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Pharmacognostical, Preliminary Phytochemical and Anti Bacterial Studies of the Stem of *Zanthoxylum rhetsa* (Roxb.) Dc.

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ABSTRACT

In worldwide available various famous medicinal systems like traditional and modern medicinal systems in various disciplines. According to India one of the traditional medicinal system is Ayurveda medicinal system. Based on this medicinal system medicine may be a powder, tablet, semisolid or liquid. Among the powdered drugs, churna which are the powdered herbs, bhasmas which are vegetable & mineral acids and ashes. *Zanthoxylum rhetsa (Roxb) Dc.* belongs to the family - **Rutaceae** is the medicinal plant, according to "*kanikkar's"* it is known as "Malvapoo" and "veerasingi". The kanikkar tribe, inhabitants of the Agashthiyarmalai Biosphere reserve, Western Ghats, Tamilnadu. They are using this plant stem as medicinal purposes for increase lactation, saliva secreation, Digestic issues, rubbing paste using for pain reliever and some mouth and tooth problems. But no literature survey was found proper pharmacognostical, physiochemical, antibacterial studies. So the present investigation deals with the studies above mentioned. Pharmacognostical study which was estimated Macroscopial, Microspial, Ash value, Loss on Drying (LOD) and purity of the drug. Physiochemical study was Confirmed the presence of Aleurone grains, Alkaloids, Amino Acids, Lignin, Volatile oils, Fats & Fixed Oils, Mucilage's, Protein, Steroids & Triterpenoids and Flavonoid's. Antimicrobial studies carried out by plate hole diffusion method or agar well diffusion, It screens the results was observed that the Petroleum Ether, Ethanol and Aqueous extracts have significant effect comparable to the standard drug Tetracycline.

INTRODUCTION:

Medicinal plants which are the backbone of traditional medicine have been in the last few decades very intense in the pharmacological studies. The system of therapy was founded based on the concept that disease can be treated with drugs. Drugs where the chemical substance, known structure and properties, which, when administered into a living organism it produce some biological effect. One of ancient medicine system of India is Ayurveda medicine, It is a system of Hindu traditional medicine. The word Ayurveda has been derived from two words Ayurveda is a knowledge (or) science of life. Some of the oldest known Ayurveda text include the susruthasamhita and charakasamhita, which are written in Sanskrit.

Zanthoxylum rhetsa – It is a lofty, deciduous tree, up to 35m tall with pale corky bark covered with conical spines on stems and branches. Commonly known as mullilam found in shaded moist localities of tropical regions of India at an altitude up to 1,800m.Commonly found in the evergreen monsoon forests of the foothills of Assam and Meghalaya and in the Eastern and Western Ghats in peninsular India [1]. ***Zanthoxylum*** (including fagara) is a genus of about 250 species. The genus is a rich source of various chemical such as alkaloids, amides, flavonoids, lignin, sterols and terpenes etc. Bark, pounded and mixed with oil, used as externally as remedy for stomach pains. Decoctions of bark taken internally for chest pains. Bark chewed and applied to snake bites. Fruit used for urinary complaints and complaints and dyspepsia caused by atrabilis (the melancholic humor) also used in same forms of diarrhea. Bark in considered bitter aromatic and aphrodisiac. Fruit, mixed with honey, taken for rheumatism.

MATERIAL AND METHODS

Collection and Identification of plant material:

The stem of *Zanthoxylum rhetsa* (Roxb.) DC. was collected from Kollihill Nadi vaithiyar. The plant identification done at Institute of herbal Botany Plant Anatomy Research Centre, West Tambaram, Chennai. The herbarium of the plant stem part was saved in Pharmacognostical Museum of Vinayaka Missions College of pharmacy, Salem.

Apparatus, Chemical reagents and Micro organism

Soxhlet's apparatus, Paper Chromatography, Tetracycline API as standard drug, Formalin, Acetic acid, Ethyl Alcohol, Petroleum ether, Ethanol, Water and all other chemical, reagents

were of analytical grade. Instrument were used microscope. Microorganism- *Escherichla coli, Streptococcus pyrogens* and *Shigella flexneri.*

Processing:

The stem material was dried in the shade for two month. Then shade dried plant was subjected to get coarse powder by using grinding machine and the coarse powder was properly stored in airtight container for further phytochemical experimental purposes.

The required samples for pharmacognostical experimental were fixed in FAA (Farmalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs the specimen were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by Sass, 1940[3]. 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.

Solvent extraction

The coarse powder of research drug was subsequently extracted sequentially in Petroleum ether (60-80^{O}C), Benzene (79-81^{o}C), Chloroform (50.5-61.5^{o}C), Ethyl Acetate (77^{o}C), Alcohol, and Aqueous in soxhlet's apparatus and then filtered. The extract was concentrated under the vacuum drier to yield semi-solid. The residue was stored in refrigerator under 10°C for subsequent experiments.

Sectioning:

The paraffin enveloped specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12µm. Dewaxing of the sections by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by O'Brien et al. (1964). Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and Fast-green and IKI (for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel 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 (Sass, 1940) were 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 component were studied and measured.

Pharmacognostic studies:

Fresh dried stem part of the plant authenticated from the Institute of herbal Botany Plant Anatomy Research Centre were taken for morphological and histological studies. The above processed sectioned specimens were used for find out the different pharmacognostical characteristics according to the established procedures [4], [5].

Physiochemical parameters

Various physiochemical parameters such as Total ash value, Water soluble ash, and Acid soluble ash, Loss on drying, Water soluble extractive value, Alcohol soluble extractive value, and Crude fiber content of the powdered stem were estimated using standard methods [6-8].

Preliminary phytochemical screening

The powder and various extracts of the plant were subjected to chemical tests for identification of its active constituents [6-9]. Aleurone grains, Test for alkaloids, Amino acids, Lignin, Volatile oil, Fats & Fixed oils, Flavonoids, Glycosides, Tannins, Proteins and Steroids & Triterpenoids.

Chromatography

The chromatography paper was used in the size of 2.5 x 10cm. And the followed the procedures [10] by using mobile phase in the ratio of solvent composition was N-Butanol: Glacial acetic acid: Water - 4: 1: 5 respectively. The solvent systems were selected according to the Phyto - Constituents present in the extracts. One mobile phase was selected on the basic of separating Amino acids. Prepared 1% solution of Petroleum Ether, Benzene, Chloroform, Ethyl Acetate, Ethanol (or) Alcohol, Aqueous extracts in separate test tubes. Applied 2-5µl volume of the solution on the paper. According to the mobile phase and phyto-constituents separate chromatogram, for each extracts were prepared [10], [11].

The RF value was calculated by using the standard formula.

Rf = Distance traveled by solute / Distance traveled by solvent front.

Antimicrobial study

Antibacterial study (plate hole diffusion method or agar well diffusion) assay was performed to determine growth inhibition of bacteria by plant extracts. Bacteria were maintained at 37°C on nutrient agar plate before use. Nutrient agar medium was prepared and each universal containing 20 ml was poured. The universals with the broth were inoculated with different bacteria species and incubated at 37°C for 24 hours. A total 25 ml of Molten Hinton (MH) agar was poured into sterile universals. Each universals was inoculated with 0.2 ml of different bacterial species mixed well with the MH into sterile Petri dishes and allowed to set.

A well prepared plates with the help of a cork-borer (6mm) four holes per plates were made into the set agar containing the bacterial culture. A total of 0.2 ml plant extract were poured into the wells with concentrations as 100 mg/ml for each bacterial strain controls were maintained where pure solvents, instead of extract. The plates were incubated overnight at 37°C. The results were obtained by measuring the zone diameter of petri dish. The results were compared with standard antibiotic drug as tetracycline[12].

RESULTS AND DISCUSSION

Microscopic characters

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures were indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books [13].

Transverse section of stem and stem bark shows:

The stem bears thick conical, woody thorns. The surface of the stem bark is pale brown and shallow fissured. (Fig.1). The stem consists of thin superficial periderm, wide cortical zone with sclerenchyma masses, wide, continuous secondary phloem and thick and dense secondary

xylem. (Fig.1.1) [14]. The surface of the periderm was waved with varying thickness and deep narrow fissures (Fig.2). On the surface of the periderm was thick dark by stained layer of crushed epidermal and sub epidermal tissues. The intact periderm was 100µm thick. These were thin layer of phelloderm comprising three or four layers of cells.

