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Anatomical Studies of Naravelia Zeylanica

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Abstract: Plants are the principal source of raw materials for many plant based medicine since ancient times. The traditional herbal medicines are receiving great importance in the health care sector especially in Indian system of medicine *i.e,* ayurveda, one of the plant *Naravelia zeylanica* (Linn) DC belonging to family Ranunculaceae has been used in the treatment of many diseases. The present study deals with the morphology and anatomy of leaf and stem. The cross and surface sections of the leaf and stem were examined.

Key words: Anatomy · Xylem · Stomata

INTRODUCTION

Narvelia zeylanica DC (Ranunculaceae) is a climbing shrub with tuberous roots; wiry stem strong tendrils, leaves 3- foliate, opposite, terminal leaf let modified into a 3 branched tendrils, leaf lets ovate, lanceolate, serrate or crenate, prominently nerved; flowers yellow, fragrant, in axillary and terminal panicles, sepals downy, petals linear- clavate, elongate; fruit aggregate of achiness, ending in twisted feathery tales. The plant is available rich in all around South India [1]. Plant is reported to contain mainly alkaloids, flavonoids, saponins and tannins. Methanolic extract of leaves of *Narvelia zeylanica* berberine is present, sterols are also present in the ethanolic stem and leaf [2]. The Indian system of medicine Ayurvedha the plant Naravelia zeylanica (Linn.) DC belonging to the family ranunculaceae has been used in the treatment of pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers [3, 4]. The stems are reported to be used as tooth sticks to cure toothaches [5]. Root extract is used to treat headache [6]. 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 it should be carried out before any tests are undertaken [7, 8]. 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 [8, 9].

Anatomical studies on the medicinal plants lay behind other branches. Many popular and useful medicinal plants have not been studied for the anatomical aspects. Microscopic investigations become essential when identification of a fragmentary material has to be carried out. Hence, the present study was undertaken to standardize the anatomical features of leaf and stem analysis to serve as a possible tool for proper identification of *Naravelia zeylanica.*

MATERIALS AND METHODS

Specimens Collection: The plant specimen for the proposed study was collected from Kolli Hills. Care was taken to select healthy plants and normal organs. The required samples of different organs were removed from the plant and fixed in FAA (Formalin -5ml + Acetic Acid -5ml + 70% Ethyl alcohol -90ml). After 24 hours of fixing, the specimens dehydrated with graded series of tertiary –Butyl alcohol [10]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

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Sectioning: The paraffin embedded specimens were sectioned with the help of rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was done as per the procedure [11]. The sections were stained with Toluidine blue as per the method published by O'Brien [12]. Since Toluidine blue is a polychromatic stain. The staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc where ever necessary sections were also stained with safranin and Fast –green and KI (for starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared.Glycerine mounted temporary preparations were made for cleared materials with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured [13, 14, 15].

Photomicrographs: Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was used. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale – bars [16, 17].

RESULT AND DISCUSSION

Leaf: The leaf consists of thick midrib and thin lamina. The midrib hangs down on the abaxial side of the lamina (Figure 1). It is planoconvex with flat adaxial side and prominently projecting abaxial part. It is 1mm thick and 1mm wide. It consists of thick epidermal layers comprising squarish, thick walled cells with prominent cuticle. The ground tissue on the abaxial side is parenchymatous the cells being wide, angular, fairly thick walled and compact (Figure 1.1, 2.1 and 2.2). The cells towards the adaxial side of the midrib are collenchymatous with thick walls. The vascular system is multistranded. There is a large, elliptical, central bundle which is collateral with, wide cluster of circular thick walled xylem elements and semicircular mass of phloem elements on the lower side. There is a thick arc of sclerenchyma elements abutting the phloem (Figure 2 and 3). Apart from the median main bundle there are smaller, accessory bundles in the adaxial portion of midrib. These bundles vary in size and are mostly collateral with conical segment of wide, xylem elements and thick mass of phloem elements (Figure 4). The lateral vein is also fairly prominent and planoconvex in sectional view. It consists of large, epidermal cells with thick walls. There is a single vascular bundle which is elliptical in outline. It consists of small, cluster of xylem elements and a few phloem elements. The vascular strand is surrounded by 2 layers of large, thick walled, bundle sheath cells which extend towards the adaxial epidermis. The lateral vein is 400µm thick and 350 µm wide (Figure 1.2).

