Original Article

Pharmacobotanical study of Hypericum thymopsis

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A B S T R A C T

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, sepals, 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, sepals 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.

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antimicrobial activity of *H. hyssopifolium* Chaix var. *microcalycinum* (Boiss. & Hildr.) Boiss. was studied by Toker et al. (2006), and myeloperoxidase activity of *H. empetrifolium* Willd. was studied by Külüktür (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 Özkân et al. (2009, 2013). Özkân et al. (2013) studied composition of essential oils of five endemic *Hypericum* species for Turkey (*H. uniglandulosum* Hauskn. ex Bornm., *H. scabrodes* Robson and Poulter, *H. kotschyanum* Boiss., *H. sal-sugineum* Robson and Hub.-Mor. and *H. thymopsis*). According to their study, α-pinene, baceekol, 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 acetycholinesterase 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; Gudzić et al., 2002, 2004).

In the field works, by interviewing local peoples under an ethobotanical 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 Akgın, 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 sepal and ovaries, length of filaments, anthers, pistils and pedicels of *H. thymopsis* are not reported. Despite of some essential oil studies on *H. thymopsis* (Özkân 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 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 mounted 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 microscope 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 lanceolate-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, pentamers, 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).
Anatomical and histological aspects

Stem

The stem is in the secondary growth stage and circular shaped in transverse section (Fig. 2A, E). The epidermis is uniseriate and its cells are small, oval or usually irregular shaped, and are surrounded by thick cutinized wall at all around the cell. Cuticle layer is thick. There are two small wings and glandiferous emergences on stem (Fig. 2A). Cortex consists of 3–7 parenchymatous cell layers. Cortex cells are oval or circular-shaped and there are some intercellular spaces between them. There is a thin collenchyma which compose a ring in cortex. Endodermis is uniseriate and cells are dorsoventrally depressed. The pericycle is conspicuous with thickened laterally cell walls, just beneath the endodermis (Fig. 2B, F). Vascular bundles are located in the major portion of the stem transverse section. In phloem, there are lots of type A secretory canals which are composed of four secretory cells. Cambium is indistinguishable. Secondary xylem is ring-porous. Pith rays are parenchymatous and uniseriate, occasionally biseriate (Fig. 2C, G). Pith cells are parenchymatous and are oval or circular shaped. Size of pith cells enlarge toward center and the cell walls are lignified and prominently thickened in this region (Fig. 2D, H).

Leaf

Leaf is “3” shaped in transverse section. There are uniseriate epidermis on both surfaces of the leaves. Adaxial epidermis cells are very large, oval or rectangular shaped and usually papillose especially near lamina margins, conversely, abaxial epidermis cells are small, rectangular or occasionally squarish shaped and are all non-papillose (Fig. 3A, D). Epidermal cells have a smooth cuticle on both sides. There is conspicuous epicuticular wax on abaxial epidermis while it is lack of on adaxial surface (Fig. 4C–F). The cell wall of adaxial epidermis is very thick, despite that it is thin in abaxial epidermis cells (Fig. 3D). In addition, margins of adaxial epidermis cells are distinct and slightly wavy, while margins of abaxial epidermis cells are obscure. Stomata are anisocytic and sunken, occurring in both on the adaxial and predominantly abaxial sides of the leaf lamina (Figs. 3F and 4). The mesophyll is dorsiventral and is formed by 2–3 layers of palisade parenchyma and 2–3 layers of spongy parenchyma. While, the palisade parenchyma cells are generally cylindrical, occasionally rectangular, squarish or ovoid-shaped, the spongy parenchyma cells are usually cylindrical occasionally ovoid or irregular-shaped (Fig. 3B, D). There are large translucent glands that positioned between just beneath the adaxial epidermis and the middle area of spongy parenchyma (Fig. 3A, D). Small
collateral vascular bundles are observed immersed into the mesophyll, associated with one or a few type A secretory canals in the phloem (Fig. 3E). The midrib is oval-shaped, particularly large and protrude from the abaxial surface. The midrib surrounded by a uniseriate parenchymatous bundle sheath. There are a collenchyma cap which cells are usually ovoid or circular-shaped and which make the midrib even more prominent and conspicuous (Fig. 3B). The cambium is clearly distinguishable between xylem and phloem, so vascular bundle type is open collateral. In vascular bundle, there are numerous type A secretory canals which are
Sepal

The transverse section of sepal was taken from calyx tube. Epidermis is uniseriate in both sides. Adaxial epidermis cells are rectangular or rectangular ovoid-shaped, but they are larger and irregular shaped especially above the main vascular bundles region. Abaxial epidermis cells are rectangular, squarish or oval shaped and are usually larger than adaxial epidermis cells (Fig. 5A). Mesophyll consists of 7–12 parenchymatous cell layers. These cells are usually oval, rarely circular-shaped and they have intercellular spaces. The midrib is almost circular-shaped and lacks bundle sheath (Fig. 5B). There are type A secretory canals in phloem of vascular bundles and these canals consist of 4–6 secretory cells (Fig. 5C). Beneath the abaxial epidermis, there are large type B secretory canals which alternate with the vascular bundles and immersed in mesophyll (Fig. 5D).