Cortex is wide and parenchymatous. These are wide, circular or elliptic secretary cavities sitated in the cortical zone (Fig.1.2). In the inner part of the cortex are irregular masses of sclereids and fibers (Fig: 1.2; 2). Secondary phloem is wide and well preserved. It consists of compact parallel lines of small, polygonal sieve elements and phloem parenchyma cells [15].

Secondary xylem is a thick and dense hollow cylinder enclosing the parenchymatous pith. It includes vessels, xylem fibers and xylem rays (Fig.1.1). The vessels are mostly in radial multiples of two or solitary's. They are elliptical or circularizes cross-sectional outline; the vessel walls are less thick. The vessels are 30-50µm in diameter. The axial (vertical) parenchyma is scanty; it forms thin incomplete sheath around the vessels. (Fig.1.2) [16].

Xylem fibers are narrow, thick walled and lignified. They are in compact radial rows. The lumen of the fibers is much reduced. Xylem fibers are thin and they run straight or bend when they cross the vessels. The ray cells are radially elongated, thick walled and lignified.

In TLS view of the secondary phloem, the structures of the rays were studied. The ray are non-storied (Fig.4.1, 2). The rays are multi seriate and less frequently lei seriate (Fig.4.2). The rays are hetero cellular. Then ray consists of middle part of horizontally oriented procurement cells and end cells of vertically oriented upright cells. (Fig.4.3). Ray-frequency is 6 / mm. Ray height is up to 700µm; Ray thickness in 30-50µm [17].

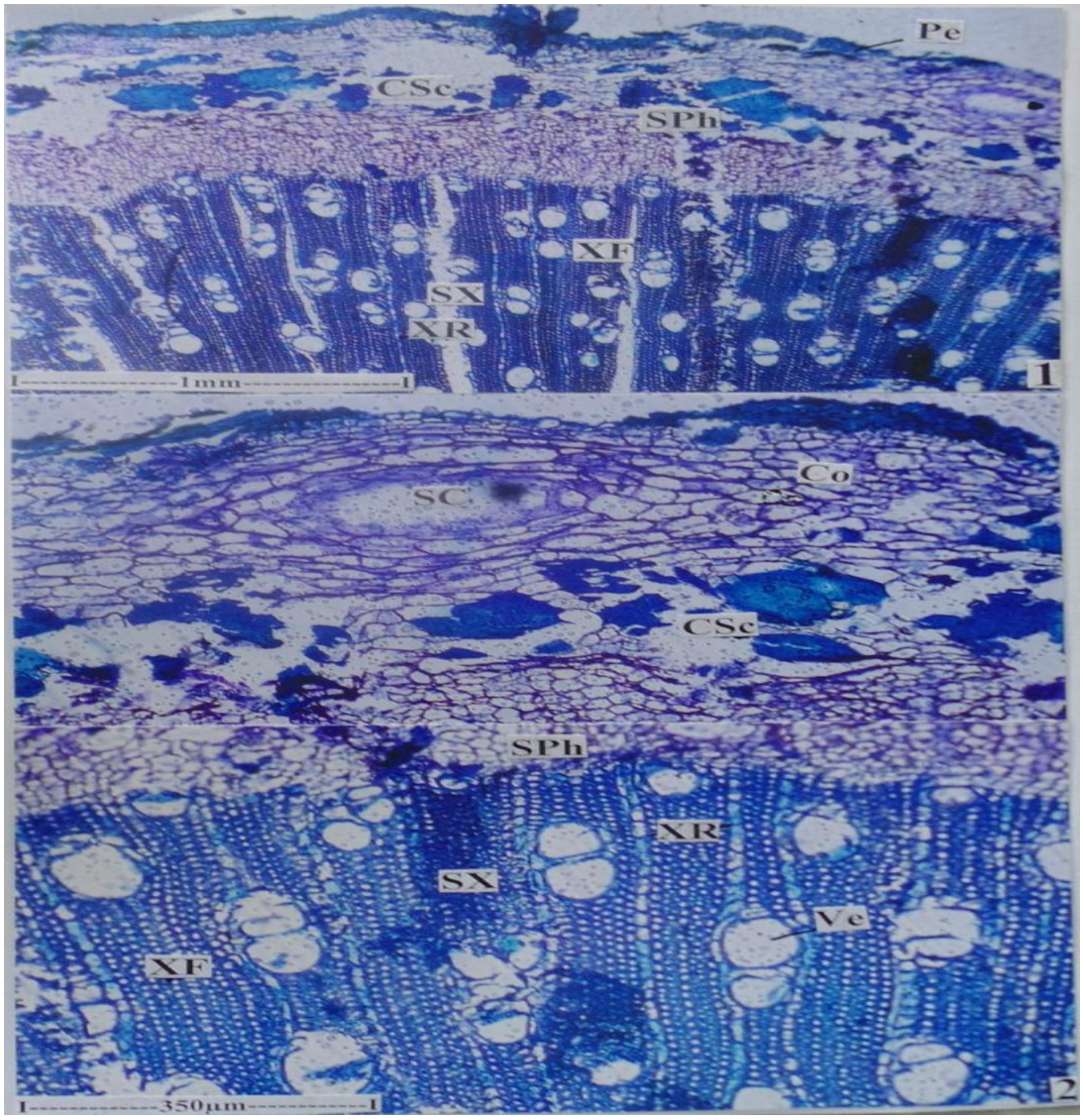


Fig 1.1: T.S of thin stem – A Sector

Fig 1.2: T.S of thin stem – Enlarged view

(Csc-cortical Sclerenchyma; Co: cortex; Pe.peviderm; Sc: secretory cavity; Sph. Secondary phloem; Sx: secondary xylem; Ve: vessel; XF: xylem fibres; XR: xylem ray;).

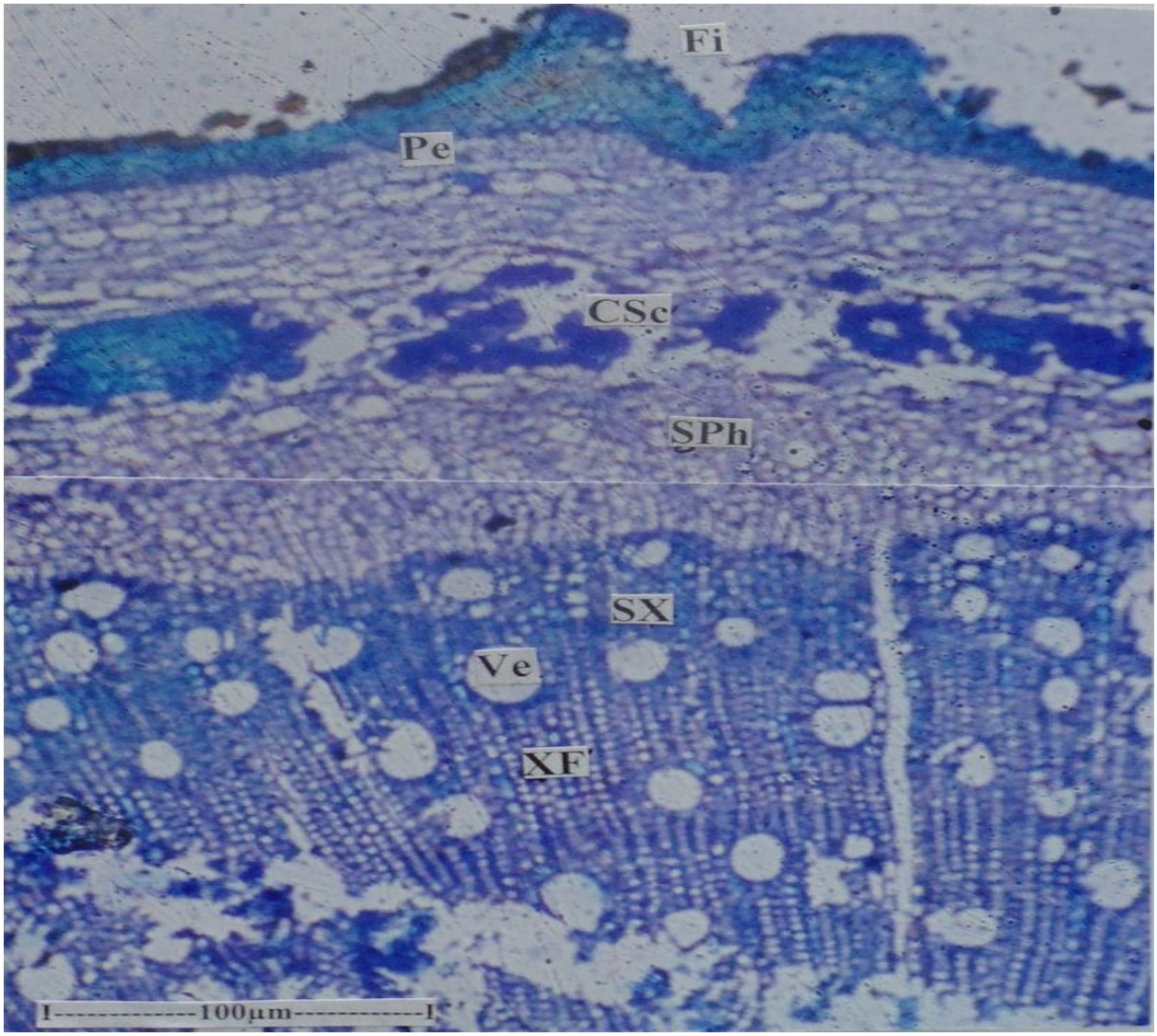


Fig. 2. T.S of thick stem – A sector.

