

MICROMORPHOLOGICAL AND HISTOCHEMICAL LOCALISATION STUDIES ON AERIAL PARTS OF *CENTRATHERUM PUNCTATUM* Cass. A TRADITIONAL DRUG SOURCE

CHITRA B¹, RAVICHANDRAN NATESAN² AND BRINDHA P^{2*}

¹Department of Biotechnology, Srimad Andavan Arts & Science College, Trichy-5, ²Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur, Tamilnadu, India. Email:brindha@carism.sastra.edu

Received: 23 Nov 2013, Revised and Accepted: 25 Dec 2013

ABSTRACT

Centratherum punctatum Cass., belonging to the family Asteraceae is rich in bioactive molecules that could be useful as anti cancer, antimicrobial, analgesic and wound healing agents. Besides the flowers and leaves were used in hair oil preparations and as skin blackening and antiageing agents. The present study was focused to analyse the micromorphological features of this drug source and to localise the active molecules histochemically. Micromorphological characterization studies were carried out employing standard sectioning and staining methods as per standard texts. Bioactive secondary metabolites specifically, flavonoids and terpenoids were localised histochemically in the aerial parts of plant using specific reagents. The cell arrangements, size and shape of cells, presence of idioplasts, shape and size of the crystals, starch grains, trichome types, stomatal types, stomatal index, stomatal frequency and other specific micromorphological characters were studied using Carl Zeiss microscope and photo micrographs were taken with the help of Prog Res digital camera. The presence of flavonoids and terpenoids were confirmed through color development due to the reaction of the cells with specific reagents. Micromorphological features observations reveals the standardisation of this traditional drug source. Histochemical observations can help in identifying the major chemical constituents. This can be further confirmed by carrying out qualitative tests and developing TLC profiles. These data can contribute in determining the standards for the plant drug under study and can contribute to the Ayurvedic Pharmacopeia of India.

Keywords: *Centratherum punctatum*, Histochemical localization, Idioplasts, Calcium oxalate crystals and stomata.

INTRODUCTION

Nowadays herbal drugs are in great demand than ever before. The public were also of general awareness in regarding the safety and efficacy of herbal drugs. Hence, development of new drugs without any side effect is the urgent need of the society [1]. Quality control studies on plant material are essential to ensure the reproducible quality of the herbal products. The initial step to ensure quality of any starting material is authentication [2]. WHO suggests that the macroscopic and microscopic description of a plant material is the primary step in establishing its identity [3]. Thus, the notch of wholesomeness of plant material should be proved before developing any formulation or using in any disease condition.

Collection and Authentication of Plant Material

The leaf, stem, and seeds of *Centratherum punctatum* were collected from herbal garden of Srimad Andavan college, Thiruchirappalli. The plant was identified with the help of Flora of Presidency of madras and authenticated at RAPINAT HERBARIUM, St Joseph's College, Trichy, Tamil Nadu, India. After authentication, the different parts were collected, dried in shade and then milled into crude powder by a mechanical chopper and subjected to botanical and histochemical studies.

Micromorphological and Histochemical analysis

Fresh free hand sections leaf, stem, and seeds of *C.punctatum* were taken. The fresh sections of the plant (leaf, stem, and seeds) used for the histochemical study were treated with the respective reagents to localize the presence or absence of metabolites like oil, mucilage, starch grains, phenol, lignin, chitin, suberin, alkaloids, flavanoids and tannin. Toluidine Blue used to detect lignin(4), Sudan black to detect oil globules, Iodine potassium iodide to detect starch grains and, ruthenium red to detect carbohydrates (5) DPH to detect terpenoids(6) and Neu's reagent to detect flavonoids(7). The stained sections were viewed under Carl Zeiss microscope and photo micrographs were taken with the help of Prog Res digital camera. The stained sections were photographed and observed at different magnifications for their characteristic features.

RESULT AND DISCUSSION

The occurrence and distribution of various metabolites such as

terpenoids, flavonoids, oil stem, leaf and seeds of *C.punctatum* were studied and presented (Fig. 1-12). Transverse section of the leaf observed in transverse sections showed central and two lateral wings (Fig.10.). The cells of outer layer are thin walled, smooth bearing appendages called trichomes. They are both multicellular and uniseriate (Fig.1). Terpenoids are copiously present in the outer layer of the epidermis and in trichome (Fig.2&5). The central part of the leaf in transverse section showed thin walled parenchymatous and oblong thick walled cells. Leaf cells also revealed the presence of terpenoids (Fig.6). In xylem regions flavonoids were located using NEU reagent and by observing through fluorescent microscope at 365nm (Fig.3). Parenchyma cells and angular xylem vessels (Fig.7) were observed in the T.S of stem. Calcium oxalate crystals were also seen in the phloem regions (Fig.8). When sudan black was used some of the parenchyma cells stained black (Fig.9). TS of seeds revealed the presence of oil containing cells (Fig.11).. Enlarged view of seed cells with oil globules (Fig. 12.). Lignin containing pith cells were abundant in pith region.

