

PHARMACOGNOSTIC EVALUATION OF *STROBILANTHUS IXIOCEPHALA* BENTHRUPALI SARPATE<sup>\*a</sup>, DR. SURESH TUPKARI<sup>a</sup><sup>a</sup> Smt. Suresh Patil College of Pharmacy, Chopada, Dist: Jalgoan, State: Maharashtra, Country: India. \*Email: rupali\_sarpate@yahoo.co.in

Received: 20 Jun 2012, Revised and Accepted: 29 July 2012

## ABSTRACT

*Strobilanthus ixiocephala* Benth, Family Acanthaceae (Ruellia family) is a small straggling shrub found in Konkan, the Deccan and Kanara in India. It is scarcely found in Khandala and Brahmagiri hills of Nashik in Maharashtra at an altitude of 500-900 m. Its Botanical name is *Strobilanthes ixiocephalus* and Synonym is *Thelepaepale ixiocephala*. Its common name is Sky Blue Karvy and in Marathi it is called as Patri, Waiti. It flowers once in seven years. Scientific information on their pharmacognosy, phytochemistry is very scant. Hence the current study describes some pharmacognostical, physiochemical and phytochemical investigations undertaken on the stem and leaves of species namely *Strobilanthus ixiocephala* Benth. The samples for research were collected from Trimbakeshwar, Nasik, India and authenticated in Botanical Survey of India and then subjected for morphological, microscopical, phytochemical and physicochemical analysis. The parameters from the above were recorded with an objective of drawing an attention on those species as well as a reference for further scientific investigations.

**Keywords:** Acanthaceae; Sky blue Karvi; *Strobilanthus ixiocephala* Benth; *Thelepaepale ixiocephala*.

## INTRODUCTION

The term plietesial has been used in reference to perennial *monocarpic* plants "of the kind most often met with in the *Strobilanthinae*" (a sub tribe of *Acanthaceae* containing *Strobilanthes* and allied genera) that usually grow gregariously, flower simultaneously following a long interval, set seed, and die. It flowers once in seven years.<sup>1</sup> The genus has around 250 species, of which at least 46 are found in India. *Strobilanthus ixiocephala* Benth, Family Acanthaceae (Ruellia family) is a small straggling shrub found in Konkan, the Deccan and Kanara in India. It is barely found in Khandala and Brahmagiri hills of Nashik in Maharashtra at an altitude of 500-900 meter.<sup>2</sup> Its Botanical name is *Strobilanthes ixiocephala* and Synonym is *Thelepaepale ixiocephala*. Its common is Sky Blue Karvy and in Marathi it is called as Patri, Waiti. It flowers once in seven years.<sup>3</sup> While the leaves of *Strobilanthes callosus* are poisonous, toxic and unfit for human consumption it is used as a traditional medicine herb by the local adivasi tribal's and villagers for the treatment of inflammatory disorders. Its leaves are crushed and the juice obtained is believed to be a sure cure for stomach ailments.<sup>4</sup>

The plant has been the subject of scientific research which confirms its use in folk medicine as a valid anti-inflammatory and antimicrobial herbal drug with anti-rheumatic activity.<sup>5, 6</sup> The assignments such as macroscopy, anatomical studies, micro measurements, preliminary phyto chemical screening and physiochemical analysis were performed since the species was not noted for its pharmacognosy and bioactivity in the past. The perusal of literature also revealed that limited pharmacological, phytochemical and limited pharmacognostical work has been done on the plant of the genus *Strobilanthus* namely *Strobilanthus ixiocephala* Benth.

## MATERIALS AND METHODS

## Plant material

The plant *Strobilanthus ixiocephala* Benth was procured from Trimbakeshwar, Nasik, India and authenticated in Botanical Survey of India (BSI) and voucher specimen (RSI-2) was kept at departmental herbarium of BSI. Drug material was powdered and stored at 25 °C in an air tight container. Fresh material was shade dried and made into 60 mesh powder and then was used for physiochemical, phytochemical analysis and powder characteristics. Fresh leaves were preserved in formaldehyde-acetic acid-ethanol (1:1:1).

