Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(4): 13-16
Received: 01-05-2016
Accepted: 02-06-2016

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Morpho-anatomical study on *Cleome viscosa* L. (Cleomaceae)

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Abstract
About 80% of world populations still use herbal drugs, for treatment of various diseases. Now a day's misidentification of plants, lack of standardization, contamination, substitution and adulteration of herb is very common which has given rise to many reports describing adverse health effects and variable quality, efficacy and contents of herbal products. Evaluation of herbal drug is an important tool in the formulation of high quality herbal products.
The aim of this research is to understand the method for analysis and to apply the method in evaluating crude drugs and their products as well as take part in the standardization of herbal medicine.
Morphological studies were determined by using simple microscope. The shape of leaf, fruit, odor and type of flower were determined. Microscopic studies were done by preparing thin hand section of upper and lower epidermis of leaf, T.S of Stem and Powder of *Cleome viscosa* Linn. The prepared slides were observed for microscopical characters at X4, X10 and X40 objective.
In the microscopic studies, the lower epidermis (Abaxial) and upper epidermis (Adaxial) contains anomocytic stomatas (45 µm at X40 objective) and glandular trichomes (480µm at X10 objective) as the main observable features. The powder microscopy contains calcium oxalate crystals, phloem parenchyma, fibres.
The pharmacognostic characteristics of this plant and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification and determination of its quality and purity and detection of nature of adulteration.

Keywords: *Cleome viscosa*, Adulteration, Quality, Microscopy.

Introduction
In recent time, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics (Alyaiev, 2007) [1]. About 80% of world populations still use the herbal drugs for treatment of various diseases. The rising popularity of herbal products, both as food and feed supplements and as phytotherapeutic drugs, has also given rise to many reports describing adverse health effects and variable quality, efficacy and contents of herbal products **(**Taylor, 2004) [17]. Side effects of herbal products may consist of allergic reactions, interactions with conventional drugs or intrinsic toxicity. Other reasons are related to preparation and manufacturing of the herbs, such as misidentification of plants, lack of standardization, contamination, substitution and adulteration of plants, failure of good manufacturing practice, and incorrect preparations and/or dosages (Calixo, 2000) [3]. Microscopic evaluation involves histological characters of organized crude drugs in its entire and powdered form with the help of a microscope (Kokate, 2005; WHO, 1998) [9, 18]. With the aid of a microscope cellular inclusions of organized crude drugs are been detected such as stomata, trichomes, calcium oxalate crystals, starch granules, aleurone grains are some of important qualitative parameters which play important role in identification of certain crude drug. Crude drug can also be identified microscopically by cutting the thin transverse and longitudinal section especially in case of stem, root and leaf base and by staining them with the appropriate staining reagents like saffranin, chloralhydrate, glycerin. Quantitative aspects of microscopy includes study of stomatal number and index, palisade ratio, vein-islet number, size of starch grains, length of fibers etc which play important role in the identification of drug. Adulteration in market samples is one of the greatest drawbacks in promotion of herbal products. Adulteration it is a practice of substituting the original crude drug partially or fully with other substances which is either free from or inferior in therapeutic and chemical properties or addition of low grade or spoiled drugs or entirely different drug similar to that of original drug substituted with an intention of enhancement of profits (Ansari, 2011; Kokate, 2004) [2, 8].

Cleome viscosa Linn. (Cleomaceae) is a weed distributed throughout the tropics of the world and plains of India. The plant is an annual, sticky herb with a strong penetrating odour, yellow flower, superior ovary, alternate leaves, digitately compound with 3–5 leaflets; petiole up to 6 cm long; leaflets obovate-lanceolate, reticulate veination, glandular hairy and long slender pods containing seeds (reddish brown in colour). In Ayurvedic system of medicine, the plant is used for fever, inflammations, liver diseases, bronchitis and diarrhea (Chatterjee *et al*., 1991) [4]. The leaves are useful in healing wounds and ulcer (Nadkarni, 1982 and Kirtikar *et al*., 1984) [11, 7]. *Cleome viscosa* is highly effective in a wide spectrum of diseases and reported to possess antipyretic activity (Devi *et al*., 2003) [5], analgesic (Parimaladevi *et al*., 2003) [14], antidiarrhoeal (Devi *et al*., 2002) [6], psycho-pharmacological, anti-microbial properties incuding *in vitro Helicobacter pylori* and wound healing activity (Devi *et al.,* 2002, Parimaladevi *et al*., 2003, Sudhakar *et al*., 2006, Parimala Devi *et al*., 2004, Mahady *et al*., 2006, Panduraju *et al*., 2011) [6, 14, 16, 13, 10, 12], also against *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* (Sudhakar *et al*., 2006) [16].

