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PHARMACOGNOSTICAL EVALUATION OF *IXORA PAVETTA* (ANDR.) BARK

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ABSTRACT

Ixora pavetta Andr. (*I.Pavetta*) is commonly known as torch tree, belongs to family Rubiaceae. This species is found in South Asia and commonly distributed in Ballari district of Karnataka, India. It belongs to order Gentianales, Genus *Ixora* and Species *Pavetta*. The tree is about 10 m of height; bark is thick, leaves are dark brown or blaze pink; branchlets are woody. The bark is used in treatment of various disorders by folk healers. It has been scientifically evaluated for its antimicrobial and antioxidant properties. Thereafter, pharmacognostical studies of bark have not been carried out so far and hence the present study was undertaken to evaluate pharmacognostical characters of bark of *I.Pavetta*. The study was mainly focussed on morphological, microscopical and physicochemical parameters using standard methods. The study revealed the presence of many essential anatomical characteristics in the bark and established as the first report on pharamacognostical evaluation of *I.Pavetta*. The study indicated towards the future research and is also useful for identification and authentication of the plant.

Keywords : *Ixora pavett*(Andr.) Morphology, Microscopy, Physicochemical, Pharmacognosy.

Introduction

Plants are used as medicine to maintain human health from ages (Upadhya *et al*., 2012). Plants are also major natural sources of medicinal compounds in current Pharmacopoeias (Kingston, 2011). Indian Materia Medica includes about 2000 drugs of natural origin and most of them are derived from different traditional system and folklore practices (Narayana *et al*., 1998). However, there are large numbers of plants, which have not been mentioned in these reports, in spite of their usage in the traditional and folk medicinal systems. Every plant has their unique nature with respect to its botany, chemistry and therapeutic potency and it is essential to study pharmacognostic characters of a medicinal plant, not only for its proper identification, but also to understand its structure and biology (Khandelwal, 2003).

Of late, bark of *I.Pavetta* plant was selected in the present investigation. It belongs to kingdom-plantae, Division-tracheophyta, Class-magnoliopsida, Order-gentianales, Family-Rubiaceae, Genus-*Ixora*, Species-*pavetta*. It is Evergreen tree, to 10 m high, bark 5-6 mm thick, dark brown; blaze pink; branchlets woody. Leaves are simple, opposite, decussate; stipules interpetiolar, ovate-acuminate; petiole 4-8 mm long, stout, glabrous; lamina 6-14 cm x 3.5-7.5 cm, obovate, elliptic, elliptic-obovate, obovate-oblong, base subcordate, obtuse or round, apex obtuse, margin entire, glabrous, thick coriaceous; lateral nerves 10-15 pairs, pinnate, very slender, prominent beneath; reticulate, slender. Flowers bisexual 5-6 mm long, white, in terminal panicles; calyx truncate, 4 teethed, teeth minute; corolla tube 6 mm long, lobes 4, 4 x 2 mm, oblong, recurved; stamens 4, attached to the mouth of corolla, anthers sagittate, ovary 1.7 mm, 2-celled, inferior, ovule one in each cell; style excreted; stigma bifid. Fruit a berry, 5-6 mm across, globose, succulent, black, pyrenes 2, planoconavex (Dontha *et al*., 2015).

The available literature revealed that, no such studies have been carried out for pharmacognosy details as well as phytochemical screening of bark of *I. pavetta*. Hence in the present investigation, *I. pavetta* was studied for its pharmacognostical characteristics.

2. Materials and Methods

2.1. Collection and authentication of plant

Bark of *I.Pavetta* was collected from Dandeli forest, Dharwad, Karnataka, in the month of june-2021. The plant specimen was authenticated by botanist Dr S.S. Hebbar, Department of Botony, Govt P.U.C College, Dharwad and herbarium was deposited at S.E.T.C.P.D, Dharwad, Karnataka, India for future reference (SETCPD/Ref/38/2021).

2.2. Chemicals and reagents

All chemicals, reagents and solvents used during the experimentation were of analytical grade. The plant specimens for the proposed study were collected from Dandeli forest. Care has been taken to select healthy plants and normal organs.

2.3 Preparation of plant specimens

The required samples of different organs were cut and removed from the plant and fixed in FAA (mixture of 5 ml of

Formalin, 5 ml of acetic acid and 90 ml of 70% ethylalcohol). After 24hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the procedure (Sass, 1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (M.P-58-60^{0}c) until tertiary butyl alcohol solution attains super saturation. The specimens were cast into paraffin blocks.

2.4 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 carried out by customary procedure (Johansen, 1940). The section was stained with toludine blue as per the method published by (Brien *et al*., 1964). Where ever necessary, the section were also stained with safranin and fast-green and Iodine in potassium iodide for starch.

For studying the transverse section of bark it is first cleared with 5% sodium hydroxide or by partial maceration employing Jeffrey's maceration fluid (Sass, 1940). Thin sections were selected and mounted with glycerine for cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell components were studied and measured.

2.5 Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 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 birefringent 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 were written as given in the standard anatomy books (Easu, 1964).

2.6 Macroscopic and Microscopic analysis

Key morphological features of the bark were observed during macroscopic analysis using dissecting microscope (Labomed, India). Transverse section (TS) of the bark was taken using LEICA CM (1850) cryostat. For this, fresh plant material was mounted on the specimen disk covered with tissue freezing medium. The specimen disks were kept for freezing at −18 ± 2 °C for about 30 min. Frozen plant materials were used for sectioning at a thickness of 20 ± 2 microns. Histochemical and powder studies were carried out by using reagents and stains like iodine, concentrated sulphuric acid, concentrated hydrochloric acid, ferric chloride, Sudan III, ruthenium red and phloroglucinol with Conc. HCl (1:1) (Mathew, 1983). Similarly, organoleptic characters like colour, odour and taste were determined for the bark powder.

