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Micromorphological and histochemical characterization of *Cleome rutidosperma*Wight &Arn.

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Abstract

Histochemical localization of leaf and stem of *Cleome rutidosperma* Wight &Arn. was studied by conventional photonic microscopy. Results of anatomy of leaf and stem showed cellular differentiation. Both the leaf and stem showed secondary growth. The stomata were found to be ranunculaceous type, the cortex and central pith region was occupied by ground parenchymatous cells filled with myrosin cells. Free hand sections of leaf and stem were obtained and treated with respective reagents to localize constituents such as alkaloids, starch, flavonoids, protein, tannin, reducing sugar, phenols and total lipids.

Keywords: *Cleome rutidosperma*, Histochemistry, Myrosin cells, Cleomaceae, Secondary metabolites

INTRODUCTION

Medicinal plants are used as alternate medicine for various diseases of man and other animals. Since most of them are without side effects when compared with synthetic drugs. Identification of the chemical nature of phytochemical constituents present in the medicinal plants will provide some information and their medicinal properties. The present work deals with micromorphological and histochemical studies of *C. rutidosperma*. The genus *Cleome* (Cleomaceae) is represented by 12 species in India. They are commonly found growing as roadside weeds which are reportedly used in traditional systems of medicines. *C. rutidosperma* is an annual herb reproducing solely by seeds. The native of plant is Africa, and flowering and fruiting plants of *C. rutidosperma* could be found throughout the year, although most abundantly in the rainy season. And now the plant has been introduced and become naturalized in tropical and subtropical regions of Asia. It is locally called "Kattukadku". The plant possesses medicinal properties like anthelmintic, (Lakshmi *et al.,* 2011) diuretic, laxative, antimicrobial, analgesic, anti-inflammatory, antipyretic, antioxidant, anti-arthritic and antiplasmodial activities *(*Bose *et al.,* 2010; Chakraborty and Roy, 2010) The present article deals with the microscopic characteristics and histochemistry of *C. rutidosperma.*

MATERIALS AND METHODS

Plant collection

The aerial parts of *C. rutidosperma* Wight &Arn. were collected from Thindal, Erode District, Tamil Nadu. The plant material was identified taxonomically with the help of the local floras (Matthew, 1983; Gamble and Fisher, 1956) and Botanical survey of India, Southern region center, Coimbatore, Tamil Nadu. The herbarium number provided by BSI is "BSI/SRC/5/23/2016/Tech./1343". Voucher specimen is kept in the Herbarium of Vellalar College for Women (Autonomous), Erode-638 012, Tamil Nadu, South India.

Histochemistry

Micromorphological characterization was made by employing standard sectioning and staining methods as per standard procedure described by O'Brien *et al., (*1964).The cell arrangements, size and shape of cell, cell inclusions, synthesis and distribution were localized histochemically in the leaf and stem of study plant using respective reagents and were recorded on a photonic microscope (Model Ax70 TRF, Olympus optical). Fresh free hand sections of stem of *C. rutidosperma* were taken. The fresh hand sections of the plant (leaf and stem) used for the histochemical study were treated with the respective reagents to localize the presence or absence of metabolites, synthesis and their storage area. The reagents used were Mayer's, Dragendorff's and Wagner's to detect alkaloids, 10% ferric chloride for detection of tannins, lead acetate to detect flavonoids, Lugol's iodine solution for starch (Jensen, 1962), Sudan black for total lipids (Brundett *et al.,*1991), anhydrous ferric chloride for phenolic compounds, Fehling's reagents to detect reducing sugars and Biurette for protein. Presence of metabolites

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was confirmed through colour development due to the reaction of the cells with specific reagents.

RESULT AND DISCUSSION

The morphology of *C. rutidosperma* is given in Plate-1. It shows habit of the study plant, fruiting twig and closure view of flower and fruit (Plate-1: a, b, c & d).

