

Pharmacognostic and Phytochemical Investigation of *Strobilanthes ciliatus* Nees (Bremek)

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Original Research Article

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Article History

Received: 13.04.2018

Accepted: 22.04.2018

Published: 30.04.2018

DOI:

10.21276/sjmeps.2018.4.4.8



Abstract: Plants have been one of the important source of medicine since the beginning of human civilization and it still continues as one of the major sources of drugs in modern as well as traditional medicine throughout the world. Medicinal plants are the local heritage with global importance. *S. ciliatus* of Acanthaceae family is commonly known as “Sahachara” and is used widely in ingenious Indian system of medicine as it is known to possess a range of folk and proven biologic activity such as anti-inflammatory, analgesic, anti-microbial, antifungal, anti-diabetic, antioxidant and hepatoprotective. The present study deals with the microscopic and macroscopic evaluation of the stem and leaf and also establishment of its quality parameters including the physicochemical and preliminary phytochemical investigation which were conducted. Considering the medicinal importance of *S. ciliatus* a complete study on macroscopic and microscopic characters are carried out. Histological studies were conducted by paraffin infiltration method. Anatomical features of the stem and leaves were studied in detail. Powder characters were also analysed. Physicochemical parameters like ash value and extractive value were done. Phytochemical screening shows the presence of alkaloid, saponins, glycosides, flavonoids, steroids, sterols, carbohydrate, tannins and terpenoids. The values from physicochemical parameters will serve as an identification tool for preventing adulteration of the plant. Findings from the study will be useful for compiling the monograph of *S. ciliatus* for its identification and quality control.

Keywords: *Strobilanthes ciliatus*, pharmacognostic, macroscopic features, powder microscopy, physicochemical parameters.

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis [1]. The biotic and abiotic elements of nature are all interdependent. The plants are indispensable to man for his life. Nature has provided a complete store house of remedies to cure all ailments of mankind. These herbal products are today the symbol of safety in contrast to synthetic drugs, that are regarded as unsafe to human being and environment. Pharmacognosy is a simple and reliable tool, by which complete information of crude drug can be obtained. *Strobilanthes ciliatus* belonging to the family Acanthaceae and is the second largest genus of this family. It comprises of approximately 300 species in topical Asia. The Indian sub continent has nearly 150 species, out of which 59 are seen in Peninsular India [2]. The genus is not greatly explored for economic utility. It is widely used in Ayurveda as a source of drug Sahachara. The name *Strobilanthes* is derived from the Latin word *Srobilos* meaning cone and *anthos* meaning flower or shoot. The genus *Strobilanthes* was first scientifically described by Christian Gottfried Daniel Nees Von Esenbeck in the

19th century. Some *Strobilanthes* species bloom yearly; whole others are plietesials with a cycle of 8-16 years. *S. ciliatus* was proved to have anti-inflammatory [3], anti-diabetic [4], anti-microbial [5], hepato protective [6] and cytotoxic activity [7]. Traditionally it is used in various conditions. The roots are found to be thermogenic depurative, expectorant and tonic. Also used in conditions like rheumatagia, lumbago, sciatica, skin diseases, cough bronchitis etc. The leaves and bark are used as diaphoretic, expectorant, leucoderma, leprosy, pruritus, inflammation etc [8]. Kurinji kuzhambu is a medicinal preparation given for women after delivery for good health[9]. The objective of the present study is to evaluate various pharmacognostical parameters such as macroscopy, microscopy, physicochemical and phytochemical studies of the plant. This evaluation aims at establishment of the standardization parameters of the leaf and stem of *S. ciliatus*.

MATERIALS AND METHODS

Strobilanthes ciliatus was collected from Kaduthuruthi and Palai region of Kottayam district in

the month of April to July 2013. It was authenticated by Mr. Rogimon P. Thomas, Asst. professor, Department of Botany, CMS College Kottayam. Specimen voucher No.268 and is preserved in the herbarium of CMS College Kottayam for future reference. Fresh and healthy leaves and stem were collected and cleaned well. The materials were chopped and dried in shade to get constant weight. It was then coarsely powdered and stored in polypet jars at room temperature. The leaf and stem were powdered separately and stored and is used for the extraction of active constituents and phytochemical investigation.

Macroscopic studies

Morphological studies were done using simple microscope. The shape, apex, margin, colour, odour and taste of leaves were determined.

Microscopic studies

T.S of leaf and stem

• Collection of specimens

The plant specimens for the proposed study were collected from Kaduthuruthi and Palai region of Kottayam district in the month of April to July 2013. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60⁰ C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

• Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure [10]. The sections were stained with Toluidine blue. Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and Iodine in Potassium iodide (for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, Para dermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared [11]. Glycerine mounted temporary preparations were made

for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

• Photomicrographs

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 crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books [12].

Powder microscopy of leaf and stem

Powdered materials of different parts were cleared with 5% sodium hydroxide and mounted in glycerine medium after staining. Different cell components were studied and measured.

Physicochemical Studies: - The total cash value, acid insoluble ash, water soluble ash, alcohol and water soluble extractive values were determined as part physicochemical parameters [13].

Phytochemical studies

Leaves and stems of *S. ciliatus* were collected and shade dried at room temperature to remove moisture. The leaves and stems were separately size reduced. Extraction of the powdered leaf and stem were carried out by soxhlation. The leaf was first extracted with petroleum ether followed by ethanol. All the extracts were concentrated and then dried and stored for further studies. The percentage yield of the extracts was calculated. Qualitative chemical tests were conducted in the extracts [14].

RESULTS

Macroscopic studies

- Shrubs: Slender to 2cm height.

