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Pharmacognostic Evaluation of *Simarouba glauca* DC Bark

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Authors' contributions

This work was carried out in collaboration between both authors. Author RMRP designed the study, performed the experiments, wrote the protocol, the first draft of the manuscript, and managed the analyses of the study. Author TCT guided and approved study, corrected, finalized, and communicated the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Simarouba glauca DC also known as 'Dysentery Bark' is an important ethnomedicinal plant native to Tropical America. Later introduced India as "Lakshmi-Taru." Bark is a potent anti-microbial, anti-diabetic, anti-cancer, and hemolytic drug. This study presents a pharmacognostic evaluation of *Simarouba glauca* DC bark, mainly shedding light on its macroscopic and microscopic characteristics. Identification, authentication, macroscopic observation, and TLS, RLS and Powder microscopical examinations have been carried out.

The macroscopic examination of the bark revealed distinctive features, including a rough, fissured surface, and a yellowish-brown colour. Microscopic analysis showcased the presence of a thick periderm with small flakes raised above the periderm surface. The outer part of phellem is crushed and the inner portion is modified into a thick cylinder of sclereids. The Phelloderm becomes thick walled and dark coloured and stores calcium oxalate crystals. Large prismatic calcium oxalate crystals located in uniseriate vertical row within thin parenchyma cells. TLS revealed that the

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phloem fibers in dense compact bundles. The fibers being highly thick walled and lignified with narrow cell lumen. The rays are 250-280 µm in height and 40-60µm. RLS showed the rays are rentered heterocellular. Parenchyma cells and fibers are vertical position at right angles to the rays. Bark powder microscopy exhibited the presence of narrow and wide fibers, fiber-sclereids, long sieve elements, thick brachy sclereids, and large quantity of quadrangular, rectangular, and polygonal Calcium oxalate crystals. These observations provide valuable diagnostic tools for the authentication and quality control of *S. glauca* DC bark.

The pharmacognostic characterization presented in this study serves as a foundation for further research and development of *S. glauca* DC bark-based herbal medicines and pharmaceutical formulations. The insights provided in this article contribute to the understanding and utilization of this valuable botanical resource, fostering a bridge between traditional knowledge and contemporary scientific approaches in pharmacognosy.

Keywords: Simarouba; bark macroscopy; powder microscopy; pharmacognostic characters.

1. INTRODUCTION

In the realm of natural products research, the quest for novel bioactive compounds has led scientists to explore the untapped potential of numerous plant species. Among these, *Simarouba glauca* DC, commonly known as "Paradise Tree," “Dysentery Bark” or "Aceituno," has garnered significant attention owing to its diverse pharmacological properties and rich traditional uses in various indigenous healing systems [1]. This tree, native to the American tropics, belongs to the family Simaroubaceae and has been the focus of increasing research interest due to its therapeutic promise. The bark of *Simarouba glauca* has been identified as a valuable source of bioactive compounds with potent medicinal properties. In 1960 this plant is introduced in India as “Laksmi Taru” [2].

The pharmacognostic characterization of plant materials is a pivotal initial step in exploring their therapeutic potential [3]. This process involves the detailed examination and analysis of botanical, macroscopic, microscopic, and chemical attributes of plant parts, facilitating their authentication, quality control, and utilization in traditional and modern medicine [4]. Understanding the pharmacognostic characteristics of *Simarouba glauca* DC bark is indispensable for ensuring the sustainable harvesting and standardized utilization of this valuable natural resource.

In this context, this research article presents a comprehensive pharmacognostic analysis of *Simarouba glauca* DC bark, aimed at elucidating its botanical identity, anatomical features for chemical composition. By employing a range of microscopic and staining techniques, including macroscopic examinations, this study seeks to shed light on the intricate details of this bark, which hold the key to its therapeutic potential.

The utilization of *Simarouba glauca* DC bark in traditional medicine systems is not only a testament to its historical significance but also highlights its potential as a source of novel natural products for modern pharmaceutical applications. In the wake of increasing global interest in plant-based medicines and the need for sustainable resources, this research endeavour strives to contribute valuable insights into the pharmacognostic characteristics of *Simarouba glauca* DC bark, ultimately paving the way for its responsible and effective utilization in various healthcare domains.

2. MATERIALS AND METHODS

2.1 Collection and Authentication

The *S. glauca* bark sample was collected from Mr. K. M. Hegde’s Simarouba plantation, Bhairumbe, Sirsi, Uttara Kannada; 14°41'43.0"N 74°49'27.2"E; GPS co-ordinates of the plant: 14.695268, 74.824211; Plus Code: MRWF+4M4 Ashisara, Karnataka, India; Altitude: 576.00m/1,889.65 ft.

The specimen collected was critically examined with morphological, macroscopic, and microscopic characters along with the consultation of literature. The specimen was identified and authenticated by Dr. K. Kotresha, Professor and Taxonomist, P. G. Department of Botany, Karnataka Science College, Dharwad, Karnataka, India. Fresh bark was cleaned and shade dried for ten days. Dry bark powder was used for different experiments such as powder microscopy and other biochemical tests.

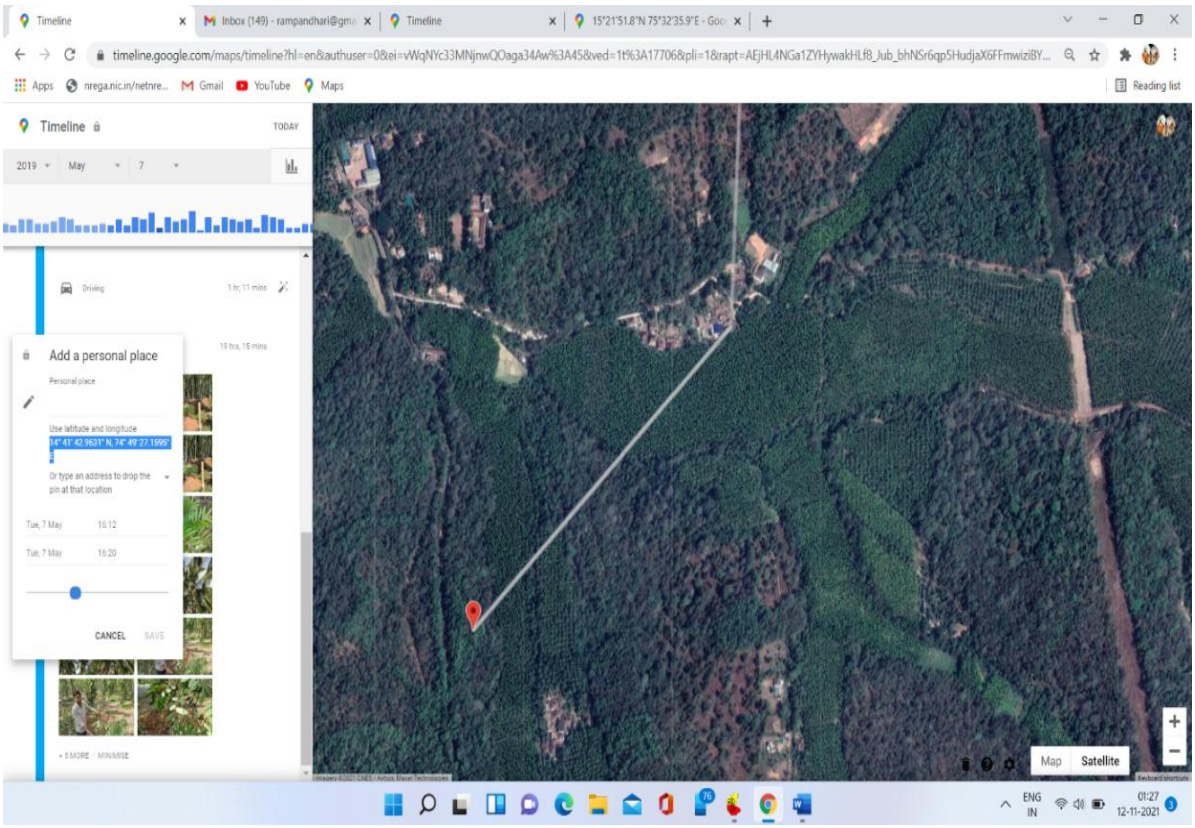

Fig. 1. Map showing the GPS location of sample collection

2.2 Pharmacognostic Characters

Disease free shade dried bark slices were fixed using FAA solution (Formalin + Acetic Acid + 70% Ethanol) [5]. After 24 hours of fixation, embedded in paraffin and sectioned in Rotary Microtome machine with a thickness of 10-12 µm [6]. Sections were stained with toluidine blue, safranin, and fast green wherever necessary [7]. Paradermal sections and bark powder were cleared in Jeffrey's maceration fluid and NaOH respectively, stained and mounted in glycerin [5]. Preparations were observed in Carl-Zeiss binocular microscope. Normal sections were observed under brightfield and birefringent materials like crystals were observed under polarized light. Photomicrographs were captured with Nikon Digital Labphoto version-2 camera [8, 9].

3. RESULTS AND DISCUSSION

3.1 Identification and Authentication

The specimen was identified as *Simarouba glauca* DC, and authenticated by its newly internationally accepted name ***Simarouba amara*** **Aubl.** belonging to the family Simaroubaceae [10-16]. The herbarium was stored with the specimen accession number **19562**.

3.2 Morphological Description

Leaves evergreen, compound, alternate, imparinnate, having 10-20 shiny glaucous leaflets, dark shiny green coloured, elliptic to oblong shaped, 1.5 to 3 inches long. Inflorescence panicle with ultimate branches bearing dichasial cymes. Flowers bisexual, polygamo-dioecious, calyx 5 unfused, greenish sepals, corolla 5 free, yellowish white petals. Male flowers with 10 stamens and without ovaries. Female flowers with 10 staminodes, pentacarpellary apocarpous ovary. Drupe fleshy, 1 inch long, greenish, ripe to dark purple colour [10-16].

3.3 Bark Macroscopy

The outer surface of the bark is brown, there are thick, longitudinal ridges which are parallel to each other (Fig. 4). The inner surface is white and smooth with thin longitudinal lines (Fig. 4).

