PHARMACOGNOSTICAL PROFILING ON THE LEAF OF VITEX PEDUNCULARIS WALL. EX SCHAUER (CHARAIGORH)

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ABSTRACT

Vitex peduncularis Wall. ex Schauer (family: Lamiaceae) is commonly known as ‘Charaigorh’ by the several ethnic groups of Odisha. The plant used by traditional healers in Malaria, Jaundice and menstrual disorders. The preliminary phytochemical analysis, HPTLC fingerprinting, and details pharmacognostical studies of its leaves revealed presence of distinctive characteristic, which help in identifying the specimen.

Keywords: Vitex peduncularis, Pharmacognosy, Phytochemistry, HPTLC, Charaigorh

INTRODUCTION

The increasing demand for herbal medicines, both in the developing and developed countries, has inevitably led for sustaining the quality and purity of herbal raw materials and finished products. WHO, therefore, acknowledged that Pharmacognostical standards should be proposed as a protocol authentication, and quality assurance of herbal drugs.Vitex peduncularisWall. ex Schauerisa medium-sized, dicotyledonous, perennial tree growing 5 – 15m tall. Bark grey, thick, exfoliating in irregular flakes. Leaves 3-foliolate; leaflets lanceolate. Flowers whitish-yellow, in axillary paniculate cymes, fruit is obovoid drupe (Figure 1). It grows in moist deciduous forests along streams and rocky slopes at an altitude up to 1000m; distributed in Eastern Himalaya and tropical region of India. Young stem bark and leaves of this plant are used traditionally as folk remedies to treat Black Water fever, Diabetes, Malaria, and Jaundice; roots used to treat excessive menstrual bleeding. Both leaves and stem bark possess antibacterial and antifungal properties. Leaves of the plant contain compounds like peduncularaside, iridiodanguside, vitexin, triterpenoids and flavonoids. It also contains triterpenoids and flavonoids, pachypodol, ursolic acid and 2-hydroxy-ursolic acid. The various parts of the plant were screened for bio-active compounds and evaluated of biological activities by some scholars. However, a comprehensive pharmacognostical work yet to be done on the species.

The present investigation was, therefore, undertaken to evaluate various qualitative and quantitative parameters on Vitex peduncularis Wall. ex Schauer, the findings of which will be helpful in setting standards for this medicinal plant.

Figure 1: Habit of Plant Vitex peduncularis Wall. ex Schauer

The taxonomical position of Vitex peduncularis Wall. ex Schauer

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Subfamily: Viticoideae
Genus: Vitex
Species: Vitex peduncularisWall. ex Schauer
Synonyms: Vitex alata Roxb., nom. illeg., Vitex morava Buch.-Ham. ex Wall., nom. nud., Vitex peduncularis f. roxburghiana (C.B.Clarke) Moldenke

Vernacular names

Sanskrit: Kakatikta; Assamese: Ahui, Khoidoi, Asohi, Sila-tita; Bengali: Ashot, Boruna, Goda, Horina (Chittagong), Awal (Sylhet), Arso; Garo: Shilangri; Hindi: Thurgorwa; Kach: Jharua, Jadghach, Shelong-phas; Kannada: Navaladi; Odiya: Charaigorh
MATERIALS AND METHODS
The plant material i.e. dried matured leaves were collected at matured stage from hilly area of Nayagarh District, Orissa, India. It was authenticated through detailed taxonomic study and confirmed from the regional flora.11 The voucher specimen of the plant was deposited to the Institute herbarium. These samples were washed under running tap water for 5 min followed by sterile distilled water for three times. Half of the drug samples used fresh for morphological study and half of them were air dried and pulverized to obtain 60 mesh size and dried in shade for 7 days and stored in airtight container to avoid any contamination due to moisture. The macroscopy and organoleptic study of the crude drug were performed in terms of its shape, size, color, odour, taste etc. For microscopy or anatomical study (transverse section), fine hand sections of fresh parts of leaf samples (petiole, midrib, lamina) were done, washed, mounted in 50% glycerine solution and observed along with Camera Lucida Drawing of each.12-15 Quantitative microscopy (palisade ratio, vein islet number, stomatal index) of leaf lamina was done as per standard method. For powder microscopy powdered samples each was treated with different solutions, stained and mounted following standard method and observed under a compound microscope at projection 10X and 40X for rapid and accurate determination of their identity following the standard methods;16-18 phytochemical analysis was done on the basis of protocols prescribed by WHO on Quality Control Methods for Herbal Materials (2011)19 and Indian Pharmacopoeia (2001).20 For chemical profiling of the plant, 100gm of the dried powder was subjected to cold extraction with ethanol and chloroform (1:1) for 7 days; the extract concentrated and then carried out High Performance Thin Layer Chromatography (HPTLC) following the method of Egon Stahl (2005).21-22