Petal

Adaxial and abaxial epidermis are uniseriate in petal transverse section. While abaxial epidermis cells are rectangular or squarish-shaped, adaxial epidermis cells are usually irregular shaped. The mesophyll consists of 5–8 layered parenchymatous cells which are usually oval-shaped and have intercellular spaces between them (Fig. 6A, C). Vascularization is made by a few small vascular bundles in the center of mesophyll. Type A and type B secretory canals may occur in same section, as they can be seen in separate sections as in Fig. 6A and C. Type A secretory canals are present in vascular bundles and they consist of four or more secretory cells (Fig. 6B). Type B secretory canals occur just beneath the adaxial epidermis and they alternate with the veins (Fig. 6C, D).

Filament

Filaments are very thin and triangular-shaped in transverse section. Uniseriate epidermal cells are large and usually irregular-shaped. Cuticle is thick and rugose. There is small single vascular

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**Fig. 3.** Photomicrographs of the leaf transverse sections of Hypericum thymois. General view (A); detail of midrib region (B); detail of vascular bundle in midrib (C); detail of lamina with translucent gland (D); detail of small vascular bundle with a type A secretory canal in mesophyll (E); detail of sunken stomata in adaxial epidermis (F). Abbreviations: ab, abaxial epidermis; ad, adaxial epidermis; as, air space; bs, bundle sheath; ca, cambium; cx, cortex; gc, guard cells; ph, phloem; pp, palisade parenchyma; sc, secretory canal-type A; sl, secretory cell; sp, spongy parenchyma; st, stoma; tg, translucent gland; vb, vascular bundle; xy, xylem.
bundle in the center of transverse section. Between epidermis and vascular bundle, there are irregular-shaped parenchymatous cells. The members of secretory structure are absent (Fig. 7A).

**Pistil**

The style is rectangular-shaped in transverse section. Epidermis is uniseriate, with rectangular, squarish or irregular-shaped cells. Outer walls of epidermal cells are thick. Cuticle is rugose and thick. There is a single vascular bundle which positioned close to epidermis. There is pollen tube transmitting tract (PTTT) in the center of transverse section. Between the region of epidermis and PTTT are filled by oval, circular or irregular shaped parenchymatous cells. The members of secretory structure are absent in transverse section of style (Fig. 7B). The ovaries have 3 locules (Fig. 7C). Adaxial epidermis cells of carpels are usually irregular, occasionally rectangular or squarish shaped. Abaxial epidermis cells of carpels are rectangular or squarish shaped. The mesophyll cells are parenchymatous and usually irregular-shaped. Just beneath the adaxial epidermis, there are numerous type C secretory canals which immersed in the parenchymatic mesophyll of each locules (Fig. 7D).

**Discussion**

Despite of its usage in folk medicine, the morphology of *H. thymopsis* is not entirely known. There are some general knowledge on morphology of the species in Flora of Turkey (Davis, 1966), but there are no report on the shape of petals, dimensions of sepals and ovaries, length of filaments, anthers, pistils, styles and pedicels of the species. These features are examined in present study. In Flora of Turkey (Davis, 1966), stems height and petals length of *H. thymopsis* are given as 3–11 cm and 5–7 mm, respectively. In present study, stems height and petals length of *H. thymopsis* were found as 3–14 cm and 4.5–7.5 mm, respectively. In addition, black glands were observed at the margins of small leaves which are positioned at the base of pedicel on stem. It is not reported in Flora of Turkey (Davis, 1966) (Fig. 1).

According to Metcalfe et al. (1950), pith rays are uniseriate, the xylem and phloem are relatively narrow, whilst there is a greater development of pith in stem of herbaceous *Hypericum*. Conversely, in *H. thymopsis* stem, phloem and especially xylem fill major part of the transverse section, pith located in the small region of the transverse section center (Fig. 2A, E). Pith rays of *H. thymopsis* are
Fig. 5. Photomicrographs of calyx tube transverse section of Hypericum thymopsis. General view (A); detail of sepal midrib region with type A and type B secretory canals (B); detail of vascular bundle and type A secretory canal in sepal (C); detail of type B secretory canal (D). Abbreviations: ab, abaxial epidermis; abw, abaxial epidermis cell; ad, adaxial epidermis; cu, cuticle; pc, parenchymatous cell; ph, phloem; scA, secretory canal-type A; scB, secretory canal-type B; sl, secretory cell; st, stoma; vb, vascular bundle; xy, xylem.