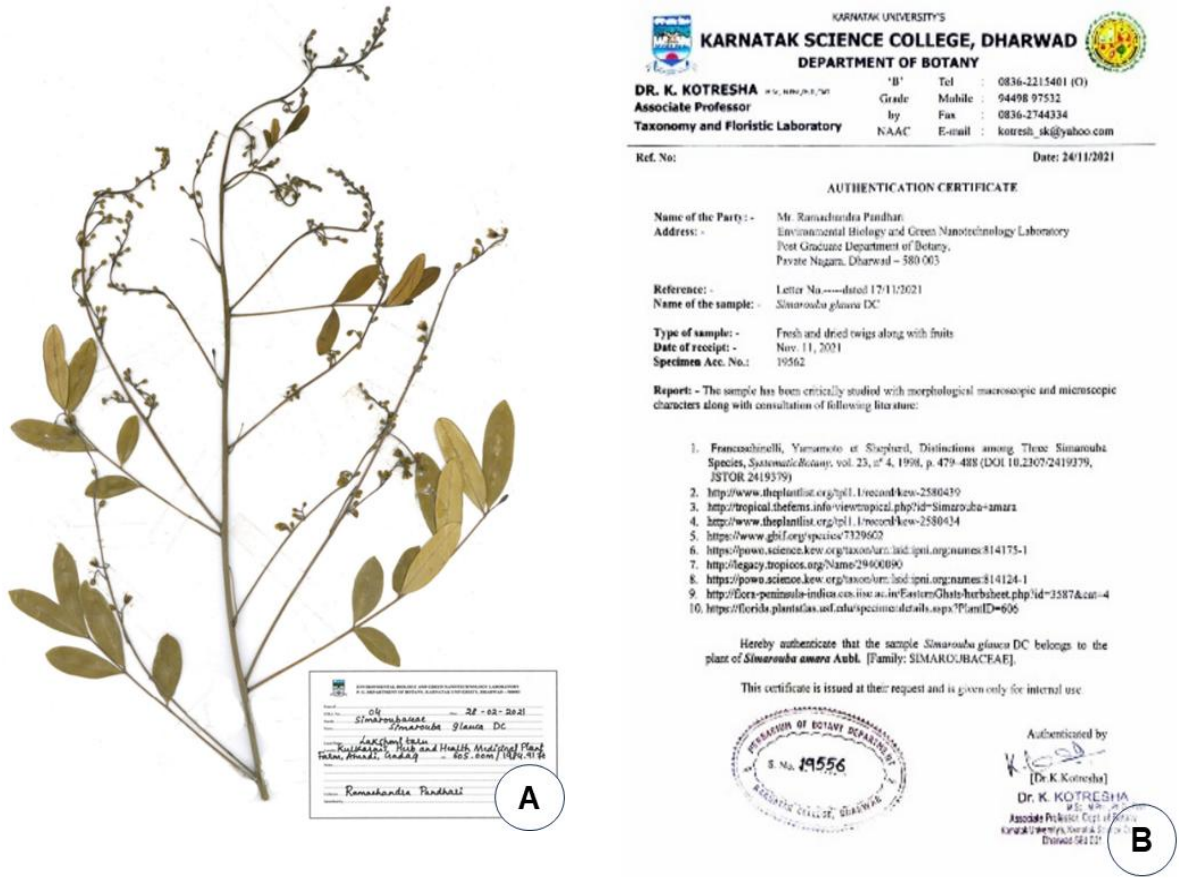

Fig. 2. A. Herbarium and B: Authentication latter from the taxonomist

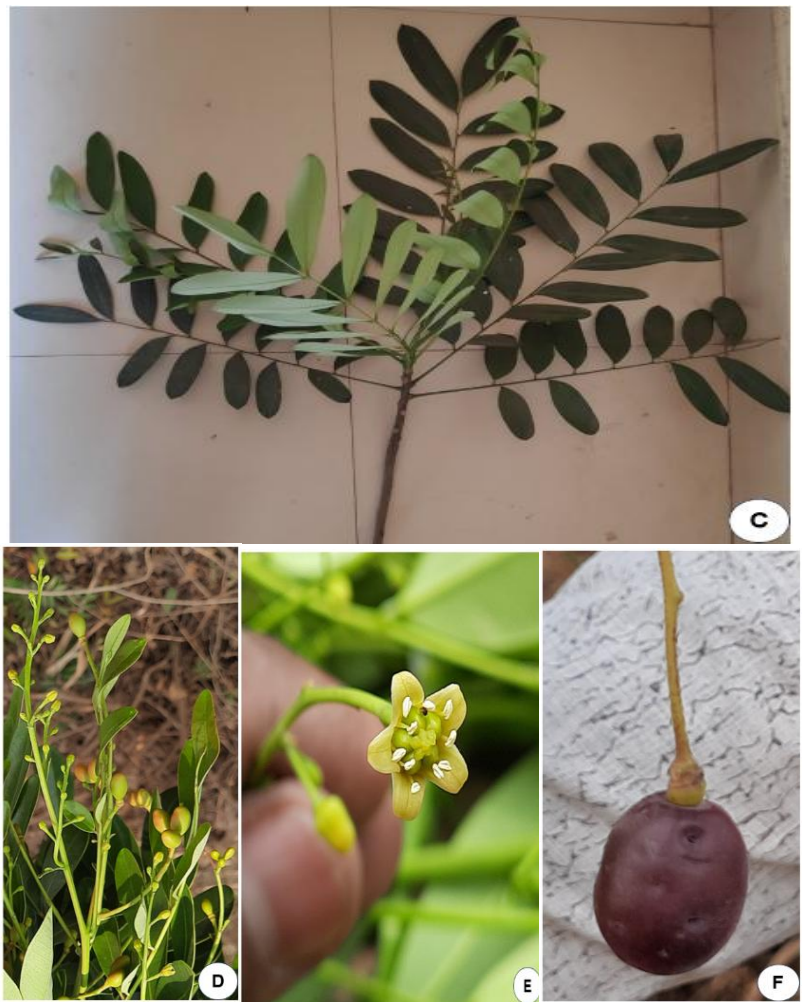

Fig. 3. A: Habitat; B: Habit; C: Leaves; D: Inflorescence; E: Flower; F: Fruit.

Fig. 4. *S. glauca* bark showing outer and inner surfaces

3.4 Anatomy of the Bark

The bark has thick **periderm** with small flakes raised above the **periderm** surface. The outer part of **phellem** is crushed and the inner portion is modified into a thick cylinder of **sclereids** (Fig. 5). The **Phelloderm** becomes thick walled and dark coloured and stores calcium oxalate crystals (Fig. 5.A, 5.B, 5.C).

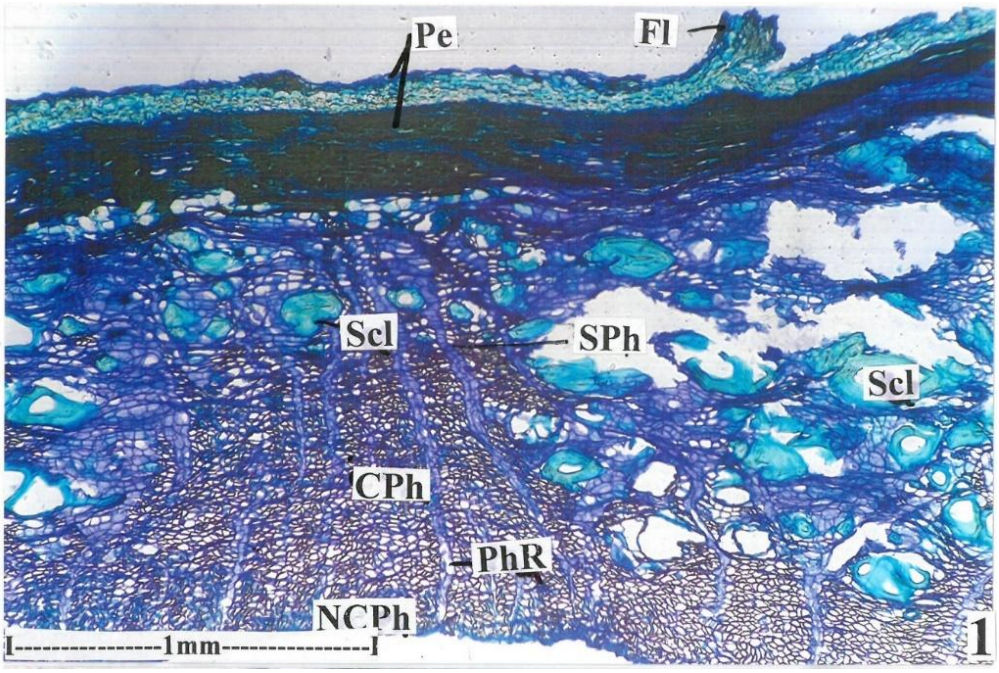

Fig. 5.A: T. S. of Bark (4X) – entire view. CPh: Collapsed Phloem; Fl: Flake; NCPh: Non-Collapsed Phloem; Pe: Periderm; Scl: Sclereids; SPh: Secondary Phloem; PhR: Phloem Ray.

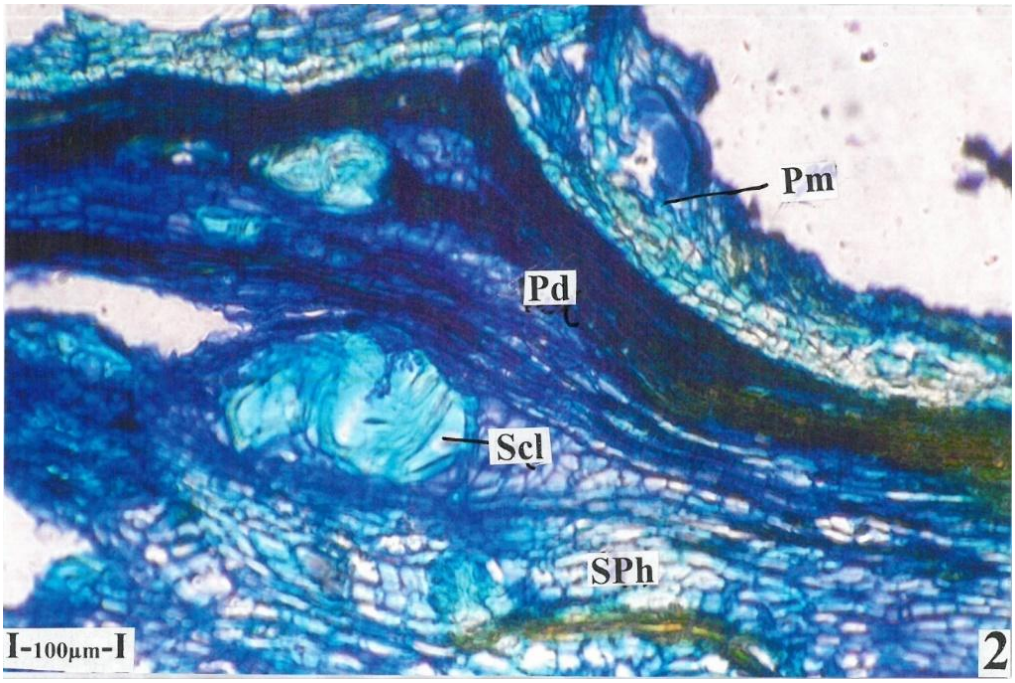

Fig. 5.B: T. S. Bark (10X) – showing periderm layers. Pm: Phellem; Pd: Phelloderm; Scl: Sclereids; SPh: Secondary Phloem

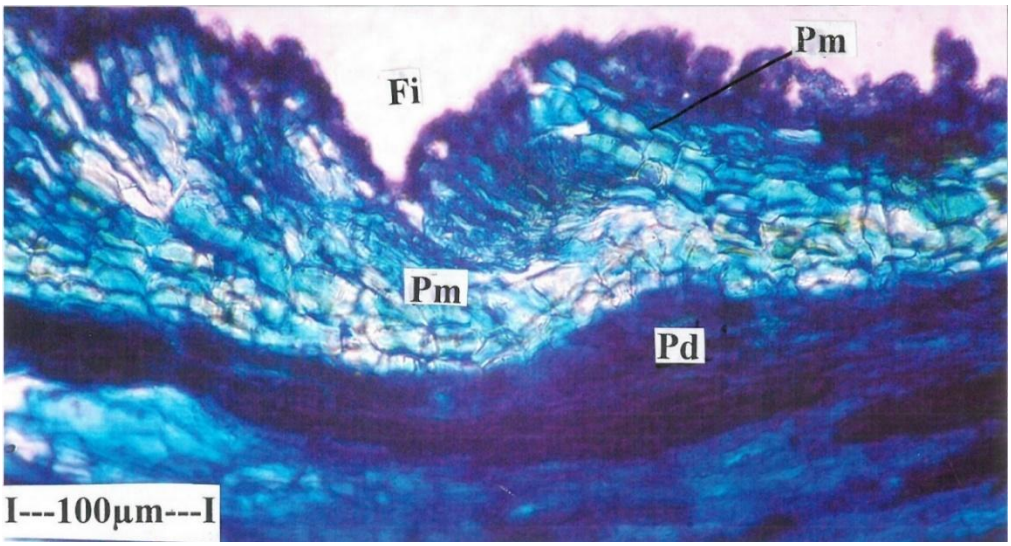

Fig. 5.C: T. S. Bark (20X) – showing periderm outer zone of Phellem. Fi: Fissured; Pm: Phellem; Pd: Phelloderm

Secondary phloem follows phelloderm. It includes outer **collapsed phloem** and inner **non-collapsed phloem** (Fig. 6.A, 6.B). These are large and wide **sclereids** with thick lamellate secondary walls scattered in the **collapsed phloem** zone (Fig. 7.A, 8.A). The collapsed sieve elements form thick dark, tangential cylinders (Fig. 7.A). Non collapsed phloem zone is narrow and includes Phloem rays, Sieve elements and Parenchyma cells. The phloem rays are radially oriented comprising two rows of elongated thin-walled cells. The sieve elements are wide, angular and have small polygonal companion cells. Phloem parenchyma cells are wide, variously shaped, and thin walled (Fig. 7.B). Large prismatic calcium oxalate crystals located in uniseriate vertical row within thin parenchyma cells (Fig. 10.B).

