

## COMPARATIVE ANATOMICAL STUDIES ON SOME SPECIES OF *HYOSCYAMUS* L. (SOLANACEAE) GROWING IN TURKEY

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### Abstract

A comparative study based on leaf and stem anatomical structure was made using light microscopy (LM) techniques on five species of *Hyoscyamus* L. (Solanaceae) in Turkey. Some characters are found important to distinguish the species within the genera. The investigated species can be divided as mesophyll type: bifacial (*H. niger* L., *H. albus* L.) and equifacial (*H. aureus* L., *H. pusillus* L., *H. reticulatus* L.). Druse crystals are recorded only in mesophyll of *H. albus*. Stomata present on both surfaces, are anisocytic (usually) and anomocytic types. *H. reticulatus* can be distinguished from other species considering types of trichomes in the stem. Vascular bundles are bicollateral types.

### Introduction

The cosmopolitan Solanaceae family includes 102 genera with 2460 species in the world. In Turkey, there are 12 genera and 36 species of the Solanaceae and most of them are wild herbs (Erik and Tarıkahya 2004; Selvi *et al.* 2009). The plants of this family are well known as a natural source of tropan alkaloids including hyoscyamine, scopolamine and atropine (Kartle *et al.* 2003) and are cultivated for their medicinal importance (Etminan *et al.* 2012). The genus *Hyoscyamus* occupies the phyto-geographical region of Sino-Japanese. *Hyoscyamus* a small herbaceous genus having 26 species all over the world (Yousaf *et al.* 2008) and is represented by 6 species in Turkey (Baytop 1978; Güner *et al.* 2000; Güner 2012). *Hyoscyamus* species have medicinal importance because of their hyoscyamine and scopolamine content (Mateus *et al.* 1998, 2000). Therefore, it is widely used as sedative and painless in folk medicine (Baytop 1999). Although the chemical aspects of *Hyoscyamus* have already been investigated, anatomical information is scarce for the species of the genera. There are systematic studies on *Hyoscyamus* taxa (Ghahreman *et al.* 1999, Sheidai *et al.* 2000). Anatomical studies carried on *Hyoscyamus* species have been shown to be insufficient and limited to a few taxa such as *H. reticulatus* (Baytop 1971, Ghassemi *et al.* 1995).

The present study describes the stem and leaf structure of five *Hyoscyamus* species growing in Turkey with the purpose of pointing out anatomical characters useful to distinguish these species.

### Materials and Methods

Materials used in this study were collected from plants in their natural habitat. Voucher specimens were kept in the Harran University, Department of Biology, Şanlıurfa and are listed in Table 1. Fresh materials were fixed in 70% alcohol. Developed middle cauline leaves from fully

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flowered plants were used in anatomical study. Five samples were taken from each specimen and free hand sectioning of stem and leaves were made for anatomical studies. Tissues were stained with phloroglusine +HCl and embedded in glycerine jelly. In addition, chlorophyll in leaves were cleared with chloral hydrate (Yakar-Tan 1982). Anatomical sections were examined by Olympus BX50 phase contrast binocular microscope and microphotographs of sections were taken by Ucmo SO5100 KPA digital camera attachment.

### Results and Discussion

The transverse section of the stem presented in Fig. 1. The epidermis consists of a single layer of isodiametric cells. Covering hairs consist of stalked glandular types in a stem. The stalked glandular hairs have usually elongated 2-3-celled stalk, and with pear-shaped unicellular head. They are recorded only with pear-shaped multicellular head in *H. reticulatus* (Fig. 1c). The collenchyma tissue is located immediately under the epidermis in some species (Fig. 1). It is also