Lamina: The lamina is 150 µm thick. It is bilateral with differentiation of adaxial and abaxial sides. The adaxial epidermis consists of thick, cylindrical cells measuring 20 µm thick. The abaxial epidermis includes circular or spindle shaped cells and the epidermis is stomatiferous. The stomata have prominently beaked guard cells (Fig. 3.1 and 3.2). The mesophyll is differentiated into short, adaxial band of compact, cylindrical palisade cells and abaxial zone of 3 or 4 layers of large, lobed cells (Fig. 3.1). Small, lateral veins with few xylem elements and wide bundle sheath cells are seen in the mesophyll tissue. Small fibers bundles are also seen in the mesophyll tissue (Fig. 3.2).

Epidermal Tissue: The epidermal tissue was studied in paradermal sections. The adaxial epidermis is apostomatic (without stomata). The epidermal cells are thin walled with highly wavy antidixal walls and the cells appear amoeboid in outline (Figure 4.1). The abaxial epidermis is densely stomatiferous. The stomata are Anomocytic type. The epidermal cells are wider, highly lobed and the cells are amoeboid. The stomata are elliptical with wide stomatal pore (Figure 4.2 and 4.3).

Venation Pattern: The venation system is densely reticulate. The veins and vein-lets are thick (Figure 5.1). The vein Islets are rectangular or polygonal in outline. The vein boundaries of the Islets are thick and straight. The terminations are well developed. They are mostly repeatedly branched and tendroid in outline (Figure 5.2). Simple, unbranched, short or long vein terminations are also seen (Figure 5.2).

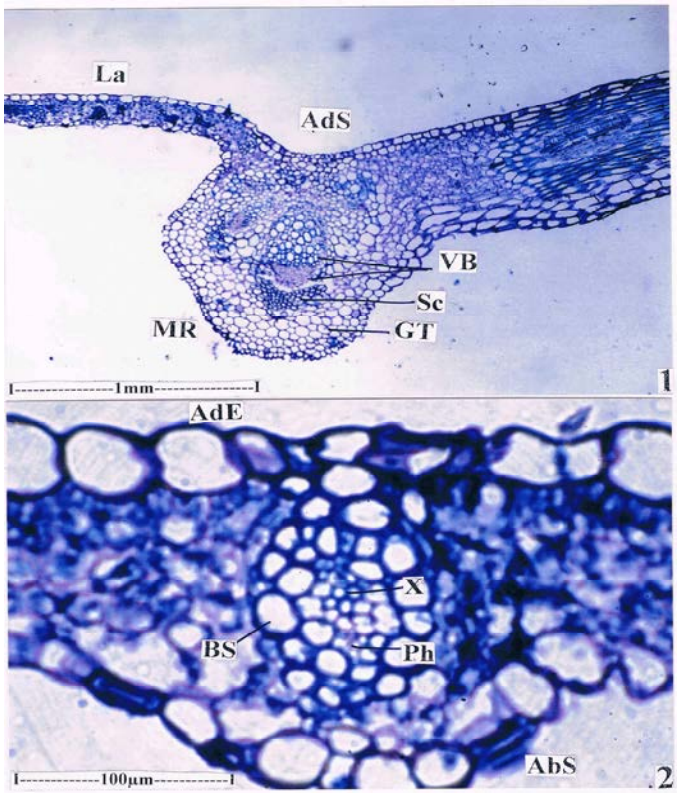


Fig. 1.1: T.S. of leaf through midrib.
1.2: T.S. of leaf through lateral vein.
(AbS-Abaxial Side, AdE- Adaxial Epidermies, AdS –Adaxial Side, BS-Bundle Sheath, GT-Ground Tissue, La Lamina, MR-Midrib, Ph-Phloem, Sc- Sclerenchyma, VB-Vascular Bundle, X-Xylem).