## Chemicals and instruments

Compound microscope, camera Lucida (prism type), glass slides, cover slips, watch glass and other common glassware's were the basic apparatus and instruments used for the study. Solvents viz. methanol (95%) and reagents viz. Safranin, glycerin, Hydrochloric

acid (HCl), Toluidine Blue, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

## Sectioning

Care was taken to select healthy plants for normal organs, the required samples of different organs were cut and removed from the plant and fixed in Formalin Aceto-Alcohol (FAA) (Formalin (5 ml) + Acetic acid (5 ml) + 70% Ethyl alcohol (90 ml)) in fresh form. After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 °C) until TBA solution attained super saturation. Then, the specimens were cast into paraffin blocks.<sup>7</sup> 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 performed by customary procedure. Since Toluidine Blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained.<sup>8</sup> The dye rendered pink color to the cellulose walls, blue to the lignified cells, violet to the mucilage and blue to the protein bodies. Wherever necessary sections were also stained with safranin and fast-green with Potassium Iodide (KI) (for starch). For studying the stomata morphology, venation pattern and trichomes distribution, paradermal 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.<sup>9</sup> Glycerin mounted temporary preparations were made for macerated/cleared materials.

Photomicrographs<sup>10, 11</sup>

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphot 2 microscopic units. 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.

Powder microscopy<sup>12</sup>

Microscopic components of significant diagnostic value were studied under different magnifications; polarized light was subjected to study the starch grains and crystals. The leaves and stem of the plant *Strobilanthus ixiocephala* Benth were dried in shade and were finely powdered and screened for the presence of its own and foreign vegetative matters. The powder was passed through a sieve no.125 and a sieve no.180, separately, to obtain fine and very fine powder respectively and then subjected for microscopic examination. The sample was treated with following

reagents and studied for their components of diagnostic value (50% glycerin as temporary mountant; phloroglucinol (2% W/V) in ethanol (90%) and conc. HCl (1:1) for lignin; 5% W/V of alcoholic ferric chloride for phenolic compounds; 2% Iodine solution for starch grains; and Ruthenium red (0.08%) in 10% lead acetate for mucilage).

#### Preliminary phyto-chemical screening <sup>7,13</sup>

The leaves and stems were dried in shade at room temperature and screened for the presence of foreign matter. The parts of interest were ground to a moderately coarse powder in a mechanical grinder. About 500g of the powder was extracted successively with petroleum ether, methanol (95%) and water using Soxhlet apparatus. The three different extracts were taken in a tarred porcelain dishes and evaporated to dryness on a rotary evaporator and dried to a constant weight. The percentage extractives were calculated with reference to air dried drug.

#### Physico-chemical analysis <sup>14</sup>

Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the methods prescribed in Indian Pharmacopoeia, 1966.

### RESULTS AND DISCUSSION

#### Morphology

Sky blue karvi is a much branched, aromatic bushy shrub; stem is viscid, glandular-hairy, swollen above the nodes. Leaves opposite, unequal, elliptic-lanceolate, crenate, sharp tip. Flowers 2.5-3 cm long, white or pale blue, in axillary or terminal condensed spikes as shown in Fig. 1. Calyx 1 cm long, connate, lobes 5, linear, obtuse. Corolla tube broad and swelling out on one side, 6-7 mm above the base. Stamens 4, filaments basally united, margin of stamina-tube hairy, anther cells oblong, Ovary 2-loculed, ovules 2, style broad at middle, pubescent upwards, stigma obscurely 2-lobed. Twigs and floral heads of the plant yield about 0.5% of an essential oil. Flowering starts from December to January.



Fig. 1: A flowering twig of *Strobilanthus ixiocephala* Benth

#### Microscopy

##### Stem

The colour of the stem is yellow brown. Height up to 1-2 meter. Shape cylindrical and branched. Characteristic or tasteless. Odorless or faint. Fibrous in inner stem.

##### Epidermis

The epidermis is uniseriate and continuous. It is 10  $\mu$ m thick, the walls of the epidermal cells are thick and the cuticle is heavily deposited on the outer walls. The epidermal cells are polygonal in shape and have cell contents. Liner to the epidermis is a hypodermal layer; the hypodermal cells are similar to the epidermal cells in shape and size, but the cell walls of the hypodermis are thin. Refer Fig. 2.

##### Cortex

Epidermis is followed by the zone of cortex consisting of 4-5 layers of elongated collenchymatous cells and 2-3 layers of parenchymatous cells as shown in Fig. 2.

##### Vascular bundle

It consists of xylem, secondary xylem, and phloem. Phloem is consisted of 4-5 layers of polyhedral parenchymatous cells. Xylem vessels are arranged in radial rows. Pith is made of polygonal, rounded parenchymatous cells with large intercellular spaces as shown in Fig. 2.

