**Pharmacognostic evaluation and phytochemical studies on leaves of *Vitex leucoxylon* Linn**

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**ABSTRACT**

**Introduction:** *Vitex Leucoxylon* Linn. (family Verbenreae) is found commonly in India. This plant is a large deciduous tree, commonly known as Songarbhi (Marathi). It is a small to large tree with a thick trunk and spreading crown found almost throughout the Indian Deccan peninsular. The trees are generally found on river banks, streams and ponds. Recent pharmacological findings indicate that the crude alcoholic extract of its leaves possess antipsychotic, anti-depressant, analgesic, anti-parkinsonian, anti-microbial activities, anti-inflammatory and wound-healing properties. However, no conclusive pharmacognostic study of its leaves has been performed yet. **Methods:** The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material and the establishment of quality parameters including physicochemical and phytochemical evaluation. **Results:** Chief macroscopic and microscopic characters include midrib, lamina, venation pattern, stomata, epidermal trichomes, sclereids, powder microscopy and phytochemical evaluation done by standard methods. **Conclusion:** The results would serve as a useful gauge of standardization of leaf material and ensuring quality formulations. **Keywords:** *Vitex leucoxylon*, microscopical characters, phytochemical screening.

**INTRODUCTION**

The World Health Organization (WHO) proves medicinal plants are the best source to obtain a variety of newer herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand the properties, safety and efficacy.¹

Standardization of natural products is a complex task due to the heterogeneous composition, in the form of the whole plant or plant part extracts obtained thereof. To ensure reproducible quality of herbal products, proper identification of the starting material is essential. The first step towards ensuring the quality of the starting material is authentication. Thus, in recent years there has been a rapid increase in the standardisation of selected medicinal plants of potential therapeutic significance.²,³ Despite modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to who, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and degree of purity of such materials and should be carried out before any tests are undertaken.⁴ *Vitex Leucoxylon* Linn (Verbenreae) is a large deciduous tree, commonly known as Songarbhi (Marathi), an excellent herbal crude drug found in nature which has the composition of the entire essential constituents required for the normal and good health of human. It is a small to large tree with a thick trunk and spreading crown and is found throughout the Indian Deccan peninsular up to an altitude of 900 meters; and extends northwards up to Jhansi and parts of Bihar. The trees are generally found on river banks, streams and ponds. The roots and bark have astringent properties and the roots are also used as a febrifuge. The leaves are smoked for relieving headache and catarrh and are also used for medicinal baths in fever and anemia.⁵

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General pharmacological studies revealed anti-psychotic, anti-depressant, analgesic, anti-inflammatory, anti-parkinsonian and anti-microbial activities of the aqueous and ethanolic extracts of leaves of *Vitex Leucoxylon* and also studied the anti-inflammatory and wound healing properties of the crude alcoholic extract of the leaves in acute inflammation model.\(^6\) \(\beta\)-sitosterol, dimethyl phthalate, vitexin, isovitexin, agnuside and aucubin were isolated from the leaves or barks of *Vitex Leucoxylon*.\(^8\)

The main objective of present study was to perform pharmacognostic investigation and preliminary phytochemical screening of the leaves of *Vitex Leucoxylon* Linn.

**MATERIALS AND METHODS**

**Collection and authentication**

The leaves of *Vitex Leucoxylon* Linn were collected in the foothills of Yercaud, Salem, Tamil Nadu, in March 2011. The plant was identified and authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. A voucher specimen [No.BSI/ SRC/5/23/2011–12/Tech] has been kept in our museum for further reference. The leaves were separated and shade dried at room temperature for 10 days and coarsely powdered with a hand-grinding mill and the powder was passed through sieve no. 60.

**Preparation of the extract**

The powdered material of *Vitex Leucoxylon* was extracted separately using the Soxhlet apparatus with different solvents\(^9\) and an aqueous solvent for cold maceration. After extraction, the extracts were concentrated under reduced pressure.

**Instruments used**

Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations, a bright field was used. For the study of crystals, starch grains and lignified cells, polarized light were employed. Under polarized light they appeared bright against dark background.\(^10\)

**MATERIALS AND METHODS**

**Collection of specimens**

The plant specimens were collected from the foothill of Yercaud, Salem, Tamil Nadu, India. Care was taken to select healthy plants with normal organs. The required leaf samples were cut and removed from the plant and fixed in FAA (Formalin-5 ml + acetic acid-5 ml + 70% Ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary–butyl alcohol as per the schedule given by Sass (1940).\(^11\) The specimens were casted 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. De-waxing of the sections was done by customary procedure. The sections were stained with Toluidine blue as per the method published by O’Brien et al.\(^12\) For studying the venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jefferey’s maceration fluid was prepared. Glycerin-mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell component were studied and measured.

