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Detailed pharmacognostical studies on Apocyanaceae family root from wild source of South India (Andhra Pradesh)

Deverakonda Ramadevi, Pachaiyappan Jayaram, Battu Ganga Rao, Neerugatti Dora Babu and Rayi Radha

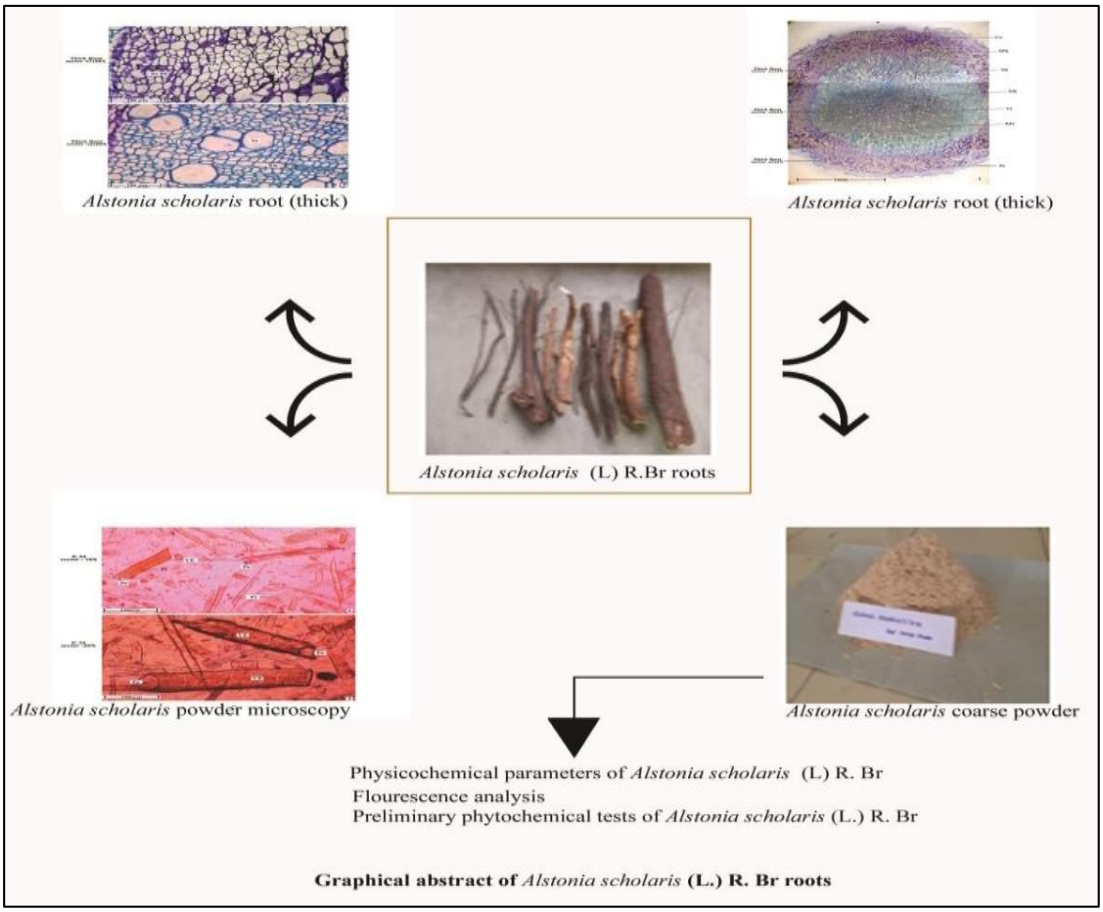
Abstract
Alstonia scholaris L R. Br plant is a tremendous powerful tropical and subtropical latex plant. There are more than 250 genera and 2500 plant species are available in apocyanaceae family. *Alstonia scholaris* (L) R. Br. (Apocyanacea) Leaf, root, fruit and flowers are useful for various conditions of ailments in traditional system of medicine since ancient times. *Alstonia scholaris* fresh roots were collected early in morning with the help of Prof. Madhavasetty and two members of tribal people who lives at forest. The present study aimed to study the detailed study on morphological, microsopical (T.S, LS and powder microscopy with different resolutions), physicochemical, florescence analysis and preliminary phytochemical screening on *Alstonia scholaris* (L) R. Br roots (Andhra Pradesh). We can easy to identify the plant with help of above characters, provided the detailed valuable information for botanical quality control, species identification and to detect the adulterations in commercial wise samples of *Alstonia scholaris* root. These studies are mainly useful for identification and standardization of *Alstonia scholaris* (L) R. Br. The plant is also helpful for the other research studies. Confocal microscope and Nikon lab photo 2 microscopic unit was used to identify the detailed parts in roots. For *Alstonia scholaris* root (T.S, L.S) and powder sectioning purpose used many reagents to get the individual part clearly. To define the purity and quality of herbal medicine used physicochemical analysis, preliminary phytochemical studies. The root anatomy contains periderm cylinder, wide cortex and largevascular cylinder, thin walled parenchyma cells, secondary phloem, secondary xylem, lighting stained phloem parenchyma cells, pith is filled with thick walled angular compact cells. These fibers are very narrow with tapering ends, the cell walls are thick the cell lumen is narrow. The wide fibers are up to 70µm wide and less than700µm long and 60µm wide. The wide fibers have thin walls and wide lumen. The fibers have vertical row of oblique elliptical simple pits. In some vessel elements, the end wall is circular and horizontal in orientation. The vessel elements have multi seriate minute ellipteal lateral cell wall boarded pits. The vessel elements are 450µm long and up to 60µm wide. In preliminary photochemical studies total ash 33.93%, water soluble ash (3.86), acid insoluble ash (5.98), foaming index (<100), swelling index (<100), Loss on Drying (10.5). extractive values with different organic solvent Hexane (0.7), N-butanol (2.9) Ethyl acetate (6), Ethanol (5.4), acetone (4.8), methanol (12), hydroalcoholic (5.62) and Distilled water (10.23). In florescence analysis at (UV 254 nm & 354 nm) observed plenty of florescence reflections so colourful compounds are present in that plant. In preliminary phytochemical tests terpenoids, aminoacids, flavonoids, tannins, glycosides are present carbohydrates. Pharmacognostic study is mainly useful for measurement of quality, safety and efficacy of herbal drugs. It is very helpful for herbal monographs.

Keywords: Standardization of *Alstonia scholaris* (L) R.Br, Apocyanaceae.

