

Studies on the foliar epidermis of *Verbascum chinense* (L.) Santapau (Scrophulariaceae).

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ABSTRACT

In the present investigation foliar epidermal features of *Verbascum chinense* (L.) Santapau have been studied in detail with emphasis on epidermal cell complex, costal cells, stomatal complex and trichome complex. The epidermal cells are predominantly irregular and anisodiametric. Their anticlinal walls are undulate to slightly sinuous in the adaxial surface and highly sinuous in the abaxial surface. The costal cells are elongated to polygonal. Leaves are amphistomatic with anomocytic and anisocytic stomata as basic type in both the surfaces. Multicellular, conical, non-glandular trichomes and simple, multicellular and uniseriate glandular trichomes occur in both the surfaces. Some of the interesting features include presence of cuticular ledges around stomatal pores, polar nodules, peristomatal rim, contiguous stomata, stomatal rings and stomatal chains.

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Introduction:

The family Scrophulariaceae comprises of 220 genera and 3000 species (Richardson, 1993). The genus *Verbascum* belongs to the tribe Verbasceae (Willis, 1966). It has a worldwide distribution with 360 species (Judd et al., 2002) and is the largest genus within the large family Scrophulariaceae. The high morphological diversity among species of the genus *Verbascum* causes problems in the delimitation of the species and hence there is a difficulty in offering a natural and well organized classification for the species and genus (Al-Hadeethy et al., 2014). Lersten and Curtis (2001) emphasized the importance of anatomical studies, whereas Al-Hadeethy et al., (2014) expressed that micromorphological characters especially those related to hairs and stomata can be considered as useful taxonomic features in the species of *Verbascum*. Earlier reports on the foliar epidermis and leaf anatomy of this genus include those of Metcalfe and Chalk, 1950; Lersten and Curtis, 1997; Kheiri et al., 2009; Al-Hadeethy et al., 2014; Ranjbar and Nouri, 2015; Balzhan et al., 2016. In this investigation characteristics of epidermal cell complex, costal cells, stomatal complex and trichome complex of the foliar epidermis of *Verbascum chinense* (L.) Santapau have been studied in detail and are presented here.

Material and Methods:

Leaf material of *Verbascum chinense* was collected from the Herbarium (Voucher specimen no.1563), Department of Botany, Visakhapatnam. Epidermal scrapes were prepared by following Theunissen's technique (1989). The material was pretreated in Glycerin (97%) and Ethanol (1:1) for 24 hours. A portion of the leaf to be examined was placed on the slide with the epidermis to be investigated facing downwards. The specimen was covered with a thin layer of Glycerin that acts as a lubricant. The unwanted epidermis was gradually scrapped away using a razor blade to expose the mesophyll. A piece of the specimen measuring 5-10 mm was placed in 60% solution of Nitric acid in a 35 ml test tube and warmed over a flame, agitating the test tube gently for 2 to 3 minutes. The mesophyll turned yellow, disintegrated and thus liberating the remaining epidermal layer. The acid was decanted and the isolated epidermis was rinsed 4-5 times with distilled water. The peels were stained in 1% aqueous solution of Safranin and mounted in Glycerin.

The Stomatal Index (S.I) was calculated by following Salisbury's method (1927) using the formula.

$$S.I = \frac{\text{No. of Stomata}}{\text{No. of Stomata} + \text{Epidermal cells}} \times 100$$

Epidermal Cell Frequency, Stomatal Frequency and Stomatal Index have been calculated out of 10 readings. The photographs were taken by using RM-600 (Radical) monocular microscope fitted with MD 35 digital eyepiece, 640×480 pixel USB to Dell laptop.

Terms used for describing epidermal cells, stomata and trichomes are of Metcalfe and Chalk (1950, 1979), Inamdar et al., (1986), Raja Shanmukha Rao (1987), Prabhakar and Leelavathi (1989) and Prabhakar (2004).

Results and Discussion:

Cuticle: A thin cuticle is present over the epidermal cells.

Epidermal Cell Complex:

Epidermal cells: The epidermal cells are variable in their size and are variously oriented (Figs. 1, 3). Mature epidermal cells are predominantly irregular and anisodiametric in both the surfaces. The anticlinal walls are undulate to slightly sinuous in the adaxial surface (Fig. 2). These are highly sinuous with 'U' and 'V' shaped sinuses in the abaxial surface (Fig. 4). The surface of the epidermal cells is smooth without any ornamentation.

Al-Hadeethy et al., (2014) on the basis of their observations of the leaf epidermis of both the surfaces of 20 species of *Verbascum*, reported earlier 2 types of epidermal cells (described as ordinary cells surrounding the stomata) – 1. Straight walls, ribbed with rectangular to oval shape and 2. Undulate or irregular walls or wavy edge with rectangular to polygonal shape. Poonam Sethi and Priya (2016) recorded adaxial epidermal cells with polygonal outline and larger abaxial epidermal cells with wavy anticlinal walls and amoeboid outline in *Leucophyllum frutescens*.