(Csc: cortical sclerenchyma: Fi: Fissuse; Pe: Periderm; Sph: Secondary phloem;

Sx: Secondary xylem; Ve: vessel; Xf: xylem fibres;).

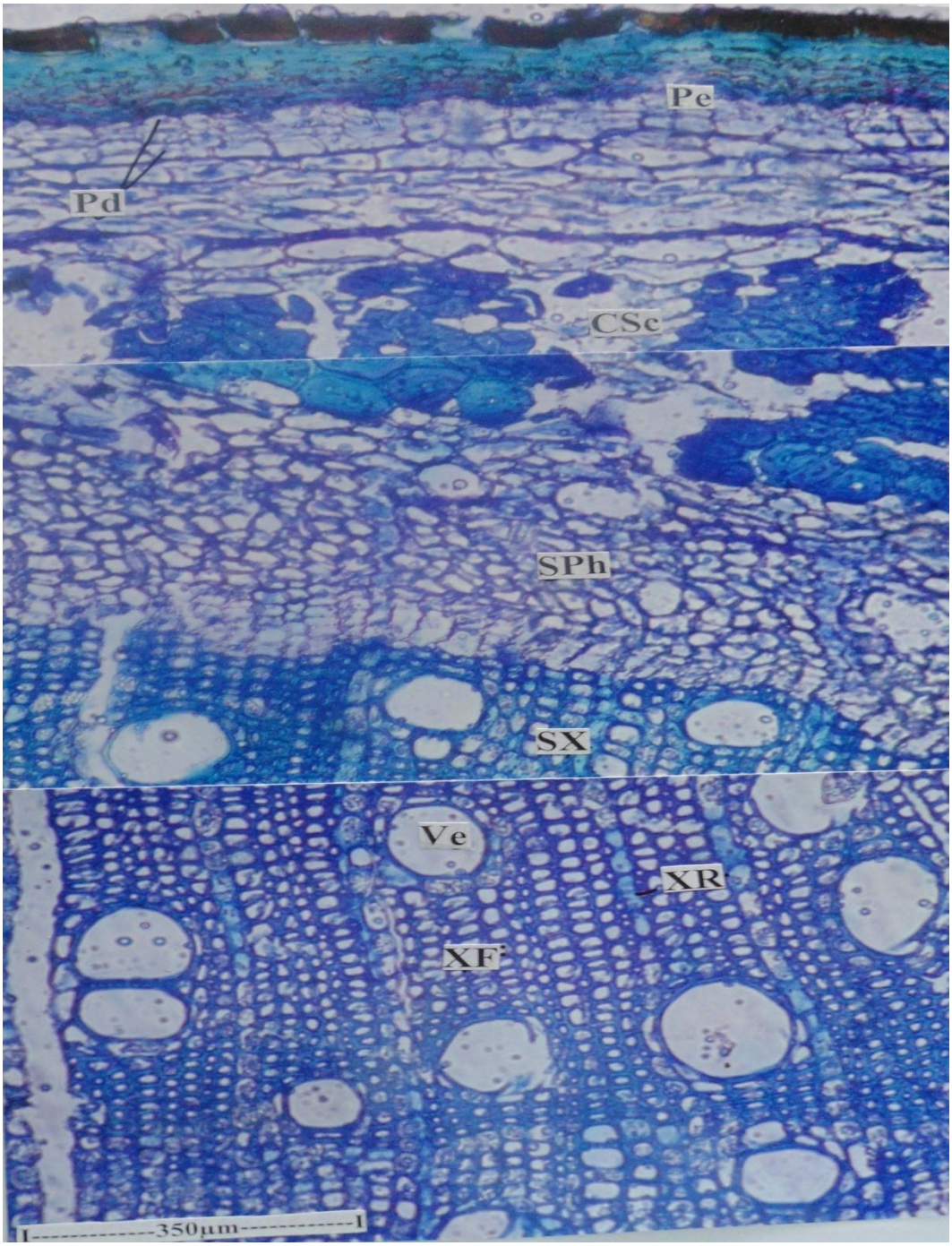


Fig. 3: T.S of old stems showing periderm, bark and secondary xylem.

(Csc: cortical sclerenchyma; Pd: phello derms, Pe: periderm; Sph: secondary phloem;

Sx: secondary xylem; Ve: vessel; Xf : xylem fibres ; Xr: xylem Ray;).

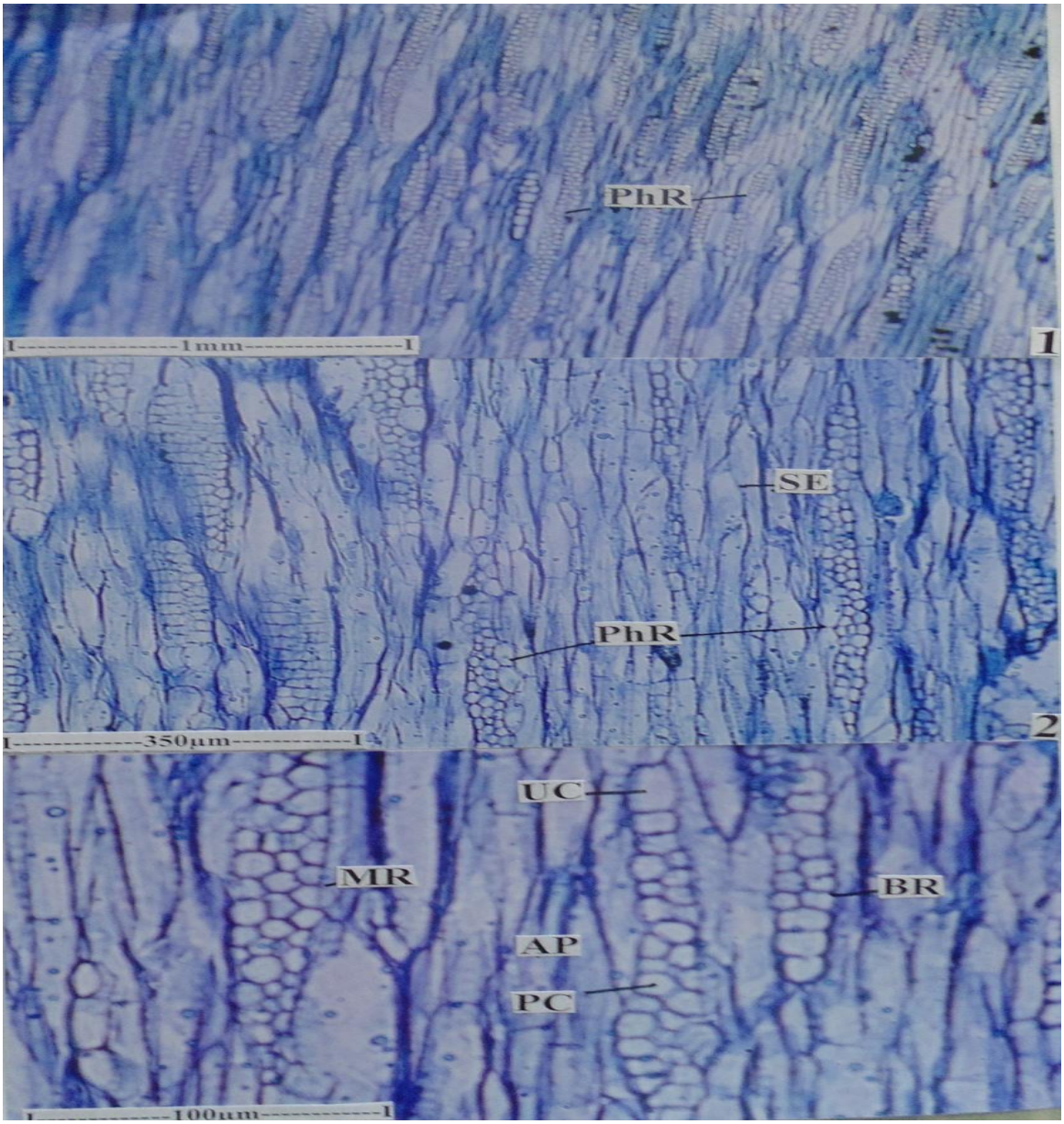


Fig. 4.1, 2, 3:- TLS view of secondary phloem.