RESULTS

Macroscopic Analysis: Leaves opposite, digitately palmately compound, 3-foliate (rarely 4); often aromatic or foetid, petiolate, petiolo up to 10 cm long, flattened, extipulate, dorsiventral, margin entire but slightly wavy or undulated towards apex, apex acuminate, venation reticulate, midrib prominent, veins are arranged alternately, veinlets prominent, middle leaflets 10-20 x 3-5 cm; petiolules 0.5-1.5 cm long; lateral leaflets 8.5-15 x 3-4 cm; petiolules 0.5-1 cm long; chartaceous, sub-glabrous or glabrous above, with dense minute rounded dotted yellow resinous glands beneath; lateral nerves 18-22 pairs, distinct beneath, texture thick on both side, dorsal side deep green and ventral side light green, glabrous type as hair absent, the lobes of the leaf blade are arranged like the claws of a bird, i.e. pedate type, leaflets lanceolate or narrow-elliptic, 15-30 cm long, 7.5-10.5 cm width, base cuneate and slender. (Figure 2)

Figure 2: Morphology of leaves with petiole of Vitex peduncularis Wall. ex Schauer

Microscopic Analysis

T.S. of midrib
Outer epidermis single cell layered, wavy, having bi and tricellular hairs or trichomes with pointed apex, cuticle present. Epidermis is followed by cortical cells more or less oval with intercellular spaces. The Central region is composed of irregular cell walled compact parenchymatous cells; thick walled polygonal sclerenchymatous cells are present as 6-9 patches or islands. The Vascular region is crescent or fulcate shaped, upper side opened. Xylem strands are prominent, 20 to 24 in numbers alternating with multiiseriated medulary rays: Protoxylem towards centre and metaxylem towards lower periphery i.e. endarch type. Phloem exists like small patches just below the xylem strands are followed by 3-6 cell layered rows of parallelly arranged bundle sheath consisting of polygonal thick walled sclerenchyma followed by the lower cortex of parenchymatous cells with no intercellular space again followed by irregular hypodermis made up of the very small oval to polygonal cells. Lower epidermis single layered with unicellular, bicular and multicellular trichomes. (Figure 3&4)

T.S. of lamina
Upper epidermis consists of single layered more or less rectangular cells with a wavy cuticle above. From upper epidermis, nonglandular bi-cellular tapering trichomes are emerged out. Epidermis is followed by two rows of elongated palisade parenchyma with chlorophyll. Spongy parenchyma consisting of the oval to elliptical loosely arranged cells with intercellular spaces beneath the palisade layers. In this region, four chambered oil glands, oil chamber, vacuoles, spiral xylem vessels, are present. Glandular trichomes with four chambered heads are emerged out from lower region. Lower epidermis is single layered having slender to tapering, bi to multicellular trichomes with pointed and curved apex. In this layer, anomocytic type of stomata and bulliform cells are found to be present. (Figure 5)

T.S. of petiole
The cellular arrangement of the petiole is mostly similar to that of the midrib. In this case, the vascular region appears like a continuous cylinder encircling parenchymatous ground tissue containing a number of sclerenchymatous patches or islands followed by central pith. (Figure 6)

Quantitative microscopy of leaf lamina
a) Palisade ratio: range is 8 to 9.75
b) Vein islet number: range is 34.5 to 44.75
c) Stomatal Index: 21.516
Powder analysis of leaf

Organoletic observations: Powder fine, greyish green in colour, slightly acrid in taste with significant odour.

Microscopic Analysis: Powder consists of two types of hairs or trichomes viz. glandular and non-glandular; glandular one is with small one celled stalk and oval multicellular (four chambered) head consisting volatile substances and nonglandular one is slender, tapering, bi to multicellular cellular with long pointed tips; many prismatic crystals of Ca-oxalate having different shapes: rectangular, triangular, irregular; aseptate, long, wavy fibres with undulated inner wall; a number of anomocytic stomata; fragmented palisade cells with chlorophyll attached with single layered epidermis; groups of spiral xylem vessels attached with fibre; three types of parenchymatous cells viz. large squarish to rectangular cells in group with rosette crystals inside, round to oval cells with rosette crystals, cell contents and small oval to polygonal light brownish opaque cells; group of small characteristic striated thick walled reddish brown cells.