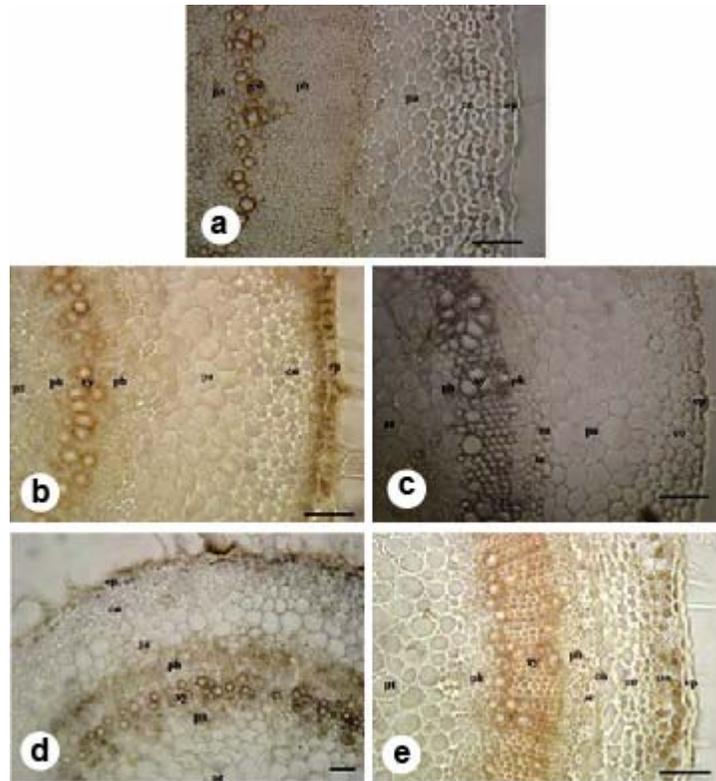


Fig. 1. Comparative stem structures of *Hyoscyamus* species. a. *H. pusillus*, b. *H. niger*, c. *H. reticulatus*, d. *H. albus*, e. *H. aureus*. eh: eglandular hair, ep: epidermis, co: collenchyma, pa: parenchyma, sc: sclerenchyma, en: endodermis, ph: phloem, xy: xylem, pt: pith parenchyma (Scale bar: 100  $\mu$ m).

located under the parenchyma in some species (Fig. 1). The collenchyma is absent in some species. Parenchyma tissue, which is 2-7-layered, is composed of usually round and ovoid or polyhedral cells. The endodermis consists of irregularly rectangular cells, or it is not distinguishable in some species. Pericycle is sclerenchymatic or parenchymatic and 1-3-layered or

not distinguishable. The vascular bundle type is bicollateral. The cambium is not distinguishable. Phloem is located on both sides of the xylem. The xylem comprises trachea and tracheids. The trachea are orbicular or ovoid while the tracheids are polyhedral. The rays are usually uniseriate, rarely biseriate. The pith consists of large round or polyhedral parenchymatic cells.

At cross-section it is observed that the epidermis consists of a single layer of cells which are isodiametric. Stomata present on both surfaces, are anisocytic (usually) and anomocytic types (Fig. 3 c,d). The both surfaces of leaves have stalked glandular trichomes. The density of the stalked glandular hairs varies in *Hyoscyamus* leaves (Fig. 3e-g). The stalked glandular hairs have usually elongated 3-celled stalk, rarely bicellular, and with pear-shaped unicellular head. They are recorded only with a pear-shaped multicellular head and elongated 2-3-celled stalk in *H. reticulatus*. The investigated species can be divided as mesophyll type: bifacial (*H. niger*, *H. albus*) (Fig. 2 b,d) and equifacial (*H. aureus*, *H. pusillus*, *H. reticulatus*) (Fig. 2a,c,e). There are some differences in the number of the mesophyll cells. Druse crystals are recorded only in mesophyll of *H. albus* (Fig. 2 d). The vascular bundle is bicollateral type. In the midrib region, there are collenchymatous tissue under the upper and lower side of vein (Fig. 3a,b).

