



Microscopic evaluation of morphotype of *Coleus malabaricus* Benth. (Lamiaceae)

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Abstract

Lamiaceae is one among the largest dicotyledon families comprises of about 236 genera and 6900-7,200 species. Most of the plant members are herbs or under shrubs with volatile oil which help them to survive in the hot summer season. *Coleus malabaricus* Benth. is a plant which is endemic to south India and Srilanka and the entire plant is used by the tribal communities for the treatment of cardiac diseases, inflammatory conditions, asthma, fever and chills and as a mosquito repellent agent. The plant exhibits extreme polymorphy and the present study focuses on the microscopic and powder microscopic evaluation of one of the morphotypes of *Coleus malabaricus* collected from Wayanad region of Kerala State of south India with the aim of taxonomic support.

Keywords: *Coleus malabaricus*, morphotype, lamiaceae

Introduction

The genus *Coleus* of the family Lamiaceae, comes under the subfamily Nepetoideae of tribe Ocimeae and subtribe Plectranthinae^[1] with about 300 species worldwide is one among the largest dicotyledonous families and consists of aromatic herbaceous plants of medicinal and traditional importance^[2]. Many species of the family produce essential oil and are therefore used as perfumes, flavourings, foods in addition to therapeutic potential. The plant *Coleus malabaricus* Benth. is globally distributed in India and Srilanka and in India it is found in the states of Kerala, Tamil Nadu and Karnataka especially in the rocky and marshy places of Western Ghats at an altitude of 700-1200 m^[3, 4]. Smitha and Sunoj reported that the plant *Coleus malabaricus* is a polymorphic species which exhibit a wide range of variations in the morphology except floral characters. Among the eight different accessions collected from different localities of Western Ghats, two of them were published as new species by Mathew *et al*^[5] According to Paton *et al.*, the currently accepted name of the plant is *Coleus malabaricus* Benth. and all the other names are considered as synonyms. Synonyms: *Plectranthus malabaricus* (Benth.) R. H. Willemse, *Coleus ovatus* Benth, *Coleus leptostachys* Benth, *Coleus macraei* Benth, *Plectranthus malabaricus* var. *leptostachys* (Benth.), *Coleus walkeri* Benth^[1]. Tribal communities of south India use the plant under different names for curing inflammation, fever, cardiac diseases, asthma etc and also the crushed leaves for repelling mosquitoes. The local names mentioned in the survey reports are Indian name: Ellambi, Tamil: Kurali & Periyathulasi, Malayalam: Parakkoorka, Kattuthulasi, & Kattappa by the tribes of Wayanad region, Kerala^[6]. According to Gamble the plant *Coleus malabaricus* is a large erect herb with purplish stem and leaves reaching a height of 3 feet,. Flowers are pale lilac in colour with dark blue upper lip^[7]. Sasidharan described the plant as herb with purplish quadrangular stem. Leaves 15 x 13 cm, puberulous, ovate, obtuse or acute, leaf base is subcordate or truncate. Inflorescence panicles terminal to 30 cm long. Calyx striate, lobes are ovate-lanceolate, upper lip is broadly ovate, lower lips 3-lobed. Corolla white with purple tinge,

1cm long. Stamens exerted, filaments glabrous^[8] According to Mathew *et al.*,^[5] *Coleus malabaricus* is a herb with a stem which is erect or prostrate and highly variable. The plant is perennial upto 150 cm tall. Stem is quadrangular, branched. Leaves obtuse or acute, base truncate or subcordate, puberulous to 15X13 cm, crenate margin, upper surface glabrous or pubescent with simple hairs, lower surface purplish with red glandular dots, petiole upto 100 mm long. Inflorescence basally branched thyrse of 30 cm long, peduncle is slightly puberulous with 5-7 flowers. (Fig 1 A) According to Gamble, the plant is found near the rocky regions inside the forest, especially in the marshy places, and the plant is mainly found in the Western Ghats, Nilgiris, Puleney hills, Hills of Travancore and Tinnavelly upto 6,000 Feet. The plant found in Srilanka was described by Bushana in the biodiversity portal of Srilanka as a large indigenous herb restricted to shady places near streams in secondary submontane forests from 640 to 1400 m elevations. Rarely occurs in wet lowland forests, and flowering is seen throughout the year^[6].

