J. Sci. Trans. Environ. Technov. 2023, 16 (4) : 12 - 19

MICROMORPHOLOGICAL AND PHYTOCHEMICAL STUDY OF *Lepidagathis cristata*, WILLD (ACANTHACEAE)

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https://doi.org/10.56343/STET.116.016.004.003
www.stetjournals.com

Article History
Received: **10.04.2023**
Revised and Accepted: **12.05.2023**
Published: **19.06.2023**

ABSTRACT

Objective : This study determines various micromorphological characters and phytochemical compounds helpful to ensure the purity, safety, and efficacy of medicinal plant *Lepidagthis cristata* .

Methods : The micromorphological study was carried out using SEM and ethanolic extracts of *L. cristata* was subjected to qualitative tests for the identification of phytochemical constituents using standard procedures.

Results : Micromorphological studies using SEM images showed the presence of scales, prismatic crystals, microhair , diacytic stomata with distinct ledges, cystolith, glandular and non-glandular trichomes in root, stem and leaves. Anther has oblong bitheca with small upper theca and large lower theca surrounded by many unicellular sharp spurs. Pollen grains images have shown tricolporate, prolate, colpus with long narrow apertures and reticulate exine ornamentation. Phytochemical analysis revealed the presence of reducing sugar, saponins, triterpenoids, alkaloids, tannins, flavonoids and ascorbic acid.

Conclusions: The results of current study could be served as a diagnostic tool for the standardization of this medicinal plant and will helpful in characterization of the crude drug.

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Keywords: Micromorphological, diacytic stomata, ledges, cystolith, tricolporate, prolate, colpus, saponins, triterpenoids, alkaloids, tannins, flavonoids and ascorbic acid.

INTRODUCTION

Medicinal plants are the richest resource of drugs of traditional system of medicine, modern medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al*., 1999). India is one of the world's 12 biodiversity centers with the presence of over 45,000 different plant species. However the primary obstacle being used of conventional medicine in the developed nations is absence of proof of documentation and stringent quality control measures. There is need of documentation for the research work carried out on traditional medicines. Standardization and quality control are essential analytical tools to assure the correct identification of herbal raw materials which will help to affirm its safety and efficacy (Akbar *et al*., 2014). For this reason we have done micromorphological and preliminary phytochemical investigations of *Lepidagathis cristata* Willd (*L.cristata*) belonging to the family Acanthaceae in the present study. It is a medicinal herb used as bitter tonic for fevers , pneumonia, flu, mouth infections (Kumar *et al*., 2014), eczema, psoriasis and other skin infections (Siva Sakthi *et al*., 2013; Abubacker and kamadevi, 2014). The ash of whole herb is applied externally on chronic wounds of pet animals (Salave, 2012). The roots of the herb are used in stomachic and dyspepsia, leaves are used for fevers and the inflorescence ash is used for itchy affections of skin and burns (Singh, 1983, Madhava chetty,2005). Leaf juice with copper sulphate is given during snakebite for gaining consciousness (Sikarwar *et al*., 2008).

P-ISSN 0973-9157
E-ISSN 2393-9249

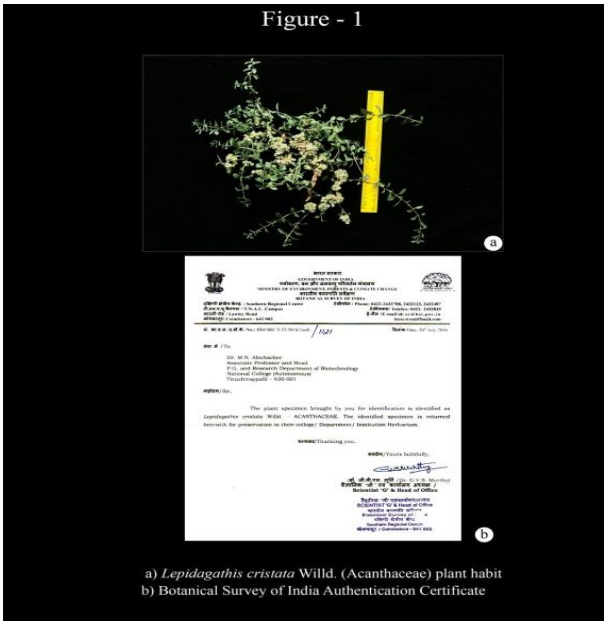
The plant is a stiff herb and the branches procumbently arise from a hard central rootstock. Leaves are alternate, elliptic, serrate and usually lineolate. Flowers are sessile, capitate, the heads terminal or axillary densely crowed at the base of the plant, fruits glucose capsule (Gamble, 1967; Pullaiah, 2002). This medicinal herb has been exploited tremendously by common people in many ways for various curative purposes. It is necessary to evaluate the herb in a scientific base for its potential use of folk medicine.