1. Introduction
India is one of the biggest resource for plants, soil, altitude, temperature, rain fall and humid condition are different from one region to another region. Plants grow in their particular region. In India more than 7500 plant species are present because of tremendous diversity [1]. Our ancestures used these plant parts for different ailments through ayurveda, homeopathy, unani, sidha, unani and aromatherapy therapy, these things are interlink to plants. Western countries importing crude drugs for different diseases and treatments. If we have to do any research either pharmacological, pharmaceutical formulations and synthetic first we need to know the standard pharmacognostic work for every particularplant. Most of the medicinal plants and their plant parts and its isolated secondary metabolites are useful for human and for different treatments. The modern medicine developed over the centuries by systemic and their observational hard work. Pharmacognostical studies are very important for identification of medicinal plants, morphological, microscopical, physicochemical, florescence analysis, preliminary phytochemical, are primary for standardization of medicinal plants.

And secondary metabolites from the separated extracts either through column, preparative TLC, Paper chromatography, HPTLC, HPLC, give beneficial therapeutic and pharmacological activities.

The obtained molecules are useful for biological activities and those secondary metabolites helps a lot into drugs. The pharmacognostic standardization and physicochemical analysis were universally believed for identification and authentication of the genuine plant. pharmacognostic standardization and physicochemical analysis were universally believed for identification and authentication of the genuine plant. Genuine identification and quality of the plant material are indispensible to ensure reproducible outcome of herbal medicines, that gives to efficacy and safety. Pharmacognostic standardisation of plant material includes mainly morphological, anatomical and biological characteristics. *Alstonia scholaris* (L) R. Br plant is a powerful tropical and subtropical latex plant [2]. The Apocyanaceae family contains more than 250 genera and 2500 species are present. It contains lot of potential secondary metabolites like alkaloids, flavonoids, saponins, terpenoids are reported [3, 4]



Graphical abstract of *Alstonia scholaris* (L.) R. Br roots

Material and methods

Plant Material Collection, Authentication and fine coarse powder

The roots of *Alstonia scholaris* were collected at nalla malai forest area, Tirupati, Andhra Pradesh during the month of December, 2018. The plant species were authenticated by Prof. K. Madhava Chetty, Plant Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati. The voucher specimen no: 1221 was deposited in the herbarium, College of Pharmaceutical Sciences, Andhra University.

Fresh material of roots were used for microscopic research study. The collected dried material was washed with water and after dried in shade at room temperature. The dried plant materials were chopped in to small pieces and gave to willy mill for coarsely powder. The obtained powdered drug material was stored in an air tight and light resistant container for the study.

Macroscopical studies

Macroscopical and organoleptic studies were done with powder materials. *Alstonia scholaris* root samples were washed, air dried in shade and observed the outline features of colour, odour, shape, taste and size. The collected fresh materials were powdered and observed for colour, odour, taste and for filter paper test.

Collection of specimens for Microscopical Characters

The plant specimens for the proposed study were collected and care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin - 5ml+Acetic acid -5ml +70% ethyl alcohol - 90ml). After 24 hrs of fixing, the specimens were dehydrated withgraded series of tertiary- butyl alcohol as per the schedule given by sass m 1940. 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.

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 by customary procedure [4]. The sections were stained with toluidine blue as per the method published by O Brien *et al.* [5]. Since toluidine blue is a polychromatic stain. The staining results were

remarkably good and some phytochemical reactions were also obtained. The dye rendered pink color to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and fast-green and IK (for starch)For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parrel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey, s maceration fluid were prepared [5]. Glycerine mounted temporary preparations were made for macerated /cleared materials. Powdered materials of different parts were cleared with NAOH and mounted in glycerine medium after staining. Different cell component were studied and measured [6].

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit.

For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have been fingerprint property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale –bars. Descriptive terms of the anatomical features are as given in the standard anatomy books [9].

Physicochemical Analysis

The total ash and acid insoluble ash content are very important to determine the purity and quality of herbal medicines. The ash value of roots were determined by igniting the powdered sample gradually increased up to 500o C until turned to white. And obtained sample was kept in to a desiccator and an half an hour weighed the obtained sample. Acid insoluble ash content was measured by solubilizing the part of total ash in hydroalcoholic acid with boiling followed by collecting and washing on filter paper, cooling in desiccator and weighing. As like we caliculated the water soluble content.

And for the extractive values, We took 2 gms of coarsely fine powdered material for maceration with help of low polar organic solvents to high polar organic solvents like petroleum ether, ethyl acetate, n-butanol, ethanol, methanol and hydroalcoholic solvents. And we stirred and shaked continuously for 10 hrs. After 10 hrs we filtered the individual extract and concentrated, dried and weighed the obtained extracts and determined the ash and extractive values as per WHO guide lines [10-12].

The florescence analysis of obtained powdered samples were carried out using Ultra Violet (UV) lamps of short wave length (254 nm) and long wave length (365nm). For florescence analysis we mixed the samples with different organic solvents and many reagents (9). The florescence analysis was carried out by the method of Chase and Pratt (13). Loss on drying values were measured by drying the airdried material (5gms) in an oven at 110 0 C.

Preliminary phytochemical screening

Freshly prepared samples for preliminary phytochemical screening, prepared the reagents like chloroform, diluted acetic anhydrate and 5% sulphuric acid for steroids and terpenoids [16, 17]. And for flavonoids Fecl3 Reagent, Ammonia and ferric chloride solutions were used for anthraquinones [14, 18, 19] and for saponins used emulsion, froth and lead acetate. [15] Phytochemical screening of alkaloids hagers, wagers, mayers, dragendorff s tests were performed. [20-22] And for tannins performed three tests like gelatin, vanillin and ferric chloride [16].

Results and Discussion

Macroscopical characters: *Alstonia scholaris* (L) R.Br roots were evaluated for morph ological studies. We noticed the size, color, shape, texture, fracture, taste and odor are tabulated in Table-1 further more we evaluated the powder sample in figure-1 (a) and (b)

Anotomy of *Alstonia scholaris* Root

Thin Root: That Thin Root was circular in cross- sectioned view. It consists of thick single periderm cylinder, wide cortex and large vascular cylinder (Fig 1.1) The periderm consists of about four layers of phellem and three layers of phelloderm. The cortical zone is homocellular with circular thin walled parenchyma cells (Fig.1.2) the vascular cylinder includes outer, continuous, compact cylinder of secondary phloem and central diac cylinder of secondary xylem (Fig.1.2). The secondary phloem consists of small, dark by stained sieve elements companian cells and wider, lighting stained phloem parenchyma cells [8]. The secondary xylem has sparely distributed wide, angular thin walled vessels narrow thick walled lignified fibers and narrow straight xylem rays. The pith is filled with thick walled angular compact cells [7].

