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# Pharmacognostic and phytochemical evaluation of *Cleome viscosa* L. (Cleomaceae)

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**ABSTRACT**

The evaluation of quality and purity of crude drugs by means of various parameters is the most important aspect of pharmacognosy. The present study deals with pharmacognostic and phytochemical evaluation of *Cleome viscosa* L. of the family Cleomaceae. The leaves of it are used by the tribal people in diarrhoea, blood dysentery, boils and also to cure swelling the body due to kidney trouble. The parameters like micromorphological, anatomical, physical constant and fluorescence analysis have been employed for the pharmacognostical evaluation of different parts of this plant. Microchemical and histochemical parameters are used for phytochemical screening. Leaves are amphistomatic and anomocytic types. Stomatal index is 21.35 on the upper surface and 24.30 on the lower surface. Palisade ratio is 5.06. Glandular multicellular, uniseriate or multiseriate trichomes with swollen tip are present on both surfaces. In T.S. of petiole, number of vascular bundles are five and arranged in semilunar manner. Methanolic extracts of leaf indicate presence of alkaloids, tannins, protein, saponins, etc. Ash value and moisture content of the leaves were found to be 04.13 % and 68.25 % respectively. This study will provide some diagnostic features by which the crude drug of this plant can easily be identified.

**Keywords:** Pharmacognostic and phytochemical studies, *Cleome viscosa* L.

## I. Introduction

Plants have been playing a vital role in curing the diseases and ailments of human being from the time immemorial. Herbal medicines are gradually being popular for primary health care of the human's world-wide because of their efficacy, easy availability, no or very negligible side effect and low price. Approximately 2,400–3,000 medicinal plants species are in use in different Indian systems of medicine including Ayurveda and many of those plants are constantly being screened for their biological activity (Bhukani, 1985). Many of the important medicinal plants in India have pharmacognostically characterized and they have been enumerated in standard literature on pharmacognosy (Mitra, 1985). It is also found that pharmacognostic studies in lesser known medicinal as well as ethnomedicinal plants are very meager. In this context, the present study has been undertaken to evaluate this lesser known ethnomedicinal plant pharmacognostically, phytochemically including its morpho-anatomy and antimicrobial activity. The leaves of the investigated plant have been considered here in this investigation because leaves are commonly used by the tribal and common people for curing the diseases. Use of micromorphology and anatomy is now a recognized tool in the field of plant systematic (Rahaman et al. 2008, 2009). Importance of epidermal characters in general and those of trichomes in particular and comparative wood anatomy are widely employed in taxonomic consideration of angiosperms (Cutler, 1984; Ogundipe and Olatunji, 1991; Parveen et al. 2000; Banerjee et al. 2002). Ontogeny and structure of stomata are now also considered as an important taxonomic character for many of the angiospermic taxa (Inamdar, 1970; Kothari and Shah, 1975; Rajagopal, 1979). Different members of the family Cleomaceae have been studied anatomically by the previous workers with special emphasis on stem and leaf epidermal micromorphology (Metcalfe and Chalk, 1950; 1979). Chemical analysis and biological assays are very important aspects in pharmacognostic evaluation of medicinal plants (Trease and Evans, 1978; 1985; Evans, 2008). The detailed pharmacognostic study of this

member of the family Cleomaceae has not yet been done. Therefore, in this investigation the foliar micromorphology, stem xylem elements, phytochemical screening and physical evaluation of this ethnomedicinally important taxon have been carried out. This investigation will provide some useful markers for identification of the crude drug obtained from the investigated plant.

