

Foliar Micromorphological Studies Of Four *Coleus* Plants (Lamiaceae)

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Abstract

Background: *Coleus* is one among the largest and most diverse genera of the family Lamiaceae comprise of aromatic medicinal plants having potential therapeutic value. Economically, the members of the genus *Coleus* are of great importance acting as a source of medicines, providing food and as ornamentals. As recorded in ancient Sanskrit texts, different species of *Coleus* have been used for treating various diseases including heart diseases, abdominal colic, respiratory disorders, painful micturition, insomnia, convulsions and skin problems. This study is a comparative foliar micromorphological characteristics analysis such as variation in the size, shape, type and abundance of leaf epidermis, stomata and trichomes of the plants *Coleus malabaricus*, morphotype of *Coleus malabaricus*, *Coleus zeylanicus* and *Coleus amboinicus*.

Methods: The type, size, abundance and structure of epidermis, stomata and trichomes were studied using Leitz Diaplan compound research microscope fitted with Leica Qwin image analyser.

Findings: The results showed the similarities and differences of the foliar micromorphological features of these plants. The epidermal pattern was quite different among the four plants but diacytic stomata and presence of both glandular and non-glandular trichomes is common. But the density and number of cells very considerably.

Novelty and applications: A comparative foliar micro morphologic characters of *Coleus malabaricus* and its morphotype with *Coleus zeylanicus* and *Coleus amboinicus* of the family Lamiaceae was conducted with a view to elucidate their taxonomic significance and presenting complementary data to aid in the identification of the species. This is a first report, especially in the case of *Coleus malabaricus*.

Keywords: *Coleus amboinicus*, *Coleus malabaricus*, *Coleus zeylanicus*, Lamiaceae, morphotype

1. INTRODUCTION

Micromorphological parameters of different plant parts have been used as an important tool in the taxonomical recognition of different plant species. From a biosystematics perspective, the foliar epidermis is one of the most notable taxonomic characteristics, and taxonomic investigations of several families are undertaken based on the leaf epidermis [1]. The leaf epidermis has several differentiated cell types such as basic epidermal cells, cells of the trichomes and cells of the stoma complexes having various functions in plants. The structure, density and pattern of distribution of stomata and trichomes on leaf surfaces differ greatly among plant species [2]. Trichomes, along with the stomata, cutin, and wax on the epidermis, perform numerous defensive tasks by synthesizing, storing, and secreting various significant chemicals [3].

Lamiaceae (earlier known as Labiatae), also known as mint or dead nettle or sage family, is the largest family among dicotyledons with strength of about 236 genera and 6,900-7,200 species. It is one of the most diverse and widespread plant families with a cosmopolitan distribution in the world and concentrated more on the Mediterranean region, Indo Malaysian region and Australia [4]. Most of the plant members are herbs or under shrubs and produce a high amount of essential oil that enables them to survive the hot summer. Owing to the ease of cultivation by the vegetative propagation method and ethnomedicinal, horticultural and culinary importance, many members of the plant family are widely cultivated around the world [5].

Coleus is one among the largest genera of the family, *Lamiaceae* (mint family) and is a close relative of basil, salvia, oregano, peppermint, spearmint, thyme and Swedish ivy. Even though the genus has importance in the horticultural and floricultural aspect, 85% of the documented uses of *Coleus* are in medicine [6]. Economically, the members of the genus *Coleus* are of great importance acting as source of medicines, providing food and ornamentals. As recorded in ancient Sanskrit texts, different species of *Coleus* have been used for treating various diseases, including heart diseases, abdominal colic, respiratory disorders, painful micturition, insomnia, convulsions, skin problems, worms etc [7].

Coleus amboinicus, *Coleus barbatus* are the two species, which constitute 68% of all traditional uses of the genus [4,6]. *Coleus amboinicus* and *Coleus zeylanicus* are mostly using in the Ayurvedic system of medicine in India for treating various diseases conditions. Ethnobotanical uses of the genera include the treatment of digestive problems, skin diseases, fever and respiratory infections genitourinary and musculoskeletal conditions. The therapeutic properties of the genus are related to flavonoids, glycosides, phenolic compounds and volatile constituents. Most of the plant species are aromatic and essential oil producing. The essential oil components are mainly responsible for different pharmacological activities and make *Coleus* important genera for drug development. Due to the tremendous medicinal value, more than 500 varieties of *Coleus* species are being cultivated world-wide nowadays [8].