Materials and methods

Collection and identification of plant materials

Fresh plants of *L. cristata*, were collected from Pachhaimalai Hills, Tiruchirappalli District, Tamil Nadu, India. The taxonomic identities of the plant were confirmed by Botanical Survey of India (BSI), Coimbatore (Figure-1).

Figure - 1



a) *Lepidagathis cristata* Willd. (Acanthaceae) plant habit
b) Botanical Survey of India Authentication Certificate

The plant material was washed under running tap water; air dried in shade and was homogenized to fine powder and stored in sterile air tight bottles for the experimental use.

Micromorphological Studies with Scanning Electron Microscope (SEM)

Micromorphological studies were conducted in *L. cristata* the plant material was dried in shade and then oven at 40 ^{o}C for 2 hours (Saraf Aparna and Samant Aruna, 2014). Then the root, stem, leaf (both upper and lower surfaces), inflorescence, flower and pollen were mounted separately on steel stabs using double side adhesive tape and sputter coated with a thin layer of gold under vacuum for 50 seconds to increase the electron conduction and to improve the quality of the integrated digital image acquisition system (Make: SEM-TESCON, Czechoslovakia).

Phytochemical analysis

The phytochemical components such as reducing sugar, saponins, triterpenoids, alkaloids, phenolics, tannins and flavonoids (Brindha *et al*., 1981) were screened using standard procedure.

Results

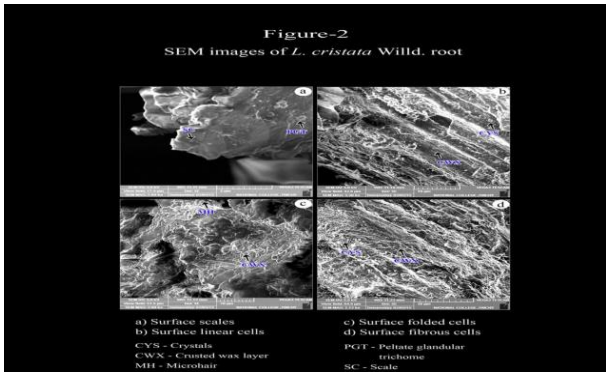
Micromorphological Study of *L. cristata* with SEM Images

In *Lepidagathis cristata*, the micromorphological characters of root, stem, leaf, infloresence, flowers, anther and pollen grains were carried using SEM images (Table 1)

Root Micromorphology

Root surface showed many scales, linear folded and fibrous cells with crusted wax layer, crystals, microhair and peltate glandular trichomes (Fig. 2a, b, c , d).

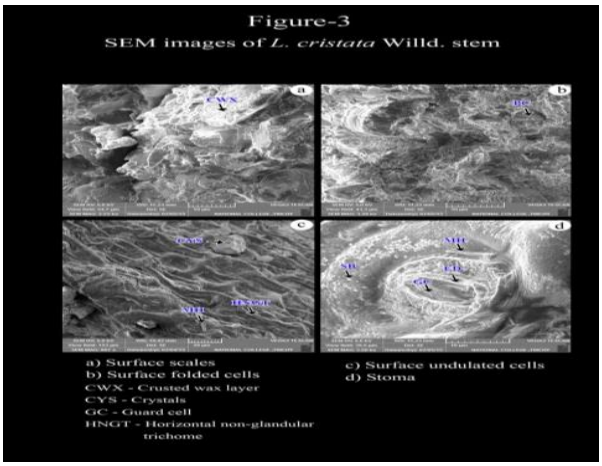
Figure-2
SEM images of *L. cristata* Willd. root



a) Surface scales
b) Surface linear cells
CYS - Crystals
CWX - Crusted wax layer
MH - Microhair
c) Surface folded cells
d) Surface fibrous cells
PGT - Peltate glandular trichome
SC - Scale

