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Morpho-Anatomical studies on *Vitex Negundo* L.

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Abstract
The present study is used to investigate the pharmacognostical screening of *Vitexnegundo* provided useful information about its correct identity and evaluation. The macroscopical studies revealed the morphological character of different parts of plant. The microscopical studies revealed the presence of lamina, mesophyll tissue, venation pattern, anomocytic stomata, Midrib, xylem, phloem and glandular trichomes etc. Various physicochemical parameters such as ash values, extractive values, foreign organic matter, and moisture content were determined. The other parameters observed are also useful for the future identification of the plant and serves as a standard monograph for identification and evaluation of plant.

Keywords: *Vitex negundo*, macroscopical studies, microscopical studies, identification and evaluation

1. Introduction
People's lives from the past up to now have been impressed by plants. In 19th century, the developmental studies could complete the anatomical perusals. Plant development studies guide us to a better understanding of the functioning of cells. Studies of growth, differentiation and organogenesis are the developmental investigation aims (Maiti, 2012) [20]. Due to the diversity of plant species, plant anatomical and developmental information can be very valuable for the classification of herbs. A cellular and tissue structure researches is very important even for physiological and molecular investigations. Therefore, the anatomical and developmental studies have major roles to a better perception in plant genotypic and phenotypic researches (Ray, 2006) [28].

Vitexnegundo L. is a woody, aromatic shrub growing to a small tree. The extract from fresh leaves exhibit analgesic and antihistaminic properties (Prasanna *et al*., 2012) [25] and its aqueous and ethanolic extract from leaf show significant hypoglycaemic activity in alloxan induced diabetic rats. It has also been reported that the extract has mosquito repellent effects, antiparasitic (Shrishailappa *et al*., 2003) [30] and antimicrobial effects. (Rasia and Srivastava 1998; Ahmad *et al*., 1998) [27, 1]. Besides it is reported that the species shows HIV type1 reverse transcriptase inhibitory activity especially the water extract of the aerial parts of *Vitexnegundo* (Tandon and Gupta, 2005) [32]. Leaves possess antiulcerogenic, antiparasitic, hepatoprotective potentials, anti-inflammatory and antifungal activities (Sathiamoorthy *et al*., 2007; Dmayanthi *et al*., 1996) [29, 11].

Most of the species of the genus *Vitex* are used therapeutically in ancient Indian systems of medicine especially, Ayurveda and Siddha. The leaves of *vitex* are used in traditional medicine for relieving headache, fever and catarrah (Chanda, 1982) [7] and are also used for medicinal baths in fever and anaemia (Nandkarni, 1976) [22]. The major compound compound such as vitexin which is a flavanoid is mainly responsible for the anti-cancer activity. These dietary constituents mostly act as anti-oxidants and may prevent from DNA damage (Althafand Sudaroli, 2012) [3]. The roots and bark are astringent and the roots are reported to be used as a febrifuge (Alluri *et al*., 2009) [2].

Plant anatomy deals with the structure, contents and development of cells and tissues. It is of primary importance for all aspects of research in plant sciences such as morphogenesis, physiology, ecology, taxonomy, evolution, genetics, reproduction etc. (Fahn, 1900) [15]. The systematic anatomy is mainly aimed towards relating structure particularly of vegetative organs, to taxonomic classification of the plants in which the characters are exemplified. Accurate microscopical and macroscopical descriptions of the medicinal plants must be carried out to maintain standards of safety and quality and to authenticate the crude drug materials properly (Cutler, 1978) [8]. Most of the drugs that are extracted from leaves, barks, roots and rhizomes may be difficult to identify from their macroscopical appearance only; they must be complemented by microscopical characterization. The microscopical features of the medicinal plants were studied for different purposes. They may be studied to outline the diagnostic features; thus helping to identify them, to classify them using the anatomical characters and to distinguish between similar species to avoid adulteration (Eltahir and Ahlam, 2002) [14]. According to the world Health Organization the macroscopic and microscopic description of medicinal plants is the first step towards the establishing the identity and degree of purity of such materials and should be carried out before any tests are undertaken (WHO, 1998) [34].

According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (Anonymous, 1996) [4]. Correct botanical identity based on the external morphology is possible when a complete plant

specimen is available. Anatomical characters can also help the identification when morphological features are indistinct (David *et al*., 2008) [9].

