



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A Comparative Study Of Epidermal Characters Of Two Species Of Genus *Adhatoda*

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Abstract: Two species of genus *Adhatoda* (*A. beddomei* L. and *A. vasica* L.) was selected for epidermal studies of leaves. 14 epidermal characteristics are studied in two species of *Adhatoda* (*A. beddomei* L. and *A. vasica* L.). These characters belong broadly to prominent structures. 1. Epidermis 2. Stomata 3. Trichomes. *Adhatoda vasica* L. differs distinctly from *Adhatoda beddomei* L. in numbers of epidermal cells. Out of 8 stomatal characteristics studied, 2 character namely average size of stomata and stomatal index show least variation in both species. Type of stomata is quite similar in both *i.e.* diacytic. However, some variations occur in average number of stomata/unit area in both species of *Adhatoda* respectively. Similarly they differ invariably in distance between stomata, guard cell index, pore area index and total pore area characteristic.

Index Terms- *Adhatoda*, Stomata, Epidermis, Diacytic, Stomatal index.

I. Introduction

Acanthaceae is divided in two subfamilies depending upon the presence or absence of jaculators, *i.e.* the curved retinacula which support the seeds. *Adhatoda* is the most important member of Acanthaceae family. Two species of genus *Adhatoda* (*A. beddomei* L. and *A. vasica* L.) was selected for epidermal studies of leaves. *Adhatoda beddomei* L. is endemic to India occurring in the Travancore hills of south western Ghat, Valparai (South Arcot), Akkamalai (Coimbatore) and Mahendragiri (Kanniyakumari dist.), Southern parts of India, Kerala. *Adhatoda vasica* L. is native to Asia. The plant is found abundantly in wild in all over India. The plant is found in throughout India, the sub-Himalayan tracts eastwards, Dehra Dun, Gujarat, Rajasthan (Awan *et al.*,2014).



Figure 1.: *Adhatoda beddomei* L. flower on twig



Figure 1.: *Adhatoda vasica* L. flowers on twig

1.1 Botanical description of *A. beddomei* L.

Habit – Perennial shrub

Stem – Stem is herbaceous, aerial, cylindrical. Stem are light green in colour. Average plant height is 147 cm . Average plant spread is 208 cm .The average length of smallest branch is 22.81 cm . Average number of node /branch is 25.21.

Leaf – Simple light green leaves are ovate, leaf margin serrate, phyllotaxy of leaf is opposite. Leaf are cauline and ramal and exstipulate. Leaves are glabrous. Leaves are petiolate. The average size of leaf is 9.68×5.26 cm and the average size of petiole 0.0136 cm.

Inflorescence- Racemose spike.

Flower – Flowers in short dense axillary pedunculate spike, towards the end of the branches, bracteate, bracteolate, bract and bracteoles are leafy. Flowers are sessils, complete, zygomorphic, bracts are three elliptic, subacute, glabrous, 5-7 nerved, closely reticulate veined. Bracteoles are oblong – lanceolate, acute with ciliolate margins. Bracteoles are one nerved, reticulate veined. The average size of bract is 1.204 × 0.35cm and light green in colour.

Calyx – Sepals are five, polysepalous, slightly connate at the base. Sepal aestivation is quincuncial and green in colour. Sepals are glabrous or slightly pubescent. Segments are oblong lanceolate, acute, three–nerved, and reticulately veined. The average size of sepal is 0.38 × 0.21 c.

Corolla – Petals are five gamopetalous. Petal aestivation is valvate. Corolla is a white with a few irregular rose – coloured bars in the throat which is called coronary outgrowth. Corolla is 2/3 bilabiate in which the upper half laterally inflated long. Ovate – oblong, curved, obtuse, notched. The lower lip as long as the upper, the lobes 1/2 inch deep, oblong, rounded. Lower lip has a groove in the centre in which style lies in bud condition. The middle lobe has the maximum width. The average size of petal is 1.084 × 0.428 cm. The average size of corolla tube also present in this species. However, it shorter than those found in flowers of *A. vasica*.