Coleus malabaricus is a plant, which is seen mostly in south India and Sri Lanka has been used by the tribal communities for the cure of various disease conditions for a long period of time. The plant is also reported to possess extreme morphological variations and almost 8 polymorphs were reported from different parts of Kerala [9].

The present study aims to compare the foliar micro morphological characters of *Coleus malabaricus* Benth. and one of its morphotypes, both collected from Thollayiram, Wayanad with traditionally valuable and well documented species of *Coleus*, *Coleus zeylanicus* and *Coleus amboinicus*. Since these plants have importance in the traditional system of medicine, the study would definitely help in the further identification and evaluation of these species.

2. MATERIALS AND METHODS

2.1 Procurement & Authentication of Plants

Fresh plant materials of *Coleus malabaricus* Benth. and morphotype of *Coleus malabaricus* (fig 1) were gathered from Thollayiram, wayanad, Kerala. Both the plant specimens were identified as *Coleus malabaricus* Benth. (*Plectranthus malabaricus* Benth.) by Dr. M. U. Sharief, Scientist E and Head of office Botanical survey of India, Coimbatore and the voucher specimen records are BSK 01 and BSK 02 (No: BSI/SRC/5/23/2019/TECH/308). The plant materials *Coleus zeylanicus* and *Coleus amboinicus* for the study were procured from cultivars near Cherthala and authenticated by Prof P. Jayaraman, Plant Anatomy Research Centre, Thambanam, Chennai with voucher specimen numbers PARC/2021/4845 and PARC/2021/4846 respectively.

2.2 Foliar microscopic studies

Leitz Diaplan compound research microscope fitted with Leica Qwin image analyser was used for the study. The study was carried out with both fresh and preserved material. Epidermal and stomatal morphology, venation pattern and trichome distribution were studied by paradermal sectioning as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [10]. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell component were studied and measured. Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit.

3. RESULTS AND DISCUSSION

3.1 Foliar Epidermal Characteristics

Among the different foliar morphological characteristics, the features of the leaf epidermis are considered as one of the most significant one for the taxonomical positioning of various plant species. Taxonomic studies of several families are based on leaf epidermal anatomy [11].

The most commonly studied characteristics of leaf epidermis are the length and width of epidermal cells, structure, orientation and undulation of anticlinal and periclinal walls [1]. Comparative micromorphological features of leaf epidermis is presented in Table 1 and Fig 1. Both abaxial and adaxial epidermal layers of *Coleus malabaricus* consist of wide epidermal cells, which possess highly wavy anticlinal walls (Fig 1 A). Sometimes cicatrix is seen on some epidermal surface (Fig 2 A). Both abaxial and adaxial epidermis of morphotype of *Coleus malabaricus* are observed with epidermis having irregular margin (Fig 1 B). Wavy anticlinal margin with much similarity to *Coleus malabaricus* in the case of *Coleus zeylanicus* (Fig 1 D). In *Coleus amboinicus* small adaxial and abaxial epidermal cells with smooth margin are seen. The epidermal cells are also angular thick walled and compact. Sometimes cicatrix are seen on the epidermal layer. (Fig 1 C). The leaf epidermal density also varies and is highest in the case of *Coleus amboinicus* and lowest in *Coleus malabaricus*. But the epidermal dimension (length X Breadth) is lowest in *Coleus amboinicus* and highest in *Coleus malabaricus* (morphotype).

Table 1. Micromorphological features of leaf epidermis

Species	Appearance	Density (no/mm ²)	Dimensions (length X width) μm ²
<i>Coleus malabaricus</i>	Irregular, appendaged	2/703376	392.9 X224.7
<i>Coleus malabaricus</i> (morphotype)	Irregular margin	17/750805.7 (14-22 in number)	404.87X215.12
<i>Coleus zeylanicus</i>	Wavy margin	17/323721.5	338.5X138.9
<i>Coleus amboinicus</i>	Smooth margin	19.25/803174(14-23 in number)	302.1X162.1

