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Original Article

The structural studies on the medicinal plant *Haplophyllum telephioides*

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ABSTRACT

Haplophyllum telephioides Boiss., Rutaceae, is an endemic herb which generally grows at the steppe region in Central Turkey. Its aerial parts in flowering stage are used by local people against influenza virus due to its antimicrobial effect. The main purpose of this study was to examine the morphology, anatomy and histology of the vegetative and reproductive organs of the *H. telephioides*, which are used in popular medicine, thereby, contributing to the pharmacognostic evaluation of the species. The species was studied morphologically in detail. New morphological features were described for them, such as the dimension of leaf, sepal and fruit, size of filament, anther and pistil, and diameter of the ovary. In addition, some deviating features were found in relation to previous published descriptions for the species, such as plant height and petal length. The anatomy of plant parts such as stem, leaf, sepal, petal, filament and pistil, were studied using light microscopy and scanning electron microscopy. Stem has incipient secondary growth. The leaf is amphistomatic and the mesophyll is equifacial. Stomata are anomocytic and sunken. In micromorphological studies, it is determined that the leaf is coated by a thick cuticle and above epicuticular wax. Schizogenous glands were found in all vegetative and reproductive organs. The structural features herein found can assist the diagnosis of *H. telephioides*.

Introduction

The Rutaceae is a large family represented by 161 genera and about 1900 species in the world (Simpson, 2006). Rutaceae representatives occur in tropical and subtropical regions, especially in Southern Africa and in Australia (Heywood, 1978; Kubitzki et al., 2011). The family has economically important species for medicine (Gözler et al., 1996; Sağlam et al., 1999), food, gardening and ornamental species (Kubitzki et al., 2011). The most distinctive feature of the family is the presence of secretory cavities, which originate schizogenous, lysigenous, or schizolysigenous. These occur particularly in the leaves and reproductive organs (Metcalfe and Chalk, 1950; Zeybek and Zeybek, 1994; Groppo et al., 2008). These secretory cavities contain volatile oils, alkaloids, resin, hesperidin and some other chemical compounds in leaf, stem, flower and fruit of the species (Metcalfe and Chalk, 1950; Kubitzki et al., 2011). *Haplophyllum* A. Juss. is a genus of Rutaceae, with *ca.* 70 species (Townsend, 1986; Soltani and Khosravi, 2005) showing the largest diversity in Irano-Turanian region, particularly in Turkey, Iran and Central Asia, and Mediterranean phytogeographical regions (Navarro et al., 2004; Salvo et al., 2011; Manafzadeh et al., 2014).

Haplophyllum has fourteen species in which eight of them are endemic to the Flora of Turkey and East Aegean Islands (Townsend, 1967). According to the recent study conducted by Güner et al. (2012), *Haplophyllum* is represented nine species in which six of them are endemic for Turkey. Many studies have examined the phytochemistry and medicinal attributes of some of *Haylophyllum*, analyzing their contents for alkaloids, lignans, glycosides etc. (Patra et al., 1984; Cantrell et al., 2005; Ulubelen and Öztürk, 2008; Parhoodeh et al., 2012). For example, phytochemical composition of *Haylophyllum buxbaumii* (Poir.) G. Don was studied and compounds such as quinoline alkaloids, kokusaginine, skimmianine and γ-fagarine were isolated from this species (Ulubelen, 1985). In addition to alkaloids, there are reports on lignans (Gözler et al., 1984), haplomyrtin, (−) haplomyrfolin (Evcium et al., 1986) and justicidin-A (Khalid and Waterman, 1981) for some *Haplophyllum*. Due to these chemical compounds, some *Haplophyllum* such as *Haplophyllum patavinum* (L.) G. Don, are used in folk medicine as antimicrobial, antimalarial, insecticidal agent. *Haplophyllum tuberculatum* Juss., which naturally grows in Saudi Arabia, is used for the cure of rheumatoid arthritis, malaria and in the cure of some gynecological problems (Ulubelen and Öztürk, 2008; Al-Yahya et al.,

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1992). *Haplophyllum hispanicum* Spach is an endemic to the East of Spain, and is known for its high skin reactivity, being employed clinically against vitiligo with some success (Massmanian and Prieto, 1996). *Haplophyllum suaveolens* G. Don and *H. buxbaumii*, which naturally grows in Turkey, are also known as medicinal plants (Cetin et al., 2012).

During field works, by interviewing with local people, we recognized that the aerial parts of *Haplophyllum telephioides* Boiss. in flowering stage are used as a microbicide against influenza, in some districts of Sivas province (Turkey). Phytochemical investigations of *H. telephioides* were studied by Ulubelen et al. (1994) and Ulubelen and Öztürk (2008). These studies found the presence of secondary metabolites such as alkaloids 7-hydroxy-9-methoxyflindersine and lignans, 4-acetyldyphyllin, in addition to haplomyrtin, dyphyllin and vanillic acid. One of these secondary metabolites, dyphyllin, a new natural compound, has been noted to potently inhibit vacular-ATPase, and thereby lysosomal acidification in osteoclasts. This leads to abrogation of bone resorption (Sorensen et al., 2007) and reduce v-ATPase expression in gastric adenocarcinoma cells (Shen et al., 2011). Dyphyllin is also reported to display significant antitumor activity (Fukamiya and Lee, 1986; Jansen et al., 2006), and shows antiviral effects on various viral strains including that of known drug-resistant strains by altering cellular susceptibility to influenza viruses through the inhibition of endosomal acidification, thus interfering with downstream virus replication (Chen et al., 2013). In addition, some dyphyllin derivatives, isolated from *Haplophyllum linifolium* (L.) G. Don which have activities against topical anti-inflammatory and antitrypanosome (Schinella et al., 2008).