Microscopic and histochemical observations

In recently application pharmacognosist have started [12-17] histochemical features in solving taxonomic problems, the identification and characterization of plant drug. The present study results confirmed the presence of terpenoids, flavonoids and oils in different parts of the *C. punctatum* which could be used as a diagnostic tool for this plant drug. Flavonoids and terpenoids are compounds which are present commonly in plants, have been reported to have a wide range of biological activities including antioxidant properties. The presence of terpenoids and flavonoids in this study were also confirmed by the qualitative analysis of *C.punctatum* and hence supported the therapeutic potentials of the plant drug under study. Finding from this study are promising as terpenoids and flavonoids compounds are effective antioxidants, therefore they could have potential therapeutic applications. The cell arrangements, size and shape of cells, presence of idioplasts, shape and size of the crystals, starch grains, trichome types, stomatal types, stomatal index, stomatal frequency and other specific micromorphological characters were studied using Carl Zeiss microscope could be used as microscopic standards for this drug Central region is concave in ventral region and slightly convex in dorsal region. The presence of alkaloids, flavonoids and terpenoids were confirmed through color development due to the reaction of the cells with specific reagents provided chemical markers that could be used in identification.

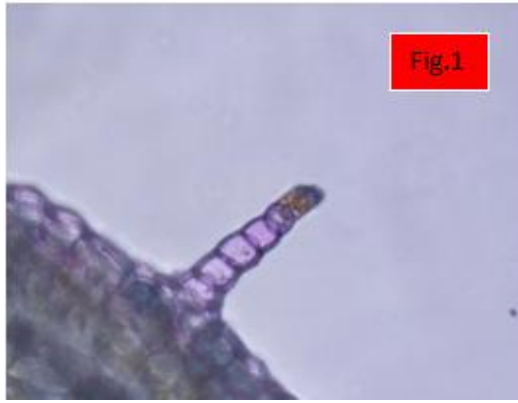


Fig. 1: Uniseriate trichome

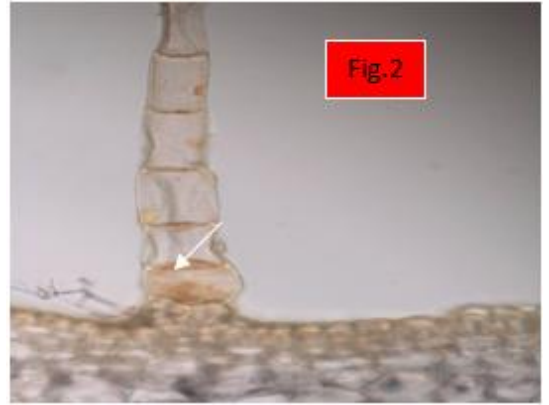


Fig. 2: Terpenoids in trichome

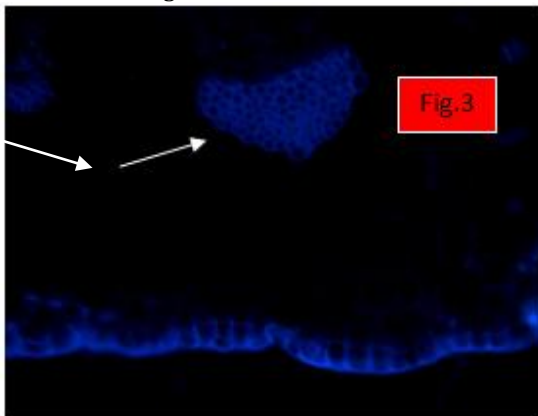


Fig. 3: Flavonoids in trichome

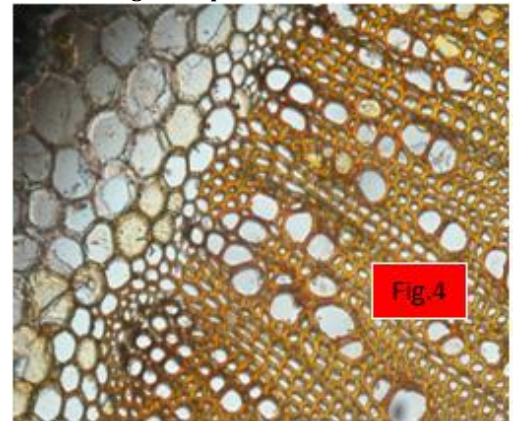


Fig. 4: T.S. of stem a portion enlarged

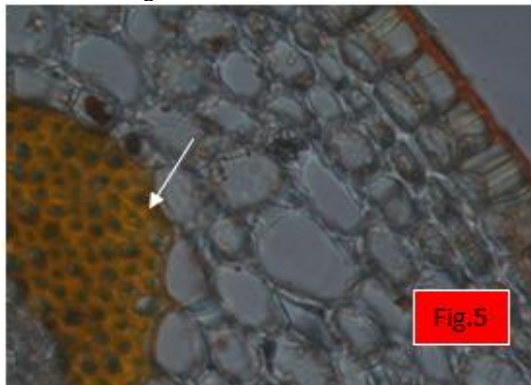


Fig. 5: T.S. of stem a portion localisation of terpenoids in cortical and vascular regions.

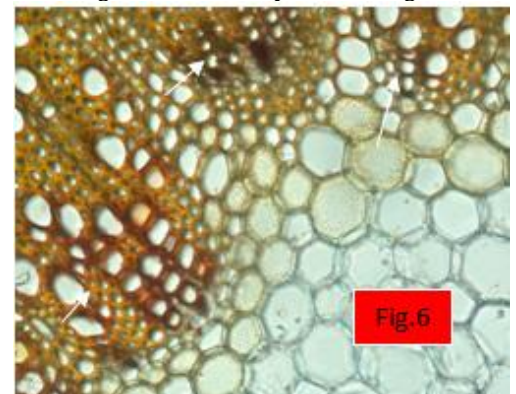


Fig. 6: Terpenoids in xylem

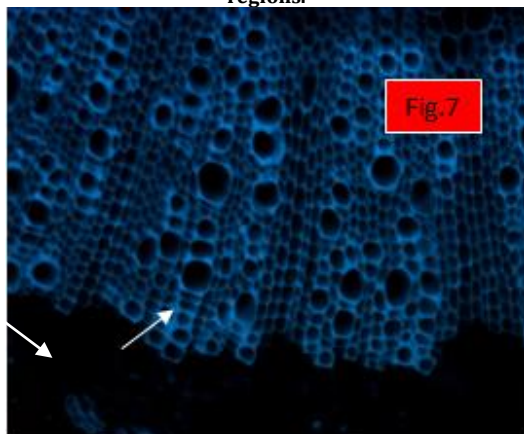


Fig.7. Terpenoids in stellar region

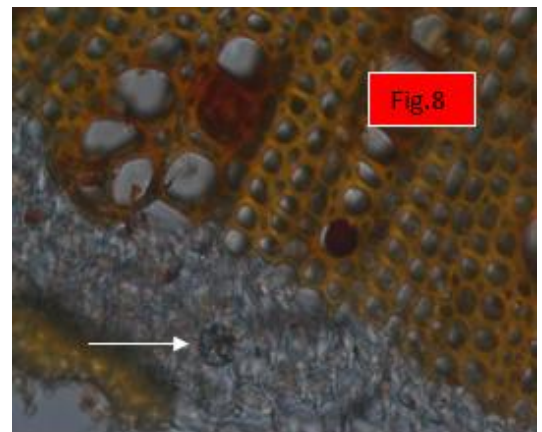


Fig.8. Phloem portion enlarged

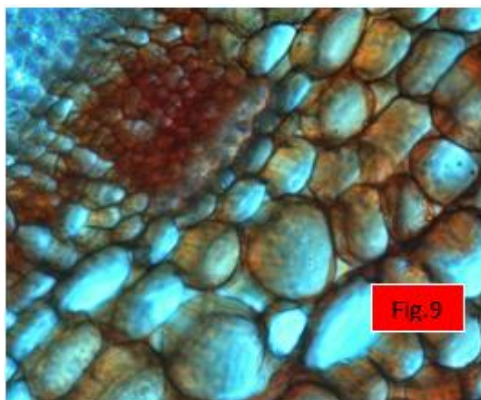


Fig. 9: Oil containing cells



Fig. 10: T.S. of Leaf

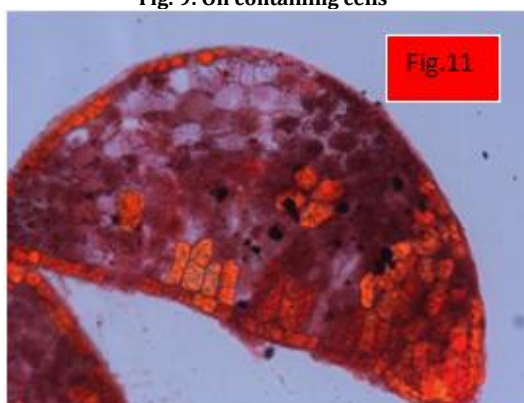


Fig. 11: T.S. of seed showing oil containing cells.