Plate - 1

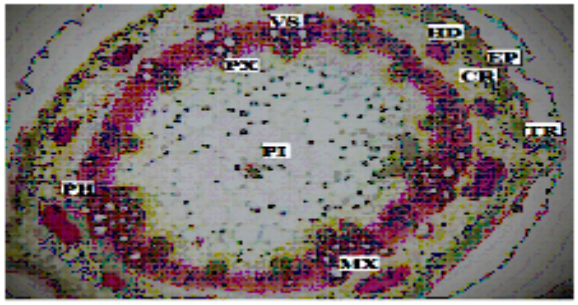
a.Habit of C rutidosperma W&A

b.Flowering twig od Crutidosperma

C.Closure view of flower

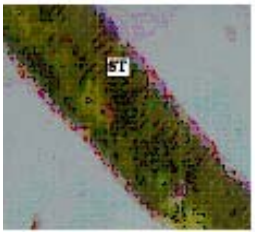
d.Closure view of fruit

Plate - 2



A.T.S OF STEM

B.T.SOFLEAF MiDRIE



C.LAMINA

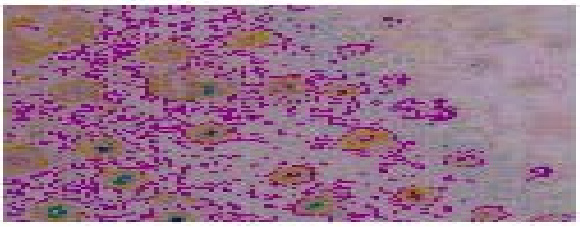
(EP: Epidermis; HD: Hypodermis; CR: Cortex; MX: Metaxylem; PH: Phloem; PX: Protoxylem; VS: Vascular bundle; PI: Pith; TR: Trichome)

(UE: Upper epidermis; LE: Lower epidermis; VB: Vascular bundle; XY: Xylem; PH: Phloem; MS: Mesophyll ST: Stomata)

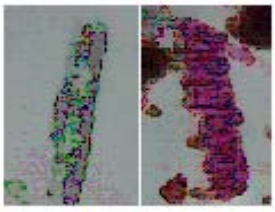
Macroscopical characters

Cleome rutidosperma Wight &Arn. is a perennial herb with angular and pubescent stem. The leaves are trifoliate, petiolate, green, elliptic and glabrous. Flowers are purple, axillary and solitary. Pedicels are slender, sepals are four, pinkish and spreading.Petals are four in number and imbricate. Stamens are 6 in number with elongated anthers,pistil is hairy and glandular. Style is elongated with disc at apex. Fruits are linear capsule, 3cm long. Seeds are eight per capsule.

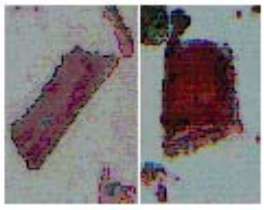
Plate - 3



A.Stomata



B.Tracheids



C.Vessels

Microscopical characters

Micro morphological characters as observed in the T.S of stem and leaves are given in Plate 2-A & B. The T.S of leaf shows the uniseriate lower and upper epidermis, the lower epidermis is pappilose in nature. The ranunculous type of stomata is present. The mesophyll is centric, which are present throughout the laminar region. The midrib shows glandular trichome with centrally located larger vascular bundle with small lateral bundles. The ground tissues of vascular region are filled with myrosin cells. The stem in primary structure shows a uniseriate epidermis and a cortex consisting of loosely arranged parenchymatous cells. The vascular cylinder is organized with proto xylem poles alternated with phloem. The well-developed vessels, medullary rays are also found in the vascular region. The cortex as well as the central pith region are occupied by ground parenchymatous tissue filled with myrosin cells (Plate-3-A,B & C). The vascular bundle is surrounded by the pericycle which represents two or three rows of parenchyma cells. The vascular cambium is composed of a continuous ring of cells with thin walls and wide rays, formed by erect parenchyma cells of various sizes. Aspects related to the morphology and anatomy of *Cissus verticillata* are found in studies on the vitaceae family. (Solereder, 1908 and Metcalfe & Chalk, 1957 and Lizama *et al.,* 2000).