**Photomicrographs**

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic unit. For the study of crystals, starch grains and lignified cells, polarized light were employed. Under polarized light they appeared bright against dark background. Magnifications of the figures were indicated by the scale–bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.

**Determination of physicochemical parameters**

The dried powdered leaves were subjected to physico-chemical analysis including fluorescence analysis,\(^13\) moisture content, total ash, water soluble ash, acid insoluble ash, sulphated ash, alcohol soluble extractive and water soluble extractive\(^15\) to determine the quality and purity of the plant materials.

**Preliminary phytochemical screening**

The dried powdered leaves were extracted with petroleum ether (60–80°C), chloroform and alcohol using Soxhlet apparatus and aqueous extraction by cold maceration. The solvents were completely removed and reduced pressure by using vacuum evaporator. All the extracts were...
screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests. 

RESULTS AND DISCUSSION

Pharmacognostic study

The *Vitex leucoxylon* Linn. plant is a large deciduous tree upto 15 m tall with 3–5 foliolate leaves and young branchlets are quadrangular, minutely pubescent and lenticellate. An attempt was made to study the pharmacognostical character of the leaves of *Vitex leucoxylon* Linn.

Macroscopical evaluation

Leaves compound, digitate or rarely trifoliate, opposite, decussate; rachis pulvinate, planoconvex in cross-section, minutely pubescent; petiolule 0.5–1.5 cm long, canaliculate, glabrous; leaflets 5 (rarely 3), lamina 7–11.5 x 2–3.5 cm, elliptic, apex acute to obtuse, base cuneate-attenuate, margin entire, chartaceous or thinly coriaceous, glaucous beneath, glabrous; midrib canaliculate above; secondary nerves 6–14 pairs; tertiary nerves reticulo-percurrent, not prominent. Inflorescence axillary corymbose cymes, minutely pubescent; flowers zygomorphic, sessile; corolla white with purplish pubescent; anther lobes purple.

Figure 1.1. T.S. of leaflet through midrib. (La-Lamina; AdS-Adaxial side; LV-Lateral Vein; VS-Vascular Strand; MR-midrib).

Figure 1.2. T.S. of midrib. (AdX-Adaxial xylem; Ph-Phloem; AbX-Abaxial xylem; Sc-Sclerenchyma; Ep-Epidermis; GT-Ground tissue).

The vascular system of the midrib is somewhat complex. There is a wide, planoconvex sclerenchyma cylinder enclosing a wide prominent arc of abaxial vascular strand and adaxial small groups of irregularly disposed adaxial vascular strands. The abaxial arc consists of several parallel lines of 3–5 xylem elements situated along the abaxial part. Phloem occurs in uniformly thick arc abutting the xylem is on the upper part of the midrib, there is a narrow, thick band of vascular strands with a few xylem elements in short parallel rows and a wide and thick segment of phloem situated on the lower end of the xylem (Fig. 2.1). In addition to this adaxial segment, there are three or four small, less

Figure No.?? Leaf of *Vitex leucoxylon* L.

MICROSCOPICAL STUDIES

Microscopic features of the leaflets

The leaflets exhibit dorsiventral symmetry with reference to the structure of the lamina and midrib (Fig. 1.1). The midrib is plano–convex; the adaxial side is flat and the abaxial side is semicircular. The midrib is 700 µm thick and 700 µm wide. The midrib consists of a thin epidermal layer of small thick-walled cells with a prominent cuticle. The ground tissue includes 5–9 layers of small, circular compact parenchyma cells (Fig. 1.2).
prominent vascular strands with small clusters of xylem and phloem, xylem being placed at the abaxial end (Fig. 2.2). Thus, these are three groups of vascular strands, one abaxial wide, deeply bowl shaped, second adaxial thick flat plate and the third one being small less prominent, three or four nests.

**Lamina**

The lamina has smooth and even surfaces. It is 330 µm thick. It exhibits xeromorphic structure. The adaxial epidermal cells are vertically elongated, thick walled and heavily cuticularized; they are 20 µm thick. The abaxial epidermis is thin; the cells are narrowly rectangular and thick walled, measuring 10 µm thick (Fig. 3.1). The palisade mesophyll is 2 layered; the cells are narrow and vertically elongated; the palisade zone is 50 µm in height. The spongy mesophyll consists of 8–10 layers of small, lobed, loosely arranged parenchyma cells with wide intercellular spaces (Fig. 3.2).

The vascular strands of the lateral veins occur in vertical pillars at regular intervals. They have small collateral vascular bundles of xylem and phloem surrounded a layer of bundle sheath fibres with adaxial and abaxial extensions (Fig. 3.3).