Thick Root: The thick root is several times larger than thin root and measures 5mm in thickness (Fig. 2). The periderm is figured deeply and irregularly forming short irregular flakes. The epidermisis olitereted; the periderm shows out wider phellem cells and inner narrow phellodermcells (Fig.3). The phellemcells are5to6 layered, thin walled and subsized.Theyare dead cells and protoactive in function. The phelloderm cells are four layered; the cells have cellulose walls and they are living cells (Fig. 4.1). Secondary phloem consists of horizontal layers, rectangular cells with companion located along coronary of the cells. Secondary xylem includeswide, thin walled angular vessels. Which are either solitary or in short radial multiples (Fig4.2). The xylem rays are narrow,straight and the cells are thin walled. Xylem fibers are narrow, angular and lignified; they are arranged in regular, radial compact lines (Fig. 4.2). The vessels are 100µm wide (Also refer Fig. 5.1.)

Powder Microscopy of the root:

The powder preparation of the root shows the fiber types. These are two types of fibers:

Narrow fibers: (Fig.6.1.) These fibers are very narrow, with tapering ends, the cell walls are thick the cell lumen is narrow. The fibers are 210-300µm long and are 10-30µm wide. Minute, slit like simple pits are less distinctly seen in narrow fibers.

Wide fibers: (Fig.6.1, 7.1.) The wide fibers are up to 70µm wide and less than700µm long and 60µm wide. The wide fibers have thin walls and wide lumen. The fibers have vertical row of oblique elliptical simple pits (Fig. 8.2) the pits are 15µmlong

Vessel elements: (Fig. 7.1, 8.1) Long cylindrical vessel elements common in the powder. The vessel elements have olique cell wall, where there is end wall perforations. The

perforations are simple and olique in walls. In some vessel elements, the end wall is circular and horizontal in orientation (Fig.8.1). The vessel elements have multi seriate minute ellipteal lateral cell wall boarded pits. The vessel elements are 450µm long and up to 60µm wide.

Determination of Physicochemical parameters

The total ash values of Roots of *Alstonia scholaris* was found to be 33.93% w/w which indicates the presence of earthy matter. The water soluble ash was found to be less i.e., 3.86% w/w than acid insoluble ash of i.e., 5.98% w/w respectively. The loss on drying values of roots of *Alstonia scholaris* was found to be 10.5%w/w which indicates the presence of moisture content. The swelling index was found to be <100. The foaming index was found to be <100. The extractive values of *A. scholaris* was found to be more for Methanol solvent. The total ash values for roots were determined and reported the percentages. total ash, water insoluble ash, acid insoluble ash values (Table-2) were wonderful significant percentage of moisture was found in air dried material of root. And for root showed good significant values of LOD. So we have to take care of stored material. Number of organic solvents like Hexane, N-butanol, Ethyl acetyate, Ethanol, Acetone, Methanol and Hydroalcoholic solutions were used to determine the extractive values, these are quality control parameters for herbal medicines. As we expected, the extractive values were more with polar solvents like methanol and hydroalcoholic and decreased the polarity decreases. Fluorescence analysis is also an important tool for the determination of constituents in herbal drugs and it provides an idea about their chemical nature [11]. The powder drug analysis was performed when treated with various chemical reagents and observations were made in visible light and UV light of short and long wavelengths [Table 3].

The preliminary phytochemical screening *Alstonia scholaris* was done by using Hexane, n-butanol Ethyl acetate, ethanol, acetone Methanol and Hydro alcoholic extracts. Ethyl acetate extract showed the presence of alkaloids, carbohydrates, proteins, glycosides, terpenoids. Hexane extract showed the presence of terpenoids. Hydro alcoholic extract showed the presence of Alkaloids, Carbohydrates, Tannins, and Glycosides. Methanolic extract showed the presence of Alkaloids, Carbohydrates, Phenols, Tannins, Flavonoids, Proteins, Glycosides [Table 4].

Conclusion

The Pharmacognostic study is established and main diagnostic characters of the obtained results are mainly useful for the measurement quality, safety, and efficacy of herbal drug. The parameters studied here are useful to identify and authenticate the traditionally more important medicinal plant of *Alstonia scholaris* and that will prove and helpful for herbal monographs and pharmacopial standards of as emphasized by WHO.

Back ground: Herbal Drugs are useful for different ailments indifferent countries. Pharmacognostical, Preliminary Phytochemical evaluation studies provide us basis to establish the quality protocols of any medicinal herbs.

Research frontiers

The pharmacognostic study provides basis for herbal remedy. This research is helpful to identify and estimate the purity of drug and can also be used to screen adulteration and it gives a drug quality.

Related reports

Introduction, Morphological, Microscopical, Preliminary phytochemical, Ash values, Extractive values, florescence analysis are the main tools to identify the purity, presence and absence of certain chemical groups in a herbal drug. Pharmacognostic studies are mainly useful for quality of a herbal drugs.

Innovations and breakthroughs

The plant *Alstonia scholaris* is mainly useful for anti malarial, anticancer etc reported. At our Andhra Pradesh no pharmacognostic research work was established so this work is a primary and important for further studies.

Applications

Alstonia scholaris root is helpful for establishing the correct identification of a plant and the plant will be main tool for standardization, characterization and identification of *Alstonia scholaris* roots. The plant is also helpful for the other research studies.

Peer review: It is a very important research work and the author had has been established the evaluation standards of a medicinal plant. This evaluations are assure the authentication of a root plant of *Alstonia scholaris* further more it is useful for both qualitative and quantities estimation of the medicinal herbal drugs.