Phytochemical analysis

Behaviour of Powder (Leaves)

The Leaves powder of *Vitex peduncularis* was treated with strong acids and bases and the behaviour of the powder was observed based on color reactions. The inference was drawn for the possible presence of specific phytochemicals. (Table 1)

Phytochemical Analysis

Preliminary phytochemical screening of methanolic, ethanolic and aqueous extracts of leaves of *Vitex peduncularis* was carried out to detect the bioactive compounds. (Table 2)
Figure 4: Detailed anatomical structure of midrib of leaf of *Vitex peduncularis* Wall. ex Schauer

tr: trichome; par: parenchyma; ue: upper epidermis; bsc: bundle sheath cells; cc: cortical cell; st: starch grain; psy: protoxylem; mr: medullary ray; mxy: metaxylem; ph: phloem; scl: sclerenchyma; lc: lower cortex; hyp: hypodermis; cu: cuticle; tr: trichome
Figure 5: T.S. through leaf lamina of *Vitex peduncularis* Wall. ex Schauer

bt: bicellular trichome; xy: xylem vessel; cu: cuticle; ue: upper epidermis; pp: palisade parenchyma; oc: oil chamber; gt: glandular trichome; gc: guard cell; bc: bulliform cell; le: lower epidermis; mt: multicellular trichome; ut: unicellular trichome; st: stomata; sp: spongy parenchyma

Figure 6: T.S. through petiole of the leaf of *Vitex peduncularis* Wall. ex Schauer
Figure 7: Powder study of leaf of *Vitex peduncularis* Wall. ex Schauer
A: striated reddish brown cells; B,C: aseptate fibre; D: spiral vessel with fibre; E,F,G,H,I: different nonglandular trichomes; J: glandular trichome; K: prismatic crystals; L,M: anomocytic stomata; N: small polygonal opaque cells; O: oval parenchyma; P: squarish to rectangular parenchyma; Q: palisade cells with epidermis

Table 1: Behaviour of Leaves powder *Vitex peduncularis* with different chemicals

<table>
<thead>
<tr>
<th>S.N</th>
<th>Chemical tests</th>
<th>Observation</th>
<th>Inference/Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder + Conc. HCl</td>
<td>No yellow colouration</td>
<td>May be absence of Quinone</td>
</tr>
<tr>
<td>2</td>
<td>Powder + Conc. H2SO4</td>
<td>Reddish to dark brown</td>
<td>Presence of Steroids</td>
</tr>
<tr>
<td>3</td>
<td>Powder + Conc. HNO3</td>
<td>Yellow coloration</td>
<td>Presence of Proteins</td>
</tr>
<tr>
<td>4</td>
<td>Powder + Aq. FeCl3</td>
<td>Blueish green color</td>
<td>Presence of Phenols and Tannins</td>
</tr>
<tr>
<td>5</td>
<td>Powder + Aq. KOH</td>
<td>No Blue color</td>
<td>May be absence of starch</td>
</tr>
<tr>
<td>6</td>
<td>Powder + Aq. NaOH</td>
<td>Yellowish coloration</td>
<td>Anthraquinone present</td>
</tr>
<tr>
<td>7</td>
<td>Powder + Aq. NaOH</td>
<td>Yellow in color</td>
<td>Flavonoids present</td>
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</tbody>
</table>