## II. Materials and methods

**Plant Material:** *Cleome viscosa* L. (Family- Cleomaceae)
**Herbarium voucher number:** U.Das 37 (Deposited at the herbarium section of departmental museum for future reference).
**Common English name:** Asian spider flower
**Local name:** Hurhure (in Bengali)
**Tribal name:** Hurhuria
**Botanical characteristics:** Erect yellowish green annual herb; stem densely clothed with glandular and simple hairs, grooved; leaves 3-5 foliate, leaflets elliptic-oblong, entire, subacute, central one largest, all subsessile; flowers yellow, axillary, growing out into a lax raceme, sepals oblong-lanceolate, glandular, hairy outside, stamens 12-20; fruits capsule linear, erect, narrowed at both ends, viscid, pubescent, striate.
**Flowering and fruiting time:** June to September
**Distribution:** South Eastern Asia, Malaysia, Tropical Australia, Tropical Africa & Southern Arabia; throughout India.
**Habit and habitat:** An erect annual herb. Terrestrial, a common weed of waste places.
**Parts used:** Leaves
**Medicinal uses:** Leaves used in diarrhoea, blood dysentery, boils, etc., decoction taken to cure swelling due to kidney trouble.
**Chemical constituents:** Whole plant contains glycoflavanone and diterpene lactones; seeds yield coumarins, e.g. umbelliferone derivatives and coumarinolignoid, cleomiscosmin A; roots contain flavonoids triterpenes and sterol.

For the study of foliar epidermis, leaf samples were cleared following the Bokhari's method (1970). The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin solution and observed under compound light microscope. For wood elements study, the stem pieces were macerated following the standard method (Johansen, 1940); washed several times, teased with needles, stained in safranin, mounted on the slides with 10% glycerine and observed under microscope. The drawings of the foliar micromorphological characters and stem xylem elements were made with the help of camera lucida and measurements were taken with standardized ocular micrometer in each case. Finally, the leaf powder was extracted (Soxhlet extraction) with 90% methanol and these extracts were used for different chemical colour reaction tests for identification of different phytochemical groups. Physical constants and the UV fluorescence nature of the powder were studied following the standard methods (Trease and Evans, 1985).

## III. Results and Discussion

### 3.1. Micromorphology

General description and measurement of the epidermal cells, stomata, trichomes and crystals are given below.

**3.2. Epidermis:** Cells are irregular in shape and cell walls are sinuous in outline. Epidermal cell size of the upper surface is 38.40 µm × 16.01µm and on the lower surface the size is 40.80 µm × 17.20 µm. Frequency on the upper and lower surface is 568.80 /mm$^2$ and 579.00 /mm$^2$ , respectively. Palisade ratio is 5.06 (**Table-1; Fig. I: A, B & E**).

**3.3. Stomatal complex:** Leaves are amphistomatic, only anomocytic types. Stomatal size is 24.00 µm × 16.40 µm on the upper surface and it is 35.65 µm × 21.98 µm on the lower surface. Stomatal frequency on the upper and lower surface is 165.60 /mm$^2$ and 143.47 /mm$^2$, respectively. Stomatal index is 21.35 on the upper surface and on the lower surface it is 24.30 (**Table- 2; Fig. I: A, B, E & F**).

**3.4. Trichomes:** Glandular multicellular, uniseriate or multiseriate trichomes with swollen tip are present on both surfaces. Size of the trichomes is 124.80 µm x 29.20 µm on the upper surface and it is 135.80 µm x 22.09 µm on the lower surface. Frequency of the trichome on the upper and lower surface is 9.60 /mm$^2$ and 7.16 /mm$^2$, respectively. Trichome index is 1.65 on the upper surface and it is 1.33 on the lower surface (**Table- 3; Fig. I: C& D**).

**3.5. Crystals:** Ca-oxalate crystals are found on both the epidermal layers.

**3.6. Wood elements**

General description and measurement of the type and size, pittations, perforation plates of vessel elements, side wall thickening of tracheids, fibres size and nature, etc. of the investigated plant have been represented in **Table- 4.**

Perforation plates are simple and transversely placed. Pits are simple. Tails are sometimes present in the vessel elements. Size of the vessel is 174.80 µm x 35.20 µm. Frequency is 4.80 /mm$^2$ (**Fig. II: c**). Tracheids are very long and spiral sidewall thickening. Diameter and frequency are 36.40 µm and 10.00 /mm$^2$ respectively (**Fig. II: e**). Fibres are long, typically libriform type; ends are narrow, pointed or sometimes blunted. Pits and septa are absent. Fibre length is very long and diameter is 19.60 µm and frequency is 26.40 /mm$^2$ (**Fig. II: d**).

