# Anatomical characterization of *Strobilanthes* (Acanthaceae) species from the northern Western Ghats of India and its implication in identification at vegetative state

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**Abstract**

A comprehensive study of stem, leaf and petiole anatomy of *Strobilanthes* from northern Western Ghats of India was carried out to identify characteristics which would enable species identification when flowering material was unavailable. In *Strobilanthes*, some species bloom annually, others are plietesials, i.e. they grow without blooming for several years and then produce huge quantities of flowers, release seeds and die. Therefore, alternative methods, such as anatomical characters, became essential to distinguish *Strobilanthes* species in their vegetative stage. During the study, ten species of *Strobilanthes* were collected from northern Western Ghats of India for anatomical characterization. Under the bright-field microscope, stem cross-sections of different species were noted as undulate, quadrangular, quadrangular-winged or terete. Study of the stem revealed a distinct outer and inner cortex, the distribution of cystoliths ($CaCO_3$ crystals), raphides ($CaC_2H_2O_5$ crystals), and sclereids which varied from species to species. Study of leaf anatomy showed structural variation and varied vascular bundle shapes between the species. Leaf epidermal characters under light and scanning electron microscopy exhibited variation in characters such as stomatal index, stomatal length and width, stomatal type and glandular or non-glandular trichomes. The petiole anatomy was distinct especially with respect to vascular bundle structure between species and distribution of structures such as sclereids, cystoliths, sphaeraphides and tannin cells varied. Hence, unique anatomical features of the stem, leaf and petiole could be used as taxonomic characters to identify *Strobilanthes* species in a vegetative state.

Keywords: Anatomy, *Strobilanthes*, Systematics, Vegetative parts

## Introduction

Blume (1826) established the genus *Strobilanthes* based on *Strobilanthes cernua* Blume and other species from Java. Nees (1832, 1847) described various additional species from India. Anderson (1867) reduced various genera recognised by Nees to synonymy under *Strobilanthes* so increasing of the genus to over a hundred species. Most authors, for example, Clarke (1884), followed Anderson treatment until. Bremekamp (1944) proposed a completely new classification. He divided *Strobilanthes* sensu Anderson (1867) into almost 50 smaller genera based principally on the structure of the pollen grain and characters of the seed testa. Bremekamp's classification was never completed to include all species of *Strobilanthes* and was never accepted by all others. Terao (1983), Wood (1994), Carine and Scotland (1998), Wood et al. (2003) rejected Bremekamp's system pointing out that it was riddled with inconsistences, separating closely related species into separate genera and using characters that were not as clear-cut as he had claimed. More recent molecular studies, especially Moylan et al. (2004) reported that the *Strobilanthinae* sensu Bremekamp formed a single monophyletic group, which could not be easily split into smaller component genera. Consequently, a single expanded genus *Strobilanthes* is generally accepted and the present study follows this approach treating *Strobilanthes* a broad, well-supported genus. The present findings have implications for the identification of *Strobilanthes* species in its vegetative state, which are often difficult to identify. Several of the genera recognised by Bremekamp that occur in the present study are *Carvia*, *Mackenziea*, *Nilgirianthus*, *Pleocaulus* and *Thelepaepale*. The species in the present study under Bremekamp's grouping are as *Carvia callosa*, *Mackenziea integrifolia*, *Nilgirianthus barbata*, *N. ciliata*, *N. heyneana*, *N. lupulina*, *N. reticulata*, *N.* sp., *Pleocaulus ritchiei* and *Thelepaepale ixiocephala*. All these segregated genera are grouped under a single genus *Strobilanthes* (Wood 1994,

Carine and Scotland 1998). Our focus is on providing anatomical characters to distinguish the species in their vegetative growth.

*Strobilanthes* Blume, the second largest genus of the family Acanthaceae, comprises perennial flowering herbs and shrubs with ca. 450 species in the tropical regions of Asia (Mabberley 2017). In India, the genus is represented by ca. 150 species (Wood 1998, Venu 2006, Karthikeyan et al. 2009) of which 59 species have been recorded from peninsular India (Venu 2006) and 64 species from the Western Ghats (Augustine 2018). Many species in the genus flower gregariously at long intervals ranging from four years (*S. integrifolia* Kuntze), seven years (*S. barbata* Nees, *S. ixiocephala* Benth., *S. sessilis* Nees var. *ritchiei* C.B. Clarke, *S. reticulata* Stapf, *S. callosa* Nees, *S.* sp.), to, twelve years (*S. kunthiana* T. Anderson ex Benth.) or even sixteen years (*S. scrobiculata* Dalzell ex C.B. Clarke), after which the seeds disperse and the plants die (Venu 2006, Mascarenhas and Janarthanam 2013, Augustine 2018). The genus *Strobilanthes* differs from other members of Acanthaceae by their combination of floral characteristics namely: filaments united to form a membranous sheath, a bifid stigma with a reduced posterior lobe, and two bundles or rows of hair on the inner posterior corolla wall that retain the style (Bremekamp 1944, Carine and Scotland 1998, 2002, Manktelow 2000, Wang and Blackmore 2003, Carine et al. 2004). The 'Stapetal curtain' is a complex structure which divides the flower due to the close synorganisation between the filaments and corolla tube and this structure is not only found in the tribe *Ruellieae* but also elsewhere in the family Acanthaceae (Moylan et al. 2004). A number of species are being cultivated for their attractive flowers and foliage as ornamental plants (*S. auriculata* var. *dyeriana* (Mast.) J.R.I. Wood, *S. anisophylla* f. *isophylla* (Nees) J.R.I. Wood while some are used as medicinal plants. *Strobilanthes ciliata* Nees, endemic to the Western Ghats, is widely used in Ayurveda as a source of drug 'Sahacharya' (Venu

2006). Distinct anatomical structural variations can also form taxonomic characters to distinguish the species. The characteristic features of the family Acanthaceae, were studied by Metcalfe and Chalk (1950), Inamdar et al. (1990), Kuo-Huang and Yen (1996), Patil and Patil (2011) in various genera and they reported the presence of acicular fibres, raphides, cystoliths and enumerated their distribution in different parts of the plant.

The studied *Strobilanthes* species are all perennial species. However *S. reticulata* and *S. sessilis* var. *ritchiei* are plants perennating from subterranean multi-headed underground rhizomes and annual aerial stems. Both these species produce annual aerial stems to complete the secondary growth by the end of the season. Although these species are annual, aerial plants show secondary growth. *Strobilanthes ciliata*, *S. lupulina*, *S. heyneana* are perennial shrubs flowering annually. Since, they are perennial shrubs their wood anatomy shows a broad range of fibres with vessels arranged uniformly in diffuse porous in vertical rows. *Strobilanthes callosa*, *S. integrifolia*, *S. ixiocephala*, *S. barbata*, *S.* sp. are pleitesials. They grow vegetatively for 4–7 years. Hence, these species shows well developed secondary growth often with annual rings. In the present study the stem selection was between 5mm to 10mm for uniformity in sections of all *Strobilanthes* species. The study was concentrated on the transverse section (t.s) and more characters related to phloem anatomy were not performed.

The ten *Strobilanthes* species in the present study are all endemic to India. The northern Western Ghats (NWG) has not been a well explored area of the Western Ghats, India with respect to *Strobilanthes* for more than a decade. Therefore documenting the species of *Strobilanthes* from NWG became important. The identification of species of *Strobilanthes* in vegetative stage is often found to be challenging due to the prolonged

intervals between blooming. Literature pertaining to anatomical studies in *Strobilanthes*, is very scanty and hence the present work has been undertaken to understand the variation among the species from the northern Western Ghats of India.

## Materials and methods

### *Study area and collection*

The present study of the anatomical features of ten species of *Strobilanthes* has been carried out in the States of Goa and Maharashtra in the northern Western Ghats of India (Fig. 1, Table 1). The northern Western Ghats (NWG) are also known as the 'Sahyadri range' in the Western Ghats, India. The NWG are densely forested with small trees, thick shrub growth and grassland plateaus and is greatly influenced by rains delivered by the south-west monsoon. There was little study with respect to *Strobilanthes* in this study area (NWG). It was important to know how many species of *Strobilanthes* were present in the area as it had not been documented in the recent years (for more than a decade). There are 10 species in the NWG at the present time. In the present study the authors have documented 10 species but one, i.e. *Strobilanthes scrobiculata* Dalzell ex C.B. Clarke could not be collected after several field visits. This species flowers once in every 16 years (Venu 2006, Augustine 2018) and was unlikely to be found in flower. The vegetative specimens were collected from Satara, Sindhudurg, Kolhapur and Mahabaleshwar (Suryanarayana 1970).

Specimens were collected, pressed as herbarium specimens using standard methods (Jain and Rao 1977) and identified using floras and earlier collections deposited at national herbaria (CAL, MH, BSI, Goa University Herbarium). Reference was made to the protologues and type specimens (Venu 2006, Mascarenhas and Janarthanam 2013, Augustine 2018). The voucher specimens were deposited in the Goa

University Herbarium Department of Botany, Goa University, Goa, India and in the Botanical Survey of India (BSI) Pune, India.

*Anatomical characterization of stem, leaf and petiole*

The samples for anatomical characterization were sliced from fresh mature samples of the stem (5mm to 10mm), leaf (middle of the lamina with main vein) and petiole (centre of the petiole) from 10 plants per species selected randomly from the entire study area. All anatomical characters were studied from six to ten free-hand sections for each of the plant parts (stem, leaf, petiole). These hand sections were stained using 0.1% Safranin and examined under bright-field microscopy (Nikon Eclipse E800 microscope). The desirable portions were photographed using a Nikon digital compact camera attached to the microscope. They were further analysed using image analyzing software (Nikon Corporation, Tokyo, Japan). Illustrations were drawn with Rötring ink pens (Rötring made, Germany, 0.1 mm nib) using prism type Camera Lucida apparatus (Weswox, India) (Figs. 9**,** 10).

*Measurements and surface morphology of leaf*

Morphometric observations include epidermal cell density ($mm^2$), stomatal index, type of stomatal complex, stomatal length and width (μm). The density and types of trichomes were determined using Scanning Electron Microscopy (SEM). The middle portion of mature leaf lamina was used uniformly for all species to avoid variation. The stomatal index was calculated for the abaxial leaf surface using Salisbury's (1927) equation.

*Scanning Electron Microscopy (SEM)*

The middle portion of mature leaf lamina were uniformly used for all ten *Strobilanthes* species and dehydrated with graded ethanol series. Dehydrated adaxial and abaxial leaf surfaces were sputter coated with gold using SC7620 Mini Sputter Coater (Quorum

Tech. Ltd. UK) under pressure and photographed using ZEISS Smart scanning electron microscope (ZEISS EVO 18 Special edition, Carl Zeiss, Germany).