Materials and Methods

Collection and authentication of plant specimen

The fresh plant samples for the proposed study were collected from Thollayiram, wayanad, Kerala and identified and authenticated by Dr. M. U. Sharief, Scientist E and Head of office Botanical survey of India, Coimbatore. Herbarium specimen no: BSK002. The herbarium specimen was deposited in Vinayaka Mission's College of Pharmacy, Salem for later reference.

Morphological studies

The fresh plant samples immediately after collection were studied for the morphological characters of root, stem, leaves and floral parts^[5, 7, 9].

Preparation for anatomical studies

Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and fixed in FAA [Formalin (5ml) + Acetic acid (5ml) + 70% Ethyl

alcohol (90ml)]. After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary -Butyl alcohol^[10]. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12.µm. After dewaxing of the sections^[11] the sections were stained with Toluidine blue^[10] and wherever necessary sections were also stained with safranin, Fast-green and Iodine reagent. 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^[11]. 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. For normal observations bright field was used. For the study of starch grains, crystals and lignified cells polarized light was employed. Descriptive terms of the anatomical features are as given in the standard anatomy books^[9, 13, 14].

Results and Discussion

Morphological studies

The plant is a small, perennial herb, bushy with profusely branching, erect or prostrate, succulent, puberulous and quadrangular stem up to 1 m tall. Root is tap root and branched.

Young stems are more puberulous and greenish instead of the pink stem of the original plant. Leaves simple, opposite, hairy, petiolate and crowded at the branches. Both abaxial and adaxial surfaces of leaves are greenish. Lamina broadly ovate, serrate, acute with semicordate /truncate base. Petiole up to 6-16cm long and 3mm diameter, fleshy, adaxial and abaxial sides greenish. Lamina broadly ovate 13.5X9.5 cm size, base truncate or subcordate, margin serrate/crenate with numerous teeth on each side of margin, apex acute, unicostate reticulate venation with veins prominent on the lower surface. 5-6 pairs of veins present. Veins more raised at the lower surface. Inflorescence is basally branched thyrse of 30 cm long. Peduncle greenish and slightly puberulous. Floral nodes 1.5-2 mm apart. Flower Hermaphrodite, and zygomorphic. Calyx green, campanulate with scattered hairs, Upper lip broadly ovate lower lip 3 lobed typical bilabiate arrangement. Corolla bilabiate, posterior (upper) lip blue coloured and anterior lip single and white. Stamens exerted, filaments glabrous and style is filiform (Fig 1 B).



Fig 1: (A) The plant *Coleus malabaricus* Benth. (B). Morphotype of *Coleus malabaricus* Benth. selected for the study

Microscopic studies

Leaf

In transverse section of the leaf, the midrib is prominently raised into conical adaxial part and thick and wider flat abaxial midrib. The lamina is thin and straight. (Fig:2 A)

The midrib is much lobed due to presence of thick multi seriate epidermal trichomes. The adaxial part of the midrib has a small cluster of thick-walled cells resembling fibres. The midrib consists of parenchymatous ground tissue. There is a single fairly prominent vascular strand with central part of the midrib. The vascular strand is collateral with abaxial phloem elements and adaxial xylem elements. The xylem elements are wide, polygonal and thin walled. The midrib is 450micrometre in vertical plane and the abaxial midrib is 200 micrometre thick.

The lateral vein also has adaxial lump and abaxial conical midrib. The adaxial hump consist of a group of thick walled cells. The abaxial conical midrib has circular collateral vascular bundle with a vertical row of xylem elements and is thin layer of abaxial phloem layer. The central core of the midrib has vertical row of thick-walled cells and outer layer of large thin walled cells.

