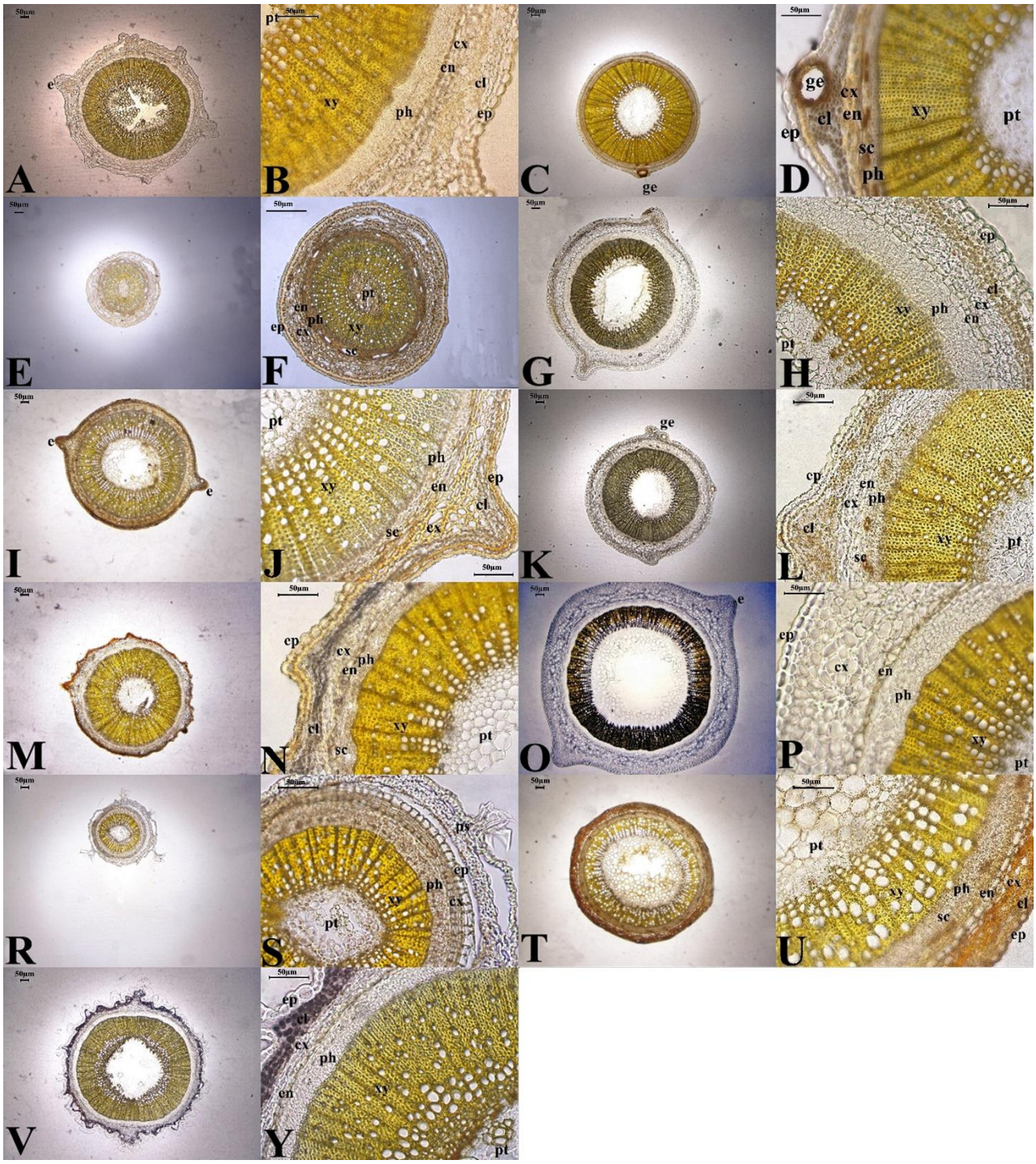

Figure 2. Photomicrographs of stem cross section. A-B *Hypericum capitatum* var. *luteum*, C-D *Hypericum lydium*, E-F *Hypericum malatyanum*, G-H *Hypericum microcalycinum*, I-J *Hypericum perforatum*, K-L *Hypericum retusum*, M-N *Hypericum scabrum* , O-P *Hypericum spectabile*, R-S *Hypericum thymopsis*, T-U *Hypericum triquetrifolium*, V-Y *Hypericum uniglandulosum* (ep: epidermis, cl: collenchyma, cx: cortex, e: emergence, en: endodermis, ge: glandiferous emergence, ph: phloem, ps: papillose sheath, pt: pith, sc: secretory canal , xy: xylem) (Scale bar 50 µm).

