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Research Article
Pharmacognostical and Phytochemical Analysis of Stems of *Vitex pinnata* Linn.

[1]S. Thenmozhi, [2]Tarafaud Tahir and [3]Subasini Uthirapathy

[1]Department of Pharmacognosy, PGP College of Pharmaceutical Science and Research Institute, Namakkal (D.T), Tamil Nadu, India
[2]Faculty of Chemistry, Environmental Electrochemistry, Koya University, Koya, Iraq
[3]Department of Pharmacology, Faculty of Pharmacy, Tishk International University, Kurdistan, Iraq

Abstract

Background and Objective: *Vitex pinnata* Linn. (Syn: *Vitex pubescens* Vahl.) is a moderately sized tree of tropical Asia. *Vitex pinnata* is a tree with trifoliate leaves, grey to brown bark. The leaves and stems are potential as an antioxidant, because of flavonoids, alkaloids and terpenoids constituents. The present study aimed to establish the pharmacognostic features of leaf, stem and powdered stem material and quality parameters including physicochemical evaluation. **Materials and Methods:** The leave and stems were investigated, which comprised macroscopic and microscopic assessment, phytochemical screening and physicochemical characterization of the stems, besides the microscopic analysis of the powder. All photographs of different magnifications were taken using a digital camera attached to the Nikon Labphot 2 Microscopic Unit. **Results:** The microscopical studies of stem and powders of *Vitex pinnata* revealed the presence of cortex, periderm, sclerenchyma, secondary phloem, vessels, xylem fibres, xylem rays, collapsed phloem cells, phellem, sclereids, phloem ray, sieve element, starch grains, prismatic calcium oxalate crystals and vessel elements, etc. Phytochemical screening revealed the presence of flavonoids, saponins, triterpenes and steroids in the crude extract. The values of the physicochemical parameters such as loss on drying 4.12%, foreign organic matter 5.60%, total Ash 09.43%, acid insoluble ash 2.34%, water-soluble Ash 3.04%, sulphated ash 3.20%, alcohol soluble extractives 15.6% and water-soluble extractives 16.8%, respectively. **Conclusion:** The information obtained with botanical, physicochemical and phytochemical studies could be used to identify *Vitex pinnata* and to certify the authenticity of commercial samples to effectively contribute to quality control of that vegetable raw material.

Key words: *Vitex pinnata* stem, morphological characters, microscopical study, powder microscopy, preliminary phytochemical screening

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Corresponding Author: Subasini Uthirapathy, Department of Pharmacology, Faculty of Pharmacy, Tishk International University, Erbil, Kurdistan Region, Iraq Tel: +964 7518732348

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbal plants are a rich source of secondary metabolites with interesting biological activities[1] and since the beginning of civilization, plants have been used for man to heal, cure or prevent diseases, they have great potential for producing new drugs of great benefit to humankind[2]. Traditional herbal medicine has been handed down for thousands of years in India. It is still widely used in the treatment of people's multiple diseases and closely related to people's health. However, with the change of natural environment and the variation of species, some characteristics of the medicinal plant from the different origin are becoming more and more similar, which led to diversity confusion and countless quality differences of the medicinal plants isolated bioactive molecules in the market and there is a large unknown hazard in medication safety. Therefore, it is very crucial to identify the varieties and assurance quality of herbal medicines[3,4].

Vitex pinnata Linn. (Family: Verbenaceae) is a small to a medium-sized evergreen tree, up to 25-30 m tall[5,6], often with a crooked bole, up to 70 cm in diameter at breast height, bark surface smooth, shallowly fissured or flaky, pale grey to yellowish-brown, inner bark pale yellow to bright orange, branches quadrangular, crown often spreading. Leaves opposite, compound, 3-5 foliolate, leaflets and petioles pubescent below, lateral leaflet sessile or nearly so, elliptic, 10-20 cm long. The plant has been reported to have various ethnomedicinal applications[7-9]. In Traditional medicine, the plant is used to expel intestinal worms, an analgesic, anti-inflammatory[10], antipyretic, wound healing[11], antioxidant, antibacterial and stomachache[12]. In Brunei, the young leaf shoots are eaten raw to treat hypertension and fever. A root-tea is taken for backache, body ache and fatigue[13]. Leaves are used to cure fever and wounds while bark scrapings are applied to wounds and used as a charm for convulsions[14]. A bark extract is taken for the treatment of jaundice.