**3.7. Stem anatomy**

The transverse section of a stem is more or less circular and wavy in outline, it shows the following anatomical features from periphery towards centre (**Fig. III: a**).

**3.7.1. Epidermis:** It consists of single layered, parenchymatous, barrel shaped cell compactly arranged and covered with thin cuticle. Multicellular hairs emerge from this layer.

**3.7.2. Cortex:** The cortex is differentiated into two distinct zones. The first zone is consists of chlorenchymatous cells of 2-3 cell layers thick, lying just below the epidermal layer. 4-5 layers of parenchymatous cells are present.

**3.7.3. Sclerenchyma zone:** Small patches of sclerenchyma cells are present to the extreme periphery of phloem layers.

**3.7.4. Vascular bundle:** They form a continuous cylinder of vascular tissue i.e. xylem and phloem. Vascular bundles are collateral, conjoint and open type. Phloems are situated on the outer side of the vascular cylinder and on the inner side of it a continuous band of xylem is present. Vessel elements are twine, cluster and radially arranged. Here cambium is indistinct.

**3.7.5. Pith:** In the centre massive pith is present with many layers parenchyma cell. Peripheral cells are smaller than the central cells

**3.8. Petiole anatomy**

The transverse section of the petiole is bean shaped, shows the following anatomical features (**Fig. IV: b**).

Epidermis consisting of single layered, compactly arranged, radially elongated, barrel-shaped cells. Multicellular hairs emerged from the epidermis. The ground tissue consists of thin walled parenchymatous cells and 5 vascular bundles are arranged in semilunar manner.

**3.9. Organoleptic features of the crude drug**

Colour: Olive green; Odour: No specific odour; Taste: Acrid; Texture: Herbaceous, glabrous, slightly hairy in fresh form.

**3.10. Microchemical evaluation of the powdered drug**

Through the phytochemical tests of the methanolic plant extract, the important phytochemical groups like alkaloids, steroids, reducing sugars, proteins, gums, tannins, flavonoids, etc. have been detected (**Table 5**).

**3.11. Histochemical study**

Histochemical study has been carried out to detect various phytochemicals localized in different tissue zones of the stem. Different histochemical localizations have been identified in the stem which contains some specific phytochemical groups (lignins, gum, proteins, alkaloids and tannins) (**Table 6**).

### 3.12. Physical evaluation

#### 3.12.1. Physical constant

Ash value:

- Total ash - 4.13 %
- Water soluble ash - 01.69%
- Acid insoluble ash - 2.25%
- Moisture content - 68.25 % (in fresh form)

#### 3.12.2. Fluorescence analysis

Here in this study it is observed that drug powder treated with different chemical reagents (powder as such, treated with dilute nitric acid, treated with dilute sodium hydroxide, treated with dilute hydrochloric acid, treated with dilute sulphuric acid, treated with antimony trichloride, methanol, ethanol and acetone) gives characteristic colourations when seen under UV light and it is compared with the colourations observed under ordinary light. In some cases there are marked differences in colour (**Table 7**).