The aim of this research is to understand the method for analysis and to apply the method in evaluating crude drugs and their products as well as take part in the standardization of herbal medicine.

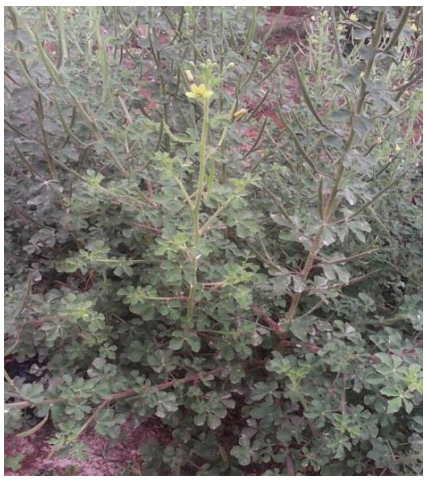


Fig 1: University of Ibadan campus premises

Materials and Methods

Materials, reagents and equipment used

Stage micrometer, Eye piece micrometer, microscope, unripped pawpaw, white tiles, razor blade, chloral hydrate, glycerin, saffranin, microscope slide, microscope cover slip, petri dish.

Plant Collection and authentication

The plant, *Cleome viscosa* Linn were collected on University of Ibadan campus. The species for the proposed study was identified and authenticated as *Cleome viscosa* (Linn.) at Forest Herbarium Ibadan, with FHI number 109669.

Pharmacognostic standardization procedures

Morphological studies were determined by using simple microscope. The shape of leaf, fruit, odor and type of flower were determined. Microscopic studies were done by preparing a thin hand section of lower and upper epidermis of leaf, transverse section of stem and powder of *Cleome viscosa* Linn. The section was cleared with chloral hydrate solution, stained with Saffranin and mounted in glycerin. The prepared slides were viewed under the microscope to determine microscopical characters at X4, X10 and X40 objective.

Calibration of the microscope

The microscope was calibrated to get the conversion factor and its associated reticle with a stage micrometer.

The following steps are followed:

Select the lowest magnification ensuring the eyepiece reticle is in sharp focus. Place the stage micrometer on microscope stage. Position and focus so the stage scale is clearly visible. Rotate the eyepiece reticle and position the stage in the field of view so that the two scales appear parallel, one positioned above the other. Then adjust the alignment of the scales on the left side of view so that the zero values correspond with the zero values aligned.

Result and Observations

Conversion Factor

Selected objective magnification X4.

For an eyepiece reticle with 100 divisions, each division will measure 25 µm at the stage for this magnification.

Selected objective magnification X10.

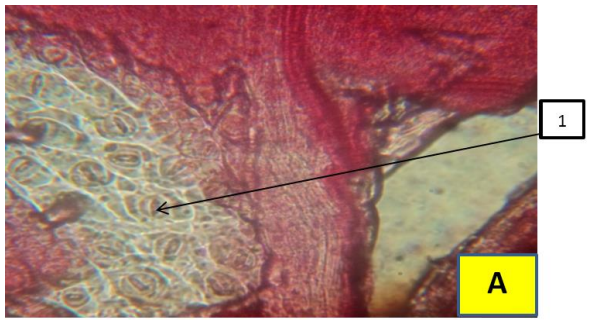
For an eyepiece reticle with 100 divisions, each division will measure 10 µm at the stage for this magnification.

Selected objective magnification X40.

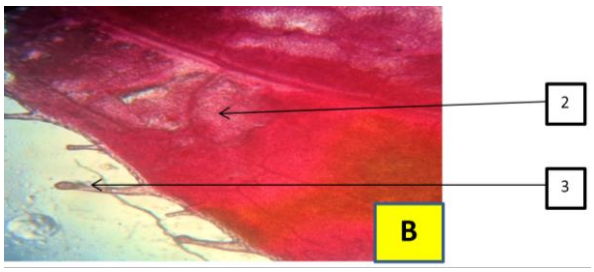
For an eyepiece reticle with 100 divisions the full length of the reticle scale equals 250 µm at the stage for this magnification.

Figure A-K: Microscopical characteristics features of *Cleome viscosa* L.