Peltate type of glandular trichomes are common on the leaf. 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 trichomes vary. (Fig: 2 B)

A part from midrib and major lateral vein, there are small veinlets which are flat on the adaxial side and triangular with conical tip. These veinlets have a group of wide circular xylem element and thin layer of phloem. Fragile and soft long multicellular non-glandular trichomes are more common on all parts of the plant. The trichomes are uniseriate and unbranched. Due to delicate cell walls these trichomes become shrunken when mounted on the slide.

Petiole

The petiole is circular in cross section with a deep irregular ridges and furrows are the adaxial side of the petiole on is a deep narrow groove. The epidermal layer has thin walled rectangular cells. The ground tissue is parenchymatous with polygonal thin-walled cells on the abaxial part of the petiole are small thick walled cells There are two groups of vascular tissue in the petiole there are two last bundles just beneath the adaxial grow on the two adaxial Wings there is a small vascular bundles in Each wing. All is bundles (wing bundles and Middle bundles) are Collateral with wide polygonal thick walled xylem elements and thin layer of phloem elements (Fig:2 C)

Stem

The stem is thin circular in trans sectional view with wide shallow adaxial concavity and less prominent ridges and furrows cell around. stem is 2.3mm thick. There is wide central pith cavity. The vascular system is multi stranded comprising larger bundles in the ridges and smaller bundles in the inter-ridge region. The bundles are collateral with outer phloem and inner xylem. (Fig:2 D) The vascular bundles in the ridges have wide cluster of vessels which are wide spaced.

The vessels are angular and thick walled and are 53 micrometre wide. The protoxylem is endarch. The inter-ridge vascular bundles are smaller with a few vessels grouped into a cluster and thin layer of phloem. The young

stem has thin epidermal layer and five and six layers of collenchyma cells.

The thick stem is also deeply bed with thick semi-circular lobes and wide deep, four grooves. Of the four grooves, the adaxial groove is wide and deep. The central part of the stem is occupied by wide pith cavity which has wavy outline. The vascular bundle of the larger wing is broadly fan shaped. It includes triangular outline of xylem elements and consists of angular thin walled vessels. The vessels in radial lines of three vessels or the vessels arc solitary. The cells in between the vessels are thick walled lignified fibres. On the outer [upper] part of the xylem strands occur

phloem. It is the form of semi-circular layer. The cells are small angular and compact. Sieve cells and companion cells are well defined. The phloem units is covered outside by an arc of fibres. (Fig:2 E)

Root

The root is circular in outline with uniform circumference. It is 2.5mm in diameter. The root consists of outer fissured periderm where the periderm cells are crushed into wavy layer. Inner to the periderm is secondary phloem, which is a continuous line all along the outline of the root (Fig:2 F).

