Research Article

PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS ON STEM AND LEAVES OF *STROBILANTHUS CALLOSUS* NEES.

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ABSTRACT

Strobilanthes callosus Nees (Synonym: Carvia callosa (Nees) Bremek) is a shrub found mainly in the low hills of the western ghats all along the west coast of India. Its standardized Hindi language name is Maruadona. This shrub belongs to the genus Strobilanthes which was first scientifically described by Nees in the 19th century. Scientific information on its pharmacognosy, phytochemistry is very scant. Hence the current study describes some pharmacognostical, physiochemical and phytochemical investigations undertaken on the stem and leaves of one of the species of the genus Strobilanthes, namely Strobilanthus callosus Nees belonging to the family Acanthaceae. The samples for research were collected from Trimbakeshwar, Nasik, India and authentificated in Botanical Survey of India and then subjected for morphological, microscopical and physicochemical and phytochemical analysis. The parameters from the above were recorded with an objective of drawing an attention on those scarce species as well as a reference for further scientific investigations.

Keywords: Strobilanthus callosus, Karvi, Pharmacognostic study, Acanthaceae.

INTRODUCTION

Plants that bloom after long intervals like *Strobilanthes callosus* are known as plietesials, the term plietesial has been used in reference to perennial monocarpic plants of the kind most often met with in the Strobilanthinae" (a subtribe of Acanthaceae containing Strobilanthes and allied genera) that usually grow gregariously, flower simultaneously following a long interval, set seed, and die[1]. The genus has around 250 species, of which at least 46 are found in India. *Strobilanthes callosus* Nees (Synonym: Carvia callosa (Nees) Bremek) is a shrub found mainly in the low hills of the western ghats all along the west coast of India[2]. Its standardized Hindi language name is Maruadona by which it is called in the state of Madhya Pradesh. In the state of Maharashtra in the Marathi language and other local dialects and in the neighboring state of Karnataka the shrub is locally known as Karvi sometimes written in English as Karvy[3]. This shrub belongs to the genus Strobilanthes which was first scientifically described by Nees in the 19th century. 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 tribals and villagers for the treatment of inflammatory disorders[4]. It's leaves are crushed and the juice obtained is believed to be a sure cure for stomach ailments. The stem bark of *Strobilanthes callosus* is used as an emollient in formulations for painful and ineffectual attempts to urinate or defecate[5]. It is used externally for mumps and flowers are used as a vulnerary[6]. Likewise, pounded leaves are rubbed on to the body during the cold period of an intermittent fever and used as a poultice to treat ague in children to alleviate coughing as an astringent and diuretic, and to treat arthritis[7].

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 lupeol and 19α-H-lupeol isolated from the roots showed anti-inflammatory and anti-arthritic activities[8]. The assignment such as macroscopy, anatomical studies, micro measurements and preliminary phytochemical screening, 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 pharmacognostical work had been done on the plant *Strobilanthes callosus* Nees.

MATERIALS AND METHODS

Plant Material

Strobilanthes callosus Nees was procured from Trimbakeshwar, Nasik, India and authentificated in Botanical Survey of India (BSI) and voucher specimen (RSC-1) 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, Chloral hydrate, Toluidine blue 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 FAA (Formalin– 5 ml + Acetic acid – 5 ml + 70% Ethyl alcohol – 90 ml) Formalin aceto-alcohol in fresh form. After 24 h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol. 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[9]. 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[10]. The dye rendered pink color to the cellulose walls, blue to the lignified cells, violet to the mucilage, blue to the protein bodies. Wherever necessary sections were also stained with safranin and fast-green with KI (for starch). For studying the stomata morphology, venation pattern and trichome 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[11]. 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. Magnifications of the figures were indicated by the scale-bars[12].

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 dried in shade were finely powdered and screened for the presence of its own and foreign vegetative matters (other than the organ selected for the research studies). 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)[13].

Preliminary phyto-chemical screening

The leaves and stem were dried at room temperature and screened for the presence of foreign matter. The leaves and stem 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[14, 15].

Physico-chemical analysis

Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the methods prescribed in Indian Pharmacopeia[16].

RESULTS AND DISCUSSION

Morphology

The morphology of the drug showed characteristic features. Leaves 4-9 by 1.5-3 inches, one of each pair often smaller than the opposite one. Leaves are elliptic-lanceolate, acute or acuminate; the margins are crenate and ciliate. They are dark green above, paler and more or less hairy on the nerves beneath, base tapering wing like into the petiole, main nerves beneath 10 to 14 pairs, slender, prominent, petioles variable in length. Flowers axillary, in simple or branched ovoid, pedunculate, subtetragonal spikes with rounded ages. Fig (2) Peduncles often with one or two pairs of orbicular sessile bracts below the spikes, bracts 1/2-3/4 inches long, often as long and broad, elliptic or obovate, rounded at the apex, concave, glabrous covered with viscous secretion of balsamic but not agreeable odour. Disc bright orange. Calyx ½ inches long in flower much enlarged in fruit, segments leathery. Elliptic- lanceolate, 1/6 to 1/3 inches broad, obtuse, softly pubescent. Corolla is long with white tube and purple limb, glabrous with yellow hairs in the mouth inside, cylindrical base. Seeds 1/2 to 3/8 inches, broadly ovate, acute and two seeded.