Conflict of Interest: We declared that we haven't any conflict of interest

Table 1: Filter pater test is for volatile oils and fixed oils

Parameters	Root
Colour	Light golden yellowish Cream
Shape	Tap root is thick and long and lateral roots are developed
Size	Medium
Odour	No characteristic odour
Taste	Bitter
Powdered Drug Study	
Colour	Light yellowish
Odour	Light characteristic
Tast	Light bitter
Filter paper test	

Table 2: Physicochemical parameters of *Alstonia scholaris* (L) R.Br

Parameters	values
Total ash	33.93
Water soluble ash	3.86
Acid insoluble ash	5.98
Foaming Index	<100
Swelling Index	<100
Loss on Drying	10.5
Extractive Values with organic solvents	-
Hexane	0.7
N-butanol	2.9
Ethyl acetyate	6
Ethanol	5.4
Acetone	4.8
Methanol	12
Hydroalcoholic	5.62
Distilled Water	10.23

± Caliculated as SEM of three reading

Table 3: Flourescence analysis

Flourescence Analysis				
Chemical Treatment	**Day Light**	**Fluorescence**	**UV Longer (365 Nm)**	**UVShort (254Nm)**
Drug powder	Brown	Peanut brown	Brunette brown	Wood brown
Ethyl acetate	Cinnamon brown	Tortilla brown	Walnut brown	Wood brown
Hexane	Light brown	Tortilla brown	Mocha brown	pecan brown
Water	brown	Peanut brown	Light brown	Wood brown
1N NaOH (aq)	Tawny brown	Tortilla brown	Pecan brown	Peanut brown
1N NaOH (alk)	Pecan brown	Umber brown	Mocha brown	Pecan brown
5% NaOH	Pecan brown	Tortilla brown	Walnut brown	Peanut brown
10% NaOH	Pecan brown	Tortilla brown	Caramel brown	Peanut brown
Con. HNO3	Light orange	Dijon orange	Ochre orange	Amber orange
Con. H2SO4	Brunette brown	Brunette brown	Walnut brown	Brunette brown
Con. HCl	Tawny brown	Tortilla brown	Walnut brown	Peanut brown
5% FeCl3	Dijon orange	Tortilla brown	Walnut brown	Peanut brown
Picric acid	Dijon orange	Amber orange	Bronze orange	Bronze orange
Methanol	Brown	Cedar brown	Walnut brown	Brunette brown
Dil. NH4	Brown	Tortilla brown	Cedar brown	Peanut brown
Acetic acid	Tortilla brown	Cedar brown	Walnut brown	Brunette brown
50% H2SO4	Cedar brown	Cedar brown	Walnut brown	Brunette brown
50% HCl	Pecan brown	Tortilla brown	Pecan brown	Peanut brown
50% HNO3	Brown	Tortilla brown	Cedar brown	Peanut brown

Table 4: Phytochemical tests for identification class of components (*Alstonia scholaris* (L.) R. Br)

Phytochemical Analysis	**Hexane**	**N- Butan ol**	**Ethylacetate**	**Ethanol**	**Acetone**	**Methanol ic**	**Hydroalcoh olic**
1. Alkaloids							
Mayer's test	-	+	++	++	+	++	+
Hager's test	-	+	++	++	+	++	+
Dragendroff's test	-	+	++	++	+	++	+
2.Carbohydrates							
Molisch's test	+	+	++	++	++	+	+
Fehling's test	+	+	++	++	++	+	+
Barfoed's test	+	+	++	++	++	+	+
Benedict's test	-	+	++	++	++	+	+
3. Glycosides							
Borntrager's test	+	+	++	++	+	++	+
b)Legal's test	+	+	++	++	+	++	+
c)Keller-Kiliani test	+	+	++	++	+	++	+
4. Phenols and Tannins							
a) Ferric chloride test	_	+	++	++	++	++	+
b) Gelatin test	-	+	++	++	++	++	+
C)Lead acetate test	+	+	++	++	++	++	+
5. Flavonoids							
a) Alkaline reagent test	_	+	++	++	++	++	+
b) Schinoda test	-	+	++	++	++	++	+
c)Zn+HCl test	+	+	++	++	++	++	+
6. Test for fixed oils							
a)Spot test	-	-	-	-	-	-	-
7) Saponins							
a) Foam test	-	-	-	--	-	-	-
8). Proteins and Aminoacids							
a)Millon's test	-	+	++	++	+	++	+
b) Biurettes test	-	+	++	++	+	++	+
c)Ninhydrin test	+	+	++	++	+	++	+
9. Terpenoids/ Phytosterols							
a)Libermann-Burchard test	++	++	++	+	+	+	+
10). Test for triterpenoids							
a)Salkowski test	++	++	++	+	+	++	++
11. Gum And Mucilages							
a) Alcoholic precipitation test	-	-	-	-	-	-	-
12. Test for lignin							
a) Lignin test	-	-	-	-	-	-	-
b) Labat test	-	-	-	-	-	-	-