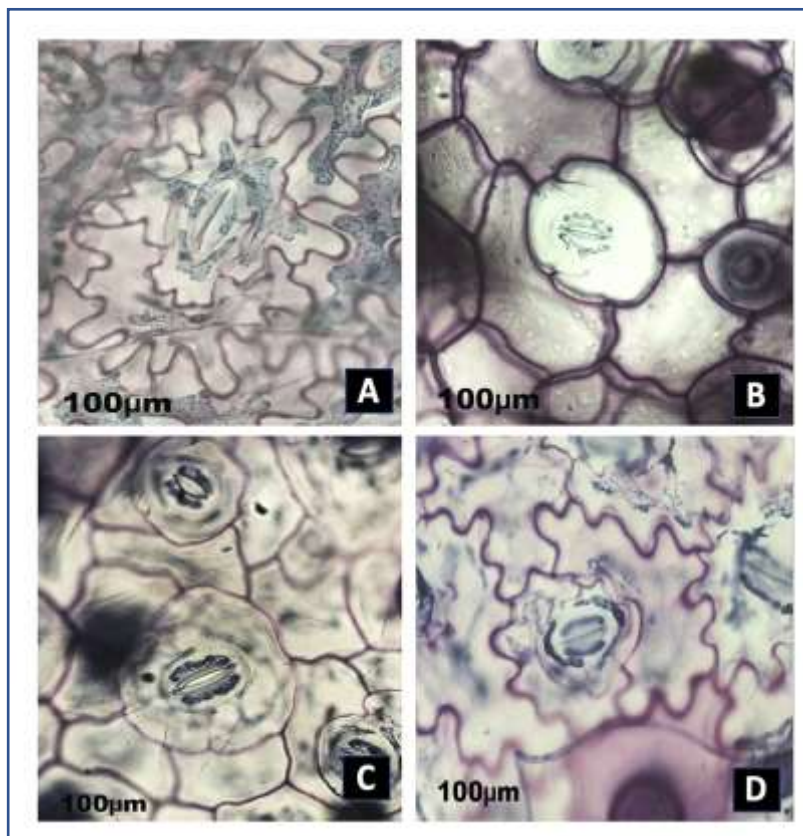


Figure 1. Foliar epidermal characters of A. *Coleus malabaricus* B. *Coleus malabaricus*(morphotype) C. *Coleus zeylanicus* D. *Coleus amboinicus*

3.2 Foliar Stomatal Characteristics

The stomatal characteristics such as size, type, distribution and frequency have been found to be specific to some taxa and are used as significant parameters in taxonomy as well as in phylogeny^[1]. Diacytic stomata are seen in all plants both on the adaxial and abaxial surfaces. Details are presented in Table 2. The abaxial and adaxial epidermis of *Coleus malabaricus* is stomatiferous. The stomata are broadly elliptical with long thick-walled guard cells.(Fig 2A). The stomatal pore is narrow and slit like. In some of the stomata, there are spherical polar nodules on either end of the guard cells (Fig 2A&B).In the case of morphotype of *Coleus malabaricus*, the stomata are large with an elliptical outline (Fig 2 C). The stomatal pores are wide and vertically oblong. The guard cells are 40×25 µm in size. In *Coleus zeylanicus* stomata is much similar to *Coleus malabaricus*. The adaxial epidermis of *Coleus amboinicus* shows stomata and cicatrix. The stomata are of the diacytic type with two subsidiary cells. Subsidiary cells are one above the other. The common wall of the subsidiary cells is at right angle to the guard cells. The abaxial epidermis has dense stomata. The guard cells are narrowly elliptical and elongated (Fig 2 E&F).The stomatal density is the highest in morphotype of *Coleus malabaricus* and least in *Coleus zeylanicus*. The dimensional analysis showed that *Coleus malabaricus* possesses stomata with large size and the smallest stomata seen in its morphotype itself.

Table 2. Micromorphological features of stomata

Species	Stomatal type	Density (no/mm ²)	Stomatal complex (length X width) µm ²
<i>Coleus malabaricus</i>	Diacytic	5.6/768123	164.6X89.25
<i>Coleus malabaricus</i> (morphotype)	Diacytic	13/583547	119.8X55.75
<i>Coleus zeylanicus</i>	Diacytic	8/805199	156.2X123.71
<i>Coleus amboinicus</i>	Diacytic	4.4/937101.9	177.16X121.92