## Results

*Anatomical characteristics of stem*

Studies on the transverse section (t.s.) of stem of ten species of *Strobilanthes* (Figs. 2, 3, 4, Table 2, 3) revealed the following structures:

*Epidermis*

An immense diversity in epidermis was found among the species of *Strobilanthes* from the study area. Four types of epidermal relief were identified and differentiated in this study: terete, undulate, quadrangular and quadrangular-winged. The epidermal cells were single-layered, parenchymatous, rectangular, cuticularised in some, with cystoliths and tannin cells in some others. The epidermal hairs were absent in *S. barbata* Nees, *S. ciliata* Nees, *S. integrifolia* Kuntze and present in the rest of the taxa (*S. heyneana* Nees, *S. lupulina* Nees, *S. ixiocephala* Benth., *S. reticulata* Stapf, unnamed *Strobilanthes* sp., *S. callosa* Nees, *S. sessilis* Nees var. *ritchiei* C.B. Clarke).

*Hypodermis*

Below the epidermis was followed by 1–many layered, sub-rounded hypodermis observed in all the ten species. Hypodermis 1 layered (*S. sessilis* var. *ritchiei*), 1–2 layered (*S. barbata*, *S. ciliata*, unnamed *Strobilanthes* sp.) and 2–3 layered (*S. callosa*, *S. heyneana*, *S. integrifolia*, *S. ixiocephala*, *S. lupulina*, *S. reticulata*). Cystoliths were seen in the hypodermal cells of many taxa.

*Cortex*

The cortex was well divided into outer and inner cortex. Outer cortex was made up of collenchymatous cells, which varied among the species. It was 2 layered in *S. sessilis*

var. *ritchiei* (Fig. 2f), 3 layered in *S. heyneana* (Fig. 4b) with cystoliths. In *S. barbata* it was 5 layered with cystoliths (Fig. 4a), however in the winged region it was 7–8 layered (Fig. 3b), 3–4 layered in *S. ixiocephala* (Fig. 2d), 5 layered in *S. ciliata* (Fig. 3g) with cystoliths, 5 layered in *S. reticulata* (Fig. 2e) and *S. lupulina* (5 layered) with cystoliths (Fig. 4d). In *S. integrifolia* 6 layered (Fig. 2b), *S. callosa* (Fig. 2c) and unnamed *Strobilanthes* sp. 8–9 layered (Fig. 4c) with cystoliths. Outer and inner cortex was separated by a distinct single layered chlorenchyma in *S. barbata*, *S. ciliata*, *S. callosa*, *S. heyneana*, *S. integrifolia*, *S. ixiocephala*, *S. lupulina*, *S. reticulata*, unnamed *Strobilanthes* sp., and 2 layered in *S. sessilis* var. *ritchiei*. Inner cortex was parenchymatous and distinctly varied among the species. It was single layered with cystoliths in *S. callosa* and *S. sessilis* var. *ritchiei*. In *S. barbata* (Fig. 3b), *S. ciliata* (Fig. 2a), *S. heyneana* (Fig. 2g) and unnamed *Strobilanthes* sp. 3 layered (Fig. 2h). In the winged region of *S. integrifolia* 9–10 layered and *S. barbata* 19–20 layered (Fig. 3b) with vascular strands, interspersed with cystoliths. *S. ixiocephala* (Fig. 2d) and *S. lupulina* were 4 layered (Fig. 3d), *S. reticulata* 6 layered (Fig. 2e). In the unnamed *Strobilanthes* sp. cystoliths were seen in the region of inner and outer cortex (Fig. 2h).

*Vascular tissues*

Xylem, diffuse porous. Xylem surrounded by phloem in ring form in all ten species of *Strobilanthes*. Vessels were arranged in vertical rows with vessel solitary or in groups of two. Phloem 3–4 layered (unnamed *Strobilanthes* sp.), 4–5 layered (*S. sessilis* var. *ritchiei*), 5–6 layered (*S. reticulata*), 6–7 layered (*S. callosa*), 6–8 layered (*S. integrifolia*, *S. lupulina*), 7 layered (*S. ciliata*), 7–8 layered (*S. heyneana*, *S. ixiocephala*), 10 layered–many (*S. barbata*). Well developed fibres were present arranged in vertical rows. The ridges show the vessels arranged in a diffuse porous

mode, arranged in vertical rows whereas in the furrows the xylem fibres were mainly devoid of vessels. The vessels end-walls were simple.

*Pith*

The innermost tissue of the stem was composed of parenchymatous cells that were heterogeneously packed. Various crystalline structures and secondary depositions, such as sclereids, brachysclereids, cystoliths, raphides, were present in pith cells and the pattern of packing varied between the species. There is hexagonal parenchyma interspersed with sclereids and cystoliths in *S. barbata* (Fig. 3a, 3b, 3c). In *S. callosa*, hexagonal pith cells with cystoliths and few sclereids were present (Fig. 2c). The pith cells in *S. ciliata* were hexagonal and distinct brachysclereids in the centre of pith were present (Fig. 2a), while in *S. ixiocephala* pith cells were hexagonal interspersed with raphides (Fig. 2d, 3e) and few sclereids (Fig. 2d). Hexagonal pith cells with uniformly scattered sclereids were present in *S. heyneana* (Fig. 2g, 3f), while the pith cells of *S. integrifolia* were hexagonal with few cystoliths (Fig. 2b, 3h). Hexagonal pith cells with sclereids were present in *S. lupulina* (Fig. 3d). The pith cells of *S. reticulata* (Fig. 2e) and *S. sessilis* var. *ritchiei* (Fig. 2f) were hexagonal in shape with few sclereids. The unnamed *Strobilanthes* sp. presented hexagonal pith cells with interspersed sclereids (Fig. 2h).

*Anatomical characteristics of leaf*

Studies on the transverse section (t.s.) of the leaf at the midrib region (Figs. 4, 5, 6, Table 4, 5) revealed the following features:

*Epidermis*

The upper epidermis was composed of thick-walled cells that were larger than those of the lower epidermis. In the majority of the species it comprised 1 layered, sub-

rectangular cells which were thickly-cuticularised on the adaxial surface whereas *S. ixiocephala* and unnamed *Strobilanthes* sp. presented with thinly cuticularised cells. It is quite interesting that the adaxial and abaxial surface were found to be pubescent in *S. callosa* (Fig. 4g), *S. heyneana* (Fig. 5c), *S. ixiocephala* (Fig. 4h), *S. lupulina* (Fig. 5f), *S. reticulata* (Fig. 5a), *S. sessilis* var. *ritchiei* (Fig. 5b), unnamed *Strobilanthes* sp. (Fig. 5d) whereas *S. ciliata* (Fig. 4e), *S. integrifolia* (Fig. 4f) and *S. barbata* (Fig. 5e) presented with a glabrous surface.

Structures such as cystoliths, sclereids, sphaeraphides were found arranged in the ground tissue of the leaf. There were quite notable structural variations in the spongy parenchyma tissue of different taxa. Cystoliths were observed in the leaf tissues of most of the *Strobilanthes* species studied. In *S. barbata* the spongy parenchyma presented with interspersed sclereids and cystoliths (Fig. 5e), *S. callosa* was observed with few cystoliths in the collenchyma cells of upper epidermis (Fig. 6f), and in the ground tissue of leaf midrib-region (Fig. 5g), *S. ciliata* shows sphaeraphides in the collenchyma of upper and lower epidermis (Fig. 5h). *Strobilanthes heyneana* was devoid of such features while *S. integrifolia* showed cystoliths arranged in the lower and upper epidermis (Fig. 6a). Cystoliths were observed in ground tissue, upper and lower epidermis of *S. ixiocephala* (Fig. 4h). Cystoliths were found in the collenchyma of upper and lower epidermis of *S. lupulina* (Fig. 5f) while *S. reticulata* and *S. sessilis* var. *ritchiei* (Fig. 6b, 6e) showed few cystoliths in the collenchyma of lower epidermis and midrib region. The unnamed *Strobilanthes* sp. showed a few cystoliths in ground tissue (Fig. 6c) and a prominent sclereid was observed in the mid-rib region (Fig. 6d).

*Palisade layer*

The palisade layer comprised palisade parenchyma cells that were closely packed, vertically elongated and chlorophyllous. There were no significant variations between

the taxa, *i.e., S. ciliata, S. integrifolia, S. sessilis* var. *ritchiei, S. lupulina* presented with 1–2 layered and *S. callosa, S. ixiocephala, S. reticulata, S. heyneana*, unnamed *Strobilanthes* sp., *S. barbata* showed 2–3 palisade layers.

*Vascular bundle*

The water conducting tissue, xylem, lies towards the upper epidermis with thick walled and a compactly arranged cell in vertical rows while the food conducting tissue, phloem, lies towards the lower epidermis and was thin-walled. The vascular tissue arrangement in *Strobilanthes* species can be categorised into three types i.e. broad U-shaped, narrow U-shaped and V-shaped. The species *S. ciliata* (Fig. 4e), *S. ixiocephala* (Fig. 4h), *S. heyneana* (Fig. 5c), *S. barbata* (Fig. 5e), *S. lupulina* (Fig. 5f) showed broad U-shaped vascular bundle; *S. integrifolia* (Fig. 4f), *S. reticulata* (Fig. 5a), *S. sessilis* var. *ritchiei* (Fig. 5b) revealed narrow U-shaped whereas *S. callosa* (Fig. 4g), unnamed *Strobilanthes* sp. (Fig. 5d) showed V-shaped vascular bundle.

*Leaf epidermal studies using Bright-field and SEM* (Figs. 6, 7, Table 6)

The stomatal characteristics such as stomatal type, length and width and, stomatal index were determined using light microscopy; the representative images are shown in Fig. 6g, 6h and similarly SEM images of a few *Strobilanthes* species are also provided (Fig. 7). All the *Strobilanthes* species studied showed mostly "diacytic" type of stomatal complex, however with variation in length and width and morphology of guard cells, subsidiary cells in each species. The stomata were concentrated on the abaxial surface. Diacytic stomata were enclosed by a pair of subsidiary cells with walls at right angle to the guard cells. On closer examination, each guard cell pair has one very small subsidiary cell, and two successively greater ones (anisocytic pattern), were observed. This observation was clearly viewed in *S. ixiocephala* (Fig. 6h). The stomatal index was

calculated for all the studied taxa of *Strobilanthes* (Table 6). The range of Stomatal Index (SI) varied between 26–33.

Trichomes were present in most of the species of *Strobilanthes* studied. The leaves of *S. lupulina*, *S. heyneana* and *S. ixiocephala*, *S. reticulata* were characterized by profusion of non-glandular types of trichomes on both adaxial and abaxial surfaces while taxa like *S. sessilis* var. *ritchiei*, unnamed *Strobilanthes* sp. and *S. callosa* showed sparse trichomes under light microscopy.

