



## Comparative foliar epidermal micromorphology of two species of *Plumbago* Linn

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### Abstract

*Plumbago* Linn. is a multipurpose medicinal plant used in Ayurveda. The paper presents epidermal micromorphology of leaf of two species of *Plumbago* namely *P. zeylanica* and *P. auriculata* by using Light and Scanning Electron Microscopy. Qualitative as well as quantitative epidermal micromorphological characteristics of both surfaces of leaf revealed by LM and SEM would help in taxonomy for identification, authentication and delimitation of species of *Plumbago*. These characters would also help in evaluation of crude drug.

**Keywords:** L.M, S.E.M, epidermis, leaf, *Plumbago zeylanica*, *Plumbago auriculata*

### Introduction

*Plumbago* which belongs to family Plumbaginaceae is used as medicinal plant in Ayurveda. It is called as 'leadwort' or 'Chitrak'. *P. zeylanica* is wild plant with good medicinal potential while *P. auriculata* is cultivated ornamental and widely available plant with least medicinal potential. Hence, there is danger of substitution and adulteration in *P. zeylanica* Linn. with *P. auriculata* Lam. particularly in sterile or fragmentary state because of close taxonomic resemblance of two species. Hence, it is necessary to distinguish these plants even at sterile fragmentary state to detect substitution. Micromorphological characters are used for identification, authentication and substitution of crude drug in addition to morphological and anatomical characters.

The relevance of epidermal morphology in taxonomy is well documented in botanical literature [1]. Taxonomically significant aspects of epidermis are epidermal cells, stomata, trichomes and glands. Taxonomically significant characters of epidermal cells are length and width of epidermal cell, its structure, orientation, undulation of anticlinal wall, curvature of periclinal wall [2], thickness of wall, sculpturing on wall as seen in surface view and inclusions of epidermal cell [3]. Features of stomata used in taxonomic considerations are number, position and structure of the subsidiary cells; level of stomata in epidermal tissues; stomatal index; structure of stomata [4].

The present investigation was undertaken to discriminate both species correctly on the basis of epidermal micromorphology of leaf which would help to identify and decide authenticity of crude drug even in fragmentary state.

### Materials and Methods

#### Light microscopy

Epidermal peels of both (upper and lower) surfaces of mature leaves were taken by direct hand peel method or simple blade scratching of leaf [4]. The epidermal peels were stained with 1% aqueous safranin [5], washed with water and mounted in glycerin [6] to make them semi-permanent. Slides were examined under light microscope at different magnifications to observe different qualitative as well as quantitative characters of epidermis.

#### Scanning electron microscopy (SEM)

Scanning Electron Microscopy of leaf epidermis (upper and lower) of *P. zeylanica* and *P. auriculata* was carried out at ICON Analytical Equipment Pvt. Ltd., Worli by using FEI Quanta 200 Environmental Scanning Electron Microscope.

#### Measurements and calculations

Measurements were taken by using LM 52-1712 Digiscope (LCD Digital Microscope) of Lawrence and Mayo. Measurements of cells were taken at the widest points. Mean values of 10 observations with standard deviation were taken for the size of guard cells, size of stomatal aperture, stomatal index, stomatal frequency but for epidermal cell size 20 observations were taken for consideration. Stomatal index was calculated by using following equation.

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

Where I = Stomatal index, S = Number of stomata per unit area, E = Number of epidermal cells in the same unit area

#### Terminology

To explain stomata type, terminology used by Wilkinson (1979) [7], Patel *et al* (1982) [8], Malvey (2004) [9], Mondal (2009) [10] is used. Terminology used for stomata shape follows Belhadj *et al* (2011) [11]. To explain other LM features of stomata and epidermal cells terminology used is in accordance with Inamdar and Patel (1970) [12], Avita and Inamdar (1980, 1981) [13, 14], Patel *et al* (1981) [15], Patel *et al* (1982) [8]. Terminology for SEM characters of epidermis follows Olowokudeja and Pereira-Sheteolu (1988) [16], Okoli (1989) [17], Olowokudeja (1990) [18], Cutler *et al* (2008) [19], Tahir and Rajput (2009) [20], Chen *et al* (2010) [21].

#### Results

Leaves of both taxa are amphistomatic with greater number of stomata on lower epidermis. All observed qualitative and quantitative features of leaf epidermis of both taxa are summarized in table 1, 2, 3, 4. Abnormal stomata observed on leaf epidermis of both taxa are tabulated in table 5. Chalk glands are found on both lower as well as on upper surface

of leaf. They are composed of 8 cells arranged in two circles-central circle of 4 secretory cells and second circle of 4 adjoining cells. Each gland is surrounded by 4 subsidiary cells. Trichomes are absent on the upper and lower surface of leaf of both species of *Plumbago*.