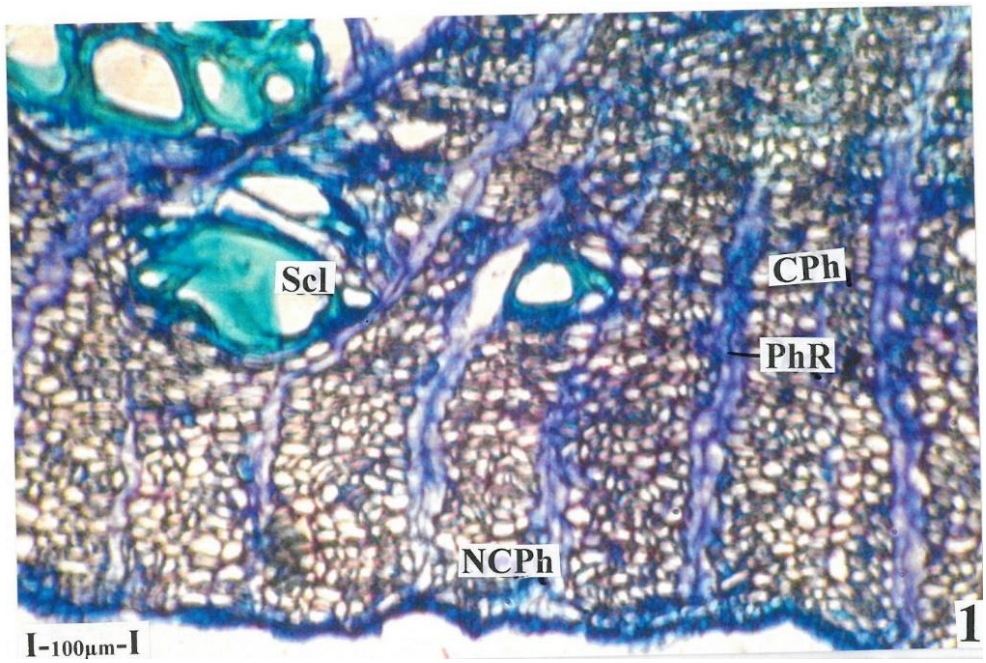

Fig. 6.A: T. S. of Bark (10X), Secondary Phloem showing Collapsed and Non-Collapsed Phloem tissues. Scl: Sclereids; CPh: Collapsed Phloem; NCPh: Non-Collapsed Phloem; PhR: Phloem Rays

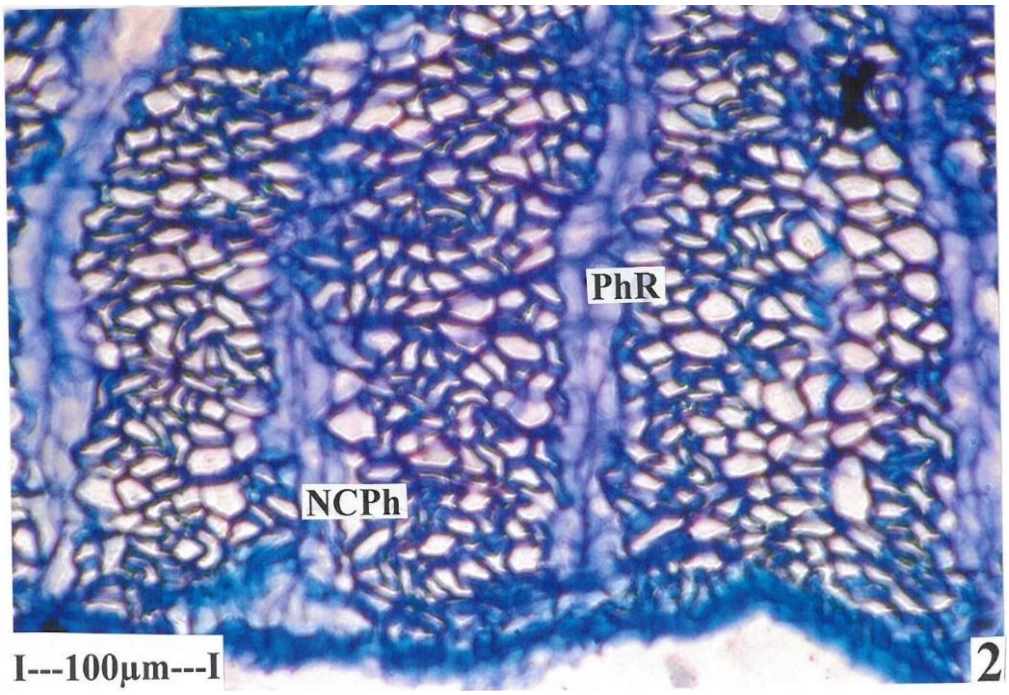

Fig. 6.B: T. S. of Bark (20X) Secondary Phloem showing Non-Collapsed Phloem Tissue. PhR: Phloem Rays; NCPh: Non-Collapsed Phloem

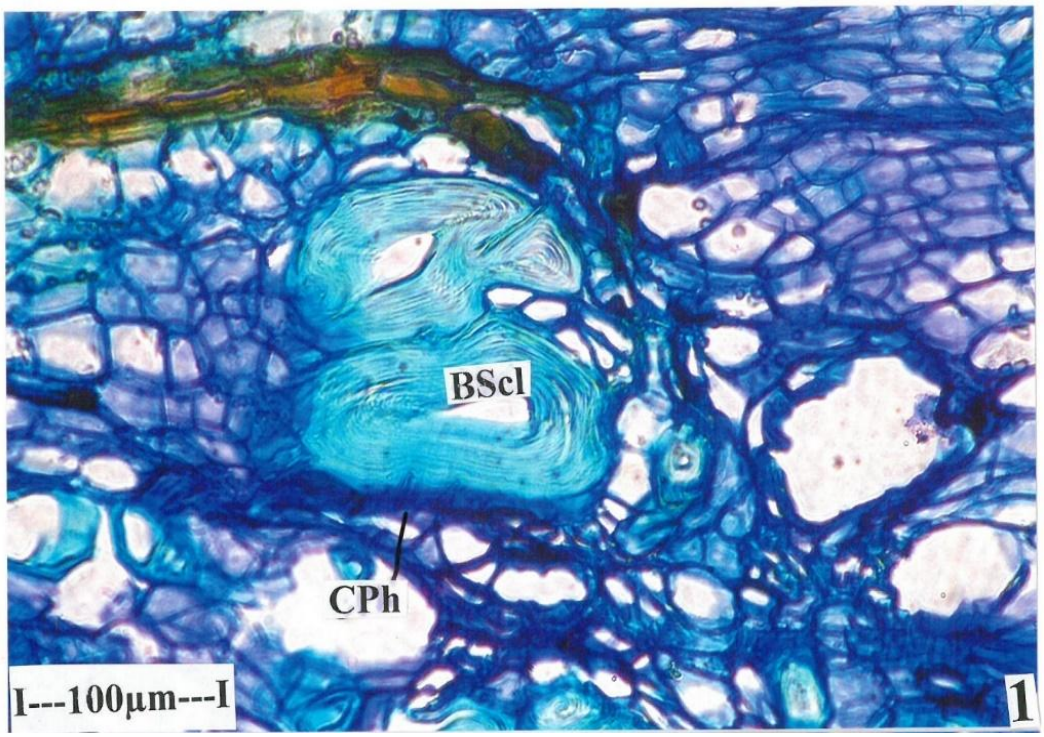

Fig. 7.A: T. S. of Bark (20X). Distribution of Brachy Sclereids in the Collapsed Phloem region. BScl: Brachy Sclereids; CPh: Collapsed Phloem

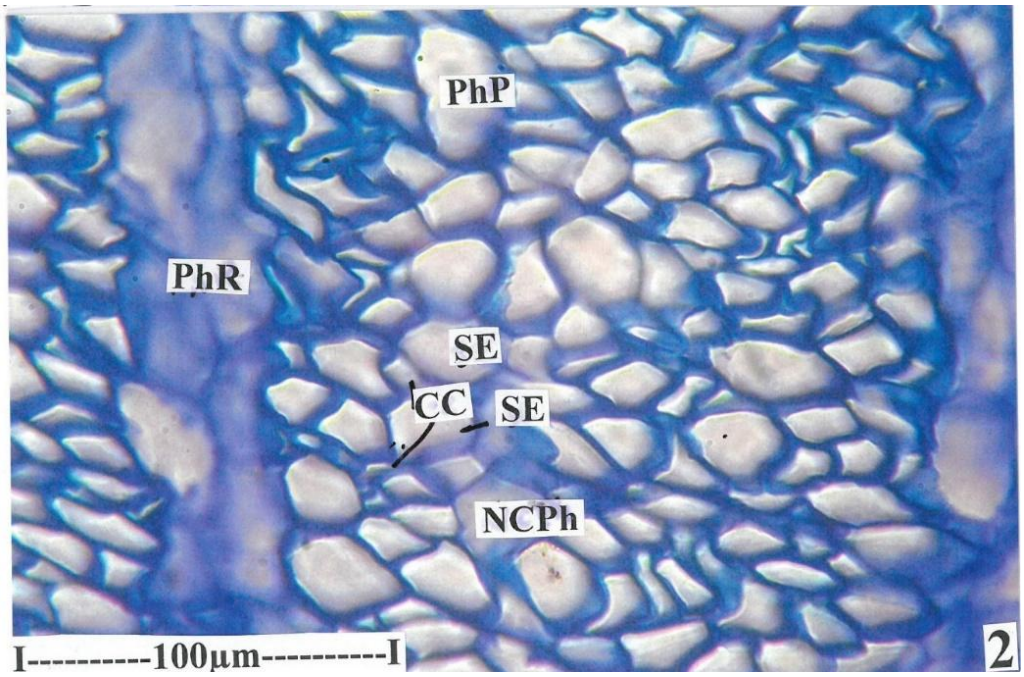

Fig. 7.B: T. S. of Bark (40X). Non-Collapsed Phloem showing Sieve Elements and Companion Cells. PhP: Phloem Parenchyma; PhR: Phloem Rays; SE: Sieve Elements; CC: Companion Cells; NCPh: Non-Collapsed Phloem

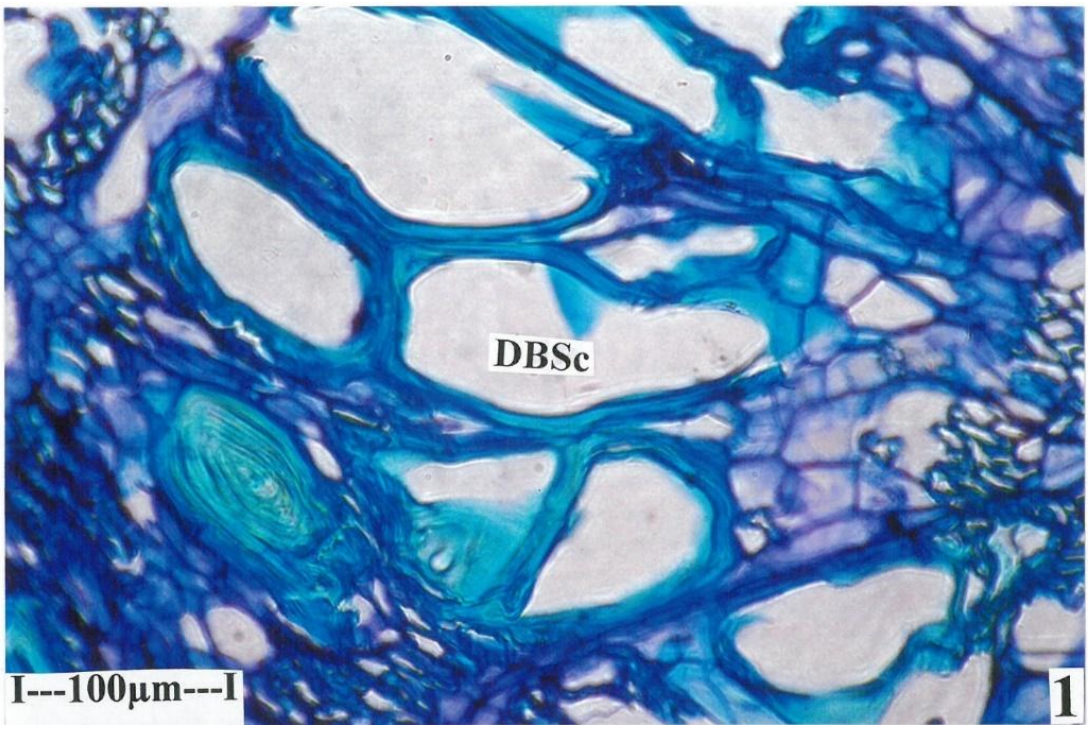

Fig. 8.A: T. S. of Bark (20X). Distribution of Brachy Sclereids in the Collapsed Phloem Zone. DBSc: Dilated Brachy Sclereids