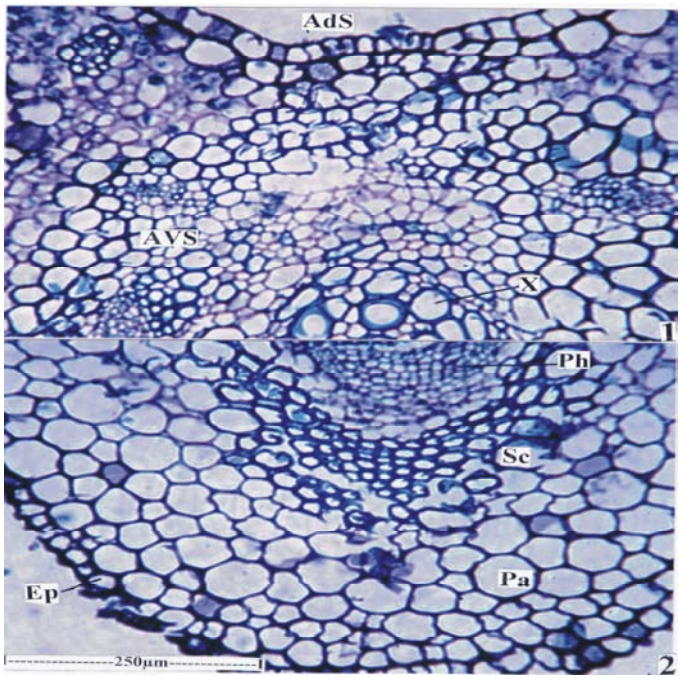


Fig. 2.1 & 2.2: T.S of mid-rib-Enlarged
(AdS –Adaxial Side, AVS- Adaxial Vascular strand,Pa-Parenchyma,Ph-Phloem, Sc- Sclerenchyma, X-Xylem).

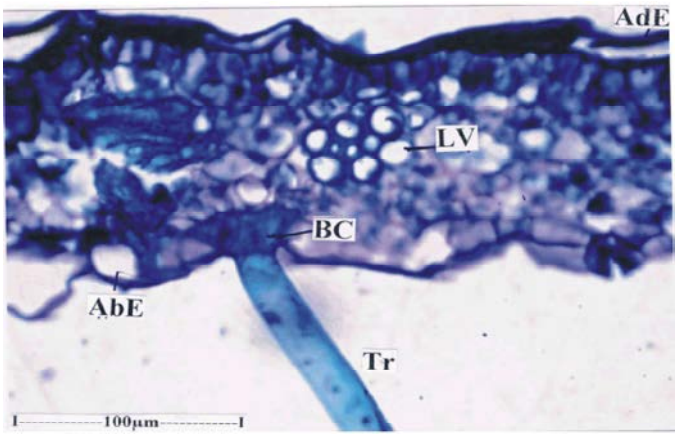


Fig. 3.1: T.S of Lamina

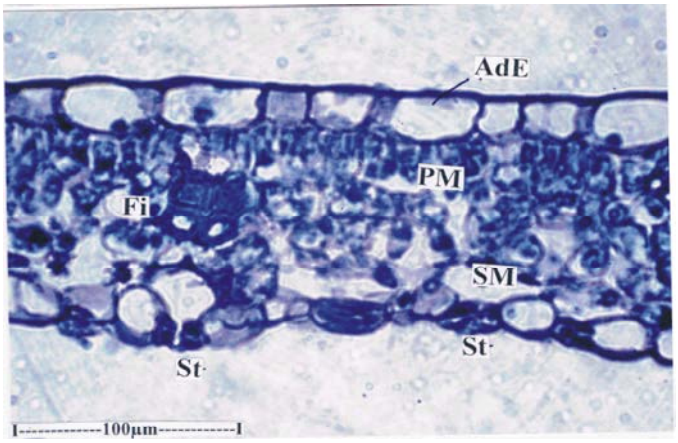


Fig. 3.2: T.S of Lamina Showing Abaxial stomata.
(AbE-Abaxial Epidermies, AdE-Adaxial Epidermies,BC-Base cells, Fi-Fibre Bundle,PM- Palisade Mesophyll,SM-Spongy mesophyll,St-Stomata,Tr-Trichome).