Table 2: Phytochemical screening of leaves of *Vitex peduncularis*

<table>
<thead>
<tr>
<th>S.N</th>
<th>Phytochemicals</th>
<th>Methanolic extract</th>
<th>Ethanollic extract</th>
<th>Aqueous extract</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Alkaloids (Dragendorff’s Test)</td>
<td>(+)ve</td>
<td>(+)ve</td>
<td>(-)ve</td>
</tr>
<tr>
<td>2</td>
<td>Cardiac glycosides (Keller-Killani test)</td>
<td>(+)ve</td>
<td>(+)ve</td>
<td>(-)ve</td>
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<tr>
<td>3</td>
<td>Flavonoids (Lead acetate test)</td>
<td>(+)ve</td>
<td>(+)ve</td>
<td>(+)ve</td>
</tr>
<tr>
<td>4</td>
<td>Tannin (L.B test)</td>
<td>(+)ve</td>
<td>(+)ve</td>
<td>Trace amount</td>
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<tr>
<td>5</td>
<td>Steroids (L.B test)</td>
<td>(-)ve</td>
<td>(-)ve</td>
<td>(-)ve</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates (Molisch’s test)</td>
<td>(+)ve</td>
<td>(+)ve</td>
<td>(+)ve</td>
</tr>
<tr>
<td>7</td>
<td>Proteins (Millons tests)</td>
<td>(+)ve</td>
<td>(+)ve</td>
<td>(+)ve</td>
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<tr>
<td>8</td>
<td>Reducing Sugar (Benedict’s test)</td>
<td>(-)ve</td>
<td>(-)ve</td>
<td>(-)ve</td>
</tr>
<tr>
<td>9</td>
<td>Phenols (Ferric Chloride test)</td>
<td>(+)ve</td>
<td>(+)ve</td>
<td>(+)ve</td>
</tr>
<tr>
<td>10</td>
<td>Saponins (Foam test)</td>
<td>(+)ve</td>
<td>(+)ve</td>
<td>(+)ve</td>
</tr>
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</table>

(*) ve :- Present , (-) :- Absent
HPTLC Profile of *Vitex peduncularis* leaves

**Sample preparation**

The dried plant (Leaves) was subjected to soxhlet extraction with methanol for 6 hours and extract was filtered and concentrated and taken for the following HPTLC profile.

**Chromatography experimental**


Mobile Phase: Hexane:chloroform:ethylacetate:formic acid (6:1:3:0.5)[G R grade solvent used, mfg. by MERCK, India]

Sample application: Applied volume 5 µL as 8 mm band and applied at 10 mm from the base of the plate. Plate size was 5X10 cm.

Development: Developed up to 80 mm in CAMAG Twin trough chamber. Plate preconditioning (temp 25°C and relative average humidity were 54%)

Table 3: Rf values observed at different wave length

<table>
<thead>
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<th>R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Colour</th>
<th>R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Colour</th>
<th>R&lt;sub&gt;f&lt;/sub&gt;</th>
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<td>0.03</td>
<td>Deep black</td>
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<td>Deep Red</td>
<td>0.03</td>
<td>Deep Grey</td>
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<tr>
<td>0.16</td>
<td>Light black</td>
<td>0.05</td>
<td>Light Blue</td>
<td>0.09</td>
<td>Light Grey</td>
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<td>0.27</td>
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<td>0.14</td>
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<td>0.33</td>
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<td>Light Blue</td>
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<td>Light Grey</td>
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<td>Light Grey</td>
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<td>Light black</td>
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<td>Deep Blue</td>
<td>0.30</td>
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<tr>
<td>0.62</td>
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<td>Deep Blue</td>
<td>0.34</td>
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<td></td>
<td>0.62</td>
<td>Light Red</td>
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</table>

**DISCUSSION & CONCLUSION**

Each medicinal plant and the specific plant part used as crude drug material contain active or major chemical constituents with a distinctive profile that can be used for chemical quality control and quality assurance. In the present study, the macroscopic microscopic study of the leaf and it’s components reveals the characteristics feature in identifying the specimen, and also reveals the presence of contaminant or deterioration in a sample. The microscopic anatomical study gives a preliminary idea about the nature and disposition of cells, tissues and cell inclusions, and thus helps understand where compounds of interest are located. The Pyrochemical screening revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, terpinols, steroids, proteins, phenols in high concentrations in theophylline and theobromine extracts; and moderate concentrations in aqueous extracts. Thus the presence of wide varieties of secondary metabolites in the leaves of *Vitex peduncularis* is proved to be effective against several diseases. HPTLC is a valuable quality assessment tool for the identification and quantification of chemical constituents present in plant drugs.

The retention factor (Rf) values obtained from it can be used to identify compounds due to their uniqueness for each compound (Table 3). In the present study, the Rf values of individual compounds appearing as spots vertically have been noted (the less polar compounds moving higher up the plates resulting in higher Rf values), which may thus be used as a quality control profile for this drug and in near future this investigation will further helps the research scholars to isolate the important compounds present in this plant species.

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