

MICROMORPHOLOGY OF THE EPIDERMIS OF STEM AND LEAF OF *CENTELLA ASIATICA* (L) URBAN

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Centella asiatica (L.) Urban, a member of family Apiaceae is well known for its medicinal value. The drug comprises fresh and dried leaves and stems of the plant. The present investigation of the epidermis of the leaves and stem of *Centella asiatica* in detail using light as well as scanning electron microscope was undertaken to enable the identification of the drug in the entire or fragmentary form. During our studies we found many important and peculiar structures in these epidermis. This becomes more useful since such an information is lacking in pharmacopoeias and related literature.

Keywords : Apiaceae; *Centella asiatica*; Drug; Epidermis; Medicinal value.

Introduction

Centella asiatica commonly known as "Brahmi" is a member of family Apiaceae and is well known for its medicinal value. The plant is herbaceous, its creeping stems bear roots and leaves on their nodes. The leaves are simple, petiolate and reniform with crenate margins. The drug Brahmi comprises fresh and dried leaves and stems of plant. The drug is used as brain tonic, wound healer and useful in the treatment of leprosy and other skin diseases. Plant contains thiancoid, asiaticoside and glycosides. The alkali asiaticoside is active in the treatment of leprosy. Asiatic acid and asiaticoside are used in creams to improve skin texture. Although epidermal characters of family Apiaceae has been studied¹⁻⁴, but *Centella asiatica* itself, has received very little attention so we have tried to study the epidermal characters of leaves and stem under light and scanning electron microscope to fill the gap.

Material and Methods

The plants of *Centella asiatica* were obtained from Itanagar, Arunachal Pradesh, India. The cuticles were prepared by macerating in a mixture of equal parts of aqueous nitric acid (10%) and aqueous chromic acid and were mounted in glycerine jelly, Epidermal

cells and orientation of stomata were also observed in transparencies made by Foster's technique⁵. For scanning electron microscopic studies the prepared cuticles were dehydrated, dried and mounted on stubs with double sided tape. Cuticle were sputter coated with a thin coat of gold palladium and examined by a JEOL SEM model L 35C. The terms used in the description of stomatal apparatus are the same as used earlier by various authors^{3,6-11}. In order to find constancy of epidermal characters cuticles were prepared from their apical, middle and basal regions. The frequency of stomata per square millimeter was determined on the basis of an average of the thirty readings in each case. The values in the rounded brackets given after the range are averages of thirty readings.

Observation

Leaves are amphistomatic but the frequency of stomata is higher in the lower epidermis than in the upper. Upper epidermis is differentiated into vein and mesh areas. Cells over vein area are rectangular, straight-walled, arranged end to end, 60 μm to 100 μm long x 15 μm to 30 μm wide (8.5 μm long x 20 μm wide). Cell wall 3 μm thick. Cells over mesh area are squarish, polygonal or rectangular in shape, slightly larger than broad,

straight-walled. Cells; 45 μm to 110 μm long x 30 μm to 55 μm wide (70 μm long x 40 μm wide), cell wall 2.5 μm thick. Stomata present over mesh areas only, stomatal frequency 12/ mm^2 . Surface striations are present over all the cells. Simple unicellular hairs are also present.

Lower cuticle of lamina is also differentiated into vein and mesh areas. Cells over vein area are rectangular and elongated, 85 μm to 200 μm long x 20 μm to 25 μm wide (135 μm long x 20 μm wide). Cell walls straight, 2.5 μm thick. Cells of the mesh area are polygonal or rectanguloid, 40 μm to 125 μm long x 25 μm to 35 μm wide (70 μm long x 30 μm wide). Cell wall thin about 1.2 μm thick. Stomata are present only over mesh areas and are scattered irregularly. Various types of stomata are observed in the same leaf. Stomata are usually rubiaceous or paracytic but anisocytic, anomocytic, diacytic and transitional stomata are also present. Besides these, some abnormal stomata like stomata having single guard cell, stomata with unequal guard cells, contiguous stomata, stomata in groups and giant stomata are also reported. Single guard cell may arise as a result of degeneration of one of the guard cells of a normal stoma or a meristemoid may directly develop into single guard cell instead of giving rise to a pair of guard cells.

Table 1. Percentage distribution of different types of stomata.