Petiole: The petiole is roughly cordate in outline with wide, shallow adaxial grove (Figure 6.1). The wings on either side of the groove are short and wide (Figure 6.1). The petiole is 2.2 mm in horizontal plane and 1.6 mm vertical plane. The petiole has thin epidermal layer of small, thin walled cells. The ground tissue is homogenous parenchymatous, thin walled, angular and compact. The vascular system is multistranded. There is a ring of about 8 vascular bundles with wide gaps in between. The bundles are collateral and variable in size. The bundles can be categorized into a largest abaxial bundle with two smaller adjacent bundles.

On the adaxial side are seen a smaller bundle beneath the adaxial groove and 1 or 2 bundles on either side of the groove (Figure 6.2). The vascular bundles have wide, angular fairly thick walled cluster of xylem elements and conical cap of phloem elements (Figure 7). The vascular bundles do not possess any sclerenchymatous cap.

Stem: The stem is circular with even outline (Figure 8.1). It is 2.3 mm in diameter. It consists of a thin epidermal layer and narrow, parenchymatous cortex. The vascular bundle is numerous and they occur towards the peripheral zone of the ground tissue. The bundles are V-shaped or triangular in outline (Figure 8.2). The bundles have wide, circular, thick walled meta xylem elements and narrow, angular, thick walled protoxylem elements. The xylem elements are surrounded by thick walled xylem fibers. Phloem occurs on the outer, concave part of the vascular bundle (Figure 8.2 and 8.3). The major centre part of the stem is wide pith. The pith is homogenous with circular thin walled, less compact, parenchyma cells. The metaxylem elements are 120 µm wide.

Powder Microscopy: The stem powder includes vessel elements, parenchyma cells and fibres:

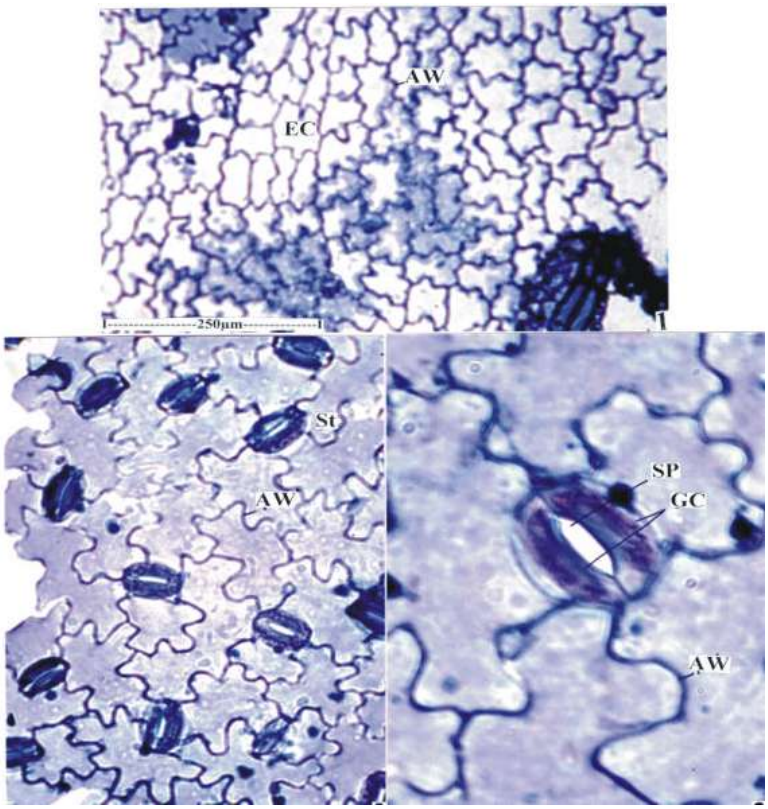


Fig. 4.1: Paradermal section of the Adaxial Epidermies.
4.2: Paradermal section of the Abaxial Epidermies showing stomata
4.3: One stomata enlarged.
(AW-Anticlinal Wall, EC-Epidermal cell, GC-Guard cell, SP-Stomatal pore, St-Stomata).