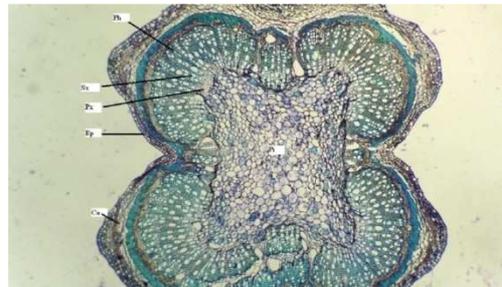


Fig. 2: T.S of Stem - *Strobilanthus ixiocephala* - a sector enlarged

[Co-Cortex, Fi - Fibers, Ph - Phloem, Pi - Pith, Px - Primary xylem, Sx - Secondary xylem, Ep - Epidermis, Ve - Vessel, Xf - Xylem fibers]

##### Fibers

These are long cells with tapering pointed ends. Some of the fibers are narrow, thick walled and narrow lumened. They are 1.5 to 2mm long; 6 $\mu$ m thick. Some other fibers are wider, shunted and wide lumened. The wide fibers are up to 600 $\mu$ m long and 15  $\mu$ m wide. Refer Fig. 3.



Fig. 3: Powder microscopy of *Strobilanthus ixiocephala*

[Axp- Axial parenchyma, VE-Vessel element, T- Tail of vessel element, PF- Perforation plate, Fi-Fibers]

##### Vessel elements

They are wide long and cylindrical cells. They have wide, circular opening at the ends, this opening are called perforation plate. Vessel elements are with axial parenchyma. The vessel elements are 200 to 500 $\mu$ m long. Refer Fig. 4.

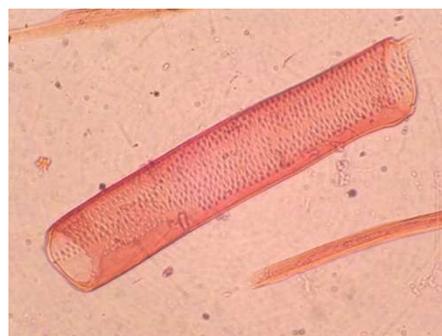
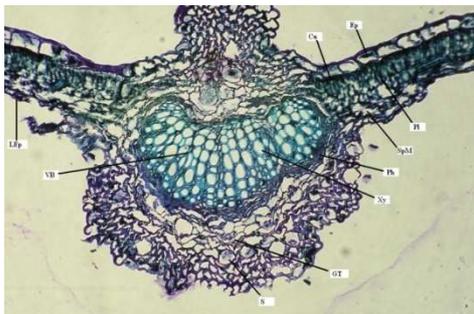


Fig. 4: Powder microscopy of *Strobilanthus ixiocephala* showing wider vessel element

## Leaves

It consists of Upper epidermis, Palisade layer, Vascular bundle, lower epidermis. Refer fig 5.



**Fig. 5: T.S of leaf of *Strobilanthus ixiocephala* - a sector enlarged**

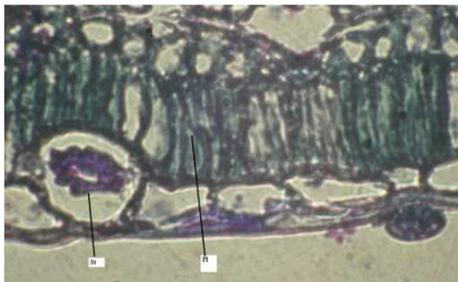
[GT-Ground tissue; Ph - Phloem; VB - Vascular bundle; X - Xylem; UEP-Upper Epidermis; S- Sclerenchyma, PI- Palisade cells, LEP-Lower Epidermis ].

### Upper epidermis

Upper epidermis is composed of tangentially arranged single layer of sub rectangular cells which are quite distinct in nature and below this the projection area with collenchmatous cells having intercellular spaces.

### Palisade layer

Palisade parenchyma is composed of elongated and capsule shaped cells which are closed together to each other which formed long axes of cells perpendicular to the epidermis and on staining they appear greenish in colour as shown in fig. 6.