Type of stomata	(%)
Paracytic	56
Anisocytic	17
Anomocytic	14
Diacytic	04
Polocytic	03
Others	06

Others 06% include giant stomata, stomata with single guard cell and

transitional stomata.

Table 2. Percent distribution of Paracytic stomata of various kinds.

Typical paracytic	31%
Hemiparacytic	07%
Axilloparacytic	07%
Paracytic with unequal subsidiaries	11%

Size of stomatal pore varies from 12 μm to 22 μm long x 2.5 μm to 6 μm wide (17 μm long x 4 μm wide). Guard cells are 20 μm to 30 μm long x 2.5 μm to 8 μm wide (25 μm long x 5 μm wide). Chloroplast are present in guard cells. Stomatal frequency is 142/ mm^2 . Surface striation are present over all the epidermal cells. Striations of subsidiary cells radiate from guard cells, Cork warts, sclereids and wound periderm are very common. *Cuticular structure of petiole:* Cuticle of petiole is differentiated into non-stomatiferous and stomatiferous bands. Cells are rectangular and longitudinally elongated. Cells over non-stomatiferous band are 75 μm to 125 μm long x 12 μm to 17 μm wide (100 μm long x 14 μm wide). Cell wall straight, 2.5 μm thick. Cells over stomatiferous band are 55 μm to 85 μm long x 12 to 18 μm wide (65 μm long x 15 μm wide). Cell wall 2.5 μm thick. Stomata are arranged in longitudinal rows and longitudinally orientated. Number of stomatal rows usually 2 to 3. Subsidiary cells are 3 to 4 usually 3 in number. Stomatal pore is 15 μm to 17 μm long x 5 μm to 7 μm wide (28 long x 6 μm wide). Stomata with unequal guard cells are also observed. Surface striations are present all over. Cork wart and wound periderm are also present.

Cuticular structure of stem: Like petiole, cuticle of stem is differentiated into non-stomatiferous and stomatiferous bands. Cells are rectangular, elongated, arranged end to end. Cells 50 μm to 115 μm long x