2.7 Microphotography

Microphotographs of the sections and powder microscopy were taken using microscope (Olympus BX 41) at different magnifications (4×, 10× and 40×) with inbuilt analogue camera (ProgRess C3-JENOPTIK). Computer images were captured using software ProgRes® CapturePro 2.1.1-JENOPTIK laser optical system.

2.8 Preparation of extracts and preliminary phytochemical analysis

The powdered bark was soxhlet extracted by using 90% alcohol and water. Bark powder (5 g) was extracted with 20 mL of respective solvent on a shaker at room temperature at 120 ± 10 rpm for overnight. The same was filtered and evaporated to dryness. The extracts were stored at 4 °C for further use. These extracts were subjected for preliminary phytochemical screening as per standard Pharmacognostic methods ((Khandelwal, 2003, 2007).

2.9 Physico-chemical analysis

Physico-chemical parameters of the powdered drug such as total ash, water-soluble ash and acid-insoluble ash were determined as per method described by (AOAC, 1990). Soluble extractive values were determined as per standard procedure. The moisture content was detected by loss on drying method (Metcalfe and Chalk, 1979, Khan and Das, 2019).

Statistical analysis

Cell dimensions were represented as RDS (radius for circle), DST (length of line) and Maj (length of large half axis for ellipse) in microscopy as defined in ProgRes®CapturePro 2.1.1-JENOPTIK, software. Standard deviation was calculated as mean of three replicates using Microsoft Excel (2007) for various physicochemical parameters.

3. Results

3.1. Morphological description

The bark is hard and brittle. The outer surface is light grey coloured with circular brownish dots. The inner surface of the bark is white, the cut end of the bark shows outer dark periderm and inner thick white portion contains secondary phloem (Figure-1). It is Evergreen tree, to 10 m high, bark 5-6 mm thick, dark brown; blaze pink; branchlets woody.

3.2. Anatomical description and powder microscopy

Transverse section of bark was taken for the anatomical study. The sections were stained with Phloroglucinol: conc HCl (1:1) and observed the cross section under the microscope. The bark exhibits outer periderm, inner cortical zone and cortex has secondary phloem. The periderm forms the outer covering zone of the bark and contains phloem cells. The phloem cells are homogenous rectangular thin walled cells. They are arranged in compact parallel vertical lines. They observe like scattered small clusters of sclereids. It occurs in the narrow region of phelloderm, which are tangentially elongated, thick walled and possess dark contents. The phelloderm cells have cellulose walls (Figure-2). Inner side of the phelloderm contains thick cylinder like sclereids, which are small, angular with lignified cells. It is also composed of two or more cylinder like sclereids (Figure-2.1; and 2.2). The cortical zone is much wider comprising mostly parenchymatous ground tissue. Some of the solitary sclereids are much wider with thick lignified walls with radial narrow simple pits (Figure-2.3).

Secondary Phloem: It has wide space and occupies major part of the bark. It is differentiated into outer wider collapsed

phloem and narrow inner noncollapsed phloem along with other cellular characters.

a. **Collapsed phloem**: The collapsed phloem consists of crushed sieves and companion cells, slightly dilated phloem rays along with few sclerides (Figure 3). Collapsed phloems appear as dark, irregular masses. The sclereids are wide, circular and have lamellate thick lignified secondary walls.

b. **Non-collapsed phloem:** It is present in innermost region of the bark. It has intact sieve elements with companion cells, narrow phloem rays and intact parenchyma cells. Wide circular secretory cavities are seen in the non collapsed region (Figures 3, 4.1). The sieve elements are wide or narrow, horizontally rectangular and thick walled cells. It is also composed of small angular companion cells which are associated with sieve elements.

c. **Brody sclerides**: These are randomly distributed in non collapsed phloem. Sclerides are isodiametric in shape, with thick lignified secondary walls with narrow radial simple pits (Figures 5.1, 2) .

d. **Calcium oxalate:** It appear as crystals, thick, isodiametric, sometimes also appear in prismatic shape (Figures 6.1, 2).

e. **Styloid crystals:** These crystals are also calcium oxalate type. They are long, narrow scale like in shape with conical (Figures 7.1, 2, 8.1,2). The styloids are up to 90 µm in length and 8-10 µm in thickness.

T.L.S of the phloem: In TLS view, the phloem rays appear in vertical parallel lines. Rays are seen at different heights, either uniseriate comprising single vertical rows of cells, or less frequently biserriate with two vertical rows of cells (Figures-9.1, 2). The rays are heterocellular, consisting of two types of cells. The cells in the middle of the ray are horizontally rectangular and are called as prominent cells. They are vertically elongated and conical in shape, these are called upright cells (Figures 10.1, 2, 3). The rays have dense of cell mutation and are 60 µm in height, and 30 µm in wide and composed of dark tannins.

R.L.S of Phloem: In RLS view, the rays appear in horizontal lines, resembling bricks of walls (Figures-11.1, 2) Cells are vertical in lines at right angles to rays. The ray cells are thin walled and possess dark, dense tannins. As in the TLS view, In RLS view also rays contain heterrocellular middle prominent cells and terminal upright cells (Figures-12.1, 2, 3).

3.2.3. Powdered microscopy of bark:

Lileriform fibers: Fibers of different forms are seen in the powdered microscopy of IP bark (Figure-13.1). Narrow and wide fibers are seen mixed in the microscopy, narrow fibers are thin, long, with the walls and narrow lumen. The fibers are 1.1 mm in length and 10 µm in thickness (Figures 13.2, 3). The fibers have dense radial lines of simple pits (Figures 14.1, 2, 3). The wide fibers are up to 450 µm in length and 15 µm wide (Figures 15.1, 2). Some of the fibers resemble sclerides, but they are long with tapering ends. The cell walls have dense, narrow with simple pits. These cells are usually known as fibrous sclerides (Figures 16.1, 2).

Crystals: Calcium oxalate crystals are long, appears scale like parallel lateral sides with conical ends. These crystals exhibit fire fringent property and appear

as bright white under polarized light (Figures 17.1,2). The styloids are up to 150 µm in length and 15 µm in thickness.