Stems: obtusely quadrangular, diffusely branched sulcate on two sides. When young, glabrous, lenticulate swollen above the nodes, nodes jointed, prominent often fimbriate.

- Leaves: opposite, broadly elliptic to elliptic lanceolate, 3-5-16 × 2-7 cm attenuate at base, subentire to serrate at margin, acute-acuminate at apex, subcoriaceous, glabrous, dark green on upper surface, glaucous on lower surface, upper surface densely covered with cystolith, secondary vein 7-9 pairs, curved upwards, raised on upper surface, petiole 0.5-8 cm long, obscure due to decurrent leaf

base. Inflorescence opposite, axillary spikes, sub-capitated to oblong, peduncles 1.5-4.5 cm long, entire to serrulate at margin, acute-acuminate at apex, bracteoles linear lanceolate.

- Flowers: 4-Seriate, calyx 5-6.5 mm, almost glabrous, densely covered with glandular hairs on outside. Corolla 13.5 mm long, glabrous outside, tube cylindrical 6-7 mm long campanulate, white with purple blotches at throat, veins clearly visible on lobes. Stamens: Stamens 4, dihynameous,

staminal tube open at one end and densely hairy. Anthers oblong, 2 mm long purple, bithecous. Disk 1-1.5 mm broad, ovary conical 1-1.5 mm long glabrous. Style 1.3 cm. Stigma 2-cleft, ovules 4, orbicular, capsules ellipsoid, seeds sub orbicular to oblong 2.5-4.5 × 2-2.5 mm, obtuse at apex [15].

Microscopic studies

Strobilianthes ciliates leaf

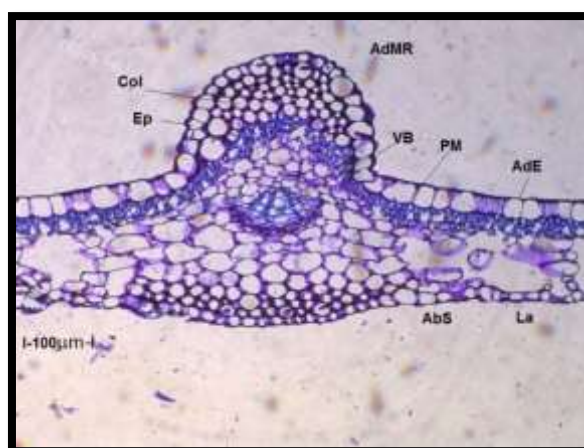


Fig-1.1: T.S of leaf through midrib

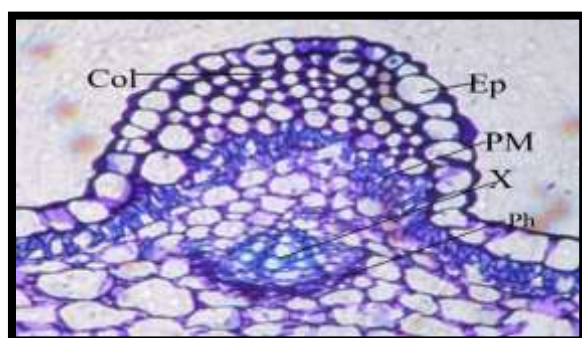


Fig-1.2: T.S of midrib enlarged

[Abs:-Abaxial side; AdE: Adaxial Epidermis; AdMr: Adaxial Midrib;
Col: Collenchyma; Ep: Epidermis; La: Lamina; PM: Palisade Mesophyll;
Ph.: Phloem; VB: Vascular Bundle; X: Xylem.]

The leaf consists of a thick plano-convex midrib and thin and smooth lamina (fig 1.1). The midrib is unusually thickened and bulged into hemispherical structure and it occurs on the adaxial side. The midrib is flat on the abaxial side. The midrib is 510 μm thick along the vertical plane and 350 μm along the

horizontal plane. The epidermal cell of the midrib is large, squarish in shape and the cells have thick cuticle (fig 1.2). Inner to the adaxial epidermal layer occur about five layers of large collenchyma cells. Such collenchyma cells occur in two layers on the abaxial end of the midrib (fig1.2). The palisade layer is horizontally Transcurrent across the adaxial collenchyma zone. The vascular strand is single, bowl shaped and small. It consists of about four short vertical rows of circular thick walled xylem elements and a thick arc of phloem elements occur on the xylem (fig 1.2).

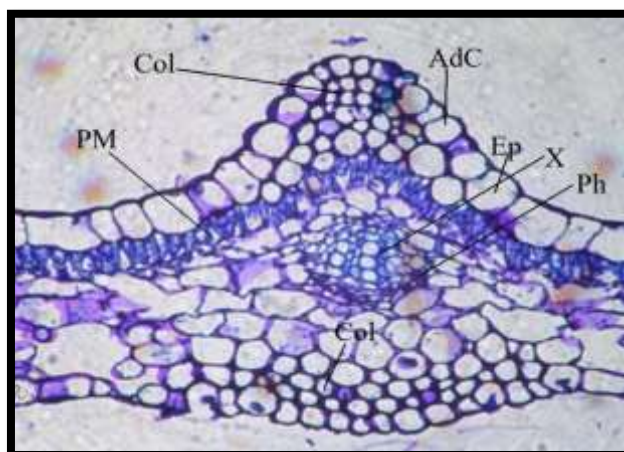


Fig-2.1: T.S of Lateral vein

The lateral vein also exhibit similar structure as the midrib. It has adaxial thick and wide con and flat adaxial side (fig2.1). The lateral view consists of small thick walled epidermal cells, hypodermal portion of collenchyma cells and horizontal portion of transcurrent

palisade layer along the adaxial cone. The vascular bundle is single and collateral. It includes three or four vertical rows of xylem elements and their arch of abaxial phloem. The lateral vein is 400 μm in vertical plane and 200 μm in horizontal plane (fig2.1).