### *Plumbago zeylanica* Linn.

#### Lower epidermis (Fig. 1 and 5)

Lower surface of leaf predominantly showed anisocytic stomata. Occasionally following types of stomata also observed

1. Amphianisocytic
2. Tetracytic
3. Staurocytic
4. Brachyparahexacytic - monopolar
5. Paracytic-The subsidiary cells may be equal or unequal
6. Stomata on one side flanked by one parallel cell and on other side flanked by two parallel cells and one wider polar cell
7. Stomata on one side flanked by one parallel cell and then surrounded by three unequal subsidiary cell
8. Stomata on one side flanked by one parallel cell and then surrounded by 4 subsidiary cells as in staurocytic stomata.

#### Upper epidermis (Fig. 2 and 6)

Upper surface of leaf predominantly showed anisocytic stomata. Occasionally following types of stomata also observed-

1. Diacytic
2. Staurocytic
3. Tetracytic
4. Amphianisocytic

### *Plumbago auriculata* Lam.

#### Lower epidermis (Fig. 3 and 7)

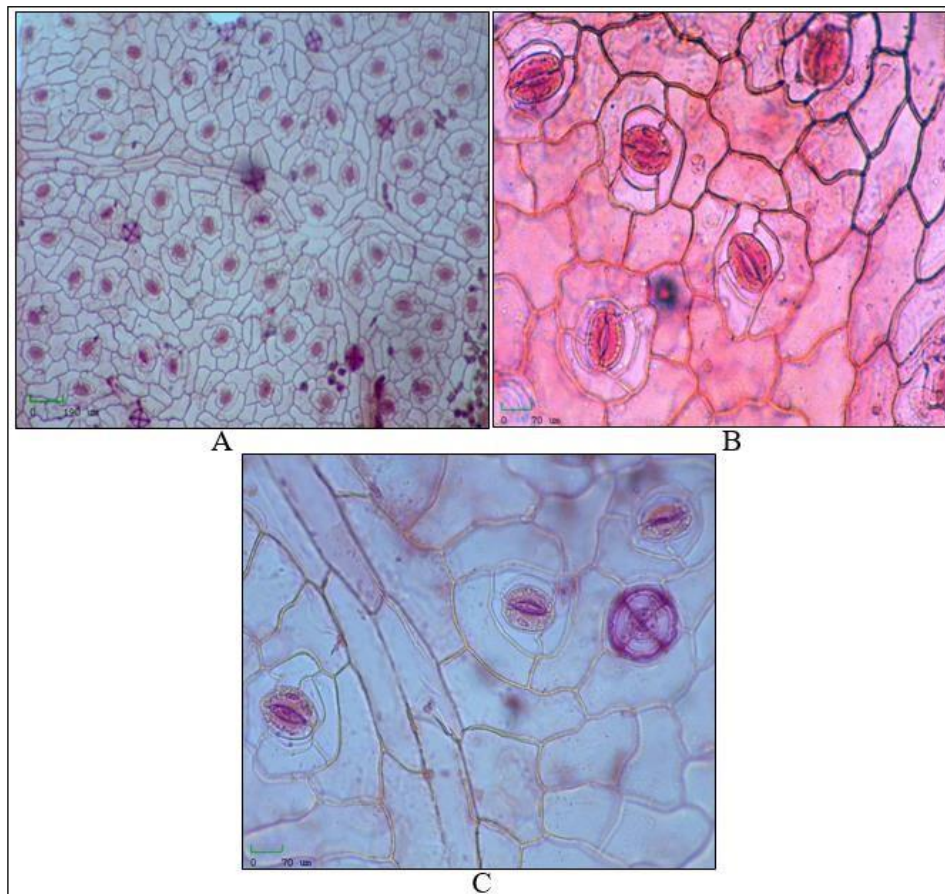
Stomata on lower epidermis of leaf are predominantly anisocytic. But following types of stomata also observed

1. Stomata on one side flanked by one parallel and then by three unequal subsidiary cells
2. Paracytic
3. Tetracytic
4. Staurocytic
5. Anomocytic
6. Hemiparacytic