The present study reveals that foliar epidermal features, stem xylem element characters, primary phytochemical screenings and physical evaluation are of some importance in identification of this investigated plant species in its fresh as well as dried form. Some of the general anatomical characters of the investigated plant conformed to the features identified in the other members of the family Cleomaceae earlier by different workers (Metcalfe and Chalk, 1950; 1979). Stomata of investigated plant are of anomocytic type. Studies in stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants (Inamdar, 1970; Kothari and Shah, 1975). There is a marked difference between stomatal indices of upper (21.35%) and lower (24.30%) surfaces of the leaf which is a distinct feature of this plant. Trichome features are also very important in proper identification of the plants and considered as one of the valuable taxonomic markers now (Leelavathi and Ramayya, 1983). Glandular multicellular, uniseriate or multiseriate trichomes with swollen tip are present on both surfaces. Trichome indices of the upper and lower surfaces are 1.65% and 1.33% respectively. Vessel elements of root of the investigated plant show a characteristic feature in respect of their size. The arrangement and number of vascular bundles in the petiole may sometimes provide the diagnostic feature for identification of the plant species. Here in the petiole, altogether 5 vascular bundles are found and they are arranged in semilunar manner which can be used as a marker for identification of this species of Cleomaceae. Chemical analysis and biological assays are considered as important aspects in pharmacognostic evaluation of the medicinal plants (Trease and Evans, 1985; Harbone and Williams, 1994). Through phytochemical tests of methanolic shoot extract of this plant, it is found that the important phytochemical groups like alkaloids, tannins, protein, saponins, etc. are present in this plant which confirms its medicinal properties. The physical constants like ash value (4.13%), moisture content (68.25%) and UV fluorescence characters of the powder drug of this plant can also be used as important characters for proper identification of crude drug of it. Finally some diagnostic features have been given below which can be employed for easy identification of *Cleome viscosa* L. in its fresh as well as dried form.

Table 1 Foliar epidermal cell characters of *Cleome viscosa* *

| **Leaf surface** | **Cell shape** | **Cell length (µm)** | **Cell width (µm)** | **Cell frequency (No./mm$^2$)** | **Cell wall outline** | **Palisade Ratio** |
|---|---|---|---|---|---|---|
| Upper | Irregular | 38.40 | 16.01 | 568.80 | Sinuous | 5.06 |
| Lower | Irregular | 40.80 | 17.20 | 579.00 | Sinuous | |

Table 2 Stomatal features of the investigated plant*

| **Leaf surface** | **Stomatal type** | **Stomatal length (µm)** | **Stomatal width (µm)** | **Stomatal frequency (No./mm$^2$)** | **Stomatal Index (%)** |
|---|---|---|---|---|---|
| Upper | Anomocytic | 24.00 | 16.40 | 165.60 | 21.35 |
| Lower | Anomocytic | 35.65 | 21.98 | 143.47 | 24.30 |

Table 3 Trichome features of the investigated species*

| Leaf surface | Types | Trichome length(µm) | Trichome width(µm) | Trichome frequency (No./mm$^2$) | Trichome Index(%) |
|---|---|---|---|---|---|
| Upper | Glandular multicellular, uni- or multiseriate with swollen tip | 124.80 | 29.20 | 9.60 | 1.65 |
| Lower | Glandular multicellular, uni- or multiseriate with swollen tip | 135.80 | 22.09 | 7.16 | 1.33 |

Table 4 Wood elements characters of the investigated plant*

| Structure | Type | Measurement |
|---|---|---|
| Vessel elements | Perforation plate | Simple |
| | Arrangement | Transverse |
| | Pits | Simple |
| | Tail | Sometimes present |
| | Length (µm) | 174.80 |
| | Breadth (µm) | 35.20 |
| | Frequency (/mm$^2$) | 4.80 |
| Tracheids | Wall thickening | Spiral |
| | Diameter (µm) | 36.40 |
| | Frequency (No./mm$^2$) | 10.00 |
| Fibres | Ending pattern | Pointed, sometimes blunt |
| | Pit | Absent |
| | Septa | Absent |
| | Length (µm) | Very long |
| | Breadth (µm) | 19.60 |
| | Frequency (No./mm$^2$) | 26.40 |

