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Pharmacobotanical study of *Hypericum thymopsis*

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ABSTRACT

Hypericum thymopsis Boiss., Hypericaceae, is an endemic herb which generally grows at the calcareous steppe regions of Central Turkey. In flowering stage, the aerial parts of this species are used for wound-healing and sedation, and its infusions are used against stomach diseases and throat infections by local people. The aim of this study, to examine and to reveal of the morphology, anatomy and histology of the aerial vegetative and reproductive organs of the *H. thymopsis*, which are used in popular medicine and thus contributing to the pharmacognostic evaluation of the species. In comparison with previous published morphological description of the species, some different findings about plant height and leaf length were found. In addition, some morphological characteristics such as dimensions of sepals and ovaries, length of filaments, anthers, pistils and pedicels were examined here for the first time. The anatomical characteristics of stem, leaf, sepal, filament and pistil were studied using light microscopy and additionally for stem and leaf using scanning electron microscopy. The stem has the secondary growth, and circular shape. The leaves are amphistomatic and the mesophyll is dorsiventral. Stomata are anisocytic and sunken. The glandiferous emergences are present on stem and translucent glands exist in leaf mesophyll. Type A secretory canals are present in stem, leaf, sepal and petal cross section. While type B secretory canals are observed in sepal and petal, type C secretory canals are observed solely in ovary cross section. All the structural features herein found can assist the diagnosis of *H. thymopsis*.

Introduction

Hypericaceae is a small cosmopolite dicot family represented by nine genera and about 500 species of tree, shrub and herb in the world (Simpson, 2006; APG III, 2009; Carine and Christenhusz, 2010). *Hypericum* is the largest genus of Hypericaceae by comprising about 400 species throughout the world except the poles, deserts and low altitude areas of tropical regions (Lewis, 2003; Meseguer and Sanmartín, 2012). In Turkey, *Hypericum* is represented by 96 species, 104 taxa in which 45 of them are endemic with the endemism ratio 43% (Güner et al., 2012). The genus *Hypericum* characterized by possess generally pale to dark yellow flowers and transparently dotted leaves with red or black colored glands (Davis, 1966, 1988; Güner et al., 2000). The genus *Hypericum* is known worldwide with its wide folk medicinal usage (Avato, 2005; Assadi et al., 2011). *Hypericum* species are known under the names “binbirdelik otu”, “kanotu”, “peygamber çiçeği” and by a majority “sarı kantaron”, and have been used as sedatives, antiseptics, anti-inflammatory, anti-astmatic, antispasmodics, wound-healing and to against stomach diseases, ulcers and diabetes in Turkish folk medicine (Bingol et al., 2011; Özkan and Mat, 2013). The taxa of *Hypericum* characterized by having extensively secretory structure such as translucent glands, black nodules and secretory canals which synthesizing and accumulating biologically active substances, so they have great phytochemical potential and composition (Siersch, 1927; Metcalfe et al., 1950; Curtis and Lersten, 1990; Ciccarelli et al., 2001a, 2001b; Onelli et al., 2002). Some of these phytochemical compositions of the Hypericaceae are naphthodianthrones (notedly hypericin and pseudohypericin), acylphloroglucinol derivatives (notedly hyperforin and adhyperforin), flavonoids (notedly quercetin, quercitrin, hyperoside and biapigenin), tannins, n-alkanes, xanthones and essential oils (Bombardelli and Morazzoni, 1995; Bruneton, 1995; Kitanov, 2001; Maggi et al., 2004). Due to their phytochemical composition and waste usage in traditional folk medicine, many studies were made on biological activities of different species extracts of *Hypericum* in Turkey and in the world. For example, analgesic activity (Öztürk, 2001), wound healing activity (Öztürk et al., 2007), hepatoprotective activity (Öztürk et al., 1992), anti-inflammatory activity (Öztürk et al., 2002), anti-*Helicobacter pylori* activity (Yeşilada et al., 1999) and antidepressant activity (Öztürk, 1997) of well-known *Hypericum*, *H. perforatum* L. were studied by different researchers. In addition, antioxidant activity of *H. venustum* Fenzl was studied by Spiteller et al. (2008),

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antimicrobial activity of *H. hyssopifolium* Chaix var. *microcalycinum* (Boiss. & Heldr.) Boiss. was studied by Toker et al. (2006), and myeloperoxidase activity of *H. empetrifolium* Willd. was studied by Kültür (2007). In addition there are some studies focused morphology, anatomy and secretory structure of some *Hypericum* species (Metcalfe et al., 1950; Toma and Rugină, 1998; Hong-Fei and Zheng-Hai, 2001; Yaylacı et al., 2013; Perrone et al., 2013a, 2013b).

Hypericum thymopsis Boiss. is an endemic species for Turkey Flora and naturally growing in Sivas, Kayseri, Malatya and Kahramanmaraş provinces (Davis, 1966). Essential oil composition of *H. thymopsis* was studied by Özkan et al. (2009, 2013). Özkan et al. (2013) studied composition of essential oils of five endemic *Hypericum* species for Turkey (*H. uniglandulosum* Hausskn. ex Bornm., *H. scabroides* Robson and Poulter, *H. kotschyanum* Boiss., *H. salsugineum* Robson and Hub.-Mor. and *H. thymopsis*). According to their study, α-pinene, baeckeol, limonene and spathulenol were identified as major components of *H. thymopsis*. Furthermore, *H. thymopsis* has greatest potential by having so much more α-pinene, within these five species. α-Pinene is one of the active substance which has bronchodilator and anti-inflammatory activity (Russo, 2011) and has bacteriostatic and bactericide effect against especially gram (+) and gram (−) bacteria with broad spectrum (Nissen et al., 2010). In addition, it has acetylcholinesterase inhibitor activity, thus it has effect that strengthens the memory of human (Russo, 2011). α-Pinene is also reported as one of the main components of the essential oils of other some *Hypericum* species (Joulain and König, 1998; Santos et al., 1999; Zeng and Zhou, 2001; Gudžić et al., 2002, 2004).