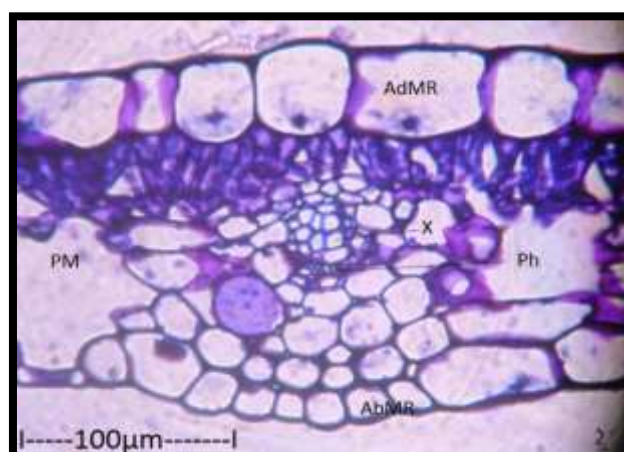


Fig-2.2: T.S of veinlet

[AbMr: Abaxial Midrib; AdMr: Adaxial Midrib; AdC: Adaxial Cone
Col: Collenchyma; Ep: Epidermis; PM; Palisade Mesophyll;
Ph: Phloem; X: Xylem.]
Veinlet (fig2.2)

The veinlet is slightly bulged but does not produce and conical structure. In the region of veinlet, the epidermal cells on the abaxial side are smaller and thick walled. The adaxial epidermis has very large thin walled squarish cells. The vascular strand is small comprising of few xylem elements and thin layer of phloem cells.

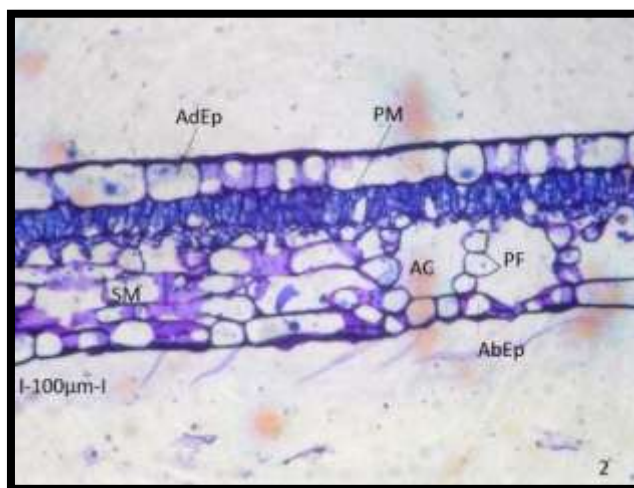
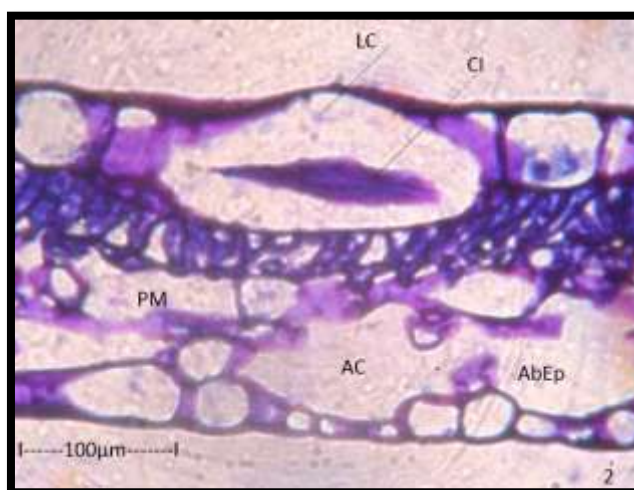


Fig-3.1: T.S of lamina

Lamina (fig3.1)

The lamina is distinctly bifacial with differentiation of adaxial and abaxial surfaces. The lamina is 190 μm thick. The adaxial epidermis has

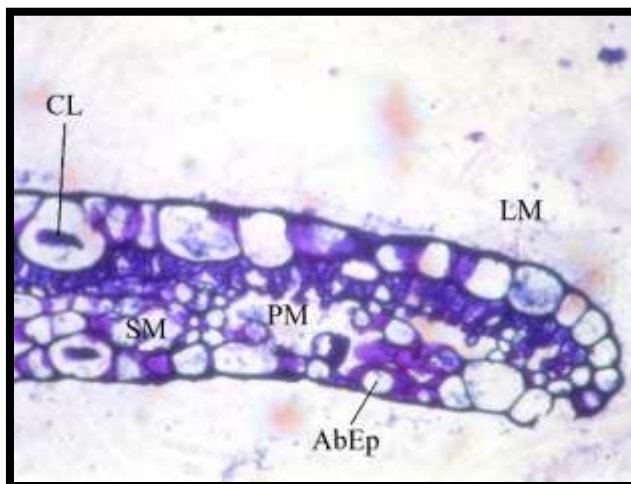
horizontally elongated with wide thin walled cells. The abaxial epidermal cells are smaller, square shaped or horizontally rectangular and stomatiferous.



3.2: T.S of lamina with cystolith

The mesophyll tissues are differentiated into adaxial single band of short conical pillar shaped columnar palisade cell and abaxial zone of wide air chambers divided vertically by uniseriate partition

filaments. Both adaxial and abaxial cells have highly dilated lithocysts which possess cylindrical cystoliths (fig3.2)



3.3: T.S of leaf margin

Leaf margins (fig3.3)

The marginal part of the lamina is slightly bent down, it is 150 μm thick. There are no much changes in the epidermal cell and mesophyll tissues. Wide epidermal cells, palisade- spongy mesophyll differentiation and reduced vascular strand are the features of the leaf margin.