However, the legislation[15] emphasizes that for the development of herbal medicines, it is necessary to describe parameters of safety, efficacy, purity and quality of both the raw material and the finished product, which also allows the inclusion in pharmacopoeias and official codes. The reliability tests aimed at qualitative and quantitative evaluation of bioactive molecules of plant material, based on characterization and phytochemical constituents of plant species. It can be started with the phytochemical analysis to identify the secondary metabolites[16]. As explained above and considering that despite the widespread popular therapeutic use of *Vitex pinnata* leaves, studies to define the parameters of quality, efficacy and safety of that plant extract. In this research, the present study reports the Pharmacognosy, phytochemical and physicochemical parameters of *Vitex pinnata* leaves, intending to effectively contribute to quality control of that vegetable raw material.

MATERIALS AND METHODS

Plant material: The stem of the selected plant was collected in July, 2018 from Tirunelveli, Tamil Nadu and was identified and authenticated by Dr. V. Chelladurai, M.Sc., PhD, (Retired Scientist), Research Officer-Botany, Central Council for Research in Ayurveda and Siddha, Govt. of India. Tirunelveli, Tamil Nadu, (the Voucher specimen No. PGP/Ph.Cog/118) has been deposited in the Department of Pharmacognosy, PGP College of Pharmaceutical Science and Research Institute, Namakkal, Tamil Nadu, India.

Macroscopic evaluation: The organoleptic and macroscopic characters of *Vitex pinnata* Linn stems like colour, odour, shape, size and taste were evaluated.

Instruments used: Photographs of different magnifications were taken with Nikon Labphot2 Microscopic Unit. For normal observations, a bright field was used. For the study of crystals, starch grains, lignified cells and polarized light was employed. Since these structures have birefringent property, under polarized light, they appear bright against a dark background.

Collection of specimens: The plant specimens were collected from Tirunelveli, Tamil Nadu, India. Care was taken to select healthy plants and for normal organs. The required samples of leaves 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. The specimens were castled into paraffin blocks.

Anatomical studies sectioning: The paraffin-embedded specimens were sectioned with the help of the Rotary Microtome. The thickness of the sections was 10-12 µm. De-waxing of the sections was done by the customary procedure. The sections were stained with Toluidine blue as per the specific method[14]. For studying the venation pattern and trichome distribution, peridermal sections (sections were taken parallel to the surface of the leaf) of a leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffery'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 components were studied and measured.

Photomicrographs: Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labophot 2 Microscopic Unit. For the study of crystals, starch grains and lignified cells, polarized light was employed. Under polarized light, they appear bright against the dark background. Magnifications of the figures are indicated by the scale-bars.

Powder microscopy: The dried stems were powered and studied under a microscope. Pinches of powder were taken in a microscopic slide and add 1-2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a coverslip. The characteristics features of cell components were observed and their photographs were taken using photomicrography.

Physicochemical analysis: The dried powdered stem was subjected to physicochemical analysis including fluorescence analysis moisture content, total ash, water-soluble ash, acid insoluble ash, sulphated ash, alcohol soluble extractive and water-soluble extractive to determine the quality and purity of the plant materials.

Preparation of extracts: The stems were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no.40 and stored in an air-tight container for further use. This dried powdered stems of *Vitex pinnata* Linn. is subjected to hydro-alcoholic extraction in the ratio of ethanol: water as 70:30, adopting the cold percolation method. The extract is concentrated under reduced pressure to yield semi-solid mass which is dried in a desiccator and then subjected to preliminary phytochemical analysis using different chemical tests.

RESULTS

The current research reveals the pharmacognostic features, physicochemical and phytochemical properties of *Vitex pinnata* Linn. These parameters could be useful in the preparation of the herbal section of the proposed Ayurveda Pharmacopoeia. Any crude drug which is demanded to be *Vitex pinnata* but whose characters knowingly deviate from the accepted standard above would then be forbidden as

Fig. 1: Leaf and stem of *Vitex pinnata* Linn

contaminated, adulterated or fake. The high content of poly-phenolic secondary metabolites (alkaloids and flavonoids) in *Vitex pinnata* and its uses in complementary medicine are indications that the plant is of great potential for a wide range of applications in ethnomedicine.

Morphological evaluation: Small to medium-sized evergreen tree up to 25-30 m in height. Pale grey to yellowish-brown colour bark smooth, shallowly fissured or flaky surface, inner surface bark pale yellow to bright orange colour. Branches quadrangular, crown often spreading. Leaves opposite, compound, 3-5 foliolate, leaflets and petioles pubescent below, lateral leaflet sessile or nearly so, elliptic, 10-20 cm long. Flowers found in terminal panicles, compact, pyramidal, blue or lilac, aromatic stamens 4, 2 longer and 2 shorter. Ovary superior, 2-4-chambered with 1 filiform style, having a bifid stigma. Fruit a drupe, subglobose, 7-13 mm in diameter, purplish-black when matured, sessile on the often enlarged calyx, 1-4 seeded. Seeds are obovoid or oblong, lacking endosperm in Fig. 1 and 2.