In this study, we investigate the structure of vegetative and reproductive organs of *H. telephioides*. This species is endemic to the region limited by Central Anatolia with Kayseri, Nevşehir, Yozgat and Sivas province in Turkey. According to IUCN threaded category, *H. telephioides* has been placed under LR-nt (lower risk-near threatened) by Ekim et al. (2000). Such as *H. telephioides*, many species of *Haplophyllum* are distributed in narrow geographic areas, which makes them more vulnerable to the risk of extinction (Salvo et al., 2011).

Though *H. telephioides* is well characterized by phytochemical studies, the knowledge on the structures of plant parts used as medicinal is incomplete. In fact, there are no data on anatomy and micromorphology for this species. Only superficial morphological descriptions are avaliable in Flora of Turkey and East Aegean Islands (Townsend, 1967). Considering these studies, presence of dyphyllin in *H. telephioides*, its usage as antimicrobial plant by local people and usage of related species as medicinal plant increases the importance of the present study. Therefore the aim of this study is to detailed describe the structure of vegetative and reproductive organs of *H. telephioides*, using morphological and anatomical procedures. This will provide new structural information for this species of well-known importance in folk medicine and phytochemical properties. This study may also provide new diagnostic features for identification of *H. telephioides*.

Materials and methods

Plant material

Haplophyllum telephioides Boiss., Rutaceae, were collected during the flowering and fruiting period from different natural populations in Sivas province of Turkey. Field works were carried out in 2015. Locality 1: B6 Sivas: Ulaş district, Ziyarettepe, 1411 m, 39°33′08.1″N; 37°01′12.5″E, 22.05.2015; Locality 2: B6 Sivas: Hafik district, Topçuyeniköy village, 1383 m, 39°46′59.3″N; 37°35′15.9″E, 26.06.2015; Locality 3: B6 Sivas: Sivas-Kangal-Gürün road intersection, roadside, 1545 m, 39°07′52.7″N; 37°14′32.6″E, 30.06.2015. Morphological description was made for specimens of each locality. For anatomical procedures, the specimens of first locality were used. Taxonomical identification was made by first author and some materials were prepared as herbarium vouchers. These were registered under collector numbers M. Tekin 1671, M. Tekin 1689, M. Tekin 1699 and are conserved at the Cumhuriyet University, Faculty of Science Herbarium (CUFH), Department of Biology, Sivas, Turkey (Fig. 1).

Structural analysis

The morphological features of *H. telephioides* were studied both in fresh and herbarium materials. Parts of the fresh material were stored in 70% alcohol–water solution for later anatomical procedures. These consisted in hand-made cross section of stem, leaf, sepal, petal, filament, ovary and style. Also paradermal hand-made sections of adaxial and abaxial surface of the leaf were taken with razor blade. The sections were stained in 1% Alcian blue (Sigma) and 1% Safranine O (Sigma), in a ratio 3/2 (Davis and Barnett, 1997). The sections were kept about 5 min in the dye. Semi-permanent slides were mounded using glycerin–gelatine (Jensen, 1962). The structural investigations of vegetative and reproductive parts of *H. telephioides* were made using Olympus BX22 light microscopy. Photomicrographs were taken using Olympus BX51 light microscopy coupled with Olympus DP70 digital camera. Stomatal index was calculated according to the study of Meidner and Mansifield (1968).

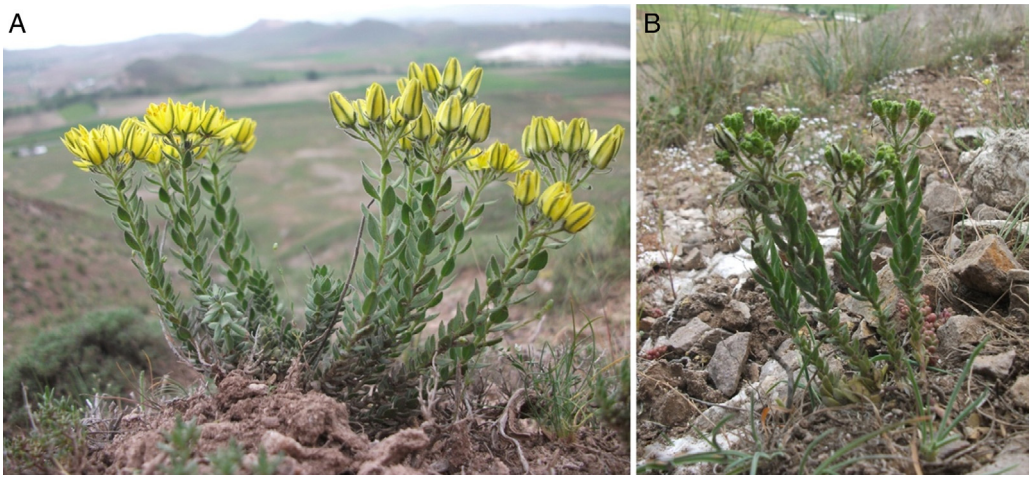

Fig. 1. *Haplophyllum telephioides* in natural habitat. At flowering stage (A), and at fruiting stage (B).

Some parts of the material were analyzed under scanning electron microscopy (SEM). For this, the plant parts were mounted on aluminum stubs and coated with gold. The micromorphological and anatomical observations were made, and micrographs were taken with a LEO 440 SEM at different magnifications.