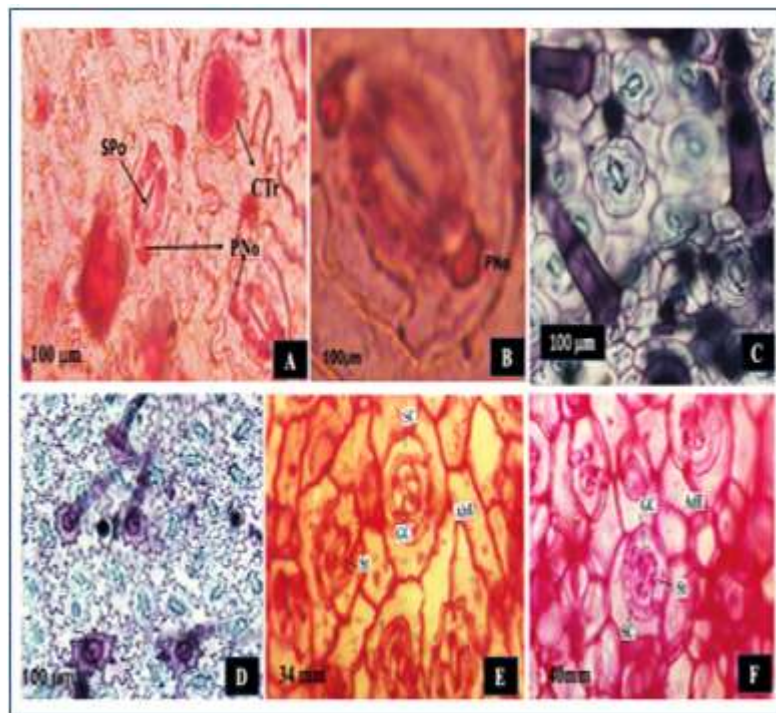


Figure 2. Stomatal pattern of A. *Coleus malabaricus* B. Stoma with polar nodules in *Coleus malabaricus* C. *Coleus malabaricus*(morphotype) D. *Coleus zeylanicus* E. Abaxial epidermis of *Coleus amboinicus* F. Adaxial epidermis of *Coleus amboinicus*.

Abbreviations: AbE-Abaxial epidermis, AdE-Adaxial epidermis, Ctr-Cicatrix, GC-Guard cells, St-Stomata, SPo-Stomatal pore, PNo-Polar nodule

3.3 Foliar Trichome Characteristics

Distinct variations in the foliar morphological structure of trichomes are visible in all species. The unique features of trichomes in each taxon are determined by environmental and genetic factors. Sometimes, different species found in the same environmental conditions exhibit dissimilar trichome morphology due to genetic reasons [12]. The four *Coleus* species possess glandular and non-glandular trichomes on adaxial and abaxial surfaces. Glandular trichomes are of the capitate and peltate type. Like other Lamiaceae species the non-glandular trichomes are of uniseriate and unbranched [13]. A multicellular basal pedestal was also seen. The raised cells of the basal pedestal provide anchoring of the trichomes to the epidermal surface.

The non-glandular trichomes are numerous in *Coleus malabaricus*. They are bent and lean slightly toward the leaf surface. The non-glandular trichomes are supported by cellular pedestal, formed by 4-6 epidermal cells arranged in a circle around the base. Some deposits can be seen on the surface of non-glandular trichomes (Fig.3A-3G) There are single or a group of epidermal cells on the leaf. The trichome is attached to the central cell. The epidermal cells that bear the trichomes are highly dilated. The cells of the trichomes are wide and thin walled. The trichomes are up to 40 µm thick at the base. On the lower surface of the lamina there are prominent circular sub sessile glandular trichomes. The gland has short stalk, which is buried in the epidermal layer. The secretory body lies above the epidermal surface. The body consists of cell wall, inside which present four secretory cells. The body of the gland is 140 µm thick (Fig 3C&D). Fragile and soft long multicellular non-glandular trichomes are present in the morphotype of *Coleus malabaricus*. The trichomes are uniseriate and unbranched. Peltate type of glandular trichomes are common on the leaf of *Coleus malabaricus* morphotype. They occur on the lamina and on the veins. The gland has short, thick one celled stalk and unicellular or multicellular, spherical and darkly stained glandular body. The size of the peltate type glandular trichomes varies (3H&I). Capitate type trichomes are seen less in both *Coleus malabaricus* and its morphotype. In *Coleus zeylanicus* leaf possesses three types of trichomes including Non-glandular and capitate, peltate type glandular trichomes. Trichomes are more distributed on the veins. The non-glandular trichomes are long, tapering, multicellular, uniseriate and unbranched. The basal cells are wide and the terminal cells gradually taper towards the tip. Some non-glandular trichomes with a hook at the terminal are also seen. (Fig 3 J).