Microscopical study of the stem of *Vitex pinnata* Linn: The stem exhibits well developed secondary growth. It shows periderm, cortex, sclerenchyma cylinder, secondary phloem and secondary xylem in Fig. 3. The periderm is superficial in position and it consists of 3 or 4 layers of phellem cells. The xylem fibres are narrow, thick-walled and lignified. The xylem rays are thin straight and the cells are also lignified in Fig. 3. Inner to the phellem occurs wide parenchymatous cortex. The

Fig. 2: Tree of *Vitex pinnata*

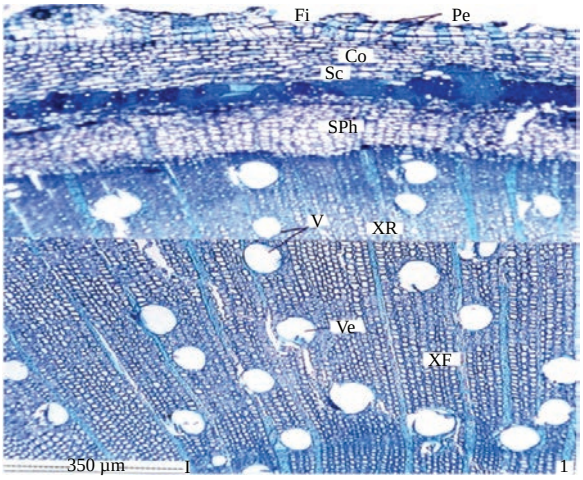

Fig. 3: Transverse section of stem showing periderm, cortex, secondary phloem and secondary xylem
Co: Cortex, Fi: Fibre, Pe: Periderm, Sc: Sclerenchyma, SPh: Secondary Phloem, Ve: Vessel, XF: Xylem Fibre, XR: Xylem Ray

cortical cells are tangentially elongated, thick-walled and compact. The inner boundary of the cortex has a thick continuous cylinder of sclerenchyma bundles. The sclerenchyma cells are fibres and sclereids. These two types of elements occur in alternate positions in Fig. 4. Inner to the sclerenchyma cylinder occurs wide well differentiate secondary phloem elements. The secondary phloem elements consist of outer collapsed elements and inner non collapsed elements. The collapsed cells are seen in the form of dark,

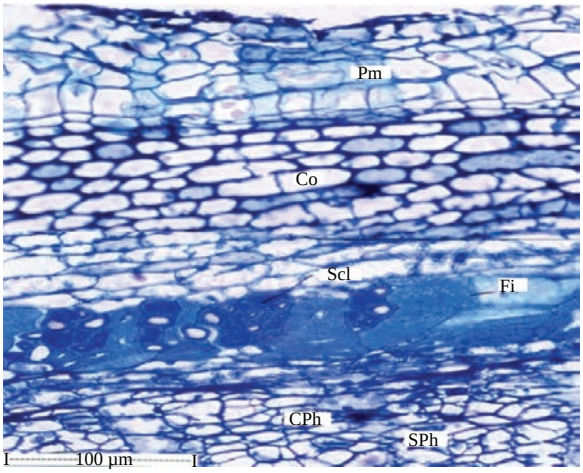

Fig. 4: Transverse section of stem showing periderm, cortex and sclerenchyma cylinder
Co: Cortex, CPh: Collapsed Phloem, Fi: Fibre, Pm: Phellem, Scl: Sclereids, SPh: Secondary Phloem

thick tangential lines in Fig. 4. In the non-collapsed sieve elements are intact, they are wide, thick-walled and polygonal in outline. The sieve elements have wide and prominent companion cells in Fig. 5a. The secondary xylem has no growth rings the vessels are in an oblique radial chain. They are solitary, circular or oval, thin-walled and 70 µm wide. The xylem fibres are narrow, thick-walled and lignified. The xylem rays are thin straight and the cells are also lignified in Fig. 5b.

Cell inclusions: Calcium oxalate prismatic crystals are abundant in the xylem ray cells. The crystals are large and occupied in the entire lumen of the fibrin Fig. 6a. The xylem fibres have a dense accumulation of starch grains. The starch grains also occur in the xylem parenchyma in Fig. 6b. The starch grains are circular and concentric.

