ANATOMICAL PROTOCOL OF BARK FOR IDENTIFICATION OF MANGROVE TREES, *EXCOECARIA AGALLOCHA LINN* *(EUPHORBIACEAE)*

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ABSTRACT

Microscopic features of plant parts have been claimed which an important tool for taxonomic diagnosis of plant. The trunk bark of Exocoearia agallocha L., of Euphorbiaceae is said to possess highly poisonous secretion. Identification of the bark through microscopic features seams relevant in this situation. The bark consists of superficial periderm with thin irregular flakes.The cortical zone is wide and possess small circular cortical (Leaf - trace) bundles and wide circular secretary cavities. The secondary phloem is differentiated into outer thin collapsed phloem and inner thin cylinder of noncollapsed phloem. The noncollapsed phloem has intact angular sieve elements and distinct companion cell; phloem parenchyma cells are also intact. Sclerenchyma elements and tannin cells absent in the noncollapsed phloem. The phloem rays are non-storied, mostly uniseriate, less frequently biseriate and heterocellular comprising middle procumbent cells and terminal upright cells. Calcium crystals of prismatic type and starch grain are also abundant in the rays. Therefore mentioned microscopic feature may be considered as protocol for diagnosis of Excoearia agallocha.

Keywords: *Anatomy, Euphorbiaceae, Exocoearia agallocha, Phloem, Taxonomic Diagnosis*

1. INTRODUCTION

Mangroves are a group of trees and shrubs that are capable of growing in marine, estuarine and to a limited degree in fresh water. They occupy the fringe of intertidal shallows between the land and the sea. The term ‘mangrove’ is used to describe individual trees or shrubs and also the general habitat, although the habitat is often called a ‘mangrove forest’ or ‘mangal’. Mangroves as a group of plants, share several highly specialised adaptations that allowed them to colonise and thrive in intertidal areas. Several studies have been attempted to quantify the economic value of mangroves to commercial, fisheries productivity and to the community in terms of their ecosystem services. Morton [1] estimated that mangroves in Morton Bay, south-east Queensland contributed approximately $8380 per hectare to commercial fisheries production based on the monetary value of catch rates of target fish caught in this habitat type.

There has been an increasing awareness among the scientific community that the mangroves are an important component of the tropical marine ecosystem and apart from the economic uses of their vegetation, the mangrove areas are potential grounds for coastal aquaculture. It is generally recognised that mangrove areas form the feeding and nursery grounds for the juvenile stages of many commercially important species of prawns and fishes. The high productivity resulting from mangrove leaf fall supports a host of detritus feeding animals such as amphipods, mysids, harpacticoid?, molluscs, crustacean larvae, prawns and fishes. The mangrove vegetation acts as a buffer against tidal currents, floods, storms and cyclones and the network of creeks and channels provide shelter to aquatic life especially in the critical stages of their life history. The vegetation also helps in preventing soil erosion in the coastal zone. Blasco [2] has made a comprehensive study of the mangroves of India.

In particular they have developed special ways of dealing with concentrations of salt that would kill or inhibit the growth of most other plants. These include salty sap (i.e. concentrations of salt in the sap), leaves with a waxy coating that limits saltwater penetration, salt-secreting pores on the leaves that allow the plant to get rid of excess salt, and removing salt by concentrating it in branches and leaves before dropping them. The most visible adaptation of mangrove plants, and the one which most distinguishes them from other terrestrial plants, is their root system. In the past mangrove forests have been considerably undervalued. The wetlands in which mangroves occur have been considered 'wastelands' or breeding grounds for nuisance insects such as mosquitoes. As a result, many mangrove forests have been cleared, dredged, reclaimed, degraded or otherwise lost. Our understanding of the value of this habitat has greatly improved over the past few decades and mangrove forests are now regarded as key fish habitats. Because of their importance as habitat for fish, mangroves are protected in New South Wales (NSW) under the Fisheries Management Act 1994.

The genus, *Excoecaria agallocha L.* of the family Euphorbiaceae comprises 37 tree species with acceptable names. Trees are distributed throughout tropical Africa, Asia and Australia of which *E. agallocha* and *E. indica* occur in the mangroves (Duke, 2006; Yin *et. al.*, 2008). Several references are of direct relevance to the present review on *E. agallocha*. There are chemical constituents of medicinal value in the genus *Excoecaria* (Yin et. al., (2008); Rajeswari and Rao, (2015) Kaliamurthi and Selvaraj, (2016); Mondal et. al., [3, 4,].*Excoecaria agallocha*trees are multi-stemmed and produce a copious amount of white latex, which is toxic, causing skin blistering and temporary blindness.