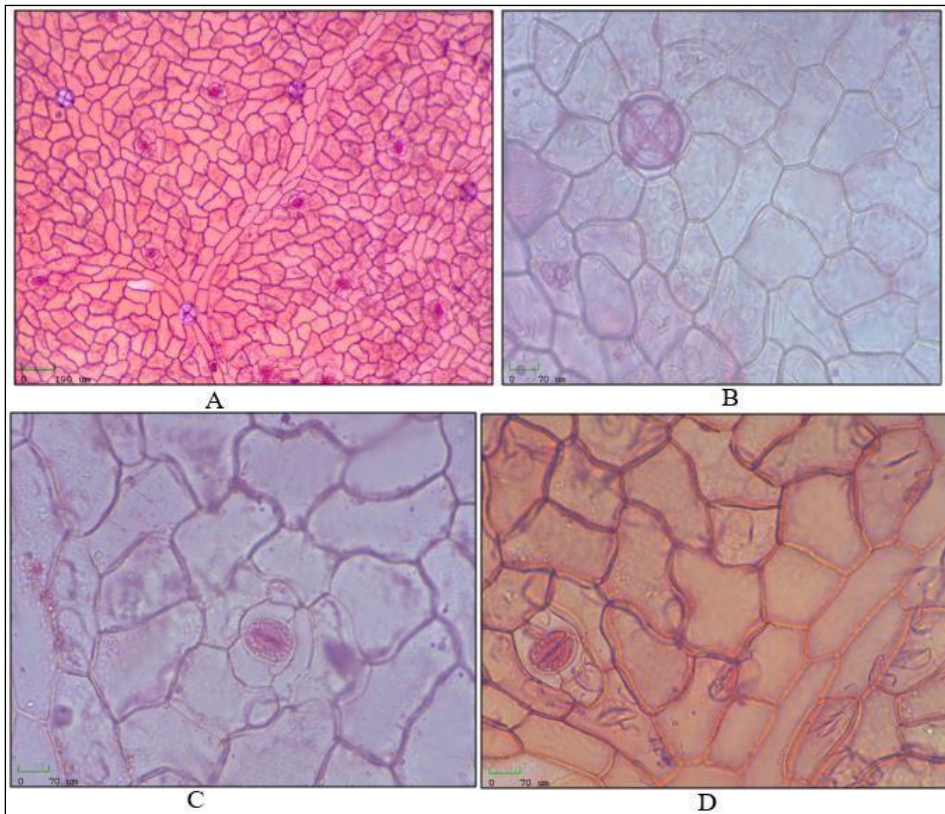
#### Upper epidermis (Fig. 4 and 8)

Stomata on upper epidermis of leaf are predominantly anisocytic. But following types of stomata also observed.

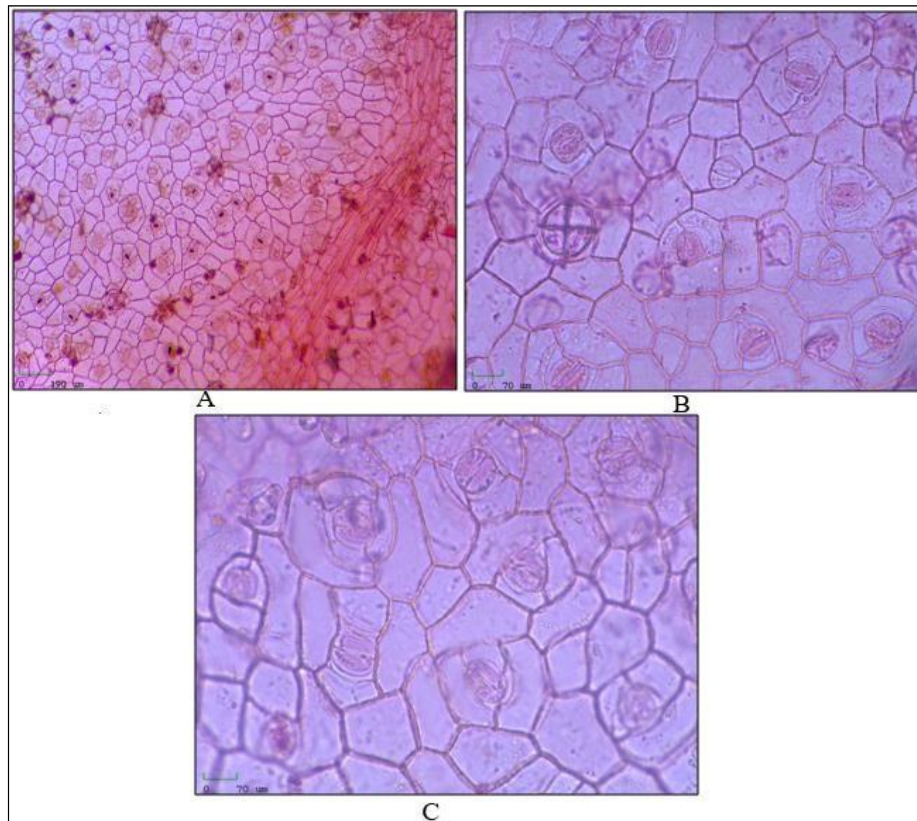
1. Polycytic
2. Staurocytic
3. Paracytic
4. Anomocytic