Powder microscopy: The powder preparation of *Vitex pinnata* stem exhibits the following inclusions.

Xylem fibres and vessel elements: The vessel elements may be tailless in Fig. 7a. The narrow fibres are longer, narrow, thick-walled with a reduced lumen and the vessel elements are long, narrow and cylindrical in Fig. 7a and b. Some of the vessel elements have oblique perforations in Fig. 7b. Long, narrow thick-walled fibres with pointed ends are abundant in the powder. The fibres may be wide or narrow. The wide fibres have thin walls and wide lumen in Fig. 8a. The vessel elements are long, narrow and cylindrical in Fig. 8b. The fibres are 620 µm long and 20 µm thick. Vessel elements are equally

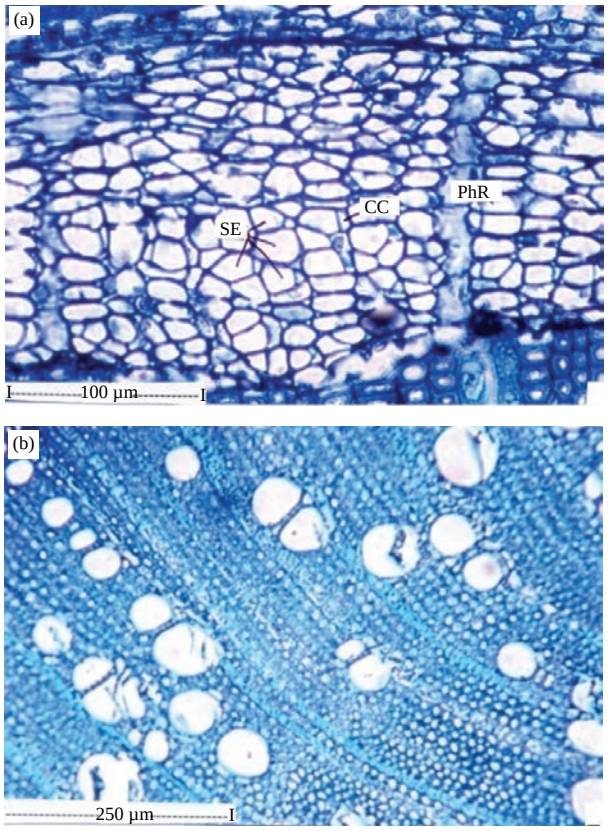

Fig. 5(a-b): (a) Phloem enlarged and (b) Secondary xylem enlarged
CC: Companion Cell, PhR: Phloem Ray, SE: Sieve Element

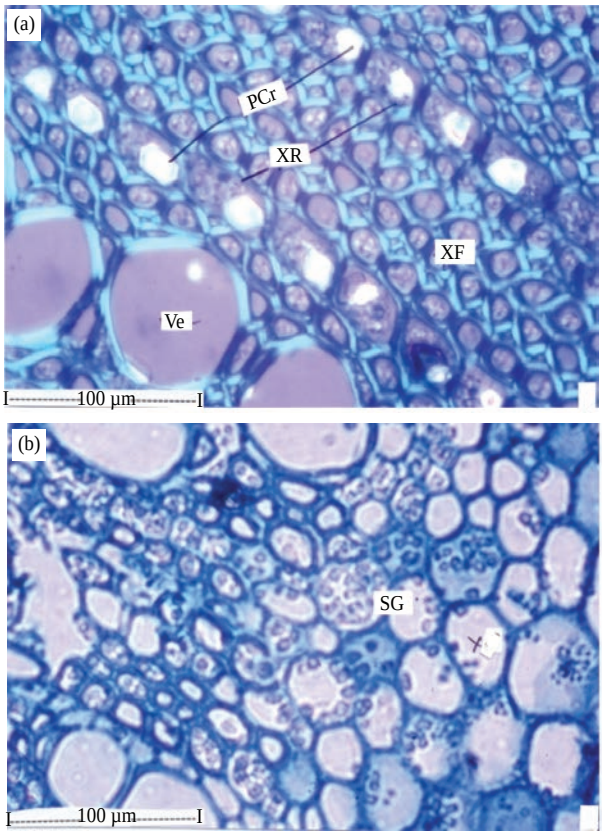

Fig. 6(a-b): Calcium oxalate crystals in the xylem rays and (b) Starch grains in the xylem parenchyma and xylem fibres
PCr: Prismatic Crystals, SG: Starch Grains, Ve: Vessels, XF: Xylem Fibres, XP: Xylem Parenchyma, XR: Xylem Ray