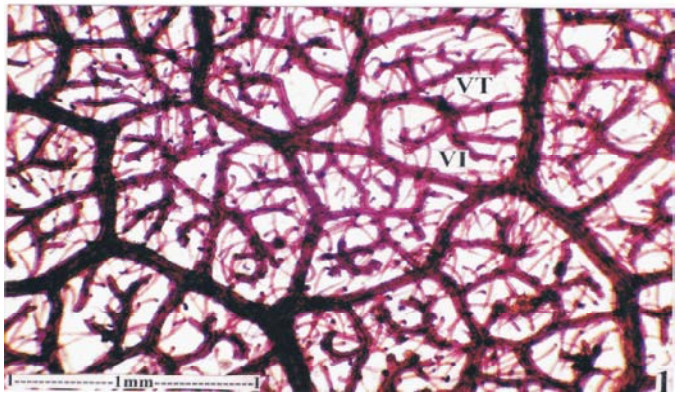


Fig. 5.1: Venation pattern of the lamina.
(Tr-Trichome, VI-Vein Islet, VT-Vein Termination).

Vessel Elements: The vessels elements are long, narrow and cylindrical (Figure 9.1, 9.2 and 9.3). They are 400-430 µm long. They have simple, circular perforations at the end walls. On the lateral walls they are dense, multiseriate, horizontally elongated and circular bordered pits (Figure. 9.3).

Parenchyma Cells: Wide, elongated, thin walled parenchyma cells are common in the powder. These parenchyma cells are wide or narrow. The wide, rectangular parenchyma cells are 160µm long and 40µm wide. The parenchyma cells have minute simple pits (Fig. 10.1).

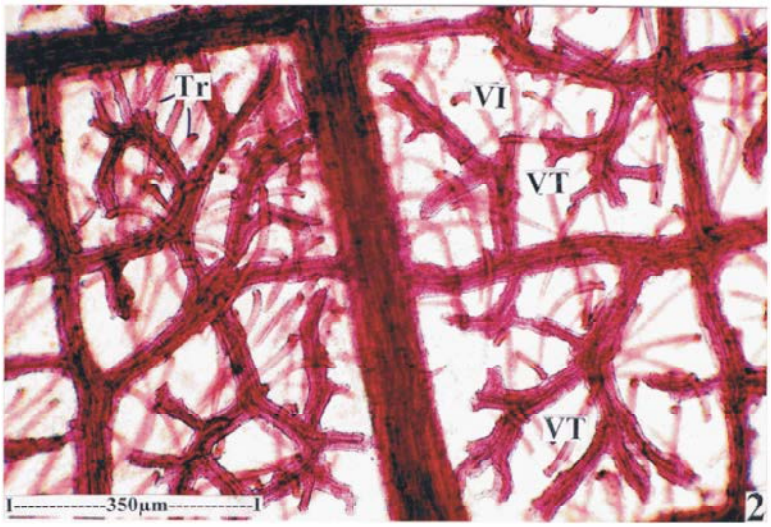
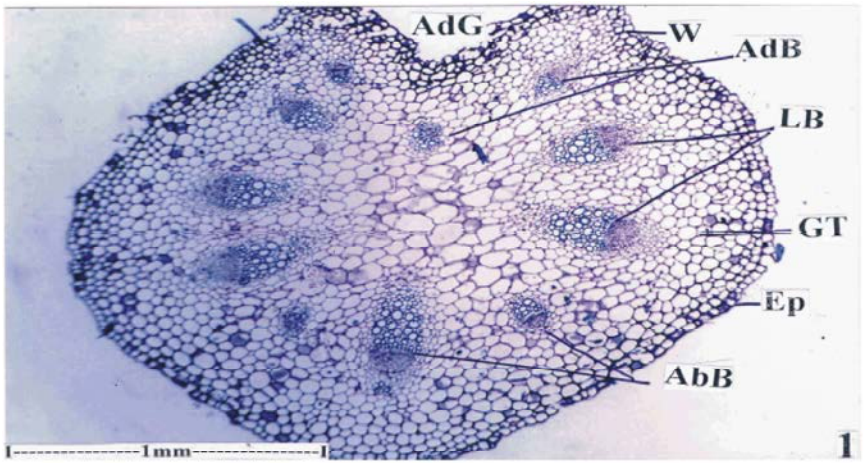


Fig. 5.2: Vein Islet and vein termination – Enlarged.