1. Phloem rays – Non storied.

2. Multiseriate and biseriate rays

3. Heterocellular rays

(BR: Biseriate Ray; AP: Axial Parenchyma; MR- Multi seriate Ray; PC: Procumbent Cells;

Phr: Phloem Ray; SE: Sieve Element; UC: Upright Cell ;).

Powder microscopic observation:

The powdered material of the plant (Stem and Bark) shows the following annexations:

1. Different types of parenchyma cells were rich in the powder. A unique type of parenchyma cells appears long, spindle shaped with tapering ends. The cell includes dark spherical bodies. (Fig.5.1). The cell is 500µm long and 80µm thick in the middle [18].

2. Fusi from sclereids, brachys dereids which are iso diametric and sclereids of fusiform shape (Fig.5.2) and rectangular sclereids (Fig.6.3) are mutual in the powder. The sclereids have wide cell lumen, thick lignified walls and canal-like simple pits. (Fig.6.3) [19].

3. Fibers (Fig.6.1):- Long narrow, thick walled fibers with tapering ends are common in the powder. They have narrow lumen. The fiber in 2.9mm long and 100µm thick [20].

4. Parenchyma cells: Rectangular and elongated narrow fiber lye shaped. Cubical and squanish epidermal cells are wide spread in the powder. (Fig.6.1, 2). The cells thin walled rectangular and squanish cells have prominent nuclear [21].

5. Vessel elements: Long cylindrical and short barrel shaped vessel elements have horizontally elongated spindle shaped, dense multi seriate lateral wall pits. The end wall perforation is circular, wide and horizontal. The length of the vessel elements, range is from 200µm to 500µm; the width is 100-150µm.

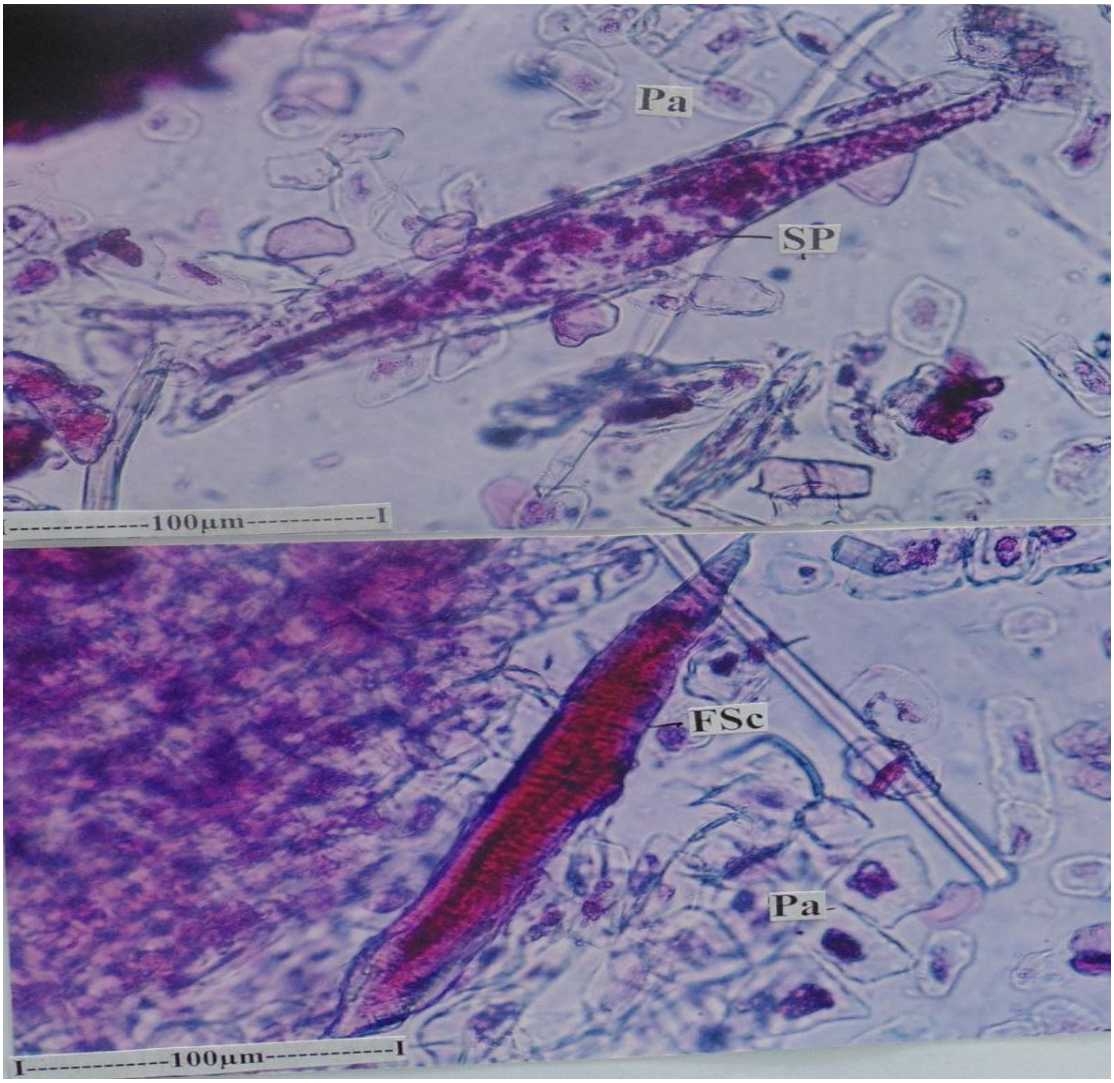


Fig. 5.1: Spindle shaped parenchyma cell seen in the powder

Fig. 5.2: Fusiform sclereid:

(FSC: Fusiform sclereid; Pa: Parenchyma cells; SP: Spindle shaped Parenchyma ;).