2. Materials and Methods

Leaf, stem and petiole of the *Vitexnegundo* were collected from Chennai, Teynampet and the plant specimen was botanically identified and authenticated by comparing the herbarium specimen.

Extraction

All the plant materials obtained were shade dried, made into coarse powder and passed through sieve#40, were successively extracted with Hexane, Ethyl acetate and Ethanol by Soxhlet extraction method (Fig-3.01).

Procedure

Few fully matured leaves, petioles and stems were preserved in fixative solution FAA (Formalin-5ml + Acetic acid5ml + 70% Ethyl alcohol-90ml) for more than 48 hours. The preserved specimens were cut into thin transverse section using sharp blade. The free hand sections were stained with safranine, Aniline blue, Eosin Y as per standard methodology. The selected diagnostic characters of the transverse section were photographed under suitable magnification using camera. The transverse sections of the stems, petioles and fresh leaves through the midrib were also cleared, mounted and observed under fluorescent microscope.

3. Result and discussion

Leaf In cross section Sectional view the leaf exhibits thick midrib and thin lamina with ridged adaxial side (Fig-1.1). The midrib is more or less flat on the adaxial side and proximately projecting into semi-circular abaxial part. The midrib is 650 µM thick and 700 µM wide. The epidermal layer of the midrib is thin and thick walled (Fig-1.2). The ground tissue consists of small, angular, thin walled and compact parenchyma cells. Some of the cells are filled with dark tissues.

The vascular system is a wide bowl shaped strand with the concavity facing the adaxial side. The vascular arc is 80µM thick. It consists of several parallel short lines xylem elements and a thin layer of phloem on the abaxial part of the xylem is (Fig- 1.2, 3). The xylem lines consist of about five circular, thick walled xylem cells; metaxylem cells are at lower end and the protoxylem elements are towards the upper side. The metaxylem elements are 20µM wide. The phloem elements are narrow, angular in outline and occur in short vertical lines mixed with phloem parenchyma cells (Fig-1.3).

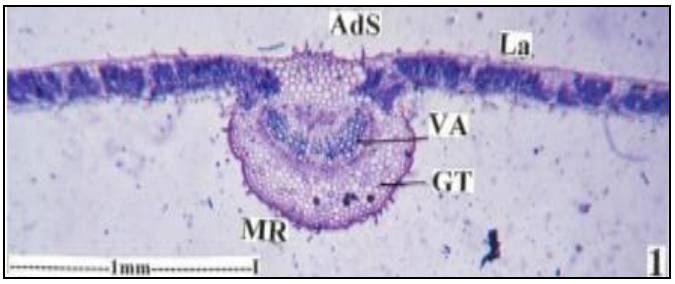

Fig 1.1

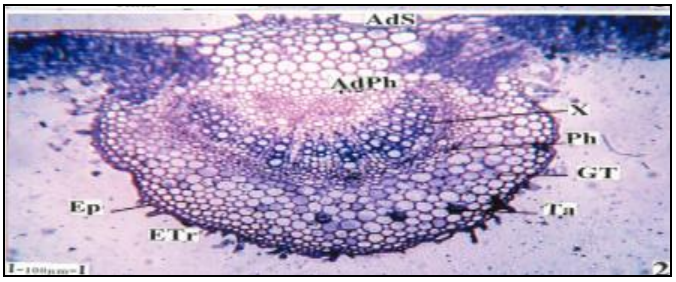

Fig 1.2

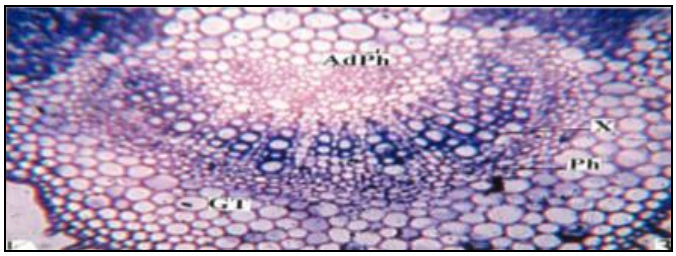

Fig 1.3

Lamina (Fig-2.1):- The lamina is smooth and even on the adaxial side and the abaxial side has prominent ridges and furrows. The abaxial surface has dense multicellular thin walled trichomes; theadaxial surface has sparsely distributed short, dagger shaped thick walled trichomes (Fig- 2.1, 2;4.2). The adaxial surface has thick smooth cuticle and wide rectangular or cylindrical epidermal cells are smaller thin walled and cylindrical.