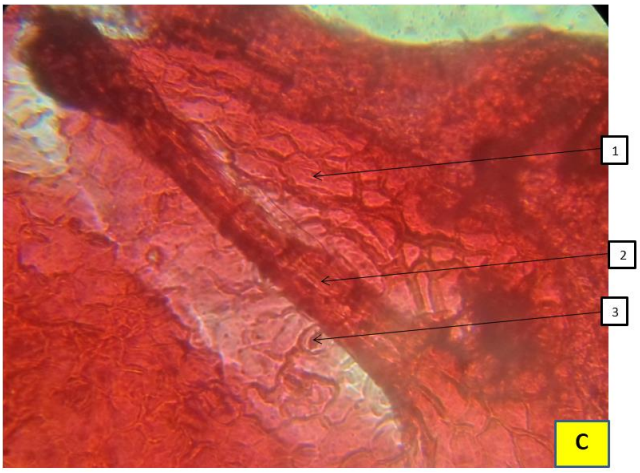
Leaf microscopy



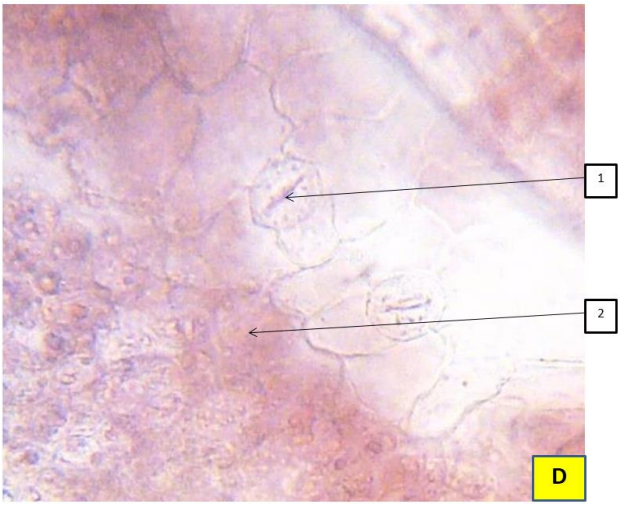
Cleome viscosa abaxial surface of leaf (X40) 1: Anomocytic stomata



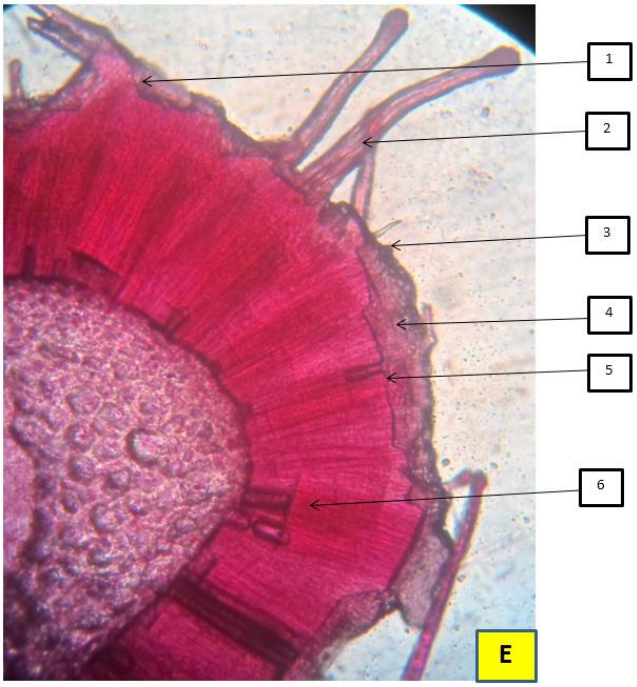
Cleome viscosa abaxial surface of leaf (X40) 2: Epidermal cell, 3: Glandular trichome



Cleome viscosa adaxial surface of leaf (X40) 1: Epidermal cell, 2: Glandular trichome, 3: anomocytic stomata.

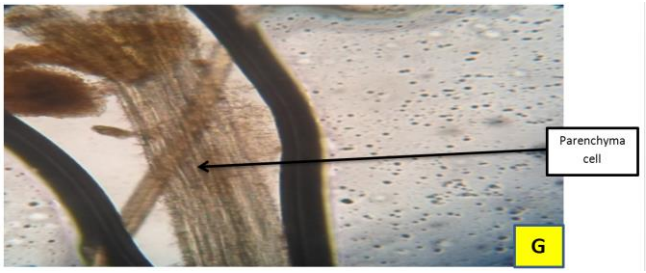


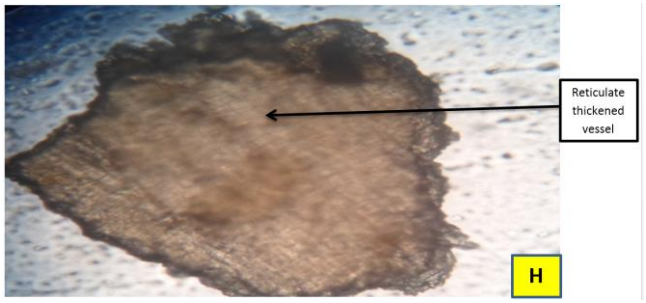
Cleome viscosa adaxial surface of leaf (X40) 1: anomocytic stomata, 2: Epidermal cell.