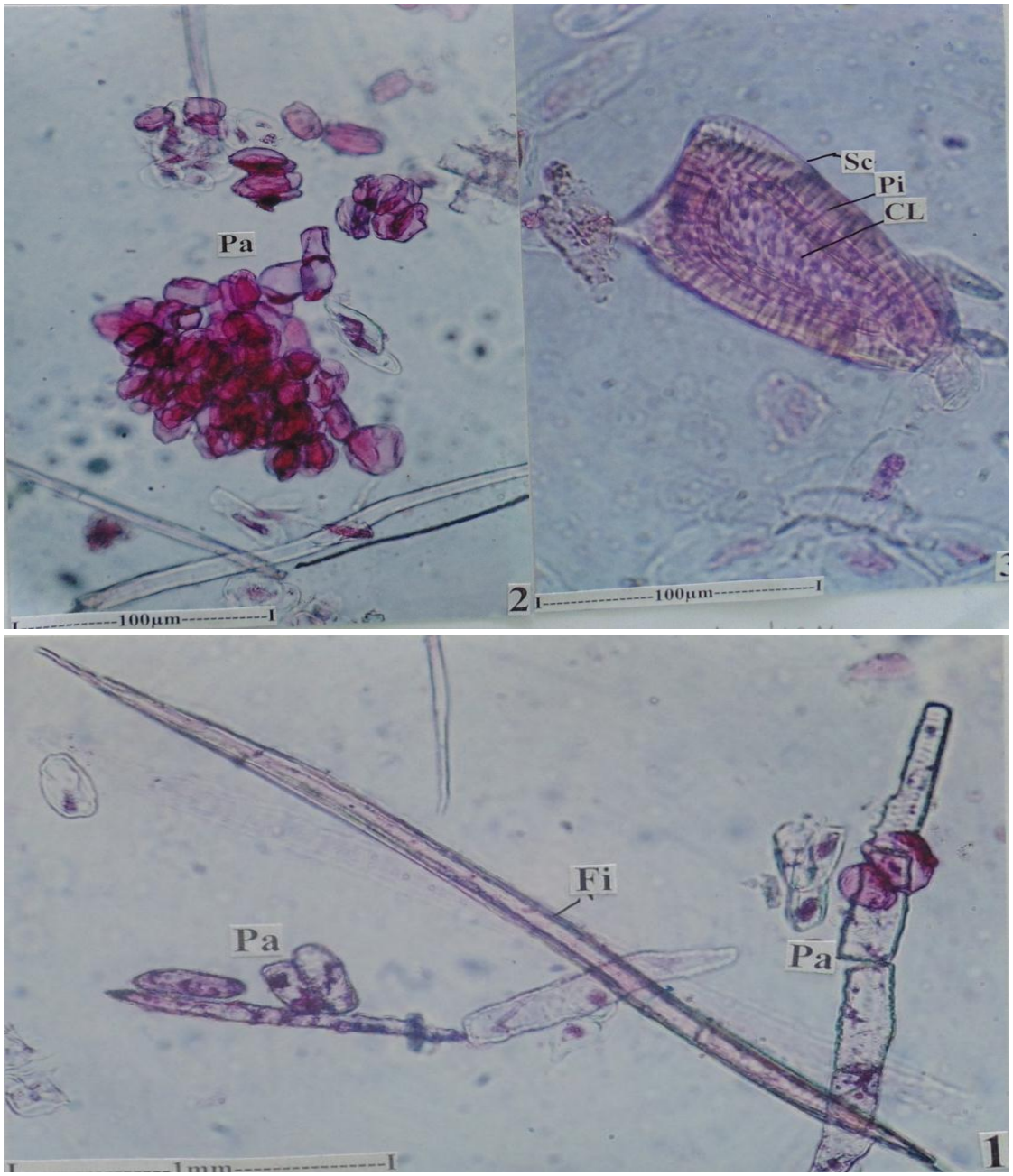


Fig. 6.1: Fiber and Parenchyma cells.

Fig. 6.2: Parenchyma cells;

Fig. 6.3: Rectangular sclereid.

(CL: Cell Lumen; Fi: Fiber; Pa: Parenchyma; Pi: Pits; SC: Sclereid;).

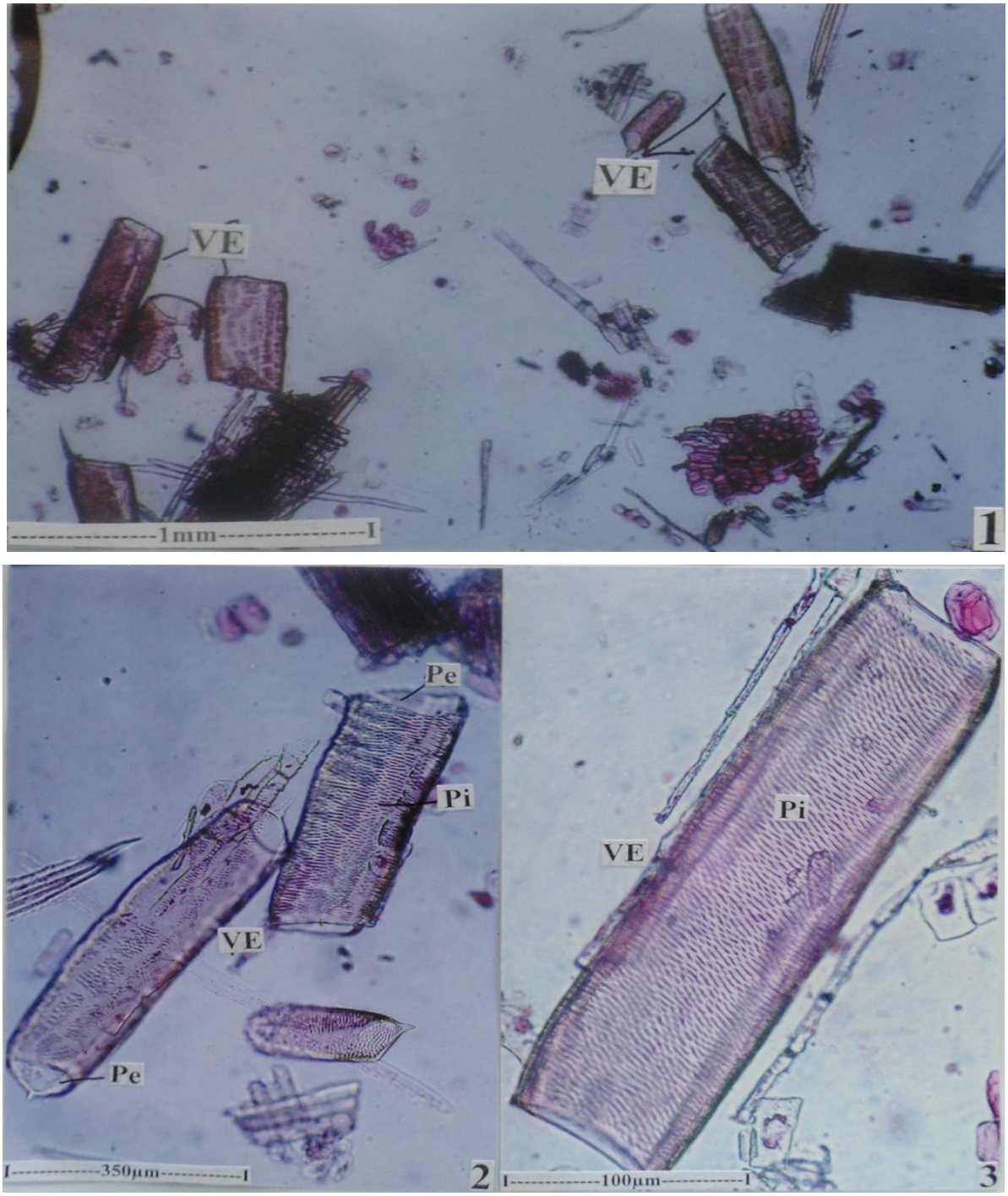


Fig. 7.1: Different types of vessel elements.

Fig. 7.2, 7.3: Long cylindrical vessel Elements.

(Pe: Perforation; Pi: Pits; VE: Vessel Elements ;).

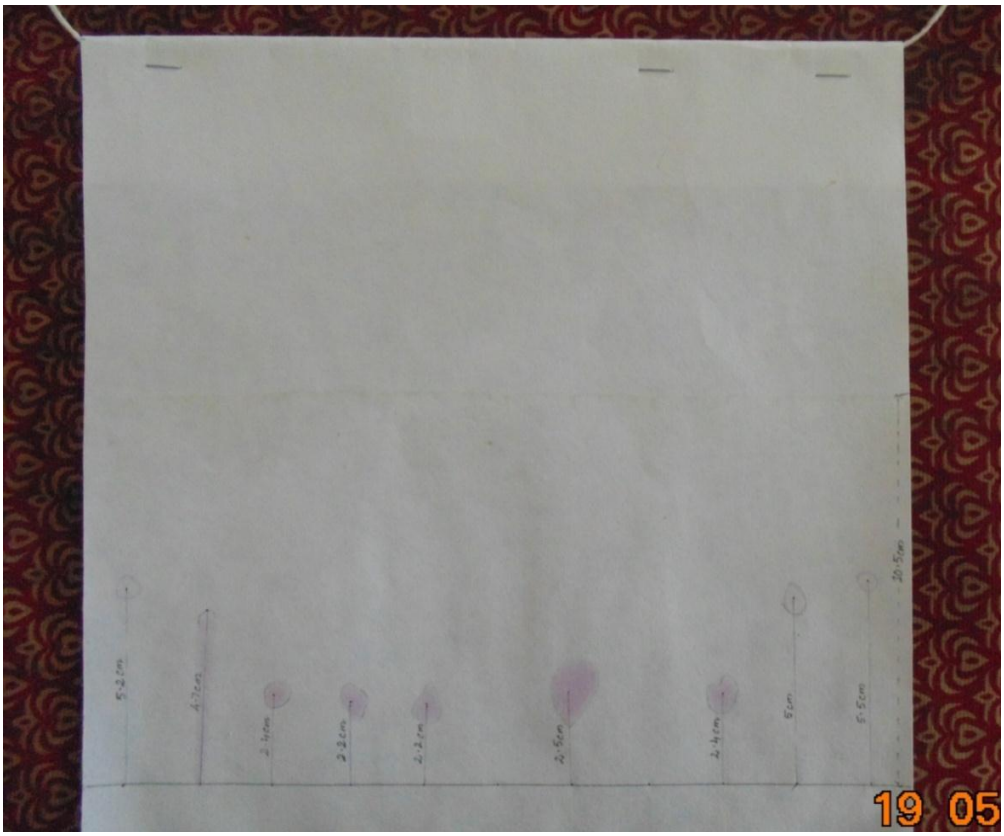
Physiochemical studies

Different parameters for the determination of standardization such as Total ash (15.85%), Water soluble ash (14.76%), Acid soluble ash(11.31%), Sulphated ash (15.4%), Loss on drying (7.76%), Water soluble extractive (3.96%), Alcohol soluble extractive (2.88%) and Crude fiber content (26%).