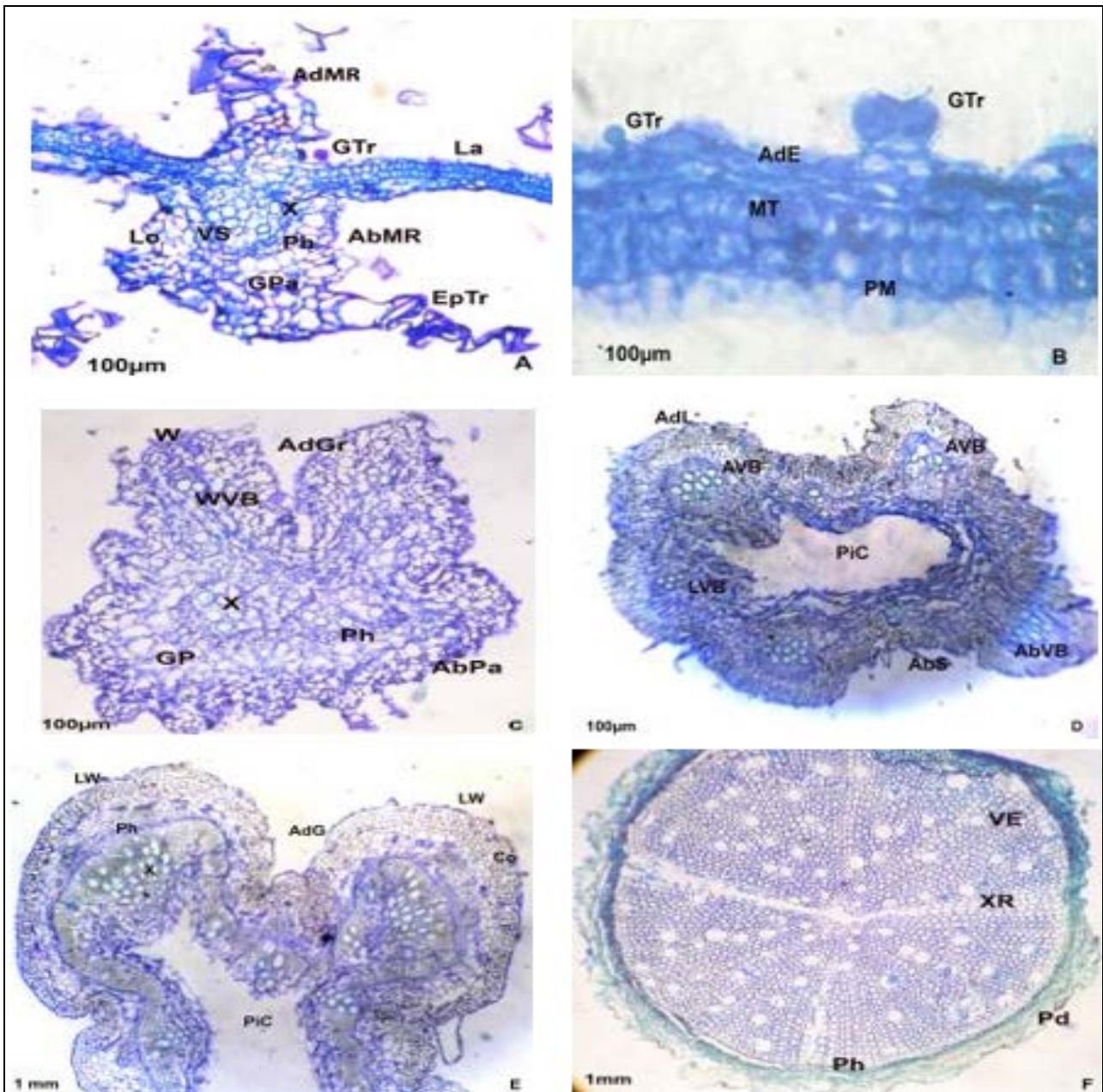


Fig 2: Microscopic characters of different plant parts of *Coleus malabaricus* (morphotype) (A) T.S of lamina through midrib 10X (B) T.S of lamina showing sessile glandular trichome 40 X (C) T.S of petiole entire view 10X (D) T.S of thin stem entire view 4X (E) T.S of thick stem partial view 4X (F) T.S of thick root entire view 4X (Explanation for abbreviations: AbMR-Abaxial midrib; Ab Pa-Abaxial Parenchyma; Abs-Abaxial side; AbVB-Abaxial vascular bundle; AbW - Abaxial wing Ad E-Adaxial Epidermis; AdG - Adaxial groove; Ad Gr-Adaxial Groove; Ad H-Adaxial Hump; Adl-Adaxial lobe AdMR-Adaxial midrib; Co - Cortex; Ep - Epidermis; EpTr-Epidermal Trichome; Fi - Fibres; GPa-Ground Parenchyma; LA-Lamina; Lw - Lateral wing; Lo-Lobe; Ph-Phloem; Tr-Trichome; VS-Vascular Strand; X-Xylem; GTr -Glandular Trichome; La -lamina; LVB - Lateral Vascular bundle; MT-mesophyll tissue; ph- phloem; Pd-Phelloderm;; PM-Palisade Mesophyll; PiC - Pith Cavity; SX-Secondary Xylem; VB- vascular Bundle; Ph-Phloem, VE-Vessel Element; W-Wing; WVB-Wing Vascular Bundle; X-Xylem; XR-Xylem Rays)