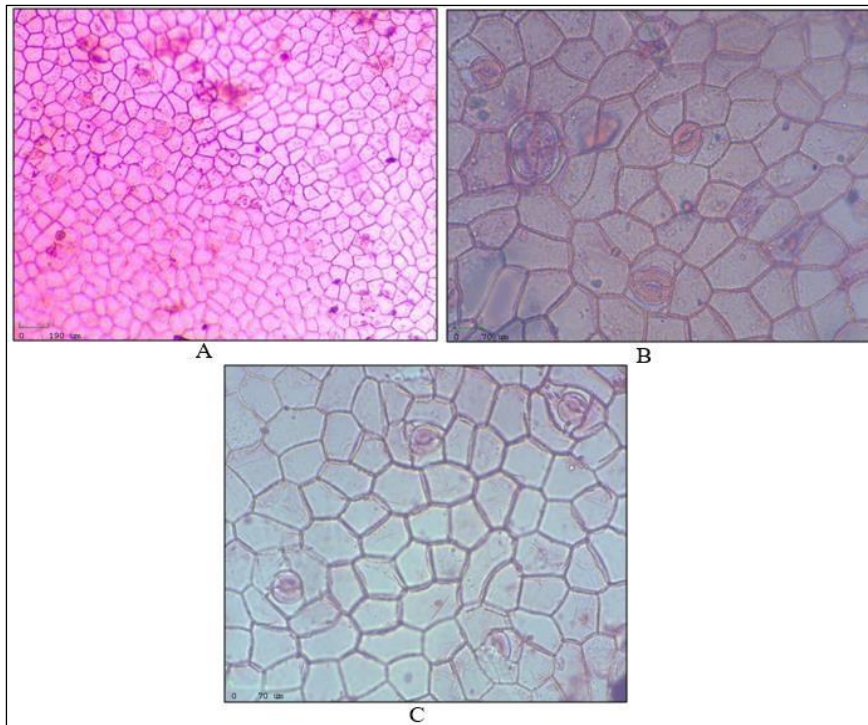
**Fig 1:** LM Photomicrographs of lower epidermis of *P. zeylanica* A. Epidermal peeling showing epidermal cells, stomata and chalk glands ( $\times 40x$ ). B. Epidermal peeling showing anisocytic stomata ( $\times 100x$ ). C. Epidermal peeling showing chalk glands, anisocytic stomata and stomata on one side flanked by one parallel cell and then surrounded by 4 subsidiary cells as in staurocytic stomata ( $\times 100x$ ).



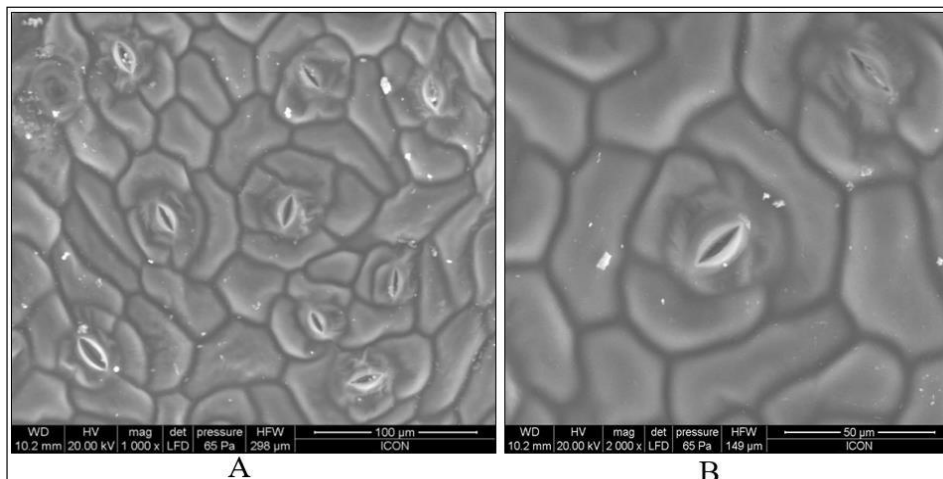
**Fig 2:** LM photomicrographs of upper leaf epidermis of *P. zeylanica* A. Epidermal peeling showing epidermal cells, stomata and chalk glands ( $\times 40x$ ). B. Epidermal peeling showing chalk glands ( $\times 100x$ ). C. Epidermal peeling showing anisocytic stomata ( $\times 100x$ ). D. Epidermal peeling showing diacytic stomata ( $\times 100x$ ).