Androecium– Stamens are two in a number. Stamens are polyandrous, epipetalous, ditheous, the anther lobes are situated at unequal heights and the lower one bears an appendage basifixed, introse. The average size of anther is 0.14×0.07 cm and white with deep red colour. Average size of anther filament is 0.82 cm.

Gynoecium- Ovary is a pubescent, bicarpellary, syncarpous, ovary superior. Ovary is a bilocular with two ovules in each locules. The placentation is axile. Style is long and curved. White style and light green stigma are present. The average size of style is 0.88 cm. Stigma is capitate.

Fruit- Fruit is capsule. Capsule is clavate, subacute, shortly and bluntly pointed. Capsule has a pubescent and solid stalk. The average size is 2.75×1.18 cm. They are hard in a texture. Fruits are light green at a young stage and after maturity it is brown in colour.

Seed – Three seeds are present in a fruit. Seeds are orbicular – oblong. The average size of seed is 0.62 × 0.58 cm. Seed are rough in a texture and brown in colour.

1.2 Botanical Discription of *Adhatoda vasica* L.

It is a perennial shrub. Stem is herbaceous, aerial, cylindrical with many long opposite ascending branches and solid. Lower portions of stem are woody, nodes swollen and flat, rough and pale yellow. The average plant height is almost 145 cm . Average plant spread is calculated as 475 cm. The average length of smallest branch is 27.61. Average number of node/ branch is 18.22. Some leaf characters are similar to previous species.

Leaves are elliptic lanceolate opposite decussate and dark green in colour. The average size of leaf is 16.73 × 6.46 cm. The average size of petiole is 1.12 cm.

A. vasica differs distinctly from *A. beddomei* in many floral and fruit characteristics. Overall floral parts in this species are quite larger than those found in *A. beddomei*. The anthers are light green in this species as compare to white and deep red anther of *A. beddomei*. Qualitatively both species resemble much with reference to flower and fruit characters. The present research work focused on epidermal variability in both plants.

II. MATERIAL AND METHODOLOGY

2.1 Plant Material

A. beddomei and *A. vasica* are the wild plant species, which are commonly grown in waste land and gardens of Jaipur city. Fresh leaves were collected from certain garden and Arboretum of the Jaipur city.

2.2 Methodology

2.2.1 Epidermal Studies

Leaves of both species of genus *Adhatoda* are collected for epidermal studies. Samples of materials for slide preparation are taken from same region of each fresh leaf, generally from midway between the leaf base and apex of lamina including the midrib. The epidermal peels are obtained using a sharp pointed forcep. A thin layer of nail polish was also spread over the leaf surface. The strips are thoroughly washed with distilled water, stained with saffranin and then mounted with a drop pure glycerin jelly on a glass slide. A cover slip was placed over the drop and sealed with nail polish. The size of epidermal cell and stomata size of pore, distance between stomata determined using ocular micrometer and stage. The data of above said aspects were carried out at X450 magnification in the compound microscope. The measurable data are presented as arithmetic mean (\bar{X}) standard error (S.E.)

2.2.2 Stomata Index: The stomatal index was calculated by the following formula.

$$I = \frac{S}{E + S} \times 100$$

Where ,

I = Stomatal index, S = Number of stomata per unit area, E = Number of epidermal cell per unit area

2.2.3 Guard Cell Index (GCI) : GCI calculated by following formula

$$GCI = \frac{\text{No. of guard cell / unit area}}{\text{No. of guard cell + subsidiary cell + no. of epidermal cell / unit area}} \times 100$$