Among the plants *Coleus malabaricus* showed longest non-glandular trichomes and capitate glandular trichomes. But the other plants including the morphotype of *Coleus malabaricus* showed almost equal number of cells in the non-glandular trichomes. In *Coleus amboinicus* epidermal trichomes occur on both abaxial and adaxial sides of lamina and on the midrib portion. The non-glandular trichomes are multicellular (3-5 cells), uniseriate and tapering at the tip (Fig 3 K). Some of the non-glandular trichomes are thick short three celled with thick partition walls. There are also non-glandular trichomes of 5 celled long with tapering ends. The cells are rectangular and thin walled. The basal cells of trichomes are wide and cylindrical. Both peltate and glandular type of glandular trichomes are seen both on the lamina and midrib.

Table 3. Micromorphological features of non-glandular trichomes

Species	Trichome type	Number of cells	Dimensions (length X width) μm^2
<i>Coleus malabaricus</i>	Multicellular, uniseriate, covering	6-14	1968.9X239.8
<i>Coleus malabaricus</i> (morphotype)	Multicellular, uniseriate, covering	3-6	360.2X51.3
<i>Coleus zeylanicus</i>	Multicellular, uniseriate, covering	3-5	578.6X91.9
<i>Coleus amboinicus</i>	Multicellular, uniseriate, covering	3-5	391.4X46.36

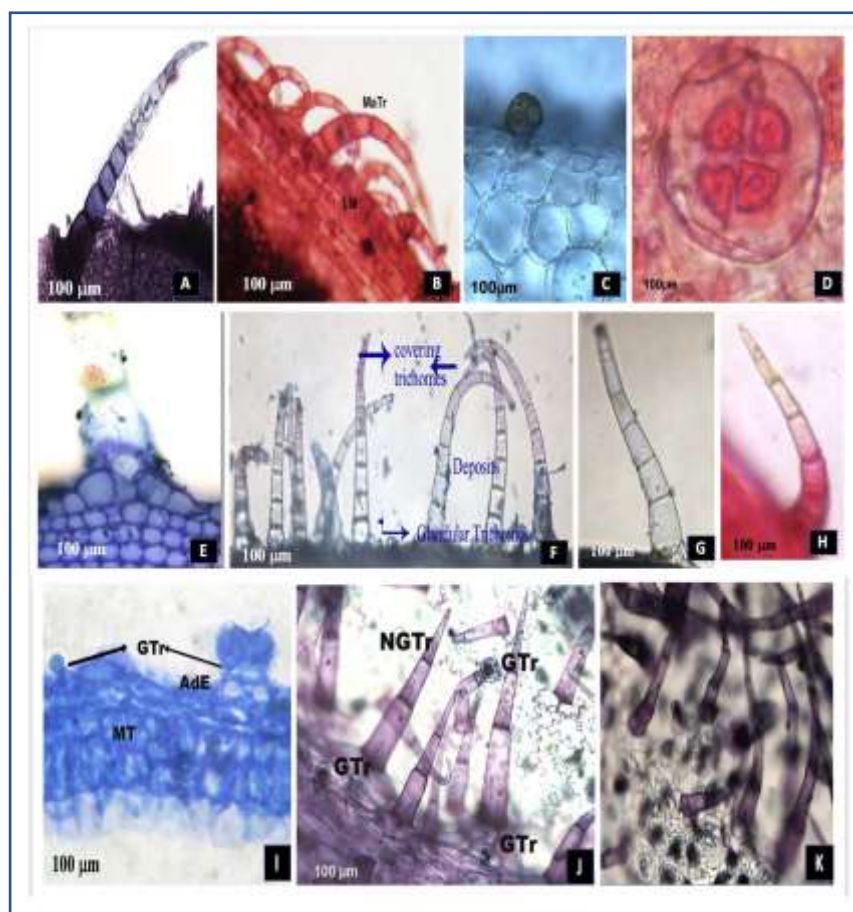


Figure 3. Foliar trichome characteristics. Figure 3A-3G: Foliar trichome characteristics of *Coleus malabaricus* Benth. 3A.Non-glandular trichome 3B.Non glandular trichomes of marginal part of lamina 3C.Peltate glandular trichome 3D.Peltate glandular trichome surface view showing four gland cells and gland wall 3E:Basal part of non-glandular trichome 3F:Non-glandular trichomes showing deposits over the surface 3G:Capitate trichome 3H:Non-glandular trichome of *Coleus malabaricus* (morphotype) 3I:Glandular trichome of *Coleus malabaricus* (morphotype) 3J: Non-glandular and glandular trichomes of *Coleus zeylanicus* 3K:Non-glandular trichomes of *Coleus amboinicus*

Abbreviations: AdE-Adaxial epidermis, GT>r- Glandular trichomes, Matr-Marginal trichomes, MT-Mesophyll tissue, NGTr-Non-glandular trichomes.