Table 1: Physicochemical analysis of stem of *Vitex pinnata* Linn

Parameters	Value obtained on dry weight basis (% w/w)
Moisture content	4.12±0.02
Foreign organic matter	5.60±0.26
Total ash value	09.43±0.26
Acid insoluble ash	2.34±0.17
Water-soluble ash	3.04±0.15
Sulphated ash	3.20±0.14
Alcohol soluble extractive	15.6±0.42
Water-soluble extractive	16.8±0.24

Table 2: Reaction of powdered drug with different reagents

Treatment	Colour
Powder as such	Light brown
Powder + conc. H_2SO_4	Brown
Powder + conc. HNO_3	Magenta brown
Powder + conc. HCl	Yellowish-brown
Powder + 5% Iodine	Reddish-brown
Powder + 5M NaOH	Brownish-yellow
Powder + Picric acid	Yellowish-brown
Powder + glacial acetic acid	Brownish-yellow

abundant in the powder Fig. 8c. The vessel elements are long, narrow and cylindrical in Fig. 9a-c. The vessel elements have wide circular, horizontal perforations at the end wall in Fig. 9a and b. Some of the vessel elements have oblique perforations in Fig. 9c. The vessel elements may be tailed in Fig. 9c. The lateral wall pits are reticulate in Fig.9a and b or circular, multiseriate and alternate in Fig. 9c. The length of the vessel elements ranges from 220-400 µm.

Sclereids and parenchyma cells: Spherical, thin-walled parenchyma cells are seen scattered in the powder. The parenchyma cells do not possess any specific inclusions in Fig. 7a and b is rarely seen in the powder. They are brachysclereids. They have very thick lignified walls and narrow canal-like pits in Fig. 8c. The sclereids are 40×90 µm in size. The parenchyma cells do not possess any specific inclusions in Fig. 9a and b.

Physicochemical analysis of crude drug: Physicochemical evaluations showed Moisture content 4.12±0.02% w/w, foreign organic matter 5.60%, total Ash 09.43%, acid insoluble ash 2.34 ±0.17%, water-soluble Ash 3.04%, sulphated ash

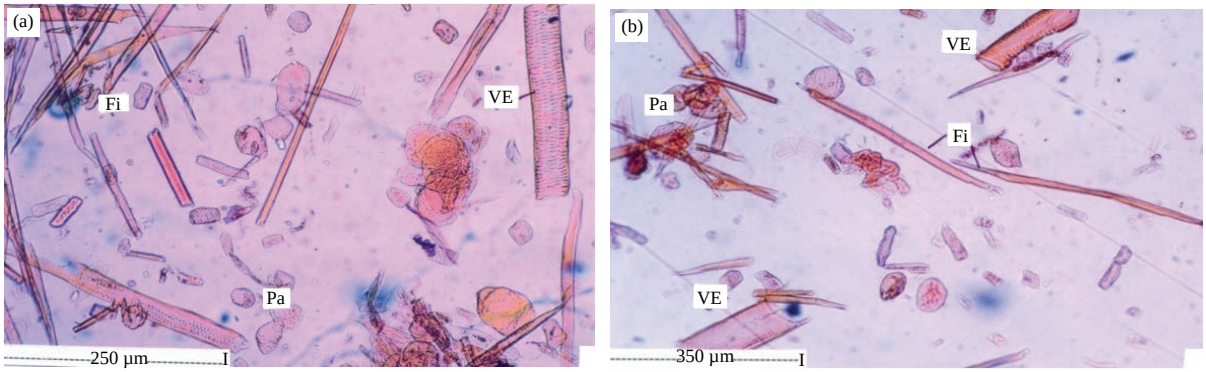

Fig. 7(a-b): Powder form of *Vitex pinnata* stem containing vessel elements, fibres and Parenchyma cells at 250 and 350 µm, respectively
Fi: Fibre, Pa: Parenchyma, VE: Vessel Element