In the adaxial surface, the epidermal cell frequency ranges from 334 to 416 per sq. mm. and in the abaxial surface it is from 437 to 500 per sq. mm. The average epidermal cell frequency is 364 per sq. mm. in the adaxial surface; 466 per sq. mm. in the abaxial surface. The size of the epidermal cells ranges from 35×25 µm to 75×50 µm in the adaxial surface and 35×25 µm to 75×40 µm in the abaxial surface. The average size of the epidermal cells is comparatively larger in the adaxial surface than in the abaxial surface. It is 54×38.5 µm in the adaxial surface and 58×31 µm in the abaxial surface.

Costal cells: The costal cells are distinct. These cells are parallelly arranged and elongated to polygonal in shape. The side walls are mostly straight to slightly wavy in both the surfaces (Figs. 5 - 8).

No information is available in the previous reports, regarding the costal cell characteristics of the species of this tribe.

Stomatal Complex:

The leaves are amphistomatic. The stomata are variously oriented, usually scattered and are distributed all over the lamina (Figs. 1, 3). The basic stomatal type is anomocytic (Figs. 9, 10) and anisocytic (Figs. 2, 10) in both the surfaces. Stomata are elliptic, circular or nearly circular and oval in this species thus making the stomata heteromorphic in shape. The stomata are heteromorphic in size (giant, large, small) also (Fig. 11).

Cuticular ledges around the stomatal pores (Fig. 4) and polar nodules (Fig. 16) are observed in both the surfaces. Peristomatal rim is noticed around the stomata in both the surfaces (Fig. 4). The guard cells are mostly equal in both the surfaces but rarely unequal in the abaxial surface (Fig. 10). Presence of stomata is observed in the surrounding cells of the trichome base (Fig. 21). Two types of contiguous stomata are observed in this species. Juxtaposed contiguous stomata are present in both the surfaces (Fig. 12), whereas obliquely posed contiguous stomata are noticed only in the abaxial surface (Fig. 13). In addition, presence of stomatal ring (Fig. 15) in the adaxial surface, stomatal chain (Fig. 16) in the abaxial surface; stomatal group (Figs. 14, 11) in both the surfaces are observed.

The frequency of stomata ranges from 78 to 136 per sq. mm. in the adaxial surface and 172 to 208 per sq. mm. in the abaxial surface. The average frequency of stomata is 107 sq. mm. in the adaxial surface and 191 per sq. mm. in the abaxial surface. The Stomatal Index is 22.72 in the adaxial surface and 29.07 in the abaxial surface. The size of the stomata ranges from 25×15 µm to 40×30 µm in the adaxial surface, 20×20 µm to 35×30 µm in the abaxial surface, with an average size of 32.5×23.5 µm and 29.5×24 µm in the adaxial and abaxial surfaces respectively.

An uniformly amphistomatic condition with anomocytic stomata in both adaxial and abaxial surfaces was reported earlier in all the 48 specimens of *Verbascum* studied by Lersten and Curtis (1997) and in all the 30 species of *Verbascum* studied by Al-Hadeethy et al., (2014). Although similar condition was reported in *V. albidiflorum* by Ranjbar and Nouri (2015), they described 3 types of subsidiary cells - 3, 4 and 5 equal or unequal in size and monocyclic with ridged walls. Balzhan et al., (2016) reported hypostomatal condition with diacytic stomata in *V. songaricum*. Poonam Sethi and Priya (2016) reported anisocytic stomata with their occurrence only on the abaxial leaf surface in *Leucophyllum frutescens*. Thus a slight variation exists in the surface distribution and type of stomata among the species of this tribe.

Al-Hadeethy et al. (2014) measured the quantitative particulars also like length, width and stomatal index both adaxially and abaxially for all the species of *Verbascum* studied by them. Poonam Sethi and Priya (2016) reported the size of the stomata in *Leucophyllum frutescens*.

Trichome Complex:

Leaves are amphitrichous. Both non-glandular and glandular trichomes are present. Slight variation is observed in their morphology. The trichomes are distributed on the margins, veins and surface of the lamina in both the surfaces (Figs. 1, 3, 5, 7).

