**Floral Micromorphological and palynological studies of medicinally important Plectranthus scutellarioides (L.) R. Br (Lamiaceae) from Assam (Udalguri) using light and scanning electron microscopy.**

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**Abstract**

The palynology and distribution of the trichomes, on the aerial parts and anatomy of Plectranthus scutellarioides from Udalguri district of Assam were studied in order to understand the significance of these features in systematics. The present work on Plectranthus scutellarioides thus provides an empirical basis for the identification of the species. Micromorphological features such as epidermal cell, stomata and trichomes were also studied under both light microscope and scanning electron microscope. The main types of trichomes - peltate, glandular and non-glandular were observed in leaves. Different features of pollen were observed and recorded. Photographs and Micrographs were also provided. All the observations were done under Light microscope and Scanning Electron Microscopy (SEM). The research provides the first detailed descriptions of floral micromorphological and palynological characteristics for Plectranthus scutellarioides, contributing to the empirical identification of the species. The findings offer valuable insights into the structure, size, and shape of stomata, as well as trichome diversity and pollen characteristics

Key words: Plectranthus scutellarioides, pollen, Micromorphological , palynological, stomata and trichomes.

**Research Highlights**

• We provided the first detailed descriptions of the floral micromorphological and palynological characters of the **Plectranthus scutellarioides (L.) R. Br (Lamiaceae) from Assam (Udalguri) using light and scanning electron microscopy**.

• Our findings provide valuable information regarding the structure, size, shape of stomata and their abundance on the leaves of the species Plectranthus scutellarioides. It also gives detailed information of types of trichomes, their size variance and also about pollengrains of the same species.

• We believe our study makes a significant contribution to the literature, as our findings will contribute to enhancing the taxonomical classification of species within the genus **Plectranthus**.

This results will be of value to researchers studying the future biology and taxonomy of species within the family **Lamiaceae**, particularly those using different microscopic techniques, including **scanning electron microscopy** (SEM).

1. **Introduction-**

Plectranthus scutellarioides (L). R.Br. is commonly called as Coleus comprising of more than 173 varieties all over the world (Astuti et al, 2019). It is considered to be native to Southeast Asia, New Guinea, and Northern Australia and was later introduced to other parts of the world for the cultivation of ornamental and medicinal aspects. The genus Plectranthus is one of the largest genera of the family Lamiaceae, belonging to the subfamily Nepetoideae, tribe Ocimeae (Musila F.M. et al., 2017) and is Paleotropical comprising around 300 species of annual or perennial herbs or subshrubs often succulent, aromatic and distributed in the tropical and warm regions of the world. (Musila F.M. et al., 2017). Lamiaceae comprises 7,800 species and is considered one of the largest botanical families. Due to the complexity in the taxonomy of the family, many changes in both delimitation and organization of constituent taxa have been made (Almeida *et. al*,202). The genus Plectranthus is one of the largest genera of Lamiaceae, belonging to the subfamily Nepetoideae, tribe Ocimeae, subtribe Plectranthinae. It comprises about 300 species distributed in both tropical and warm regions of the Old World. Plectranthus includes ornamental, edible , medicinal and ethnobotanical species ( Khalik *et. al,*2016).

The name of this genus derives from the Greek words “Plectron” meaning ‘spur’ and “anthos” meaning ‘flower’, in reference to the spur-shaped flowers of some members of the genus (Stearn, 1992). Its specific epithet refers to its perceived similarity to the Scutellaria plant, which contains a pouch on the fruiting calyx resembling ‘scutella’ (Stearn, 1992).

Plectranthus scutellarioides is an evergreen, perennial, aromatic plant growing mostly in shady regions. Plant is considered as native to Southern Asia with wide distribution throughout India, Burma, Australia, China. The plant is widely cultivated outdoors in both tropical and sub tropical regions as an ornamental gardens. The plant is 60-70 cm tall and bushy in appearance. The plant is widely grown as an ornamental plant for its unique decorative, variegated and colorful leaves. The leaves are membranous characterized by different shades of colour due to thepresence of different types of pigments. The inflorescence is typical verticillaster or irregularly branched cymes in simple branched of 7-11 cm long with, small blue to purple erect flowers. Flower are terminal racemes or panicles, bract ovate, calyx throat is oblique with lateral lobes and corolla is straight tube like, filaments of stamen usually united and style bifid.