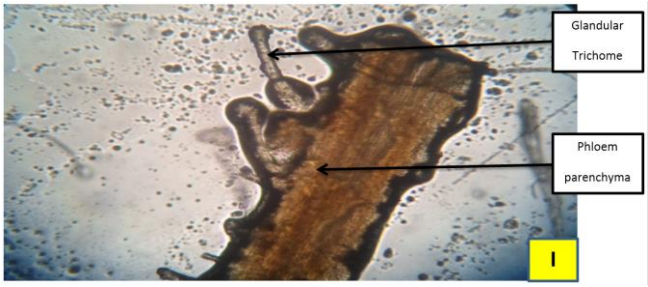


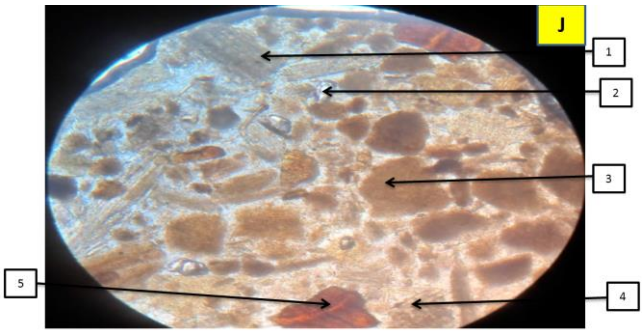
T.S of *Cleome viscosa* stem (X10) 1: Collenchyma, 2: trichome, 3: epidermis, 4: Phloem, 5: Xylem, 6: pericyclic fibre.

Powder Characteristics of *Cleome viscosa* L whole plant (X10).

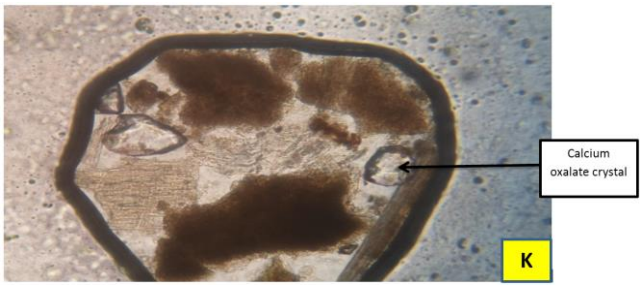








Cell inclusions in powder of *Cleome viscosa* L. whole plant. 1: parenchyma cells, 2: calcium oxalate crystals, 3: cork cell, 4: medullary rays crossing the parenchyma, 5: Reticulate thicken vessel.



Discussion and Conclusion

In recent time, sophisticated modern research tools for evaluation of the plant drugs are available but microscopic method is still one of the simplest and cheapest methods to start for establishing the correct identity of the source materials (Singh *et al*., 2010) [15]. The leaf (upper and lower eidermis), transverse section of stem and powder of *Cleome viscosa* Linn were observed under the microscope for their histological characteristics. In the microscopic studies, the lower epidermis (Abaxial) and upper epidermis (Adaxial) contains stomatas (Anomocytic) and glandular trichomes as the main observable features. The stomata size at X40 objective is 45 µm, while the trichome size is 480µm at X10 objective (Figure A and B). The transverse section of stem of *Cleome viscosa* shows the presence of centrally vascular bundles as phloem surrounds with the xylem, collenchyma, pericyclic fibre, epidermis and also trichomes (Figure E). The microscopic study of powder revealed the presence of Trichomes (480 µm at X10 objective), phloem parenchyma (>1000µm at X10 objective), fibres (900 µm at X10 objective), calcium oxalate crystals (180 µm at X10 objective), reticulate thickened vessel (900 µm at X10 objective), cork cell, medullary ray crossing parenchyma (Figure F-K). Herbal medicine can only stand in commercial market if they are evaluated properly according to modern science. Evaluation of herb involves confirmation of its identity and determination of its quality, purity and nature of adulterant. In future there is need to develop advanced technique for the evaluation of crude drugs thereby, allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities.

Pharmacognostic standardization including morpho-anatomical evaluation is meant for identification, authentication and detection of adulteration and also compilation of quality control standards of crude drugs. Since the plant, *Cleome viscosa* Linn is useful in traditional medicine for the treatment of some ailments, it is important to standardize it for use as a drug. The pharmacognostic characteristics of this plant and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

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