Fig. 6. Photomicrographs of petal transverse section of Hypericum thymopsis. General view with type A secretory canals (A) and detail view of vascular bundle and type A secretory canal (B). General view with type B secretory canals (C) and detail view of type B secretory canal (D). Abbreviations: ab, abaxial epidermis; ad, adaxial epidermis; cu, cuticle; pc, parenchymatous cell; ph, phloem; scA, secretory canal-type A; scB, secretory canal-type B; sl, secretory cell; vb, vascular bundle; xy, xylem.
commonly uniseriate, occasionally biseriate. In addition, pith ray cells of *H. thymoposis* are upright and usually oval or rectangular-shaped, whereas the examined *Hypericum* species of Metcalfe et al. (1950), they are upright and entirely square-shaped (Fig. 2C, G). The outlines of stem transverse section are circular-shaped, vascular bundles of the *H. thymoposis* stems always formed a circle which is reported for some other *Hypericum* species by Arda (1989) and Tokur and Misirdali (1989) (Fig. 2A, E).

According to Metcalfe et al. (1950), the leaves of Hypericeae are dorsiventral with hypoderm in certain species of *Hypericum* and stomata of *Hypericum* species are commonly surrounded by three or more cells. The leaves of *H. thymoposis* are dorsiventral, without hypodermis and stomata are anisocytic (Figs. 3B, D and 4A, B). The level of stomatal guard cells is lower than epidermis cells, so stomata are sunken type which is known as a special feature of the xerophytes (Fig. 3F). Prominent epicuticular waxes are present on abaxial side of the leaf and thus, the capacity of reduce the water loss is increase (Fig. 4E, F). Furthermore, presence of the wax reduces mechanical damage and prevents from fungal and insect attack (Eglinton and Hamilton, 1967). In addition, according to Briquet (1919) leaves of certain species of *Hypericum* growing arid regions in Brasil have thickened margins consisting of enlarged epidermal cells or collenchyma. The margin epidermal cells of *H. thymoposis* leaves are enlarged, the cuticle and outer cell walls of these cells are more thickened than other epidermal cells (Fig. 3A, D) so, the leaves of *H. thymoposis* show the same feature of mentioned knowledge.

Gîtea et al. (2011) were studied four *Hypericum* (*H. perforatum, H. tetraperturum* Fries, *H. maculatum* Crantz and *H. hirsutum* L.) in point of secretory structure of some vegetative organs. In their study, while type A canals are present in the phloem of all four species stems, type B secretory canals are present solely, in subepidermic area of *H. perforatum* and *H. tetraperturum*, and they are absent in *H. maculatum* and *H. hirsutum*. In stem of *H. thymoposis*, type A canals and glandiferous emergences are present, but type B canals are absent as well as *H. maculatum* and *H. hirsutum* (Fig. 2). However, there are no reports on presence of glandiferous emergences, in stems of their studied four *Hypericum* species. According to Gîtea et al. (2011), all examined species have 1–2 layered palisade parenchyma in leaf mesophyll, whereas it was observed as 2–3 layered in *H. thymoposis* (Fig. 3B, D). According to Shields (1950), having more strongly developed palisade tissue is one of the characteristic of the xerophytic plants, due to being the xeromorphic plant, *H. thymoposis* has similar feature of mentioned literature.

Recently, Perrone et al. (2013a) were studied the leaf and stem anatomy of eight *Hypericum* species (*H. aegypticum* L., *H. androsaemum* L., *H. hircinum* L., *H. perforatum* L., *H. pubescens* Boiss., *H. tetraperturum* Fr. and *H. triquetrifolium* Turra) which grow heterogeneous environments of Sicily Island. General anatomical characteristics of *H. thymoposis* are similar with those in their study. At the same time, there are some evident different characteristics for *H. thymoposis* especially in leaf anatomy. In stem anatomy, there are numerous glandiferous emergences outer of the *H. thymoposis* stem (Figs. 1B and 2A, E). There is no report on the presence of this characteristic in stems of their studied eight species. Transverse section shape of the stem and presence of slightly two wings in *H. thymoposis* are common characteristics with some species studied by Perrone et al. (2013a). Secondary xylem of *H. thymoposis* is ring-porous (Fig. 2C, G) such as their studied six species, except *H. pubescens* and *H. aegypticum* which have diffuse-porous secondary xylem. The leaves of *H. thymoposis* are dorsiventral (Fig. 3A, B, D) as with *H. perforatum, H. perforatum, H. tetraperturum, H. androsaemum* and *H. hircinum*, but the leaves of *H. pubescens, H. triquetrifolium* and *H. aegypticum* are isobilateral (Perrone et al., 2013a). The leaves of *H. thymoposis* are amphistomatic (Fig. 4) as with *H. pubescens, H. tetraperturum, H. triquetrifolium* and *H. aegypticum* while the leaves of

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![Fig. 7. Photomicrographs of the reproductive parts transverse section of flower of Hypericum thymoposis. General view of filament (A); general view of style (B); general view of ovary (C); detail of carpel (D). Abbreviations: ab, abaxial epidermis; ad, adaxial epidermis; cp, carpel wall; cu, cuticle; ep, epidermis; pc, parenchymatous cell; ph, phloem; pt, pollen-tube transmitting tract; scC, secretory canal-type C; sl, secretory cell; vb, vascular bundle; xy, xylem.](image-url)
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