The plant is used in the treatment of dyspepsia, ophthalmic, headaches, bruises etc. It is also used as abortifacient and is also used as a repellent for intestinal worms. The roots are used in treatment of diarrhea and colic. The juice from the leaf of the plant is applied in sores and also in leprosy. The plant is used in various traditional medicines (Hanelt et al., 2001) as it has plenty of medicinal properties viz. anti-inflammatory, immune-stimulating, anticancer, hepatoprotective, antioxidant, anti diabetic, antinociceptive effect and antibacterial activities (Mustarichie at el., 2017).

Most of the species of Lamiaceae consists of high content of essential oils present in the glandular hairs. Essential oil is widely used in preparations of pharmaceutical items like cosmetics and perfumery. Only a very few investigations have been reported on the types and functions of trichomes in Plectranthus (Abu-Asab and Cantino, 1987, Bhatt et al., 2010, Waly et al.,2012 and Ascensao et al.,1999). The detailed investigation on glandular and non-glandular trichomes and palynological studies were needed, to know the significance of these features in systematic purposes. The present investigation deals with micromorphological characters of leaves and pollen characters of Plectranthus to support their practicability in systematic.

**Plate I :** Plectranthus scutellarioides. (Fig:1) plant in their natural habitat, (Fig:2) Inflorescence of the plant.

**2.Materials and methods**

2.1. Plant material - Plectranthus scutellarioides leaves are obtained from natural populations of Udalguri (26.7460° N, 92.0959° E), Assam. . Determination and taxonomic identification was made from the Department of Botany, The Assam Royal Global University, Guwahati-781035, Assam, India.

2.2. Light microscopy procedure -

For Light Microscope, foliar micro morphological study of leaves is done to observe the different types of trichomes, epidermis, nature and distribution of stomata, guard cells. Fresh or preserved (formalin-acetic-ethanol -1:2:1 solution) fully matured leaves of Plectranthus scutellarioides were taken. The temporary slides leaves were prepared following the techniques of Boulos and Beakbane (1971). From the leaves of both upper and lower surface, epidermal peels were obtained either mechanically or by maceration, using a 10% aqueous solution of nitric acid. Small pieces of leaves were boiled and soaked in warm water for few minutes. From the surface of the leaves, disintegration of leaf tissue was indicated by bubbles. In the petridishes containing clean water the epidermis was transferred. Epidermis was separated with the help of forceps and needles and was passed in different grades of Ethanol to harden the cells. In 1% aqueous solution of safranin, epidermal peels were stained. Prepared slides were observed under light microscope (Olympus BX21 light) with 400× to 1000× magnifications and micrographs were taken.