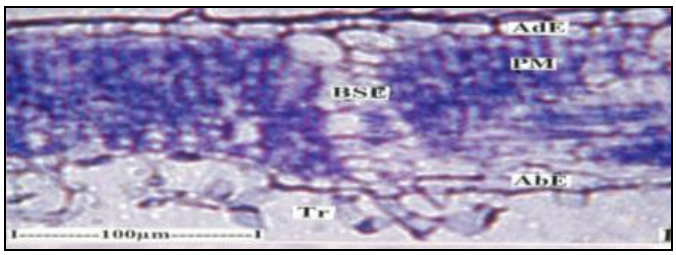

Fig 2.1

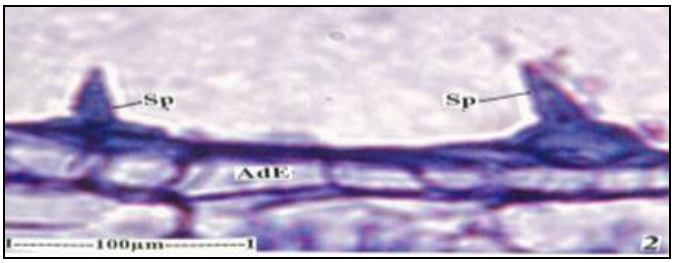

Fig 2.2

Stomata: Stomata occur on the abaxial epidermis. The stomata are broadly elliptical with prominent slit shaped stomata pores (Fig-3.1, 2). The stomata are 35x45µM in size. The abaxial epidermal cells are thin walled and slightly wavy. The epidermal cells have wide circular markings these markings have thick walls and prominent nucleus. The abaxial epidermal multicellular, uniseriate unbranched trichomes arise from the cuticular markings (Fig 3.2).

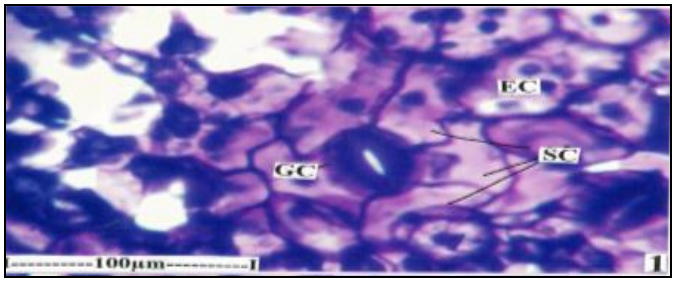

Fig 3.1

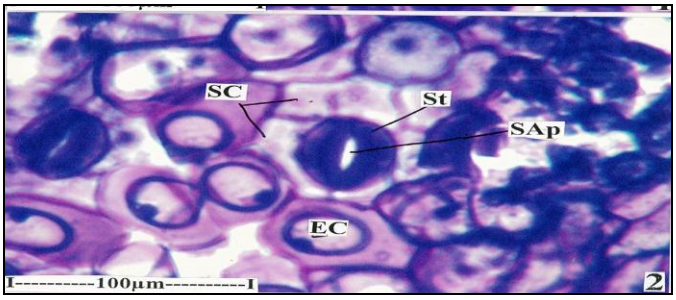

Fig 3.2

Adaxial epidermis (Fig-4.1). The adaxial epidermis is apostomatic. The epidermal cells are polygonal with straight thick anticlinal walls on the surface walls of the epidermal cells there are narrow circular markings where the trichomes were attached on the epidermis. These trichomes are unicellular unbranched broader at the base tapering and pointed at tip (Fig 4.2). These trichomes are150µM long and 12 µM thick.