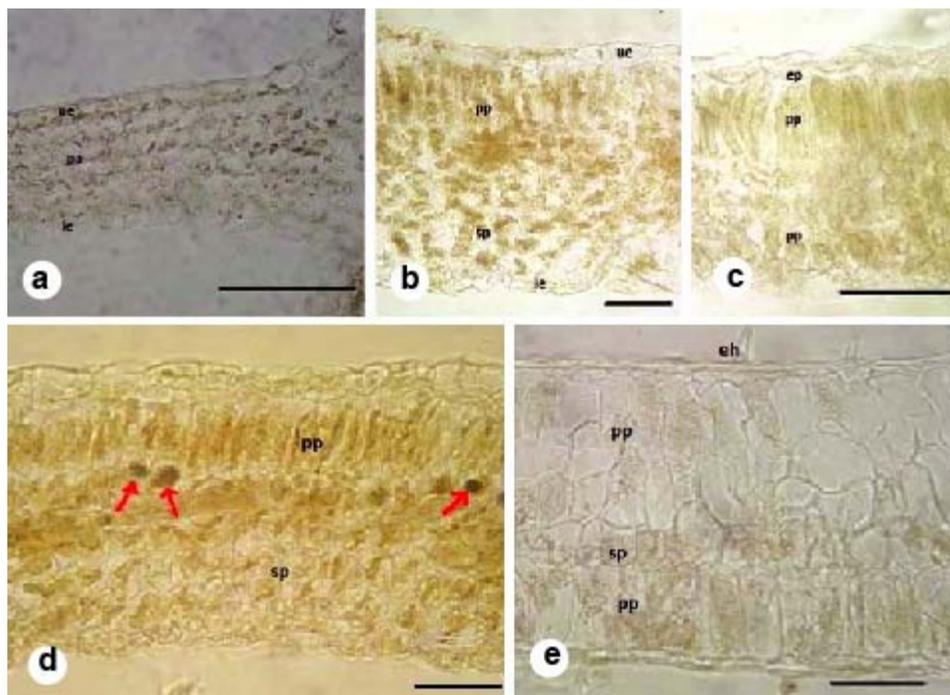


Fig. 2. Comparative mesophyll tissue of *Hyoscyamus* species. a. *H. pusillus*, b. *H. niger*, c. *H. reticulatus*, d. *H. albus*, e. *H. aureus*. eh: eglandular hair, gh: glandular hair, ue: upper epidermis, pa: parenchyma, pp: palisade parenchyma, sp: spongy parenchyma, le: lower epidermis. Druse crystals are shown by arrows. (Scale bar: 100  $\mu$ m).

The trichomes of *Hyoscyamus* show differences among the species studied as in the case of *Solanum* (Seith 1979, Seith and Sullivan 1990). The trichomes found in the Iranian *Hyoscyamus* species are divided into two hair classes; The first is unbranched hairs, with or without glandular type while second class is branched to dentritic hairs, with or without glandular type. Type of trichomes can be successfully used for the delimitation of the subgenera and species (Ghahreman

*et al.* 1999). The trichomes in the stem and leaves may be divided into two categories: I. Head multicellular, stalk 2-3-celled, II. Head unicellular, stalk 2-3-celled. The stalked glandular hairs have usually elongated 3-celled stalk, rarely bicellular, and with pear-shaped unicellular head (Fig. 4). They are recorded only with pear-shaped multicellular heads in trichomes of *H. reticulatus* (Fig. 3f). The cortex consists of only parenchymatic cells in *H. pusillus* (Fig. 2a.). The collenchyma tissue is located immediately under the epidermis in *H. niger* and *H. reticulatus*, followed by parenchyma (Fig. 2). Also, the collenchyma is located under the parenchyma in *H. aureus* (Fig. 1e). A single layer parenchyma tissue is located immediately under