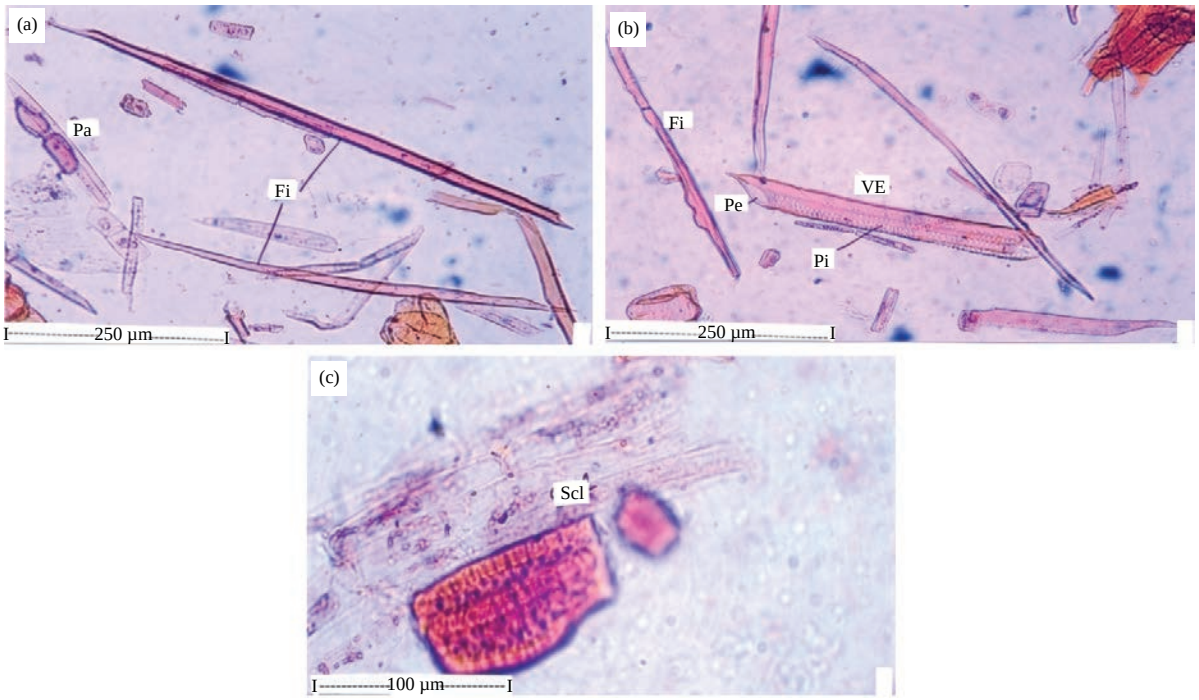

Fig. 8(a-c): Fibers in the powder of *Vitex pinnata* stem, long cylindrical vessel element in the powder of *Vitex pinnata* stem and Brachysclereids in the powder of *Vitex pinnata* stem
Fi: Fibre, Pa: Parenchyma, Pe: Perforation, Pi: Pits, Scl: Sclereids, VE: Vessel Element

3.20%, alcohol soluble extractives 15.6%, water-soluble extractives 16.8%, respectively, results were presented in Table 1.

Fluorescence analysis: The powder drug with different chemical reagents showed different colours when seen on the naked eye. The different colour observed shows the presences of different types of phytoconstituents are reported in Table 2. Many drugs fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under UV light. The fluorescence characteristics of the powdered drug were studied under UV light after treating with different chemical reagents was reported in Table 3. Different reagents under the effect of daylight and UV light showed different colours in fluorescence analysis.