3.3. Histochemical analysis

The bark was treated with different reagents to know various cell components. The results are presented in Figures-17.1 ,2. Bark showed the presence of calcium oxalate crystals soluble in Conc. HCl with lignin and cellulose. Since toluidine blue is a polychromatic stain. The staining results were remarkably good, 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.

3.4. Organoleptic characters

Dried bark of *I.Pavetta* bark and its powder has brown colour without any specific odour with astringent taste.

3.5. Physicochemical parameters

The physiochemical characters such as total ash, acid soluble ash, moisture content, and extractive values in ethanol and water of dried bark powder were calculated in terms of air dried sample. The results were presented in (Table 1). Total ash plays an important role in evaluation of purity of drugs and for IP bark powder it was 11.6% w/w. Acid insoluble ash was found to be 1.50 %, Water soluble ash 5.03 % and moisture content was found to be 8.23%, respectively. Quantitative estimation of extractive values was represented as percentage yield. Soluble extractive percentage yield for bark was higher in ethanol (15.61% w/w) than in water (10.24% w/w). The results showed greater extractive values on ethanolic extract followed by aqueous extract indicating the concentration of secondary metabolites.

3.6 Phytochemical analysis

Alcoholic and aqueous extracts were subjected to preliminary phytochemical screening and the results revealed the presence of flavonoids, tannins, alkaloids, glycosides, steroids, saponins and triterpenoids in *I.Pavetta* bark extracts (Table 2).

Table 1: Physicochemical parameters of *I. Pavetta* bark

Physicochemical parameters	Percentage yield
Ash value (% w/w)	11.6
Total ash	1.50
Acid insoluble ash	5.03
Water soluble ash	8.23
Moisture	15.61
Soluble extractive values (% w/w)	10.24
Ethanol	11.6
Water	1.50

Table 2: Phytochemical analysis of *I.Pavetta* bark extracts

(+) = Present, (-) = Absent

Sl.no	Phytochemical tests	Alcoholic extract	Aqueous extract
1	Alkaloids	+	-
2	Glycosides	+	+
3	Tannins	+	+
4	Flavonoids	+	-
5	Steroids	+	-
6	Saponins	-	+
7	Triterpenoids	+	-

(Note : + = present, - = Absent)