Mangrove swamps form a type of coastal wetland found in the tropics and subtropics. Within a mangrove forest, the most salt-tolerant species occur near the ocean. *Excoecaria agallocha*, known as a back mangrove, is found at higher elevations back away from the ocean where salinity is lower.This small tree species may grow up to 15 m high. Trees are either male or female. Male flowers form drooping tassels, while female flowers appear as shorter spikes. Pollinators such as bees commonly visit the flowers. The fruit is a small dark capsule. The milky latex of *Excoecaria agallocha* is very poisonous and powerfully irritant, which is not unusual in milky species of plant in the family Euphorbiaceae. Contact with skin causes irritation and rapid blistering; slight contact with eyes can cause temporary blindness, hence the common names that refer to blindness. Even the generic name is from the Latin for "blinder". Bligh (1977) stated that the smoke from the burning wood is poisonous and can harm the eyes, so it would not have been useful as fuel. Even dried and powdered leaves retain the poison and can kill fish very quickly. Owing to its complex chemistry, the plant may have many new medicinal uses. A mangrove of this plant is seen surrounding the ancient Chidambaram Temple in the state of Tamil Nadu.

2. MATERIALS AND METHODS

2.1 Collection of specimens

*Excoecaria agallocha L.*an evergreen mangrove species belonging to the family Euphorbiaceae was used for the present investigation. This species is naturally growing in abundance in the salt marshes of Pichavaram, Thandavarayan chozhaganpet (Fig. 1) on the east coast of Tamil Nadu, India about 10 km east of the Annamalai University campus. The mature seedlings were collected from Pichavaram. The plant wasidentified the type sample by Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai. The specimen is deposited in the herbaria of Madras Presidency College Chennai.

2.2 Infiltration

The bark cut in the small pieces to enable to prepare transverse section, tangential longitudinal and radial longitudinal section. The cut pieces were fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml).After 24 hrs of fixing; the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^{o}C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

2.3 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 (Johansen, 1940) [5 - 15] 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 colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. 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.

2.4 Photomicrographs

Microscopic descriptions of tissues are 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 are as given in the standard Anatomy books (Esau, 1964).

3. RESULTS

3.1 Barks of *Excoecaria agallocha*

Bark measuring 2.8µm thick was studied. The bark consists of a superficial periderm, wide cortex and wide secondary phloem (Fig 2). The periderm is deeply fissured forming irregular fissures. The epidermis is broken into shallow cavities. The phalloderms derivatives are seen only as few layers. The cortical zone consist of small thin walled compact parenchyma cells, some of the cortical cells possess dense masses of brownish tannin contents (Fig 2: 1, 2.). in further inner portion of the bark lies the secondary phloem zone which is the major area of the bark (Fig. 2). The secondary phloem zone is 2.5µm in radial plane. The secondary phloem is differentiated in to two distinct portions, namely collapsed secondary phloem and non-collapsed secondary phloem (Fig 4. 1). The collapsed secondary phloem includes crushed and obliterated sieve elements and companion cells, dilated parenchyma cells and phloem rays cells. The crushed phloem cells are visible in thin dark tangential lines (Fig 4:1). The dilated parenchyma cells are filled with brownish tannin. Phloem fibres are sparsely distributed in collapsed phloem zone. Wide, circular secretary cavities are rarely seem thin outer non-collapsed phloem. The cavity is lined by two circular layers of small rectangular thin walled epithelial cells (Fig 4.1; 6.2).

3.1.1Non-collapsed secondary phloem

The non-collapsed part of the phloem occursas the last part, and inner to the secondary xylem (Fig 4.1). It is 150µm thick; all the cells of the phloem are intact. The sieve elements are wide, squarish, and fairly thin walled. The companion cells are rectangular or spindle shaped. There are no tannin cells or phloem fibres in this non-collapsed phloem (Fig 4: 1, 2). There is circular vascular bundle in the cortical region. The cortical vascular bundle israre and sparse. The bundles are collateral, comprising four short uniseriate or triseriate vertical lines of xylem elements. The xylem elements are angular, narrow, thick walled and lignified. At one end of the xylem strands occurs wide, angular phloem elements (Fig 5:1, 2). The vascular bundle is surrounded by a thin layer of bundle sheath parenchyma cells.