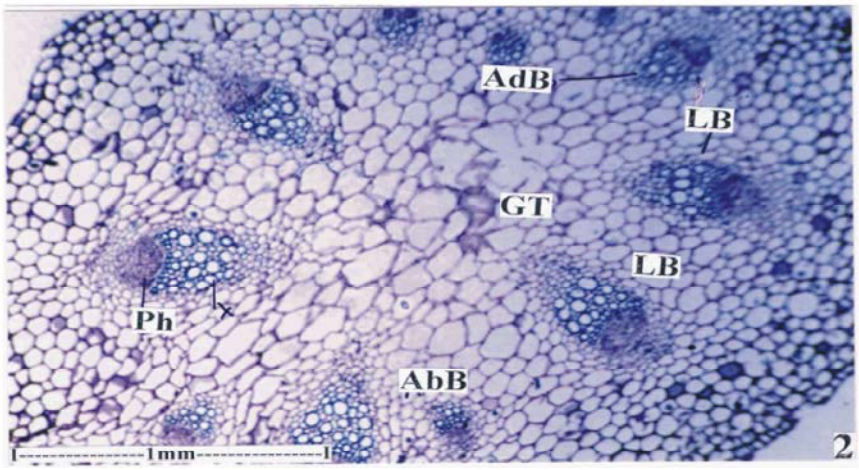


Fig. 6.1: T.S. of petiole-entire view.

6.2: T.S. of Petiole Distribution of Vascular Strands.

(AbB –Abaxial bundle, AdB –Adaxial bundle, AdG- Adaxial Groove, Ep-Epidermies, GT-ground tissue, LB Lateral bundle, Ph-Phloem, W-wing, X-Xylem).

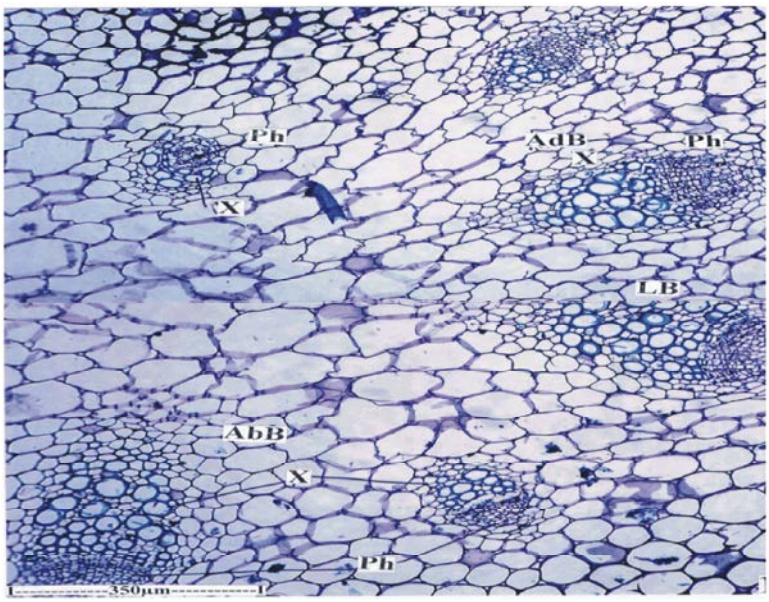


Fig. 7: Vascular strands of the petiole-enlarged.
(AbB –Abaxial bundle, AdB –Adaxial bundle, LB - Lateral bundle, Ph-Phloem, X-Xylem).