4. CONCLUSION

Recent plant taxonomy consider foliar micromorphological features of plants such as type, appearance and density of stomata, trichomes and epidermis as the key characteristics for identification of taxon. This investigation focused on foliar micro morphological analysis of four *Coleus* plants among which two are of the same species but differ phenotypically. This is the first study of foliar morphological characteristics of *Coleus malabaricus*. Since the plant exhibits extreme polymorphism, comparative studies would help in the identification of various morphotypes. This study provides a better taxonomic key to identify the species. The study revealed the presence of both glandular and non-glandular trichomes and diacytic type of stomata in all species; beyond, the glandular trichomes included capitate and peltate types. The epidermal characteristics and density of stomata and number of cells and distribution of trichomes vary in all plants.

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6. REFERENCES

1. Albert S, Sharma B. Comparative foliar micromorphological studies of some *Bauhinia* (Leguminosae) species. Turkish Journal of Botany. 2013;37(2):276-81. <https://journals.tubitak.gov.tr/cgi/viewcontent.cgi?article>.
2. Nikolić NP, Merkulov LS, Krstić BĐ, Orlović SS. A comparative analysis of stomata and leaf trichome characteristics in *Quercus robur* L. genotypes. Zbornik matice srpske za prirodne nauke. 2003(105):51-9. <https://agris.fao.org/agris>
3. Wang X, Shen C, Meng P, Tan G, Lv L. Analysis and review of trichomes in plants. BMC plant biology. 2021 Dec; 21(1): 1-1. <https://bmcplantbiol.biomedcentral.com/articles/10.1186/s12870-021-02840-x>
4. Venkateshappa SM, Sreenath KP. Potential medicinal plants of Lamiaceae. American International Journal of Research in Formal, Applied & Natural Sciences. 2013 Jun;3(1):82-7. <http://iasir.net/AIJRFANSpapers/AIJRFANS13-239.pdf>
5. Saraswathy A, Amala K and Arunmozhi D, “*Coleus vettiveroides* K.C Jacob; Botany and Pharmacognosy”, Indian Journal of Traditional Knowledge ,2011, Vol 10(4), pp 636-42. <https://www.researchgate.net/publication/339202452>.
6. Likhoba CW, Simmonds MS and Paton AJ: *Plectranthus*: A review of ethnobotanical uses. Journal of Ethno-Pharmacology 2006; 103(1): 1-24. <https://pubmed.ncbi.nlm.nih.gov/16289602/>
7. Soni H and Singhai AK: Recent updates on the genus 22. *Coleus*: A review. Asian Journal of Pharmaceutical and Clinical Research 2012; 5(1): 12-17. <https://www.researchgate.net/publication/283041762>.
8. Waldia S, Joshi BC, Pathak U and Joshi MC: The genus *Plectranthus* in India and its chemistry. Chemistry & biodiversity 2011; 8(2): 244-52. <https://pubmed.ncbi.nlm.nih.gov/21337498>.
9. Kamal BS and Balakrishnan BR: Medicinally important *Coleus* species, *Coleus malabaricus* Benth. and many others of family Lamiaceae from the Western Ghats, India - a review.
10. Int J Pharm Sci & Res 2021; 12(4): 2001-11. https://ijpsr.com/?action=download_pdf&postid=72888
11. Sasser J E. Elements of Botanical Microtechnique (McGraw Hill Book Co, New York).1940; 222.
12. Albert S, Sharma B. Comparative foliar micromorphological studies of some *Bauhinia* (Leguminosae) species. Turkish Journal of Botany. 2013;37(2):276-81. <https://journals.tubitak.gov.tr/cgi/viewcontent.cgi?article=1681&context=botany>
13. Sanoj E, Deepa P. Micromorphological variations of trichomes in the genus *Ocimum* L. Plant Science Today. 2021 Jul 1;8(3):429-36. <https://horizonpublishing.com/journals/index.php/PST/article/view/1006>
14. Kalicharan B, Naidoo Y, Nakhoda M, Dewir YH. Micromorphological evaluation of the foliar trichomes of field grown and micropropagated *Stachys natalensis* Hochst.(Lamiaceae). South African Journal of Botany. 2018 Nov 1;119:369-76. <https://www.sciencedirect.com/science/article/pii/S0254629918306562>