**Fig. 6: *Strobilanthus ixiocephala* leaf showing compact pattern of palisade cells**

[St-Stomata, PI-Palisade cells]

### Vascular bundle

It consist of xylem, secondary xylem and phloem elements. Xylems consist of parenchyma cells which are distinct and have intercellular spaces. The xylem elements are thick walled angular and compactly arranged in a radial pattern. Phloem elements consist of small compactly arranged parenchymatous cells.

### Lower epidermis

It consists of single layer of tangentially arranged sub rectangular cells with smooth cuticle. Just above the lower epidermis three to four layers of collenchmatous cells are presents.

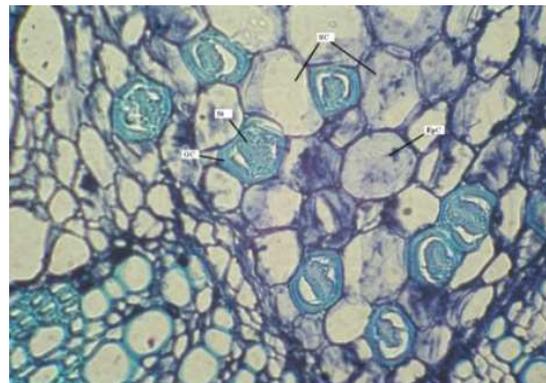
### Stomata

The stomata are mostly anomocytic in nature. The ground cells are elliptical measuring 15x20µm. The epidermal cells are large, polygonal with straight or slightly wavy thin anticline walls. Refer fig. 7.

### Venation pattern

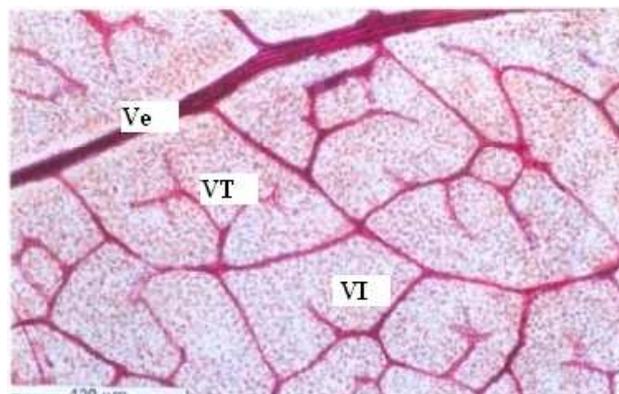
The lateral veins are fairly thick; the veinlets are thin and branched. The vein islets are distinct, wide and variable in outline. The vein

terminations are also distinct, wide and variable in outline. The vein terminations are also distinct. They are either simple or branched, twice or thrice as shown in fig. 8.



**Fig. 7: *Strobilanthus callosus* leaf showing stomatal pattern**

[SC- subsidiary cells, EpC- Epidermal Cell, St-Stomata, GC- Guard Cells]



**Fig. 8: Cleared leaf showing vein islets and vein termination**

[Ve-Vein, VI-Vein islets, VT-Vein termination]

### Powder characteristics

The powder was yellowish green in colour; with some specific odour; slightly bitter, rough in texture. The ground cells are elliptical measuring 15x20µm. The epidermal cells are large, polygonal with straight or slightly wavy thin anticline walls. The fibers are long cells with straight or slightly wavy thin anticline walls. The fibers are long cells with tapering pointed ends. Some of the fibers are narrow, thick walled and narrow lumened. They are 1.5 to 2mm long; 6µm thick. Some other fibers are wider, shunted and wide lumened. The wide fibers are up to 600µm long and 15 µm wide. The vessel elements are wide long and cylindrical cells. They have wide, circular opening at the ends, this opening are called perforation plate. Vessel elements are with axial parenchyma. The vessel elements are 200 to 500µm long. Refer fig. 3and 4.

### Physicochemical analysis

In Physicochemical analysis various parameters were studied via ash values, extractive values, loss on drying etc. The maximum loss on drying at 110 °C was 4.0 and 2.0 for leaf and stem. Percentages of physical constants are given in Table1 and Percentage of various extract is shown in the Table 2.