Fig 1(a): *Alstonia scholaris* (L)R.Br root

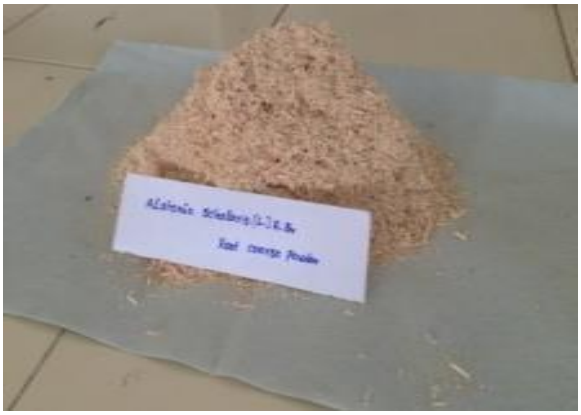


Fig 1(b): Coarse powder form of *Alstonia scholaris* (L) R.Br root

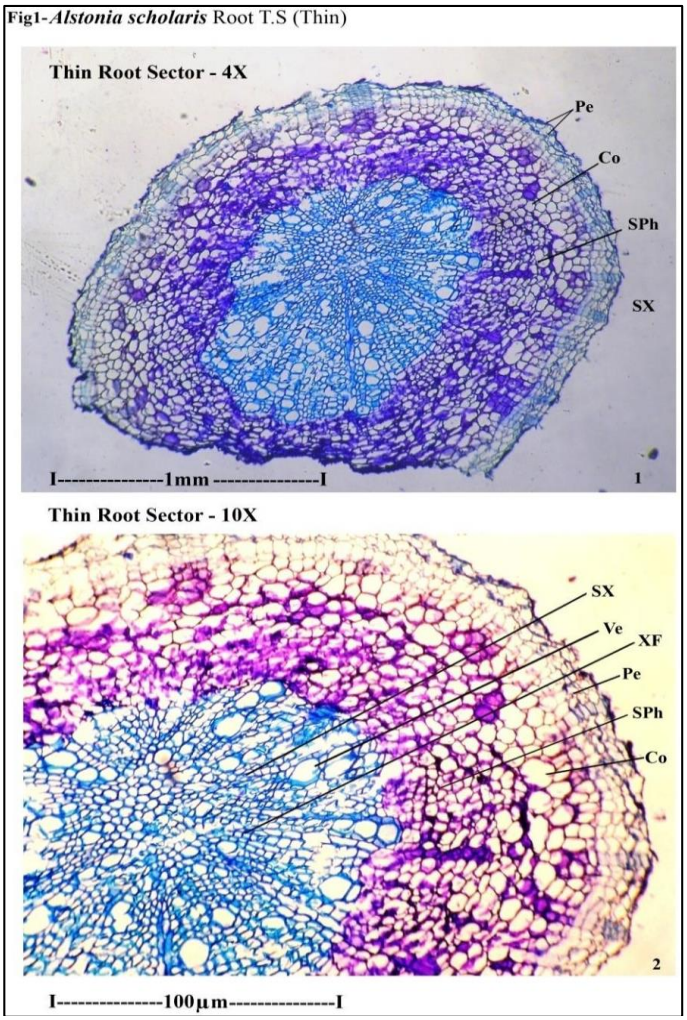


Fig 1.2: T.S of thin root A section enlarged Co-Cortex; Pe –Periderm ; Sph- Secondary phloem ; SX- Secondary xylem ;Ve- Vessel ; XF- Xylem fibres

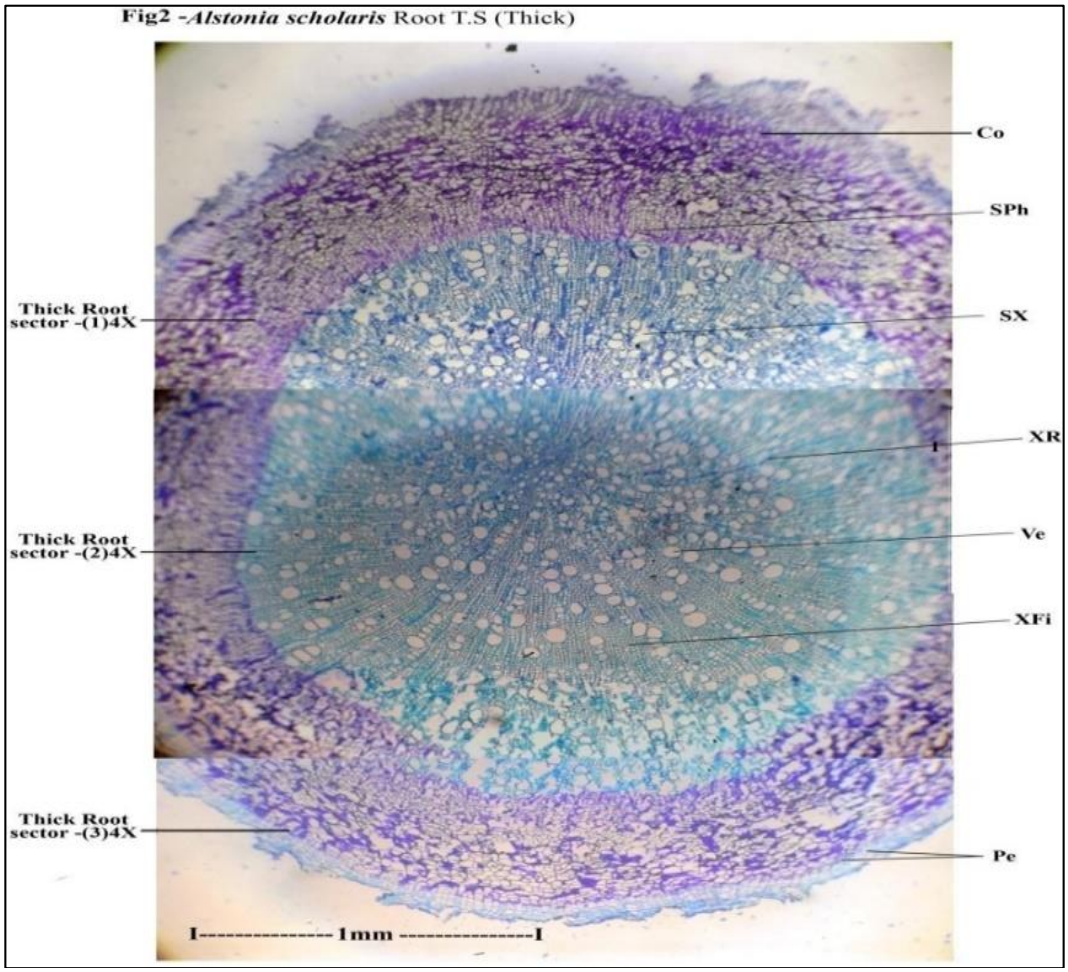


Fig 2: T.S of Thick – entire view Co-Cortex; Pe -periderm; Sph-Secondary phloem; SX-Secondary xylem; Ve- vessel; XFi- Xylem fibres; XR-Xylem Ray