1: Photograph of *I. pavetta* tree

2:Photograph of bark of *Ixora pavetta*

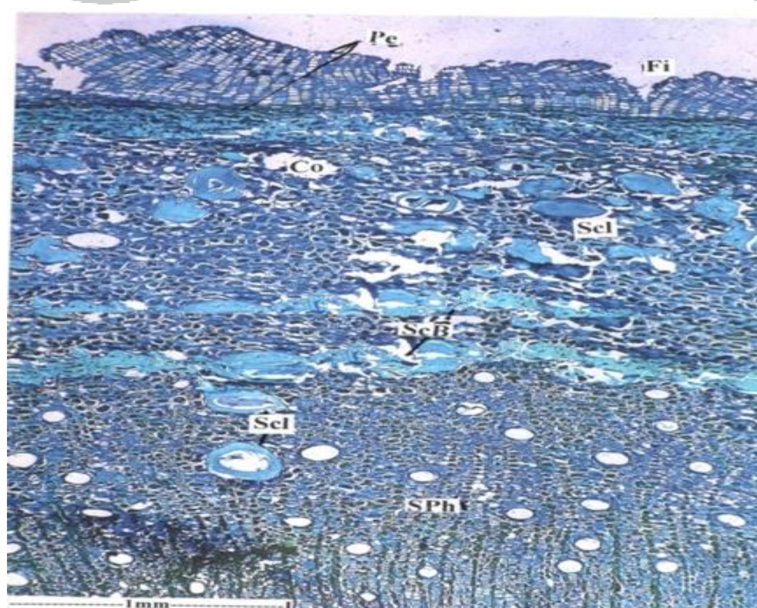


Figure 2: T.S of bark of *Ixora pavetta*

CO-cortex, FI-fissured, PE-periderm, SCB- Scleride band, SCL-sclerides,

SPH- secondary phloem

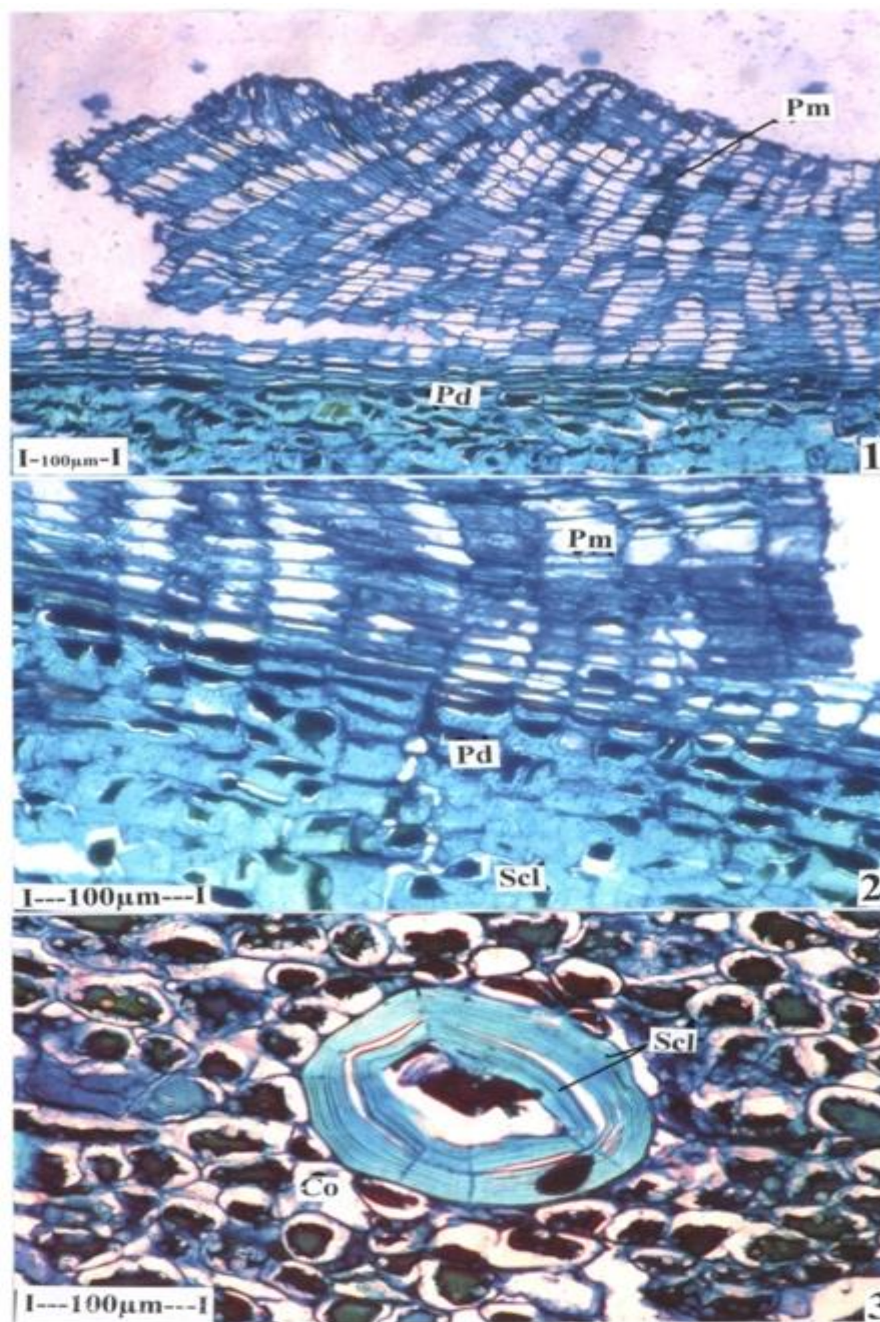
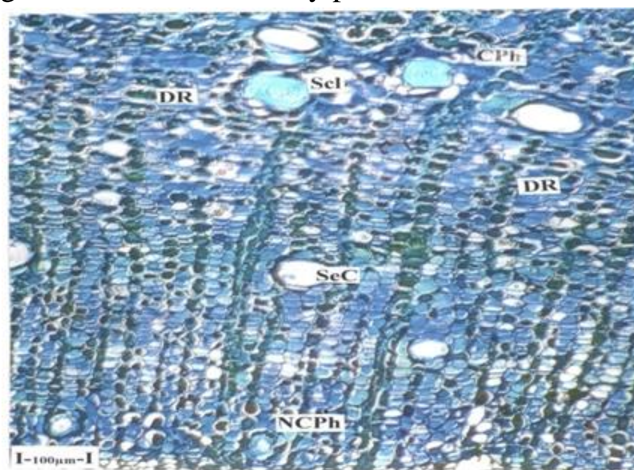


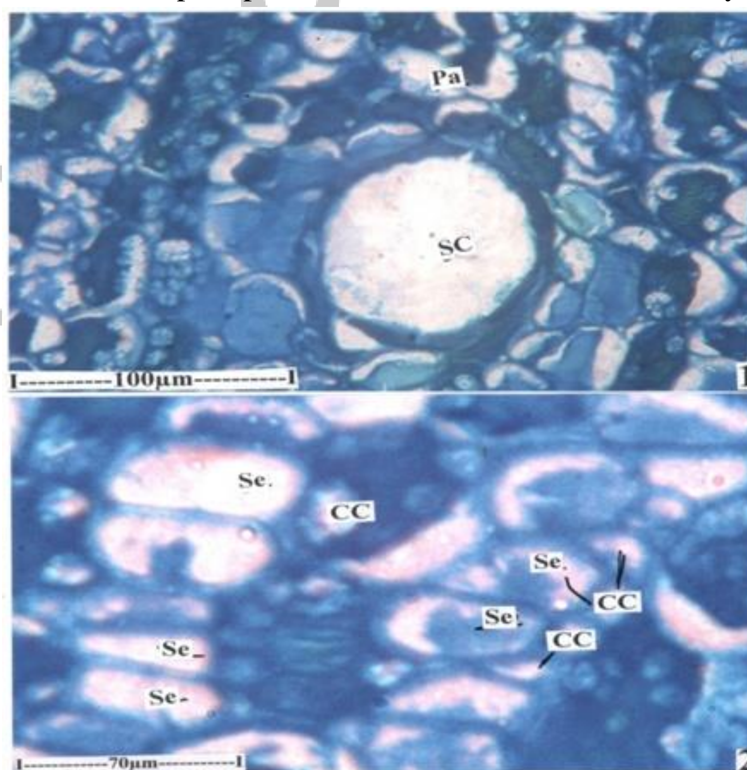
Figure 2.1, 2 ,.3: T.S of periderm showing phloem, phelloderm and sclerides