[AbEp: Abaxial Epidermis; AdEp: Adaxial Epidermis; AC: Air Chamber; Cu: Cuticle; Cl: Cystolith; LC: Lithocyst; LM; Leaf Margin; PM: Palisade Mesophyll; SM: Sponge Mesophyll; PF: Partition filament.]

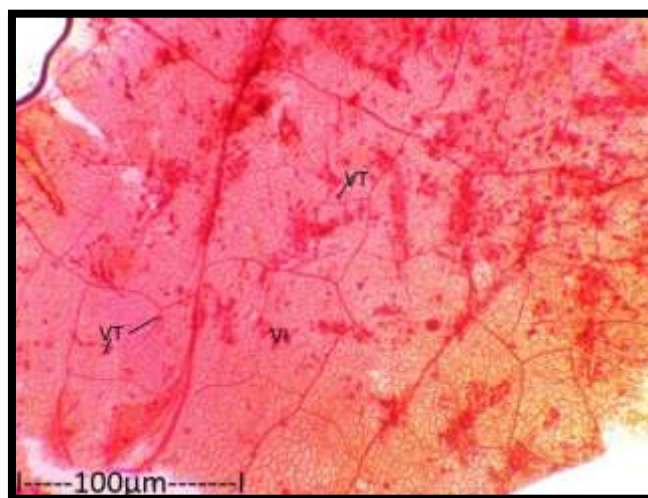
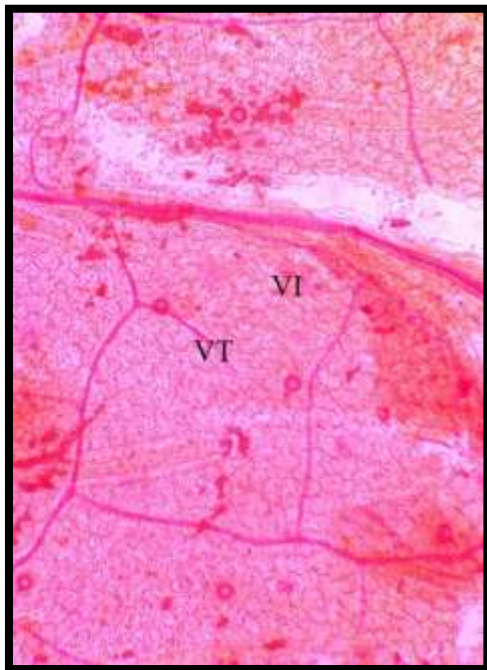
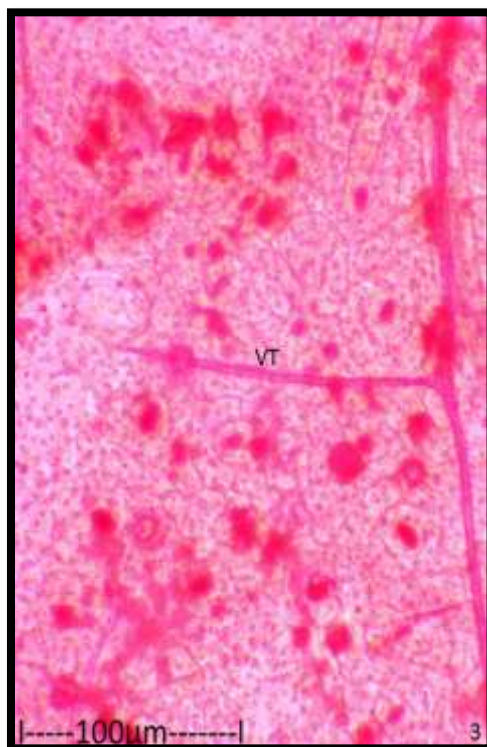


Fig-4.1: Leaf clearing to show venation



4.2: Vein islet and vein termination



4.3: Vein islet and vein termination

Venation pattern (fig 4.1, 4.2. & 4.3)

The lateral veins and veinlets are very thin and less prominent. The vein islets are wide and rectangular in shape. The vein boundaries of the islets are thin and

straight. The vein termination is almost thin and straight. They are either simple (unbranched) (fig 4.1, 4.3) or branched once (fig4.2).

[VI: Vein Islet; VT: Vein Termination]

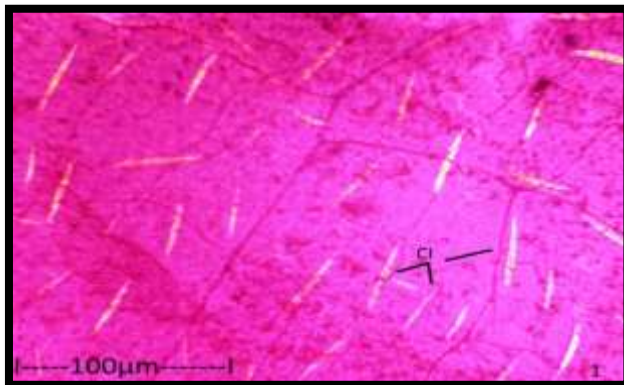
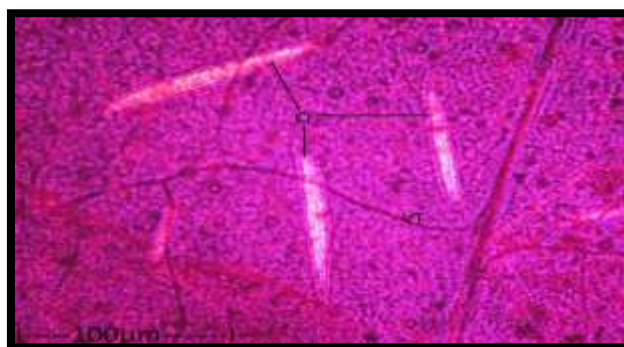


