

Pharmacognostic Estimation of Powder and Microscopic Analysis of Leaves Stem of *Vitex Castofolia*

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Abstract: Present work is to perform the microscopic evaluation of *Vitex Castofolia* fresh leaf and stem transverse section shows upper and lower epidermis with thin cuticle, cortex differentiated into collenchyma and cortical parenchyma, bi-collateral vascular bundle in midrib region; dorsiventral structure with palisade tissue lying towards upper surface, upper and lower epidermis with thin cuticle and spongy parenchyma in lamina portion with multicellular covering trichomes. Sub-epidermis, Inner coat containing Sclerenchymatous layer, Parenchymatous layer, Pigment layer and also contains endosperm and oil globules and paracytic stomata. The quantitative microscopy of leaf shows average stomatal index ~ 12.23-16.18 and 20.14-23.98 in upper and lower surfaces respectively, vein-islet number~41-44/sq.mm, vein termination number~74-77/sq.mm, palisade ratio~8-10. It can be concluded that the microscopic analysis and pharmacognostic parameters can serve as tool for developing standards for proper authentication, quality, and purity of *Vitex Castofolia* leaves and stem.

Keywords: *Vitex Castofolia*, Transverse section, Leaves, Microscope, phloglucinol, Hcl, Iodine, Chlorolhydrate

1. Introduction

Vitex Castofolia is an erect shrub or small tree growing from 2 to 8 m (6.6 to 26.2 ft) in height. The bark is blockish brown leaves are violet colour with five lanceolate leaflets, sometimes three. Each leaflet is around 4 to 10 cm (1.6 to 3.9 in) in length, with the central leaflet being the largest and possessing a stalk. The leaf edges are toothed or serrated and the bottom surface is covered in hair.

The numerous flowers are borne in panicles 10 to 20 cm (3.9 to 7.9 in) in length. Each is around 6 to 7 cm (2.4 to 2.8 in) long and are violet in color. The petal is of different lengths, with the middle lower lobe being the longest. Both the corolla and calyx are covered in dense hairs. The fruit is a succulent drupe, 4 mm (0.16 in) in diameter, rounded to egg-shaped. It is black or purple when ripe. The active constituents of the leaf juice are casticin, isoorientin, chrysophenol D, luteolin, para hydroxybenzoic acid and D-fructose. The main constituents of the oil are sabinene, linalol, terpinen-4-ol, beta-caryophyllene, α -guaiene constituting 61.8% of the oil. is an important medicinal plant and is used for treatment of a wide spectrum of health disorders in traditional and folk medicine; some of which have been experimentally validated. It is widely planted as a hedge plant along the roads. Traditionally it is reported to have multifarious activities such as analgesic, anti-inflammatory, antioxidant, insecticidal, antimicrobial, anticancer, galactogogue, tonic, febrifuge, expectorant and diuretic .(1)

Habitat

It thrives in humid places or along water courses in wastelands and mixed open forests and has been reported to occur in India, Pakistan, Afghanistan, Sri Lanka, Thailand,

Malaysia, eastern Africa and Madagascar. It is grown commercially as a crop in parts of Asia, Europe, North America and West Indies. It is used as a food crop and also as a source of timber. A large aromatic shrub, the plant is distributed throughout the greater part of India up to an altitude of 1500 m in the outer Himalayas properties.(2)

Authentication and Collection of Fresh Plant

The fresh parts of *Vitex Castofolia* were collected in August 2019, from forest area of adilabad, telangan, India. The plant was identified authenticated by the Dr. K.Raju , professor, Department of Botany; Kakatiya university warangal collection number1005 . For further confirmation, the microscopic characters of this plant was studied and compared with available literature as mentioned above.

Pharmacognostical Studies

The morphology or macroscopical description of *Vitex Castofolia*. Including size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc were studied and compared with available literature. The leaves were dried in shade and stored at 27°C. It was powdered, passed through 40# and stored in air tight containers. Microscopy (T.S) and powder characters of leaf drug were done using compound microscope (Inco-Ambala), inbuilt light microscope (Metzer-Metzer Optical Instrument, Mathura), and photographs were taken using photographic microscope (Motic-Image-2003). Quantitative microscopical measurements were made using eye piece, stage micrometer (Erma-Japan), and camera lucida (Prism type—Swift-Ivis).