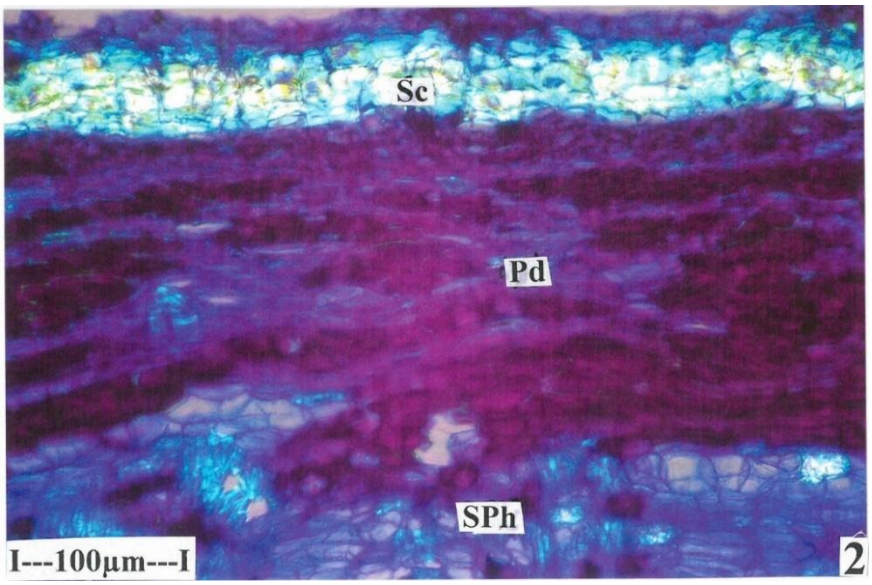

Fig 8.B: T. S. of Bark (20X). Secondary Phloem region showing Sclerenchyma layer and Phelloderm. Pd: Phelloderm; SPh: Secondary Phloem

3.5 Tangential Longitudinal Sectional View of Phloem Rays (TLS)

The Phloem rays are non-storied and are arranged at different levels (Fig. 9.A). The rays are mostly multiseriate, less frequently uniseriate or biseriate. The multiseriate rays have more than three vertical rows of cells. The ray cells are angular and fairly-thick walled. The cells in the rays are heterocellular i. e., the rays have two types of cells, namely, **Upright cells** and **Procumbent cells**. The cells at the two ends of the ray are vertically elongated and wide; the cells at the middle of the rays are squarish or circular and isodiametric. The terminal cells and the middle cells constitute the heterocellular native (Fig. 9.B). The phloem **parenchyma** cells are seen in vertical rows, with each row having vertically elongated rectangular cells. The phloem fibers in dense compact bundles. The fibers being highly thick walled and lignified with narrow cell lumen (Fig. 9.B, 9.C). The rays are 250-280 µm in height and 40-60µm.

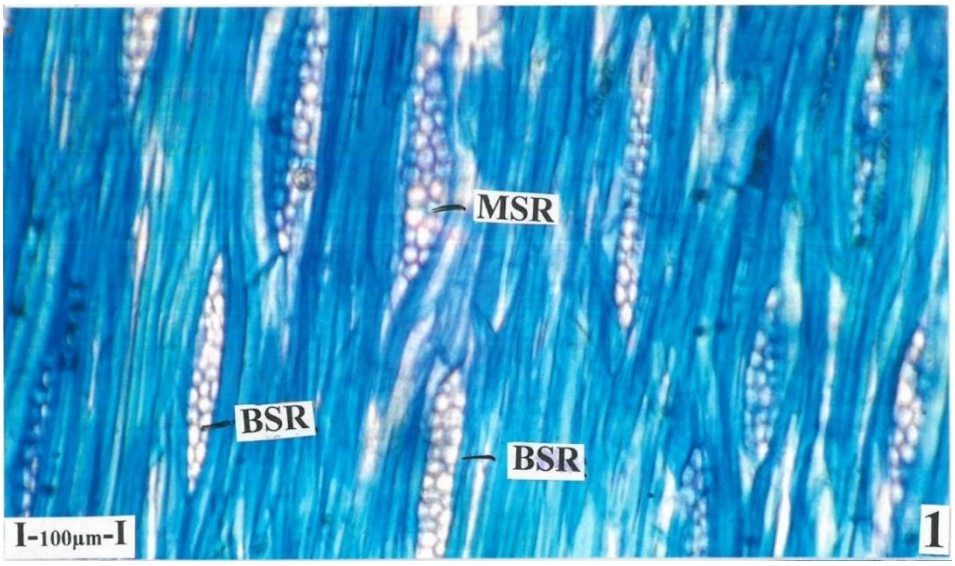

Fig. 9.A: T. L. S. of Bark (10X) showing Phloem Rays. MSR: Multiseriate Ray; BSR: Biseriate Ray

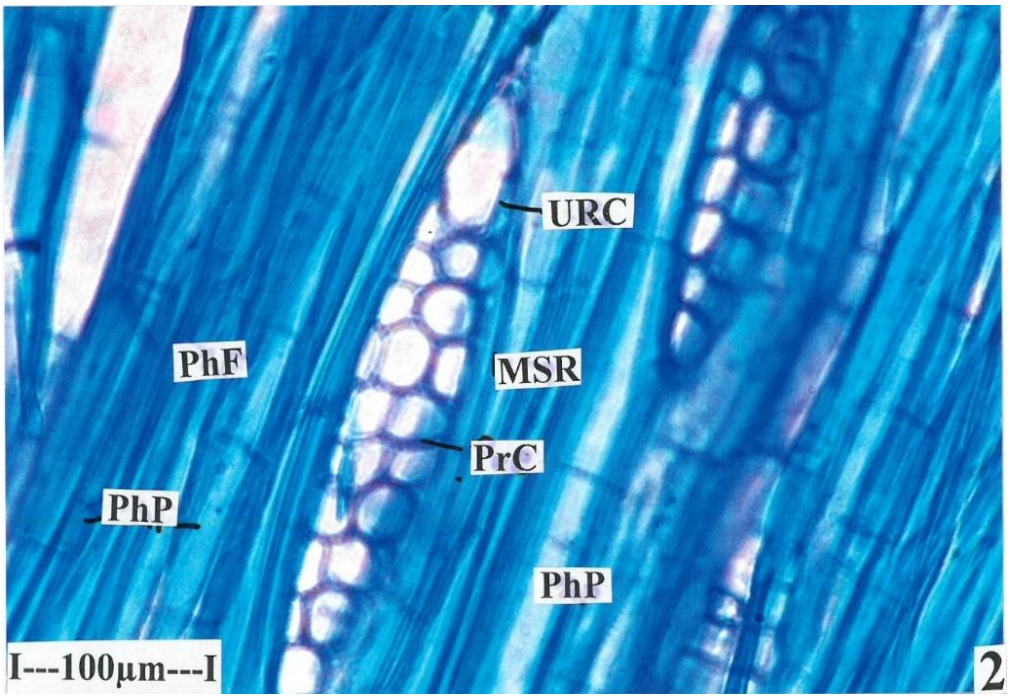

Fig. 9.B: T. L. S. of Bark (20X) showing Phloem Rays. PhF: Phloem Fibre; PhP: Phloem Parenchyma; URC: Uniseriate Ray; MSR: Multiseriate Ray; PrC: Procumbent Cell.

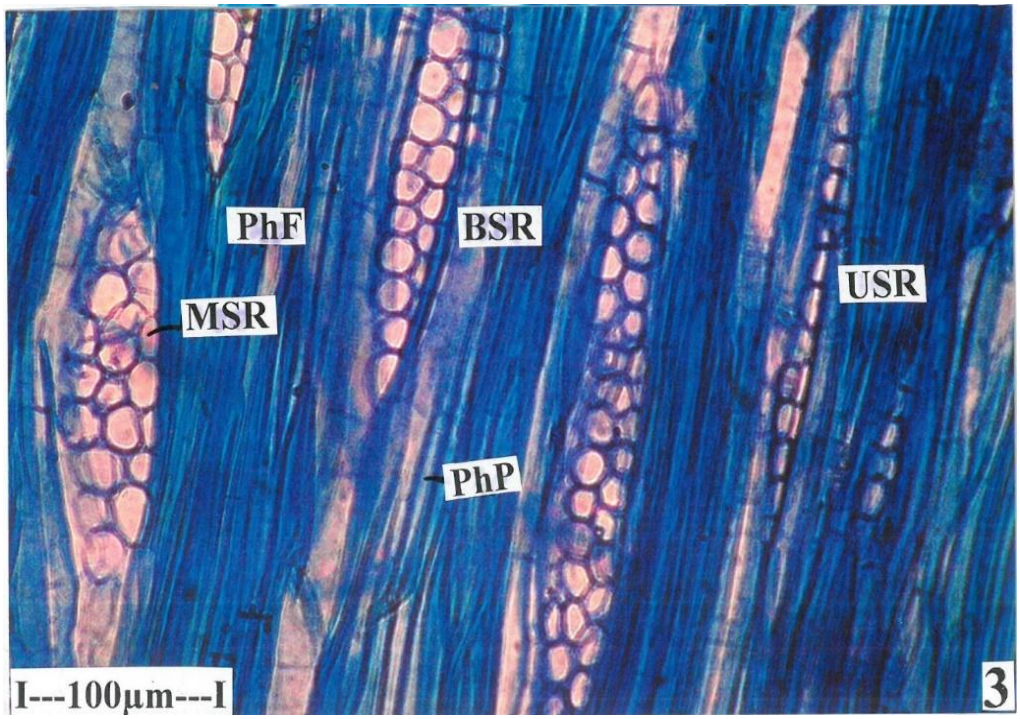

Fig. 9.C: T. L. S. of Bark (20X) showing Phloem Rays. PhF: Phloem Fibres; MSR: Multiseriate Rays; BSR: Biseriate Rays; USR: Uniseriate Rays; PhP: Phloem Parenchyma.

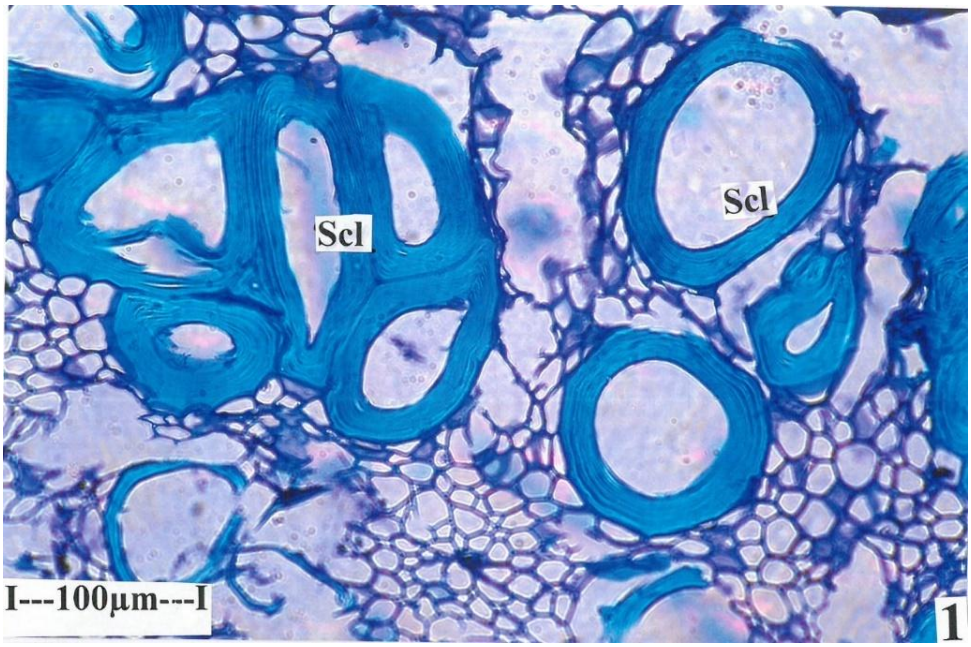

Fig. 10.A: T. L. S. of Bark (20X). Distribution of Brachy Sclereids in Collapsed Phloem. Scl: Sclereids.