Hypericum retusum Aucher

The root has 2-3 layers of periderm, canals are not observed. The stem has two wings, epidermis is biseriate, the phloem has secretory canals. The leaves are bifacial, phloem has 4-6 secretory canals, the epidermis has straight to curved cell walls, stomata are anisocytic and sunken.

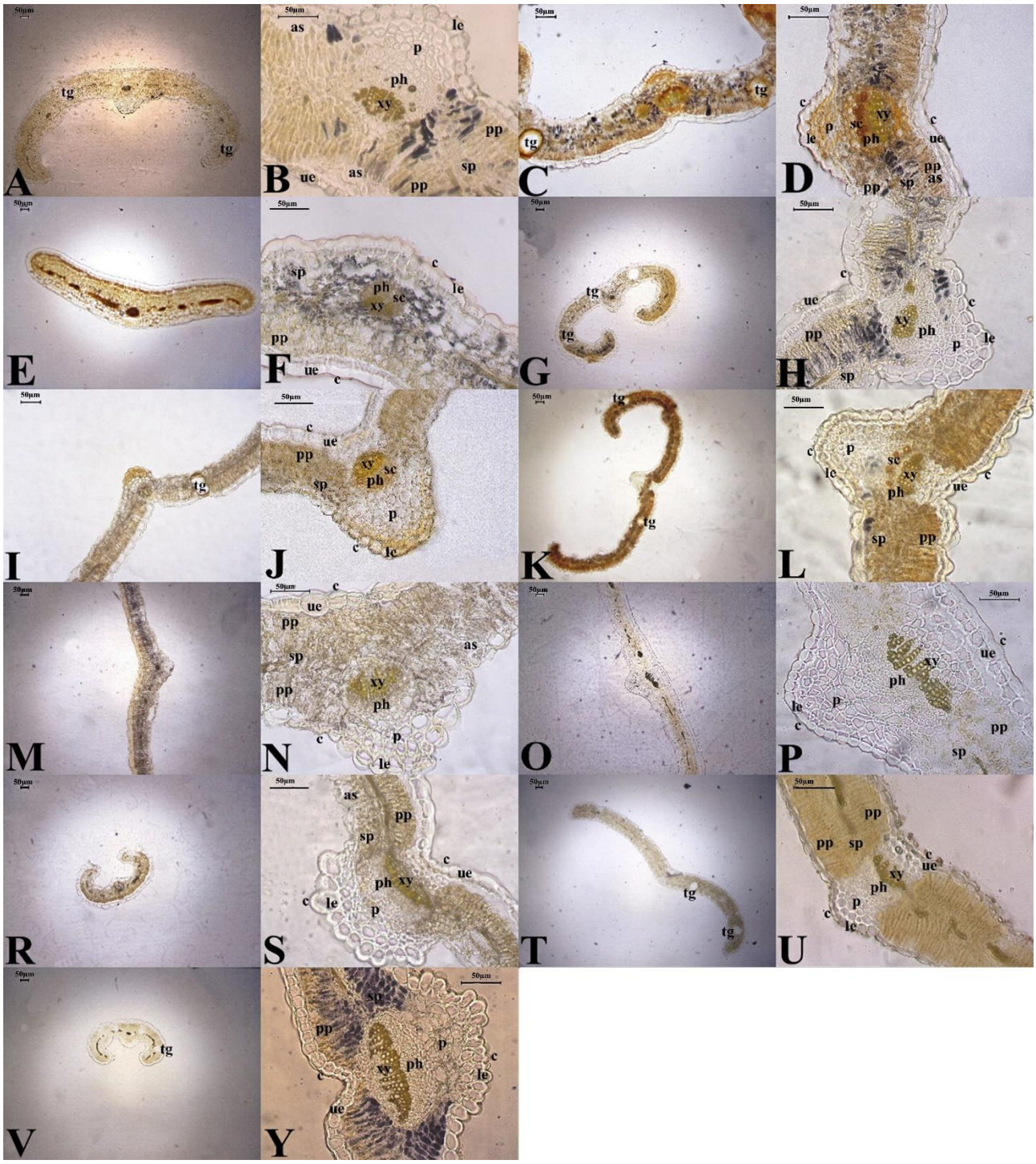

Figure 3. Photomicrographs of leaf cross section. A-B *Hypericum capitatum* var. *luteum*, C-D *Hypericum lydium* E-F *Hypericum malatyanum*, G-H *Hypericum microcalycinum*, I-J *Hypericum perforatum*, K-L *Hypericum retusum*, M-N *Hypericum scabrum*, O-P *Hypericum spectabile*, R-S *Hypericum thymopsis*, T-U *Hypericum triquetrifolium*, V-Y *Hypericum uniglandulosum* (as: air space, c:cuticle, le: lower epidermis, p: parenchyma, ph: phloem, pp: palisade parenchyma, sc: secretory canal, sp: spongy parenchyma, tg: translucent gland, ue: upper epidermis, xy: xylem) (Scale bar 50 µm).

Hypericum scabrum L.

The root has 2-3 layers of periderm, canals are numerous, with wide lumen. The stem has undulate border, epidermis is biseriate, the phloem has secretory canals. The leaves are equifacial, phloem has 4-6 secretory canals, the epidermis has straight to curved cell walls, stomata are anisocytic and sunken.