Fig-5.1: Distribution of cystoliths in Surface view



5.2: Distribution of cystoliths in Surface view

Cystolith (fig5.2)

Calcium carbonate crystals called cystoliths are abundant in the mesophyll tissue of the lamina. The cystoliths are long, cylindrical with tapering ends and

are random in distribution. They occur mostly in the dilated specialized cells of the lamina, called lithocyst. [Cl: Cystolith; VT: Vein Termination]

Strobilianthes ciliates stem

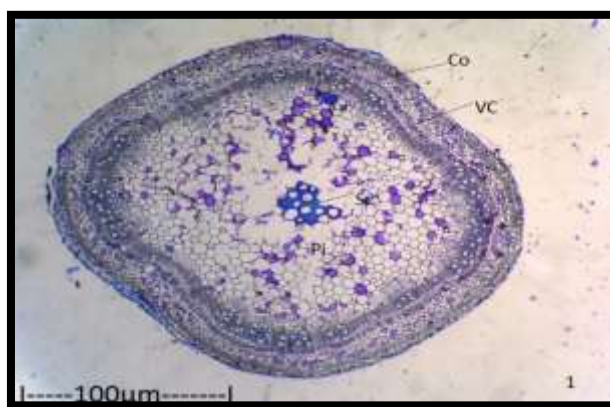
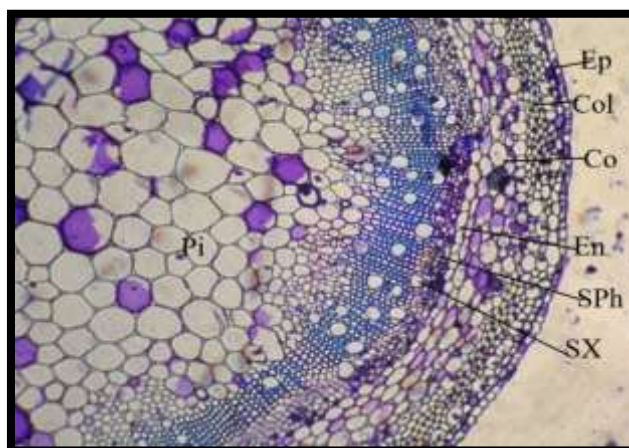


Fig-6.1: T.S of Stem entire view



6.2: T.S stem sector

The stem appears four angled in sectional view. It consists of thin epidermal layer, cortical zone, secondary phloem, secondary xylem and pith (fig6.1). The epidermal layer consists of thin, elongated thick walled cells. The cortical layer consists of about four layer of elliptical compact parenchyma cells. In the part

of the cortical zone there is a discontinuous two cell thick sclerenchyma layer (fig6.2).

[Co : Cortex ; Col : Collenchyma ; En : Endodermis ; Ep : Epidermis ; En : endodermis ; SX : Secondary Xylem ; SPh : Secondary Xylem ; Pi : Pith]

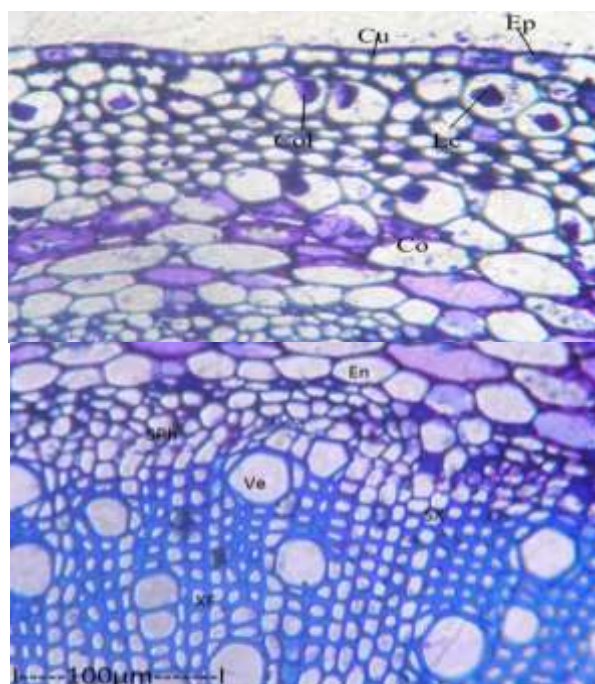


Fig-7: T.S of Stem showing Cortex secondary phloem and secondary xylem

The phloem tissue occurs in thick continuous cylinder. The phloem element include sieve elements which are wide, angular and thick walled with small companion cell located around the corner of the sieve element (fig 7). Secondary xylem is a thin continuous cylinder comprising solitary, wide, angular thick walled vessel, wide, thick walled lignified radial compact layer

of fiber and thick walled lignified radially oblong xylem ray cells.