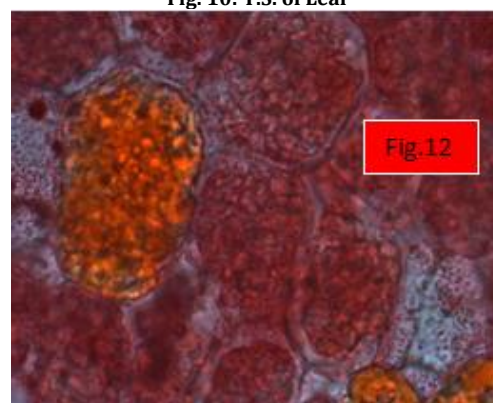


Fig. 12: Oil globules.

In the present investigation, we have reported the data obtained on histochemical studies on *C.punctatum* leaf, seed and stem and concluded the presence of polyphenols, terpenoids, flavonoids, tannins and lignin, which are of great medicinal value and finds extensive use in the drug and pharmaceutical industry. Results from this work therefore supports that the *C.punctatum* could be used as an alternative in the management of various ailments such as anticancer and wound healer.

REFERENCES

- Hussain SZ and Sheikh SA, Ethno-medicinal survey of plants from salt range of Pakistan. Pak. J. Bot. 2008; 40(3): 1005-1011.
- Lalithrani S, Mohan VR and Maruthupandian A, Pharmacognostic investigations on *Bulbophyllum albidum* (Wight) Hook.f. International Journal of Pharm Tech Research. Pharmtech., 2011; Vol.3(1): 556-562.
- Anonymous, Macroscopic and Microscopic examination: Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1998.
- O'Brien Tp, Feder N and Mc Cull ME, Polychromatic staining of plant cell walls by toluidine blue-O. Protoplasma.1964; 59: 364-373.
- Johansen DA . Plant microtechnique. 1st edn. (New York, London McGraw-Hill), 1940.
- Ganter P, Jollés G .Histochimie normale et pathologique. (Gauthier-Villars, Paris), 1969.
- Neu R. A new reagent for differentiating and determining flavones on paper chromatograms. Naturwissenschaften, 1957; 43: p. 82.
- RR Sederoff, JJ MacKay, J Ralph, RD Hatfield. Curr Opin Plant Biol 1999; 2: 145-152.
- Jorin, E De Ruck J, Serghini K, Perez De Luuque A, Munoz-Garcia J, Garcia-Torres L, Castejon M. Biochemical aspects of the parasitism of sunflower by Orobanche. In Oreno, MT, 1996; 551-8.
- Heldt HW. Phenylpropanoids. Oxford University Press. Oxford, UK, 1997; pp. 377-393.
- Mbagwu FN, Unamba CIN, Onuoha CI, Ezeibekwe IO. Histochemical Studies on Five Variants of Viscum L. (Loranthaceae). Research Journal of Biological Sciences 2009; 4(3): 254-257..
- Edeoga HO, Omosun G, Osuagwu GGE, Emezue OO. Micromorphology of the Leaf Cuticle in Mimosa Species (Leguminosae-Mimosoideae). Asian Journal of Plant Sciences 2007; 6(4): 688-691.
- Hassan EA, El-Akkad SS, Moustafa SM, El-Awadi ME. Histochemical aspects of penetration and vascular connection of *Broomrape haustoria* in the host root, and the possible implication of phenylpropanoids. International Journal of Agriculture & Biology 2004; 6(3):430-434.
- Senthamarai R, Ismail AM, Shri Vijaya Kiurbha T, Balasubramanian P. Pharmacognostical and phytochemical studies on leaves of *Stephania japonica* Linn. Journal of Chemical and Pharmaceutical Research 2012; 4(3):1457-1464.
- Shri Vijaya Kirubha T, Senthamarai R, Mariya P, Praveen Mani. Pharmacognostical and phytochemical standards of *Cyclea peltata* (Lam) Hook.f & Thomson Leaves. Journal of Chemical and Pharmaceutical Research 2012; 4(3):1465-1469.
- Balasubramanian M. Study on phytochemical screening and antibacterial activity of *Nyctanthes-arbor tristis*. Journal of Chemical and Pharmaceutical Research 2012; 4(3):1686-1695.
- Suganya S, Bharathidasan R, Senthilkumar G, Madhanraj P, Panneerselvam A. Antibacterial activity of essential oil extracted from *Coriandrum sativum* (L.) and GC-MS analysis. Journal of Chemical and Pharmaceutical Research 2012; 4(3):1846-1850.