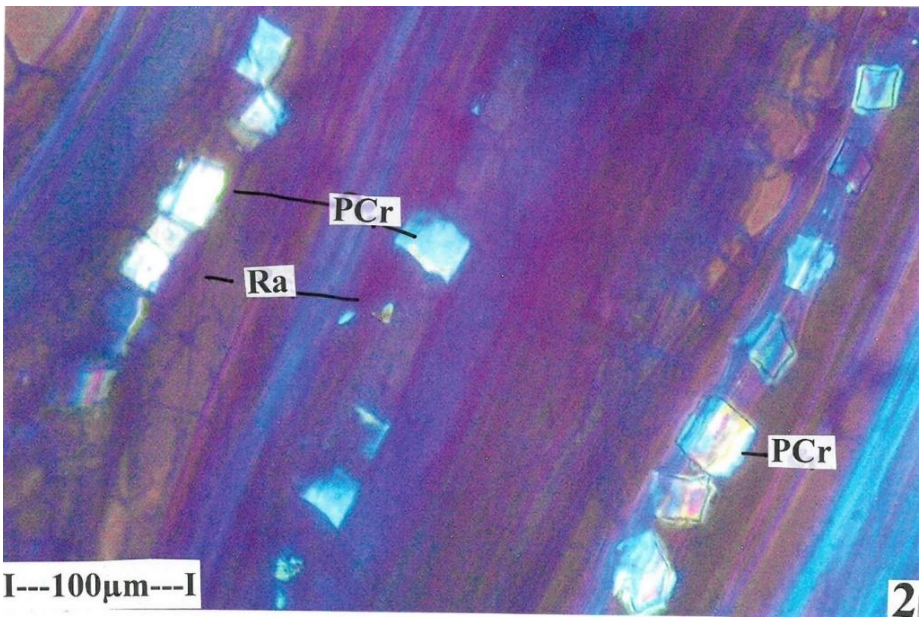

Fig. 10.B: T. L. S. of Bark (20X). Distribution of Calcium oxalate crystals in the Phloem Rays. Ra: Rays; PCr: Prismatic Crystals.

3.6 Radial Longitudinal Sectional View of Phloem Rays (RLS)

In RLS view, the rays appear in horizontal orientation, the appearing as in bricks of a wall. The cells are rectangular and they are in flat layers (Fig. 11.A, 11.b and 12.A, 12.B). The cells are wide and thick walled. The cells in the middle part of the ray are prostrate and are procumbent cells. The cells at the upper, lower parts of the ray are standing straight and called Upright cells. Thus, the rays are rentered heterocellular (Fig. 12.A, 12.B). Parenchyma cells and fibers are vertical position at right angles to the rays.

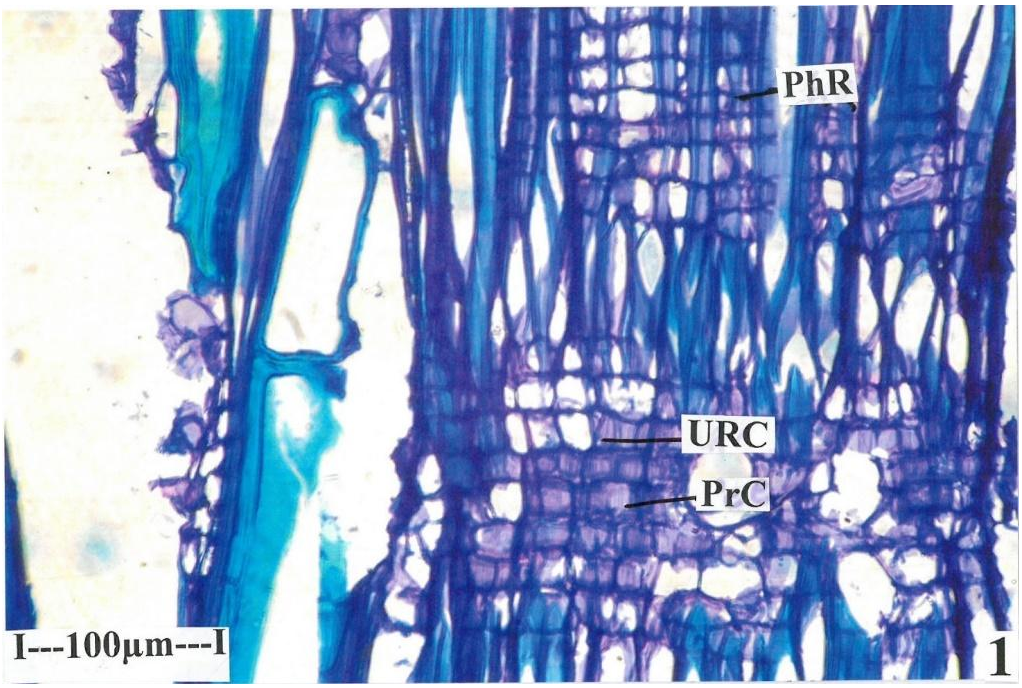

Fig. 11.A: R. L. S. of Bark (20X) showing Phloem Rays. PhR: Phloem Rays; URC: Upright Cells; PrC: Procumbent Cell

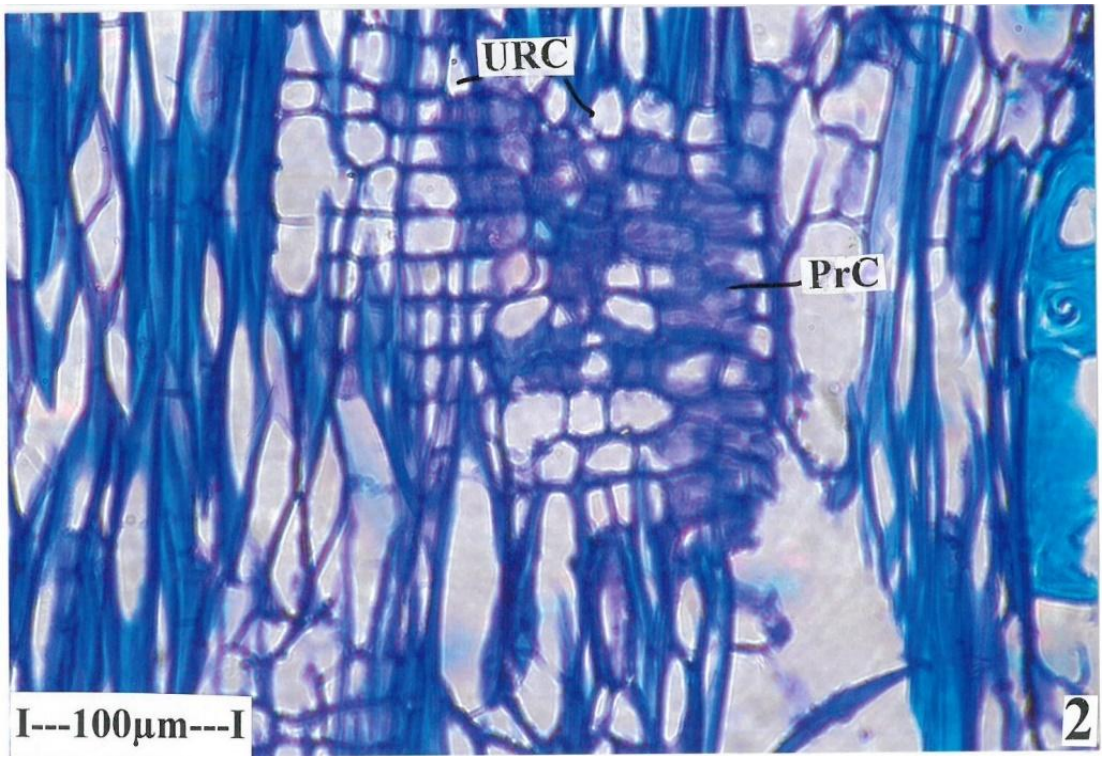

Fig. 11.B: R. L. S. of Bark (20X) showing Phloem Rays. PrC: Procumbent Cells; URC: Uniseriate Cells

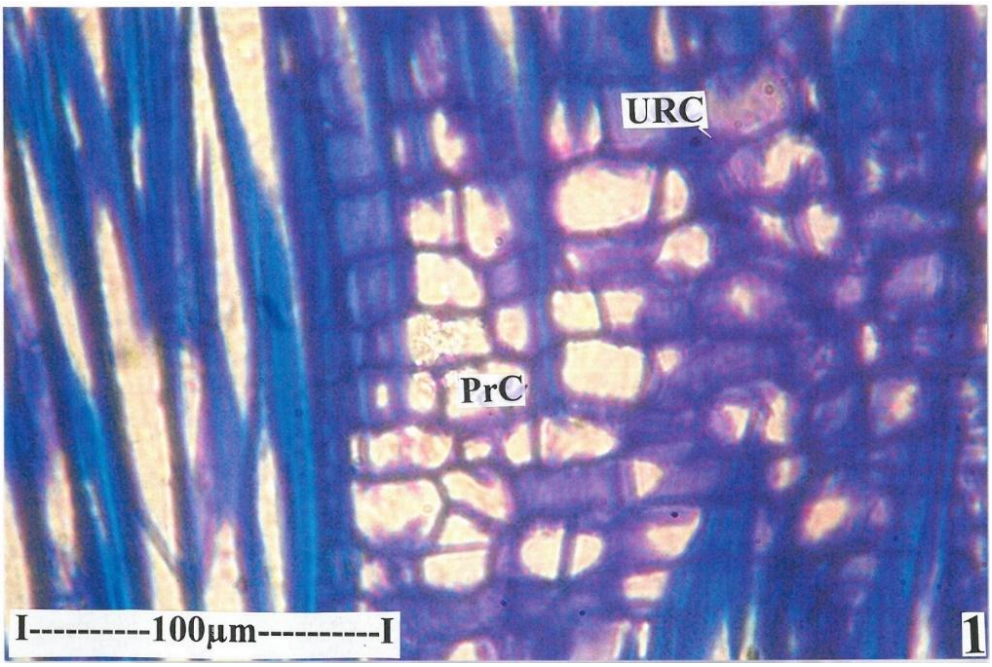

Fig. 12.A: R. L. S. of Bark (40X) showing Phloem Rays enlarged. URC: Upright Cells; PrC: Procumbent Cell

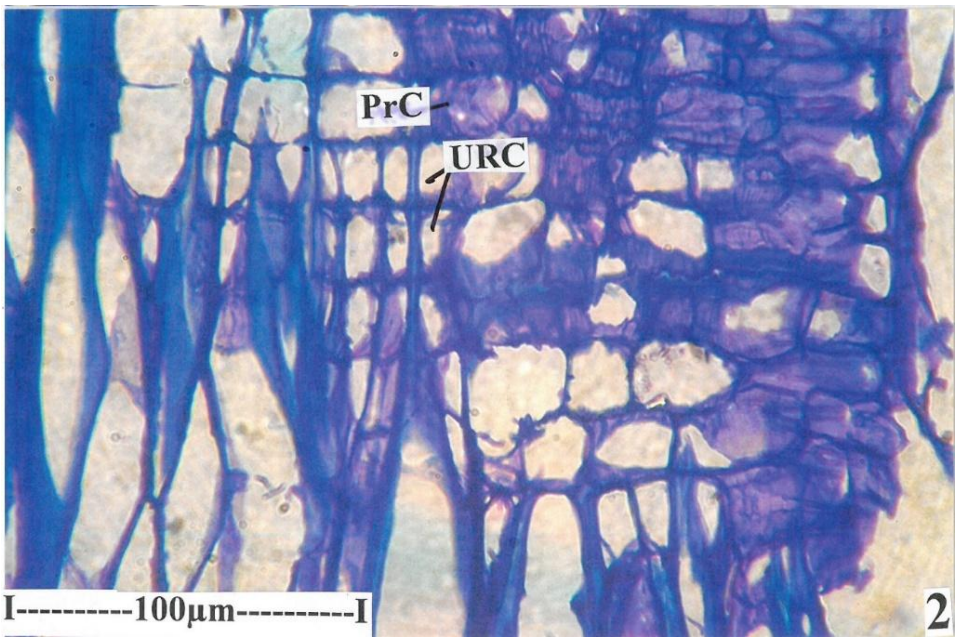

Fig. 12.B: R. L. S. of Bark (40X) showing Phloem Rays enlarged. PrC: Procumbent Cell; URC: Upright Cells

3.7 Bark Powder Macroscopy

Bark powder is rough crystalline fibrous, pale white in colour, strong woody odour, pleasant, woody bitter taste, non-mucilaginous in nature, and when observed under the microscope following fragments of tissues were observed.