[Col : collenchyma ; Co : Cortex ; Cu : Cuticle ; Ep : Epidermis ; En : Endodermis ; Lc : lithocyst ; SPh : Secondary phloem ; SX : secondary xylem ; Ve : Vessel ; XF: Xylem Fiber]

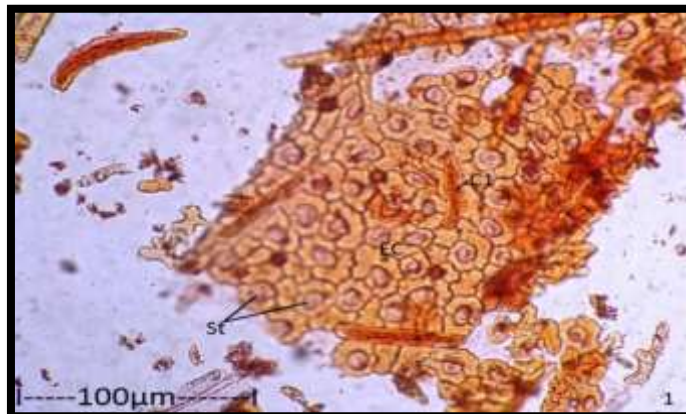


Fig: 8.1

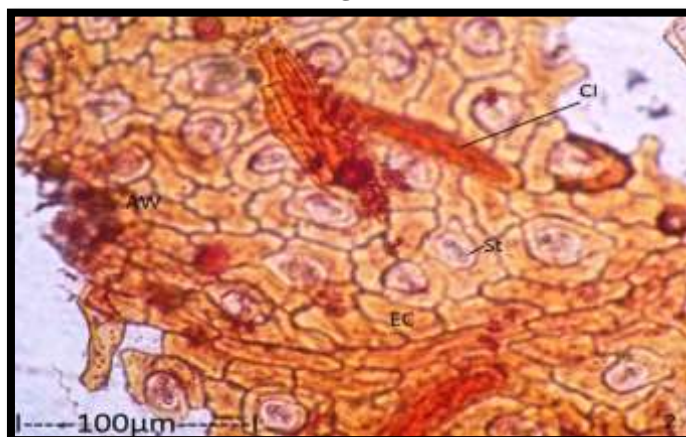


Fig: 8.2

Fig: 8.1 & 8.2: Adaxial Epidermal Cells – Surface View Showing Stomata

[AW: Anticlinal Wall; Cl: Cystolith; EC: Epidermal cell; St: Stomata]

Powder microscopic observation

The powder preparation of the leaf and stem shows the following inclusions:-

- Fragments of epidermal peeling are common in the powder. The epidermal cells have wavy anticlinal walls. Stomata are abundant in distribution. The stomata are paracytic type with parallel subsidiary cells associated with the guard cells.

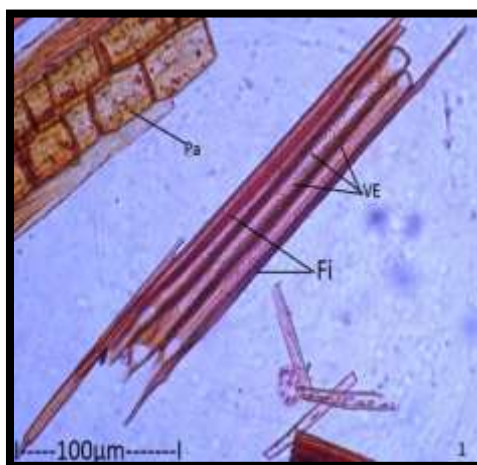
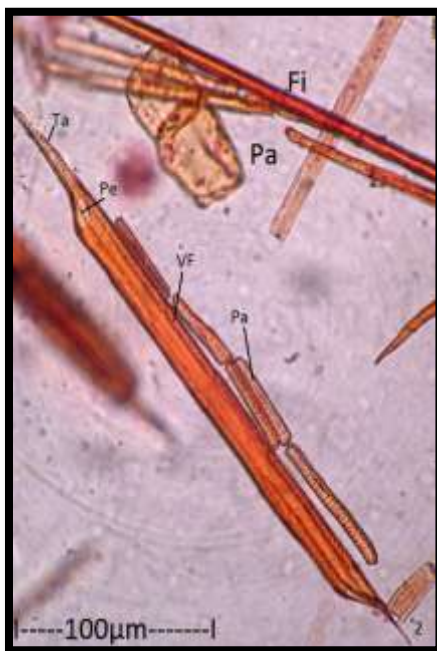
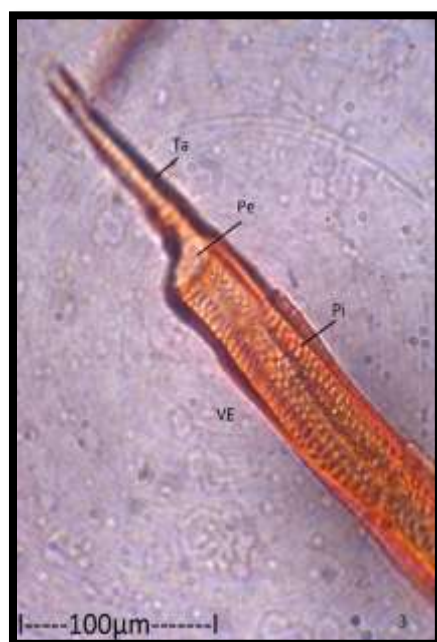


Fig-9.1: A Bundle of vessel Elements, fibers and Parenchyma cells



9.2: A vessel element



9.3: A vessel element showing end wall perforation and lateral wall pits

Vessel elements (fig9.1, 9.2, 9.3)

Vessel elements are common in the powder. They are long, narrow cylindrical cells. They have long, thin straight tails at both ends of the vessel elements. The end wall perforations wide, elliptical and oblique in orientation. The lateral walls have horizontally

elongated bordered pits which are multiseriate and densely crowded (fig9.3). The vessel elements are 600 μm long and 30 μm wide.

[i: Fiber; Pa: Parenchyma; Pe: Perforation; Pi: Pits; VE: Vessel Element.]