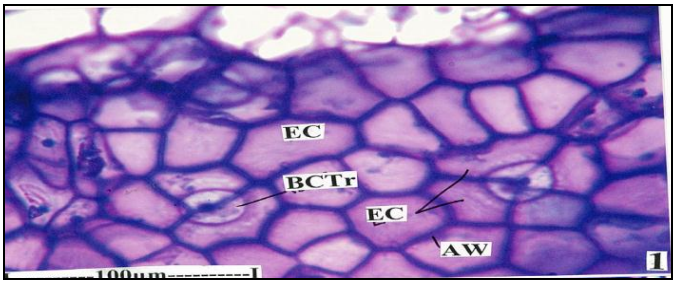

Fig 4.1

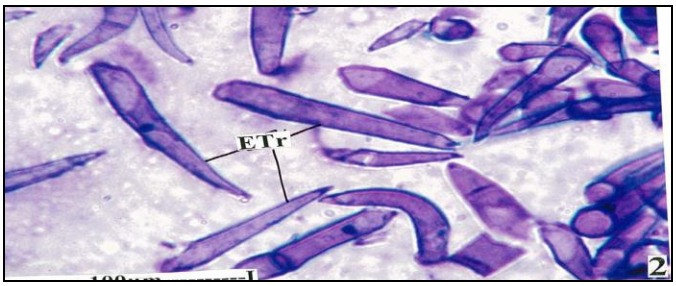

Fig 4.2

Petiole (5.1; 6.1):- Both distal part and proximal part of the petiole were studied. The distal part of the petiole was studied. The distal part of the petiole is circular is sectional outline with two thick spherical wings, one on either side of the petiole (Fig 5.1).

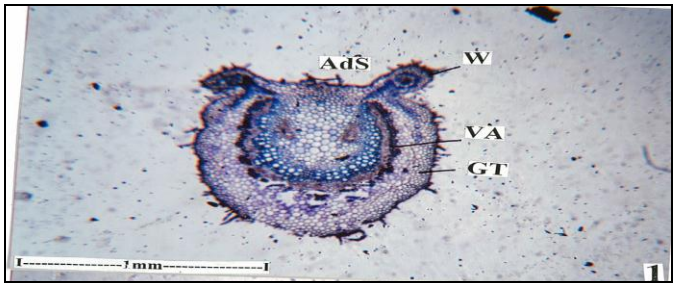

Fig 5.1

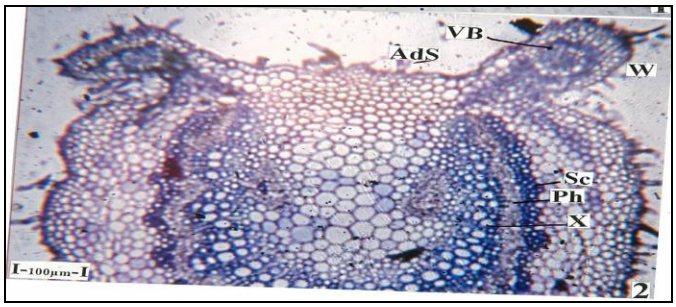

Fig 5.2

The petiole is 1 mm thick. The wings are 200µM long and 150µM thick. The petiole consists of three single layer of epidermis: the epidermal cells are small and darkly stained. The ground tissue includes about four layers of small thick walled compact cells. The remaining ground tissue is the outer and central portions consists of wider, polygonal, angular thin walled compact parenchyma cells (Fig- 5.2).

The Vascular bundle in the wing of the petiole is single, circular with control core of xylem, surrounded thin layer of phloem and a single layer parenchymatous bundle sheath. The vascular system of the petiole is in the form of deep and wide bowl shaped xylem, phloem strands. The strands have inner layer of short parallel lines three to five xylem elements. The xylem cells are circular or angular, thick walled with narrow cell lumen. Along the outer part of the xylem strand occurs a prominent continuous layer of sclerenchyma elements abutting the phloem (Fig- 5.2).

Proximal petiole (Fig- 6.1, 2): The proximal part of the petiole is 1.8 mm thick It is circular with even and smooth outline except adaxial part which somewhat flat (Fig- 6.2). Inner to the epidermis, there are two or three layers of small thick walled cells and the cells gradually become larger thin walled angular while reaching central part of the petiole. The vascular system includes a wide and shallow bone shape outline with small arc of vascular strands located at the adaxial ends of the main vascular strand (Fig 6.1). The vascular strands are collateral with inner xylem elements and outer layer of phloem elements (Fig- 6.2; 7.1, 2). The xylem elements are in compact parallel lines with four to seven cells in each row. The xylem cells are circular and thick walled. The meta xylem elements are 20µM wide. There is an outer layer continuous cylinder of phloem en sheathing the xylem layer. The phloem includes short rows of sieve elements and phloem parenchyma. Inner to the xylem strand there are a few discrete circular masses medullary phloem or inner phloem. In the central core of the medullary phloem there are group of

xylem elements (Fig 7.2). On the outer part of the vascular are, there are several independent clusters of fibres adjacent to the outer phloem layer (Fig-6.2).

