PHARMACOGNOSTIC EVALUATION OF STROBILANTHUS IXIOCEPHALA BENTH

RUPALI SARPATE⁎, DR. SURESH TUPKARI⁎

⁎ Smt. Suresh Patil College of Pharmacy, Chopada, Dist: Jalgoan, State: Maharashtra, Country: India. *Email: rupali_sarpate@yahoo.co.in

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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 Strobilanthus ixiocephala 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, Nashik, 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 plectial is 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 Strobilantes and allied genera) that usually grow gregariously, flower simultaneously following a long interval, set seed, and die. It flowers once in seven years. 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. Its Botanical name is Strobilanthus ixiocephala 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. While the leaves of Strobilanthus callosus are poisonous, toxic and unfit for human consumption it is used as a traditional medicine herb by the local adavasi 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.

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. 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, Nashik, 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 physicochemical, 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. The paraffin embedded specimens were sectioned with the help of Rotary Micromote. The thickness of the sections was 10-12 μm. Dewatering of the sections was performed by customary procedure. Since Toluidine Blue is a polychromat stain, the staining results were remarkably good, and some cytochemical reactions were also obtained. 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. Glycerin mounted temporary preparations were made for macerated/cleared materials.

Photomicrographs

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

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**

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 tared porcelain dishes and evaporated to dryness on a rotarary evaporator and dried to a constant weight. The percentage extractives were calculated with reference to air dried drug.

**Physico-chemical analysis**

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.

**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 µm 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.
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, Pl- 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 collenchymatous 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, Pl-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 collenchymatous cells are present.

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.

Fig. 7: *Strobilanthus callosus* leaf showing stomatal pattern
[SC- subsidiary cells, EpC- Epidermal Cell, St-Stomata, GC- Guard Cells]

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.

![Vein terminations](image)

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 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 ºC was 4.0 and 2.0 for leaf and stem. Percentages of physical constants are given in Table 1 and Percentage of various extract is shown in the Table 2.

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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Leaf</th>
<th>Stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total Ash</td>
<td>0.121</td>
<td>0.137</td>
</tr>
<tr>
<td>2.</td>
<td>Acid insoluble ash</td>
<td>0.042</td>
<td>0.054</td>
</tr>
<tr>
<td>3.</td>
<td>Water insoluble ash</td>
<td>0.167</td>
<td>0.129</td>
</tr>
<tr>
<td>4.</td>
<td>Loss on drying at 110 ºC</td>
<td>4.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Table 2: Extractive Values of *Strobilanthus ixiocephala* stem and leaves

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of extract</th>
<th>Extractive value of Stem % w/w</th>
<th>Extractive value of leaf % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pet ether</td>
<td>0.56</td>
<td>0.93</td>
</tr>
<tr>
<td>2.</td>
<td>Methanol</td>
<td>2.22</td>
<td>4.54</td>
</tr>
<tr>
<td>3.</td>
<td>Aqueous</td>
<td>7.42</td>
<td>9.22</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Chemical Test</th>
<th>Pet. Ether extract</th>
<th>Alcoholic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dragendorf’s reagent</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mayer’s reagent</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hager’s reagent</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Wagner’s reagent</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannic acid Glycosides</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fehling</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Legal</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Keller kiliani</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>50% Nitric acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ammonia</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
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<td>-</td>
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</tr>
<tr>
<td>Alkali</td>
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<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
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<td>+</td>
</tr>
<tr>
<td>Molisch</td>
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<td>+</td>
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</tr>
<tr>
<td>Fehling</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ninhydrin</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Millon’s Biuret</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ 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 pietesials. *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, physiochemical, 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