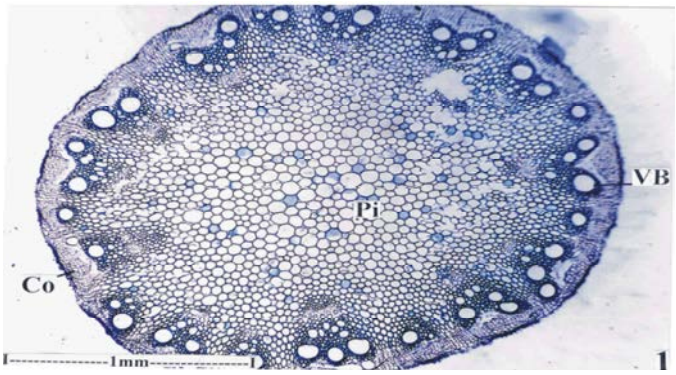


Fig. 8.1: T.S. of Stem (Entire view).

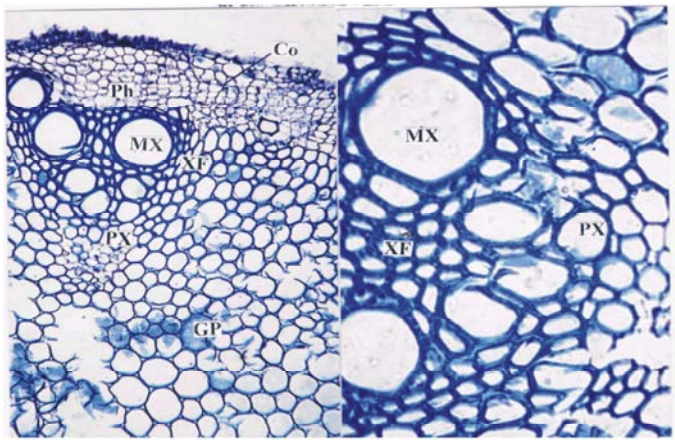


Fig. 8.2: A sector enlarged.
Fig. 8.3: Xylem strand enlarged.
(Co-Collenchyma, Gp- Ground parenchyma, Pi-pith, MX-Metaxylem, Ph-Phloem, PX-Proto Xylem, VB-Vascular bundle, XF-Xylem fibre).

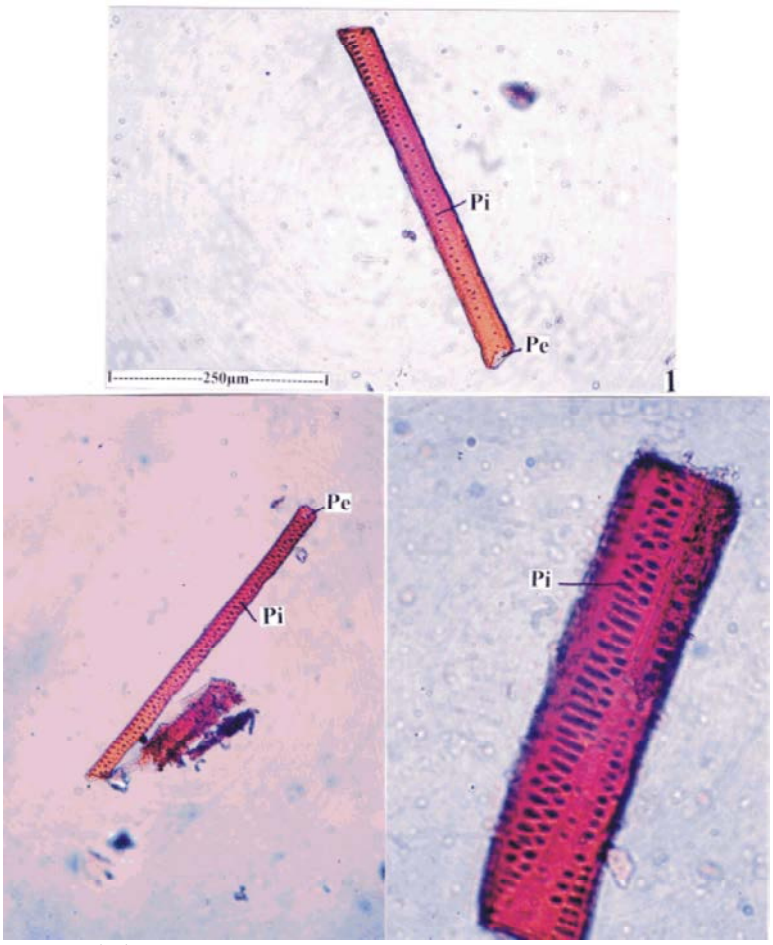


Fig. 9.1 and 9.2: Narrow vessel elements.
Fig. 9.3: Vessel element showing lateral wall pits
(Pe-Perforations, Pi-Pits).