2. Microscopical Study

Transverse sections of leaf *Vitex Castofolia*. Microscopic evaluation of leaf was carried out. The material to be sectioned was held between the thumb and four finger of the left hand. Using sharp razor blade held in the right hand, thin section was made the razor blade across the object in quick successions. Transferred the sections in to watch glass containing water, added chloral hydrate to these sections, boiled, filtered and the sections were stained with phloroglucinol and hydrochloric acid (1:1) and the same mounted in glycerin and observed under low power and high power of the microscope. Surface preparation of leaf of *Vitex Castofolia*. Stomata, trichomes and epidermal cells are important identifying characteristics of leaf drug. In transverse sections their exact nature can't be studied hence exposure of surface/epidermis becomes must for the detail microscopical study. Type of stomata present, nature of epidermal cell wall, type of trichomes and their details can be studied only after exposing the epidermis. This technique has significance in the determination of leaf constants, identification of crude drug and detection of adulterants. The leaf of *Vitex Castofolia* was placed on a glass slide and tissues were scrapped off with the sharp edge of razor with care. Water was slowly and continuously added and scrapping was done till transparent and colourless epidermis was exposed. The scrapped material was then placed on a glass slide, mounted in glycerin and examined microscopically.(3)

Quantitative microscopy: The leaf of *Vitex Castofolia* was done and stomatal index, vein-islet number, vein termination number and palisade ratio were determined(4)

Determination of stomatal number

Stomatal number is the average number of stomata per square mm of the epidermis of the leaf. Cleared the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peeled out upper and lower epidermis separately by means of forceps. Kept it on slide and mounted in glycerin water. Arranged a camera lucida and drawing board for making the drawings to scale and drew a square of 1 mm by means of stage micrometer. Put the slide with cleared leaf (epidermis) on the stage. Traced the epidermal cells and stomata then counted the number of stomata present in the area of 1 sq. mm. Include the cell if at least half of its area lies within the square found out the result for each of the ten fields and calculated the average number of stomata per sq. mm. Stomatal number is affected by various factors like age of the plant, size of the leaf, environmental conditions etc.. Hence it is more significant in the evaluation of a leaf drug.

Determination of Stomatal Index

Epidermal cells, each stomata being counted as one cell. Stomatal index can be calculated by using following equation.

$$I = \frac{S}{E+S} \times 100$$

Where, I= Stomatal index

S= No. of stomata per unit area

E= No. of epidermal cells in the same unit area

Cleared the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peeled out upper and lower epidermis separately by means of forceps. Kept it on slide and mounted in glycerin water. Arranged a camera lucida and drawing board for making the drawings to scale and drew a square of 1 mm by means of stage micrometer. Put the slide with cleared leaf (epidermis) on the stage. Traced the epidermal cells and stomata. Counted the number of stomata, also the number of epidermal cells in each field. Calculated the stomatal index using the above formula. Found out the values for upper and lower surface (epidermis) separately.

Determination of Palisade Ratio

Palisade ratio is the average number of palisade cells beneath one epidermal cell of a leaf. It is determined by counting the palisade cells beneath four continuous epidermal cells. Cleared the piece of the leaf by boiling with chloral hydrate solution. Arranged the camera lucida and drawing board for making drawings. Using the 4 mm objective, traced off the outlines of four cells of the epidermis. Then, focused down to palisade layer and traced off sufficient cells to cover the tracings of the epidermal cells. Completed the outlines of those palisade cells, which were intersected by the epidermal walls. Counted the palisade cells under the four epidermal cells. (Included the palisade cell in the count when more than half was within the area of epidermal cells and excluded it when less than half was within the area of epidermal cells.) Calculated the average number of cells beneath a single epidermal cell, this figure was the 'palisade ratio'. Repeated the determination for five groups of four epidermal cells from different parts of the leaf and found the average of the results for the five groups. This average was the 'palisade ratio' of the leaf.

Determination of Vein-islet Number

Vein-islet is the small area of green tissue surrounded by the vein-lets. The vein-islet number is the average number of vein-islets per square millimeter of a leaf surface. It is determined by counting the number of vein-islets in an area of 4 sq. mm. of the central part of the leaf between the midrib and the margin. Cleared the piece of the leaf by boiling with chloral hydrate solution for about thirty minutes. Arranged the camera lucida and drawing board for making drawings to scale. Put stage micrometer on the microscope and using 16 mm objective, drew a line equivalent to 1 mm as seen through the microscope and constructed a square on this line. Moved the paper so that the square was seen in the eyepiece, in the centre of the field. Put the slide with the cleared leaf (epidermis on the stage). Traced off the veins, which were included within the square, completing the outlines of those islets, which overlapped two adjacent sides of the square. Counted the number of vein islets in the square millimeter. Where the islets were intersected by the sides of the square included those on two adjacent sides and excluded those islets on the other sides. (To obtain a critical result for a leaf, 4 sq. mm. should be used, preferably in one large area of 4 sq. mm). Found the average number of vein islets from the four adjoining squares, to get the value for one sq. mm.