Results

Morphological aspects

Perennial herb, 4–18 cm. One or generally numerous stems, erect or ascendens, pale dark-brown at base, green at the middle and upper parts (Fig. 1). The stem is covered with densely fine white hairs. Leaves are simple, dense, alternate, sessile and ovate to elliptic or subovate, and covered by short simple non-glandular hairs. Leaves are 2–18 mm × 1–9 mm and containing numerous tiny black dots which corresponds to the secretory cavities. The inflorescences are terminal, with 2–16 flowers, or occasionally with a solitary flower. Inflorescence is cymose, small and compact, and the branches are covered white wooly non-glandular hairs. Flowers are usually pentamerous, and occasionally hexamerous. Sepals are 2–3.5 mm × 0.7–1.5 mm, linear-lanceolate or triangular-shaped, dark green when fresh and blackish green when dry, outer side densely whitish hairy, inner side glabrous. Petals are 6–9 mm × 3–4.5 mm, usually ovate to lanceolate, occasionally elliptic-shaped, slightly concave, entire, bright yellow. There is median longitudinal strip which is pale to dark-green colored and is more distinct with whitish hairs abaxially and glabrous adaxially. Filaments are 4–6 mm long and thick, gradually attenuate from base to apex and are covered with whitish hairs at base. Anthers are 1–2 mm long, dorsifixed and longitudinally opening. Pistils are 5–6 mm long, ovaries are *ca.* 2 mm in diameter. Ovary composed of five carpels, and each carpel bearing four ovules. Carpels are with a prominent and sharply pointed conical apical tubercles. Capsules are 5 lobed, 3–4 mm × 4–5 mm, pale green to yellow colored. Surface of capsule is covered short hairs with pointed tubercles and convex or tuberculate glands at below. Seeds are reniform, 1.1–1.5 mm × 1–1.3 mm, coal-black colored and covered by transverse ridges, on lateral and dorsal side (Fig. 1 and Box 1).

Anatomical and histological aspects

Stem

The cross section taken from middle area of the stem was observed. The shape of sections is circular (Fig. 2A, D). The epidermis is uniseriate and its cells are small, rectangular or squarish in shape. The outer cell wall of the epidermis is thick but, the cuticle layer on these cells is thin. There are unicellular non-glandular hairs on stem epidermis (Fig. 2A, D). Cortex consists of 8–13 cell layers. Cortex cells are oval or circular-shaped and there are some intercellular spaces between them. The cells in the outer cortex layers have chloroplast and are thus photosynthetic. These cells are smaller than the non-photosynthetic inner cortex cells. There are also few schizogenous glands in the cortex layer, just below the epidermis (Fig. 2B, E; Fig. 3). Inner side, there are isolated patches of fibers, with 1–3 cell layers, between inner cortex layers and phloem, thus probably of phloematic origin (Fig. 2B, E). The phloem consists of 3–5 cell layers, cambium is distinct and consists of 1–3 cell layers. The xylem has vessel elements, sclerenchymatic cells and a uniseriate parenchymatous rays. The pith is large and its cells are oval or circular-shaped and they have intercellular spaces (Box 1, Fig. 2C, F).

Leaf

There are uniseriate epidermises on both surfaces of leaf. Epidermal cells are oval or rectangular-shaped. There are unicellular hairs on adaxial and abaxial epidermises (Figs. 4A, B and 5). The outer cell wall of the both epidermal cells is thick. Epidermal cells have a rugose cuticle and apparently covered by wax (Fig. 5C, D). The leaf is amphistomatic and the mesophyll is equifacial. Adaxial and abaxial palisade parenchymas have 2–3 cell layers. The cells of palisade parenchyma are generally cylindrical or rectangular, occasionally ovoid-shaped. The spongy parenchyma has 1–3 cell layers, positioned between the two palisades. Spongy parenchyma cells are circular, ovoid or irregular-shaped. In the leaf mesophyll, there are large schizogenous glands close to both epidermises (Fig. 4A, C). The midrib is oval-shaped (Fig. 4B). There is one main central vascular bundle, which is surrounded by a uniseriate parenchymatous bundle sheath. The stomata are anomocytic and sunken, occurring in both sides of the leaf (Figs. 4D and 5). Stomatal index and stoma

Box 1
Comparison of some diagnostic morphological and anatomical features of *Haplophyllum telephioides* and three *Haplophyllum* species (*H. myrtifolium* Boiss., *H. vulcanicum* Boiss. et Held. and *H. megalanthum* Bornm.) in study of Ulukış et al. (2016).

	H. telephioides	*H. myrtifolium*	*H. vulcanicum*	*H. megalanthum*
Morphological features				
Leaves shape	Ovate, elliptic or subovate	Lanceolate, lanceolate-elliptic, rarely ovate	Lanceolate, ovate-lanceolate	Lower lanceolate-elliptic, the upper distinctly rhomboid-lanceolate
Leaves size (mm)	2–18 × 1–9	8–23 × 3–7	2–17 × 2–9	7–20 × 2–8
Sepals shape	Linear-lanceolate or triangular	Lanceolate-oblong, oblanceolate-ovate	Deltoid-ovate to lanceolate	Lanceolate-elliptic
Sepal size (mm)	2–3.5 × 0.7–1.5	1–3.5 × 0.5–1.5	2–4 × 1.5–2	3–4 × 1.25–1.75
Petal shape	Ovate to lanceolate, occasionally elliptic	Oblong-ovate, ovate	Oblong-ovate, clavate	Oblong-ovate or elliptic lanceolate
Petal color	Bright yellow	White to sulphur-yellow	Creamy-white	White or creamy-white
Petal size (mm)	6–9 × 3–4.5	6–8.5 × 3–4.5	8–11 × 4–5	7–14 × 4–5
Filaments length (mm)	4–6	4.5–5	4.5–5.5	4–6
Capsule size (mm)	3–4 × 4–5	2–3 × 4–5	3–3.5 × 5–6	3–3.5 × 5–6
Anatomical features of stem				
Epidermis cell shape	Rectangular or squarish	Rectangular and oval	Rectangular	Rectangular
Cortex cell layers	8–13	7–9	6–12	7–12
Shape of cortex cells	Oval or circular	Oval, cylindrical or rectangular	Oval or rectangular	Oval or rectangular
Phloem sclerenchyma cell layer	1–3	2–4	5–9	1–4
Cambium cell layer	1–3	1–3	1–4	3–5
Shape of pith cells	Oval, orbicular	Hexagonal or orbicular	Hexagonal or orbicular	Hexagonal or orbicular