CO- cortex, PD- phelloderm, PM-phloem, SCL- scleride

Figure 3: T.S of secondary phloem entire view

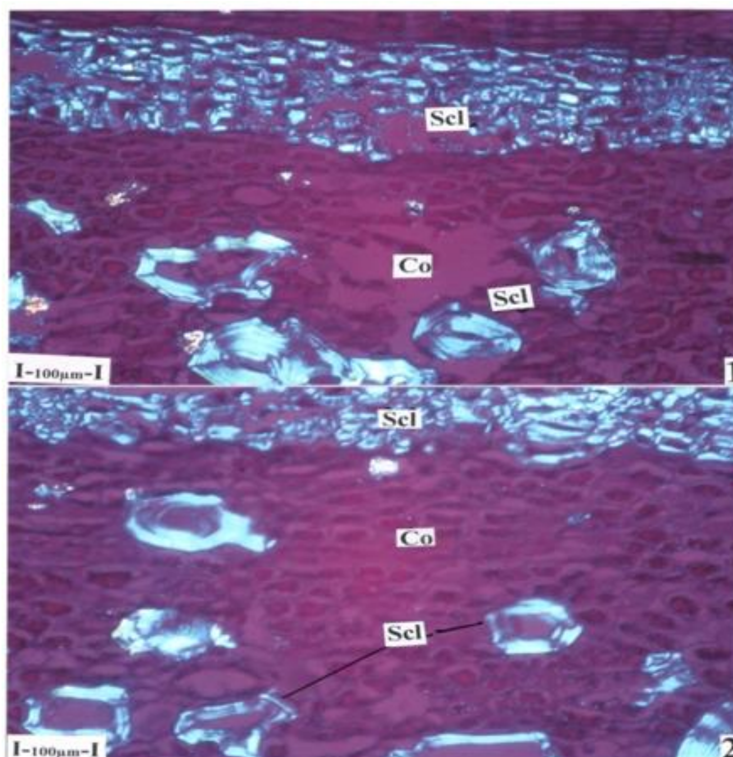


CPH-collapsed phloem, NCPH- non-collapsed phloem, SCL- sclerid, DR- Dilated ray, SC- secretary cavity

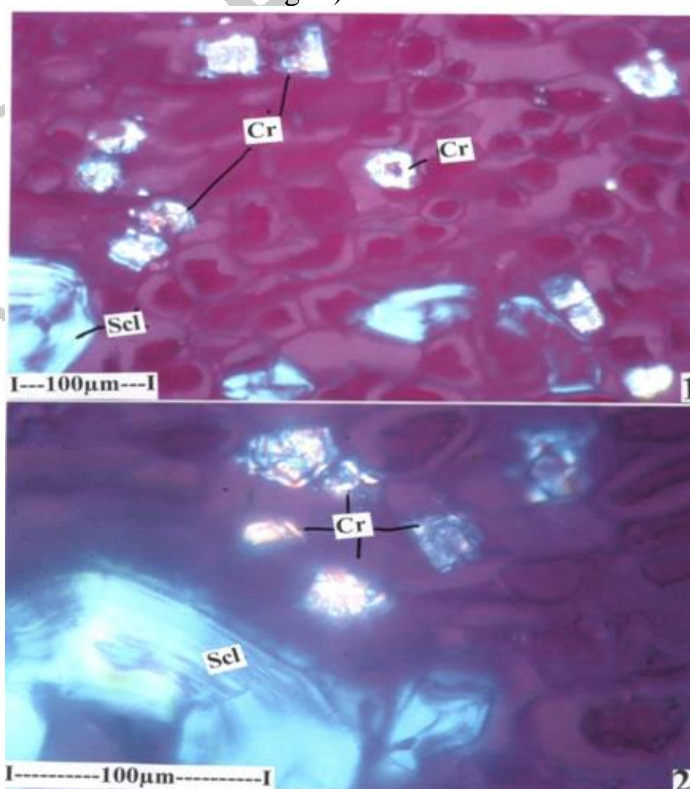


Figures:4.1, 2: T.S of phloem showing secretary cavity

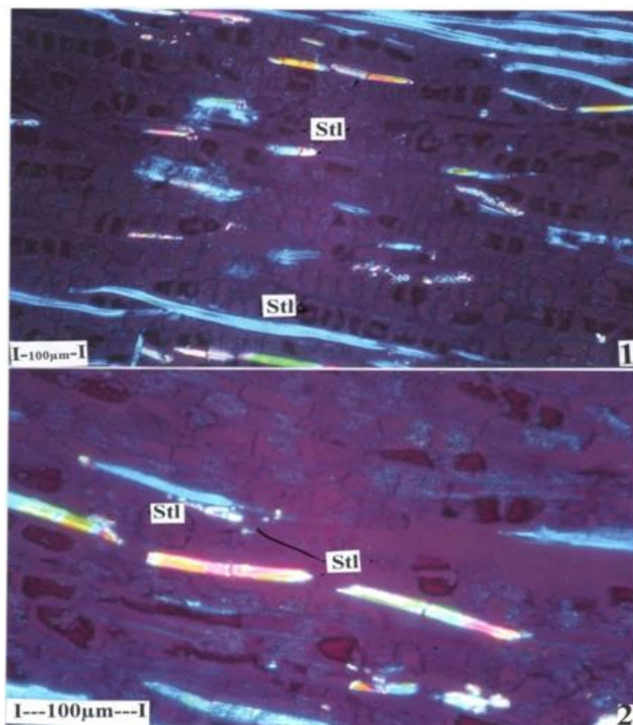
CC- companion cells, SE-sieve elements, SC- secretory cavity, PA- parenchyma.



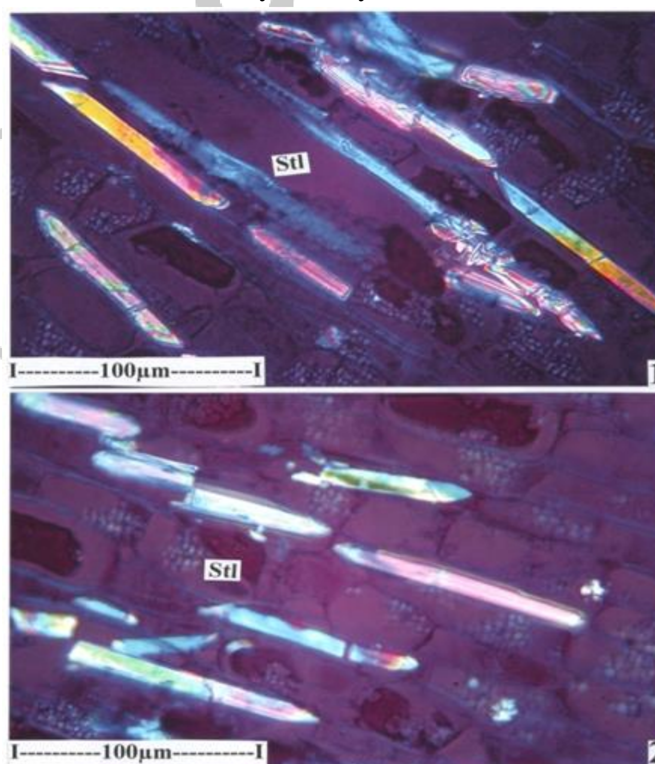
Figures 5.1, 2: T.S of bark showing sclereids band and scattered sclereids in the cortical zone (Polarized light)