Determination of Vein-let Termination Number

Vein-let termination number is defined as the number of vein-let termination per sq. mm of the leaf surface, midway between midrib of the leaf and its margin. A vein termination is the ultimate free termination of vein-let. Cleared the piece of the leaf by boiling with chloral hydrate solution for about thirty minutes. Arranged the camera lucida and drawing board for making drawings to scale. Put stage micrometer on the microscope and using 16 mm objective, drew a line equivalent to 1 mm as seen through the microscope. Constructed a square on this line. Moved the paper so that the square was seen in the eye piece, in the centre of the field. Put the slide with the cleared leaf (epidermis on the stage). Traced off the veins, which were included within the square, completing the outlines of those islets, which overlapped two adjacent sides of the square. Counted the number of vein-let terminations present within the square. Found the average number of vein-let termination number from the four adjoining squares to get the value for one sq.mm.

Diameter of Starch Grains

Eyepiece micrometer was calibrated using stage micrometer to determine the value for one division of eyepiece micrometer (3.152 at high power and 14.87 at low power). Powder sample was gently spread over clean glass slide with the help of muslin cloth, warmed near the flame with 2-4 drops of clearing solution (chloral hydrate) for 2-3 times. Slide was stained with few drops of N/50 Iodine solution, excess stain was removed and cover slip was placed with 1-2 drops of glycerin water solution. Faint blue colored starch grains were observed and none of divisions of eyepiece micrometer occupied by individual starch grain were noted. In all group of 25 starch grains were counted and such four groups were analyzed from different slides. From the data obtained, average diameter of starch grains was determined using formula
 where = number of grains and = number of divisions

3. Result and Discussion

Macroscopic evaluation

Table 1: Morphology of *Vitex Castofolia* Leaves

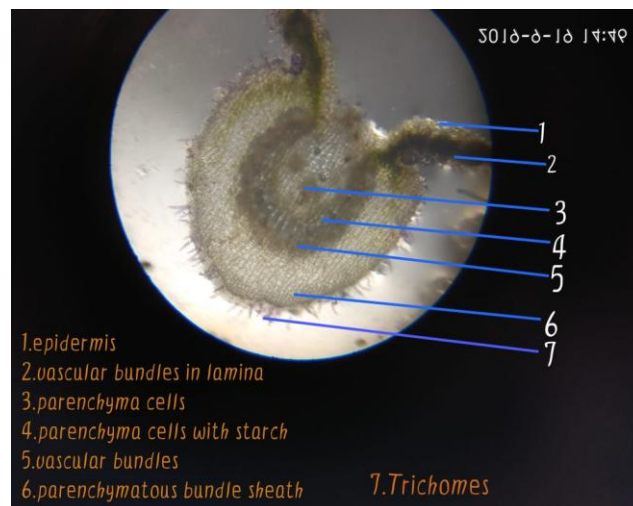
S.No	feature	Observation
1	Upper Surface	Dull Dark Green
2	Lower surface	Violet
3	Odour	charectristic
4	Shape	ovate or suborbicular, broadly cordate to ovate with oblique base
5	Size	7-10 cm
6	Texture	smooth
7	Colour of leaflet	blockish brown

Microscopic evaluation

Free hand transverse sections (T.S) of fresh leaf and stem of *Vitex Castofolia* were taken and studied for their histological characters T.S of leaf shows midrib and lamina portion. Lamina is dorsiventral in nature. Upper Epidermis: Straight walls, 600 to 1100 cells/sq. mm, single layered, rectangular. Trichomes : Covering , uniseriate, waxy, blunt tip, Glandular Trichomes: stalk unicellular, head 2-4 celled. Stomata, Palisade: Single layered, compact celled, radially