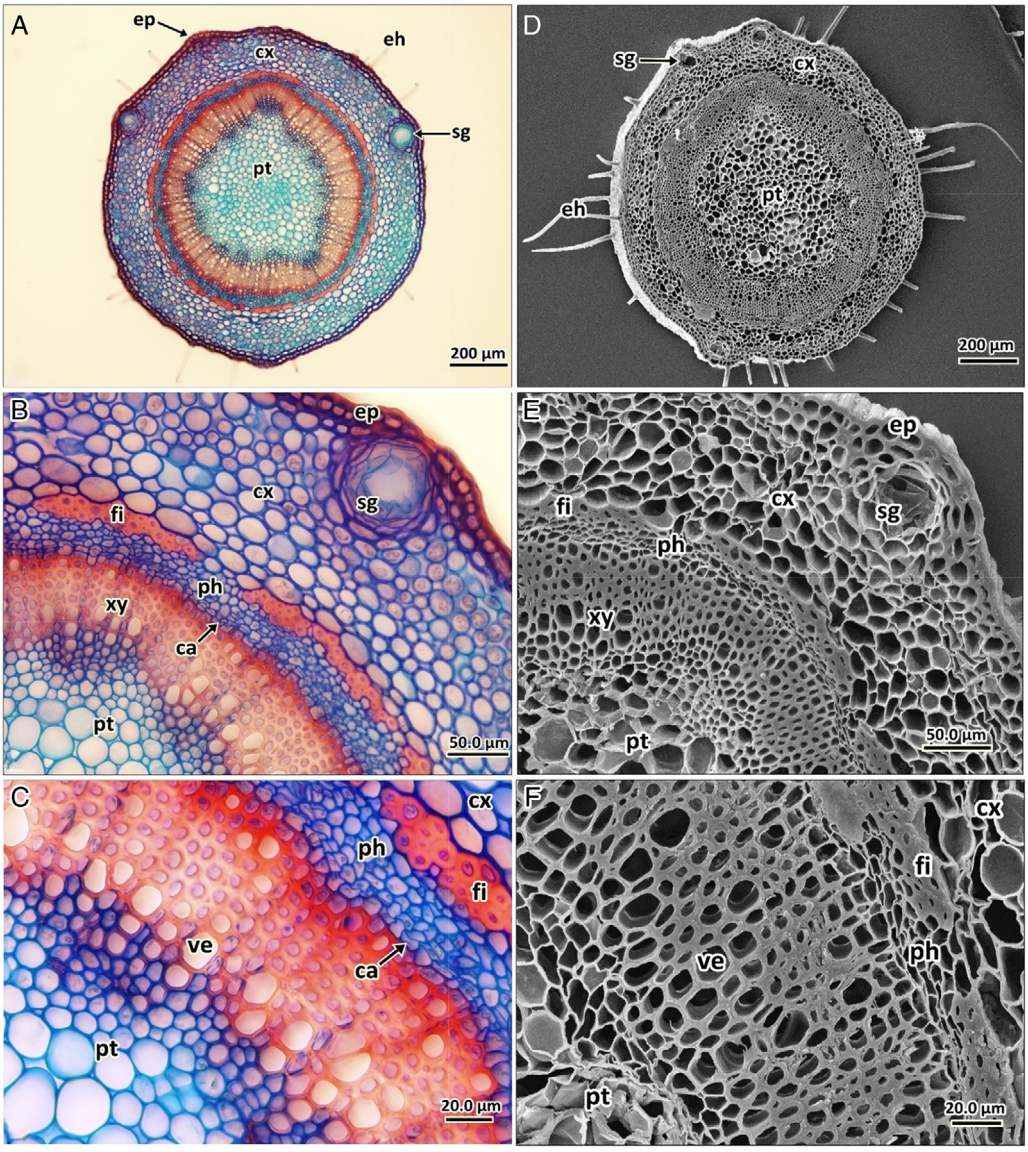

Fig. 2. Photomicrographs of stem cross section of *Haplophyllum telephioides*. General appearance (A and D), detail of stem (B and E), detail of vascular system (C and F). A, B and C under light microscopy (LM); D, E and F under scanning electron microscopy (SEM). Abbreviations: cambium (*ca*), cortex (cx), non-glandular hair (eh), epidermis (ep), fibers (fi), phloem (ph), pith (pt), schizogenous gland (sg), vessel element (ve), xylem (xy).

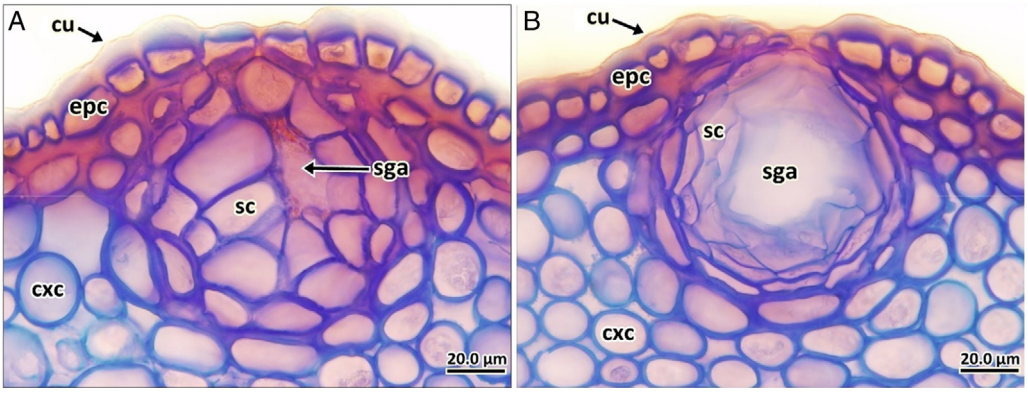

Fig. 3. Photomicrographs of the schizogenous gland development in stem cross section of *Haplophyllum telephipides*. An early phase (A), late phase (B). Abbreviations: cuticle (cu), cortex parenchymatous cell (cxc), epidermis cell (epc), secretory cell (sc), schizogenous sac gap (sga).