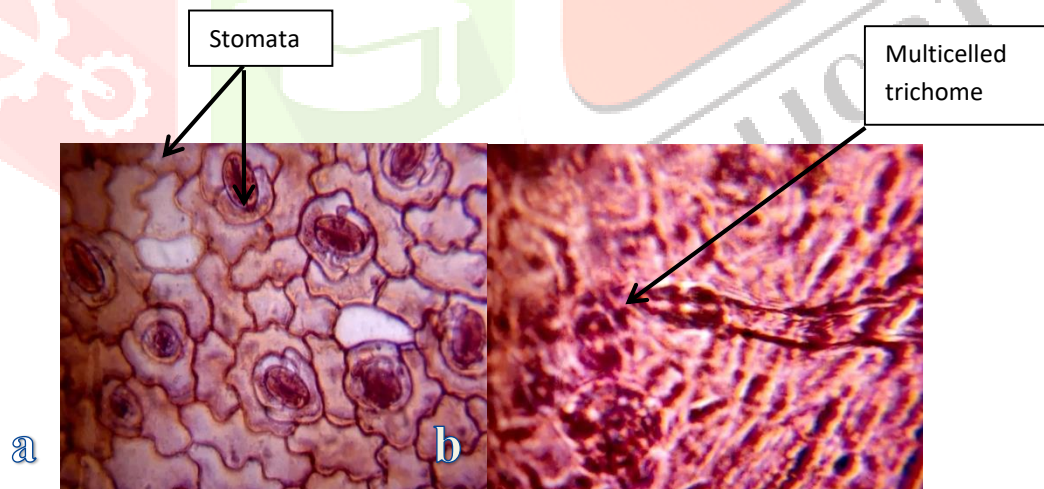
2.2.4 Pore Area Index (PAI): PAI calculated by following formula

$$PAI = \frac{\text{Mean pore area}}{\text{mean l} \times \text{b of stomata}} \times 100$$

2.2.5 Total Pore Area (TPA): TPA calculated by following formula

$$TPA = \frac{\text{Mean pore area} \times \text{mean no. of stomata / unit area}}{10^6} \times 100$$