3.2 Crystal distribution

Calcium oxalate crystals of prismatic type are characteristically distributed in single line around the wide brachy sclereids of the phloem (Fig 6: 1). The crystals areadhering along outer walls of the sclereids.

3.2.1 Tangential longitudinal sections of the phloem (TLS).

In TLS view the phloem rays appear in vertical lines. The rays are spindle shaped (Fig 7: 1), nonstoried and mostly uniseriate. The rays are hetero cellular comprising middle portion of procumbent cells and terminal portions of upright cells. The procumbent cells are short and rectangular in shapes; the upright cells are long and narrowly conical (Fig 7: 2; 8: 1, 2). The biserisate rays are less common and they are 200µm long and 20µm thick. The uniseriate rays are upto 110µm long and 10µm thick. Some of the rays possess calcium oxalate prismatic crystals (Fig 8: 2). The crystals are large and occur in single in each cell.

3.2.2 Radial longitudinal section (RLS)

In RLS view (Fig 9 & 10). The phloem rays appears horizontal, ribbon shaped layers. They consist of middle portion of horizontally elongated rectangular cells and terminal portion of vertically elongated upright cells. The middle cells are called procumbent cells. Starch grains are abundant rays cells (Fig 10: 1, 2).

4. DISCUSSION

Wood and bark are two most essential components of trees, providing mechanical stability, conduction of metabolites and water and storage of mainly chemical substances which are necessary for growth and reproduction plant. Identification of the plants is the first step for any type of studies of the plants. Taxonomist mostly rely up on the vegetative and floral morphology of plants for taxonomic identify of taxa if the plant samples are available in fragmentary form, especially when obtained from crude drug markets, one can most expect the sample having the flower, fruits and leaves. In such circumstance, the investigator has to depend up on the anatomical parameters of the leaf, petiole, stem, root, bark and wood. The microscopic feature of mentioned plant parts are highly specified well defined in their anatomic parameter eminent anatomic like Metcalfe & chalk (1950). Esau (1964; 1979), Arber (1961) and others have expressed the view that plant anatomy plays crucial role in taxonomic diagnosis of plants.

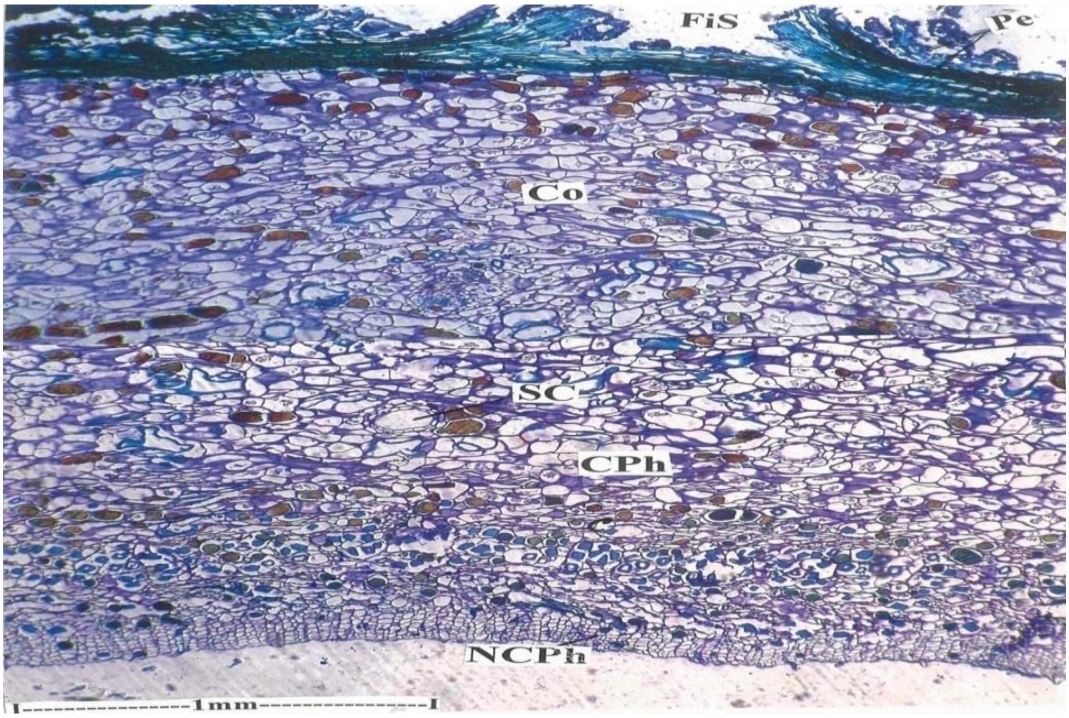
Mangrove plants, as mentioned elsewhere, live in for saline water saturated soil. But it seems that each mangrove species has specific microscopic feature not shared by other species. The bark also exhibits diversity of in the structural architecture. The present study, the structure of bark of *Excoearia agallocha* is study in detail and the result of the study will from protocol of anatomical feature to identify that plant either in fragmentary from or in a tree standing in the field without floral parts. The bark of *Excoearia agallocha* has thin narrow vertical fissures leading to formation of the irregular periderm flakes. This surface feature of the bark has been given much importance for preliminary diagnosis of the standing trees. Bark has distinct cortical zone where the cells are parenchmatous and possess brownish tannin content. The cortical tissue is much reliable diagnostic features for species studied (Fig 2: 2).The secondary phloem which is major parts of the bark has outer collapsed phloem and inner non collapsed phloem. The collapsed phloems consist of crushed dark thick streaks of sieve elements and dilated phloem parenchyma cells. The non-collapsed phloemisdistinctly marked by its intact sieve elements and phloem parenchyma which is regular compact vertical files (Fig 4:1, 2)distinct delimitation of collapsed and noncollapsed phloem tissues provides another indelible clue for diagnosis of the species.