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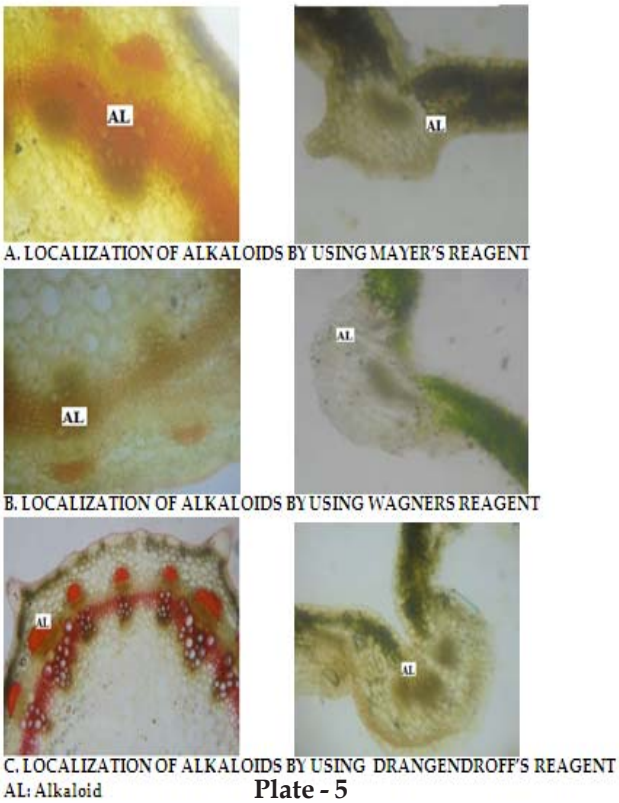
Plate - 4