3.8 Bark Powder Microscopy

3.8.1 Fiber

Fibers are abundant in the fiber. They are long with thick lignified walls and tapering ends and narrow lumen (Fig. 13.A, 13.B, 14.A; 15.B, 18.A).

The fibers appear in types: **Narrow fibers** and **Wide fibers**.

3.8.2 Narrow fibers

Narrow fibers are thin with thick walls and narrow cell lumens (Fig. 13.A). The narrow fibers up to 1.6 mm long and 20 µm thick.

3.8.3 Wide fibers

Wide fibers are short and wide. The cell lumen is wide; cell walls are thin. The wide fibers are 1.2 mm long, 40 µm thick.

3.8.4 Ground parenchyma

Bark parenchyma cells are often seen in the powder. The cells are polygonal, compact, and thin walled. The anticlinal walls of the cells are straight and smooth (Fig. 15.A).

3.8.5 Fiber-Sclereid

Long, fiber like cells with secondary lignified walls, are often seen in the powder. The cell lumen is wide. The cell resembles the fiber, but it is originally sclereid (Fig. 15.B).

3.8.6 Crystals

Calcium oxalate crystals are seen in large number in the powder. The crystals are prismatic type. They are quandular, rectangular and polygonal in shape (Fig. 16.A, 16.B).

3.8.7 Sieve elements

Long, tubular cells, which are sieve tube members (sieve elements) are common in the powder. The sieve elements have thin walls, wide lumen, and oblique end walls (sieve plates). The sieve elements are 540 µm long and 50-60 µm wide (Fig. 17.A, 17.B).

3.8.8 Brachy sclereids

Different forms of brachy sclereids are found in the powder. Some sclereids are cubical or squarish with thick lignified walls (Fig. 13.A). Other types of sclereids are long and cylindrical or lobed (Fig. 18.B, 18.C). Brachy sclereids have numerous canal-shaped, simple pits.

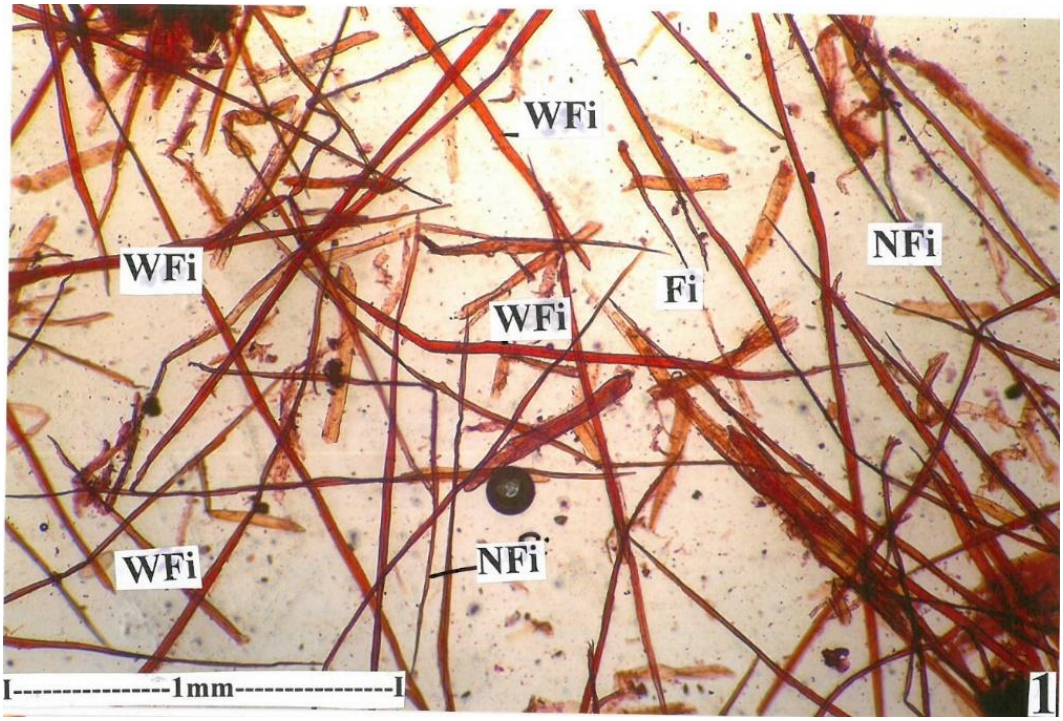

Fig. 13.A: Bark powder Microscopy (4X). A large number of fibre found in Bark powder. Fi: Fibre; NFi: Narrow Fibre; WFi: Wide Fibre

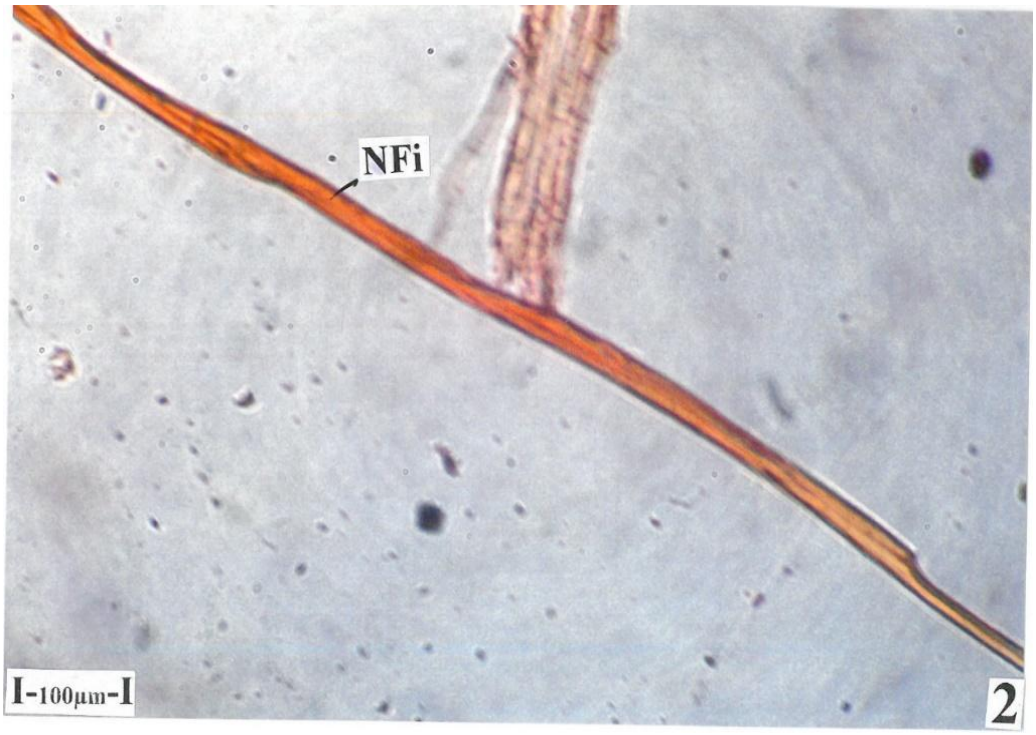

Fig. 13.B: Bark powder Microscopy (10X). A Single Narrow Fibre enlarged. NFi: Narrow Fibre.

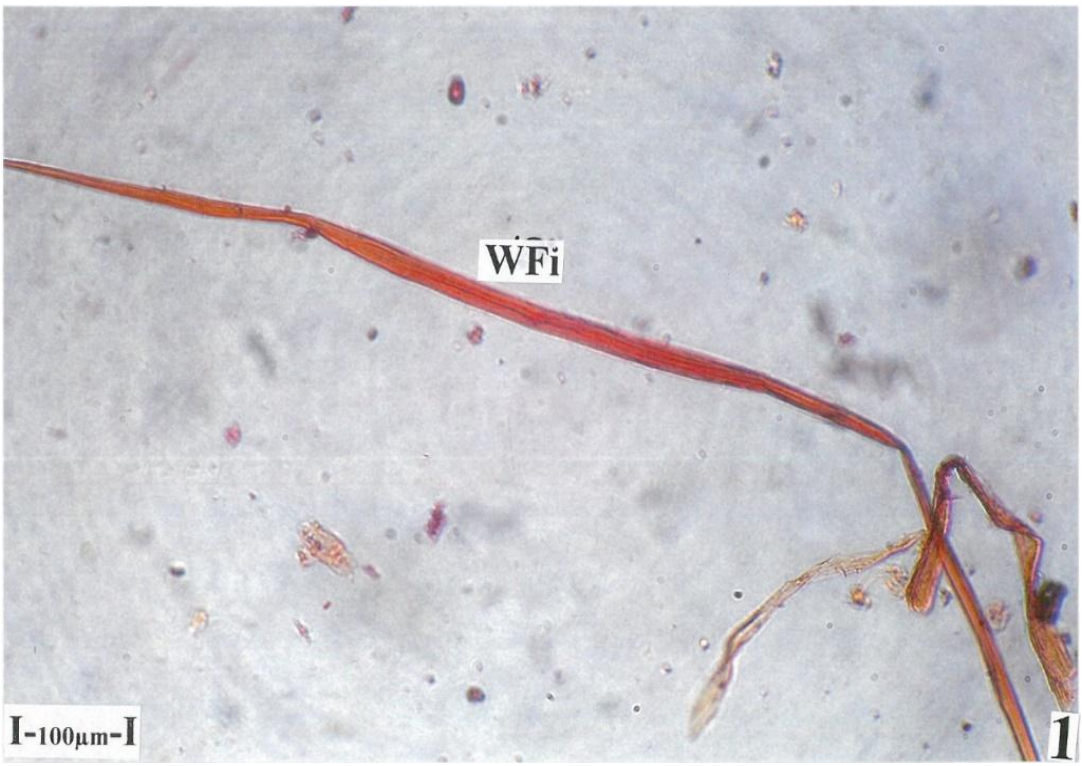

Fig. 14.A: Bark powder Microscopy (10X). A Wide FIbre enlarged. WFi: Wide Fibre

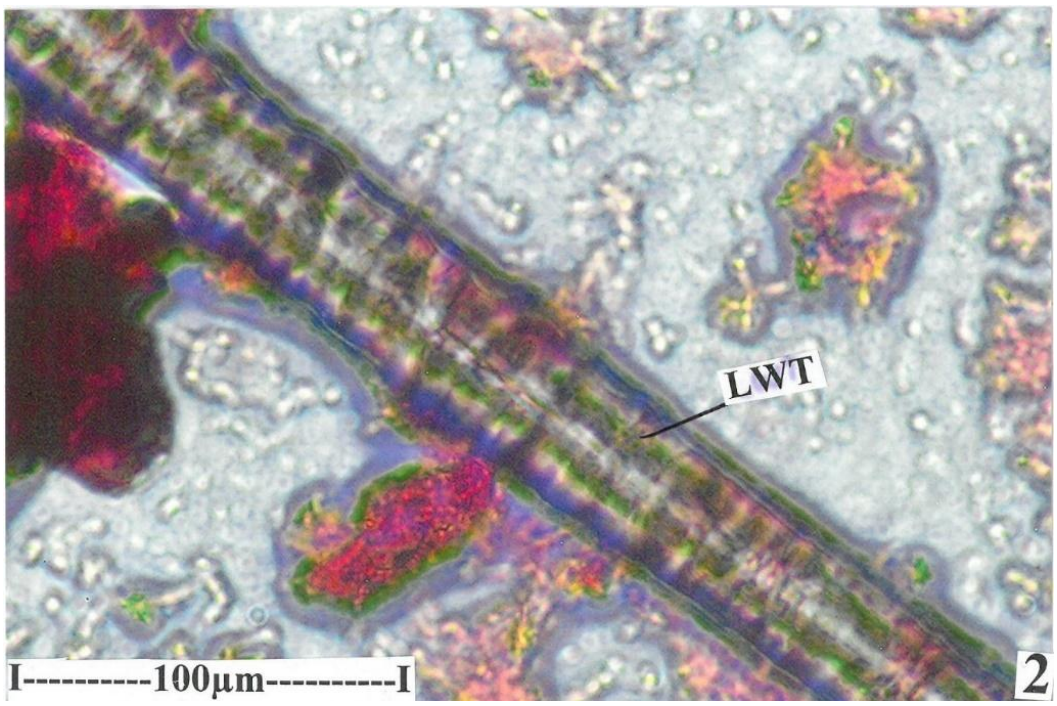

Fig. 14.B: Bark powder Microscopy (40X). A Wide Fibre tracheid showing Scalariform, lateral wall thickening. WFi: Wide Fibre; LWT: Lateral Wall Thickening