1. Non-glandular trichomes:

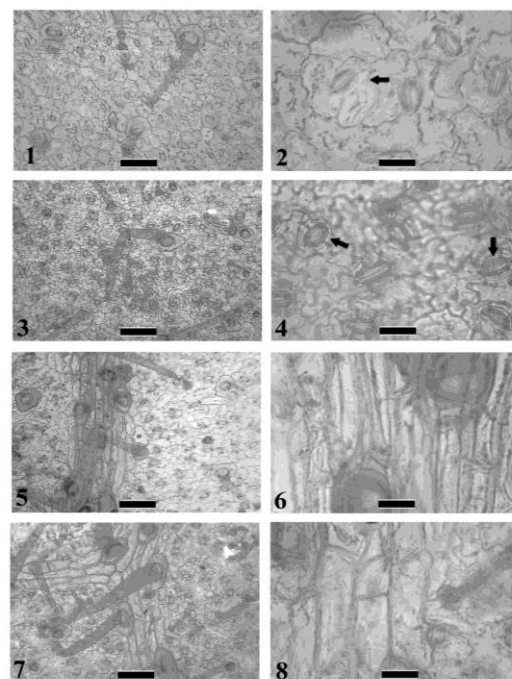
Multicellular, conical trichomes: Multicellular, uniseriate, conical trichomes are found. The number of cells in the trichome slightly varies from surface to surface. The trichomes are 4 to 5 celled in the abaxial surface (Fig. 19) and only 5-celled in the adaxial surface (Fig. 17). The cuticle is thick and exhibits ornamentation over the surface of the trichomes (Figs. 18, 20).

2. Glandular trichomes: These are observed in both the surfaces of the leaf. The glandular trichomes are simple, multicellular and uniseriate (Figs. 12, 16). The foot of the trichome is embedded in the epidermis. The body is distinguishable into a stalk and head. The stalk is 2 to 3 celled in both the surfaces (Figs. 21, 22). It is usually constricted at the cross walls and the cells are longer than broad. The head is uniformly 2-celled and capitate (Fig. 23, surface view, 24).

Occurrence of non-glandular and glandular trichomes was also reported in the members of this tribe studied by earlier workers. However some variation existed in their sub-types. Presence of branched, multicellular trichomes in the species of *Leucophyllum* and *Verbascum* and glandular hairs with unicellular or uniseriate stalks of varying length and heads usually composed of 1-4 cells in the species of *Celsia* and *Verbascum* were reported earlier (see, Metcalfe and Chalk, 1950). Lersten and Curtis (1997) reported a mixture of dendroid trichomes and tiny sessile capitate forms in the different species of *Verbascum* studied by them. They recorded only dendroid trichomes in 5 species; long, uniseriate trichomes with small cap cells and sessile capitate trichomes in 2 species; long uniseriate trichomes with pointed ends and no cap cells and tiny, sessile capitate trichomes in 3 species and only uniseriate pointed trichomes in 1 species. Al-Hadeethy et al., (2014) recorded the presence of both glandular and unglandular trichomes in both the surfaces of all the species of *Verbascum* studied by them except *V. agrimonifolium*, in which the trichomes were observed only on the lower surface. They mentioned that among the unglandular trichomes two types occurred - 1. The dendroid type, which are present in all the species and 2. The asteroid or stellar trichomes present in 8 species viz., *V. alceoides*, *V. alepense*, *V. calvum*, *V. laetum*, *V. oreophilum*, *V. sinaiticum*, *V. songaricum* and *V. thapsus*. They described glandular trichomes having a multicellular head (Peltate) of four cells with a multicellular long (*V. carduchorum*, *V. damascenum*) or short (*V. alepense*, *V. cheiranthifolium*) neck. They also recorded the quantitative measurements for glandular (length of neck

and frequency), unglandular, dendroid and stellar (frequency) trichomes. 3 types of hairs were described in *V. albidiflorum* by Ranjbar and Nouri (2015). These are - 1. Eglandular, stellate hairs with unicellular or multicellular, single stalk; 2. Eglandular, many branched dendroid hairs and 3. Glandular hairs, simple or rarely branched with a short neck and a broad 4-celled head. They also measured the length of the neck of the multicellular glandular hairs. Balzhan et al., (2016) reported the following types of non-glandular and glandular hairs in *V. songaricum* - 1. Multicellular stellate and capitate hairs on the surface of leaves and 2. Little branching hairs and multicellular stellate hairs in the edges of leaves.

Presence of single - celled subepidermal foliar idioblasts in some of the species of *Verbascum* (Lersten and Curtis, 1997); in the mesophyll of leaves of *V. oreophilum*, *V. szovitsianum*, *V. agrimonifolium* and *V. macrocarpum* (Kheiri et al., 2009) and epithelium - lined oil cavities in *Leucophyllum* (Lersten and Curtis, 2001) was reported earlier. However, such structures are not found in *V. chinense* worked out now.



Figures 1 - 8: *Verbascum chinense*.

1, 2, 5, 6: Adaxial (Upper) epidermis,
3, 4, 7, 8: Abaxial (Lower) epidermis.