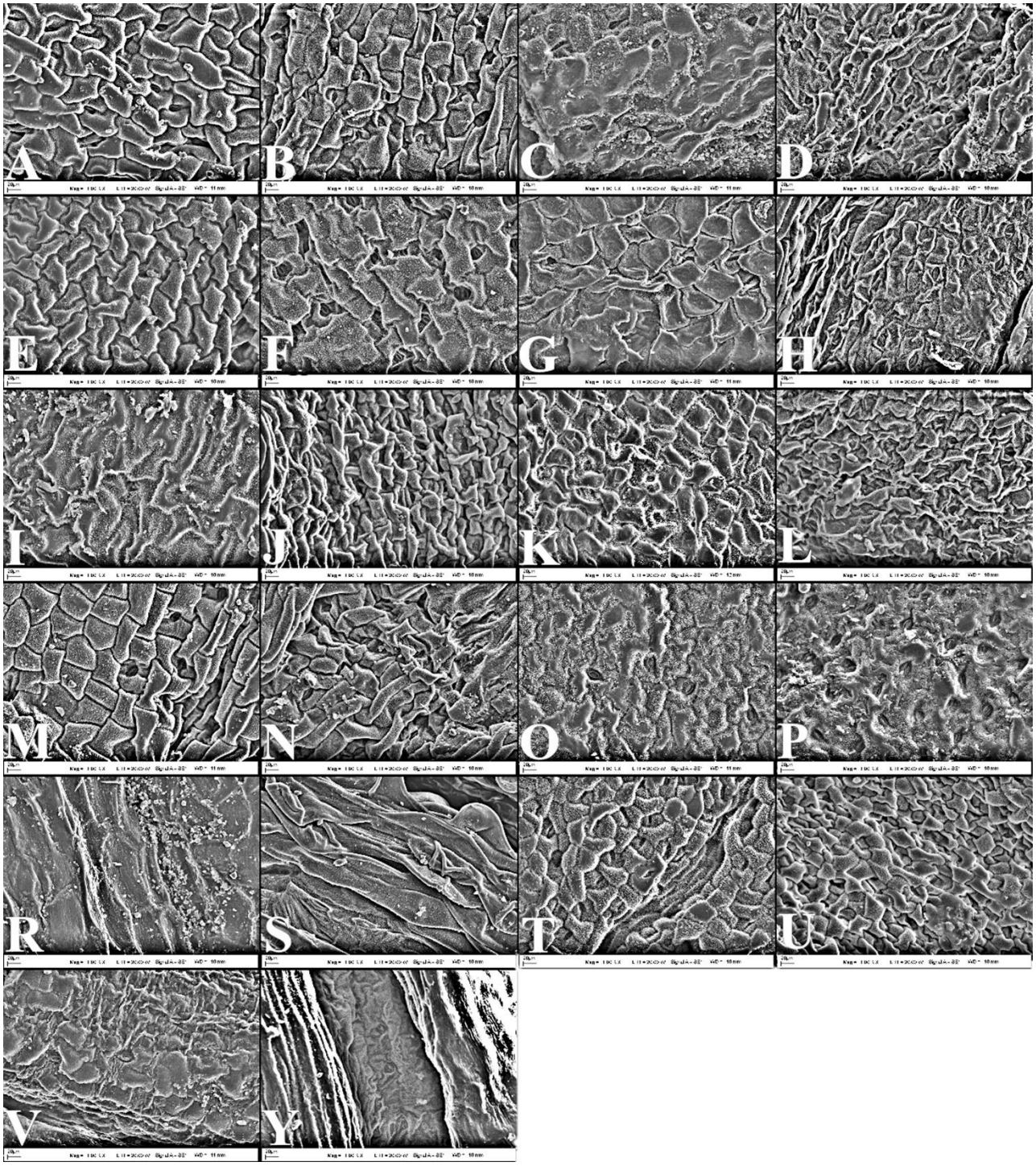

Figure 4. Leaf blade epidermis scanning electron microscopy (SEM) images. *H. capitatum* var. *luteum*; A-adaxial surface, B-abaxial surface. *H. lydium*; C- adaxial surface, D- abaxial surface. *H. malatyanum*; E- adaxial surface, F-abaxial surface. *H. microcalycinum*; G-adaxial surface, H-abaxial surface. *H. perforatum*; I-adaxial surface, J-abaxial surface. *H. retusum*; K-adaxial surface, L-abaxial surface. *H. scabrum*; M-adaxial surface, N-abaxial surface. *H. spectabile*; O-adaxial surface, P-abaxial surface. *H. thymopsis*; R-adaxial surface, S-abaxial surface. *H. triquetrifolium*; T-adaxial surface, U-abaxial surface. *H. uniglandulosum*; V-adaxial surface, Y-abaxial surface (Scale bar 20 µm).

Hypericum spectabile Jaub. & Spach

The root has 4-5 layers of periderm, canals are not observed. The stem has two wings, epidermis is biseriate, the phloem has secretory canals. The leaves are bifacial, phloem has secretory canals, the epidermis has undulate cell walls, stomata are anisocytic and slightly sunken.

Hypericum thymopsis Boiss.

The root has 2-3 layers of periderm, canals are observed. The stem has trichomes on the epidermal layer, epidermis is uniseriate, the phloem has secretory canals. The leaves are bifacial, phloem has 4-6 secretory canals, the epidermis has straight to curved cell walls, stomata are anisocytic and facial.

Hypericum triquetrifolium Turra

