Research Article

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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.^{1,2} WHO, therefore, acknowledged that Pharmacognostical standards should be proposed as a protocol authentication, and quality assurance of herbal drugs.³ *Vitex peduncularis* Wall. ex Schauer is a medium-sized, dicotyledonous, perennial tree growing 5 – 15m tall. Bark grey, thick, exfoliating in irregular flakes. Leaves 3-foliate; leaflets lanceolate. Flowers whitish- yellow, in axillary panicle 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.⁴ 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.⁵ Both leaves and stem bark possess antibacterial and antifungal properties.⁶ Leaves of the plant contain compounds like peduncularaside, iridoidanguside, vitexin, triterpenoids and flavonoids.^{7,8} It also contains triterpenoids and flavonoids, pachypodol, ursolic acid and 2-hydroxy-ursolic acid.⁹ The various parts of the plant were screened for bio-active compounds and evaluation of biological activities by some scholars.⁶⁻¹⁰ 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 peduncularis* Wall. 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, Ashoi, Sila-tita; Bengali: Ashot, Boruna, Goda, Horina (Chittagong), Awal (Sylhet), Arsol; Garo: Shilangri; Hindi: Thiurgorwa; Kach: Jharua, Jadhgach, Shelong-phang; 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, petioles up to 10 cm long, flattened, exstipulate, 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 tri-cellular 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 multiseriated medullary 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, bicellular 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

- Palisade ratio: range is 8 to 9.75
- Vein islet number: range is 34.5 to 44.75
- Stomatal Index: 21.516

Powder analysis of leaf

Organoleptic 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. (Figure 7)

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 3: T.S. through midrib of leaf of *Vitex peduncularis* Wall. ex Schauer

prt:protuberance; btr:bicellular trichome; mtr:multicellular trichome; ue:upper epidermis; bs:bundle sheath; pi:pith; xy:xylem; ph:phloem; scl:sclerenchyma; par:parenchyma; hy:hypodermis; tr:trichome

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; **pxy:** protoxylem; **mr:**medullary ray; **mxy:** metaxylem; **pi:**pith; **xyv:**xylem vessel; **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:bicellulartrichome; **xy:** xylem vessel; **cu:**cuticle; **ue:**upper epidermis; **pp:**palisade parenchyma; **oc:**oil chamber; **gt:**glandulartrichome; **gc:**guard cell; **bc:** bulliform cell; **le:**lowerepidermis;**mt:**multicellulartrichome ;**ut:**unicellulartrichome ;**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

S.N	Chemical tests	Observation	Inference/Remarks
1	Powder+Conc.Hcl	No yellow colouration	May be absence of Quinone
2	Powder+Conc.H ₂ SO ₄	Reddish to dark brown	Presence of Steroids
3	Powder+Conc.HNO ₃	Yellow coloration	Presence of Proteins
4	Powder+Aq.FeCl ₃	Blueish green color	Presence of Phenols and Tannins
5	Powder+I ₂ Solution	No Blue color	May be absence of starch
6	Powder+Aq.KOH	Yellowish coloration	Anthraquinone present
7	Powder+Aq.NaOH	Yellow in color	Flavonoids present

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

S.N	Phytochemicals	Methanolic extract	Ethanolic extract	Aqueous extract
1	Alkaloids (Dragendorff's Test)	(+)ve	(+)ve	(+)ve
2	Cardiac glycosides (Keller-Killani test)	(+)ve	(+)ve	(-)ve
3	Flavonoids (Lead acetate test)	(+)ve	(+)ve	(+)ve
4	Tannin	(+)ve	(+)ve	Trace amount
5	Terpenoids (L.B test)	(+)ve	(+)ve	(+)ve
6	Steroids (L.B test)	(-)ve	(-)ve	(-)ve
7	Carbohydrates (Molisch's test)	(+)ve	(+)ve	(+)ve
8	Proteins (Millons tests)	(+)ve	(+)ve	(+)ve
9	Reducing Sugar (Benedict's test)	(-)ve	(-)ve	(-)ve
10	Phenols (Ferric Chloride test)	(+)ve	(+)ve	(+)ve
11	Saponins (Foam test)	(+)ve	(+)ve	(+)ve

(+) 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

Stationary Phase: Precoated (support on Aluminum Sheets) Silica Gel Plate. Specification: TLC Silica Gel 60F254, Mfg. by Merck, 26.09.2011, Batch No. 1.05554.0007.

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%)

Derivatising Reagent: Dipped in 20% aqueous Sulphuric acid and charred at 105°C for 10 minutes.

HPTLC Fingerprints observed at 254 nm (Plate 1), 366 nm (Plate 2) and white light (Plate 3)

Table 3: Rf values observed at different wave length

Observed at 254 nm		Observed at 366 nm		Observed at white light	
R _f	Colour	R _f	Colour	R _f	Colour
0.03	Deep black	0.03	Deep Red	0.03	Deep Grey
0.16	Light black	0.05	Light Blue	0.09	Light Grey
0.27	Deep black	0.08	Light Blue	0.14	Light Grey
0.33	Light black	0.11	Light Blue	0.20	Light Grey
0.40	Light black	0.14	Deep Blue	0.24	Light Grey
0.52	Light black	0.19	Deep Blue	0.30	Light Grey
0.62	Light black	0.24	Light Blue	0.34	Light Grey
		0.28	Light Red	0.39	Light Grey
		0.34	Deep Red	0.56	Light Grey
		0.40	Deep Red	0.62	Light Grey
		0.45	Light Red		
		0.48	Light Red		
		0.52	Light Red		
		0.55	Light Red		
		0.62	Light Red		

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.²³ In the present study, the macroscopic microscopic study of the leaf and its components reveals the characteristics feature in identifying the specimen, and also reveal the presence of contaminant or deterioration in a sample. The microscopical or 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 Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, terpenols, steroids, proteins, phenols in high concentrations in methanolic and ethanolic 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 (R_f) values obtained from it can be used to identify compounds due to their uniqueness for each compound (Table 3).²⁴ In the present study, the R_f values of individual compounds appearing as spots vertically have been noted (the less polar compounds moving higher up the plates resulting in higher R_f 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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