Figure 1: Entire plant of *Strobilanthus callosus*

Figure 2: A flowering twig of *Strobilanthus callosus*

Microscopy

Stem: The colour of the stem is Greenish yellow. Height up to 1-2 m. Shape cylindrical and branched. Tasteless, odorless or faint. Fibrous in inner stem.

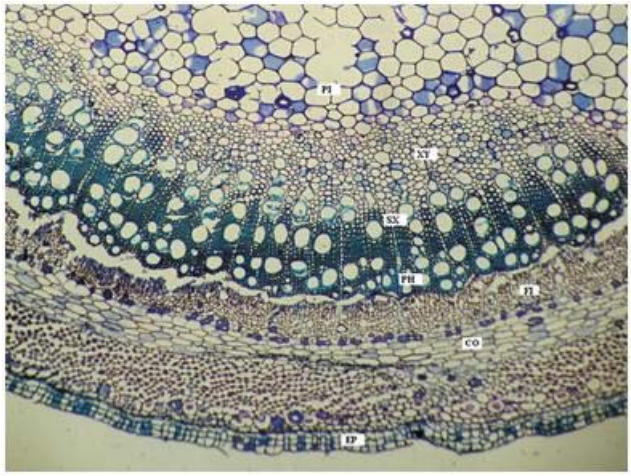

Figure 3: T.S. of *Strobilanthus callosus* Stem - a sector enlarged

[CO-Cortex; FI - Fibers; PH - Phloem, XY- Xylem, PI - Pith; SX - Secondary xylem; EP-Epidermis]

Epidermis (Fig. 3): The epidermis is multiseriate and continuous. It is 40 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

Cortex: Epidermis is followed by the zone of cortex consisting of 2-3 layers of elongated collenchymatous cells and four layers of parenchymatous cells.

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.

Fibers: These are long cells with tapering pointed ends. Some of the fibers are narrow, thick walled and narrow lumened. They are 1.25 to 2mm long; 8µm thick. Some other fibers are wider, shunter and wide lumened. The wide fibers are up to 700µm long and 12 µm wide. (Fig 7)

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 270 to 400µm long. (Fig 7)

Leaves: It consists of Upper epidermis, Palisade layer, Vascular bundle, lower epidermis (Fig 4).

Upper epidermis: Upper epidermis is composed of tangentially arranged single layer of sub rectangular cells below this the projection area with collenchmatous cells is present.

Palisade layer: Palisade parenchyma is composed of elongated and capsule shaped cells which are closed together to each other which form long axes of cells perpendicular to the epidermis.

Vascular bundle: It consist of xylem, secondary xylem and phloem elements. Xylems consist of parenchyma cells. The xylem elements are thick walled angular and compactly arranged radially. 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 presents.

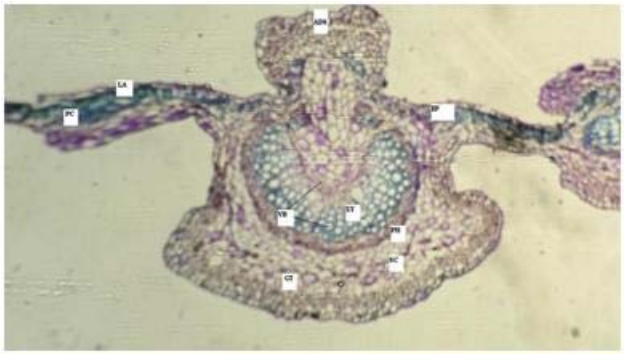

Figure 4: T.S. of *Strobilanthus callosus* Leaf- a sector enlarged

[ADS: Adaxial Side; GT-Ground tissue; PH - Phloem; VB - Vascular bundle; XY – Xylem; EP-Epidermis; SC-Sclerenchyma, PC- Palisade cells, LA- Lamina].

The stomatas are mostly paracytic. (Figure 5). The ground cells are elliptical measuring 20x15µm. The epidermal cells are large, polygonal with straight or slightly wavy thin anticline walls. Stomular frequency 23/mm^2.

The lateral veins are mostly thick; the veinlets are thin and profusely 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 (Fig 6).

