

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*.

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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 (Metcalf 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 (Metcalf 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 *Haplophyllum*, 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 *Haplophyllum 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 jisticidin-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 vacuolar-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 anti-tumor 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 available 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% Safranin 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 Mansfield (1968).

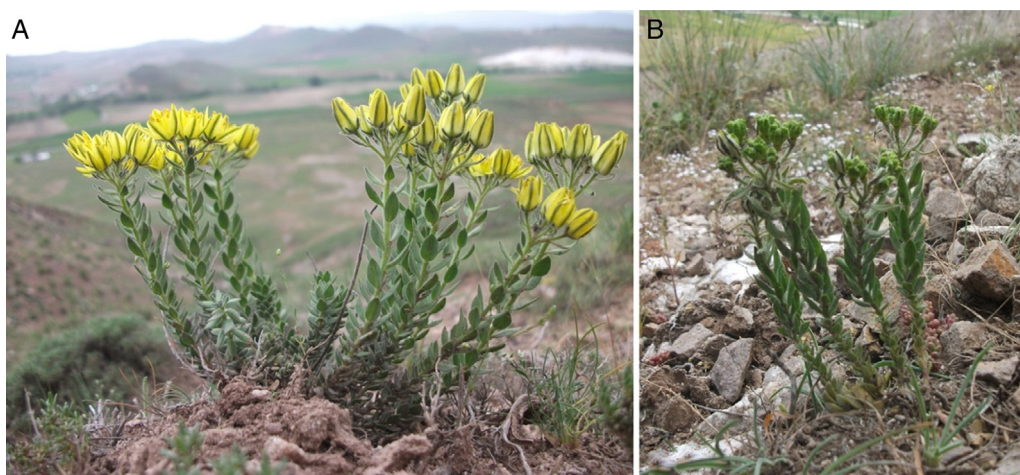


Fig. 1. *Haplophyllum telephioides* in natural habitat. At flowering stage (A), and at fruiting stage (B).

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