Preliminary phytochemical screening

The various extracts of crude drug was subjected in to the preliminary phytochemical analysis using chemical standard methods which mainly exposed the presence of Aleurone grains, Test for alkaloids, Amino acids, Lignin, Volatile oil, Fats & Fixed oils, Flavonoids, Glycosides, Tannins, Proteins and Steroids & Triterpenoids.

Chromatography technique



Prepared the solution of 1% various extracts of crude drugs in separately. That were applied on the paper and achieved the chromatogram by using the mobile phase solvent.

Fig. 8: Paper chromatography results of various extracts and standard amino acids

STANDARD:

1. DL – Valine
2. L - Tyrosine
3. L - Glutamic acid
4. DL – Serine
5. DL – Lysine

SAMPLES:

6. Ethyl Acetate
7. Aqueous
8. Benzene
9. Pet. Ether
10. Ethanol
11. Chloroform

The number of spots, color changes and which were matched all reported in the table.

Table 1: Extracts contain Amino acids

S. NO:	EXTRACTS	STANDARD SOLUTION (Amino acids)
1.	**Benzene**	L - GLUTAMIC ACID
2.	**Ethyl Acetate**	L - GLUTAMIC ACID
3.	**Ethanol**	DL – VALINE
4.	**Aqueous**	L – TYROSINE

Amino acids are present in the given extraction of **Benzene, Ethyl Acetate, Ethanol** and **Aqueous.**

Antimicrobial study

Antimicrobial study of various extracts of research plant compared with Tetracycline.

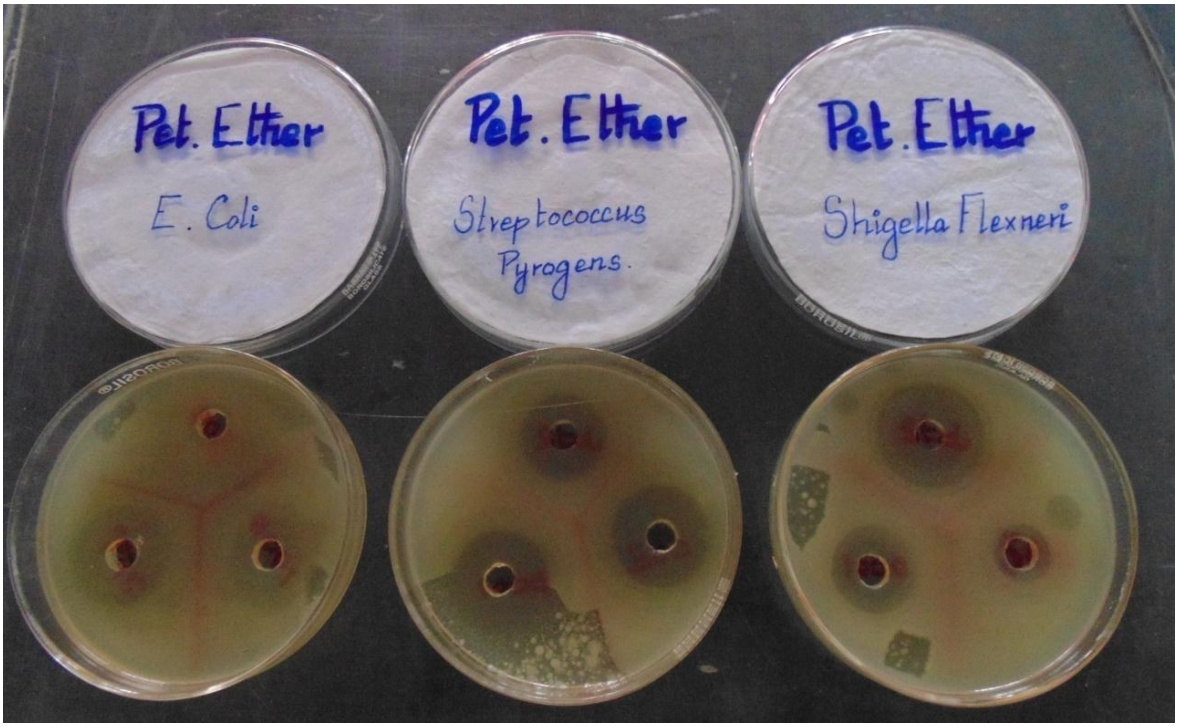


Fig. 9: The Antibacterial activity of *Zanthoxylum rhetsa* (Roxb.) DC Pet. Ether extract.

Table 2: Antibacterial Response of Pet. Ether extract

ORGANISM	ZONE OF INHIBITION		
	STANDARD	PET. ETHER EXTRACT	
		500 µg/ml	1000 µg/ml
E-coli	25mm	10mm	28mm
Streptococcus pyrogens	28mm	20mm	30mm
Shigella flexneri	35mm	16mm	24mm

Results of antibacterial screening the **1. Pet. Ether** extracts of ***Zanthoxylum rhetsa* (Roxb.) DC.** at the concentration of 1000µg/ml, confirmed antimicrobial activity against ***E-coli*.**

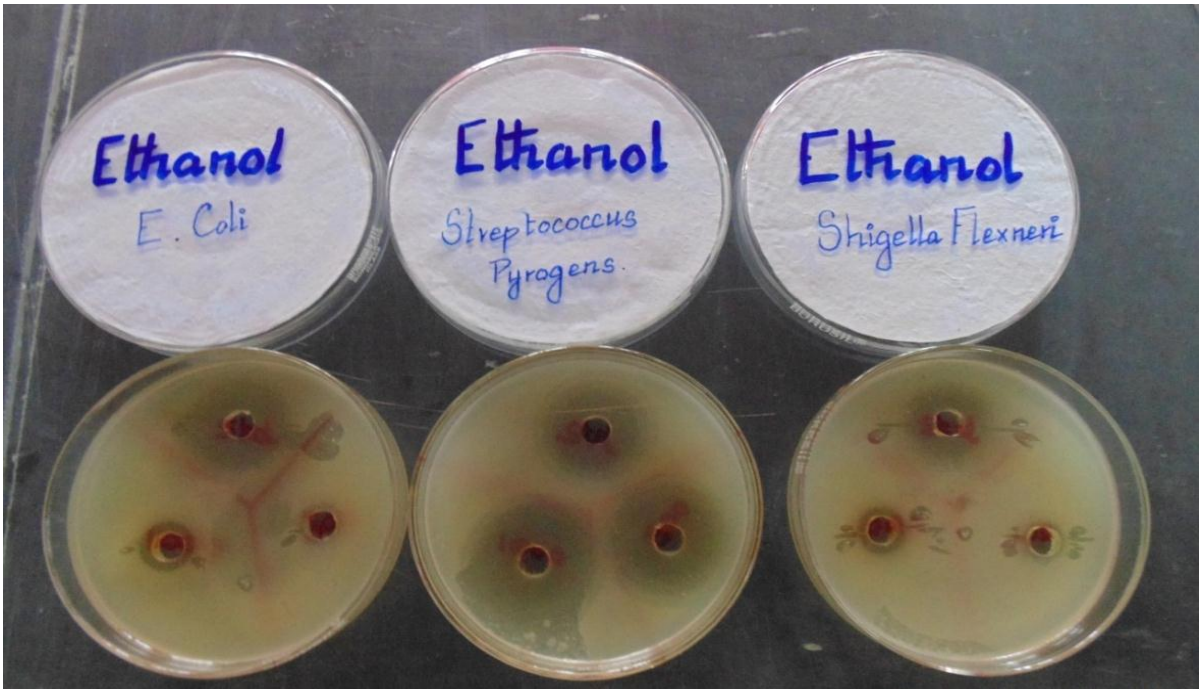


Fig. 10: The Antibacterial activity of *Zanthoxylum rhetsa* (Roxb.) DC Ethanol extract.