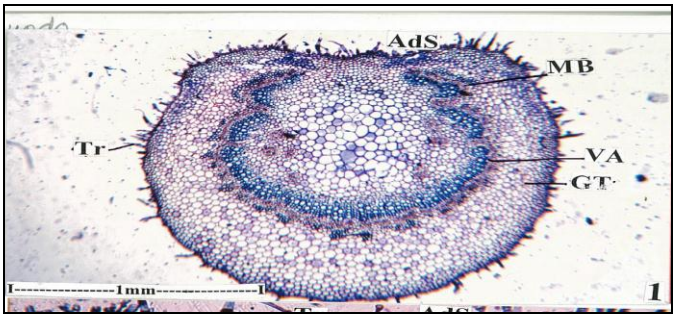

Fig 6.1

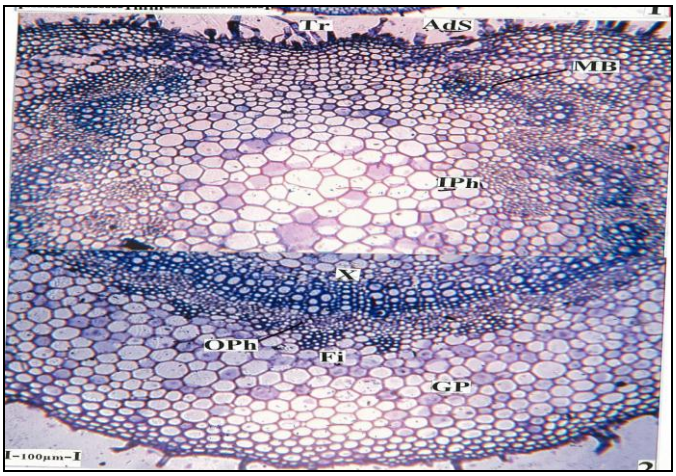

Fig 6.2

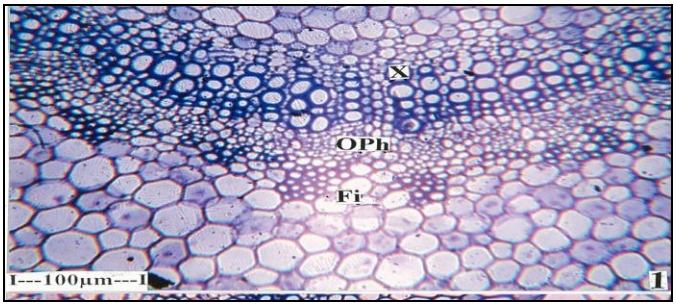

Fig 7.1

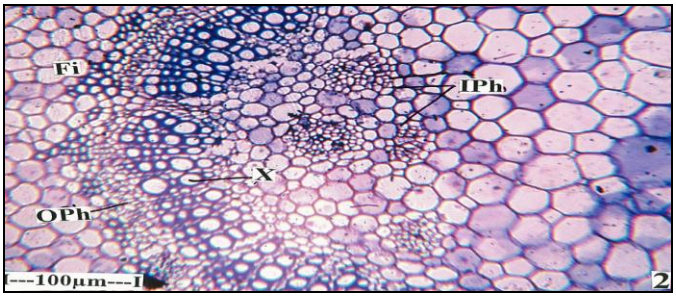

Fig 7.2

Venation pattern of the Lamina

The venation of the lamina is densely reticulate. The veins become gradually thin starting from the midrib and ending in the vein lets (Fig- 8.1). These are dense vein-islets of various shape and size. The vein islets are ultimate area surrounded by the vein lets. Within the vein-islets, there is vein- termination which represent the final endings of the small vein lets. The vein- terminations are either simple and un branched: they may be forked on the (Fig-8.3) or they branch more than once (Fig- 8.2). The vein terminatious is long and slightly wavy.