When observed under SEM, trichomes in *Strobilanthes* species can be categorized into two type's, *viz*., simple non-glandular trichomes and peltate glandular trichomes with 1-celled apical head. They differ in their morphology and density between the taxa. Nevertheless, different patterns of density were observed on the adaxial and abaxial surfaces. *Strobilanthes callosa* and *S. integrifolia* showed peltate glandular trichomes on the adaxial surface (Fig. 7g, 7i). *Strobilanthes barbata* (Fig. 7j) showed sparse peltate glandular trichomes with a wrinkled head on both surfaces whereas *S. ixiocephala* showed peltate glandular trichomes on the adaxial surface (Fig. 7h) and four-celled non-glandular trichomes on both leaf surfaces. The adaxial surface of *S. heyneana* had 1-celled trichomes with nine basal cells surrounding a non-glandular trichome (Fig. 7k) and the abaxial surface also showed non-glandular trichomes. *Strobilanthes reticulata* exhibited a profuse distribution of non-glandular trichomes on both the adaxial and abaxial surfaces (Fig. 7l). In the case of *S. sessilis* var. *ritchiei*, non-glandular trichomes were sparsely present on the adaxial surface (Fig. 7m). The unnamed *Strobilanthes* sp. showed simple non-glandular trichomes sparsely on both surfaces, predominantly seen in the vein region of the leaf (Fig. 7n). *Strobilanthes lupulina* possessed non-glandular trichomes and peltate glandular trichomes densely distributed on both the adaxial and

abaxial surfaces (Fig. 7o). *Strobilanthes barbata* showed a single type of trichome, peltate glandular trichomes on both the surface of leaf.

*Anatomical characteristics of petiole*

Studies on transverse section (t.s.) of the petiole (Figs. 8, 9, 10, Table 7, 8) revealed the following features:

The petiole shape varied from dorsi-ventrally undulate to flat to terete in cross-section. Two lateral wings were present in most of the species, but varied in size. In *S. ciliata*, the petiole was dorsi-ventrally flattened with the abaxial surface convex and the adaxial surface shallowly concave (Fig. 8c). The petioles of *S. integrifolia* (Fig. 8e), *S. ixiocephala* (Fig. 8f) and the unnamed *Strobilanthes* sp. (Fig. 8j) were sub-terete with the abaxial surface convex and the adaxial surface flat to slightly convex or shallowly concave. The petioles of *S. heyneana* (Fig. 8d) and *S. lupulina* (Fig. 8g) were terete, being convex on both adaxial and abaxial surfaces. The petiole of *S. callosa* (Fig. 8b) showed a distinct sub-terete shape with the abaxial surface convex and the adaxial surface slightly concave in the center and deeply concave at the wings. The petioles of *S. reticulata* (Fig. 8h) and *S. sessilis* var. *ritchiei* (Fig. 8i) showed an undulate abaxial surface while the adaxial surface was slightly concave. In *S. barbata* (Fig. 8a) the petiole showed terete convex on the abaxial surface and adaxial surface circular at the centre and deeply concave at wings.

The epidermis consisted of a single layer of cells with thin cutinized outer walls that were more or less rectangular in shape. Trichomes were found in most of the species except in *S. ciliata*, *S. callosa*, *S. barbata* which were glabrous. The trichomes were simple, unicellular to multicellular, occurring abundantly on both the adaxial and abaxial surfaces in *S. heyneana* and were non-pigmented whereas in, *S. lupulina* the

trichomes showed pigmentation. They were sparsely distributed in *S. sessilis* var. *ritchiei*. A well-developed hypodermis made up of collenchyma cells was present beneath the epidermal cells of both surfaces of the petiole of all studied taxa. The individual hypodermal cells were more or less cubical to round in shape. The ground tissue consisted of numerous parenchymatous cells which were either hexagonal or irregular in outline and structures such as sclereids, cystoliths, sphaeraphides and tannin cell were seen with varied packing patterns (Figs. 9, 10).

In all ten species of *Strobilanthes* examined, the vascular structure consisted of a single principal bundle accompanied by subsidiary strands in the wings. They were always supported by layers of interspersed collenchyma and sclerenchyma. On the basis of their shape, four main types of vascular bundles were found in the species examined. Crescent-shaped bundles were found in *S. reticulata* (Fig. 10c) and *S. sessilis* var. *ritchiei* (Fig. 10d). *Strobilanthes callosa* showed U-shaped vascular bundles (Fig. 9b) while, *S. barbata* showed circular vascular bundle (Fig. 9a). Deeply crescent-shaped bundles were present in most of the species, including the unnamed *Strobilanthes* sp. (Fig. 10e), *S. ciliata* (Fig. 9c), *S. integrifolia* (Fig. 9e), *S. ixiocephala* (Fig. 10a), *S. heyneana* (Fig. 9d) and *S. lupulina* (Fig. 10b).

## Discussion

The comprehensive goal of the current study was to find and document anatomical characters and its implication for the identification of *Strobilanthes* species in vegetative stage.

### *Stem anatomy*

The present study revealed the unique distinguishing characters in each *Strobilanthes* species that could be employed to identify the taxa at their vegetative stage. For the

Stem, the stem outline, the cortical layers in the outer and inner cortex, the presence of cystoliths and their distribution in the stem, the shape of pith cells and the intervention of sclereids (stone cells), types of sclereids, cystoliths, raphides, presence and absence of trichomes, etc. Metcalfe and Chalk (1950), Inamdar et al. (1990), Kuo-Huang and Yen (1996), Patil and Patil (2011), Tripp and Fekadu (2014), Fernandes and Krishnan (2019) reported the presence of acicular fibres, raphides and cystoliths enumerated their distribution within the regions of the stem and stated that these features were notable to this family.

*Strobilanthes* species studied showed the presence of sclereids in the pith cells of the stem except in *S. ciliata*, which revealed the presence of brachysclereids, reported here for the first time. Sclereids were reported to provide mechanical support and protection, aiding plants with supplementary vigour and firmness (Rao 1957). According to Fahn (1982) brachysclereids were produced in plants as a response towards physiological disturbances. The stem of *Strobilanthes* species was observed to be quite firm in spite of their herbaceous nature, owing to the distribution of sclereids. Many of the anatomical features are genetically fixed and characteristics of the respective species (Schweingruber et al. 2011). Thus, anatomical features were gathered to find valuable comparison, to distinguish the *Strobilanthes* species in their vegetative stage.

*Leaf anatomy*

The leaves are variable with respect to the packaging of features such as sclereids, raphides, cystoliths in the spongy parenchyma of the *Strobilanthes* species examined. Variations in the arrangement of the shape of vascular bundles were observed in the present study. The systematic significance of vascular bundle arrangement is significant and is proven in the family Rhizophoraceae (Nurnida 2012) and Dipterocarpaceae

(*Parashorea*) (Noraini and Cutler 2009). During this study, 3 types of vascular bundles were identified i.e. broad U-shaped, narrow U-shaped and V-shaped. The most common type of vascular bundle shared by the *Strobilanthes* species was broad U-shaped. The comprehensive study on nodal and leaf anatomy of Bonnetiaceae provided important characters such as foliar vascular bundles enveloped by a sheath composed of two concentric regions, as in, an inner region consisted of multiple layers of fibres and an outer specialized endodermis composed of thin-walled cells with Casparian strips, which helped in segregation of Bonnetiaceae from Theaceae (Dickison and Weitzman 1996).

The SEM studies showed that the trichomes were of two types in the present study: Non-glandular and peltate type glandular trichomes. They serve as antiherbivory function (Valverde et al. 2001), in the production of aromatic compounds (Gersbach 2002). It is also hypothesized that aromatic and ethereal oils may have lower evaporation and transpiration rates (Fahn 1990). Hair types cannot serve directly to define groups, they can be characteristic of individual species (Moylan et al. 2004).

The diacytic type (cross-walled) stomata were observed in all the studied species of *Strobilanthes*. They are mainly confined to the lower side of the leaf, are always caryophyllaceous (Solereder 1908, Metcalfe and Chalk 1950, Inamdar 1970). Nevertheless, other stomatal type was rarely mixed with diacytic type. For example, anisocytic stomata were observed in most of the *Strobilanthes* species studied. Similar rare observations were made in the other members of Acanthaceae (Paliwal 1969, Inamdar et al. 1983). The stomata are diacytic type when the subsidiary cells are consistently two however, there can be variation of other types of stomata on the same foliar surface. This type of variations was also documented in the other dicot families (Solereder 1908, Inamdar and Patel 1969, Rajagopal 1973, Shisode and Patil 2008).

Studies have shown that the stomatal index of leaves differs with the light intensity encountered by the plant, the lower the light intensity lesser the Stomatal Index (Schoch et al. 1980). The highest Stomatal Index (SI) was observed in *S. barbata* and the lowest in *S. reticulata*. As *S. reticulata* is densely covered with trichomes on both surfaces, this could be a factor for its lower SI.

*Petiole anatomy*

Metcalfe and Chalk (1950) pointed out the importance of petiolar anatomy along with other anatomical data which can be used as in systematics. It was indicated that the vascular pattern in petiole of some genera of Acanthaceae can be effectively used in taxonomic significance. In the present study, transverse section, the very distinct shape of the petiole and vascular bundles has yielded some interesting data (Figs. 13–22). In the present study, the parenchymatous cells of ground tissue showed structures such as sclereids, cystoliths, sphaeraphides and tannin cells seen with varied packaging and their distribution is unique for any particular species. The formation of cystoliths are of four phases: a pure silica phase; a silica phase which becomes an Mg-rich silica phase; a silica phase overlaid with a relatively stable Amorphous Calcium Carbonate (ACC) phase; and a bulky and less stable ACC phase which superimposes the first stable ACC phase (Gal et al. 2012). These phases of cystoliths and minor calcium oxalate reflections were elucidated in the present *Strobilanthes* species studied using X-ray Diffraction analysis (Fernandes and Krishnan 2019).

The vascular nodal patterns such as crescent-shaped, deeply crescent-shaped, U-shaped and circular vascular bundles were reported in the present study. Among these, deeply crescent-shaped was found as the most common type of vascular bundle in the petiole anatomy of ten species of *Strobilanthes*. Howard (1962, 1979) proposed the

terminology and ontogeny of petiolar vascular systems. The systematic significance of types of vascular bundles was used for grouping of species of *Dipterocarpus* (Ruzi et al. 2009). On the basis of these characteristics the petiole anatomy provides new taxonomically informative characters (Metcalfe and Chalk 1950, Olowokudejo 1987, Patil and Patil 2012, Talip et al. 2017, Song and Hong 2018).

In conclusion, this study of the anatomical characteristics of stem, leaf and petiole of the *Strobilanthes* species of northern Western Ghats of India revealed unique anatomical characters which could be of immense taxonomic importance. Although the flower acts as a significant structure for species identification, it poses a greatest challenge in the case of *Strobilanthes* due to the prolonged intervals between flowering. Hence the above findings using vegetative anatomical characters could form a basis which will play a vital role in distinguishing the species of *Strobilanthes* in their vegetative state.