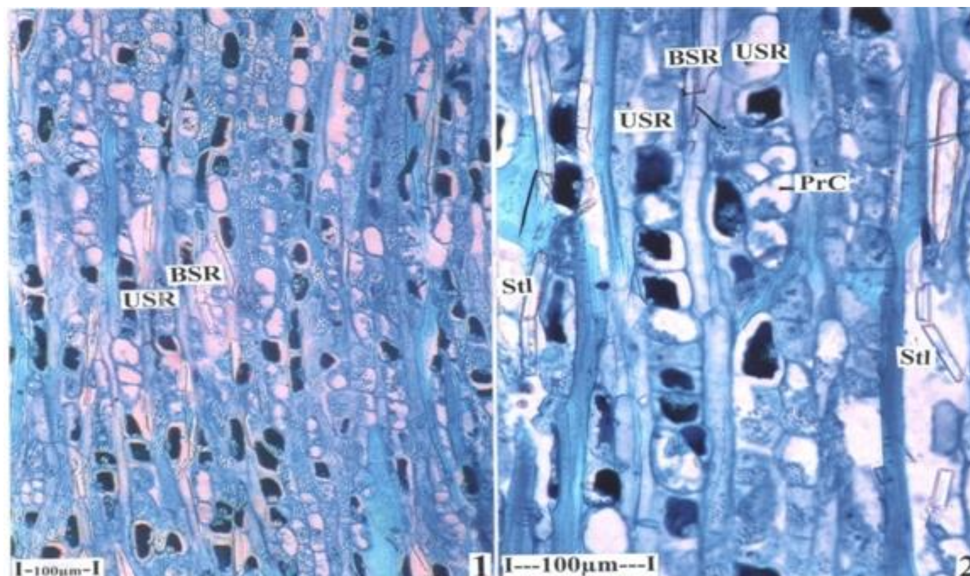
Figures 6.1, 6.2: Distribution of crystals in the ground tissue (Under polarized light)
CO-cortex, SCl-Sclerides



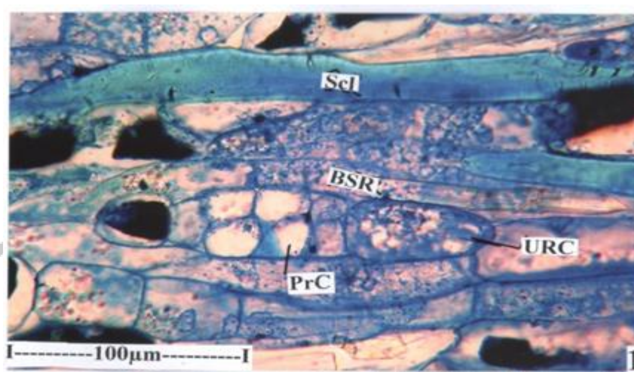
Figures 7.1, 2: T.S of secondary phloem showing styloid crystals in the parenchyma cells (Polarized light)
STL- styloid crystal



Figures 8.1, 2 : T.S of phloem showing styloid crystals in the parenchyma cells
STL- styloid crystal



Figures 9.1, 9.2: Tangential longitudinal section of phloem rays
BSR- Biseriate ray, PRC- prominent cell, PHR- phloem ray, STL- styloid crystal, USR- uniseriate ray, URC- upright cell



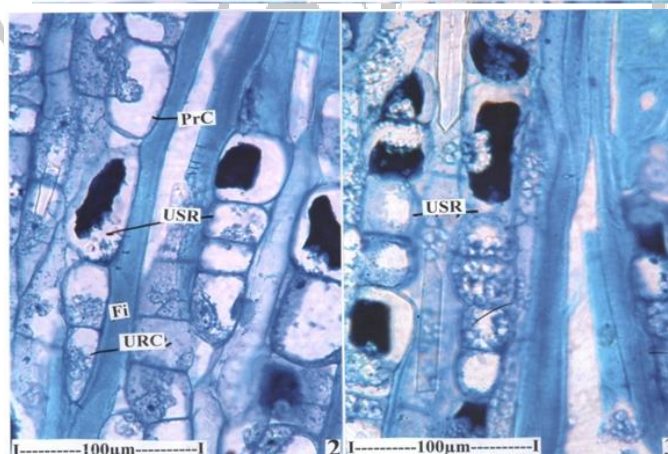


Figure 10.1, 2, 3: TLS of phloem ray enlarged
BSR- Biseriate ray, PRC- prominent cell, PHR- phloem ray, STL- styloid crystal, USR- uniseriate ray, URC- upright cell