At certain places epidermal cells are intact and the periderm cells are also well preserved. The secondary phloem consists of thin dark cylinder of narrow tangential elongated phloem elements. Secondary xylem is a wide solid cylinder comprising three xylem rays and dense xylem fibres with scattered solitary circular thin walled vessels. The vessel elements are also slightly polygonal thin walled and measure upto 40µm in diameter.

Powder Microscopic Observations

Leaf

Small fragment of epidermal peeling of the leaf are seen in the powder. The abaxial epidermal peeling shows stomata and epidermal cells. The epidermal cells have highly wavy and amoeboid outline. The stomata are large with elliptical outline. The stomatal pores are also wide and vertically oblong. The guard cells are 40×25µm in size. The stomata are diacytic type. There are two subsidiary cells. Which are situated along the two pores of guard cells; the common walls of the subsidiary cells are at right angles to the long axis of the guard cells.(Fig 3A&B)

Stem

The stem powder includes vessel elements, fibres and parenchyma cells. The fibres are long, cylindrical thick-walled lignified cells tapering ends. Some of the fibres are narrow with reduced cell lumen and thick wall. The narrow

fibres are 870 µm long and 20 µm thick. The wide fibres are 30-50 µm thick. Their length varies from 550 µm to 1.2 µm. Their walls are thin; cell lumen is wide; pits are not evident. The narrow fibres and wide powders occurs in compact bundle along with other vascular bundles. Vessel elements of the different types are frequent in the powder. The elements are long cylindrical cells with thick lignified walls. The lateral walls have dense circular small or wide bordered pits. The end walls may be either horizontal or deeply oblique. The end walls have wide circular opening called end wall perforation. many of the vessel elements have long conical tails at the end. Some vessel elements do not have tails. The end walls are flat and plane. The length and thickness of the vessel elements vary much; they range in length from 180 µm to 670 µm and 20-70 µm in thickness.

Parenchyma cells are seen in solitary or in clusters. They are spherical or in vertical rows of rectangular cells. The cells have minute, dark spherical bodies (Fig:3C&D).

Root

The root powders are so has different types of vessel elements, fibres and parenchyma cells. The vessel elements are rarely very narrow, very long with dense multiseriate circular pits

They are also known very long narrow cylindrical septate vessel elements with horizontal perforation plate