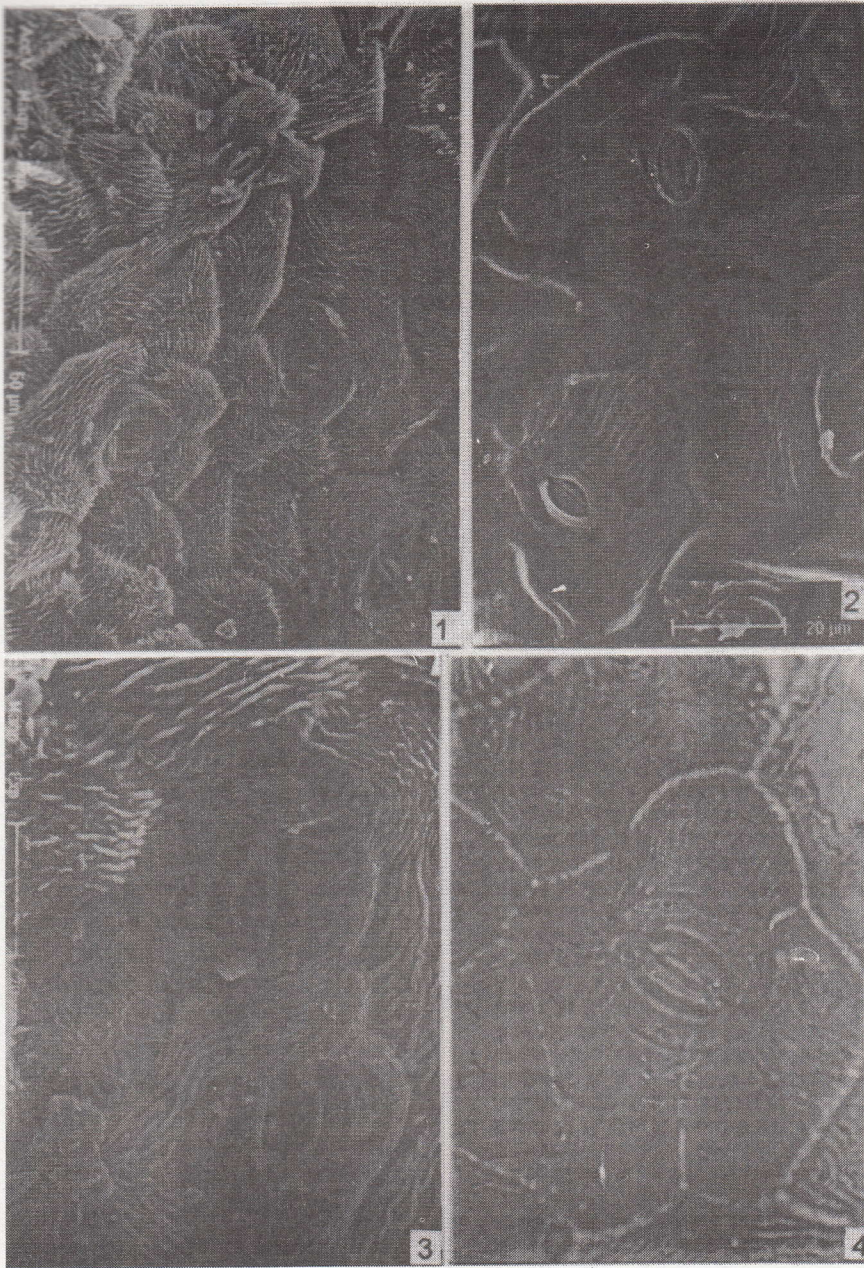


PLATE - 1

Centella asiatica

- Fig.1. SEM photograph of upper epidermis of lamina showing paracytic and anomocytic stomata. Epidermal cells and subsidiary cells show characteristic surface striations. x 750.
- Fig.2. SEM photograph of lower epidermis showing striations and stomata. Striations are radiating from the subsidiary cells. x 1500.
- Fig.3. SEM photograph of lower epidermis showing striations and paracytic stomata. x 1800.
- Fig.4. LM photograph of anomocytic stoma with radiating striations. x 1200.



PLATE - 2

Centella asiatica

- Fig.1. Lower cuticle of leaf showing a hemiparacytic stoma on the left and an axilloparacytic stoma on the right. x 1200.
- Fig.2. Upper cuticle of lamina showing unicellular hairs. x 120.
- Fig.3. A hemiparacytic stoma. x 1200.
- Fig.4. An axilloparacytic stoma. x 1200.
- Fig.5. A wound showing radially arranged thick-walled cells. x 120.



PLATE - 3

Centella asiatica

- fig.1. A typical anisocytic stoma. x 1200.
- Fig.2. An anisocytic stoma. x 1200.
- Fig.3. An axilloparacytic stoma with two parallel subsidiary cells and one polar encircling cell. x 1200.
- Fig.4. A paracytic stomata with unequal subsidiary cells. x 1200.