**Acknowledgements**

We are grateful to Dr. John R.I. Wood, Department of Plant Sciences, University of Oxford, UK, for useful discussions, motivation and guidance. The study was supported by the University Grants Commission, New Delhi, India for the financial support under SAP-II at the level of DRS–III (No.F.3-9/2015/DRS–III (SAP–II) in the Department of Botany, Goa University, India. The first author is thankful to Maulana Azad National Fellowship, New Delhi, India, for providing Research Fellowship Award. We wish to thank the two anonymous reviewers for comments to improve the manuscript.

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**Figure Legends**

Figure 1. *Strobilanthes* species from northern Western Ghats of India. (a) *S. callosa* Nees, (b) *S. heyneana* Nees, (c) *S. ixiocephala* Benth., (d) *S. sessilis* Nees var. *ritchiei* C.B. Clarke, (e) *S. integrifolia* Kuntze, (f) *S. ciliata* Nees, (g) *S. barbata* Nees, (h) *S. lupulina* Nees, (i) *S.* sp., (j) *S. reticulata* Stapf.

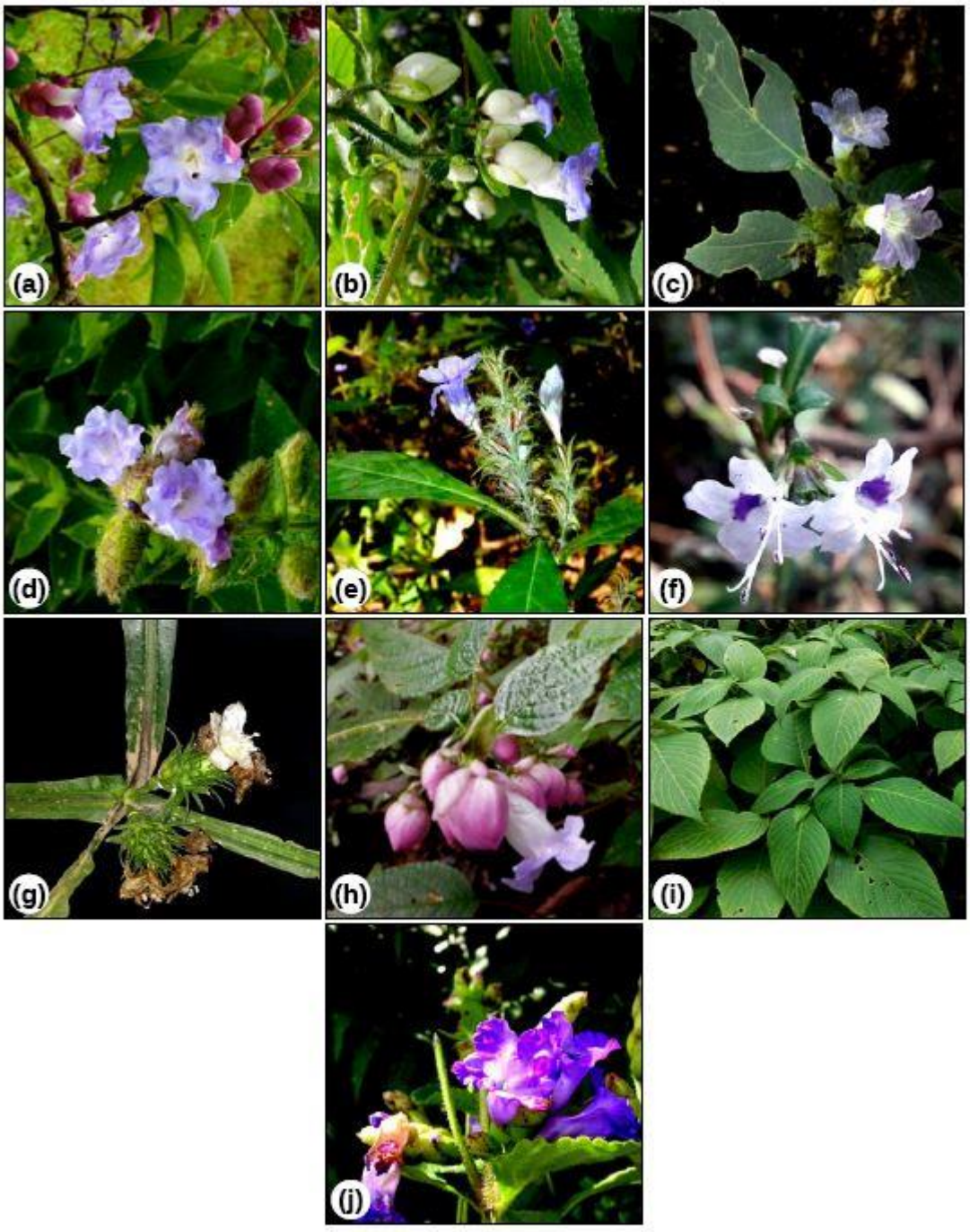

Figure 2. Stem anatomy of *Strobilanthes* spp. (a) *S. ciliata* (showing distinct brachysclereids in the centre of pith), (b) *S. integrifolia* (few cystoliths in pith cells), (c) *S. callosa* (sclereids distributed in pith cells), (d) *S. ixiocephala* (raphides concentrated in centre of pith cells), (e) *S. reticulata* (hexagonal shaped pith cells with few sclereids), (f) *S. sessilis* var. *ritchiei* (hexagonal shaped pith cells with sclereids), (g) *S. heyneana* (sclereids and raphides in the hexagonal pith cells, cystoliths in the cortex), (h) *S.* sp. (cystoliths in the cortex, sclereids in pith cells). BSc = brachysclereids, Cyt = cystoliths, E = epidermis, H = hypodermis, iC = inner cortex, Oc = outer cortex, P = pith, Ph = phloem, X = xylem, Sc = sclereids (10x).

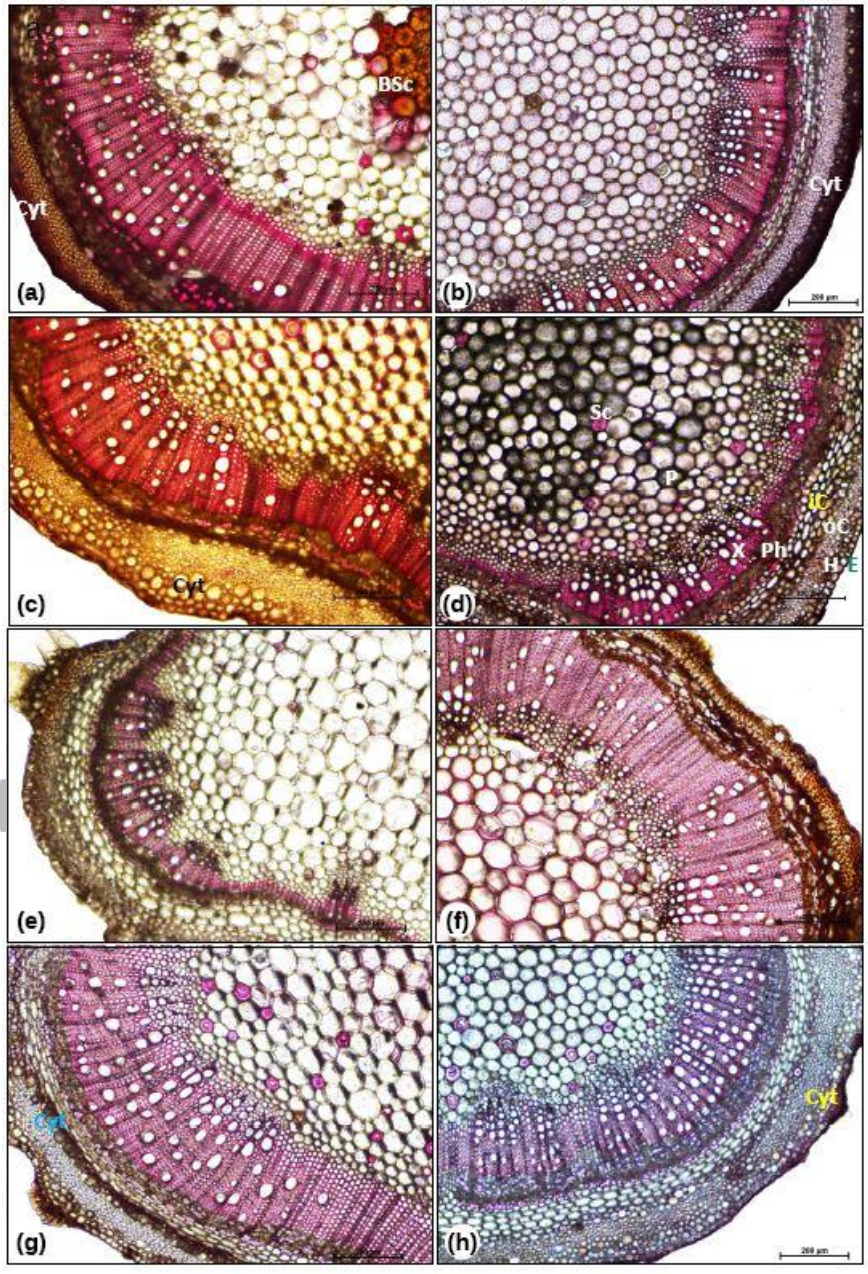

Figure 3. Stem anatomy of *Strobilanthes* spp. (a) *S. barbata* (hexagonal pith cells with cystoliths and sclereids (4x), (b) & (c) *S. barbata* (showing magnified view of winged stem) (10x), (d) *S. lupulina* (hexagonal pith cells with sclereids) (10x), (e) *S. ixiocephala* showing raphides in the pith cells (40x), (f) *S. heyneana* (hexagonal shaped pith cells with uniformly distributed sclereids) (40x), (g) Cystoliths in hypodermal cells and outer cortex of *S. ciliata* (40x), (h) Pith cells of *S. integrifolia* showing cystoliths (40x). Cyt = cystoliths, E = epidermis, H = hypodermis, iC = inner cortex, oC = outer cortex, P = pith, Ph = phloem, X = xylem, R = raphides, Sc = sclereids.