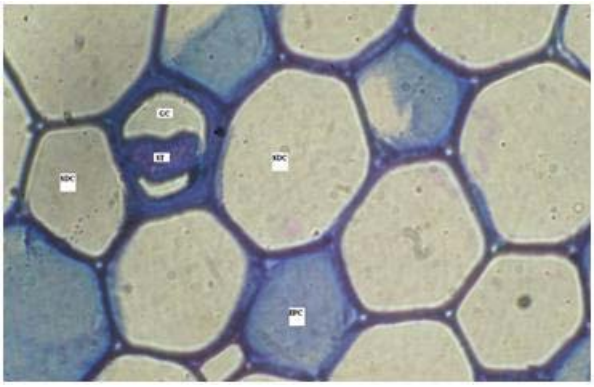

Figure 5: *Strobilanthus callosus* leaf showing stomatal pattern

[ST-Stomata; EPC-Epidermal cell; GL- Guard cell; SDC-Subsidiary cells].

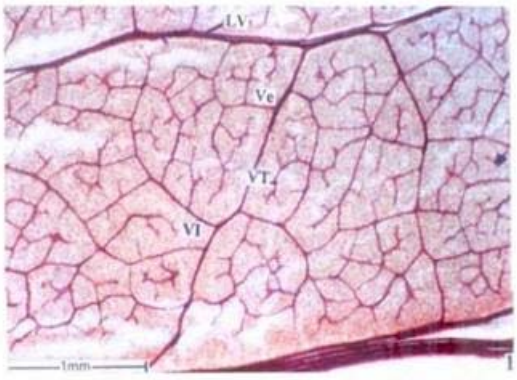

Figure 6: Cleared leaf showing vein islets and vein termination

[LV- Lateral vein; Ve-Vein; VI-Vein Islets; VT-Vein Termination].

Powder characteristics

The powder was pale green in colour; with no any specific odour; slightly bitter; smooth and slippery in texture. The epidermis has stomata, which are either diacytic or anisocytic in nature. The epidermal cells are polygonal and rectangular with thin, straight, anticlinal walls. Fibers are the long cells with tapering pointed ends. Some of the fibres are narrow, thick walled and narrow lumened. They are 1.25 to 2mm long; 8µm thick. Some other fibres are wider, shunter and wide lumened. The wide fibres are up to 700µm long and 12 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 270 to 400µm long. (Fig 7).

Physicochemical analysis

In Physicochemical analysis various parameters were studied viz ash values, extractive values, loss on drying etc. The maximum loss on drying at 110°C was 5.0 and 3.0 for leaf and stem. Percentage of physical constants is given in Table 1. Percentage of various extract is shown in the Table 2.

Figure 7: Powder microscopy of *Strobilanthus callosus* stem

[AP - Axial parenchyma; PF - Perforation plate, SF - Septate Fibre, WF - Wide fibres, VE- Vessel Element]

Table 1: Physicochemical observations of *Strobilanthus callosus* leaves and stem

Parameters	Leaf	Stem
Total Ash	0.141	0.127
Acid insoluble ash	0.052	0.038
Water insoluble ash	0.124	0.110
Loss on drying at 110°C	5.0	3.0

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

Type of extract	Extractive value of stem % w/w	Extractive value of leaf % w/w
Pet ether	1.56	0.95
Methanol	3.22	2.54
Aqueous	7.25	9.20

Preliminary phytochemical studies

Various extracts of *Strobilanthes callosus* Nees plant analysed systematically for the different chemical groups to assess one active constituents present and nature of polarity of the constituents like alkaloids, steroids, carbohydrates, fixed oils, Tannins, phenolic compounds, proteins, glycosides. The extracts showed the presence of phytoconstituents such as alkaloids, tannins, flavanoids, carbohydrates, saponins, steroids, terpenoids, phenolics, coumarins and fixed oil. The results were shown in Table 3.

CONCLUSION

The research paper encompasses on the comparative and the multidisciplinary approach to the study of *Strobilanthes callosus* plant that usually grow gregariously, flower simultaneously following a long interval, set seed, and die. The genus has around 250 species, of which at least 46 are found in India. The perusal of literature also revealed that limited pharmacological, phytochemical and pharmacognostical work had been done on the plant of *Strobilanthes callosus* Nees. The present work concludes the macroscopy,

microscopy, physiochemical, preliminary and phytochemical analysis. The phytochemical analysis reveals the presence of alkaloids, steroids, flavonoids, glycosides and carbohydrates. The objective of the present investigation is the ease to identification of the species both in whole and powdered form.

Table 3: Phytochemical test of different extracts of *Strobilanthes callosus* 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	-	-	-
Flavonoids			
Alkali	+	+	+
Carbohydrates			
Molisch	-	+	+
Fehling	+	+	+
Amino acids			
Ninhydrin	-	+	-
Millon's	-	+	-
Biuret	-	+	-
*+ Present; - Absent			

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