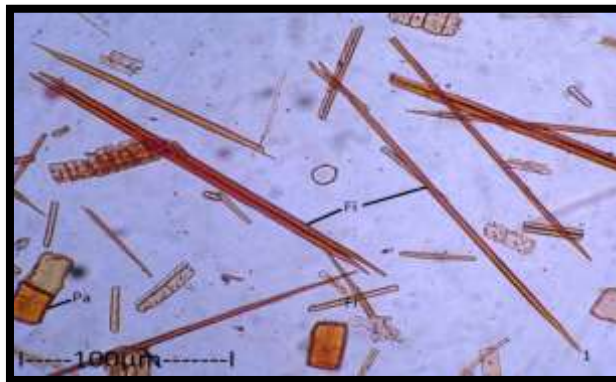


Fig 10.1: Fibers

Fibers

They are more abundant in the powder. The fibers are long narrow thick walled cells with pointed ends. Their walls are lignified. The cell lumen is wide

and there are many cross wall septae in the fiber. Apart from septate fibers there are also non-septate fibers. The fibers are 500-800 μm long and 20 μm thick.

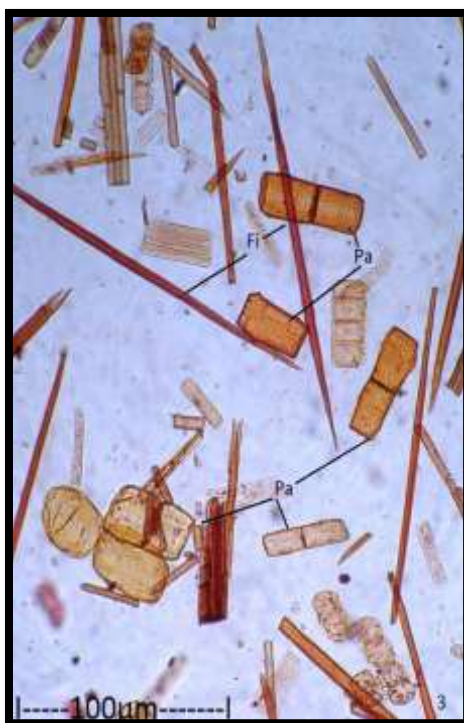


Fig-10.2: Fibers and Parenchyma Cells

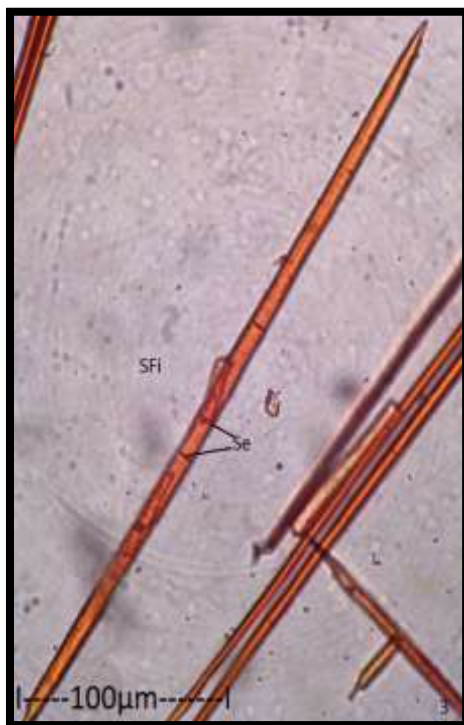


Fig-10.3: Fibers

[Fi: Fiber; Pa: Parenchyma cells; Se: Septa; Spi: Septate Fiber]



Fig-11.1: Parenchyma cell and Cystolith

Parenchyma cells

Parenchyma cells of different shape and size are seen scattered in the powder (fig10, 11, 12). The parenchyma cell is short, wide, thin walled and is 50×80 µm in size (fig10.2). There are other parenchyma cells which is 80×180 µm in size (fig11.1). Cells measuring 50×100 µm are also seen (fig12.1)

Cystoliths (fig11.2)

Lithocyst, a modified parenchyma cell possessing calcium carbonate, cylindrical body is fairly common. These cystoliths seen in the powder are 250µm long and 30 µm thick.

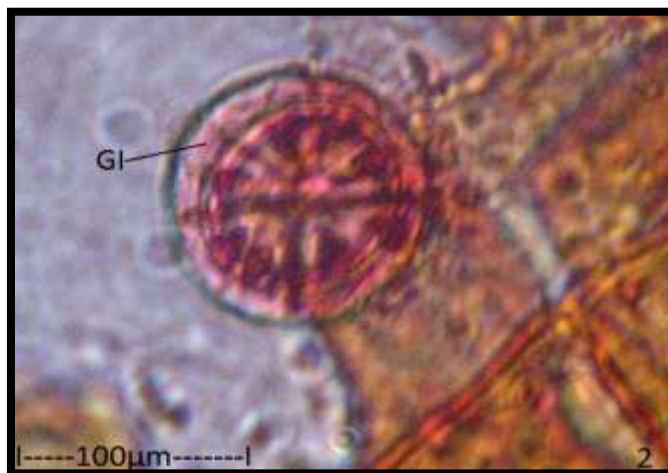


Fig-11.2: Glandular Trichomes

Glandular trichomes (fig11.2)

Circular, sessile glandular trichomes are occasionally seen on surface of lamina. The gland is multicellular, made up of four cells in tetrad. The cells

have dense and dark cell inclusion. The gland measures 100 μm in diameter

[CL: Cystolith; GI: Gland; Pa: Parenchyma]