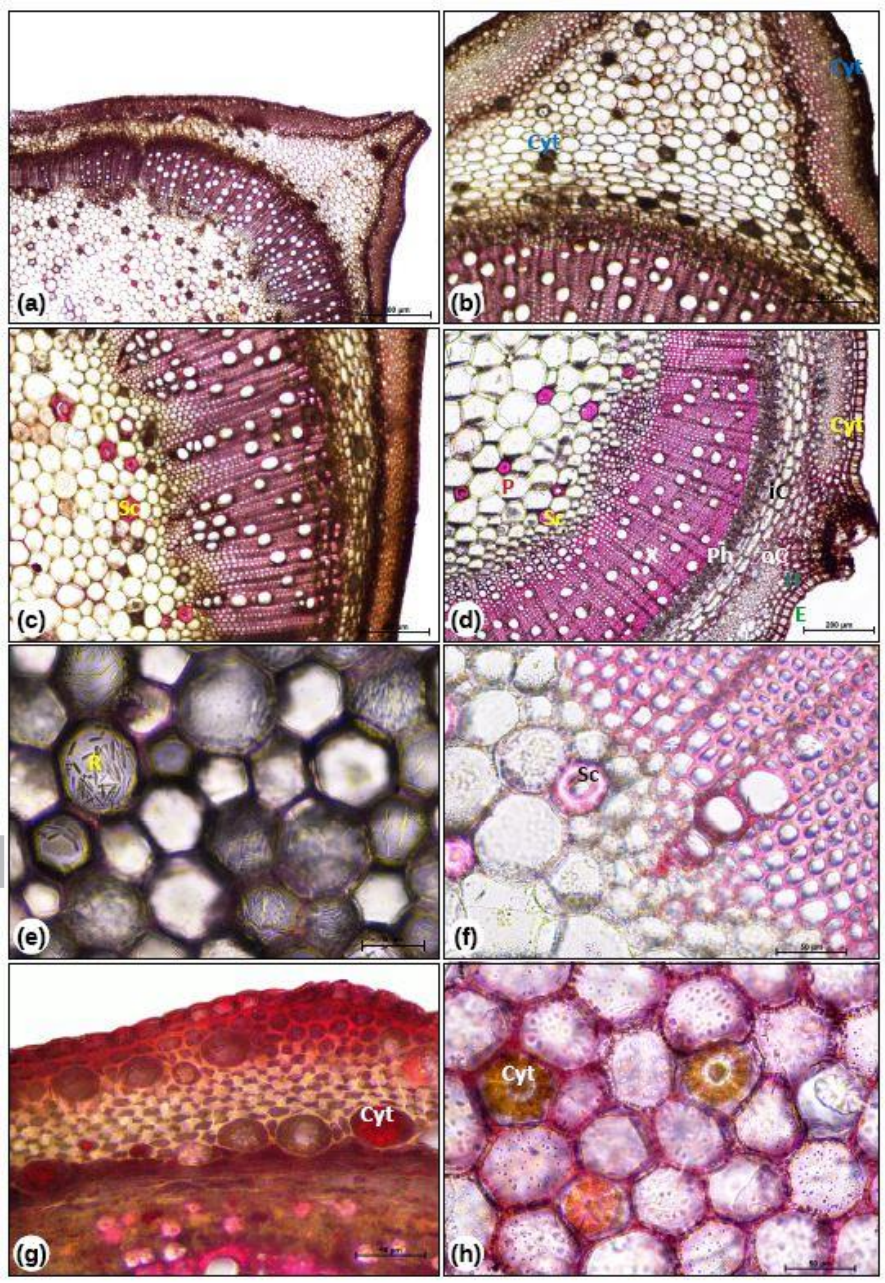

Figure 4. Stem and Leaf anatomy of *Strobilanthes* spp. cystoliths in the hypodermal cells and outer cortex of stem (40x) (a) *S. barbata*, (b) *S. heyneana*, (c) *S.* sp., (d) *S. lupulina*, (e) *S. ciliata* leaf (broad U-shaped vascular bundle) (4x), (f) *S. integrifolia* leaf (narrow U-shaped vascular bundle) (4x), (g) *S. callosa* leaf (v-shaped vascular bundle) (4x), (h) *S. ixiocephala* leaf (broad U-shaped vascular bundle) (4x). Cyt = cystoliths, Pp = palisade parenchyma, R = raphides, Sc = sclereids, Sph = sphaeraphides.

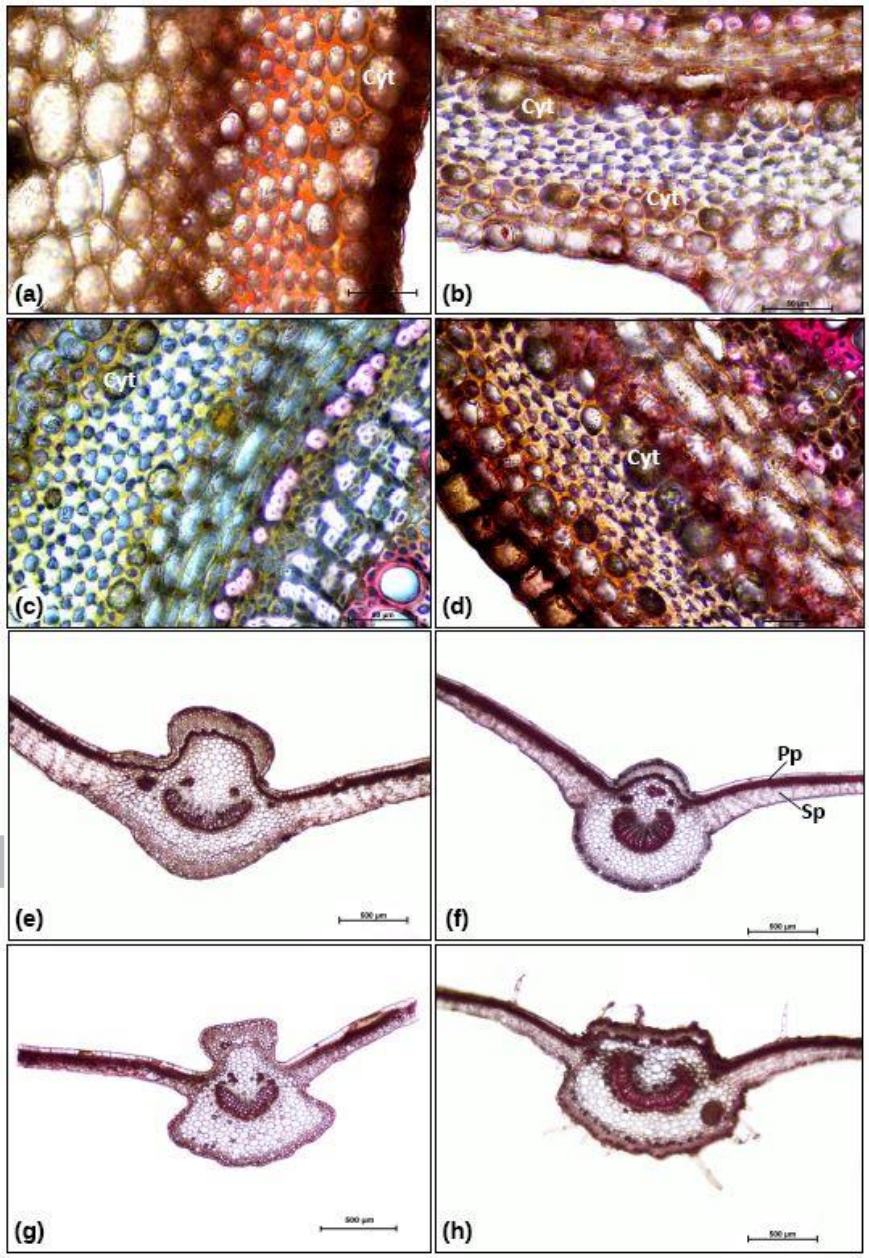

Figure 5. Leaf anatomy of *Strobilanthes* spp. (a) *S. reticulata* leaf (narrow U-shaped vascular bundle) (4x), (b) *S. sessilis* var. *ritchiei* leaf (narrow U-shaped vascular bundle) (4x), (c) *S. heyneana* leaf (broad U-shaped vascular bundle) (4x), (d) *S.* sp. leaf (V-shaped vascular bundle) (4x), (e) *S. barbata*, (f) *S. lupulina* leaf (broad U-shaped vascular bundle) (4x), (g) *S. callosa* ground tissue of leaf midrib region showing cystoliths (40x), (h) *S. ciliata* collenchyma cells of lower epidermis of leaf showing sphaeraphides (40x). Cyt = cystoliths, Pp = palisade parenchyma, Sc = sclereids, Sp = spongy parenchyma, Sph = sphaeraphides.

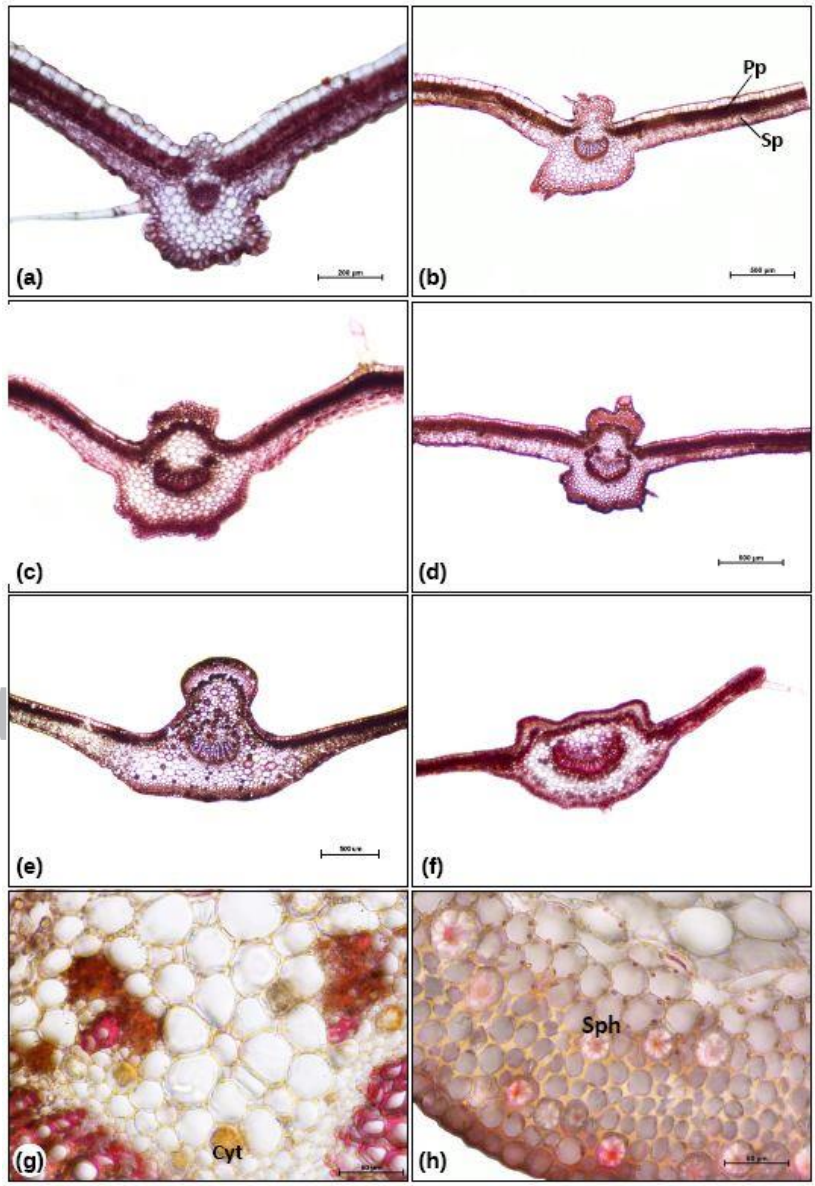

Figure 6. Leaf anatomy and stomata type of *Strobilanthes* spp. (a) *S. integrifolia* showing the arrangement of cystoliths in collenchyma cells of upper epidermis of leaf (40x), (b) *S. sessilis* var. *ritchiei* collenchyma cells of lower epidermis of leaf showing fewer cystoliths (40x), (c) *S.* sp. ground tissue with cystolith (40x), (d) *S.* sp. observed prominent sclereid in the mid-rib region of leaf (40x), (e) *S. sessilis* var. *ritchiei* collenchyma cells of upper epidermis and the epidermal cells of leaf showing cystoliths (40x), (f) *S. callosa* leaf showing cystoliths in the collenchyma cells of upper epidermis (40x), (g) *S. callosa* (Diacytic type of stomata), (h) *S. ixiocephala* (Diacytic & anisocytic type of stomata ). Cyt = cystoliths, Sc = sclereids.