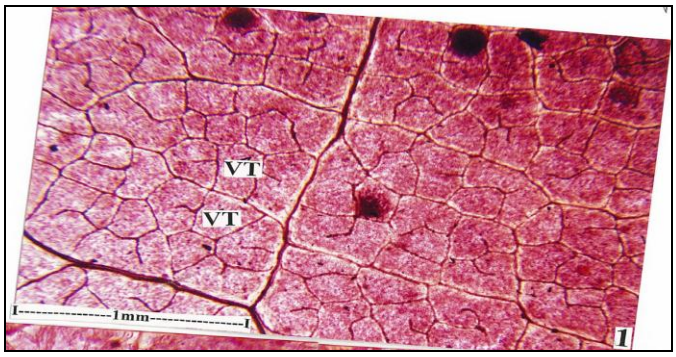

Fig 8.1

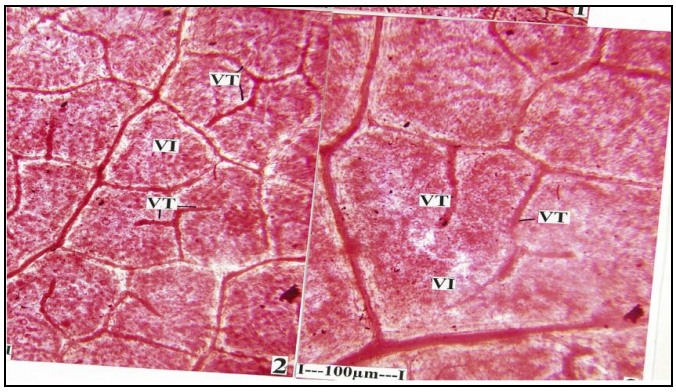

Fig 8.2

Stem

The stem is circular in sectional view with smooth and even surface. The stem is 5.6 mm thick. It consists of thick periderm, fairly wide cortex, a thin layer of cortical sclerenchyma, secondary phloem and hollow cylinder of secondary xylem with central cortex (Fig-9.1).

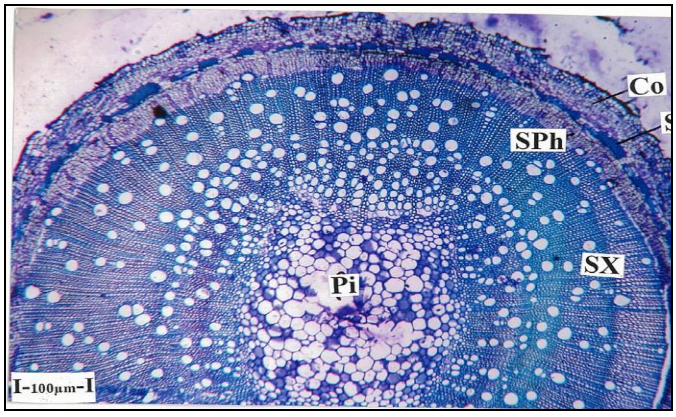

Fig 9.1

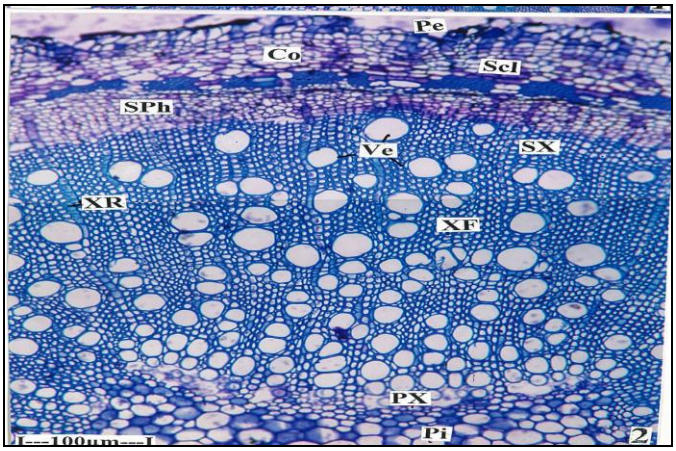

Fig 9.2

The epidermal layer is disintegrated and replaced by the epiderm, is located two or three layers inner to the cortex. It consists of four layers of rectangular thin walled and suberized phellem cells (Fig-10.1). The cortical zone is about five layered. The cells are angular or elliptical, thin walled and parenchymatous (Fig-9.2; 10.1). The inner boundary of the cortex includes a discontinuous layer of three or four layers of sclerenchyma cells, which are angular thick walled and lignified (Fig-10.2).