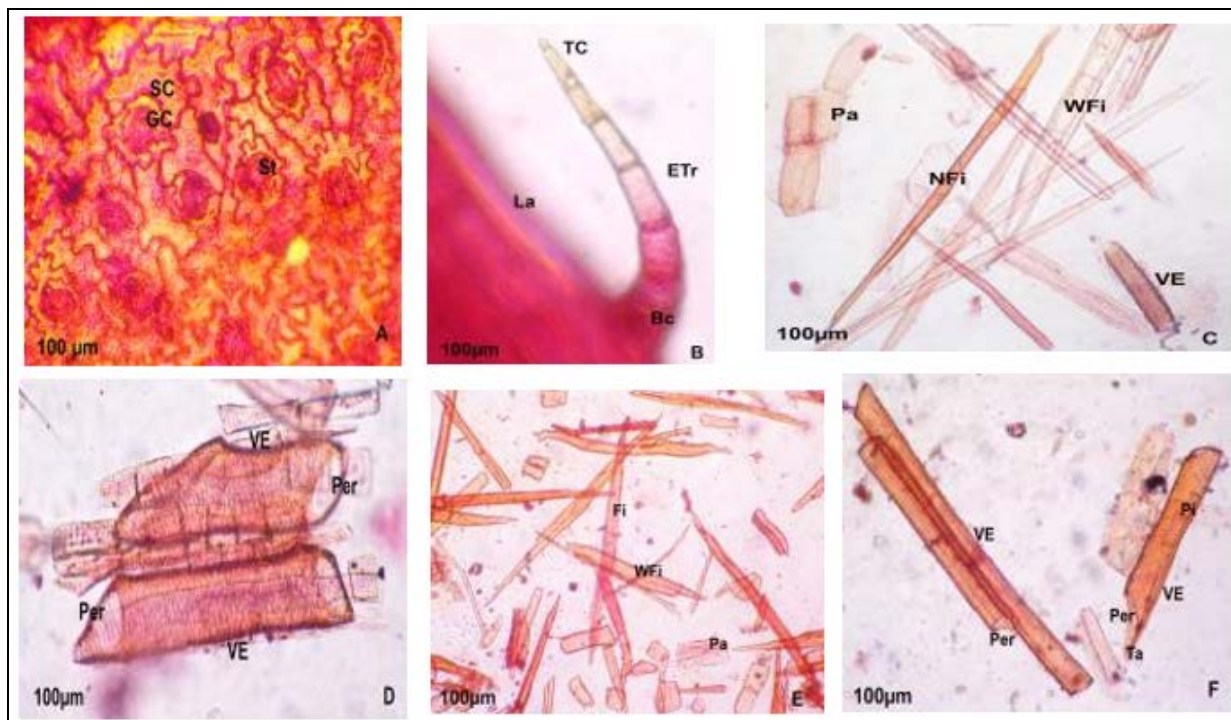


Fig 3: Microscopic observations of leaves, stem and root powder of *Coleus malabaricus*(morphotype) (A) Abaxial epidermis showing stomata 40X (B) Lateral margin of the leaf with non-glandular trichomes 40X (C) Stem powder showing vessel elements, wide fibres, narrow fibres and parenchyma cells 10 X (D) Stem powder showing vessel elements 20X (E)Root powder showing narrow fibres and parenchyma 10X (F)Root powder showing long narrow and short tailed vessel element (Explanations of abbreviations: AW-Anticlinal wall ; BC-Basal cell ; CL- Cell Lumen; CW- Cell Wall ;EC-Epidermal Cell; ETr-Epidermal Trichome; Fi-Fibres; FiB-Fibre Bundle; GC-Guard cells; SC-Subsidiary cells St-Stoma; Stp-Stomatal pore; EC-Epidermal cells; GC-Guard cells; la-Lamina; NFi-Narrow Fibres; St-stoma;Pa-Parenchyma ; ;Per- Perforation; Ta-Tail ;TC-Terminal cell; VE-vessel element; WFi-wide fibres; WFi-wide fibres)

Many of the vessels elements have oblique perforation plate with predominant tails and these vessel element have circular wide vertical rows of bordered pits.The fibres are also abundant in the root powder fibres are mostly wide and long and thick walled A thick block of compact fibres of

darkly stained periderm cells also seen in the powder. The cells are rectangular with dark dense contents. The cell are both in horizontal and vertical files (Fig 3 E&F). 5A). These cells have walls.no cell inclusions are seen. The cells are 525 µm long and µm wide.

Conclusion

The plant family Lamiaceae is well known for its wealth of aromatic herbaceous plants of traditional value. The Genus *Coleus* of the family is enriched with valuable medicinal plants and so many members are still to be exploited. In this context the plant *Coleus malabaricus* Benth. and its morphotype seen especially found in the Western Ghats area also have to be studied more considering the therapeutic importance, to find out the chemical moieties and as a base for taxonomic support. This study would definitely give a limelight for the comparative evaluation of microscopy of the authentic plant and its morphotype. The study also emphasise the need of further research works of the authentic plant and its morphotypes

Conflict of interest

The authors declare that the article content has no conflict of interest.

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