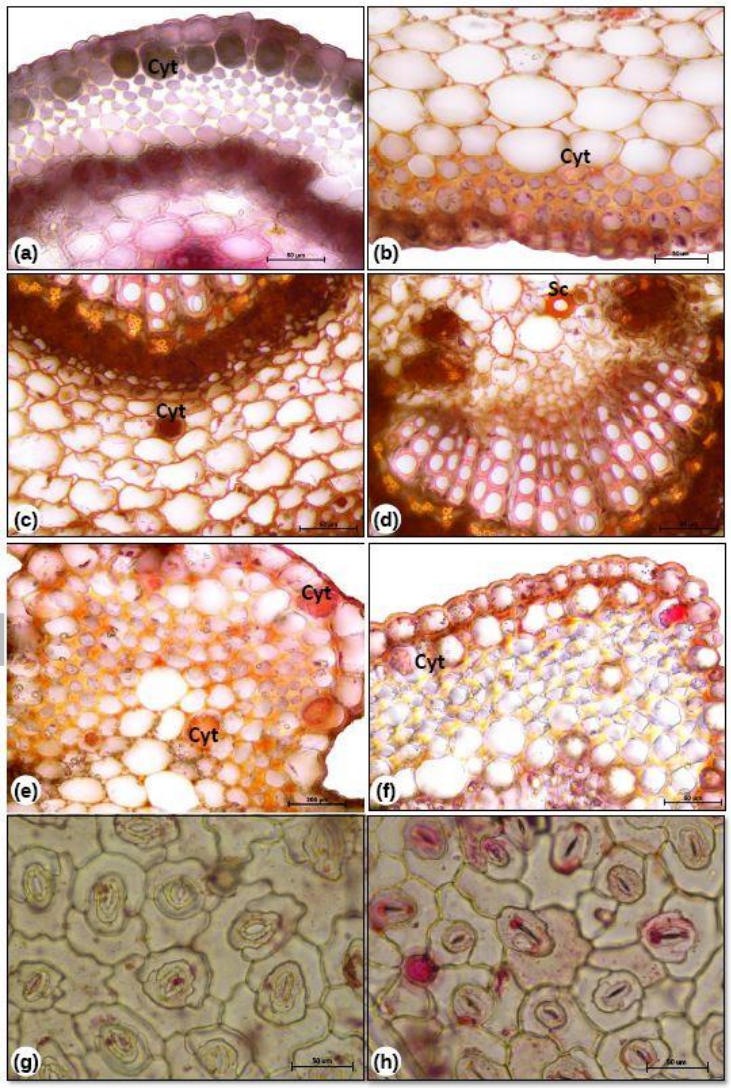

Figure 7. Scanning Electron Micrographs of *Strobilanthes* spp. showing stomata and trichomes. (a) *S. barbata* adaxial surface (2000x), (b) *S. barbata* abaxial surface (1000x), (c) *S. callosa* adaxial surface (2000x), (d) *S. callosa* abaxial surface (1000x), (e) *S.* sp. adaxial surface (2000x), (f) *S.* sp. abaxial surface (1000x), (g) *S. callosa* adaxial surface with peltate glandular trichomes (1000x), (h) *S. ixiocephala* adaxial surface showing epidermal cells with peltate glandular trichome (1000x), (i) *S. integrifolia* adaxial surface showing epidermal cells with a wrinkled head of a peltate glandular trichome (1000x), (j) *S. barbata* adaxial surface showing epidermal cells with a peltate glandular trichome (1000x), (k) *S. heyneana* adaxial surface with 9 basal cells surrounding the non-glandular trichome (1000x), (l) *S. reticulata* adaxial surface with non-glandular trichomes (1000x), (m) *S. sessilis* var. *ritchiei* adaxial surface showing epidermal cells with non-glandular trichome (1000x), (n) *S.* sp. adaxial surface with a 4-celled non-glandular trichome (1000x), (o) *S. lupulina* adaxial surface showing epidermal cells with a peltate glandular trichome and striated non-glandular trichomes (1000x).

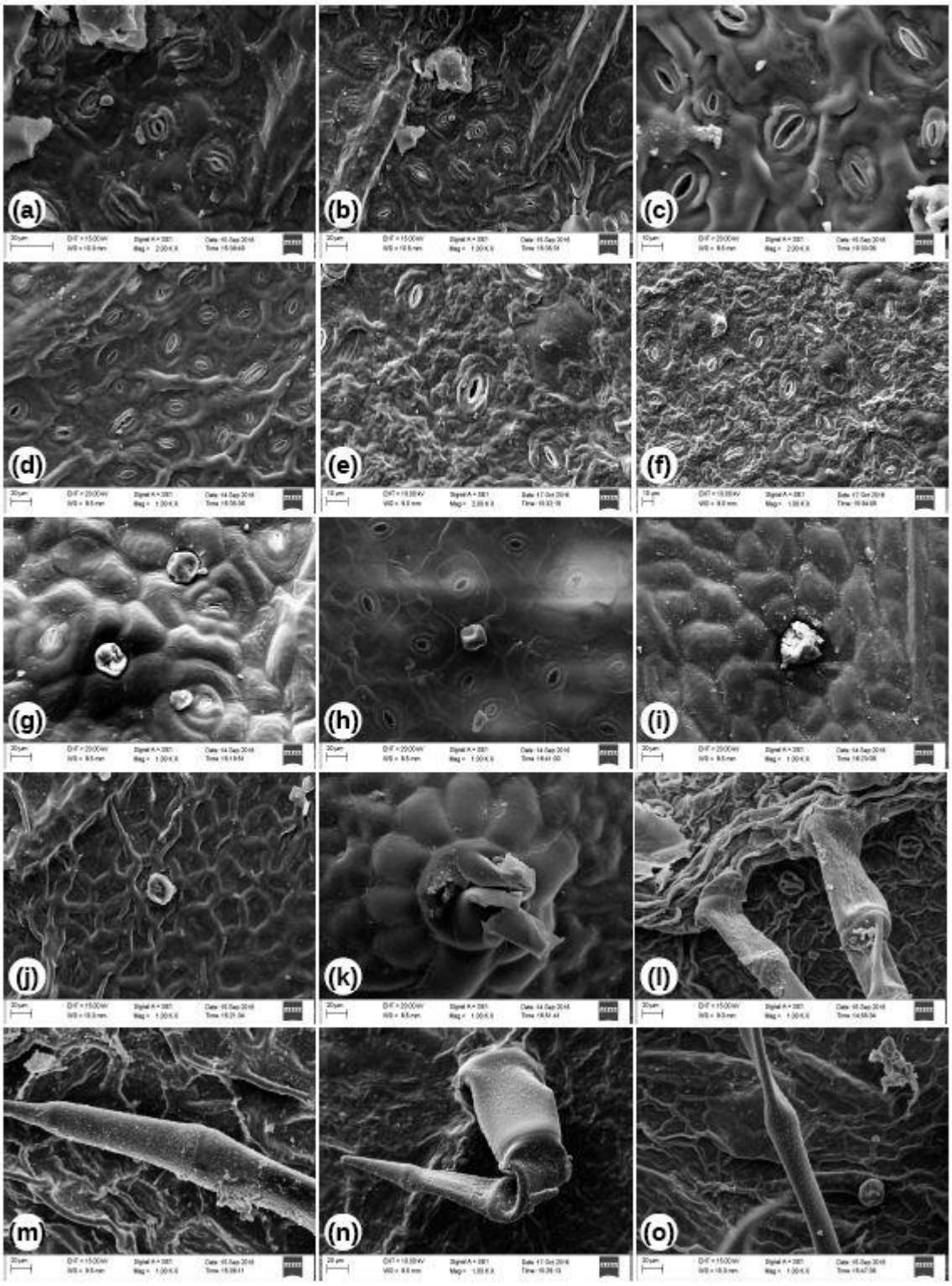

Figure 8. Petiole anatomy. (a) *S. barbata*, (b) *S. callosa*, (c) *S. ciliata*, (d) *S. heyneana*, (e) *S. integrifolia*, (f) *S. ixiocephala*, (g) *S. lupulina*, (h) *S. reticulata*, (i) *S. sessilis* var. *ritchiei*, (j) *S.* sp. (4x).