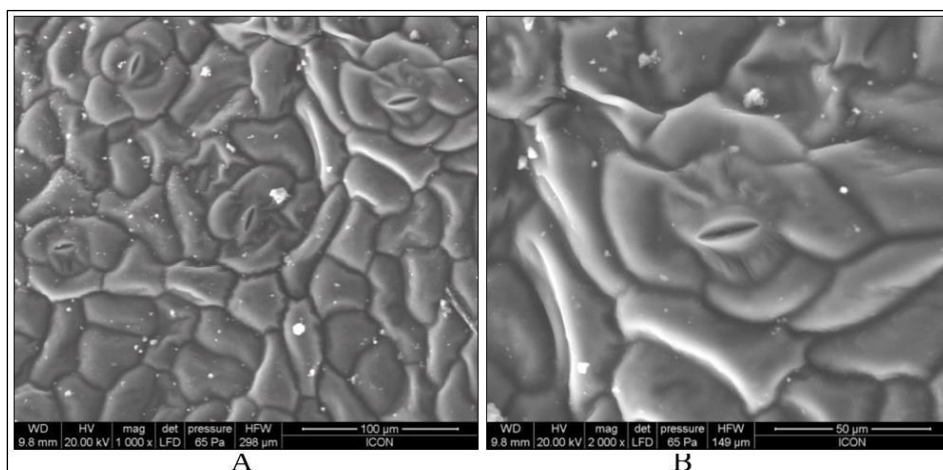
**Fig 3:** LM Photomicrographs of lower epidermis of *P. auriculata* A. Epidermal peeling showing epidermal cells, stomata and chalk glands ( $\times 40x$ ). B. Epidermal peeling showing anisocytic stomata and chalk glands ( $\times 100x$ ). C. Epidermal peeling showing paracytic and anisocytic stomata ( $\times 100x$ ).



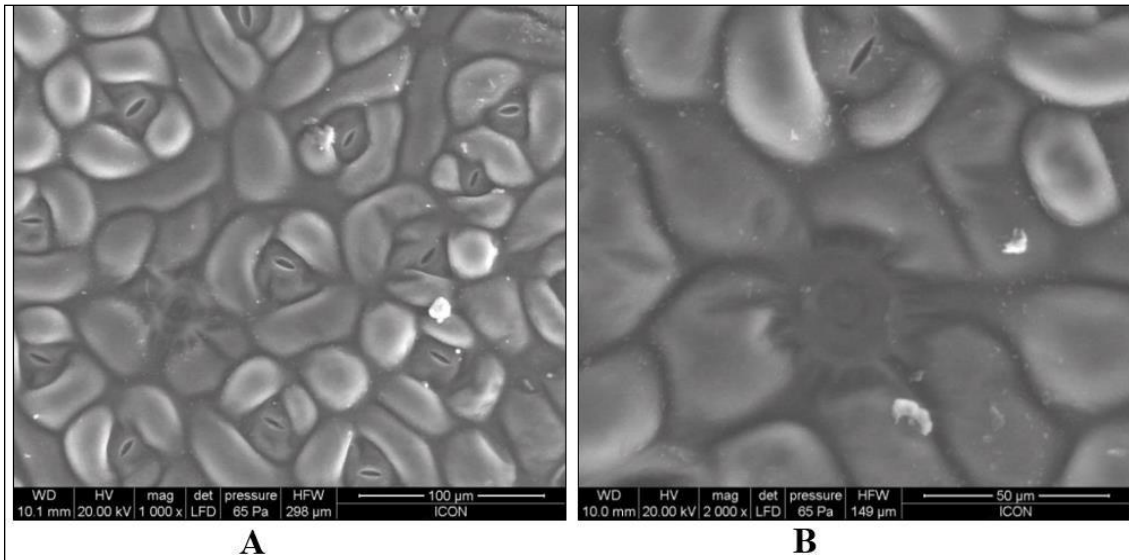
**Fig 4:** LM Photomicrographs of upper epidermis of *P. auriculata* A. Epidermal peeling showing epidermal cells, stomata and chalk glands ( $\times 40x$ ). B. Epidermal peeling showing anomocytic stomata, anisocytic stomata and chalk gland ( $\times 100x$ ). C. Epidermal peeling showing stoma with single guard cell, stoma with unequal guard cells and anisocytic stoma ( $\times 100x$ ).