As seen in radial and tangential section of the phloem, the phloem rays are non storied uniseriate, rarly biseriate, and the rays cell are heterocellular having in the middle part of the rays' procumbent cells and in the terminal part upright cells (Fig 7:2; 8:1and 9: 1,2). Calcium oxalate prismatic crystals and starch grain are abundant in the cells. Rays. Tannin content and sclerenchyma elements are absent in the non-collapsed phloem. These anatomical features will be helpful in identifying the plant species studied.

5. CONCLUSION

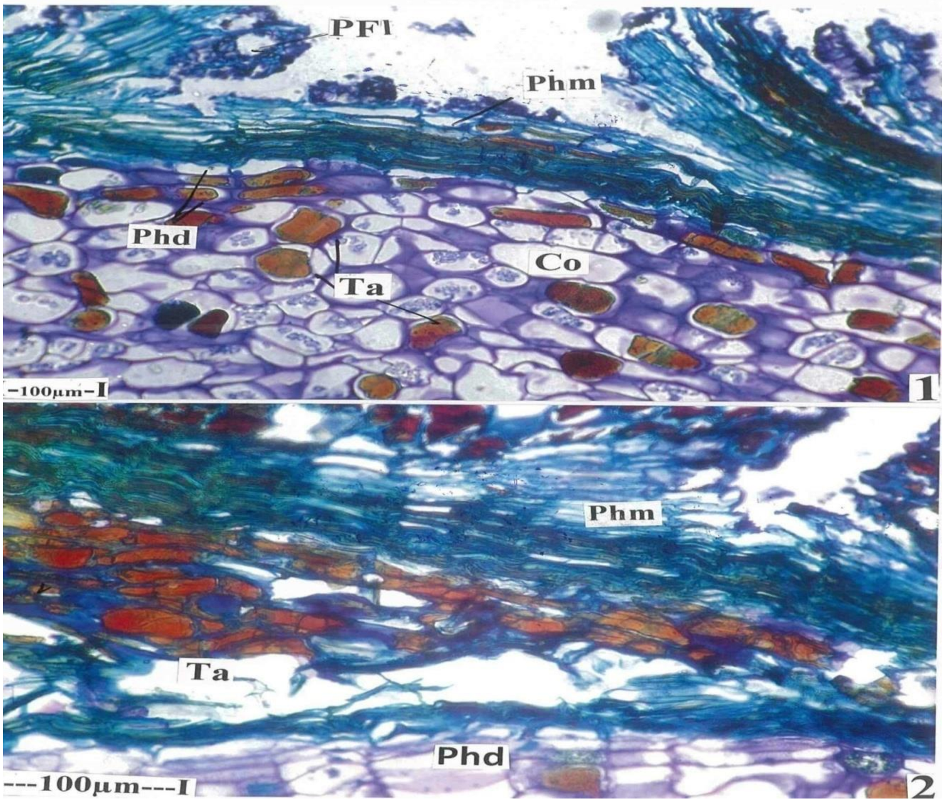
Since the bark of*Excoecaria agallocha* has highly poisonous secretion, their diagnosis with help of microscopic features will provided precocious mesheres for handling bark sample for medicinal treatments with much care.