** Data presented in the tables are averages of 20 observations*

Table 5 Microchemical tests of *Cleome viscosa*

| Tests/ Reagents | Tests for | Nature of changes | Degree of changes |
|---|---|---|---|
| Dragendroff's reagent | Alkaloids | Orange brown ppt. | +++ |
| Wagner's reagent | Alkaloids | Orange brown ppt. | +++ |
| Mayer's reagent | Alkaloids | Crème colour | + |
| Millon's reagent | Proteins | Yellow to brown | ++ |
| Benedict's test | Reducing sugars | Brick red ppt. | - |
| Fehling's test | Reducing sugars | Brick red ppt. | - |
| 10% aq. $K_2Cr_2O_7$ Solution | Tannins | Yellowish-brown ppt. | +++ |
| 10% aq. Lead acetate solution | Tannins | Yellow ppt. | + |
| 5% aq. Ferric Chloride solution | Tannins | Greenish – black | + |
| Lugol's reagent | Proteins | Yellowish- brown | +++ |
| Keddy reagent | Glycosides | Violet- blue | - |
| Molish's test | Gum | Red- violet ring | - |
| 1% Lead acetate solution | Saponins | White ppt. | ++ |

- = Absent; + = Present

Table 6 Histochemical localization test

| **Reagents** | **Colour change** | **Tissue zones** | **Compounds detected** |
|---|---|---|---|
| Dagendroff's reagent | Orange- brown | Sclerenchyma cells, few cells of xylem and pith | Alkaloids |
| 5% $FeCl_3$ | Greenish- black | Pith, cortex, few cells of xylem | Tannins |
| Lugol's reagent | Yellowish- brown | Sclerenchyma patch | Amino acid |
| Phloroglucion + Hcl | Reddish brown to rose red | Sclerenchyma patch, vascular bundles | Lignin |
| Wagner's reagent | Orange- brown | Cortex and pith | Alkaloids |
| Millon's reagent | Yellow to brown | Sclerenchyma patch, xylem cells | Amino acid |
| Molish's test | Red-violet | Sclerenchyma patch, few cells of xylem | Gum |

Table 7 UV fluorescence nature of the investigated plant

| **Materials and treatment** | **In UV light** | **In ordinary light** |
|---|---|---|
| Powder as such | Fluorescent-green | Deep green |
| Treated with dilute nitric acid | Brownish-green | Orange-brown |
| Treated with dilute sodium hydroxide | Greenish-brown | Brownish black |
| Treated with dilute hydrochloric acid | Greenish-brown | Greenish-black |
| Treated with dilute sulphuric acid | Brownish-green | Black |
| Treated with antimony trichloride | Deep green | Deep brown |
| Treated with concentrate methanol | Light green | Greenish-black |
| Treated with concentrate ethanol | Light green | Greenish-black |
| Treated with concentrate acetone | Deep green | Brownish-green |