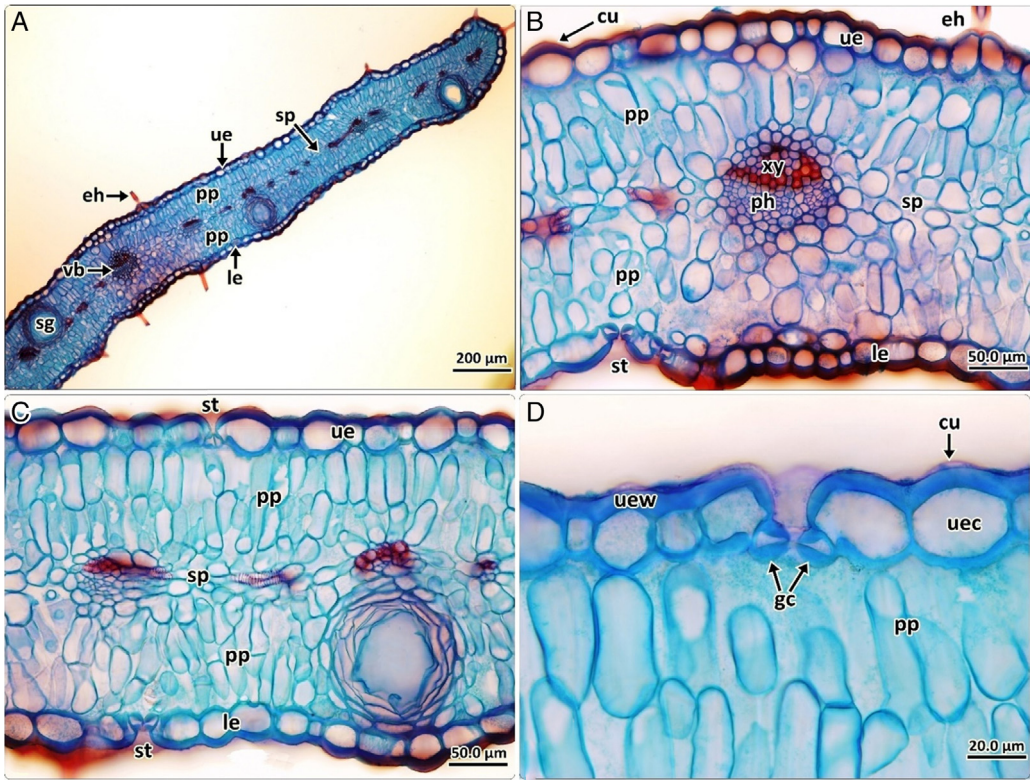

Fig. 4. Microphotographs of the leaf cross sections of *Haplophyllum telephioides*. General view (A), detail of midrib region (B), detail of mesophyll and schizogenous gland (C), detail of sunken stomata in adaxial epidermis (D). Abbreviations: cuticle (cu), epidermal hair (eh), guard cell (gc), abaxial epidermis (le), phloem (ph), palisade parenchyma (pp), schizogenous gland sac (sg), spongy parenchyma (sp), stoma (st), adaxial epidermis (ue), adaxial epidermis cell (uec), adaxial epidermis cell wall (uew), vascular bundle (vb), xylem (xy).

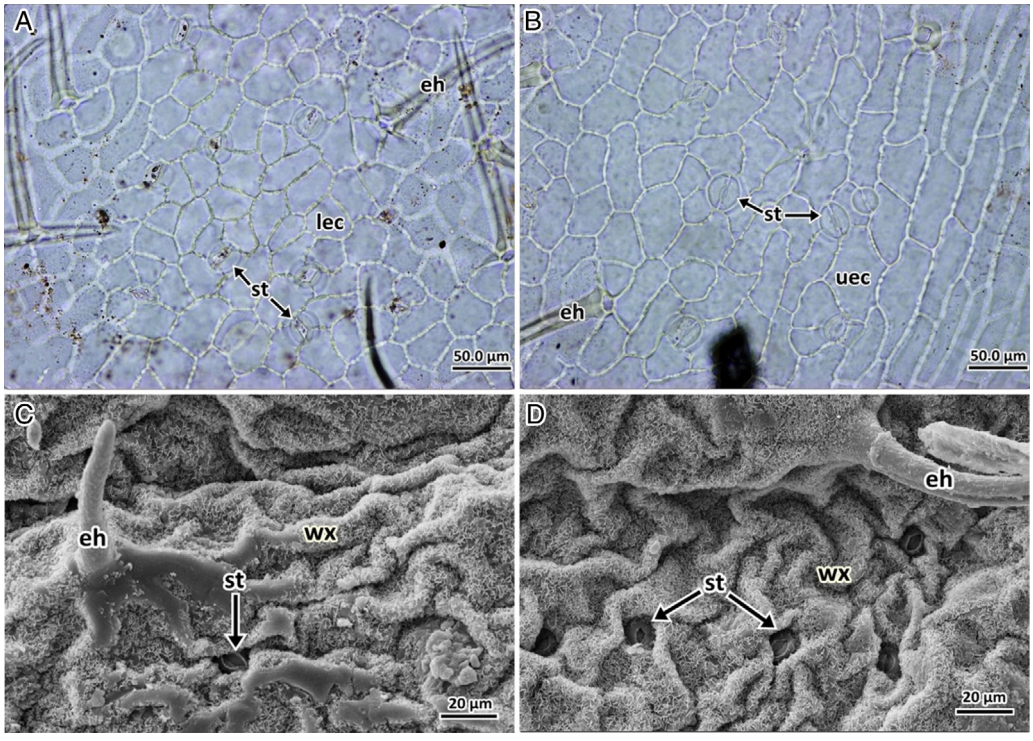

Fig. 5. Microphotographs of the leaf surface section on light microscopy (A–B) and micrographs of the leaf surface on scanning electron microscopy of *Haplophyllum telephioides* (C–D); abaxial surface (A–C), adaxial surface (B–D). Abbreviations: non-glandular hair (eh), epicuticular wax (wx), abaxial epidermis cell (lec), stoma (st), adaxial epidermis cell (uec).