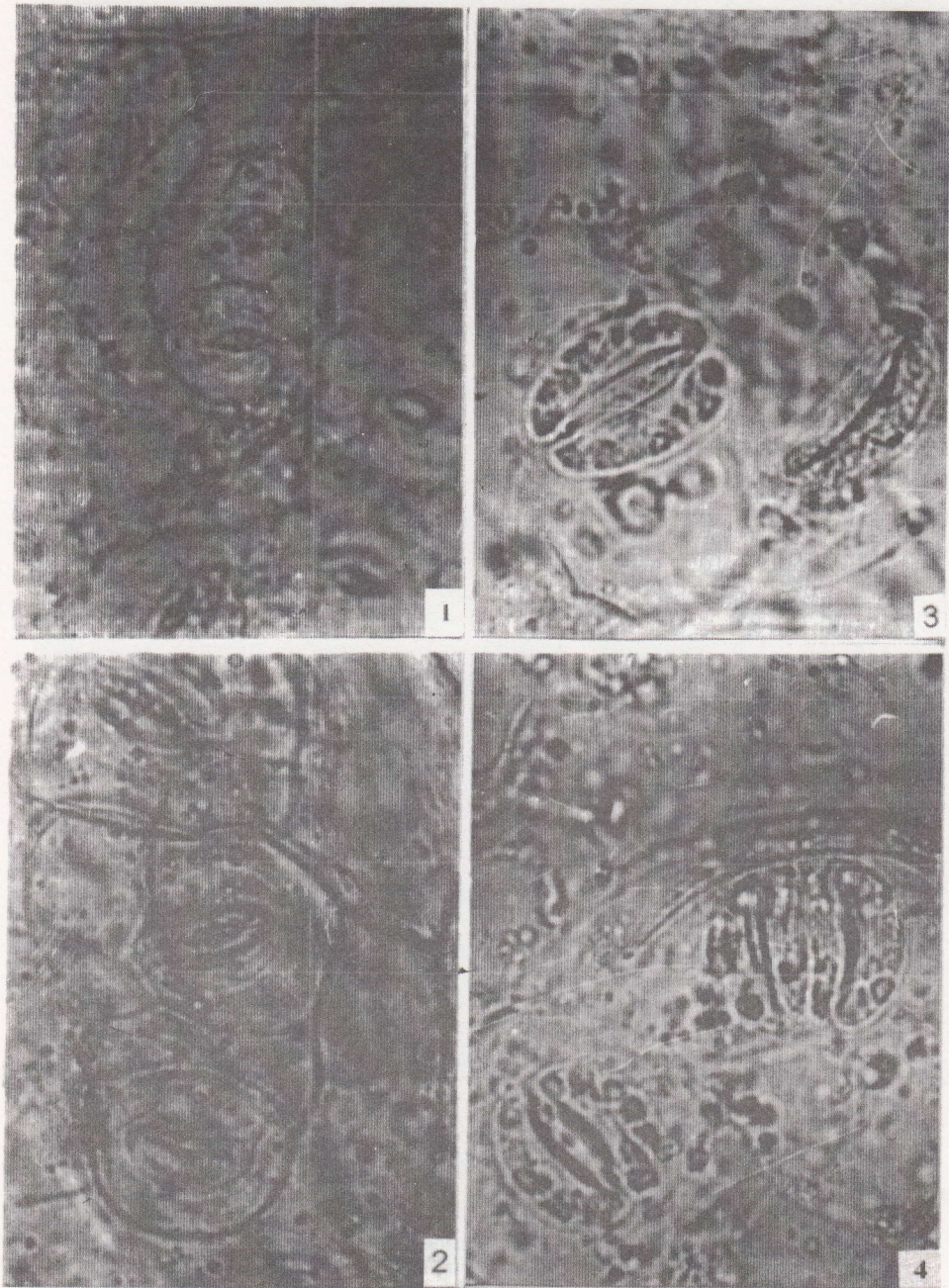


PLATE - 4

Centella asiatica

- Fig.1. Lateral contiguous stomata where subsidiary cells are contiguous. x 480.
 Fig.2. A paracytic stoma with single guard cell (top). x 480.
 Fig.3. A stoma with unequal guard cells (left). x 1200.
 Fig.4. Lateral contiguous stomata. Guard cells showing chloroplasts x 1200.