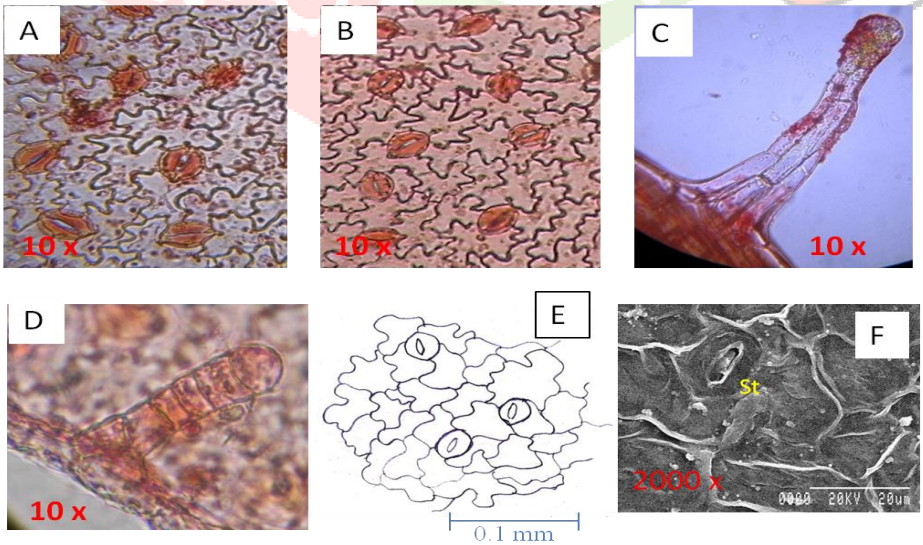



*Fig. I:* **Epidermal micromorphology: A-** Upper epidermal cells with stomata**; B** & **E–** Lower epidermal cells with stomata**; C**& **D –** Multicellular glandular trichome**; F-** Anomocytic stoma
*Fig. I:* A, B, C & D- **LMP;** F - **SEMP;** E – **CLD**
[St - Stoma]

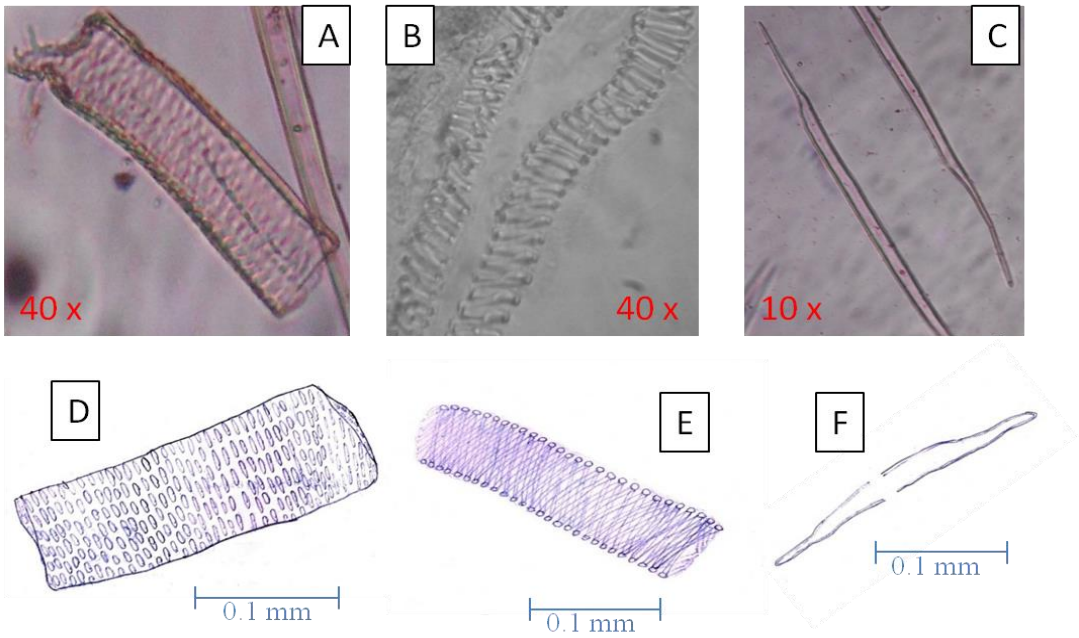



*Fig. II:* **Wood elements: A** & **D**-Vessel elements; **B** & **E**– A portion of tracheid; **C** & **F**- Fibres (Portion)
*Fig. II:* A, B & C - **LMP**; D, E & F- **CLD**