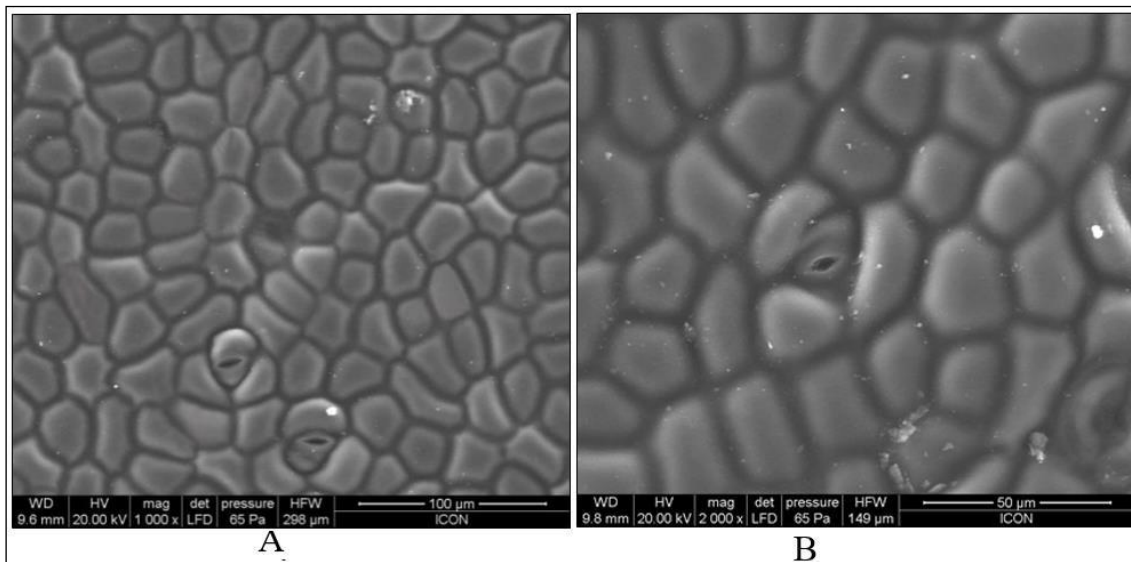
**Fig 5:** SEM photomicrographs of lower leaf epidermis of *P. zeylanica* A. Lower leaf epidermis showing epidermal cells, stomata and chalk glands ( $\times 1000x$ ) B. Lower leaf epidermis showing epidermal cells, stomata ( $\times 2000x$ )



**Fig 6:** SEM photomicrographs of upper leaf epidermis of *P. zeylanica* A. Upper leaf epidermis showing epidermal cells, stomata ( $\times 1000x$ ) B. Upper leaf epidermis showing epidermal cells and tetracytic stomata ( $\times 2000x$ )



**Fig 7:** SEM photomicrographs of lower leaf epidermis of *P. auriculata* A. Lower leaf epidermis showing epidermal cells, stomata and chalk glands (×1000x) B. Lower leaf epidermis showing epidermal cells, stomata and chalk glands (×2000x)



**Fig 8:** SEM photomicrographs of upper leaf epidermis of *P. auriculata* A. Upper leaf epidermis showing epidermal cells, stomata and chalk glands (×1000x) B. Upper leaf epidermis with stomata (×2000x)

**Table 1:** Qualitative features of leaf epidermis of two species of *Plumbago* Linn.as revealed by LM

Sr. No.	Name of Plant	Occurrence of stomata on leaf epidermis	Surface of Leaf	Feature of epidermal cells			Feature of Stomata			
				Shape	Arrangement	Anticlinal wall	Type	Distribution	Orientation	Shape
1	<i>Plumbago zeylanica</i>	Amphistomatic	Upper	Polygonal, occasionally rectangular, or elongated in various directions	No definite pattern of arrangement or sometime arranged in linear fashion	Slightly sinuous or straight	Predominantly anisocytic	Distributed unevenly	Oriented irregularly in any direction	Elliptic
			Lower	Polygonal, occasionally rectangular, or elongated in various directions	No definite pattern of arrangement or sometime arranged in linear fashion	Slightly sinuous or straight	Predominantly anisocytic	Distributed uniformly (equidistant) or unevenly	Oriented irregularly in any direction	Elliptic
2	<i>Plumbago auriculata</i>	Amphistomatic	Upper	Polygonal, or rarely rectangular elongated in various directions,	No definite pattern of arrangement	Straight	Predominantly anisocytic	Distributed unevenly	Oriented irregularly In any direction	Somehow circular or elliptic
			Lower	Polygonal, or rarely rectangular elongated in various directions,	No definite pattern of arrangement	Straight	Predominantly anisocytic	Distributed uniformly (equidistant) or unevenly	Oriented irregularly In any direction	Somehow circular or elliptic