**Table 1. Collection data and collectors's number of studied *Hyoscyamus L.* species.**

Taxa	Collection data
<i>H. pusillus</i> L.	<b>B7 Tunceli:</b> 2 km north of Pertek port, crevices and fissures on rock faces.13.06.2004, 1200 m, Aslan 1529. <b>B8 Elazığ:</b> Harput Castle, Stony or rocky places, waste places, roadsides, 15.05.2004, 1400 m. Aslan 1539. <b>C5 Niğde:</b> 2 km north of Niğde, roadside verge, dumping ground, 25.05.2003, 1000 m. Aslan 1451.
<i>H. niger</i> L.	<b>B8 Batman:</b> in downtown of Hasankeyf, on the castle wall, 19.05.2004, 800 m., Aslan 1448. <b>C6 Kahramanmaraş:</b> 10 km south of Kahramanmaraş, the margins of arable fields, 14.05.2004, 550 m., Aslan 1534. <b>C8 Mardin:</b> Waste places, roadsides, 19.05.2004, 1350 m., Aslan 1536.
<i>H. reticulatus</i> L.	<b>B8 Diyarbakır:</b> Ergani, Şölen village, Steppe, Stony or rocky places, waste places, 14.05.2004, 1100 m, Aslan 1521. <b>C5 Niğde:</b> North of Ulukışla, along forest margins, open habitats, 20.06.2003, 1100 m., aslan 1399., <b>C7 Şanhurfa:</b> Küçüksergen village, stony steppe, 19.06.2003, 550 m., Aslan 1411.
<i>H. albus</i> L.	<b>C6 Gaziantep:</b> Antep castle, rock crevices on the wall, 31.07.2003, 650 m., Aslan 1352, <b>C7 Şanhurfa:</b> Urfa Castle, Stony or rocky places, Foot of rocks and walls, 11.04.2004, 600 m., Aslan 1501. <b>C8 Mardin:</b> Mardin castle, in fissures on limestone, 13.05.2004, 1300 m., Aslan 1528,
<i>H. aureus</i> L.	<b>C6 Gaziantep:</b> Nizip, Rum castle, crevices and fissures on rock, 04.04.2003, 550 m, Aslan 1254. <b>C7 Şanhurfa:</b> Birecik castle, crevices and fissures on rock, 24.05.2003, 650 m., Aslan 1356, <b>C8 Mardin:</b> Castle, Rock crevices, old walls, 14.05.2004, 1250 m, Aslan 1525.

**Table 2. Anatomical characteristics of studied *Hyoscyamus L.* species.**

Characters	Investigated taxa				
	<i>H. pusillus</i>	<i>H. niger</i>	<i>H. reticulatus</i>	<i>H. albus</i>	<i>H. aureus</i>
	Stem				
Stalked glandular hair	Unicellular head 2-3(-6) celled stalk	Unicellular head 1-6(-8) celled stalk	Multicellular head 2-3- celled stalk	Unicellular head 2-4(-8) celled stalk	Unicellular head 2-3- celled stalk
Collenchyma	-	4-6	4-6	3-5	2-3
Parenchyma	5-7	2-4	3-6	4-6	2-4
Endodermis	Rectangular	Distinguishable	Distinguishable	Distinguishable	Rectangular
Pericycle	1-2 sclerenchymatic	1-3 parenchymatic	1-3 parenchymatic	1-3 parenchymatic	1-3 sclerenchymatic
Pith cells	Polyhedral	Polyhedral	round	Polyhedral	Polyhedral
	Leaves				
Stalked glandular hair	Unicellular head 2-3(-6) celled stalk	Unicellular head 3-4(-6) celled stalk	Multicellular head 2-3- celled stalk	Unicellular head 3-4(-6) celled stalk	Unicellular head 2-3- celled stalk
Mesophyll type	Equifacial	Dorsiventral	Equifacial	Dorsiventral	Equifacial
Druse crystals	Abscent	Abscent	Abscent	Present	Abscent

the epidermis, followed by collenchyma and parenchyma in *H. niger* and *H. reticulatus*. Pericycle is 1-3-layered and sclerenchymatic in *H. pusillus*, *H. aureus*. It is parenchymatic in *H. niger*, *H. reticulatus* and *H. albus* (Table 2). Stomata are present on both leaf surfaces (amphistomatic leaves), which are anisocytic (usually) and anomocytic types in studied species (Fahn 1990;

Yentür 2003). According to Metcalfe and Chalk (1950), amphistomatic leaves are common in Solanaceae, although Cosa de Gastiazoro (1994) described hypostomatic leaves in some species of the family. The investigated species can be divided as mesophyll type: bifacial (*H. niger*, *H. albus*) and equifacial (*H. aureus*, *H. pusillus*, *H. reticulatus*). Bifacial mesophyll is the most