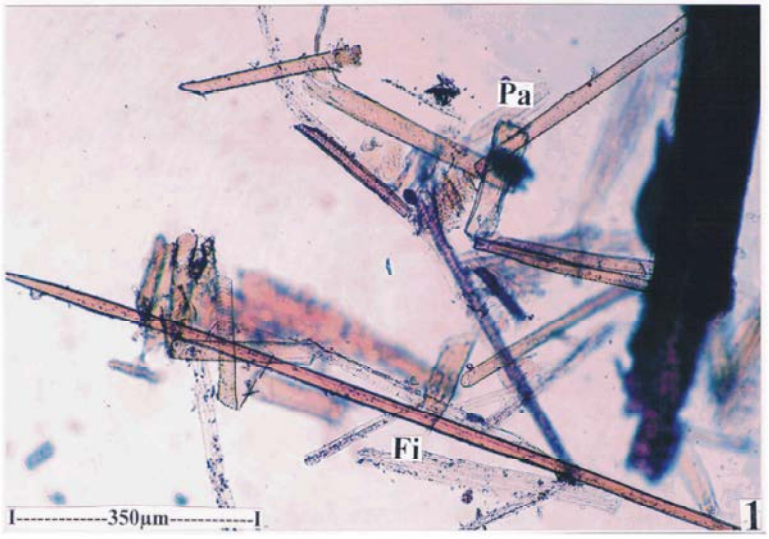


Fig. 10.1: Fibres and parenchyma cells in the powder.
(Fi- Fibres, Pa- Parenchyma).

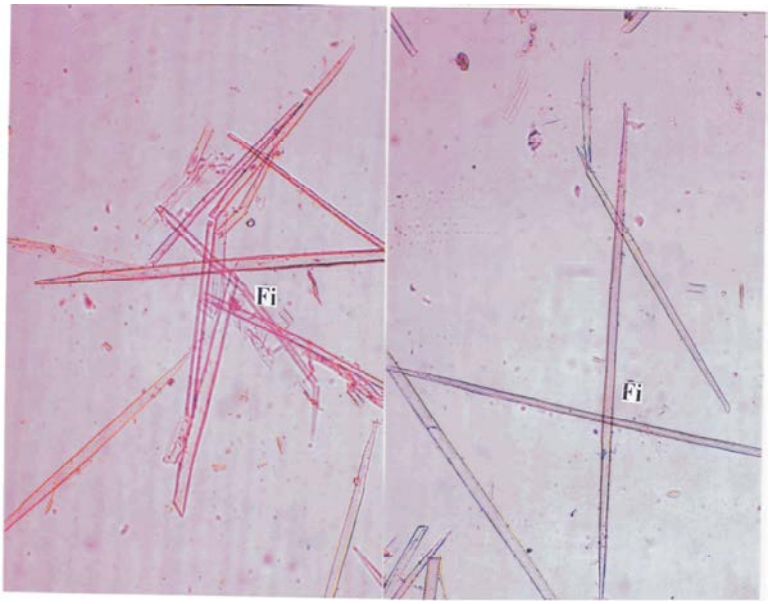


Fig. 10.2: Wide fibres.
Fig. 10.3: Narrow fibres.
(Fi- Fibres, Pa- Parenchyma).

Fibres: Fibres are abundant in the powder. The fibres are of two types. Some are narrow, longer and thick walled. These narrow fibres are 750 µm long and 10µm thick. The second type of fibres is wide fibres. They are comparatively thin walled with wide lumen. They are less than 700µm long and 20µm wide (Figure 10.2 and 10.3). Pits are not evident on the walls of the fibres.

CONCLUSION

Naravelia zeylanica widely used in Indian system of medicine has tremendous medicinal values owing to its biological functions. However, there are no detailed anatomical studies on this plant to help in the proper identification.This study provides referential botanical information for correct identification of these plants.

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