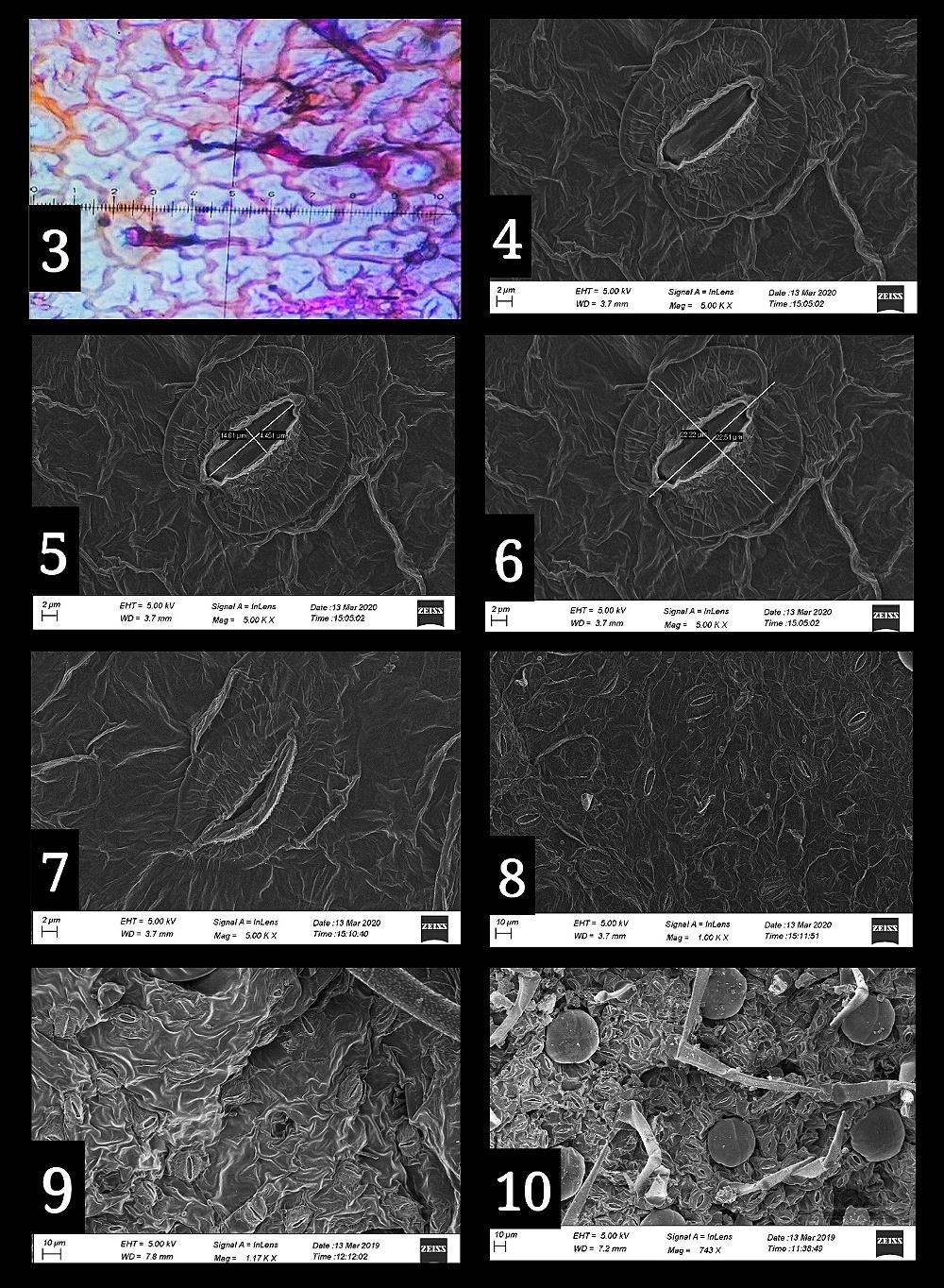
For the study of pollen grains, the fresh anther head were collected from just open fresh flowers to avoid the risk of dispersal of pollen grain with the help of clean forceps. The pollen grains were transferred to 70% alcohol vials for preservation and future study of pollen grains. For preparation of permanent slides, acetolysis method followed by procedure and terminology of pollen grains were adopted from Erdtman (1952) and Nair (1970b).Type of exine stratification, polar length and equatorial length measured and the ratio of polar to equatorial axis (P/E) was determined to find out the size and shape of pollen grain.