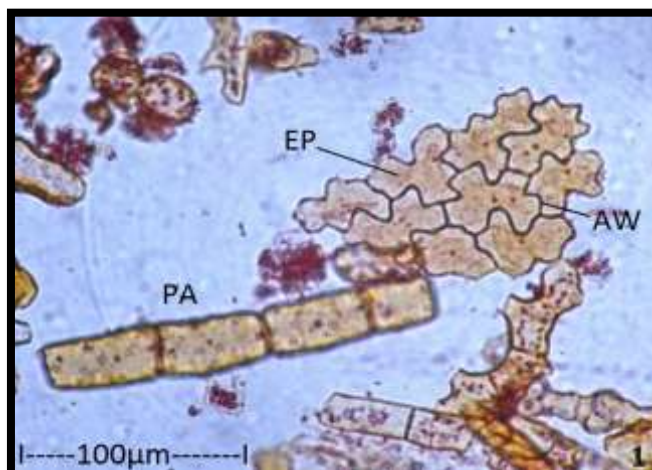


Fig-12.1: Parenchyma Strand and Epidermal Cells

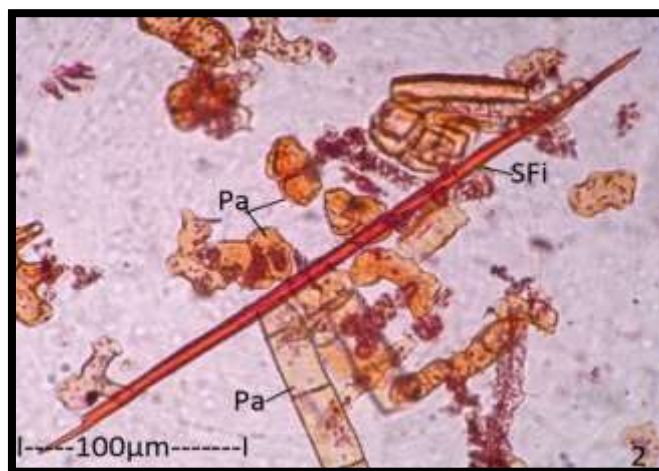


Fig-12.2: Septate Fiber and Parenchyma Cells

[AW: Anticlinal Wall; Ep: Epidermis; Pa: Parenchyma; SFi: Septate Fiber]

Physicochemical evaluation

Table-1: The morphological characters were shown

Properties	Observation
Colour	Leaf-dark green on the upper and lower side
Odour	Characteristic and aromatic
Taste	Bland

Table-2: The ash values and extractive values were studied and tabulated

Sl. No.	Parameter (% w/w)	<i>S.ciliatus</i> leaf	<i>S.ciliatus</i> stem
1	Total ash	12.3±0.2	15.4±0.3
2	Water soluble ash	6.1±0.4	8.2±0.5
3	Acid insoluble ash	2.2±0.3	2.4±0.4
4	Water soluble extractive	8.8±0.5	11.4±0.2
5	Alcohol soluble extractive	9.5±0.4	8.7±0.6

Table-3: Percentage yield of the three extracts, Petroleum ether extract of leaf (LPE), Alcoholic extract of leaf (LAE) and Alcoholic extract of stem (SAE) were tabulated

Sl. No.	Name of extract	Colour & Consistency	Percentage yield
1	Petroleum ether extract of leaf (LPE)	Dark green Waxy	7.2%
2	Ethanol extract of leaf (LAE)	Dark brown Semi solid	9.5%
3	Ethanol extract of stem (SAE)	Brownish yellow Semi solid	8.7%

Preliminary phytochemical evaluation

Table-4: Qualitative chemical test were carried out the three extracts and the results were depicted

Sl.No	Phytoconstituent	Chemical test	LPE	LAE	SAE
1.	Alkaloid	Dragendorff test	-	+	+
		Mayer's test	-	+	+
		Hager's test	+	+	+
		Wagner's test	-	+	+
2.	Carbohydrates	Molisch's test	+	+	+
		Fehling's test	+	+	+
3.	Glycosides	Bontrager's test	-	+	-
4.	Saponins	Foaming test	-	+	-
		Haemolytic test	-	+	-
5.	Protein & aminoacid	Biuret test	-	-	-
		Ninhydrin test	-	-	-
6.	Phytosterols	Liebermann-Buchard test	+	+	+
7.	Terpenoids	Salkowski test	+	+	+
8.	Flavanoids	Shinoda test	+	+	+
9.	Tannins	Ferric chloride	+	+	+
		Lead acetate	+	+	+
10.	Fixed oils and fat	Spot test	-	-	-
11.	Gums and mucilages	Precipitate formation in alcohol	-	-	-

DISCUSSIONS

Strobilanthes is one of the most interesting genera in the family Acanthaceae known for its diversified habits, gregarious nature and infrequent but elegant flowering. It is the second largest genus in the family with approximately 300 species entirely restricted to hills of tropical Asia. *Strobilanthes ciliatus*

are one of the species endemic to Western ghats. The plant has get several therapeutic properties, has a strong aroma and used medicinally.

The present studies revealed that pharmacognostic screening can serve as a basis for the preparation of the herbal monograph for proper

identification, authentication and standardisation of drugs. It also helps to identify the correct species of the plant. The qualitative analysis of various extracts of *Strobilanthes ciliatus* were carried out and the extract showed the presence of bioactive constituents like alkaloids, carbohydrates, saponins, phytosterol, terpenoid, flavonoids and tannins. The leaf contain more active constituents than the stem.

CONCLUSION

Standardisation of a crude drug is an integral part for establishing its identity, therefore the data gathered here as the pharmacognostic features will be useful in identifying this drug and differentiating it from other related species. The physicochemical parameters found out are useful in determining the quality and purity of commercial samples and detection of adulterants.

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