Fig. 1. Habit of *Excoecaria agallocha*



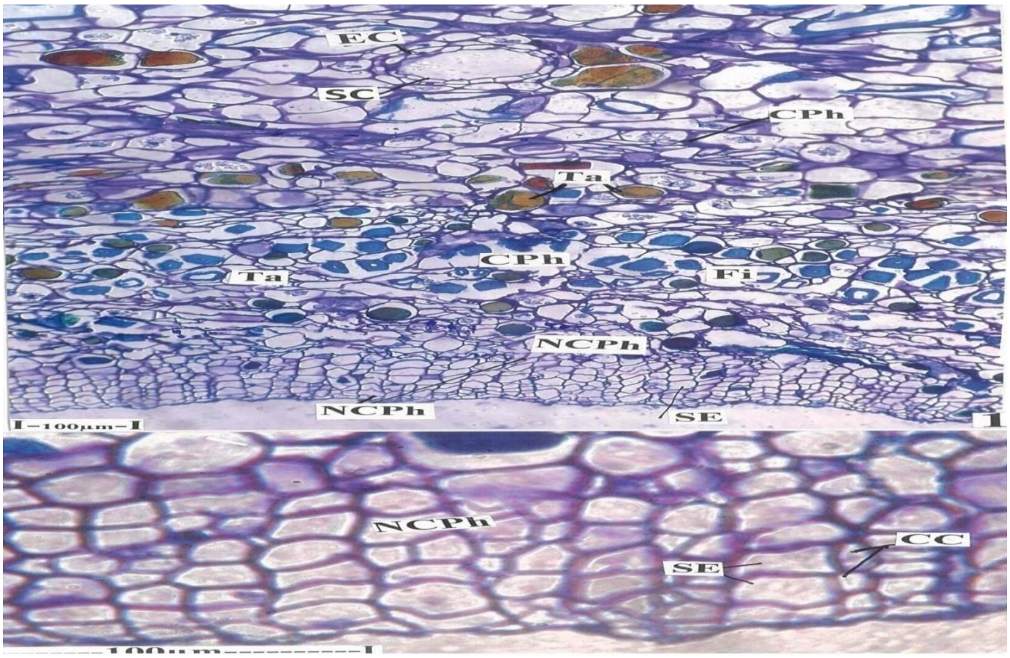
Co – cartex; Cph – Collapsed phloem; Ncph – Non collapsed phloem; Pe – Peiderm; Sc – Secretory cavity.

Fig. 2. T. S. of Bark of *Excoecaria agallocha*



Co – Cortex; Pfl – periderm flakes; Phm – Phelloderm; Ta – Tannin.

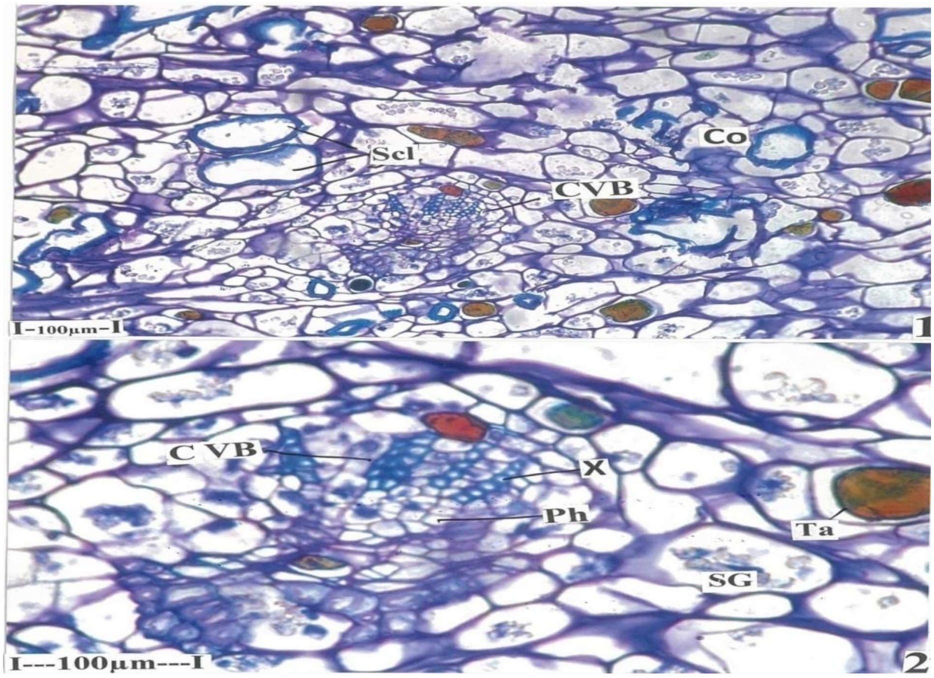
Figure :3. 1. *Excoecaria agallocha* T.S Of Periderm of the bark showing tissues and inner phellem.