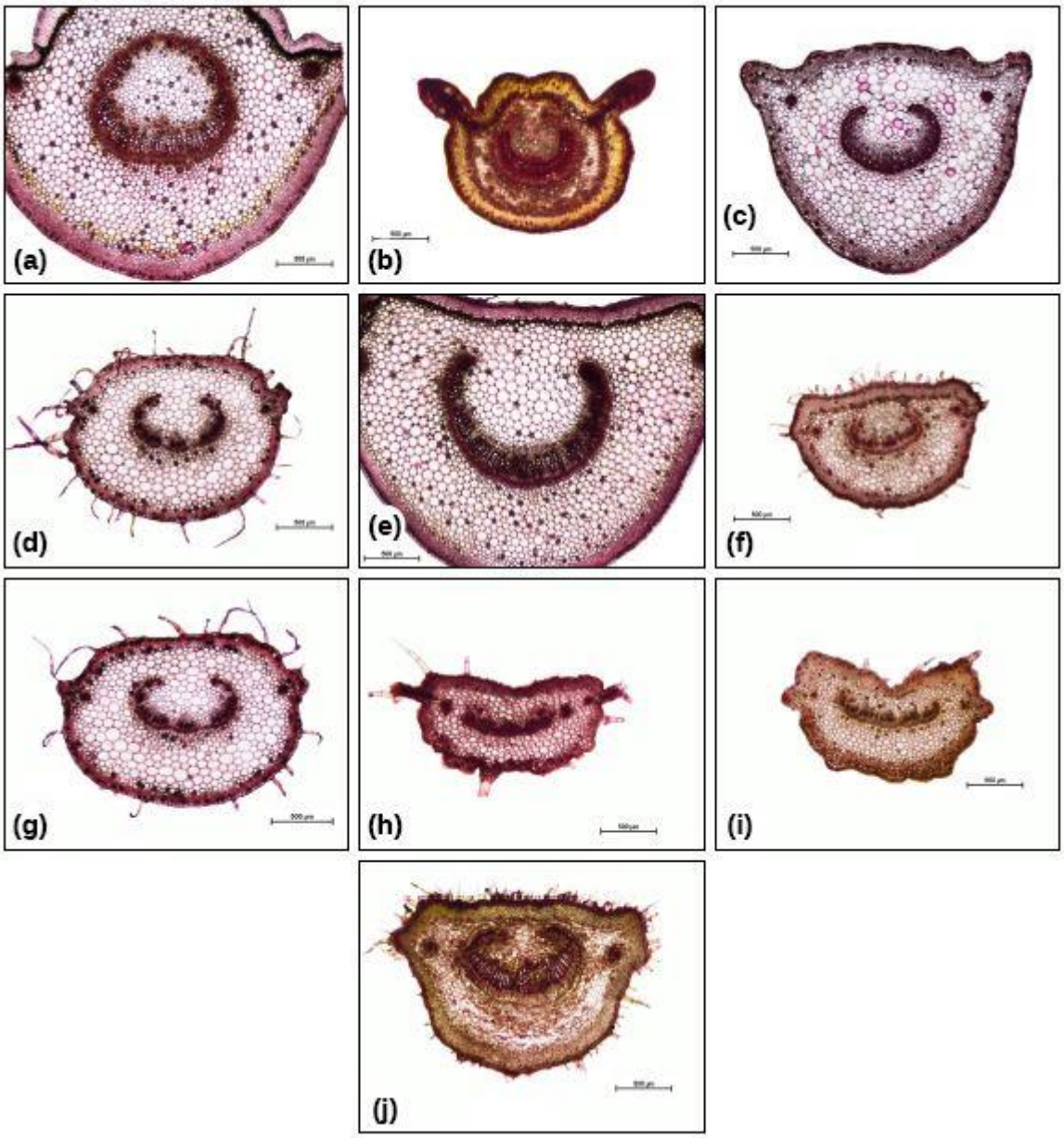

Figure 9. Petiole anatomy of *Strobilanthes* spp. showing structure of main vascular bundle. (a) *S. barbata* (circular), (b) *S. callosa* (U-shaped), (c) *S. ciliata*, (d) *S. heyneana*, (e) *S. integrifolia* (deeply crescent-shaped). Col = collenchymas, Cyt = cystoliths, Sc = sclereids, Sph = sphaeraphides, Tc = tannin cell.

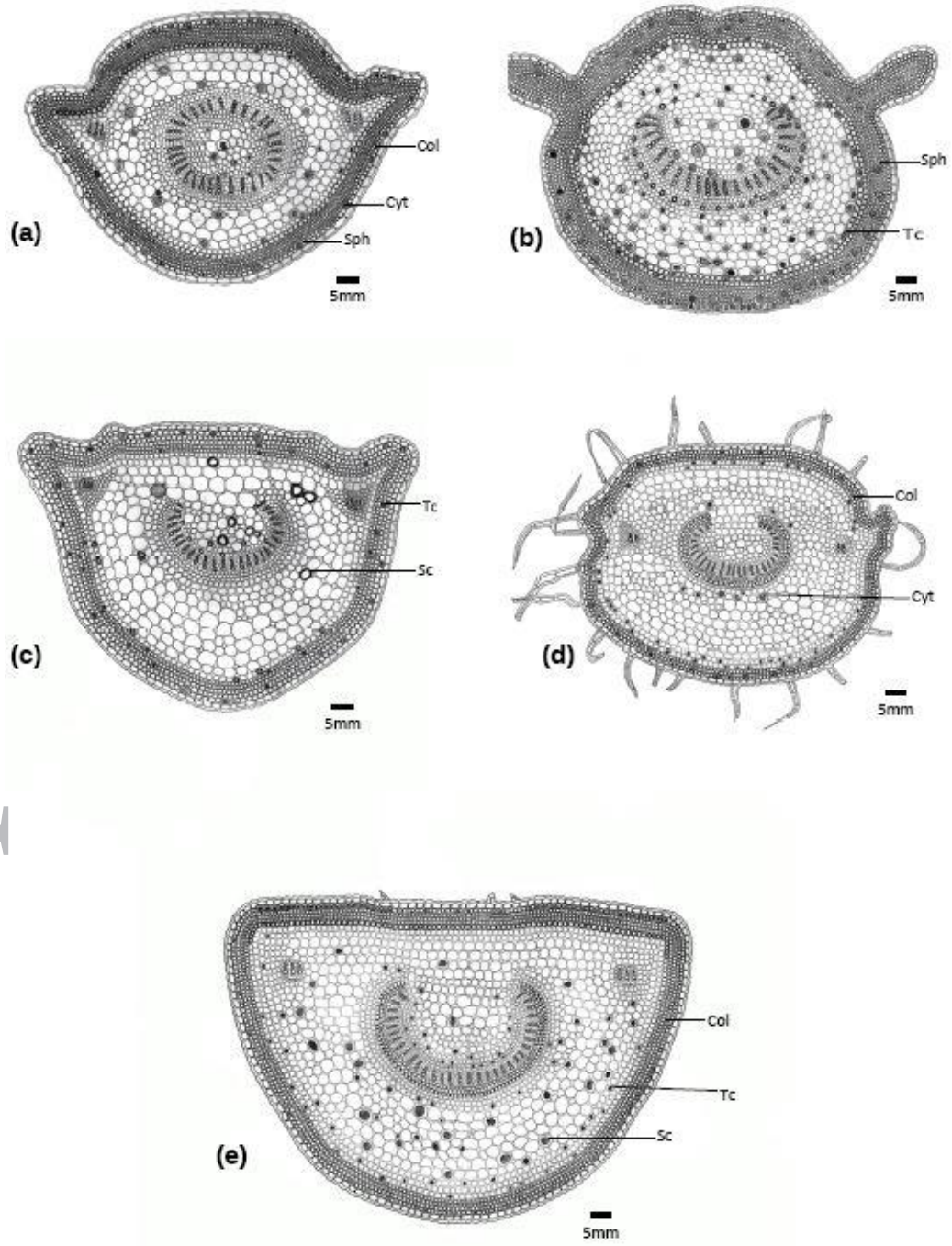

Figure 10. Petiole anatomy of *Strobilanthes* spp. showing structure of main vascular bundle. (a) *S. ixiocephala* and (b) *S. lupulina* (deeply crescent-shaped), (c) *S. reticulata* and (d) *S. sessilis* var. *ritchiei* (crescent-shaped), (e) *S.* sp. (deeply crescent-shaped). Col = collenchymas, Cyt = cystoliths, Sph = sphaeraphides, Tc = tannin cell.

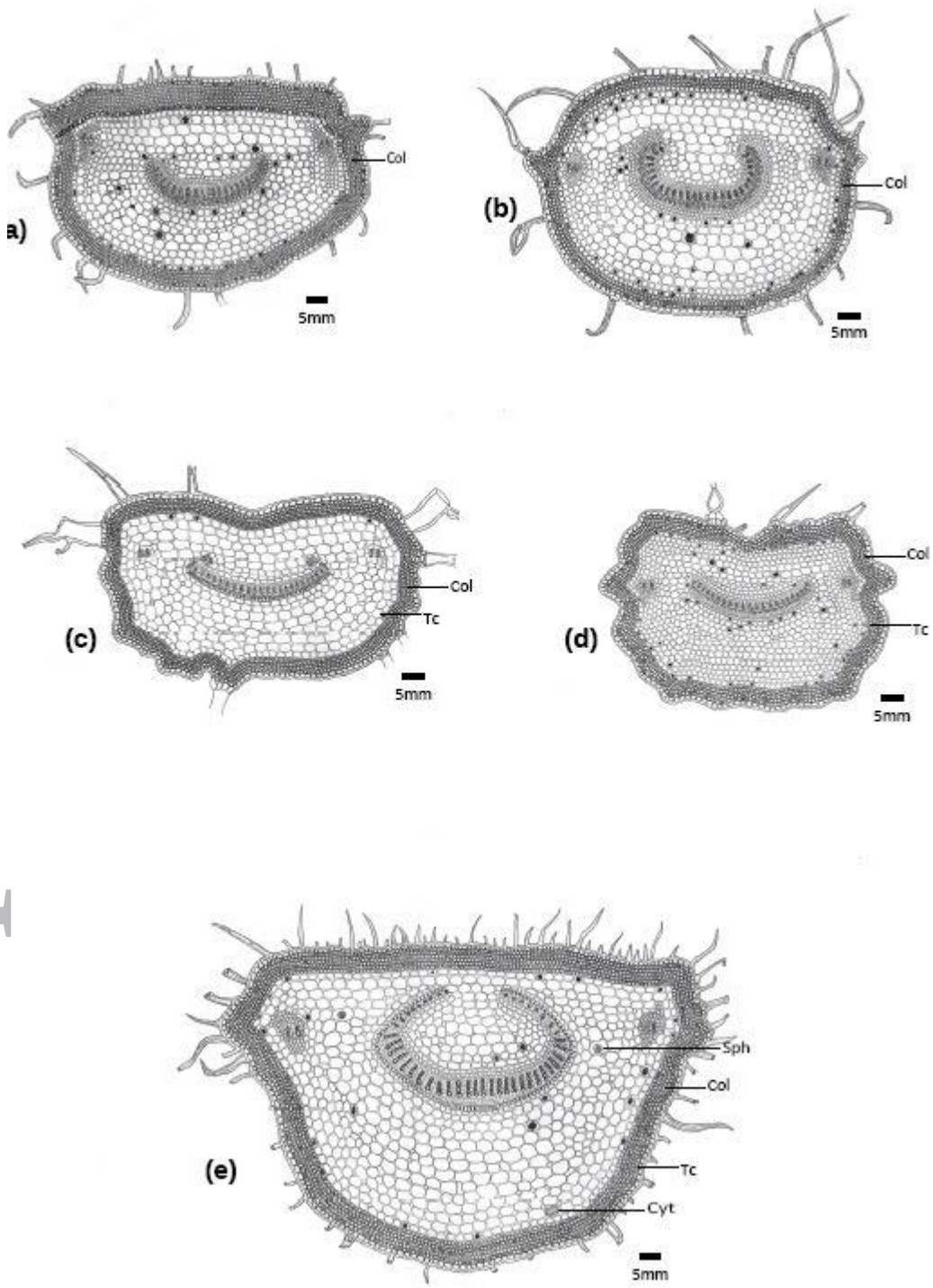

**Table Legends**

Table 1. *Strobilanthes* species studied from northern Western Ghats of India.

| Taxon | Place of collection | Geographic coordinates | Elevation (m) | BSI accession numbers |
|---|---|---|---|---|
| *S. callosa* Nees | Amboli, Maharashtra | N15°57‘05.2" E73°59'45.3" | 702.3 | 137255 |
| *S. heyneana* Nees | Amboli, Maharashtra | N15°57'32.8" E73°59'50.3" | 729 | 137256 |
| *S. ixiocephala* Benth. | Bondla, Goa | N15°25'957" E74°06'611" | 167 | 137253 |
| *S. sessilis* Nees var. *Ritchiei* | Amboli, Maharashtra | N15°56'15.2" E74°00'04.8" | 815.2 | 137252 |
| *S. integrifolia* Kuntze | Bondla, Goa | N15°27'066" E74°05'755" | 62 | 137251 |
| *S. ciliata* Nees | Bondla, Goa | N15°26'485" E74°05'946" | 157 | 137254 |
| *S. barbata* Nees | Nagargao, Goa | N15°35'706" E74°11'929" | 108 | 137257 |
| *S. lupulina* Nees | Amboli, Maharashtra | N15°56‘53.6" E74°00'02.5" | 790.3 | 137258 |
| *S.* sp. | Amboli, Maharashtra | N15°56'10.9" E74°00'19.3" | 809.3 | 137262 |
| *S. reticulata* Stapf | Mahabaleshwar, Maharashtra | N17°56'16.9" E73°42'08.3" | 1285 | 137260 |

*BSI, Botanical Survey of India.