III. RESULT AND DISCUSSION



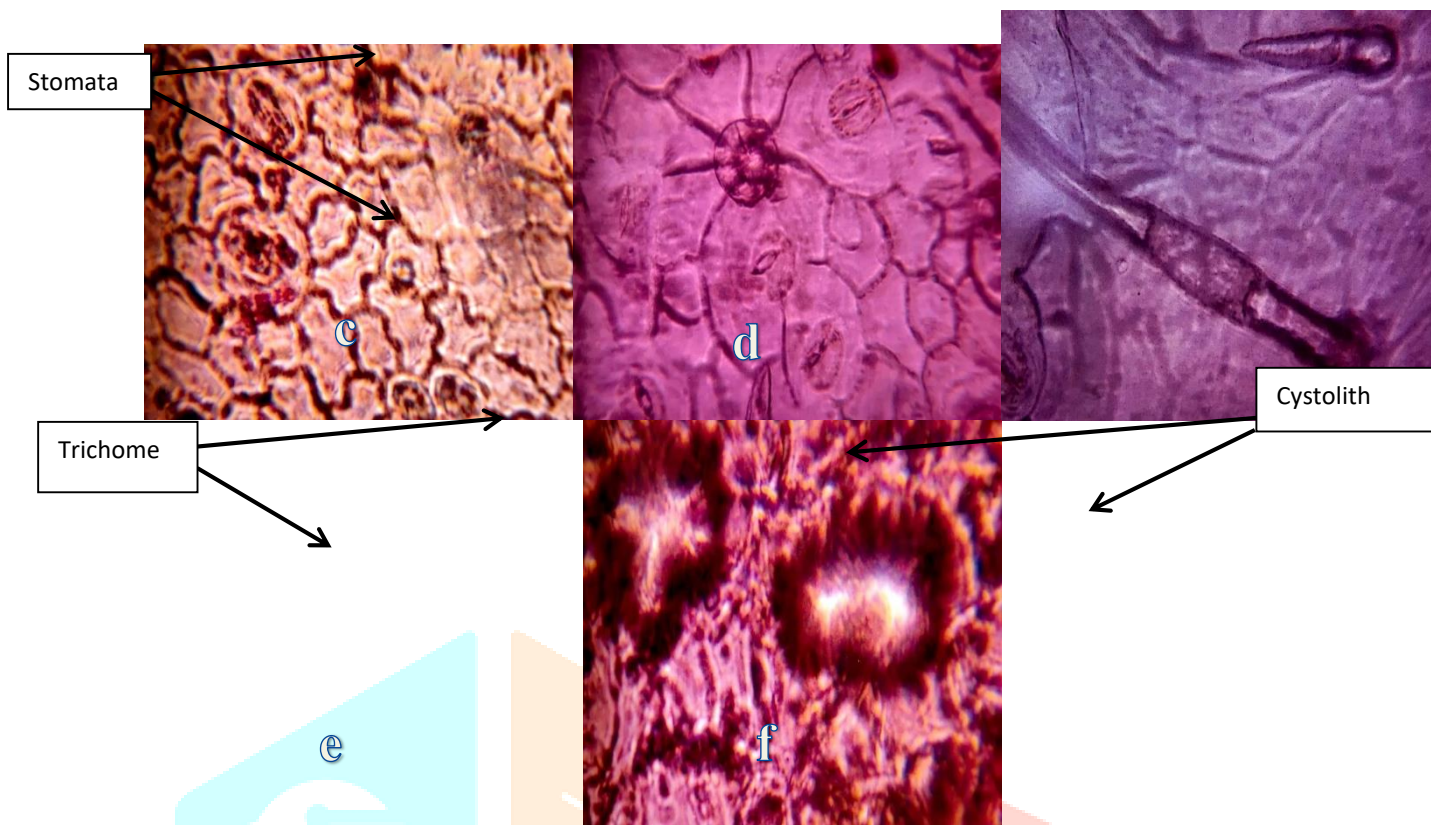


Figure 3:(a)Lower epidermis of *A. beddomei* (X200) (b) Multicelled long trichome of *A. beddomei* (X400) (c) Upper epidermis of *A. vasica* (X200) (d) Part of lower epidermis of *A. vasica* (X200)(e) Trichomes of *A. vasica* (X400) (f) Lower epidermis showing elongated cystolith (X400)

Many parameters related to epidermal studies of *Adhatoda* species are described epidermal characters are studied as number of epidermal cells (per unit area), size of epidermal cell, type of epidermal cell, number of stomata, size of stomata, type of stomata, stomatal index, distance between stomata, number of guard cell (per unit area), size of pore (L × B), number of subsidiary cell, size of trichomes, type of cystoliths. The details of above said characteristics are given in Table 1 and Figs. 3(a)-3(f).

These epidermal characters have been described plant wise.

Table 1 : Comparative data of epidermal characters of *Adhatoda* spp.

S. No	Plant species Character	<i>Adhatoda beddomei</i> L.		<i>Adhatoda vasica</i> L.	
		Upper	Lower	Upper	Lower
1.	Average number of epidermal cell	Absent	104±17.862	95.3±6.013	92.5±10.625
2.	Av. size of epidermal cell (µm)	Absent	34.301×26.601 ±6.640×2.480	28.31±36.63 ±1.019×3.231	30.1×42.7 ±3.278×5.761
3.	Type of epidermal cell	Absent	Sinuuous	Straight	Straight
4.	Av. number of stomata	Absent	27±0.531	32±1.639	39±0.735