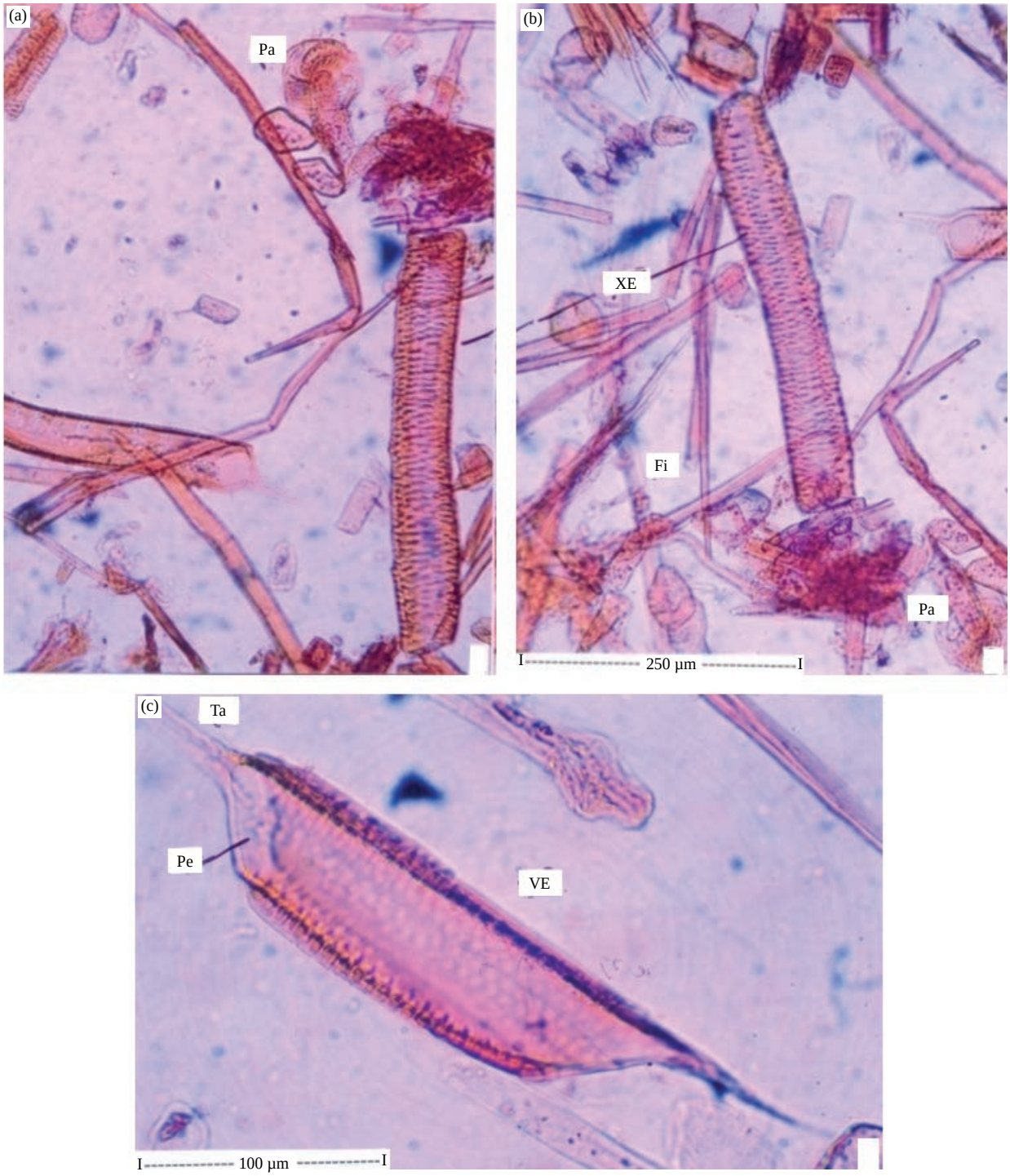

Fig. 9(a-c): Vessel elements with reticulate lateral wall thickening showing Pa, I and XE, Fi, Pa, short, wide vessel element with long tails

Fi: Fibre, Pa: Parenchyma, Pe: Perforation, Ta: Tannin, VE: Vessel Element, XE: Xylem Element

Preliminary phytochemical screening: The percentage yield of hydro-alcoholic extract of *Vitex pinnata* Linn stem was found to be 28.46% with semi-solid masses in dark brown colour. The hydro-alcoholic extract was subjected to phytochemical screening which reveals the presence of various pharmacological active compounds such as

Table 3: Fluorescence analysis of the powdered drug under UV light

Reagents	Day light	UV light
Drug powder	Light brown	Dark brown
Drug powder+1 M NaOH	Orange-brown	Brownish black
Drug powder+alc. 1 M NaOH	Dark brown	Pale green
Drug powder+1 M HCl	Magenta brown	Brown
Drug powder+50% HNO_3	Magenta brown	Yellowish-brown
Drug powder+5% $Fecl_3$	Brownish black	Dark brown
Drug powder+80% H_2SO_4	Brown	Black
Drug powder+water	Light brown	Dark brown
Drug powder+conc. H_2SO_4	Dark brown	Black
Drug powder+ethanol	Light brown	Dark brown

Table 4: Preliminary phytochemical screening of stem of *Vitex pinnata* Linn

Plant constituents	Identification tests	Hydro alcoholic extract
Alkaloids	Mayer's test	-
	Hager's test	+
	Wagner's test	+
	Dragendorff's test	-
Carbohydrates	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
	Barfoed's test	-
	Seliwanoff's test	-
Glycosides	Borntrager's test	-
	Legal's test	-
	Keller killiani test	-
	Conc.H_2SO_4	-
Phenolic compounds and tannins	Ferric chloride test	+
	Lead acetate test	+
	Ellagic acid test	-
Protein and amino acids	Millon's test	+
	Ninhydrin test	-
	Biuret test	+
	Xanthoproteic test	-
Saponins	Foam test	+
Steroids and triterpenoids	Liebermann-burchard test	+
	Salkowski test	+
Fixed oils and fats	Spot test	-
	Saponification test	-
Flavonoids	Shinoda test	+
	Alkaline reagent test	+
	Conc.H_2SO_4	+
	Ferric chloride test	+
	Fluorescence test	+
Mucilage and gums	With 90% alcohol	-

+: Present, -: Absence

carbohydrates, alkaloids, phenolic compounds, tannins, proteins and amino acids, saponins, steroids, triterpenoids and flavonoids shown in Table 4 and revealed the presence and absence of hydro-alcoholic extract.