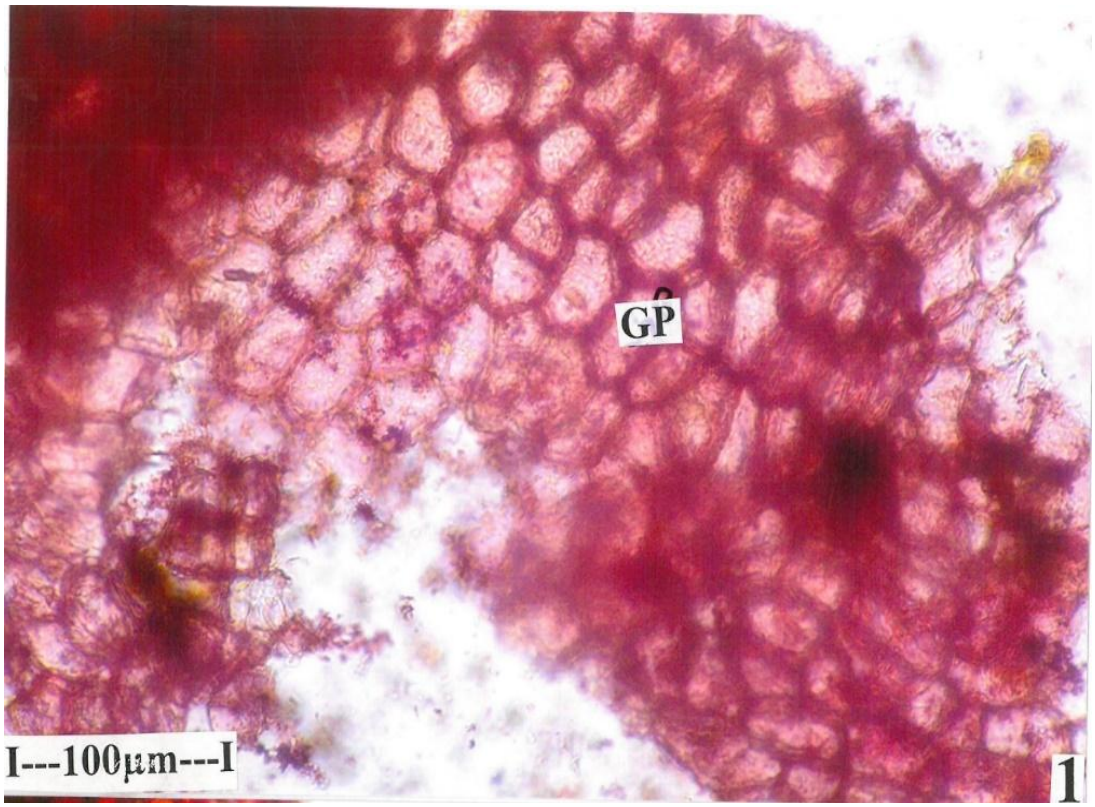

Fig. 15.A: Bark powder Microscopy (20X). Parenchymatous ground tissue of the Bark.GP: Ground Parenchyma

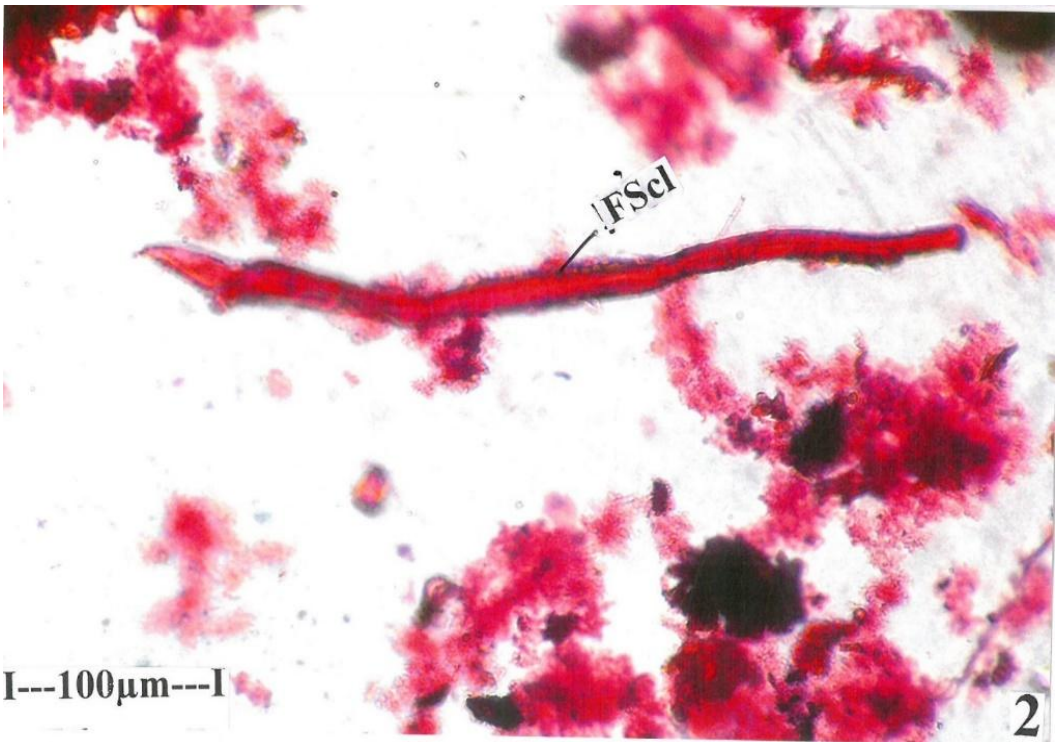

Fig. 15.B: Bark powder Microscopy (20X). A Fibre Sclereid. FScl: Fibre Sclereid

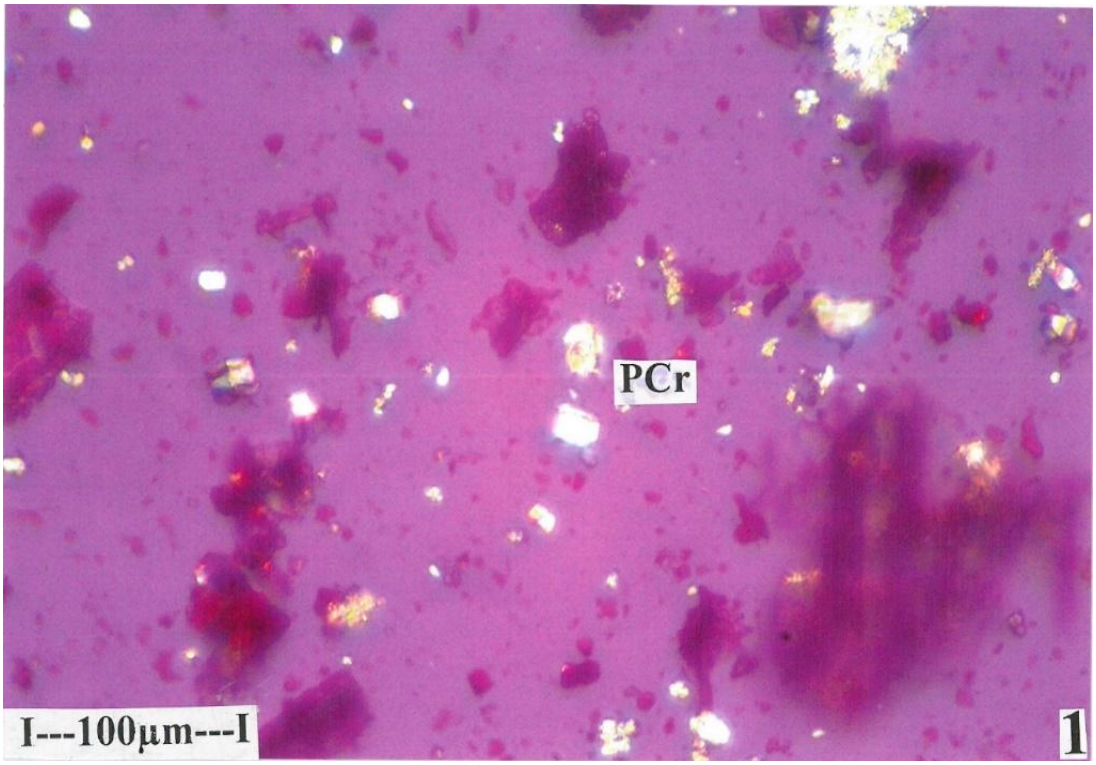

Fig. 16.A: Bark powder Microscopy (20X). Prismatic Calcium Oxalate Crystals. PCr: Prismatic Crystals

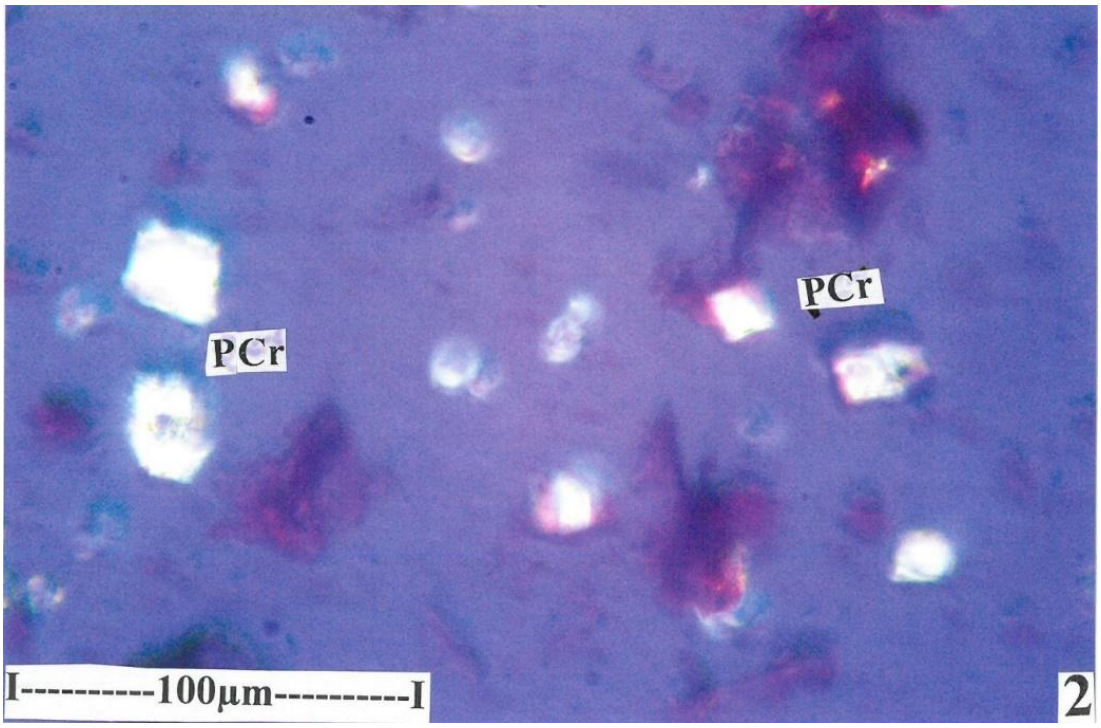

Fig. 16.B: Bark powder Microscopy (40X). Prismatic Calcium Oxalate Crystals. PCr: Prismatic Crystals.