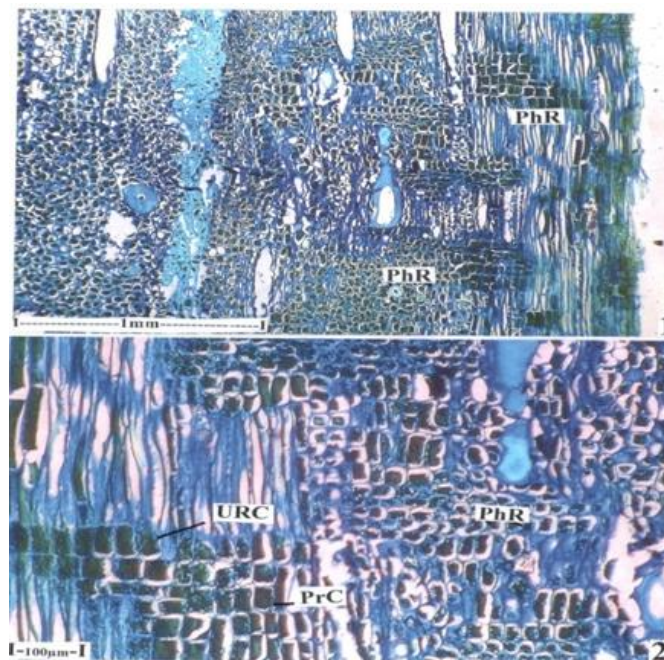
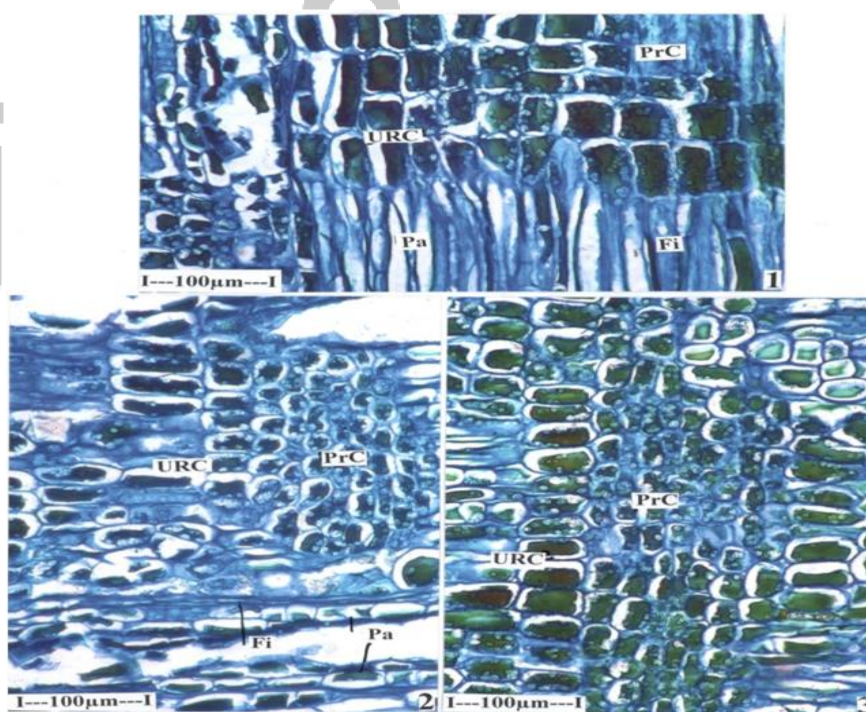
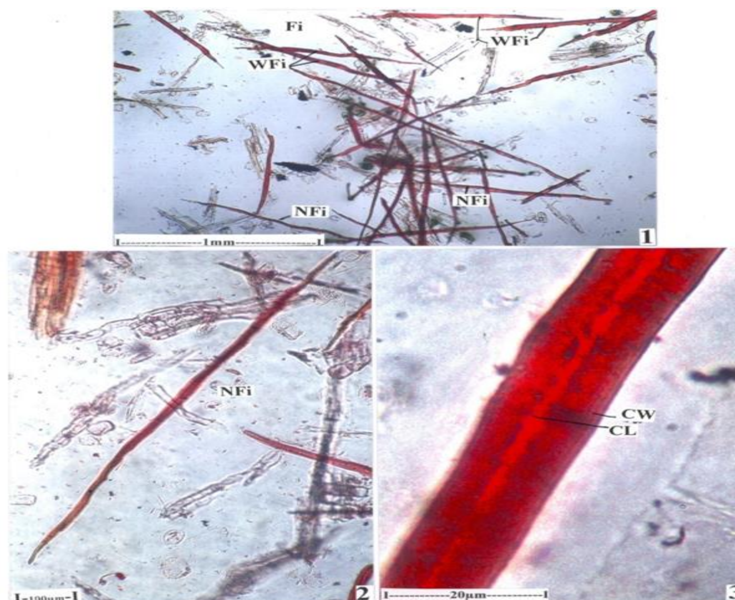


Figure 11.1, 2: Radial longitudinal sections of phloem rays
PHR- phloem ray, PRC- prominent cell, URC- Upright cell

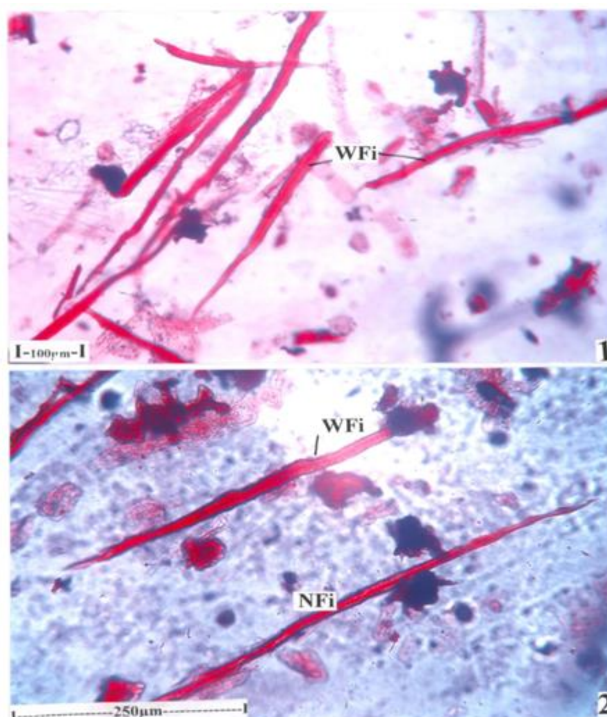


Figures 12.1, 2, 3: RLS of phloem rays enlarged
FI- Fibre, PA-parenchyma, PRC-prominent cell, URC- Upright cell