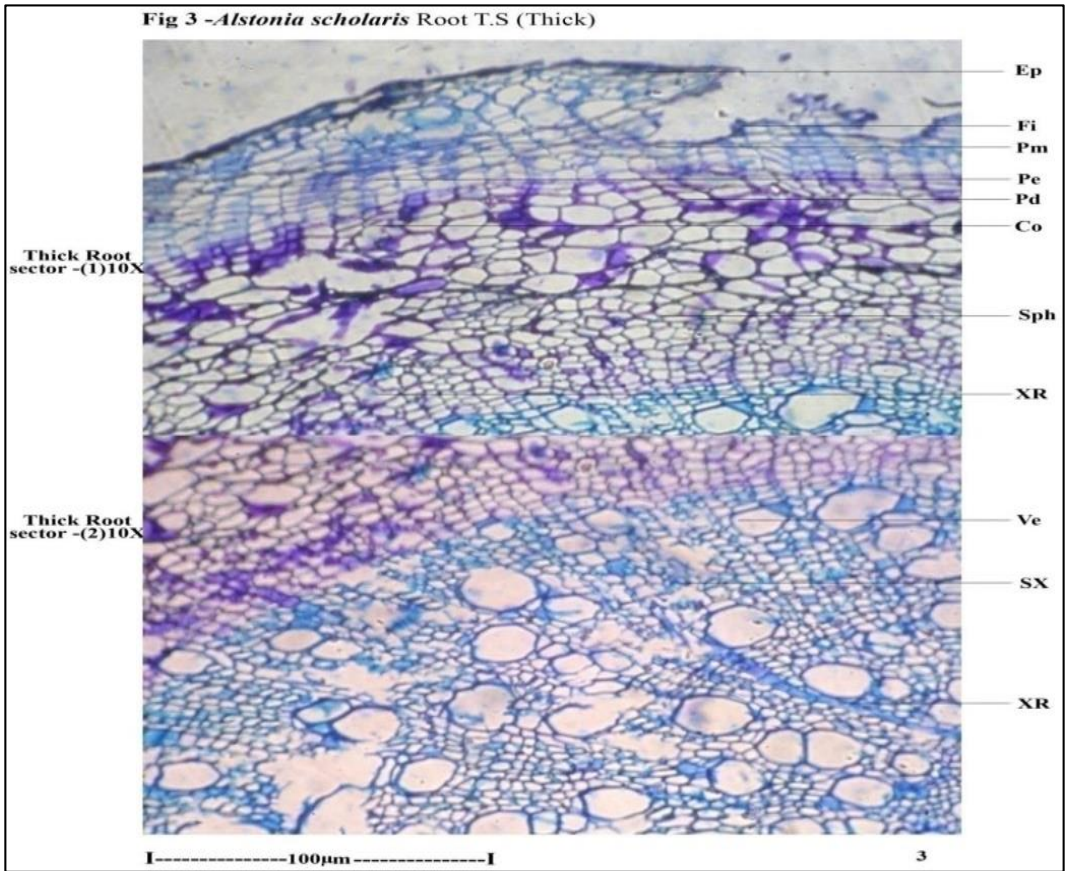


Fig 3: T.S of thick root –A Section enlarged Co-Cortex; Ep-Epidermis; Fi-Fibres;PD- Phelloderm; Pe-periderm ;Pm-phellem;Sph-Secondary Phloem Sx-Secondary xylem Vessel ; XR-Xylem ray

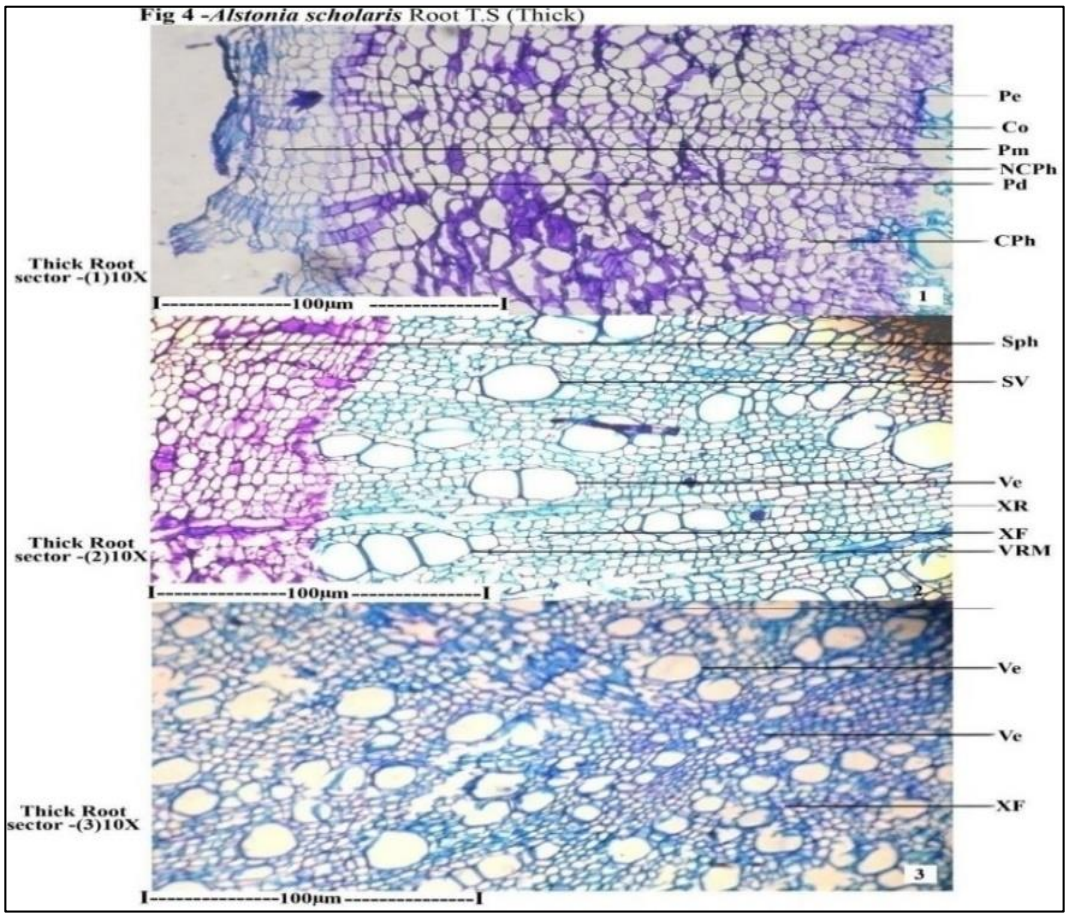


Fig 4.1: T.S of thick root – periderm, cortex and secondaryphloem **Fig 4.2:** Secondary phloem and secondaryxylem
Fig 4.3: Secondary xylem elements Co-Cortex; CPh-Collapsed phloem ; Pd-Phelloderm ; pe-periderm ; Pm-phellem; Ncph- Non collapsed phloem; Sph –Secondary phloem ; SV-Solitary vessel; V-Vessel; VRM- Vessel in radial multiplies ; XR-Xylem rays; XF- Xylem Fibres