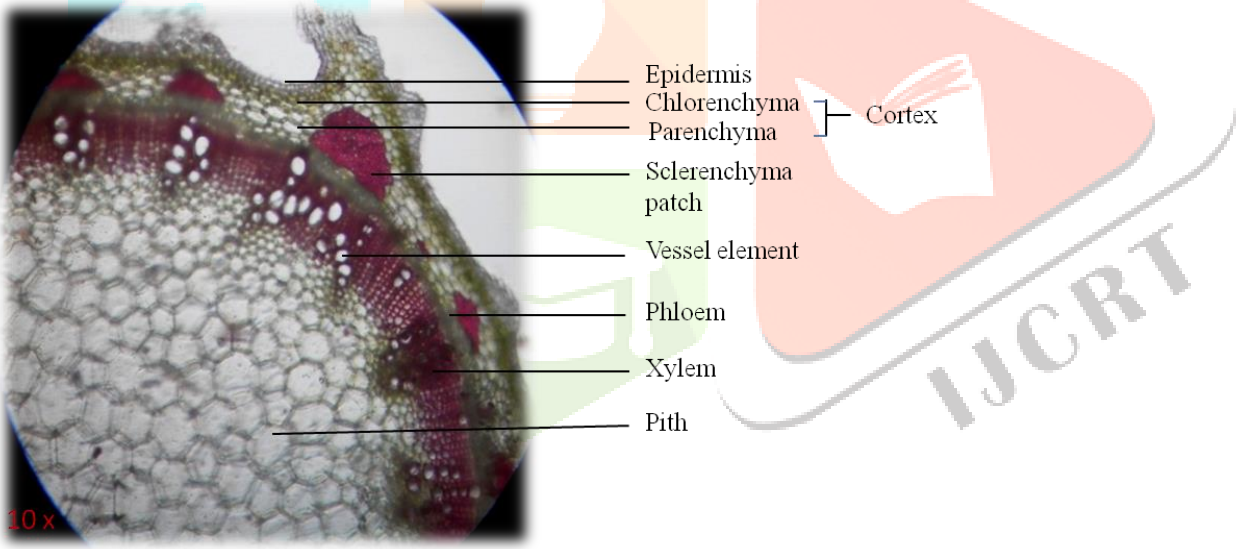
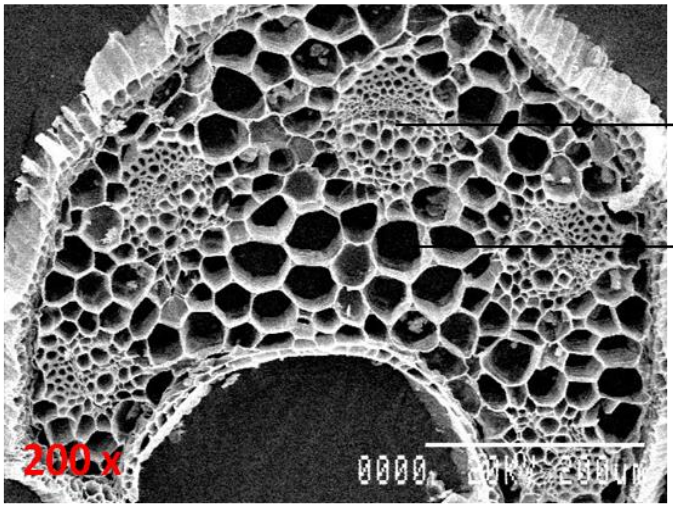



*Fig. III:* **a- T. S. of Stem** (Snap taken by Light Microscope, ZEISS, AXIOSTAR plus, 176045)




**b- T. S. of Petiole** (Snap taken by SEM: Hitachi-S530, Japan)

3.13. **Diagnostic features**

- Stricly anomocytic types of stomata are present.
- Stomatal indices of upper and lower surfaces are 21.35 and 24.30 respectively.
- Multicellular glandular trichomes with swollen tip are present on both surfaces.
- In petiole,number of vascular bundles is 5 and arranged in semilunar manner.
- Ash Value-total ash- 4.13%

## IV. Conclusions

The present work was undertaken with an aim of pharmacognostic and phytochemical investigation of *Cleome viscosa* L. providing useful information, which could be useful to detect the authenticity of this medicinal plant. Pharmacognostic evaluation can be useful to substantiate and authenticate the drug.