elongated, covering 25 th of lamina. Spongy parenchyma: 6-8 layers, loosely arranged, intercellular spaces, cluster crystals (sphaeraphides), Micro-spheroidal crystals and vascular strands are found in upper layers. Lower Epidermis: Similar to upper epidermis, paracytic stomata and numerous multicellular trichomes are seen; wavy walls and cuticle. Mid Rib: Epidermis: Epidermal layers are continuous. Collenchyma: Below upper and above lower epidermis, Thick walled cellulosic cells. Cortical Parenchyma containing prisms of calcium oxalate and micro-phenoidal crystals. Vascular Bundle: Bicollateral vascular Testa: Outer coat: Epidemis: Single layered, polygonal tabular cells with thin anticlinal walls filled with mucilage. Sub – epidermis: contains cylindrical collenchymas. Inner coat: Sclerenchymatous layer: Longitudinally elongated, lignified sclerides, thick walled, pitted, very small lumen. Parenchymatous layer: One or two layers, thin, tangentially elongated, collapsed parenchymatous cells. Pigment layer: Single layer of flattened polygonal pigment cells with reddish brown contents.

Microscopical evaluation of leaf T.S *Vitex Castofolia* (Fig.1)



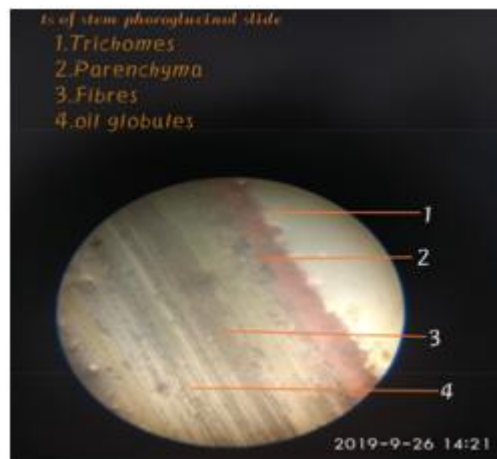
Epidermis, Vascular bundles in lamina, Parenchyma cells, Starch grain, Cluster sheath, Trichomes



Trichomes

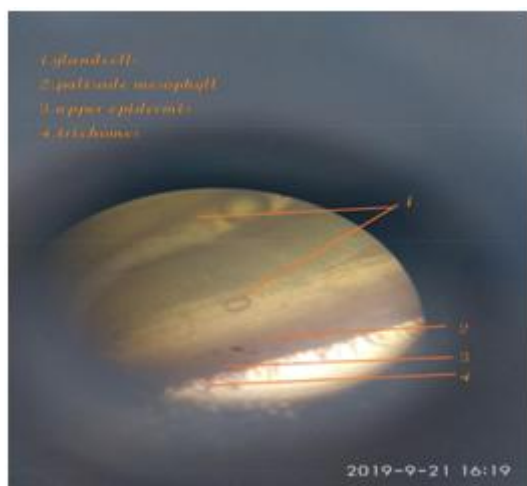


T.S of leaf with lignin

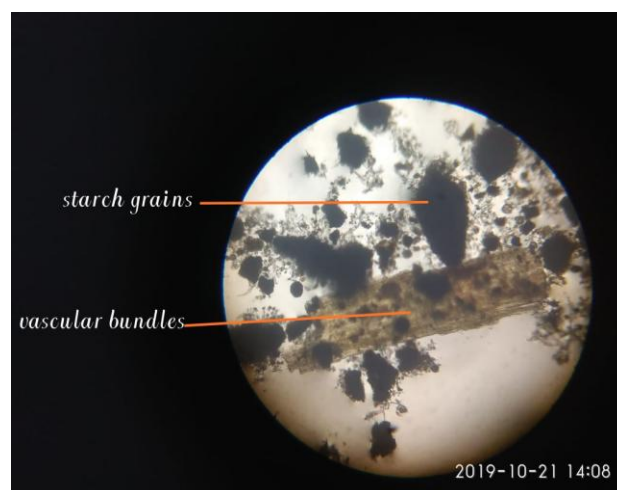


(1) Trichomes (2) Parenchyma (3) Fibres (4) Oil globules

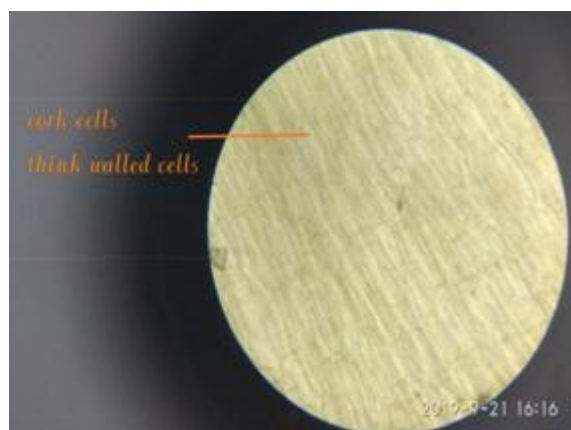
Microscopical evaluation of stem T.S *Vitex Castofolia* (Fig.2)