In the field works, by interviewing local peoples under an ethnobotanical studies, it was learned that above ground parts of *H. thymopsis* are used for wound-healing and sedation, and its infusions and decoctions as tea are used also against stomach diseases and throat infections. By considering great usage of various *Hypericum* taxa in folk medicine, their anatomical features are not well-known except *H. perforatum*. In Turkey, there are some anatomical studies on other Turkish *Hypericum* taxa (Yaylacı et al., 2013; Altıntaş and Akçin, 2015). The morphological characteristics of all Turkish *Hypericum* taxa are more or less known. But there is some deficiency on some endemic species such as *H. thymopsis*. For example, in Flora of Turkey (Davis, 1966), dimensions of sepals and ovaries, length of filaments, anthers, pistils and pedicels of *H. thymopsis* are not reported. Despite of some essential oil studies on *H. thymopsis* (Özkan et al., 2009, 2013), there is no report on anatomical and histological structure of the species. The aim of this study, revealed entirely morphological characteristics, and anatomical and histological structure in all above ground parts and their secretory structures which make up biologically active substances of *H. thymopsis*.

Materials and methods

Plant material

The specimens of *Hypericum thymopsis* Boiss., Hypericaceae, were collected during the flowering and fruiting time from different natural populations in Sivas province of Turkey. Field works were carried out in between the years 2012–2014. Locality 1: B6 Sivas: Ulaş district, Ziyarettepe, 1444 m, 39°33′06.7″ N; 37°01′11.9″ E, 26.06.2013; Locality 2: B6 Sivas: Ulaş district, Kurtlukaya village to Boğazdere village, 1458 m, 39°23′02.1″ N; 36°55′48.3″ E, 05.06.2014; Locality 3: B6 Sivas: Sivas-Kangal-Gürün road intersection, 1560 m, 39°07′53.1″ N; 37°14′32.9″ E, 06.08.2012, ibid. 05.06.2014, ibid. 05.07.2014.

These were registered under collector numbers M. Tekin 1309, 1470, 1562, 1566, 1625 and are conserved at the Cumhuriyet University, Faculty of Science Herbarium (CUFH), Department of Biology, Sivas, Turkey. Taxonomical identification was made according to Flora of Turkey (Davis, 1966) by author.

Structural analysis

Morphological description of *H. thymopsis* was made using both fresh and herbarium samples collected from different localities. Parts of the some fresh material were stored in 70% alcohol–water solution for later histo-anatomical procedures. For this reason, hand-made transverse sections of stem, leaf, sepal, petal, filament, ovary and style were taken. Also superficial hand-made sections of adaxial and abaxial surface of the leaf blade 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. thymopsis* were made using Olympus BX22 light microscopy. Photomicrographs were taken using Olympus BX51 light microscopy coupled with Olympus DP70 digital camera. The anatomical terminology used is that according to Esau (1965). For secretarial analyses, the study of Ciccarelli et al. (2001b) was carried out: (a) *Translucent glands*. These are pale glands or glandular pockets (Robson, 1981) and are spherical or oblong glands delimited by two layers. (b) *Three types of secretory canals*. Type A: the lumen of the canals is usually surrounded by four (or more in especially flowers parts) polygonal cells which have very thin wall toward canal lumen. Type B: they have a same structure with translucent glands with their wide lumen, but they seem elongated and pale which differ from the translucent glands. Type C: type C canals composed of wide cavity surrounded by one or more cell layers which have densely stained and thin walls.

For scanning electron microscopy (SEM) analyses, the some plant parts were mounted on aluminum stubs and coated with gold. The micromorphological and anatomical observations were made, and micrographs were taken at different magnifications by using LEO 440 SEM.

Results

Morphological aspects

Perennial herbs. Stems 3–14 cm, erect, numerous, glabrous, scabrid with glandiferous emergences (Fig. 1B). Leaves 5–18 × 0.6–1.6 mm, linear, revolute with obtuse or rounded apex, glabrous, usually papillose at adaxial surface and especially at margins. Black glands present at the margins of small leaves which positioned at the base of pedicel on stem. Inflorescence corybose, up to 17 cm in fruiting time, soliter or 20 flowered. Sepals 1.9–2.8 × 0.8–1.1 mm, united at base, lanceolate to lanseolate-elliptic with sessile or subsessile black glands at margins and acute apex (Fig. 1D). Sepals reddish when flowers in bud stage, green in flowering time (Fig. 1A, D). Petals 4.5–7.5 × 2–3.8 mm, pentamerous, elliptic-oblanceolate to obovate and without black glands (Fig. 1C, E). Filaments 4–5.5 mm, thin and numerous. Anthers 0.35–0.45 mm, dorsifixed and longitudinal dehiscence (Fig. 1F). Pistils 4–5 mm, ovaries 1.5–2.2 × 0.9–1.4 mm, 3 loculed and ovoidal shaped. Style 3, free and selender, 2.5–3.2 mm (Fig. 1G). Placentation axillar, ovules anatropous. Pedicels 2–8 mm. Fruit 4.5–5.5 × 2.7–3.1 mm, ovoid, reddish-brown, septicidal capsule rostrate and with 3 locule, each locule bear usually 2, occasionally 1 seed. Seeds 2–2.3 × 0.7–1 mm, light brown, oblong-cylindrical and covered by densely short thick white hairs (Fig. 1).

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