Table 2. Comparative stem anatomical characteristics of *Strobilanthes* spp.

| Characters | *S. ciliata* | *S. integrifolia* | *S. callosa* | *S. ixiocephala* | *S. reticulata* |
|---|---|---|---|---|---|
| Epidermal relief | Terete | Quadrangular winged | Terete | Quadrangular | Undulate |
| Epidermis | Single layered, rectangular cells with tannin | Single layered, rectangular cells with tannin | Single layered, rectangular cells with tannins | Single layered, rectangular cells with tannins | Single layered, rectangular cells |
| Trichomes | Not present | Not present | Rare | Numerous | Numerous |
| Hypodermis | 1–2 layered | 2–3 layered | 2–3 layered | 2–3 layered | 2–3 layered |
| Chlorenchyma | Single layered | Single layered | Single layered | Single layered | Single layered |
| Cortex (outer) | 5 layered with cystoliths | 6 layered | 8–9 layered | 3–4 layered | 5 layered |
| Cortex (inner) | 3 layered | 9–10 layered (winged region) | Single layered | 4 layered | 6 layered |
| Xylem | Diffuse porous | Diffuse porous | Diffuse porous | Diffuse porous | Diffuse porous |
| Growth rings | Not distinct | Not distinct | Not distinct | Not distinct | Not distinct |
| Vessel arrangement | Vertical rows | Vertical rows | Vertical rows | Vertical rows | Vertical rows |
| Vessel grouping | Solitary or groups of two | Solitary or groups of two | Solitary or groups of two | Solitary or groups of two | Solitary or groups of two |
| Phloem | 7 layered | 6–8 layered | 6–7 layered | 7–8 layered | 5–6 layered |
| Pith cells | Hexagonal cells with distinct brachysclereids in the center | Hexagonal cells with few cystoliths | Hexagonal cells with few sclereids and cystoliths | Hexagonal cells with few sclereids | Hexagonal cells with few sclereids |

Table 3. Comparative stem anatomical characteristics of *Strobilanthes* spp.

| Charac | *S. sessilis* var. *ritchiei* | *S. heyneana* | *S.* sp. | *S. barbata* | *S. lupulina* |
|---|---|---|---|---|---|
| Epiderm al relief | Quadrangular | Quadrangular | Undulate | Quadrangular winged | Quadrangular |
| Epidermis | Single layered, cuticularised, rectangular cells | Single layered, rectangular cells with cystoliths | Single layered, cuticularised, rectangular cells | Single layered, cuticularised, rectangular cells | Single layered, rectangular cells |
| Tric mes | Rare | Numerous | Few | Not present | Numerous pigmented (red) |
| Hypodermis | Single layered | 2–3 layered | 1–2 layered | 1–2 layered | 2–3 layered |
| Chlorenchyma | 2 layered | Single layered | Single layered | Single layered | Single layered |
| Cortex (outer) | 2 layered | 3 layered with cystoliths | 8–9 layered | 5 layered and winged region 7–8 layered | 5 layered with cystoliths |
| Cortex (inner) | Single layered | 3 layered | 3 layered | 19–20 layered (winged region) | 4 layered |
| Xylem | Diffuse porous | Diffuse porous | Diffuse porous | Diffuse porous | Diffuse porous |
| Growth rings | Not distinct | Not distinct | Not distinct | Not distinct | Not distinct |
| Vessel arrangement | Vertical rows | Vertical rows | Vertical rows | Vertical rows | Vertical rows |
| Vessel grouping | Solitary or groups of two | Solitary or groups of two | Solitary or groups of two | Solitary or groups of two | Solitary or groups of two |
| Phloem | 4–5 layered | 7–8 layered | 3–4 layered | 10 layered–many | 6–8 layered |
| Pith cells | Hexagonal cells with few sclereids | Hexagonal cells with uniformly scattered sclereids | Hexagonal cells with sclereids and cystoliths | Hexagonal cells with sclereids and cystoliths | Hexagonal cells with sclereids and cystoliths |

Table 4. Comparative leaf anatomical characteristics of *Strobilanthes* spp.

| Characters | *S. ciliata* | *S. integrifolia* | *S. callosa* | *S. ixiocephala* | *S. reticulata* |
|---|---|---|---|---|---|
| Upper Epidermis | Single layered, sub-rectangular cells, cuticularised | Single layered, sub-rectangular cells, cuticularised | Single layered, sub-rectangular cells, cuticularised | Single layered, sub-rectangular cells, thin cuticularised | Single layered, sub-rectangular cells, cuticularised |
| Palisade layer | 1–2 layered | 1–2 layered | 2–3 layered | 2–3 layered | 2–3 layered |
| Shape of vascular bundle | Broad U-shaped | Narrow U-shaped | V-shaped | Broad U-shaped | Narrow U-shaped |
| Epidermal outgrowths | Absent | Absent | Present (Few) | Present (Numerous) | Present (Profuse) |
| Lower epidermis | Single layered, rounded-rectangular with sphaeraphides | Single layered, sub-rectangular, smooth cuticle with cystoliths | Single layered, sub-rectangular, smooth cuticle with cystoliths | Single layered, sub-rectangular with cystoliths | Single layered, rounded-rectangular with few cystoliths |

Table 5. Comparative leaf anatomical characteristics of *Strobilanthes* spp.

| Characters | *S. sessilis* var. *ritchiei* | *S. heyneana* | *S.* sp. | *S. barbata* | *S. lupulina* |
|---|---|---|---|---|---|
| Upper Epidermis | Single layered, sub-rectangular cells, cuticularised | Single layered, sub-rectangular cells, cuticularised | Single layered, sub-rectangular cells, cuticularised | Single layered, sub-rectangular cells, thin cuticularised | Single layered, sub-rectangular cells, cuticularised |
| Palisade layer | 1–2 layered | 2–3 layered | 2–3 layered | 2–3 layered | 1–2 layered |
| Shape of vascular bundle | Narrow U-shaped | Broad U-shaped | V-shaped | Broad U-shaped | Broad U-shaped |
| Epidermal outgrowths | Present (few) | Present (numerous) | Present (Few) | Absent | Present (numerous) |
| Lower Epidermis | Single layered, rounded-rectangular with few cystoliths | Single layered, sub-rectangular, smooth cuticle | Single layered, sub-rectangular | Single layered, rounded-rectangular | Single layered, rounded-rectangular with numerous cystoliths |

Table 6. Stomatal characteristics of abaxial leaf surface of *Strobilanthes* spp.

| *Strobilanthes* species | Leaf surface | No. of stomata/ mm-$^2$ | Stomatal length (µm) | Stomatal width (µm) | No. of cells/ mm-$^2$ | Stomatal Index (SI) |
|---|---|---|---|---|---|---|
| *S. barbata* | Abaxial | 336.66±1.52 | 5.0±0.84 | 3.0±0.16 | 1013.66±2.08 | 33.26 |
| *S. callosa* | Abaxial | 216.33±3.21 | 14.60±3.07 | 6.26±2.54 | 730.66±3.05 | 29.45 |
| *S. ciliata* | Abaxial | 212±3.60 | 16.11±0.40 | 8.18±1.61 | 723.66±1.52 | 29.63 |
| *S. heyneana* | Abaxial | 207±6.24 | 19.18±0.57 | 8.39±0.70 | 728±2.64 | 28.63 |
| *S. integrifolia* | Abaxial | 288.33±2.08 | 16.01±2.41 | 3.89±0.65 | 948.33±2.08 | 30.52 |
| *S. ixiocephala* | Abaxial | 303±2.64 | 17.82±0.38 | 9.65±0.13 | 1005±2 | 30.34 |
| *S. lupulina* | Abaxial | 207.66±3.21 | 6.98±1.29 | 3.08±0.79 | 740±2.64 | 28.30 |
| *S. reticulata* | Abaxial | 208.33±2.08 | 11.36±2.02 | 2.02±0.44 | 780±5 | 26.92 |
| *S. sessilis* var. *Nilghiei* | Abaxial | 347.66±2.51 | 18.43±2.33 | 6.83±2.75 | 1108±7.21 | 31.81 |
| *S.* sp. | Abaxial | 309±2.64 | 8.82±1.33 | 2.95±0.18 | 1016±4 | 30.39 |

*Each value expressed as mean± S.D of 10 replicates from 5 different leaves.

Table 7. Comparative petiole anatomical characteristics of *Strobilanthes* spp.

| Characters | *S. ciliata* | *S. integrifolia* | *S. callosa* | *S. ixiocephala* | *S. reticulata* |
|---|---|---|---|---|---|
| Petiole shape | Dorsi-ventrally flattened with abaxial surface convex and adaxial surface shallowly concave | Sub-terete with abaxial surface convex and adaxial surface flat to slightly convex or shallowly concave | Sub-terete with abaxial surface convex and adaxial surface slightly concave in the center and deeply concave at the wings | Sub-terete with abaxial surface convex and adaxial surface flat to slightly convex or shallowly concave | Undulate abaxial surface and adaxial surface slightly concave |
| Trichomes | Absent | Present (Sparse) | Absent | Present | Present |
| Vascular bundle shape | Deeply crescent-shaped | Deeply crescent-shaped | U-shaped | Deeply crescent-shaped | Crescent-shaped |

Table 8. Comparative petiole anatomical characteristics of *Strobilanthes* spp.

| Characters | *S. sessilis* var. *ritchiei* | *S. heyneana* | *S.* sp. | *S. barbata* | *S. lupulina* |
|---|---|---|---|---|---|
| Petiole shape | Undulate abaxial surface and adaxial surface slightly concave | Terete with both adaxial and abaxial surface convex | Sub-terete with abaxial surface convex and adaxial surface flat to slightly convex or shallowly concave | Terete abaxial surface convex and adaxial surface circular at centre and deeply concave at the wings | Terete with both adaxial and abaxial surface convex |
| Trichomes | Present- sparse | Present | Present | Absent | Present- pigmented |
| Vascular bundle shape | Crescent-shaped | Deeply crescent-shaped | Deeply crescent-shaped | Circular | Deeply crescent-shaped |