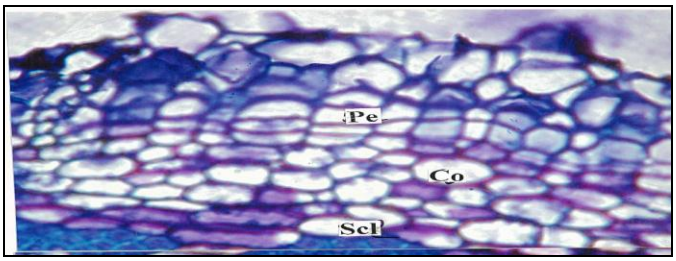

Fig 10.1

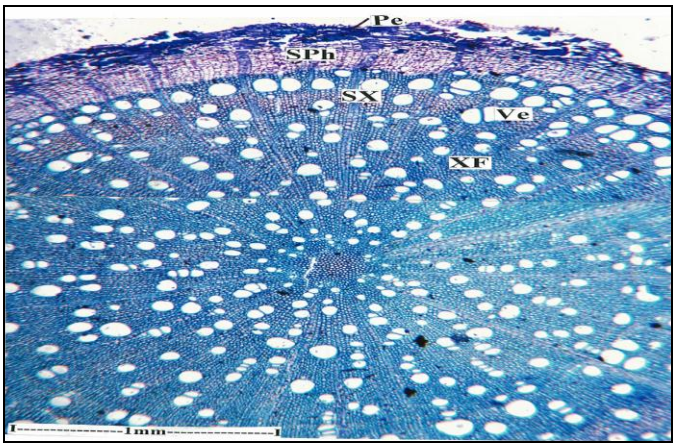

Fig 10.2

The vascular cylinder has thick continuous layer of secondary phloem enclosing the secondary xylem (Fig-10.2). Secondary phloem consists of wide angular, thick walled sieve elements with companion cells located on the lateral side. Phloem parenchyma cells are comparatively smaller and thin walled. Phloem rays are uniseriate and straight (Fig-10.2).

Secondary xylem consists of vessels and xylem fibres. The vessels are mostly solitary and diffuse in distribution (Fig-9.2). Both wide and narrow vessels are randomly mixed. They are circular and thin walled. Both wide and narrow vessels are randomly mixed. They are circular and thin walled.

The diameters of the vessels range from15 to 30 µM- xylem fibres are angular very thick walled with narrow cell lumen. The fibres are in compact radial lines. Xylem rays are narrow with the radially elongated, thick walled lignified cells. Along the inner boundary of the xylem cylinder occurs the primary xylem. It consists of one or two wide metaxylem elements and protoxylem lacuna

Root

The root shows circular outline with shallow fissures on the surface (Fig-11). Periderm and cortical tissue are not clearly visible. Secondary phloem is wide and continuous all around the xylem cylinder. The secondary phloem is 150 µM thick. It consists of small rectangular cells arranged radial files; the companion cells are seen along the lateral part of the cell wall (Fig-13.1). Phloem rays are prominent and darkly stained. They are one or two cell wide. The cells are horizontally elongated and rectangular in shape.

Secondary xylem (Fig-11; 12).

The Secondary xylem is a thick solid and dense cylinder measuring 1.8 radius. There is narrow core of pith in the centre. The vessels are dense and are distributed in numerous radial lines or in solitary condition (Fig-11). The xylem includes both narrow and wide vessels. The vessels are mostly circular in outline or less frequently elliptical. The walls of the vessels are thin (Fig-12:13). The narrow vessels are 30µM in diameter: the wide vessels are 150µM in diameter. The xylem fibres polygonal in outline, thick walled and lignified. Starch grains are densely filled in some of the fibres. Xylem rays are not evident.