**Table 2:** Qualitative features of leaf epidermis of two species of *Plumbago* Linn.as revealed by SEM

Sr. no.	Name of plant	Surface of Leaf	Stomata			Epidermal cells			Trichome
			Position	Striae associated with stomata	Cuticular rim of stomata	Anticlinal Wall	Periclinal wall	Cuticular ornamentation	
1	<i>Plumbago zeylanica</i> .	Upper	Stomata present at the same level of epidermal cell	Inconspicuous striae present	Pronounced cuticular rim of uneven width	Position is indicated by narrow shallow groove	Convex	Absent	Absent
		Lower	Stomata raised above epidermal cell	Inconspicuous striae present	Pronounced cuticular rim of uneven width	Position indicated by broad shallow groove	Flat or slightly convex	Absent	Absent
2	<i>Plumbago auriculata</i>	Upper	Stomata present at the same level of epidermis or slightly sunken	Absent	Narrow cuticular rim	Position indicated by broad shallow groove	Flat or slightly convex	Absent	Absent
		Lower	Stomata slightly sunken	Absent	Narrow cuticular rim	Position indicated by broad shallow groove	Convex or low domed, sometime periclinal wall is shallow depressed	Absent	Absent

**Table 3:** Quantitative characters of leaf stomata of two species of *Plumbago* Linn. as revealed by LM

Sr. No.	Name of Plant	Leaf Surface	Stomata																	
			Number per mm <sup>2</sup>			Stomatal Index			Size of Stomatal aperture						Size of guard Cell					
			Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD	Length in (µm)			Breadth in (µm)			Length in (µm)			Breadth in (µm)		
									Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD
1	<i>Plumbago zeylanica</i>	Upper	12	24	15.60 ± 4.789	2.564	5.607	3.298 ± 0.963	12.996	19.760	15.970 ± 2.155	2.410	5.054	3.648 ± 0.885	21.548	33.860	27.201 ± 3.632	6.334	19.551	10.345 ± 3.974
		Lower	68	140	112.8 ± 29.078	10.366	14.706	12.578 ± 1.330	12.291	17.634	14.952 ± 1.900	2.342	5.115	3.612 ± 0.903	24.848	37.500	31.130 ± 3.893	7.430	15.730	10.755 ± 2.620
2	<i>Plumbago auriculata</i>	Upper	48	68	57.200 ± 7.554	3.647	4.570	3.943 ± 0.316	6.432	13.120	9.095 ± 1.976	2.414	5.121	3.524 ± 0.948	15.626	21.433	19.155 ± 1.852	5.147	8.974	7.415 ± 1.135
		Lower	140	252	184 ± 31.496	11.945	16.423	14.892 ± 1.565	9.320	15.150	11.983 ± 2.018	2.301	5.930	3.994 ± 1.322	15.768	21.643	18.931 ± 1.870	5.351	6.666	5.955 ± 0.430

**Table 4:** Quantitative characters of leaf epidermal cells of two species of *Plumbago* Linn.as revealed by LM

Sr. No.	Name of Plant	Leaf surface	Epidermal cell								
			Number per mm <sup>2</sup>			Size					
			Min.	Max.	Mean ± SD	Length in (µm)			Breadth (µm)		
						Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD
1	<i>Plumbago zeylanica</i>	Upper	380	732	465.2 ± 114.014	50.579	89.640	64.571 ± 10.894	15.163	49.291	33.914 ± 7.956
		Lower	592	1048	776 ± 157.186	35.028	87.375	60.505 ± 13.112	15.339	47.705	29.489 ± 8.679
2	<i>Plumbago auriculata</i>	Upper	1252	1500	1389.600 ± 94.998	20.608	54.834	36.102 ± 8.076	17.800	28.583	24.166 ± 3.589
		Lower	880	1316	1052.4 ± 149.232	29.565	70.502	41.923 ± 11.235	11.188	36.862	23.845 ± 6.611

**Table 5:** Abnormal stomata observed on leaf epidermis of two species of *Plumbago* Linn.

Sr. No.	Name of plant	Surface	Abnormal Stomata
1	<i>Plumbago zeylanica</i>	Upper	Stomata with single guard cell
		Lower	Two neighbouring stomata sharing one subsidiary cell
2	<i>Plumbago auriculata</i>	Upper	1. Two contiguous stomata which are right angle to each other 2. Stomata with single guard cell 3. Stomata with unequal guard cells 4. Stomata without pore
		Lower	1. Two stomata sharing common subsidiary cells 2. Two contiguous stomata which are obliquely oriented 3. Stomata with unequal guard cells