Table 3: Antibacterial Response of Ethanol extract

ORGANISM	ZONE OF INHIBITION		
	STANDARD	ETHANOL EXTRACT	
		500 µg/ml	1000 µg/ml
E-coli	24mm	10mm	14mm
Streptococcus pyrogens	30mm	35mm	36mm
Shigella flexneri	36mm	10mm	15mm

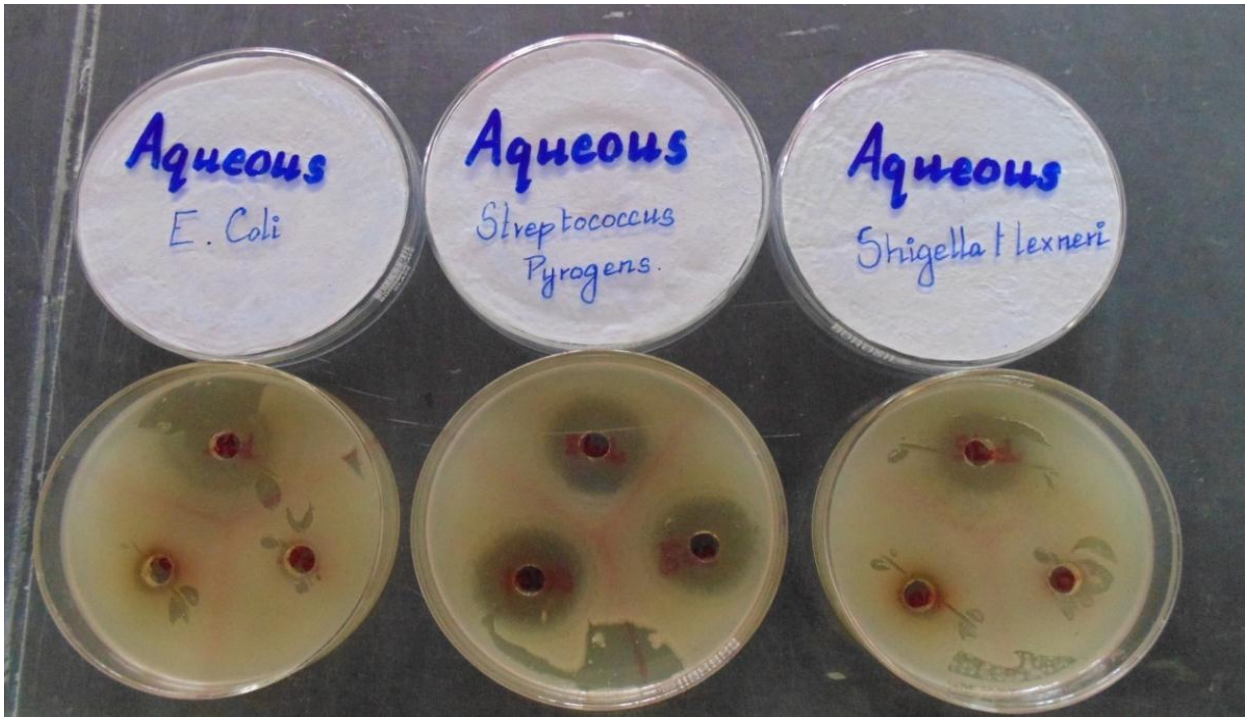


Fig. 11: The Antibacterial activity of *Zanthoxylum rhetsa* (Roxb.) DC Aqueous extract.

Table 4: Antibacterial Response of Aqueous extract

ORGANISM	ZONE OF INHIBITION		
	STANDARD	AQUEOUS EXTRACT	
		500 µg/ml	1000 µg/ml
E-coli	28mm	11mm	14mm
Streptococcus pyrogens	32mm	33mm	36mm
Shigella flexneri	36mm	8mm	21mm

Results of antibacterial screening the **1. Ethanol, 2. Aqueous** extracts of ***Zanthoxylum rhetsa* (Roxb.) DC**. at the concentration of 500µg/ml, confirmed antimicrobial activity against ***Streptococcus pyrogens*.**

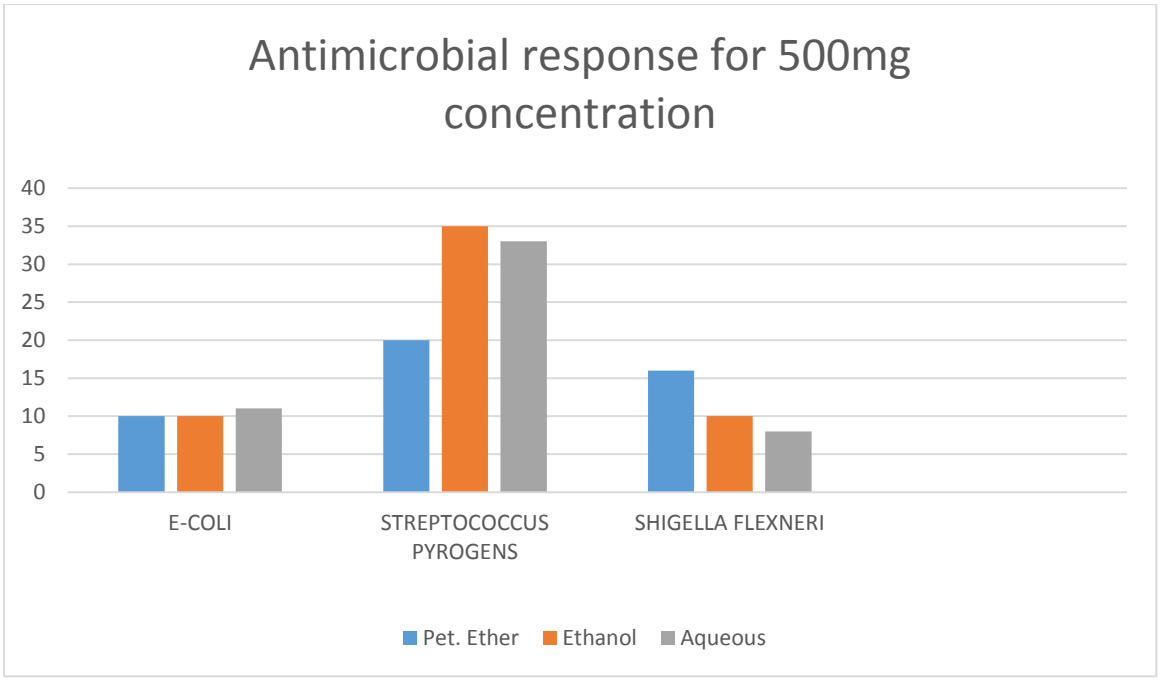


Fig. 12: Antimicrobial Screening response of 500mg concentration of Extract drug

Results of antibacterial screening the **1. Pet. Ether, 2. Ethanol, 3. Aqueous,** extracts of ***Zanthoxylum rhetsa* (Roxb.) DC**. at the concentration of 1000µg/ml, confirmed antimicrobial activity against ***Streptococcus pyrogens*.**