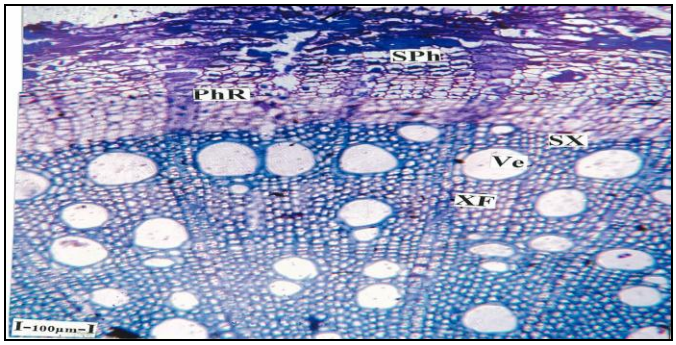

Fig 11

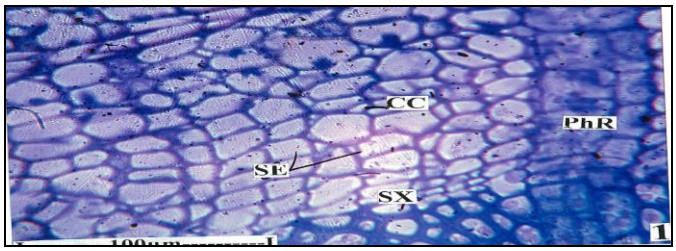

Fig 12

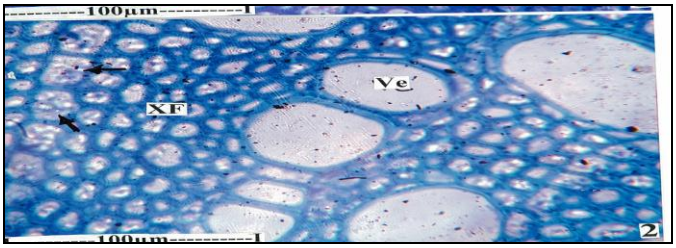

Fig 13

Crystals

Calcium oxalate crystals are abundant in the leaf and less so in the stem. In the leaf druses type of crystals occurs all along the veins in continuous vertical line (Fig-14.1). The druses are spherical spiny bodies formed by aggregation of minute triangular crystals. In the stem crystals occur in the pith parenchyma cells. The crystals core druses or occasionally prismatic type (Fig-14.2).

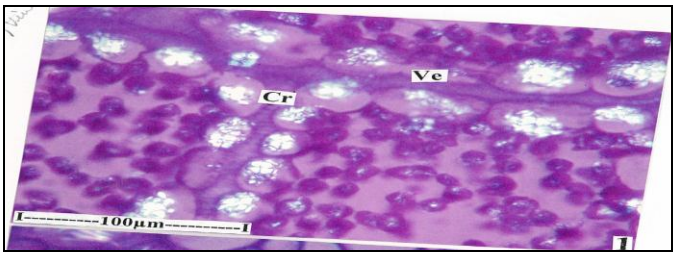

Fig 14

Anatomical study of medicinal plants is significant in pharmacognosy and to prevent adulteration as well as evolve the specific parameters for authenticity and quality control of raw drugs (Bernerjee and Mukherjee, 2001; Gupta *et al*., 2001) [5, 17]. Earlier contributors to similar studies include Edeoga (1991) [12], Ugboroghoet *al*. (1992), Obute and Omotayo (1999) [23], Edeoga and Eboka (2000) [13], Idu*et al*. (2000), Gill and Mensah (2001) [16]. Studies on the morphology of *B. diffusa* have been done by Bhargava (1932), Maheshwari (1930) [19] and anatomical studies by researchers like Metcalf and Chalk (1983) [21], Pant and Mehra, (1961) [24], and Rajput, Rao, (1998) [26]. Anatomical Studies on *Vitex Leucoxylon* and *Vitex Negundo* (Verbenaceae) was reported by Silvy*et al*., 2014.

The present study is used to investigate the pharmacognostical screening of *Vitexnegundo* provided useful information about its correct identity and evaluation. The macroscopical studies revealed the morphological character of different parts of plant. The microscopical studies revealed the presence of lamina, mesophyll tissue, venation pattern, anomocytic stomata, Midrib, xylem, phloem and glandular trichomes etc. Various physicochemical parameters such as ash values, extractive values, foreign organic matter, and moisture content were determined. The other parameters observed are also useful for the future identification of the plant and serves as a standard monograph for identification and evaluation of plant.

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