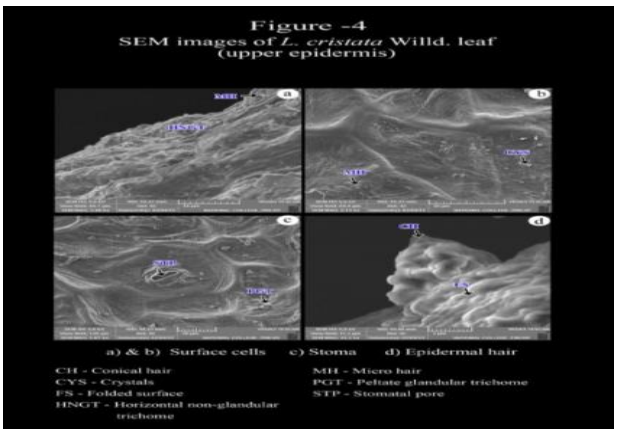
Stem Micromorphology

Stem surface was revealed with rough crust of wax layer with crystals, prismatic crystals, horizontal unicellular non-glandular trichomes, microhairs and various size crystals were scattered. Diacytic stomata with two subsidiary cells situated at the two poles of the guard cells. The cuticular surface extended to form ledge or lip forms around the stomatal pore and small microhairs distributed around the stomata, they were slightly sunk on the epidermal cells (Fig.3a, b, c , d).

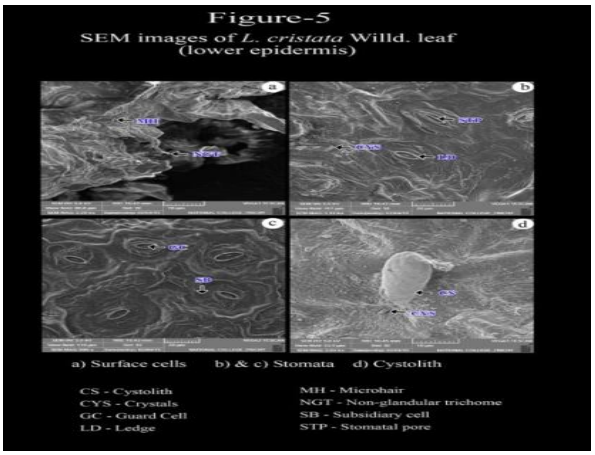


Leaf Micromorphology

Upper Epidermis Horizontal unicellular non-glandular trichomes, microhairs and crystals were scattered on the upper surface of leaf. Diacytic stomata with stomatal pore surrounded by ledge were present. Peltate glandular trichomes and single conical hair were distributed (Fig. 4a, b, c ,d).

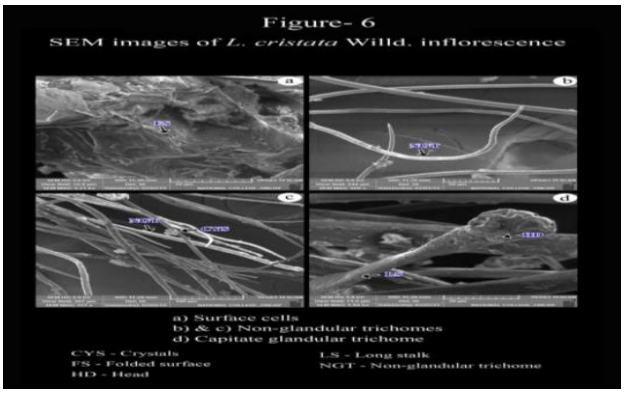


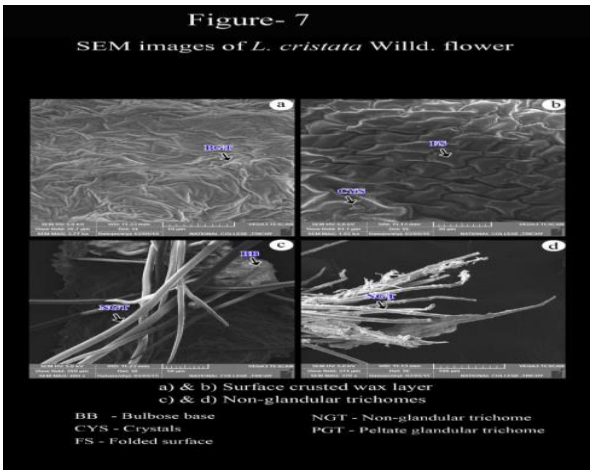
Lower Epidermis Non-glandular unicellular trichome, microhairs, stomata with ledges and crystals were present. Two diacytic stomata with cuticular ledge and crystals of various size were observed on the lower epidermis. Narrow, elongated, solitary cystolith with one blunt extremity attached at the right angle to the leaf axis (Fig.5a, b, c, d).