5.	Av. size of stomata (μm)	Absent	$32.91 \times 21.00 \pm 3.130 \times 2.097$	$28.43 \times 20.31 \pm 1.013 \times 0.396$	$30.52 \times 22.01 \pm 12.01 \times 2.228$
6.	Type of stomata	Absent	Diacytic	Diacytic	Diacytic
7.	Stomatal index	Absent	20.610 ± 1.671	25.137 ± 1.612	29.657 ± 3.013
8.	Distance between stomata (μm)	Absent	46.090 ± 8.932	59.544 ± 4.313	54.610 ± 6.031
9.	Guard cell index (GCI) (μm)	Absent	24.602 ± 5.463	31.637 ± 2.613	34.625 ± 4.312
10.	Pore area index (PAI)	Absent	20.561 ± 6.432	10.932 ± 2.679	13.397 ± 1.131
11.	Total pore area (TPA)	Absent	0.358 ± 0.009	0.289 ± 0.013	0.187 ± 0.002
12.	Av. number of trichome	Absent	12.01 ± 0.395	8.32 ± 1.913	17.41 ± 0.868
13.	Av. size of trichome (μm)	Absent	$102 \times 12.41 \pm 24.981 \times 1.318$	$122 \times 16.10 \pm 22.101 \times 2.101$	$119 \times 15.40 \pm 24.861 \times 1.578$
14.	Av. size of cystolith (μm)	$45.65 \times 19.76 \pm 15.737 \times 1.653$	$48.35 \times 21.32 \pm 16.507 \times 1.769$	$53.76 \times 20.10 \pm 17.019 \times 1.009$	$55.41 \times 21.55 \pm 9.322 \times 1.093$

3.1 *Adhatoda beddomei* L.

The average number of epidermal cells / unit area are observed 104 ± 17.862 Fig.3(a) . The average size of epidermal cells are $34.301 \times 26.610 \mu\text{m} \pm 6.640 \times 2.480$ (Table 1). The average number of stomata are 27 ± 0.531 . The average size of stomata is $32.91 \times 21.00 \mu\text{m} \pm 3.130 \times 2.097$. Stomatal index is calculated as 20.610 ± 1.671 (Table 1). Distance between stomata and guard cell index are $46.090 \mu\text{m} \pm 8.932$ and $24.602 \text{ cm} \pm 5.463$. Pore area index (PAI) and total pore are (TPA) are 20.561 ± 6.432 and 0.358 ± 0.009 respectively. The average number of trichome / unit area is 12.01 ± 0.395 . The average size of trichome is $102 \times 12.41 \mu\text{m} \pm 24.981 \times 1.318$. In this species solitary semi round cystolith are present. Their average size is $48.35 \times 21.32 \mu\text{m} \pm 16.507 \times 1.769$ for lower epidermis. Similarly on upper epidermis the average size of cystolith is quite similar (Table1).

3.2 *Adhatoda vasica* L.

The average number of epidermal cell / unit area is lesser in comparison to those in *Adhatoda beddomei* L. Fig.3(c). However, in this species amphiblastic type of stomata occur. The number of upper epidermal cells and lower epidermis cells are calculated as 95.3 ± 6.031 and 92.5 ± 10.625 . The average size of upper and lower epidermis are calculated as $28.31 \mu\text{m} \times 36.63 \mu\text{m} \pm 1.019 \times 3.231$ and $30.1 \times 42.7 \mu\text{m} \pm 3.278 \times 5.761$. Straight type of epidermal cells are observed in *A. vasica* L. Fig.3 (d). The average number of stomata / unit area of upper epidermis and lower epidermis is 32 ± 1.639 and 39 ± 0.735 respectively (Table 1).

Average size of stomata of upper epidermis and lower epidermis is $28.43 \times 20.31 \mu\text{m} \pm 1.013 \times 0.396$ and $30.52 \times 20.01 \mu\text{m} \pm 12.012 \times 2.228$. The stomatal index of upper and lower epidermis is quite similar (Table 1).

Distance between stomata of upper and lower epidermis is $59.554 \mu\text{m} \pm 4.313$ and $54.610 \mu\text{m} \pm 6.031$. Guard cell index of upper and lower epidermis has not much variation (Table:1). Pore area index of upper and lower epidermis is 10.932 ± 2.679 and 13.397 ± 1.131 respectively.