**Venation pattern**

Venation was studied by paradermal sectioning (Fig. 4.1.). The venation is densely reticulate. Both major and minor veins are thick and straight with prominent bundle sheath cells (Fig. 4.2). The vein islets narrow, polygonal in outline and dense. The vein terminations are absent in most of the islets. When present, the terminations are short, thick and stumpy.

**Stomata**

Stomata are crowded in shallow depression and within the boundary of the vein–islets (areoles) the stomata are

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**Figure 2.2.** T.S. of midrib – abaxial vascular strands enlarged. (Abx- Abaxial xylem; Ph-Phloem; Sc-Sclerenchyma).

**Figure 3.1.** T.S. of midrib as seen under polarized light to show the lignified cells. (AdX-Adaxial xylem; Sc-Sclerenchyma; AbX-Abaxial xylem).

**Figure 3.2.** T.S. of lamina. (PM-Palisade mesophyll; AdE-Adaxial Epidermis; LV-Lateral vein; SM-Spongy mesophyll).

**Figure 3.3.** Vascular strand of the lamina enlarged. (AdE-Adaxial epidermis; PM-Palisade mesophyll; BSE-Bundle sheath epidermis; SM-Spongy mesophyll).

**Figure 4.1.** Paradermal sectional view of the venation pattern of the lamina. (VT-Vein termination; VI-Vein islet).

**Figure 4.2.** Vein-islets enlarged. (St-Stomata).
mostly cyclocytic type; they are surrounded by a circle of 4–6 subsidiary cells (Fig. 5.1). The guard cells are broadly elliptical, slightly thick walled, with wide stomatal pore. The stoma is 30 µm in length and breadth. The epidermal cells are narrow, thick walled with smooth walls.

Powder microscopy

Powdered preparation of the leaf exhibits the following elements when examined under the microscope:

Fragments of lamina

Small broken pieces of leaf-blades are frequently seen in the powder. They exhibit the venation. The venation is is closely reticulate with small and dense areoles (vein islets). They are variable in outline and very thick short and straight. There are primary areas with borders of thicker veins. Within such primary areas are the vein-islets with comparatively their veinlets. The vein terminations are not usually seen in most of the islets. Occasionally short, thick and simple (unbranched) terminations are seen in some of the islets (Figs. 6.1 and 6.2).

Epidermal peelings

Epidermal layers are often seen in the powder, both adaxial epidermal peeling and abaxial peelings are seen. The adaxial epidermal layer, as seen in surface view (Fig. 7.1) exhibits fairly thick anticlinal walls which are highly wavy. The cells appear amoeboïd in outline (Fig. 9.2). The epidermis is apostomatic, having no stomata. The abaxial epidermal peelings are also seen in the powder. The abaxial layer is stomatiferous (having stomata). The stomata are seen in shallow areole and are highly crowded. They are cyclocytic type with circle of upto 6 subsidiary cells enclosing the guard cells.

Epidermal trichomes

Nonglandular epidermal trichomes are fairly common in the powder. They are 2 or 3 celled, uniseriate, unbranched, and pointed at the tip. The walls are thick and the lumen is wide. The trichomes are 220–300 µm long and 20 µm thick (Fig. 7.2).

Sclereids

Sclereids of different shape and size are seen in the powder. They are broad, short elongated, fibre like cells. They may straight or curved. The walls are thick and the lumen is wide (Fig. 7.3) some of them have cell inclusions. The sclereids are 200–300 µm long and 20 µm wide.
Physicochemical parameters

Fresh leaves of *Vitex leucoxylon* were collected and subjected to various physicochemical parameters such as moisture content, foreign matters, total ash, acid insoluble ash, water soluble ash, sulphated ash and the various extractive values are shown in Table 1.

Preliminary phytochemical studies

Qualitative chemical investigation of all the extracts of the selected plant was carried out to check the presence of several phytoconstituents. It revealed the presence of alkaloids, carbohydrates, tannins, glycosides, flavanoids, saponins, protein and amino acids, steroids and gums and mucilage, etc (Table 2).

CONCLUSION

From the above discussion, it was concluded that the results would serve as a useful gauge standardization of the leaf material and ensuring quality formulations. The presence of phenol, tannin, carbohydrate, flavanoid and glycosides shows that this plant is potent neutraceutical agent. There is an urgent need for the documentation of the presence of these compounds in *V. leucoxylon* leaves.
of herbal drugs, systematic phytochemical and pharmacognostical studies of medicinal plants and their natural products.

**REFERENCES**


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**Table 4. Fluorescence Analysis.**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Day Light</th>
<th>UV Light</th>
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<tr>
<td>Drug powder</td>
<td>Pale green</td>
<td>Pale green</td>
</tr>
<tr>
<td>Drug Powder + 1M NaH</td>
<td>Greenish yellow</td>
<td>Green</td>
</tr>
<tr>
<td>Drug Powder + alcoholic 1M NaH</td>
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