Inflorescence and Flower Micromorphology

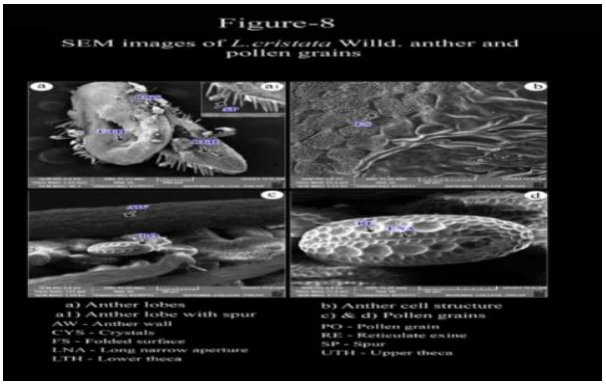
Inflorescence have shown the presence of many surface cells with folded surface, many unicellular non-glandular trichomes with crystals were distributed. A single capitate glandular trichome with long stalk and terminal multicellular head were present, which is characterized with peltate surface. Flower showed crusted wax layer with many irregular foldings and very short stalked peltate glandular trichomes with multicellular terminal. Many unicellular non-glandular trichomes with bulbose base were distributed (Fig. 6a, b, c, d and 7a, b, c, d).





Anther and Pollen Micromorphology

Anther bithecate, theca oblong, small upper theca and lower large theca surrounded by many unicellular spurs and crystals. Anther wall with folded surface was observed. Many tricolporate, prolate, colpus pollen grain with long narrow apertures with coarsely reticulate ornamentation were present (Fig.8a, b, c , d).



Phytochemical	Result
Reducing sugar	+
Saponins	+
Triterpenoids	+
Alkaloids	+
Phenolics	+
Tannins	+
Flavonoids	+

Table- 1 Results of the phytochemical constituent test of *L. cristata* extract

Discussion

According to World Health Organization, the macroscopic and microscopic descriptions of a medicinal plant is the first step towards establishing the identity and degree of purity of such materials should be carried out before any tests are undertaken (Venkatesh *et al*., 2004; WHO, 1998). Herbal drugs play an important role in healthcare programs in developing countries. Use of micromorphology and anatomy is now recognized tool in the field of plant systematics (Sudipa Nag *et al*., 2013). Scanning Electron Microscope (SEM) studies strengthen morphological description of surface characteristics of stem, leaves, pollen, floral parts, fruits and seeds revealing both conservative and variable data thus helping in studying various aspects of biodiversity. Moreover, it acts as tools for correct identification of crude drugs to ensure quality control and prevention of adulteration (Ambarish Mukherjee and Jaydeep Acharya, 2014). Root surface of *L. cristata* showed many scales, linear, folded and fibrous cells with crusted wax layer, crystals and prismatic crystals. Stem surface was revealed with rough crust of wax layer with crystals. Prismatic crystals were distributed. Horizontal unicellular non-glandular trichomes, microhairs and various size crystals were scattered. In *L. cristata* stomata are distributed in stem and leaves. In leaves stomatal distribution is amphistomatic; nevertheless, the stomata on upper epidermis were very sparsely scattered. In both stem and leaves stomata were diacytic with cuticular ledges. It prevents water loss by sealing the pore when stomata is closed and prevent water droplets entering when the pore is open. It tilts its orientation to help to open and close stomatal pore (Fricker and Wilmer, 1996; Zhao and sack, 1999; Kozma and Jenks, 2007). Similar observations were noticed in many Acanthaceae plants with two subsidiary cells situated at the two poles of the guard cells covered with cuticular ledges. Diacytic stomata were reported in *Phlogacanthus thyrsiflorus*(Barnali Dutta and Borthakur, 2014); *Acanthus illicifolius*(Poompozhil and Kumarasamy, 2014) and *Dipteracanthus prostrates*(Iyyappan and Mounnissamy, 2011). Cystoliths are silicified bodies with cellulose skeleton or occasionally not encrusted. They are generally found in vegetative parts in several Acanthaceae species. They vary in nature, shape, size, colour and occurrence throughout the family (Costa *et al*., 2009).