- 1: Epidermal cells, stomata and trichomes,
- 2: Epidermal cells with irregular outline, slightly sinuous anticlinal walls and anisocytic stomata,
- 3: Epidermal cells, stomata and trichomes,

4: Epidermal cells with irregular outline, sinuous anticlinal walls with 'U' and 'V' shaped sinuses and stomata with cuticular ledges and peristomatal rim,

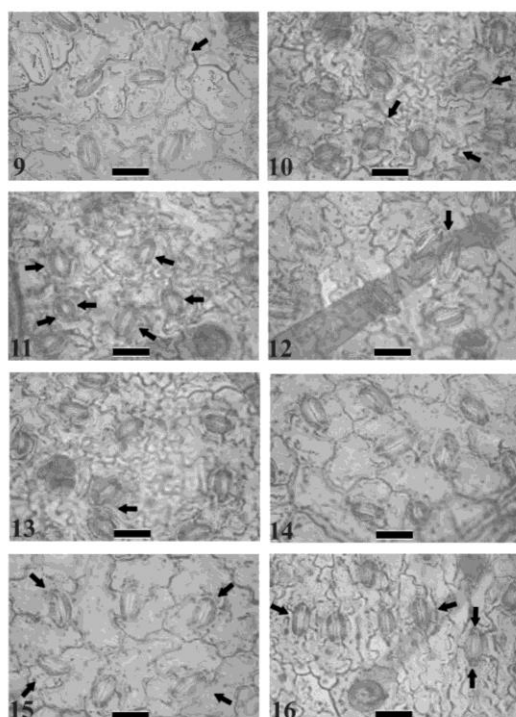
5: Epidermal cells, costal cells, stomata and trichomes,

6: Elongated, polygonal costal cells with parallel arrangement and straight to slightly curved side walls,

7: Epidermal cells, costal cells, stomata and trichomes,

8: Elongated, polygonal costal cells with parallel arrangement and straight to slightly curved side walls.

Scale bar: 1, 3, 5, 7:  = 60µm, 2, 4, 6, 8:  = 15µm.



Figures 9 - 16: *Verbascum chinense*.

9, 12, 14, 15: Adaxial (Upper) epidermis,

10, 11, 13, 16: Abaxial (Lower) epidermis.

9: Anomocytic stomata,

10: Enlarged portion showing anisocytic, anomocytic stomata and stomata with unequal guard cells,

11: Stomatal group with heteromorphic stomata in shape (elliptic, nearly circular, circular, oval) and size (giant, large, small),

12: Contiguous stomata (juxtaposed pairing) and glandular trichome,

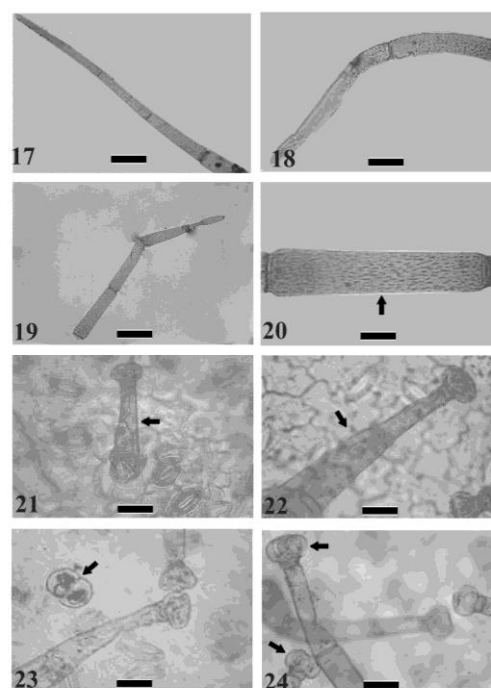
13: Contiguous stomata (obliquely posed pairing),

14: Stomatal group,

15: Stomatal ring,

16: Stomatal chain (transverse), polar nodules and glandular trichome.

Scale bar: 9 - 16:  = 15µm.



Figures 17 - 24: *Verbascum chinense*.

17, 18, 22 - 24: Adaxial (Upper) epidermis,

19 - 21: Abaxial (Lower) epidermis.

17: Multicellular (5-celled), non-glandular trichome,

18: Non-glandular trichome with cuticular ornamentation,

19: Multicellular (4-celled), non-glandular trichome,

20: Part of the non-glandular trichome with cuticular ornamentation,

21: Glandular trichome with 2-celled, uniseriate stalk (1 long cell and 1 short cell), capitate head and stomata near trichome base,

22: Glandular trichome with 3-celled, uniseriate stalk (2 long cells and 1 short cell) and capitate head,

23: 2-celled head of glandular trichome (surface view) and glandular trichomes,

24: Glandular trichomes with 2-celled capitate head.

Scale bar: 17 - 24:  = 15µm.

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