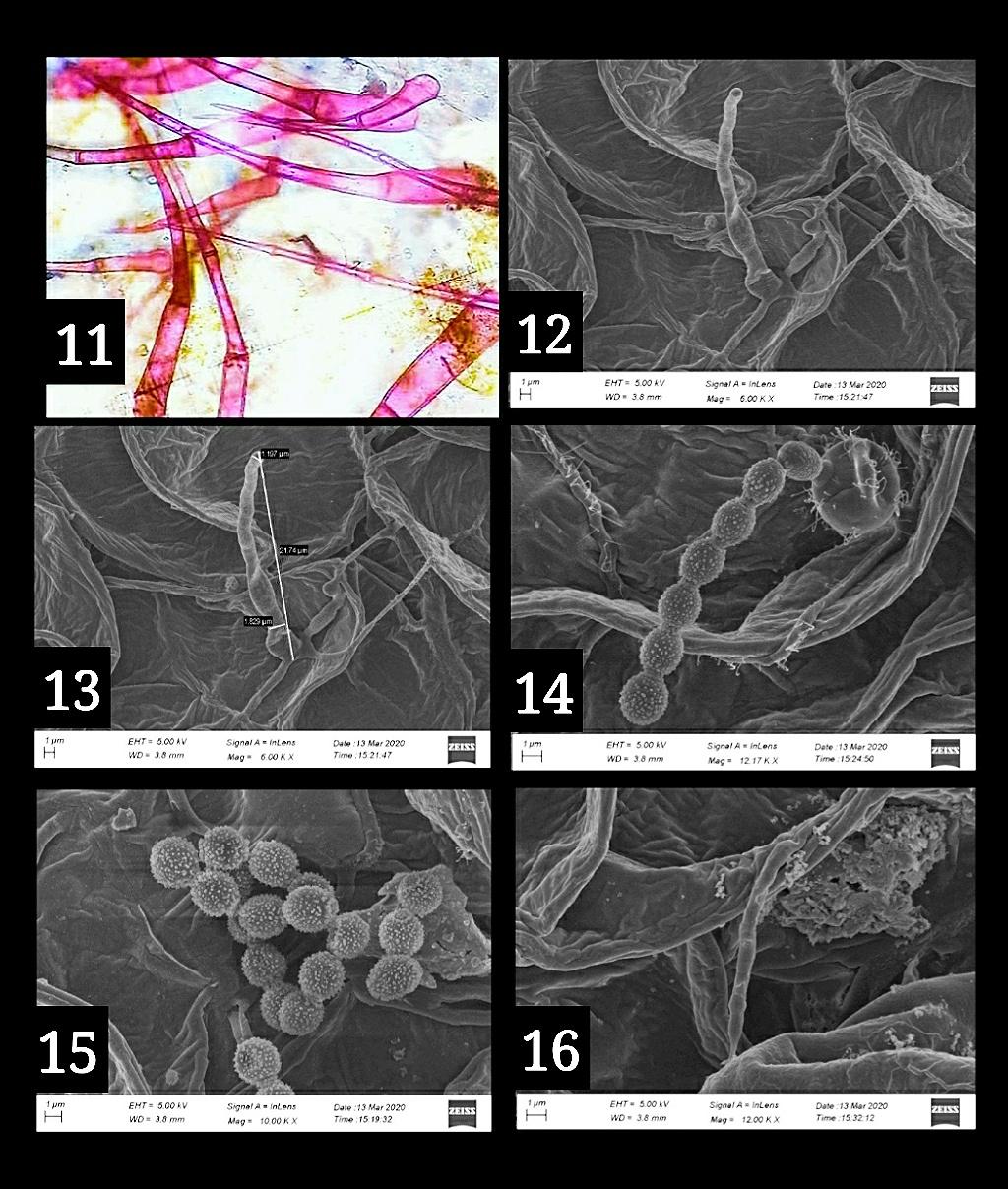
2.3. Scanning electron microscopy procedure- For Scanning Electron Microscopy Investigations, dried leaf specimen was taken, where about 1cm of dried leaf section was dissected and mounted directly on stubs with adaxial and abaxial surfaces separately facing upwards using double sided adhesive tape. Dried pollen grains were mounted on a specimen stubs. Both the stubs of leaf and pollen were coated with a thin layer of gold. The specimens were then examined in the scanning electron microscope (SEM) model Gemini Sigma 300 (ZEISS-EDAX).The micrographs were taken for stomata, trichomes and pollen.