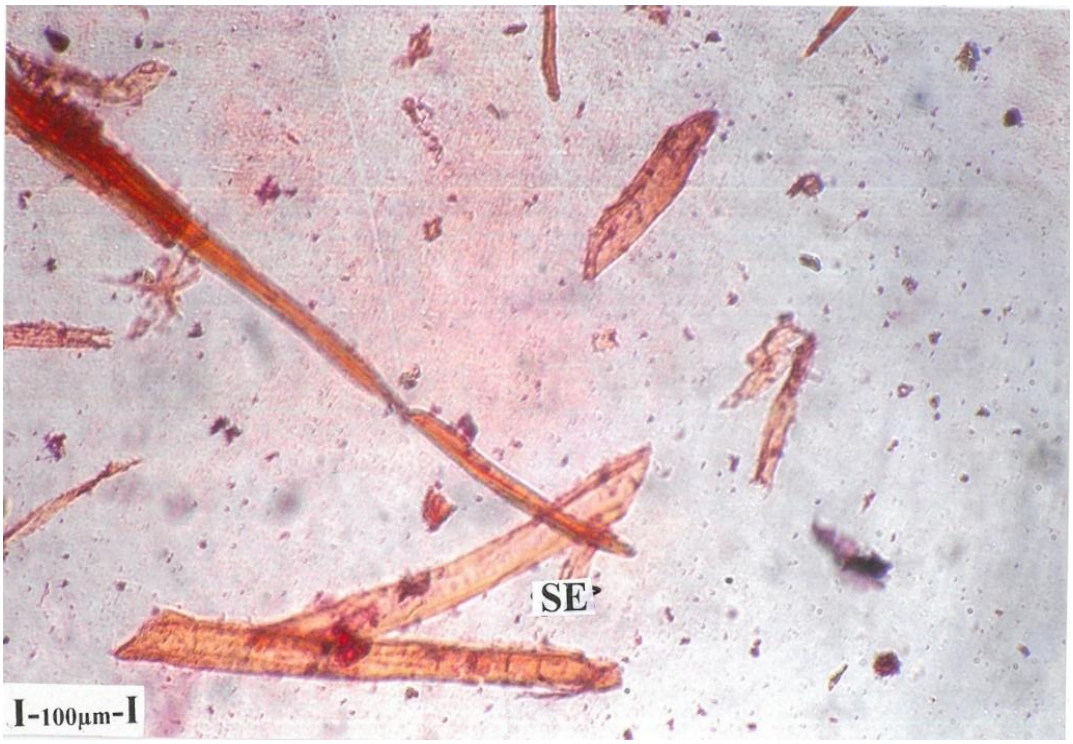

Fig. 17.A: Bark powder Microscopy (10X). Sieve Elements found in the Bark powder. SE: Sieve Element

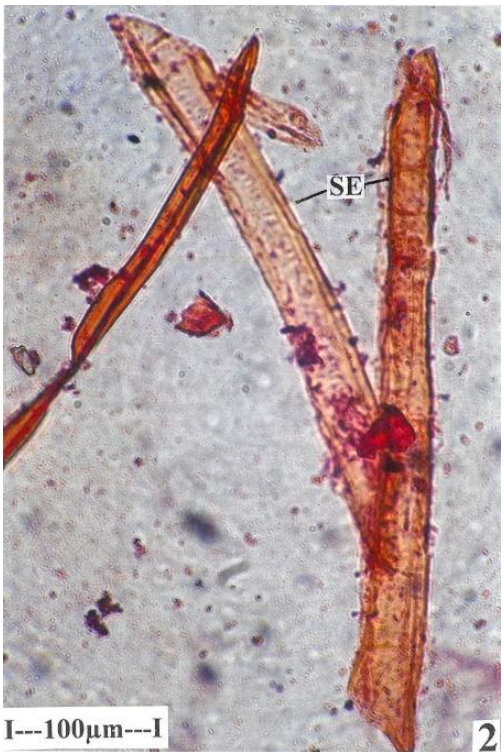

Fig. 17.B: Bark powder Microscopy (20X). Sieve Element enlarged. SE: Sieve Element.

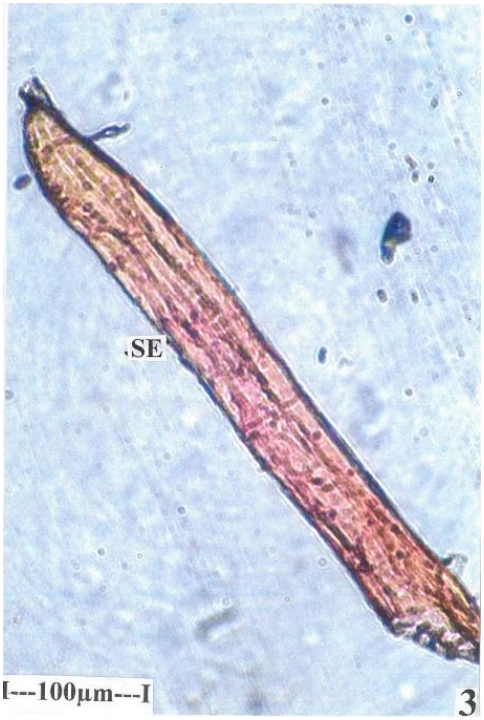

Fig. 17.C: Bark powder Microscopy (20X). Sieve Element enlarged. SE: Sieve Element.

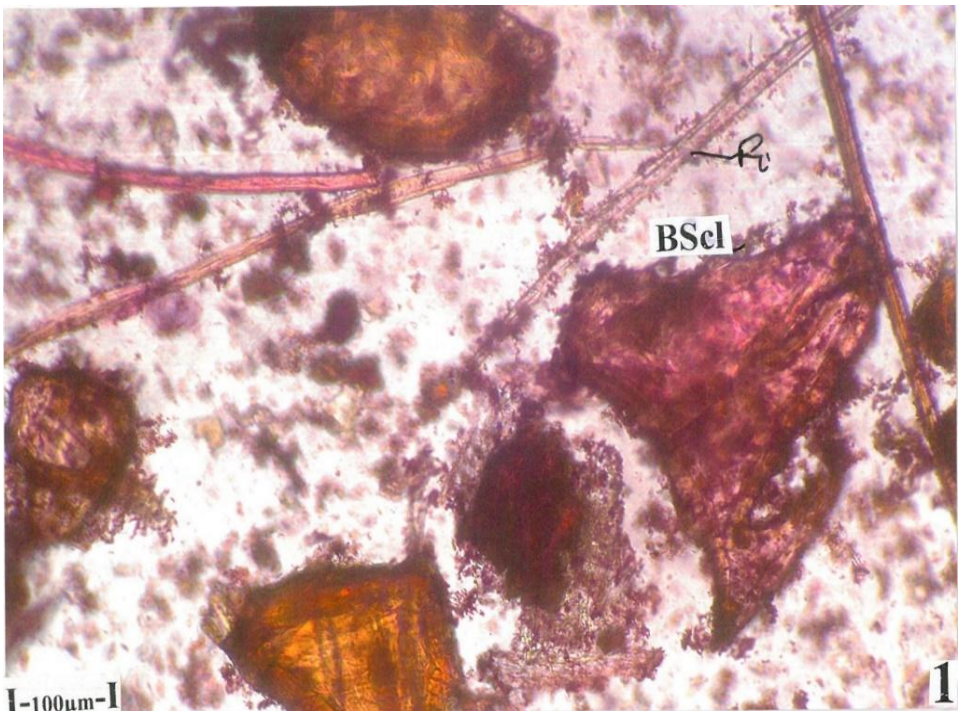

Fig. 18.A: Bark powder Microscopy (10X). Small Parenchymatous Sclereids (Brachy Sclereids). BScl: Brachy Sclereids; Fi: Fibre

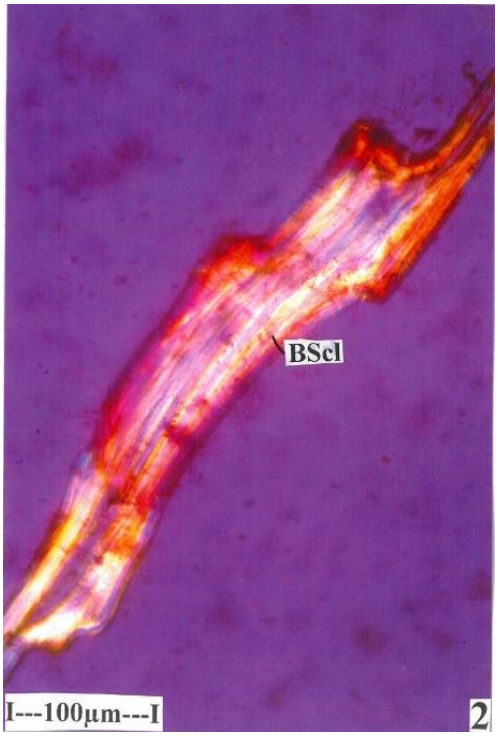

Fig. 18.B: Bark powder Microscopy (20X). Elongated Brachy Sclereid. BScl: Brachy Sclereid

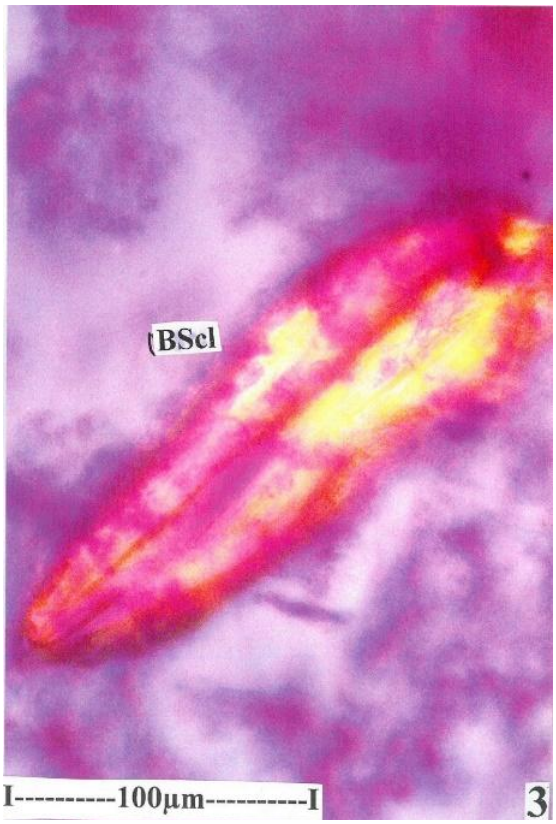

Fig. 18. C: Bark powder Microscopy (40X). Elongated Brachy Sclereid. BScl: Brachy Sclereid

The Simaroubaceae family consists of 32 genera and nearly 170 species of pantropical distribution. Among six subfamilies, Simarouboideae is the major subfamily that includes 22 genera [17]. Alternate compound leaves, axial inflorescence, flowers showing free sepals and petals, stamens double in number of petals, filaments with appendix, pentacarpellary superior apocarpous ovary, fleshy drupe fruits are key taxonomic markers for species level identification [18].

Botanically, the Simaroubaceae family is related to the Rutaceae, Meliaceae, and Burseraceae families. However, within this group, it is more related to the Rutaceae family, primarily in terms of chemical composition, wood structure, the absence of resin ducts in the bark, and the presence of free stamens. It differs exclusively from the other families by the absence of secretory cavities containing aromatic oils in leaves and floral parts.

The thick periderm, calcium oxalate crystals stored in dark coloured thick phelloderm, multiseriate phloem rays, distribution of Brachy Sclereids in Collapsed Phloem, distribution of Calcium oxalate crystals in the Phloem Rays, distribution of Calcium oxalate crystals in the Phloem Rays, presence of narrow and wide fibers, and mainly large quantity of quadrangular, rectangular, and polygonal Calcium oxalate crystals are key anatomical markers for the species level differentiation [19].

Pharmacognostic standardization plays a crucial role in assessing herbal drugs to ensure their identity, purity, safety, and overall quality. Among various techniques available, microscopic evaluation stands out as a cost-effective and straightforward method for accurately identifying the source material. Microscopy provides valuable anatomical information that aids in selecting high-quality herbal sources and assessing the purity of different parts of the herbal drug [20].

4. CONCLUSION

From centuries, *Simarouba glauca* has been used as a potent traditional medicine against many diseases. Leaves are mainly used as medicine but other parts of the plant including bark, root, seeds, seed oil are potential drug

parts. Quassinoids being major class of phytochemicals found in this plant are isolated from seeds, bark, and root. Pharmacognostical and the pharmacological activities have been conducted on *S. glauca* leaves by many researchers, but as per the review of literature, the Pharmacognostical studies on the bark has not been carried out till date. The present investigation establishes the Pharmacognostical characteristics of bark that identify and differentiate between *S. glauca* and other members of Simaroubaceae family and to check the purity of the drug.

CONSENT

It is not applicable.

ETHICAL APPROVAL

An approval letter from the Biodiversity Management Committee for the sustainable harvest (only when it involves multiple plant parts in large quantity) of cultivar bioresource for research purpose should be taken as per the Biological Diversity Act 2002, section 41 (1A) of amended Principal Act in 2023. Both the authors declare that 'written ethical approval has been collected from the Biodiversity Management Committee, Gram Panchayat, Bhairumbe, Sirsi, Uttara Kannada, India, for the collection of *Simarouba glauca* DC leaf and bark samples for research purpose from the field of Mr. Krishna M. Hegde.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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