Total pore area of upper and lower epidermis is 0.289 ± 0.013 and 0.187 ± 0.002 . Average number of trichome per unit area on both surface have variation i.e. on upper epidermis 8.32 ± 1.913 and on lower epidermis 17.41 ± 0.868 . Average size of trichomes of upper and lower epidermis is $122 \times 16.10 \mu\text{m} \pm 22.101 \times 2.101$ and $119 \times 15.40 \mu\text{m} \pm 24.861 \times 1.578$ non-glandular and multicelled trichome are observed Fig. 3(e). Most important character is many elongated single cystoliths with blunt extremities are observed Fig. 3(f). The average size of cystolith $55.41 \times 21.55 \mu\text{m} \pm 19.322 \times 1.093$ respectively for lower epidermis but there is no significant variation on upper epidermis observed Fig. 3(d).

IV. DISCUSSION

Some variation were observed in many epidermal characters in both species of *Adhatoda*, i.e., average number of epidermal cell per unit area, type of epidermal cell, stomatal index, distance between stomata, size of trichome type of trichome and type of cystolith. Verma and Murty (1979), Grewal (2000) and Cutler *et al.* (2007) have reported the Diacytic type of stomata in *Adhatoda* species. We have also observed diacytic (caryophyllaceous) type of stomata which are surrounding by two subsidiary cells, the common wall of which is at right angle to the longitudinal axis of the stomata. We have found more distance between stomata in comparison to less distance between stomata as reported by (Prakash and Kumar, 1995). *Adhatoda vasica* L. are amphiblastic in nature where is the general epidermal characters on upper and lower sides of the leaf in *A. vasica* are concerned, there are usually have not much variation in these characters except that stomata less in number of upper surface as compared to lower surface. Akhtar and Syed (2006) reported amphiblastic stomata of *Adhatoda* species. In both above said plant species the leaf is amphiblastic. Trichomes are long with tapering end and uni, multicelled. They occur singly are condensed in *Adhatoda vasica*. In *Adhatoda vasica* L. the average number of trichomes/unit area is the highest among all four species studied by me. However, these species differ from each other whereas the average size of trichome is concerned. Akhtar and Syed (2006) have reported the different type of trichome in above said species. We have found non glandular trichome in *Adhatoda* species. Inamdar (1970), Kumar and Paliwal (1978) and Shendage and Yadav (2009) have studied trichome in *Adhatoda* spp. We have also observed unicellular, shaggy, glandular shaped trichome in *Adhatoda* species. This view of also supported by Srivastava and Choudhary (2006), Somprasong *et al.*, 2014. A prominent structures occur in all four species in cystolith observed by me. Cystolith consist of cellulose and are impregnated with calcium carbonate and they are irregular in shape. In both *Adhatoda* spp. elongated cystoliths with blunt extremities are observed. Previously, Shendge and Yadav (2009) have reported solitary elongated and round cystoliths in *Adhatoda* spp. The presence of cystoliths is also confirmed by Labhane (2011), Patil and Patil (2012). Gabel *et al.* 2021 documented distribution of cystoliths in the leaves of *Acanthaceae* and its effect on leaf surface anatomy in 28 species. Khan *et al.*, 2020 study the phylogeny of the various selected genera of *Acanthaceae* was investigated based on macromorphological characters, chloroplast DNA (cp-DNA) genes *rbcl*, *matK* and intergenic spacer *trnH-psbA* sequences.

V. CONCLUSION

Present work gives a brief detail about the epidermal characters of both plant species of *Adhatoda* genus. The given data suggested that both plant species have many similar characters. However, they have many distinguished characters. The epidermal characters are very important because it gives an elementary idea about functional position of a leaf. As well as it may also help in plant physiology, respiration mechanism and

pollution condition of a particular area. The work underscores the significance of stomatal features in delineating various taxonomic levels within plant groups. The variability in stomatal frequency can be particularly informative at the genus level, aiding in the differentiation of different genera. The presented work may be a stack holder for further studies.

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