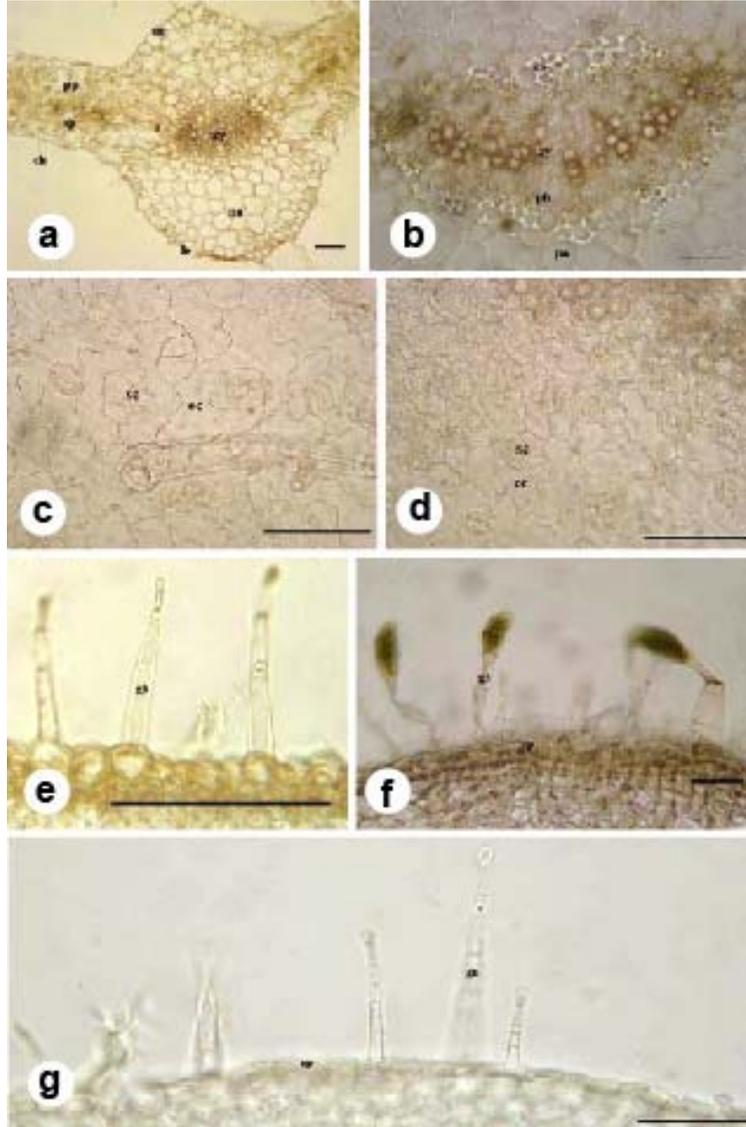


Fig. 3. General anatomical structures of *Hyoscyamus* species. a-b. Middle vascular tissue observed on *Hyoscyamus* leaves ( a. Leaf middle region (*H. albus*), b. Leaf middle vascular bundle (*H. niger*); c-d Stoma types observed *Hyoscyamus* species (c. Anisocytic stomata (*H. aureus*); d. Anomocytic stomata; (*H. reticulatus*); e-g. Trichomes observed in *Hyoscyamus* species (e. *H. albus*, f. *H. reticulatus*, g. *H. pusillus*). (Scale bar: 100  $\mu$ m).

frequent in Solanaceae, but equifacial mesophyll for some species of the family is also reported (Cosa de Gastiazoro 1994). Shape, structure and distribution of druse crystals are important from the taxonomic point of view (Metcalf and Chalk 1950, Fahn 1990). These are recorded only in mesophyll of *H. albus* in our studies. Druse crystals, as described for some species of Solanaceae (Ogundipe 1992), were observed in the mesophyll of *Calibrachoa sellowiana* and *C. caesia*. Baytop (1971) has found high number of druse crystals in the leaf mesophyll during the anatomical study of *H. leptocalyx*, and the similar high density druse crystals in the leaf mesophyll. The reports of Ghassemi (1995) on the leaf anatomy of *H. reticulatus* are similar in general, except the mesophyll layer, while he has observed a dorsiventral layer, we have seen an equifacial layer (Fig. 2-c). In a phylo-genetical study of Iranian species of *Hyoscyamus*, Sheidai *et al.* (2000) reported that *H. niger* is closely related to *H. reticulatus*. The present investigation has shown that these two species are anatomically similar.

As a result of anatomical studies carried on *Hyoscyamus* taxa; presence or absence of druse crystals, as well as their shape, structure and distribution, the presence of trichome on stem and leaf, number of collenchyma and parenchyma layer, sclerenchymatic or parenchymatic of pericycle, mesophyll structure, and stomata types (anisocytic and anomocytic) were found to be important characters for identification of *Hyoscyamus* species.

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