Crystals in cystoliths, may serve as a deterrent against grazing by large herbivores and insects. Shape, size, location and quantity of crystals determine defense efficiency and resilience (Patil and Patil, 2011). Occurrence of cystoliths in the vegetative parts is considered characteristic for the family Acanthaceae. Even their presence is also marked out in some taxonomic accounts (Lerstern and Horner, 2006). The present study has revealed that the presence of solitary cystoliths on the lower leaf surfaces. Crystallization is the most common way by which plants neutralize abundant calcium absorbed in ionic solutions and these crystals remain even when water vapour transpires and are a common phenomenon in higher plants (Barthlott *et al*., 1998). The micromorphological images reveal such crystals in root, stem, leaf and anther surface in *L. cristata*. SEM images have shown crusted wax layers in root and stem in *L. cristata.* The epicuticular wax structures shown a considerable ultrastructural and chemical diversity and also have taxonomic significance (Nurul-Aini *et al*., 2014). Crust wax layers were distributed in both upper and lower surfaces in leaves of *Acanthus ebracteatus*,*A. ilicifolius*, *A. montanus, Andrographis paniculata, Asystasia gangetica* subsp. *micrantha* and *Peristrophe roxburghiana* (Dipa and Daniel, 2011).

The trichomes have their own taxonomic significances as viable taxonomic markers (Werker, 2000). Their morphological features, location and mode of secretion are varied (Bhatt *et al*., 2010). It has been reported that the type and density of trichomes differ among species and can vary in organs of the same plant (Metcalfe and Chalk, 1950). Glandular trichomes produced essential oils in order to protect the aerial parts of the plants against herbivores and pathogens (Werker, 1993). The secretory products in peltate trichomes remain trapped in a large sub-cuticular cavity, and this will rupture if there are external factors such as high temperatures, low air humidity or animal aggression (Ascensao *et al*., 1996). *L.cristata* has glandular and non-glandular trichomes distributed in root, stem, leaf and inflorescence with different shapes and sizes.

Pollen morphology is presently a global accepted tool in consideration of plant taxonomy and evolution, and with the SEM images of pollen providing information on finer architecture, the application of pollen in comparative morphology has become possible in gaining new knowledge at varietal and even ecosystem levels (Nair, 1964). The constant features and the sculpturing of the exine make pollen grains a highly recognizable object by which parent genera or even species may be recognized (Harris, 1995). Acanthaceae is a eurypalynous family, there is a remarkable diversity in the pollen morphology in various characters such as pollen size, shape, aperture (colpi or pore, simple or compound) and tectum (Radlkofer, 1883, Lindau, 1893, Lindau 1895).

There are 26 different pollen types within the family Acanthaceae (Muller *et al*., 1989). The tribal classification of the family Acanthaceae is also well correlated with pollen morphology (Anjum and Qaiser, 2010). The SEM image of micromorphological characters of pollen of *L. cristata* have shown reticulate exine and long narrow aperture are very much similar to the pollen of *Lepidagathis incurve, L. trinervis, L. lutea* and *Eranthemum pulchellum* (Anjum and Qaiser, 2010).

Phytochemical screening was carried out to assess the chemical composition of root, leaf and inflorescence extracts of *L. cristata* by preliminary phytochemical test and found that reducing sugar, saponins, terpenoids, alkaloids, phenolics, tannins and flavonoids are present. Phytochemical constituents and several other aromatic compounds are secondary metaboloites of plants. Similar results were obtained in various Acanthaceae plants, *Acanthus montanus* (Andrew and Chinedum, 2018), *Phaulopsis imbricate* (Kengne *et al.,* 2016), *Asystatia travancorica* (Ouedraogo *et al.,* 2017*)* and *Odontonema strictum* (Komalavalli *et al.,* 2014).

Conclusion

In this current study, the micromorphological and preliminary phytochemical screening of *Lepidagathis cristata* was carried out. The results of current study could be served as a diagnostic tool for the standardization of this medicinal plant and will helpful in characterization of the crude drug.

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