3.**Results**

3.1**. Micromorphological studies:**

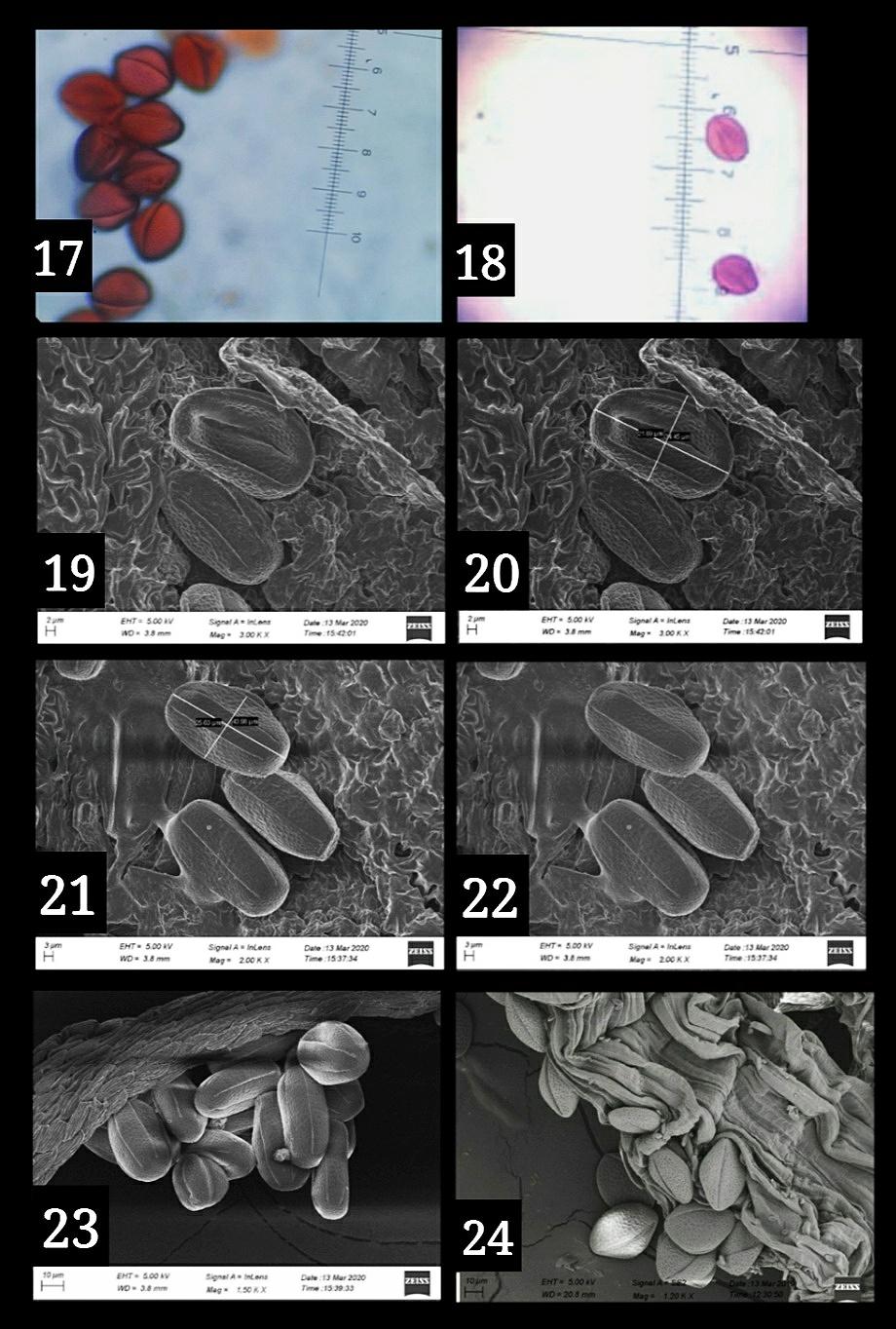
The stomatal structures of Plectranthus scutellarioides were observed under SEM micrographs. The abaxial surface of the leaf contains a greater number of stomata as compared to the adaxial surface. The guard cells of the open stomata on the outer surface measures to be 22.22 m in length and 22.51 m in width whereas, the inner surface measures to be 14.61 μm in length and 4.451 μm in width. The stomata are paracytic and were recorded to be semi open or closed and are randomly distributed. Epidermal cells and cell wall is sinus and guard cell is equal. In Plectranthus scutellarioide, both the glandular and non glandular trichomes were observed. The types of trichomes observed were peltatae glandular trichomes in a form of long beaded shape, 2-celled and 3-celled non- glandular trichomes. Glandular trichomes mainly include peltate type consists of a basal epidermal cell, a very short stalk and a round broad secretory head. The glandular trichomes secrete essential oils, that give leaves their fragrance which have great medicinal and commercial value. The non-glandular trichomes is of 21.74 μm in length, 1.829 μm in width at the base and 1.197 μm in width at the tip. Both long and short non-glandular trichomes were dominantly present on adaxial surface of Plectranthus scutellarioides. The non-glandular trichomes consist of one basal epidermal cell. It is unicellular to multicellular, uniseriate, unbranched and are quite variable in length.