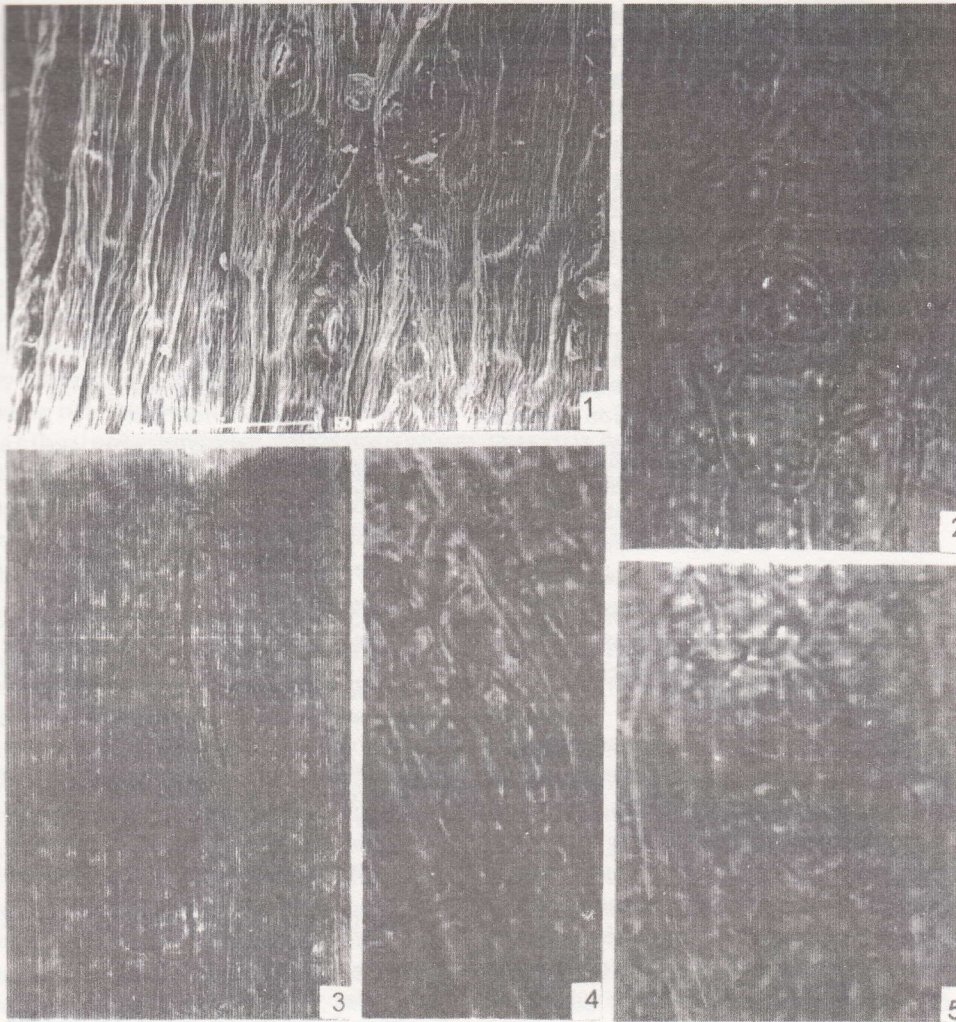


PLATE - 5

Centella asiatica

- Fig.1.SEM photograph of epidermis of petiole showing stomatiferous and non-stomatiferous bands. Cells showing surface striations. x 750.
- Fig.2. A paracytic stoma of petiole. x 480.
- Fig.3. Cuticle of stem showing rectangular elongated cells and stomata. x 480.
- Fig.4. A stoma of stem having single guard cell, other guard cell remains small and undeveloped. x 1200.
- Fig.5. Cuticle of petiole showing a stoma. x 1200.

μm to 12 μm wide (75 μm long x 12 μm wide), cells wall straight, 3 μm thick. Stomata are arranged in longitudinal rows and longitudinally orientated. Subsidiary cells are 2 to 6 usually 4 looking like other epidermal cells. Stomatal pore 14 μm long x 2.5 μm wide. Guard cells 25 μm to 35 μm long x 5 μm to 8 μm wide (30 μm long x 7 μm wide).

Discussion

Kim *et al.*² and Metcalfe and Chalk³ have reported paracytic stomata in the genus. But in the present investigation we have observed paracytic, anisocytic, anomocytic, polocytic, diacytic and numerous transitional types of stomata in the same leaf. It is also interesting that stomata have radiated striations on subsidiary cells. These surface striations appear to radiate from the guard cells and are comparable to the striations of *Angelica decursiva*².

The typical paracytic stomata of *Centella asiatica* appear like mesogenous and are comparable to that of syndetocheilic stomata¹² or to that of the paracytic stomata of *Drimys* and *Linum*¹⁴. The subsidiaries in most of the paracytic stomata are extended up to the poles of two guard cells and meet each other while in some paracytic stomata the subsidiary cells fall short at one side of the stomatal poles and are flanked by perigeneous neighbouring cells. Such stomata are termed here as axilloparacytic. These axilloparacytic stomata are comparable to anisocytic stomata of *Notonia grandiflora*⁸. A few stomata showed only

single parallel subsidiary cell in one side of the guard cell while the opposite side had been replaced by ordinary epidermal cell, these stomata are termed as hemiparacytic type.

The epidermal cells of *Centella* are polygonal with straight anticlinal walls. The sculpturing of epidermal cells is very characteristic. Striations are usually parallel but some cells show radiate type of striations. The striations are radiated from the centre towards the margins of the cell. The cells of petiole also show continuously running longitudinal striations. The pattern of sculpturing in the cells of lamina and petiole and the type of stomata are the diagnostic features of the leaves of *Centella asiatica*.

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