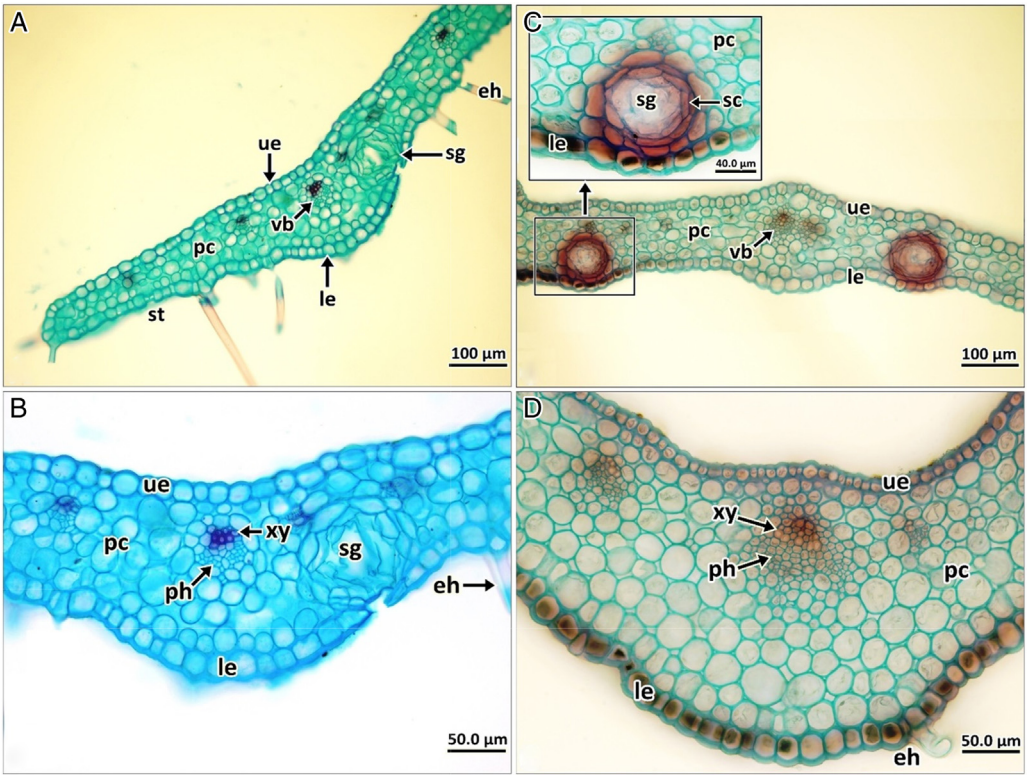

Fig. 6. Microphotographs of perianth parts cross section of *Haplophyllum telephioides*. General view of sepal (A) and detail of sepal midrib region (B), general view of petal (C) and detail of petal midrib region (D). Abbreviations: non-glandular hair (eh), abaxial epidermis (le), parenchymatous cell (pc), phloem (ph), secretory cell (sc), schizogenous gland (sg), stoma (st), adaxial epidermis (ue), vascular bundle (vb), xylem (xy).

density found were 10.57 and 264 stoma per 1 mm^2 for abaxial side of leaf and 11.67 and 292 stoma per 1 mm^2 for adaxial side of leaf, respectively (Figs. 4 and 5).

Sepal

Sepals are slightly concave in adaxial surface and convex in abaxial surface. Epidermal cells are rectangular, oval or squarish-shaped in both surfaces. There are non-glandular hairs solely on abaxial epidermis. Mesophyll is composed of 2–4 parenchymatous cell layers which presented conspicuous intercellular spaces. There are occasionally schizogenous glands in sepal mesophyll. Vascularization is made by small collateral vascular strands, positioned close to the adaxial epidermis. The main vascular bundle is circular-shaped (Fig. 6A, B).

Petal

Petals are "V" shaped in cross section. Epidermal cells are rectangular, oval or squarish-shaped. The mesophyll consists of 4–8 layers of oval-shaped, parenchymatous cells. In the mesophyll, there are occasionally schizogenous glands located close to abaxial epidermis. Vascularization is made by a wide central collateral strand and by a number of smaller ones which positioned close to the adaxial epidermis (Fig. 6C, D).

Pistil

The style epidermis is uniseriate, with rectangular or squarish-shaped cells. The style have 5 (6) stylar canals, each with a secretory epidermis and a narrow lumen filled with secretion (Fig. 7A, B). There are lots of vascular bundles that forms a ring close to epidermis. Occasionally schizogenous glands occur laterally to the stylar canals, especially the part of style which close to ovary side (Fig. 7B, C). The ovaries have 5 locules, and beneath the outer epidermis each locule wall has 2–3 schizogenous glands (Fig. 7D).

Filament

Filaments have an elliptical shape, a seen in cross sections. Their epidermal cells are rectangular or squarish-shaped. Filaments have sparse non-glandular hairs and schizogenous glands closer to the epidermis. Vascularization is made by a wide single amphicribral bundle which located at the center of each filament in cross section. There are circular and oval-shaped parenchymatous cells with intercellular spaces between epidermis and vascular bundle (Fig. 7E, F).

Discussion

Despite of its usage in folk medicine due to its antimicrobial effect, the morphology of *H. telephioides* is not known entirely and this study is the most comprehensive investigation on morphology of the species after Townsend (1967). However, this is the first study conducted to determine the anatomical and some micromorphological characteristics of aerial parts in flowering time of *H. telephioides*.