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## References

[1] Banerjee, A. Rahaman, CH. Kar, RK. and Mandal, S. 2002. Micromorphology of foliar epidermis of some tropical tree legumes. Phytomorphology, 52(2-3): 223-230.

[2] Bhukani, DS. 1985. Biologically activecompounds from some Indian plants, in Proc 5th Int Soc Hort Sci Med Arot Sp Plts. Ed. SK Chatterjee. Darjeeling, India, 1-4.

[3] Bokhari, MH. 1970. Morphology and Taxonomic significance of foliar sclereids in Limonium. Notes Royal Bot Gard, 30: 43-53.

[4] Choudhury, S. Rahaman, CH. and Mandal, S. 2008. Studies on leaf epidermal micromorphology and wood elements of four medicinally important species of *Cassia* L. J Botan Soc Beng, 62 (2): 67-80.

[5] Choudhury, S. Rahaman, CH. and Mandal, S. 2009. Studies on foliar epidermal micromorphology and preliminary phytochemical screening of three medicinally important taxa of the family Vitaceae. Sci and Cult, 75 (7-8): 294-300.

[6] Cutler, DF. 1984. In VH Heywood and DM Moore. Systematic anatomy and embryology, recent development. Current Concepts in Plant Taxonomy. London, UK, Academic Press, 107 –131.

[7] Evans, WC. 2008. Trease and Evans' Pharmacognosy. 15th ed. London Bailliere Tindall, English Language Book Society.

[8] Harborne, JB. and Williams, CA. 1994. Recent advances in the chemosystematics of the Monocotyledons. Phytochemistry,37(1):3-8.http://dx.doi.org/10.1016/0031-9422(94)85004-6

[9] Inamdar, JA. 1970. Epidermal structure and ontogeny of Caryophyllaceous stomata in some Acanthaceae. Bot Gaz, 131: 261-268.http://dx.doi.org/10.1086/336540

[10] Johansen, DA. 1940. Plant Microtechnique. New York:Mc Graw-Hill.

[11] Kothari, MJ. and Shah, GL. 1975. Epidermal structure and ontogeny of stomata in the Papilionaceae. Bot Gaz, 136: 372-379.http://dx.doi.org/10.1086/336829

[12] Leelavathi, PM. and Ramayya, N. 1983. Structure, distribution and classification of plant trichomes in relation to taxonomy II. Caesalpinioidae, Indian J For., 6: 43-56.

[13] Metcalfe, CR. and Chalk, L. 1950. Anatomy of Dicotyledons.Vol.2. Oxford, UK, Clarendon Press.

[14] Metcalfe, CR. and Chalk, L. 1979. Anatomy of Dicotyledons.Vol.1, 2ndedn. Oxford, UK, Clarendon Press.

[15] Mitra, R. 1985. Bibliography on Pharmacognosy of medicinal plants. Economic Botany Information Service, National Botanical Research Institute. Lucknow, India, 1-735.

[16] Ogundipe, OT. and Olatunji, OA. 1991. The leaf anatomy of the species of Cochlospermum Kunth. (Cochlospermaceae) in West Africa. Feddes Repertorium, 102:183-187.http://dx.doi.org/10.1002/ fedr.19911020306

[17] Parveen, NS. Murthy, KSR. and Pullaiah, T. 2000. Leaf epidermal characters in Crotalaria sp. (Papilionoideae) from Eastern Ghats. Phytomorphology, 50: 205-212.

[18] Rajagopal, T. 1979. Distributional patterns and taxonomic importance of foliar stomata. Indian J Bot. 2: 63-69.

[19] Trease, GE. and Evans, WC. 1978. Pharmacognosy. 11th ed. London Bailliere Tindall, English Language Book Society.

[20] Trease, GE. and Evans, WC. 1985. Pharmacognosy.12th ed. London Bailliere Tindall, English Language Book Society.