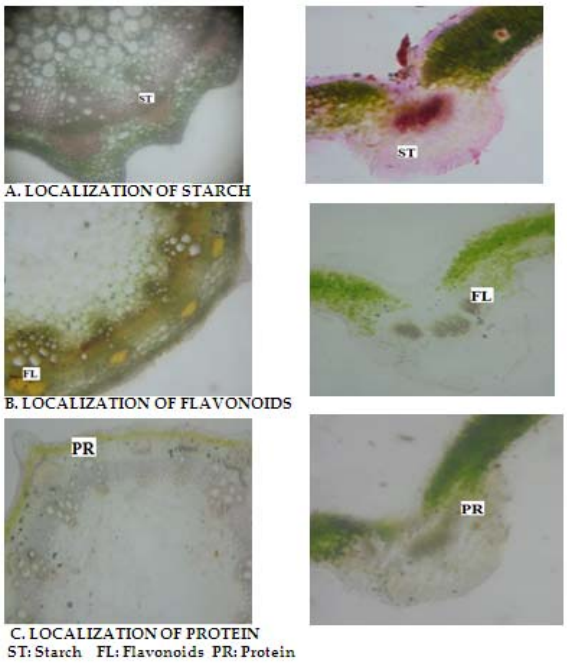
A. LOCALIZATION OF ALKALOIDS BY USING MAYER'S REAGENT

B. LOCALIZATION OF ALKALOIDS BY USING WAGNERS REAGENT

C. LOCALIZATION OF ALKALOIDS BY USING DRANGENDROFF'S REAGENT

AL: Alkaloid

Plate - 5



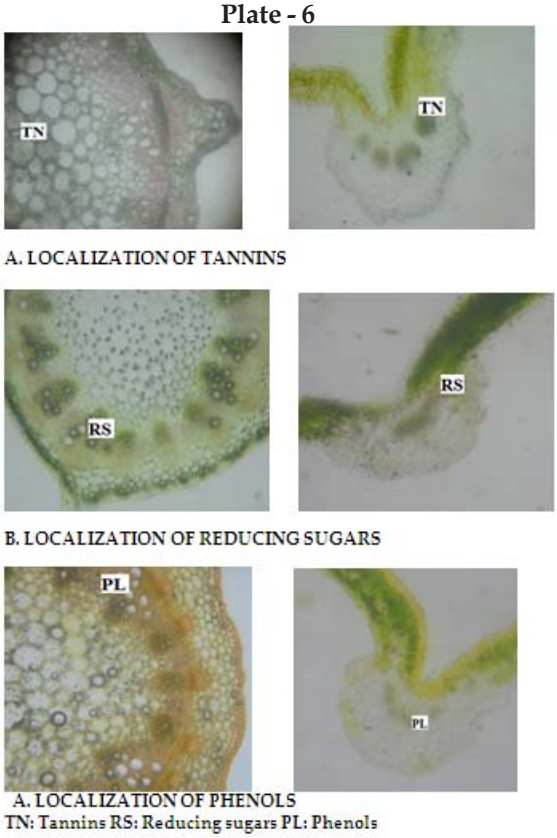
A. LOCALIZATION OF STARCH

B. LOCALIZATION OF FLAVONOIDS

C. LOCALIZATION OF PROTEIN

ST: Starch FL: Flavonoids PR: Protein

Plate - 6



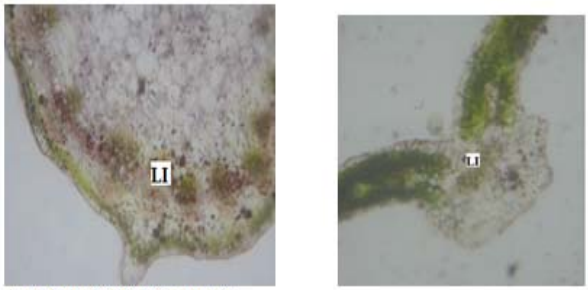
A. LOCALIZATION OF TANNINS

B. LOCALIZATION OF REDUCING SUGARS

A. LOCALIZATION OF PHENOLS

TN: Tannins RS: Reducing sugars PL: Phenols

Plate - 7



A. LOCALIZATION OF LIPIDS

LI: Lipids

Histochemistry

Histochemical localization studies showed a positive reaction to alkaloids, starch, flavonoids, tannins, reducing sugars, phenols and total lipids. The Plate 4-A shows the histochemical localization of alkaloids in the cortical vascular bundles and also secondary tissues in stem. The alkaloid containing cells are turned into reddish brown. It indicates the presence of alkaloids in these cells. The Plate 4-B shows the presence of alkaloids in the region of vascular tissues. The alkaloids containing cells become orange brown. Alkaloids can be found in many plant organs such as roots, stems, leaves, fruits and seeds. Dhale (2011) and Kadam *et a. (*2013) studied the alkaloids in three species. It was shown that alkaloids existed in the epidermis, mesophyll and parenchyma cells of leaf veins. In Plate 4-Cshows the presence of alkaloids, in the epidermal cells and secondary tissues. The alkaloid containing cells are reddish orange, which proves the presence of alkaloids. These results corroborate the results of Marin *et al.(*2008, 2010). The occurrence of starch in stem and leaf is illustrated in Plate 5-A.The localization of flavonoids by lead acetate solution (Plate 5-B) is indicated by the yellow colour

of the cells Plate 5-C shows the localization of protein. Plate 6-A shows the tannin which is evident by the black colour of the cells in the pith region of the stem, hypodermis and vascular region in leaf. Tannins are defined as the naturally occurring plant poly phenolic compounds and are found widespread in terrestrial plants (Waterman and Mole, 1994). The Plate 6-B, shows the distribution of reducing sugar in the epidermal region and vessels, sugar containing cells turn into pale blue. This shows the presence of reducing sugar. Plate 6-C shows the presence of phenols. Reports have revealed that phenolic compounds are the most effective antioxidants in brown algae (Nagai *et al.*, 2003). It has been reported that there was high accumulation of phenolic compounds in the cuticle of *Zuccagnia punctate* which was developed in response to U V radiation as a defense mechanism (Mercado *et al.*, 2013) . Plate 7-A, shows the lipids containing cells in the region of pith and cortex. These secondary metabolites could be involved in various physiological processes such as defense against bioaggressors, redox homeostasis and UV radiation. (Agati*et al*., 2013; Beckmam, 2000; Caldwell *et al*., 1983; Smith and Markham, 1998).

CONCLUSION

Histochemical localization of secondary metabolites in solving taxonomic problems is now a common practice for the identification and characterization of a taxon. The results of the present study confirmed the presence of phenols, alkaloids, starch, tannins, reducing sugars and total lipids in stem and leaf of total lipids in stem and leaf of *C. rutidosperma*, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Hence this study offers a base of using *C. rutidosperma* as herbal alternative for the synthesis of active compounds.

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