Figures 13.1,2,3: A cluster of fibers
CW- cell wall, CL- cell luman, NFI- Narrow fibre, WFI- wide fibre, FI- Fibre

Figures 14.1,2,3:Three narrow fibre attached with each other
CL- cell lumen, CW-cell wall, FI- fibres, NFI- Narrow fibres, PI-Pits



Figures 15.1, 2: A group of wide fibres
NFI- Narrow fibres, WFI- Wide fibre

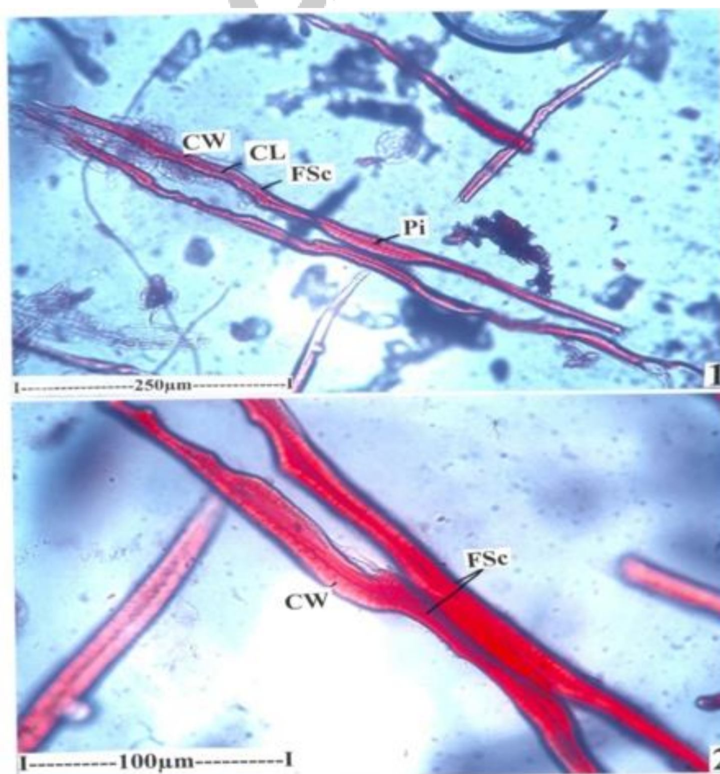


Figure 16.1,2: showing Fibre sclerides
Cl- cell lumen, CW- cell wall, FSC- Fibre sclereids, PI- pits

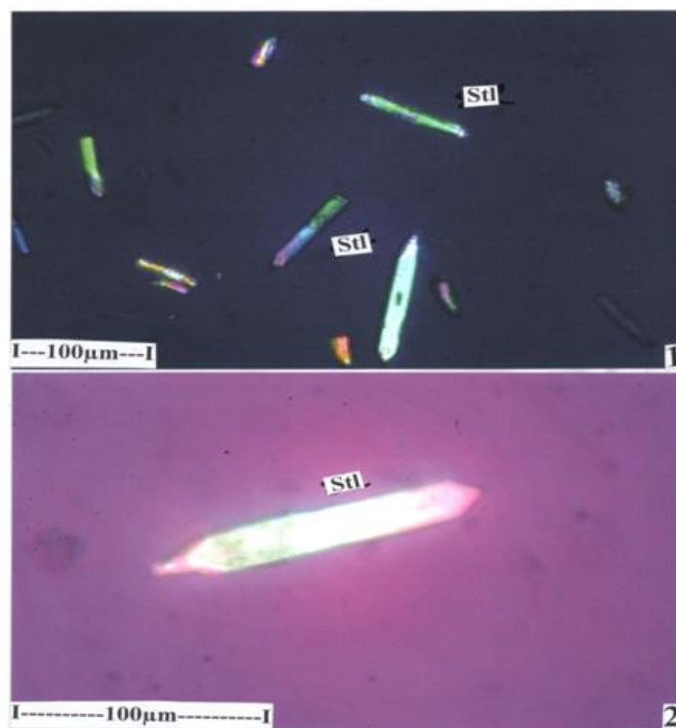


Figure-17.1, 2: Showing Styloid crystals under polarized light
STL- styloid crystals

4. Discussion

In traditional system of medicines (like Siddha, Homeopathy or Ayurveda) *I.Pavetta* used to cure various disorders (Brien *et al.*, 1964). Medicinally the plant is used as hepatoprotective, chemoprotective, antimicrobial, anti-oxidant, antinociceptive, anti–mitotic and anti-inflammatory activities (Glossary, 1992). Pharmacognostical and physicochemical studies, being reliable and inexpensive, play an important role in quality control issues of the crude drug samples (Sass, 1940; Yadav *et al*., 2020). The macro and micro characters observed in the bark of *I.Pavetta* serve as basis for the identification of original plant. Morphologically the bark is hard and brittle. The outer surface is light grey coloured with circular brownish dots. However, the T.S of bark showed the presence of Collapsed phloem, Non-collapsed phloem, Brody sclereids, Calcium oxalate and styloid crystals and

powder microscopy of bark showed the presence of Lileriform fibers and calcium oxalate crystals with conical ends.

Total ash, acid insoluble ash and water soluble ash parameters indicate the presence of inorganic and silica components in the sample studied (Wallis, 1985). The ash content of *I.Pavetta* lies within the range as reported in Ayurvedic pharmacopeia (YogaNarasimhan, 2000). The results of the water and alcohol extractive studies revealed the presence of secondary metabolite in the powder sample with considerable amount of total phenolic content in the bark of *I.Pavetta*, which was further reported to be responsible for *in vitro* antioxidant and hepatoprotective activities (Das *et al*., 2017; Malik *et al*., 2020).

5. Conclusion

Pharmacognostical studies plays important role in plant identity and standardization parameters help to prevent adulterations. Such studies will also assist in authentication of the plants and enables to produce quality herbal products which lead to safety and efficacy of natural products.

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Conflict of Interest

Authors declare no conflicts of interests in a relevant article.

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