DISCUSSION

Macroscopic and microscopic evaluation is an indispensable tool for the identification of medicinal herbs and is one of the essential parameters in Ayurveda monograph. In this regard, the important macroscopic study is the morphological description of the stem parts which are seen by the naked eye or magnifying lens. The microscopical studies of stem part and powders of *Vitex pinnata* exposed the presence of cortex, periderm, sclerenchyma, secondary phloem, vessels, xylem fibres, xylem rays, collapsed phloem cells, phellem, sclereids, phloem ray, sieve element, prismatic calcium oxalate crystals and vessel elements, etc, each distinguishing character can be noted down, some of which are retained in the powder study also. Some of the chemicals which are used in obtaining clear sections are phloroglucinol, chloral hydrate, safranine, methyl orange,

etc[17-20]. The preliminary phytochemical analysis revealed the presence of carbohydrates, alkaloids, flavonoids, saponins, phenolic compounds, tannins, steroids, protein and amino acids. The parameters which are studied are moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values, etc. Ash values are used to determine the quality and purity of the crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate. The water-soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid-insoluble ash consists mainly of silica and indicates contamination with earthy material. The moisture content of drugs should be at a minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The crude drugs extracted in different solvents are tested for various phytoconstituents present in them by standard procedures[21,22]. They are generally tested for the presence of alkaloids, flavonoids, tannins, phenols, cardiac glycosides, triterpenes, steroids and saponins. Some constituents show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs[23,24].

The macroscopic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. Therefore, the macroscopic characters of *V. pinata* studied can serve as diagnostic parameters especially its organoleptic characteristics[25,26]. The organoleptic characteristics of the leaf were unique being bitter and greenish with an unpleasant odour. Microscopic evaluation is one of the simplest and cheapest methods to establish the correct and accurate identity for a plant drug[27].

The transverse section of the stem indicated that is periderm, cortex, sclerenchyma cylinder, secondary phloem and secondary xylem. When there are no data regarding the transverse section of the stem of the plant species of interest, Calcium oxalate crystals were observed and they may be involved in the dispersing of light to the chloroplasts in the photosynthetic parenchyma cells of the leaves. The crystals were thought to have a physiological role in sequestering excess calcium within plant cells[28]. Starch granules were observed and appeared oval. Although starch is the main form in which plants store carbon, it is sometimes converted into sugar by amyloplast when the plant needs energy[29]. Chemo-microscopic evaluation of the leaf showed proteins, starch, cellulose and tannins including calcium oxalate crystals and lignin's which are indications of the presence of alkaloids, flavonoids and glycosides in the leaves[30].

CONCLUSION

The present study is used to investigate the pharmacognostical, physical constants and preliminary phytochemical screening of *Vitex pinnata* Linn. stems provided useful information about its correct identification and evaluation. The macroscopical studies discovered the morphological character of different parts of the plant. The microscopical studies of stem part and powders of *Vitex pinnata* exposed the presence of cortex, periderm, sclerenchyma, secondary phloem, vessels, xylem fibres, xylem rays, collapsed phloem cells, phellem, sclereids, phloem ray, sieve element, prismatic calcium oxalate crystals and vessel elements, etc. Various physicochemical parameters such as ash values, extractive values, foreign organic matter, moisture content and fluorescence analysis were determined. Phytochemical screening is also useful to isolate the pharmacologically active secondary metabolites are present in the hydro-alcoholic extract of *Vitex pinnata.* The other parameters also observed, it is useful for the future identification of the plant and serves as a standard monograph for the identification and evaluation of this plant.

SIGNIFICANCE STATEMENTS

The information obtained with pharmacognostical, physicochemical and phytochemical studies could be used to identify *Vitex pinnata* and to certify the authenticity of commercial samples of this vegetal raw material. This study will help the researchers to uncover the critical areas for the compilation of a monograph and help in identifying this plant in its crude form and prevent it from adulteration and ensure its therapeutic efficacy plant from other Vitex species. Any crude drug which is demanded to be *Vitex pinnata* but whose characters knowingly deviate from the accepted standard above would then be prohibited as contaminated, adulterated or fake.

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