In this study, some different morphological characteristics have been determined from its description given for *H. telephioides* in Flora of Turkey and East Aegean Islands (Townsend, 1967). The height of plant and length of petal were determined as 4–18 cm and 6–9 mm, respectively while these measurements are given as 7–12 cm and 7–9 mm in the study of Townsend (1967). The leaf shape was determined as ovate, elliptic or subovate in this study, while this feature was specified as only ovate in the study of Townsend (1967). The dimension of leaves, sepals and fruits, the length of filaments, anthers and pistils, and diameter of ovaries are new characteristics which defined in the present study for the first time. Other new findings are the morphological features of seeds, such as shape, size and color of testa. Comparisons of some morphological measurements of *H. telephioides* and three

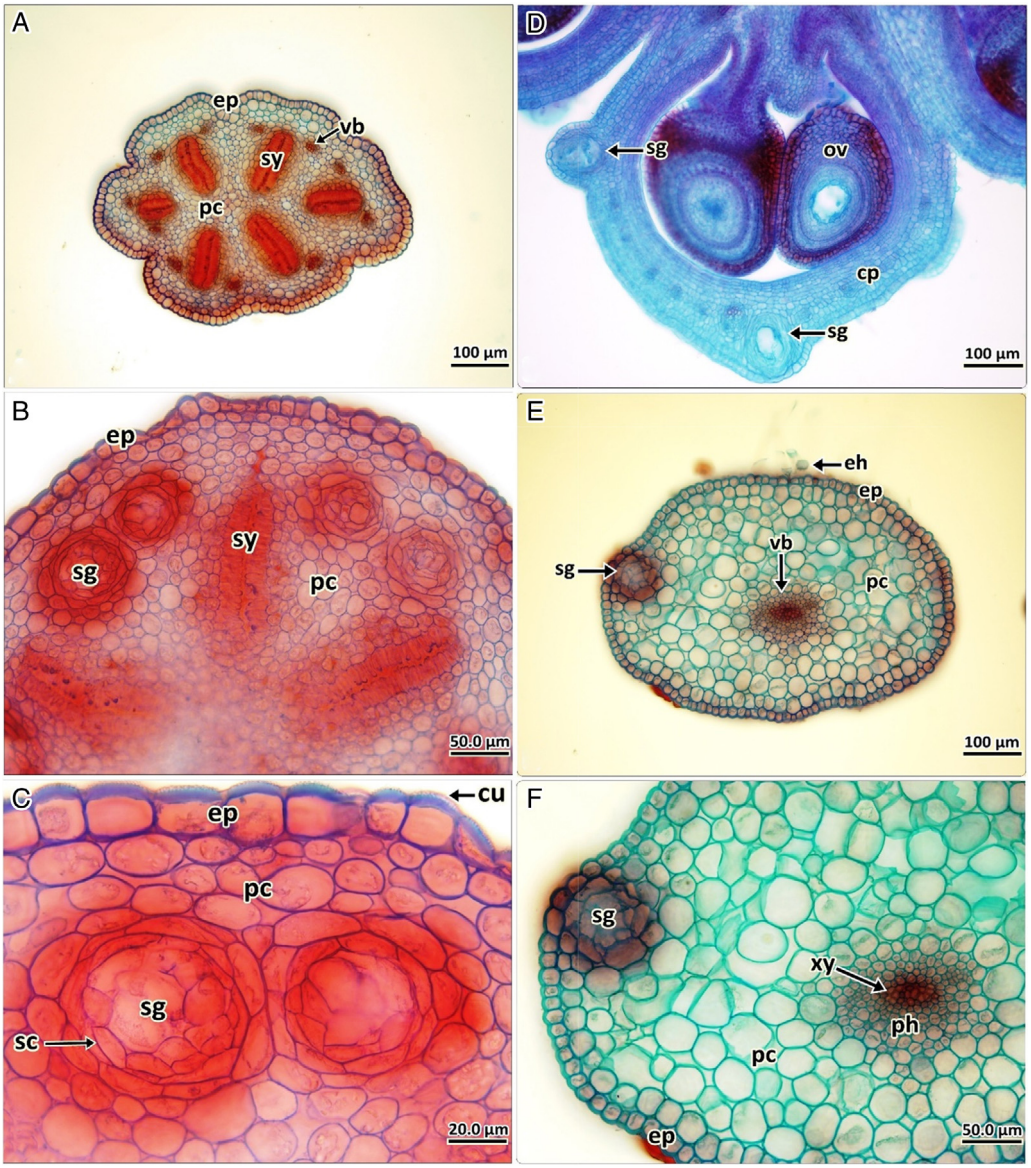

Fig. 7. Microphotographs of the reproductive parts cross section of flower of *Haplophyllum telephioides*. General view of style (A), detail of style (B) and detail of schizogenous glands in style (C), general view of ovarium locule (D), general view of filament (E), detail of filament (F). Abbreviations: carpel wall (cp), cuticle (cu), non-glandular hair (eh), epidermis (ep), parenchymatous cell (pc), phloem (ph), ovulum (ov), secretory cell (sc), schizogenous gland (sg), stylar canal (sy), vascular bundle (vb), xylem (xy).

allied *Haplophyllum* species (*Haplophyllum myrtifolium*, *Haplophyllum vulcanicum* and *Haplophyllum megalanthum*) in study of Ulukış et al. (2016) were given in Box 1.

There are some anatomical studies on Rutaceae (Metcalfe and Chalk, 1950; Saunders, 1934; Moore, 1936; Souza et al., 2004; Muntoreanu et al., 2011; Cruz et al., 2015), but anatomical studies on *Haplophyllum* are deficient.