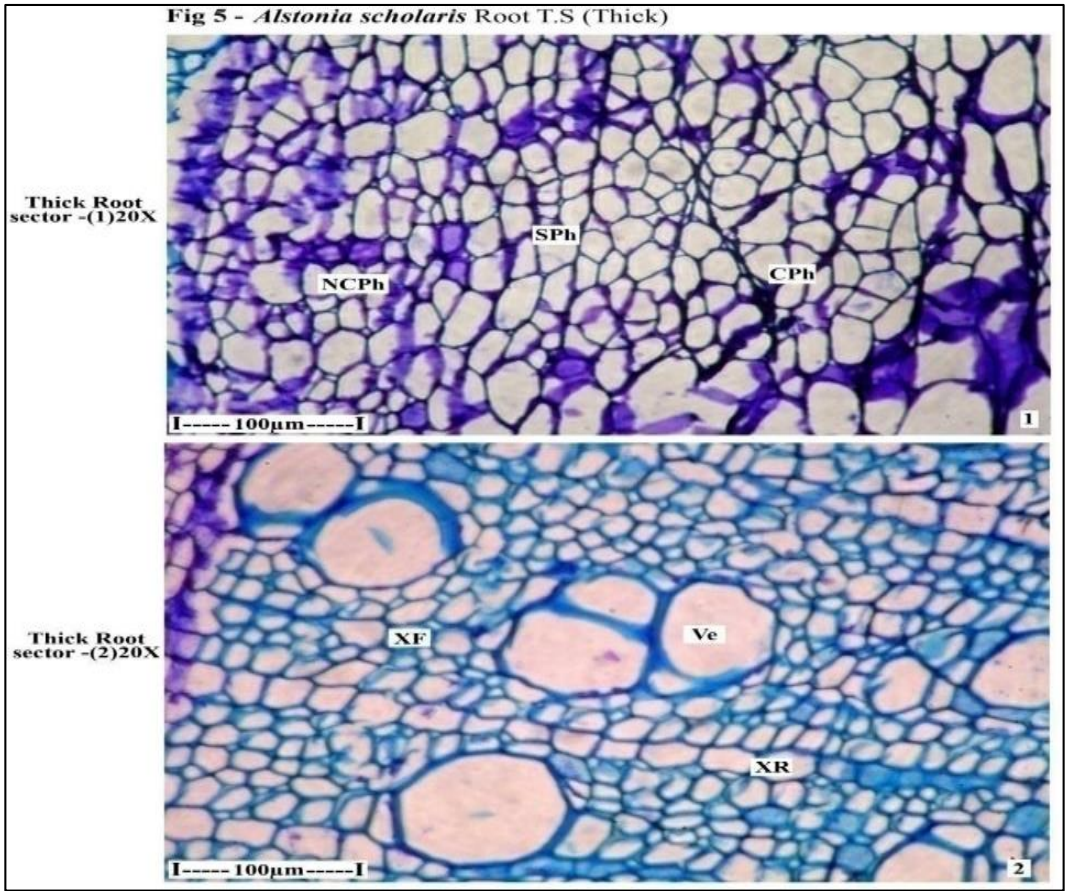


Fig 5.1: T.S of thick root – Secondaryphloem with collapsed Noncollapsed phloem elements 5.2: T.S of Secondary xylem elements Cph- Collapsed phloem ; NCPh - Non collapsed phloem ; SPh- Secondary phloem ; Ve- Vessel; XF-Xylem fibre; XR-Xylem Ray.

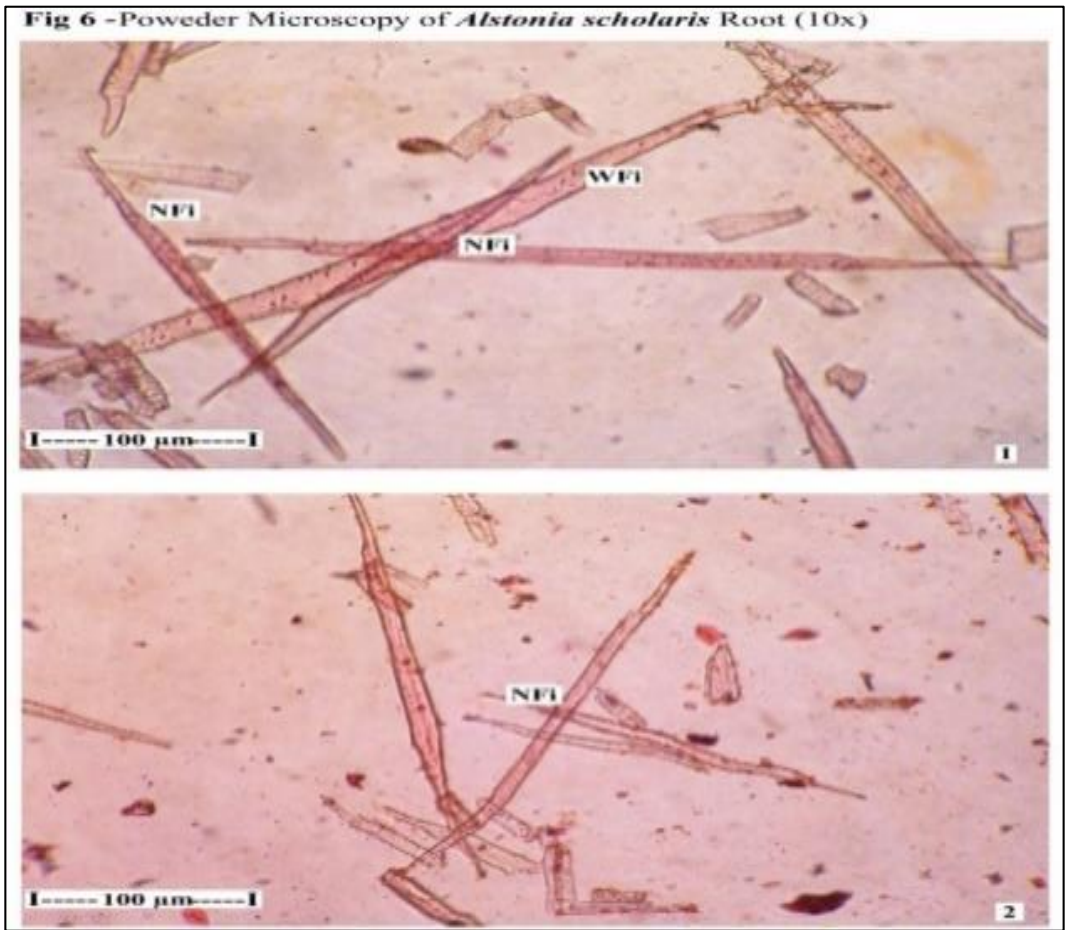


Fig 6.1, 6.2: Powder microscopy showing the narrow and wide fibres NFi- Narrow fibres; Wfi-Wide fibres