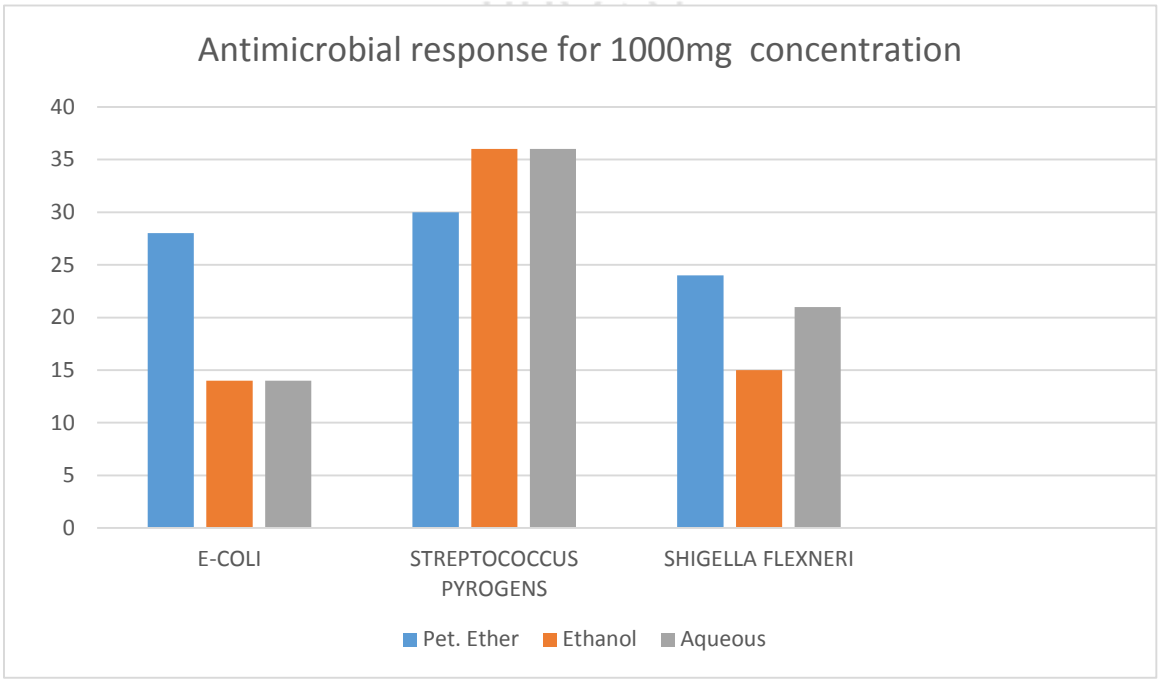


Fig. 13: Antimicrobial Screening response of 1000mg concentration of Extract drugs

CONCLUSIONS

Pharmacognostical investigation

The stem stands thick conical, woody thorns. The surface of stem bark is pale brown and shallow fissured. The stem consists of thin superficial periderm, wide cortical zone with sclerenchyma masses, wide, continuous secondary phloem and thick and dense secondary xylem. On the surface of periderm was darken by stained layer of crushed epidermal and sub epidermal tissues. The intact periderm was 100µm thick. These are thin layer of phelloderm containing three or four layers of cells. Cortex is wide parenchymatous. These are wide, circular or elliptic secretory cavities situated in the cortical zone. Secondary phloem is wide and well preserved. In consists of compact parallel lines of small, polygonal sieve elements and phloem parenchyma cells. Xylem fibers are narrow, thick walled and lignified. They are in compact circular rows. The lumen of fibers are much reduced. The powdered materials of the plant (Stem and Bark) shows different types of parenchyma cells were abundant in the powder. Fusi from sclereids, brachys derides which are iso diametric and sclereids of fusiform shape and rectangular sclereids were common in the powder. Fibers are long narrow, thick walled fibers with tapering ends were common in the powder. Parenchyma cells rectangular and elongated narrow fiber lye shaped. Vessel elements are long cylindrical and short barrel shaped vessel elements have horizontally elongated spindle shaped, dense multi seriate lateral wall pits.

Physico – chemical standards

Physico - chemical standards such as total ash, water soluble ash and sulphated ash, loss on drying, water soluble extractive, alcohol soluble extractive and crude fiber content in percentage were estimated.

Phyto – chemical investigation

The dried powder of stem was extracted by continuous hot percolation (**Soxhlet** apparatus) with different solvents by increasing polarity and percentage of extracts were calculated. The various extracts were subjected to Phyto-chemical staining the extracts were confirmed the presence of **Aleurone grains, Alkaloids, Amino Acids, Lignin, Volatile oils, Fats & Fixed Oils, Protein, Steroids & Triterpenoids, Flavonoids** and **Glycosides.** All the extract

subjected to Paper Chromatography **(PC)** was confirmed the presence of Amino acids are present in the given extraction was found to be **Benzene, Ethyl Acetate, Ethanol, Aqueous.**

Antibacterial activity

From the obtained results it was observed that the Pet. Ether, Ethanol and Aqueous extracts have significant effect which is comparable to that of standard antibacterial drug Tetracycline. The observations will stimulate further researches in these fields and also in the clinical applications of the phytochemical constituents of this drug.

Acknowledgments

The authors are very thankful to Parents for keep on supporting and project guide, Institute of herbal Botany Plant Anatomy Research Centre, West Tambaram, Chennai and Vinayaka Mission's College of Pharmacy, Salem for availed the necessary facilities to carry out the work.

Conflicts

We declare that we have no conflict of interest.

REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Vol.I India: International book distribution; 1995.
2. The Wealth of India: A dictionary of Indian Raw Materials and Industrial Products. Raw Materials. Vol VII. Publication & Information Directorate CSIR New Delhi; 2003.
3. Sass, J.E. (1940) Elements of Botanical Microtechnique. McGraw Hill Book Co., New York, 222.
4. Kokate CK. 4th ed. New Delhi: Vallabh Prakashan; 2005. Practical Pharmacognosy; P.No: 107–111.
5. Vasudev Pai*, Rudraprabhu.V.Savadi, Anant.Bhandarkar, Pharmacognostic and Phytochemical Investigation of stem bark of *Zanthoxylum rhetsa*, Pharmacognosy journal, Vol, 1, Issue 1, 33-36, 2009.
6. WHO-Geneva, Quality control methods for medicinal plant materials, (A.I.T.B.S.Publisher and Distributors, New Delhi, 2002) 8-24.
7. The Herbal Pharmacopoeia, Revised ed. (Indian Drug Manufacturers Association, Mumbai, 2002) 491-502.
8. Vinod D. Rangari, Pharmacognosy & Phytochemistry, Vol-1, (Pag:7,10,12,13,14,16, 20,21,22,23,24-27) 3rd Edition, Jan- 2012.ISBN : 978-81-88739-98-1.
9. Dr. Mity Thambi, Phytochemical investigation of conical prickles on the stem bark petroleum ether extract of Zanthoxylum rhetsa; Journal of Chemical, Biological and physical science: Sec A, Nov 2014- Jan 2015;Vol.5,No:1; 129 – 133 : ISSN: 2249 – 1929.
10. Bryan M. Ham, Aihui MaHam, 1st ed. Text book of analytical chemistry, ISBN-13: 978-1118714843.
11. A.H. Beckett J.B. Stenlake practical pharmaceutical chemistry, IIIrd Edition, Vol-II, P.86-108
12. Adegoke, Anthony, A., Adebayo-Tayo., Bukola, C., 2009. Antibacterial activity and photochemical analysis of leaf extracts of Lasienthera Africantum. African Journal of Biotechnology 8 (1), Page: 077-080.
13. Easu, K. 1964. Plant Anatomy John Wiley and Sons. New York. PP.767. Easu, K. 1979. Anatomy of Seed Plants. John Wiley and Sons. New York. PP. 550.
14. Gamble, J.S 1935. Flora of the Presidency of Madras. Vol. I, II, & III. Botanical Survey of India, Calcutta, India.
15. Johansen, D.A. 1940. Plant Microtechnique. Mc Graw Hill Book co; New York. PP.523.

16. Mathew, K.M. 1983. The flora of Tamil Nadu karnatic Vol-I. Polypetalae. PP.688. Vol.3. Gamopetalae & Monochlamydae PP. 689-1540. The Ranipa Herbarium, St. John's College, Tiruchirappalli, India.
17. Metcalfe, C.R. and Chalk, L. 1950. Anatomy of the Dicotyledons. Vol. I & II. Clarendon Press, Oxford.
18. Metcalfe, C.R. and Chalk, L. 1979. Anatomy of the Dicotyledons. Vo1.I. Clarendon Press, Oxford. PP.276.
19. O'Brien, T.P; Feder, N. and Mc Cull, M.E. 1964. Polychromatic staining of plantcell walls by toluidine blue-O. Protoplasma; 59:364-373'
20. Sass, J.E. 1940. Elements of botanical microtechnique. Mcgraw hill book co; New York. PP.222.
21. Wallis, T.E.1985. Text Book of Pharmacognosy, CBS publishers and distributors, shahdara, Delhi, India.
22. Trudy H.Grossman, Tetracycline antibiotics and Resistance, journal of Cold spring harbor perspectives in medicine, 2016 Apr; 6(4): a025387.