In Rutaceae, stomata are confined to the abaxial side in most species (Metcalfe and Chalk, 1950). Similarly, one of the other genus of Rutaceae, *Pilocarpus* Vahl and related genera have hypostomatous leaves, with stomata at the same level of the epidermis cells (Duval, 1903; Marquete, 1981; Spegazzini et al., 2002; Gallardo and Jiménez, 2002). Conversely, in *H. telephioides*, stomata are exist both sides, so leaves are amphistomatic. Furthermore, stomata of *H. telephioides* are at the low level of the epidermis cells which known as sunken type. According to Metcalfe and Chalk (1979), in the leaf, the anticlinal walls of epidermal cells may be straight or wavy in some members of Rutaceae. In the leaves of *H. telephiodes*, we observed that solely straight anticlinal walls in adaxial and abaxial surfaces (Fig. 5A, B).

For Turkey flora, *H. megalanthum* is another related endemic to *H. telephioides* and the morphological, anatomical and palynological properties of *H. megalanthum* were investigated by Akyol et al. (2012). When we compare the results on *H. megalanthum* with our results on *H. telephioides*, the anatomical characteristics of both species, there are some considerable differences between them. Such as, in stem cross section, the cambium is composed of 1–3 cell layers, phloem is narrow and pith region is wide in *H. telephioides*. Whereas, the cambium is composed of 3–5 cell layers, phloem is wide and pith region is narrow in *H. megalanthum*. At the more outside of stem, there are unicellular nonglandular hairs, and in cortex layer, there are occasionally schizogenous glands in *H. telephioides*, whereas for these properties there is no report in *H. megalanthum* (Akyol et al., 2012). In the leaf cross section, the shape of epidermis cells is oval or rectangular in *H. telephioides*, while it is solely rectangular in *H. megalanthum*. There is significant epicuticular wax layer and sunken type stomata on adaxial and abaxial surfaces of the leaf in *H. telephioides*, while there is no report for these characteristics in *H. megalanthum*. The presence of wax and sunken type stomata could be an indicator of the adaptation of habiting in arid

region for *H. telephioides*. Recently, Ulukış et al. (2016) have studied comparatively detailed morphology, stem and leaf anatomy and pollen features of morphologically related Turkish endemics, *H. myrtifolium*, *H. vulcanicum* and *H. megalanthum* with systematic aspect. The morphological and anatomical characteristics of *H. telephioides* and three species of Ulukış et al. (2016)'s study were given comparatively in Box 1. Ulukış et al. (2016) were found that the number of cortex layers and sclerenchymatic cell layers on phloem in stem anatomy are to be important as species specific characters for their studied three species. When we compare the stem anatomy results of *H. telephioides* with the results of *H. myrtifolium*, *H. vulcanicum* and *H. megalanthum*, we found that the significant difference is the number of sclerenchymatic cell layer on phloem, just as between three species in study of Ulukış et al. (2016) (Box 1). In leaf anatomy of *H. telephioides*, the numbers of upper and lower palisade and spongy parenchyma cell layers are identical with *H. myrtifolium*, *H. vulcanicum*, and *H. megalanthum*. There are some differences in midrib anatomy of *H. telephioides* and between these three species. In comparison the form and size of vascular bundle in the midrib region, the main difference is midrib shape which is oval in *H. telephioides* whereas, it is adaxially concave and abaxially convex in three species of the study of Ulukış et al. (2016). Additionally, *H. telephioides* has compact and small vascular bundle, whereas, *H. myrtifolium*, *H. vulcanicum*, and *H. megalanthum* have large midrib and in all three species it forms a projecting parts.

Liu and Hu (1998) studied morphology and anatomy of secretory cavities in the leaves of 22 genera, 40 species and 2 varieties of Rutaceae. As a result of this study, authors suggested that there are three types of secretory cavities in Rutaceae: a notch type (between leaf teeth), a mesophyll type and a mixed type (with both notch and mesophyll cavities), suggesting that the mixed type of cavities developed from the notch and mesophyll types. Considering the study of Liu and Hu (1998), schizogenous glands of *H. telephioides* leaves could be classified as mesophyll type (Fig. 4A, C).

Flowers of Rutaceae species are not well-known in their morphological and anatomical aspects (Souza et al., 2004). There are some studies which focused on floral bud development on some members of Rutaceae (Souza et al., 2003; El Ottra et al., 2013). Souza et al. (2004) were studied morphology and anatomy of the flower of *Metrodorea nigra* St. Hill. from Rutaceae. They were indicated secretory glands which immersed in the parenchymatic mesophyll of sepals, petals and ovaries. This feature is similar to *H. telephioides*. In addition, Souza et al. (2003) studied flower anatomy of another Rutaceae, *Pilocarpus pennatifolius* Lem. and they indicated secretory glands in petal, ovary and style cross section, as *H. telephioides*. Furthermore, in cross section of petal of *P. pennatifolius*, Souza et al. (2003) determined druse crystals which are not observed any organs of *H. telephioides* as in petals. In Rutaceae, thick filament was cited by Lersten (2004). The filaments are thick in *H. telephioides* and almost equal with styles width. In addition, the filament has single and large amphicribral vascular bundle in middle area (Fig. 7E, F). Conversely, style has lots of small vascular bundles which located between epidermis and stylar canals (Fig. 7A).

As a conclusion, as can be seen from the literature studies, this study seems to be the first and extensive research on morphology and anatomy of the stem, leaf, sepal, petal, filament and pistil, in addition to the micromorphology of *H. telephioides*. In this study, some different morphological results and new characters were determined, and by using these characters, morphological description of the *H. telephioides* was possibly extended. All anatomical characteristics which belong to the parts used in folk medicine were investigated in detail. The presence of schizogenous glands which produce biologically active substances were shown anatomically in vegetative and reproductive organs. In addition, micromorphological properties of some of these organs were studied. Therefore, some results given in this research were compared with the results of other members of *Haplophyllum* in the literature records and differences between them were stated in detail. All these results can be used to evaluate of the diagnostic features of *H. telephioides* as well as the genus *Haplophyllum*.

Authors' contributions

MT contributed to collecting and identifying plant samples, preparing the slides, data analysis and drafting the paper. NE contributed to writing and critical reading of the manuscript. Both authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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