**Table 1: A physicochemical observation of *Strobilanthus ixiocephala* leaves and stem.**

S. No.	Parameters	Leaf	Stem
1.	Total Ash	0.121	0.137
2.	Acid insoluble ash	0.042	0.054
3.	Water insoluble ash	0.167	0.129
4.	Loss on drying at 110 °C	4.0	2.0

**Table 2: Extractive Values of *Strobilanthus ixiocephala* stem and leaves**

S. No.	Type of extract	Extractive value of Stem % w/w	Extractive value of leaf % w/w
1.	Pet ether	0.56	0.93
2.	Methanol	2.22	4.54
3.	Aqueous	7.42	9.22

**Table 3: Phytochemical test of different extracts of *Strobilanthus ixiocephala* plant**

Chemical Test	Pet. Ether extract	Alcoholic extract	Aqueous extract
Alkaloids			
Dragendorff's reagent	-	+	-
Mayer's reagent	-	+	-
Hager's reagent	-	+	-
Wagner's reagent	-	+	-
Tannic acid	-	+	-
Glycosides			
Fehling	+	+	+
Legal	-	+	+
Keller kiliani	-	+	-
Resins			
Sulphuric acid	-	-	-
50% Nitric acid	-	-	-
Tannins			
Gelatin	-	-	+
Lead acetate	+	+	+
Coumarins			
Ammonia	-	-	-
Flavanoids			
Alkali	+	+	+
Carbohydrates			
Molisch	-	+	+
Fehling	+	+	+
Amino acids			
Ninhydrin	-	+	-
Millon's	-	+	-
Biuret	-	+	-

+ Present; - Absent

**CONCLUSION**

The genus *Strobilanthus* belonging to the family Acanthaceae has around 250 species, of which at least 46 are found in India.

*Strobilanthus ixiocephala* Benth, Family Acanthaceae (Ruellia family) is a small straggling shrub found in Konkan, the Deccan and Kanara in India. Plants that bloom at long intervals once in seven years are known as plietesials. *Strobilanthus ixiocephala* Benth belongs to this category. The perusal of literature also revealed that limited pharmacological, phytochemical and pharmacognostical work has been done on the plant *Strobilanthus ixiocephala*. The present work encompasses macroscopy, microscopy, physicochemical, preliminary and phytochemical analysis. The phytochemical analysis reveals the presence of alkaloids, steroids, flavanoids, glycosides, carbohydrates. The objective of the present investigation is the ease of identification of the species both in whole and powdered form and this can rectify the authenticity of the drug for future investigation.

**REFERENCES**

1. Hooker ID. The Flora of British India, Reer and Co. London, Vol I; 1875,p.525-27
2. Santapau H. The Flora of Khandala on the Western Ghats of India. Calcutta: Govt. of India Press, 2<sup>nd</sup> edn , Vol 16; 1960, p.227
3. Anonymous. The Wealth of India-Raw Materials, New Delhi: Publication and Information Directorate, Vol X; 1976.
4. Agarwal R, Rangari V. Anti-inflammatory and anti-arthritis activities of lupeol and 19 $\alpha$ -H lupeol isolated from *Strobilanthus Callosus* and *Strobilanthus Ixiocephala* roots. Ind J Pharm. 2003; 35: 384-7.
5. Singh B, Sahu PM, Sharma MK. Anti-inflammatory and antimicrobial activities of triterpenoids from *Strobilanthus Callosus* and *Strobilanthus Ixiocephala* roots. The Free Library Short Communication. 2002; 1.
6. Agarwal R, Rangari V. Phytochemical investigation and evaluation of anti-inflammatory and antiarthritic activity of essential oil of *Strobilanthus Ixiocephala*. Ind J of Exp Bio. 2003; 41: 890-4.
7. Khandelwal K R. Practical Pharmacognosy Techniques and Experiments. India- Nirali Prakashan; 16<sup>th</sup> edn; 2006, p.165.
8. Lala PK. Practical Pharmacognosy. India: Lina Guha Publication; 1<sup>st</sup> ed; 1981, p.135-155.
9. Johansen DA. Plant Microtechnique. New York: Mc Graw Hill Book Co; 1940, p. 523.
10. Sass JE. Elements of Botanical Micro-technique. New York: Mc Graw Hill Book Co; 1940, p. 222.
11. Henry AN. Flora of Tamilnadu. Botanical Survey of India. Coimbatore: Southern Circle; 1981, p.228
12. O'Brien TP, Feder N, Mc Cull ME. Polychromatic staining of plant cell walls by toluidine blue-O. Protoplasma. 1964; 59: 364-3.
13. Easu K. Plant Anatomy. New York: John Wiley and Sons; 1964, p. 767.
14. Anonymous. Indian Pharmacopoeia, Government of India: Ministry of Health and Welfare, New Delhi: Controller of Publications, Vol. 2; 1996: A53-A54.