(1) Oil glands (2) Polysade cells with mysophyll (3) Upper epidermis (4) Trichomes



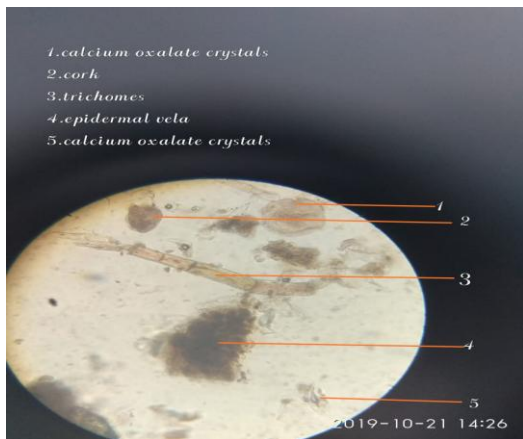
A. (1) Starch grains (2) Vascular bundles



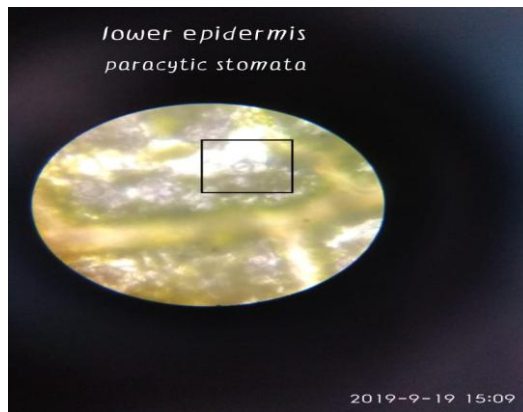
(1) Thickwall corkcells (2) Polygonal tabular cells



B. (1) Calcium Oxalate crystals (2) collenchymatus



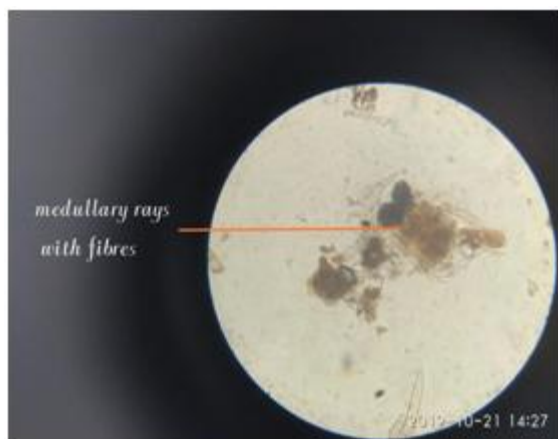
C. (1) Calcium oxalates (2) Cork (3) Trichomes



Palisade cells Parasitic stomata



Vein islet and vein terminations



D. (1) Medullary rays with fibres (2) Sclerenchyma



Bundles of trichomes

Quantitative microscopy of leaf of *Vitex Castofolia*

Quantitative microscopy of leaf of *Vitex Castofolia* was done and stomatal index, vein-islet number, vein termination number and palisade ratio were determined which are given in Table

Table 2: Quantitative microscopy of leaf

S. No	Determination	Value (per sq. mm)
1	Stomatal index	
	Lower epidermis	17.24-20.52
	Upper epidermis	24.22-26.91
2	Vein-islet number	40-45
3	Vein-termination number	65-68
4	Palisade ratio	10-14

4. Discussion

Pharmacognostic studies are not part specific. All parts of the plant are important and show therapeutic efficacy, though their efficacy varies. Hence pharmacognostic studies should be done for the part of the plant which is under investigation. Identified diagnostic characters are essential for the synthesis of secondary metabolites. Some of the examples of pharmacognostic studies of different parts reported in the literature are vijaybhaskar et al, leaf stem and root of *Trianthema portulacastrum*. The organoleptic, macroscopic, microscopic characters results of this study could be used for the quality control of the crude drug. They will also help to maintain the efficacy and identity of the drug and will prevent mishandling of the drug.

5. Conclusion

Pharmacognostical studies of the *Vitex Castofolia* leaves are useful to researches for further study on plant.

6. Acknowledgement

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