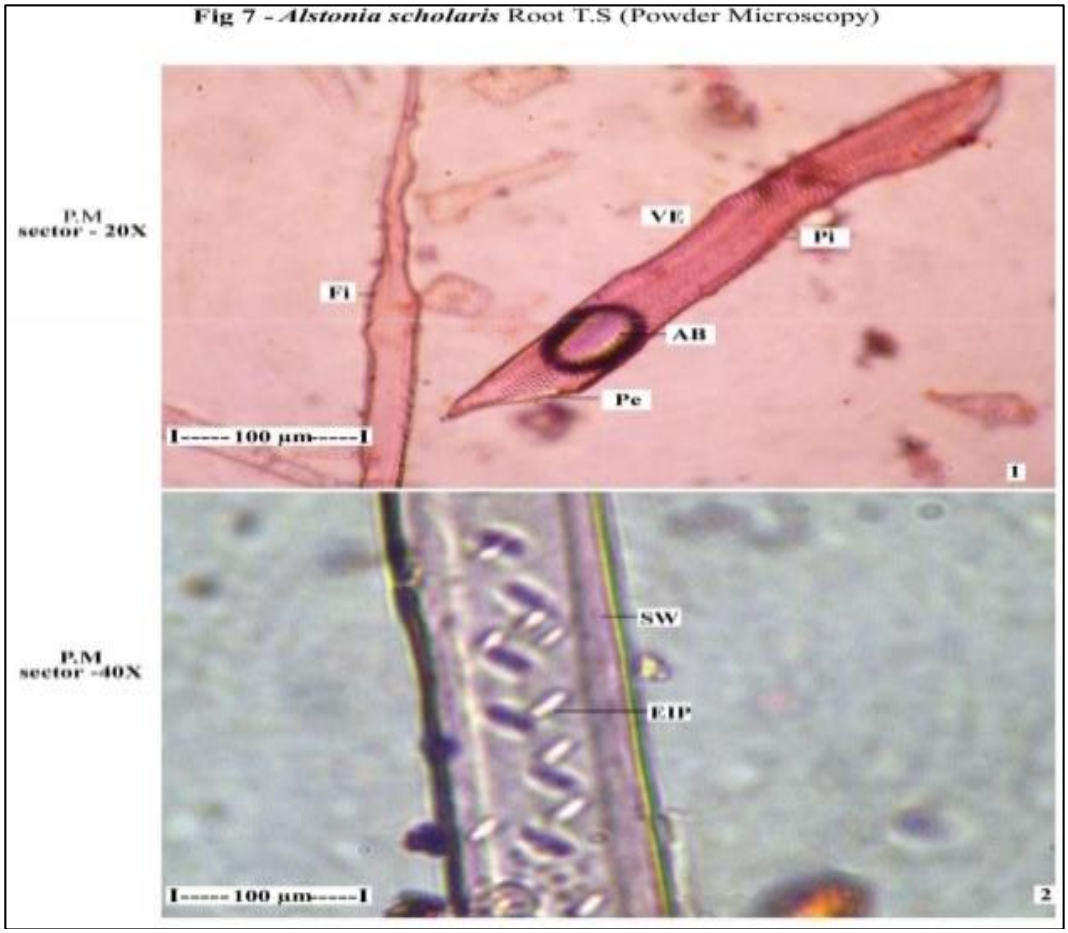


Fig 7.1: Powder showing fibre and avessel element with air bubble A.B – Air Bubble ; EIP-Elliptical simple pits ; Fi-Fibres; Pe- Perforation ; Pi-Pits; Fi-Fibres; Pe- Perforation; Pi-Pits;SW- Secondary wall; VE-Vessel Element

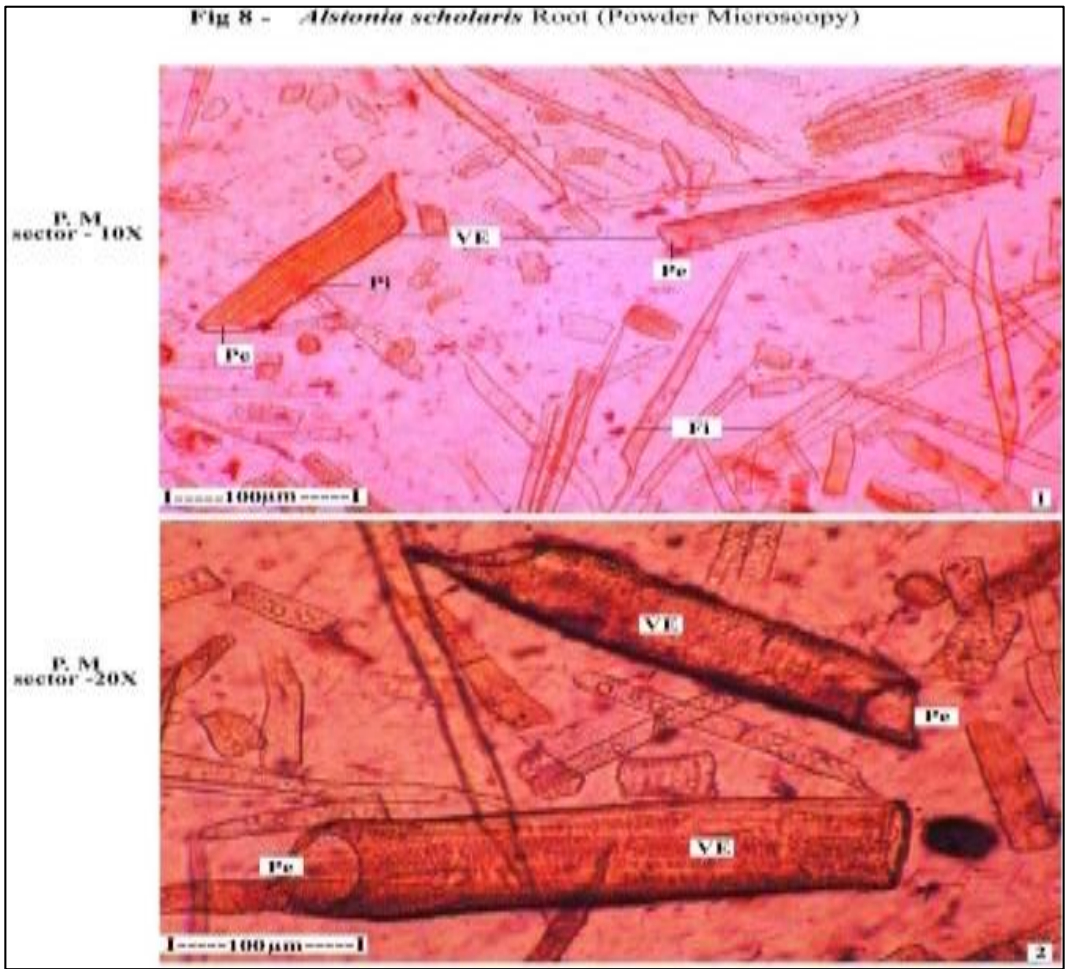


Fig 8.1: Vessel Elements and fibres; **Fig. 8.2** Wide cylindrical vessel elements with circular horigental end wall perforation. Fi-Fibres; Pi-Pits; Pe-Perforation; V

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