The root has 4-5 layers of periderm, canals are observed. The stem is unwinged, epidermis is uniseriate, the phloem has secretory canals. The leaves are equifacial, phloem has 2-4 secretory canals, the epidermis has straight to curved cell walls, stomata are anisocytic and sunken.

Hypericum uniglandulosum Hausskn. ex Bornm.

The root has 2-3 layers of periderm, canals not observed. Stem with numerous thin wings, epidermis is uniseriate, secretary canals are absent in phloem, The leaves are equifacial, phloem has no secretory canals, the epidermis has undulate cell walls, stomata are anisocytic and slightly sunken.

4. Conclusions and discussion

The anatomical properties of *Hypericum capitatum* var. *luteum, H. lydium, H. malatyanum, H. microcalycinum, H. perforatum. H. retusum, H. scabrum, H. spectabile, H. thymopsis, H. triquetrifolium* and *H. uniglandulosum* speciemens from Türkiye were examined.

The cross-section of the roots in the examined species show similarities. A thick cuticle layer is take place on the outer surface. Below cuticle layer; 2-5 layer of periderm, 2-3 layer of cortex tissue, a phloem layer and a xylem are located respectively (Table 2). Secretory canals are observed in *H. capitatum* var. *luteum, H. lydium, H. perforatum. H. scabrum, H. thymopsis* and *H. triquetrifolium.* Erkara and Tokur (2004) reported that a thick cuticle, 4-5 layer of periderm, a single layer of endodermis and 2-3 layer of phloem in the roots of *H. montbretii* Spach., *H. origanifolium* Willd. and *H. perforatum* L.[13].