## Discussion

### Epidermal cells

Inamdar and Patel (1970) [12], Ayodele (2000) [22] reported straight anticlinal wall of leaf epidermal cells in *P. zeylanica* Linn. But in the present investigation slightly sinuous anticlinal wall of leaf epidermal cell was observed in addition to straight anticlinal wall which is in the conformity of Metcalfe and Chalk (1950) [23] who recorded sinuous or straight wall in family Plumbaginaceae. Observation of straight anticlinal wall of epidermal cells of leaf of *P. auriculata* Lam. is in the conformity with Ayodele (2000)

[22]. Inamdar and Patel (1970) [12] recorded polygonal epidermal cells with irregular arrangement in leaf surface of *P. zeylanica* Linn. Ayodele (2000) [22] also recorded polygonal shape of epidermal cells in the leaf of *P. zeylanica* Linn. In the present investigation occasionally rectangular epidermal cells arranged in linear fashion were also observed in addition to polygonal epidermal cells with irregular arrangement in the leaf surface of *P. zeylanica* Linn.

Observation of polygonal epidermal cells of *P. auriculata* Lam. is in accordance with Ayodele (2000) [22].

Epidermal cells are larger in *P. zeylanica* Linn. and smaller in *P. auriculata* Lam. This observations is in accordance with Ayodele (2000) [22].

### Stomata

Anisocytic stomata on leaves of *P. zeylanica* were recorded by Metcalfe and Chalk (1950) [23] and Ayodele (2000) [22]. Inamdar and Patel (1970) [12] recorded predominantly anisocytic stomata (surrounded by ring of 6 subsidiary cells) but additionally they recorded paracytic (flanked by 4 subsidiary cells), tetracytic (surrounded by 8 subsidiary cells on 4 sides) and rarely anomocytic and cyclocytic stomata in *P. zeylanica* Linn. Ayodele (2000) [27] also observed anisocytic stomata on the leaves of *P. auriculata* Lam.

### Keys to *Plumbago* species

**1. Key based on LM characteristics of epidermal cells (size, number per 1mm<sup>2</sup>, anticlinal wall) of leaf**  
Epidermal cell larger (60.505 ± 13.112µm × 29.489 ± 8.679 µm on lower surface and 64.571 ± 10.894 × 33.914 ± 7.956µm on upper surface), number of epidermal cells per 1 mm<sup>2</sup> less (776 ± 157.186 on lower surface and 465.2 ± 114.014 on upper surface), anticlinal wall of epidermal cells slightly sinuous or straight

-*Plumbago zeylanica*

Epidermal cell smaller (41.923 ± 11.235 × 23.845 ± 6.611 µm on lower surface and 36.102 ± 8.076 × 24.166 ± 3.589 µm on upper surface), number of epidermal cell per 1mm<sup>2</sup> more (1052.4 ± 149.232 on lower surface and 1389 ± 94.998 on upper surface), anticlinal wall of epidermal cells straight

-*Plumbago auriculata*

**2. Key based on LM characteristics of stomata (stomatal aperture size, stomatal index, number per 1mm<sup>2</sup> and shape) on leaf**

stomatal aperture larger (14.952 ± 1.900µm × 3.612 ± 0.903µm on lower surface and 15.970 ± 2.155 × 3.648 ± 0.885µm on upper surface), stomatal index less (12.578 ± 1.330 on lower surface and 3.298 ± 0.963 on upper surface), stomata number per 1mm<sup>2</sup> less (112.8 ± 29.078 on lower surface and 15.60 ± 4.789 on upper surface), elliptic shape of stomata

-*Plumbago zeylanica*

Stomatal aperture smaller (11.983 ± 2.018 µm × 3.994 ± 1.322 on lower surface and 9.095 ± 1.976 × 3.524 ± 0.948 on upper surface), stomatal index more (14.892 ± 1.565 on lower surface and 3.943 ± 0.316 on upper surface), stomata number per 1mm<sup>2</sup> more (184 ± 31.496 on lower surface and 57.200 ± 7.554 on upper surface), somehow circular or elliptic shape of stomata

-*Plumbago auriculata*

**3. Key based on SEM characteristics of stomata on leaf**  
Stomata raised above epidermal cell (on lower surface) or at the same level of epidermis (on upper surface) with inconspicuous striae and pronounced cuticular rim of uneven width

-*Plumbago zeylanica*

Stomata slightly sunken (on lower surface) or at the same level of epidermis (on upper surface) and striae absent, narrow cuticular rim

-*Plumbago auriculata*

### Conclusions

Qualitative as well as quantitative epidermal micromorphological characteristics as revealed by LM and SEM would help in taxonomy for identification, authentication and delimitation of species of *Plumbago*. These characters also help in evaluation of crude drug.

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