**Plate II:** *Plectranthus scutellarioides.* (Fig:3-10) LM and SEM micrographs of stomata. (Fig: 3) stomata in LM. (Fig: 4-8) stomata on the abaxial surface. (Fig: 9-10) stomata on the adaxial surface.

**Plate III:** Plectranthus scutellarioides. (Fig:11-16) LM and SEM micrographs of different types of trichomes.

**3.2 Palynological studies:**

The pollen grains of Plectranthus scutellarioides were radially symmetrical and isopolar. The size of the pollen grain are medium (Erdtman, 1945). Apperture simple and syncolpate. Exine appeared as heterocolpate in equatorial view and tillete in polar view, sculpturing element absent, surface pitted. The shape of the pollen grain in equatorial view appeared to be non-angular, elliptic and truncate whereas it appears to be angular, quadrangular and convex in the polar view. The polar axis of pollen is measured to be 43.98 μm and the equatorial diameter is measured to be 25.63 μm. Shape of the pollen is Prolate.

**Plate IV:** *Plectranthus scutellarioides*. (Fig;17-24) LM and SEM micrographs of pollen.

**4. Discussion :**

In *Plectranthus scutellarioides,* trichomes are not uniformly distributed in both the leaf surface. Non-glandular trichomes are more than the glandular trichomes in the adaxial surface. Both the trichomes are densely present in the leaf surfaces, that the stomata were not prominently observed under Light microscope. However, stomata are distinct in Scanning electron microscope due to high magnification. Pollen found in *Plectranthus scutellarioides* is very medium and Prolate.

In conclusion, Plectranthus scutellarioides exhibits a non-uniform distribution of trichomes on both the adaxial and abaxial leaf surfaces, with a greater abundance of non-glandular trichomes compared to glandular trichomes on the adaxial surface. This high density of trichomes may account for the difficulty in observing stomata on the leaf surfaces under a light microscope, but their distinctness can be observed using a scanning electron microscope at high magnification. Additionally, the pollen of Plectranthus scutellarioides is characterized as being medium-sized and prolately shaped. These findings provide valuable insights into the morphology and anatomy of Plectranthus scutellarioides, which may have implications for its ecological and evolutionary adaptations. Further studies are needed to explore the functional significance of these morphological features and their potential interactions with other environmental factors.

**5. Acknowledgement :**

We would like extend a sincere word of gratitude to The Assam Royal Global University, Guwahati for providing us necessary laboratory facilities and giving us the opportunity to carry out this project.We would like to thanks our Dean, Royal School of Life Sciences (RSLSC) and Head of the Botany Department for their support during the project.

**6. Conflict of interest:**

The authors disclose that there are no conflicts of interest that could potentially influence the impartiality of this research.

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