Table 2. Anatomical characteristics of the *Hypericum* taxa

Taxa	Root		Stem			Leaves			
	Periderm	Canal	Wing	Epidermis	Secretary canals in phloem	Structure	Secretary canals in phloem	Epidermis cell walls	Stomata
Hypericum capitatum var. *luteum*	4-5 layers	observed	4-5 wings	biseriate	not observed	equifacial	not observed	straight to slightly undulate	anisocytic, sunken
H. lydium	4-5 layers	observed	1 wing	biseriate	observed	equifacial	10-12	straight to curved	anisocytic, slightly sunken
H. malatyanum	-	-	unwinged	uniseriate	observed	bifacial	4-6	straight to slightly undulate	anisocytic, sunken
H. microcalycinum	4-5 layers	not observed	2 wings	uniseriate	not observed	bifacial	not observed	straight to curved	anisocytic, slightly sunken
H. perforatum	2-3 layers	observed	2 wings	uniseriate	observed	bifacial	4-6	straight to undulate	anisocytic, sunken
H. retusum	2-3 layers	not observed	2 wings	biseriate	observed	bifacial	4-6	straight to curved	anisocytic, sunken
H. scabrum	2-3 layers	observed	undulate border	biseriate	observed	equifacial	4-6	straight to curved	anisocytic, sunken
H. spectabile	4-5 layers	not observed	2 wings	biseriate	observed	bifacial	4-6	straight to undulate	anisocytic, slightly sunken
H. thymopsis	2-3 layers	observed	trichomes on the epiderma l layer	uniseriate	observed	bifacial	4-6	straight to curved	anisocytic, facial
H. triquetrifolium	4-5 layers	observed	unwinged	uniseriate	observed	equifacial	2-4	straight to curved	anisocytic, sunken
H. uniglandulosum	2-3 layers	not observed	with numerous thin wings	uniseriate	not observed	equifacial	not observed	straight to undulate	anisocytic, slightly sunken

The stem has a cuticle, epidermis, collenchyma, and parenchymatic cortex layer from outside to inner. The epidermis is single or double layered. The stem consists from 2-3 rows of phloem layer, a large xylem layer and a parenchymatic core. The stems are circular in outline. The wings of species are various in shape and number. *H.*

malatyanum and *H. triquetrifolium* are unwinged, *H. lydium* have one wing, *H. microcalycinum, H. perforatum. H. retusum, H. spectabile* have 2 wings, *Hypericum capitatum* var. *luteum* has 4-5 wings and *H. uniglandulosum* has numerous thin wings. The stem of *H. scabrum* consist from undulate border. *H. thymopsis* is characterized by trichomes on the epidermal layer (Table 2). Tekin (2017) reports two small wings and glandiferous emergences on the stem of *H. thymopsis* [16]. The outline of stem found as unwinged in *H. pubescens* Boiss. and *H. aegypticum* L., slightly wavy and with two small wings in *H. perforatum* and *H. triquetrifolium*, two evident wings in *H. hircinum* L. and *H. androsaemum* L., and four triangular wings in H. *tetrapterum* Fr. in the previous study [10].

Secretary canal in phloem was not observed in *Hypericum capitatum* var. *luteum, Hypericum microcalycinum* and *Hypericum uniglandulosum* both in stem and leaf cross-sections.

The leaves are equifacial or bifacial. The micro-anatomy of leaf surface show that the epidermis cell walls are straight to slightly undulate, straight to undulate or straight to curved. The stomata were found as anisocytic in the surface sections of the examined leaves (Table 2). This result is in well-agreement with previous studies [16, 17]. In addition, paracytic and diasitic stomata were also reported in some *Hypericum* species [10, 13]. The stomata are sunken in *Hypericum capitatum* var. *luteum, H. malatyanum, H. perforatum. H. retusum, H. scabrum, H. triquetrifolium*, slightly sunken in *H. microcalycinum, H. lydium, H. spectabile, H. uniglandulosum* and facial in *H. thymopsis.*

The results obtain from this study are compared with literature records. It is aimed to make contributions to the diagnostic features of genus *Hypericum* in this study.

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