CPh – Collapsed phloem; CC – Companion cell; EC – Epithelial cell; Fi – Fiber; NCPh – Noncollapsed phloem; Sc – Secretary cavity; SE – Sieve Element; Ta – Tannin.

Figure :4. 1 T.S of secondary pholem showing outer collapsed and inner noncollapsed phloem.

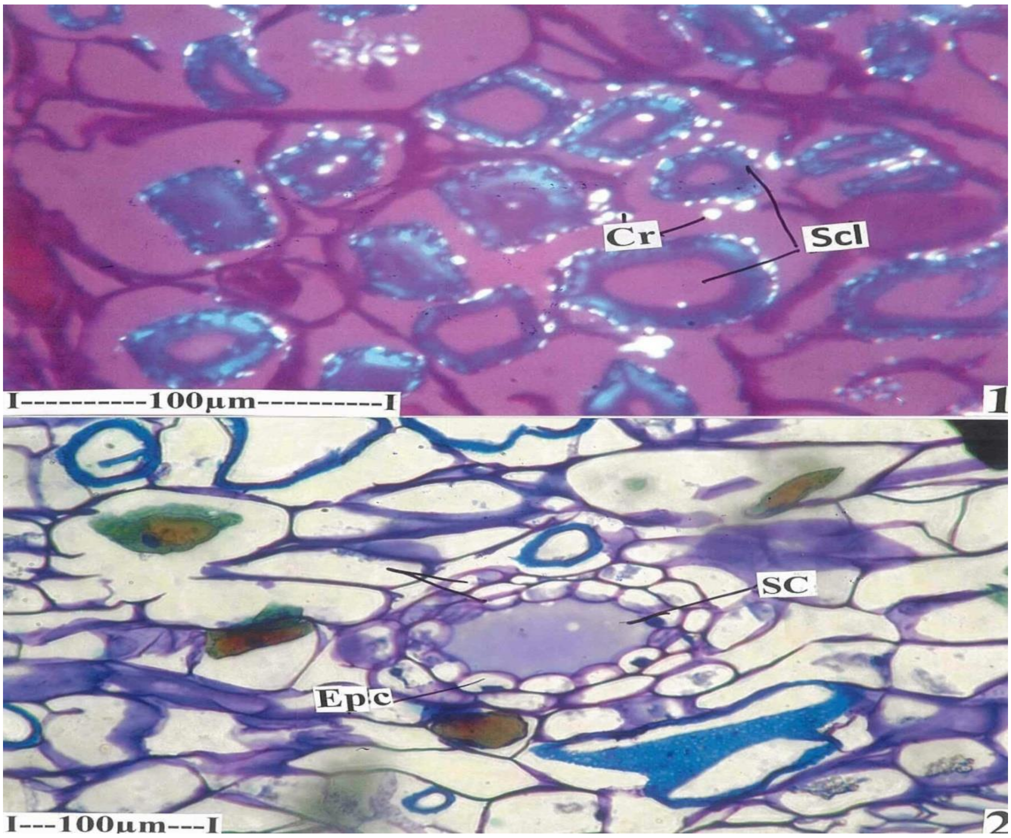
Figure : 4.2 T.S of non collapsed phloem.



Co – Cortex; CVB – Circular vascular bundle; Ph – Phloem; SG – Starch grain;SCL – Scleroids; Ta – Tannin; X – Xylem.

Figure 5.1. *Excoecaria agallocha* T.S of collapsed phloem and cortical vascular bundle

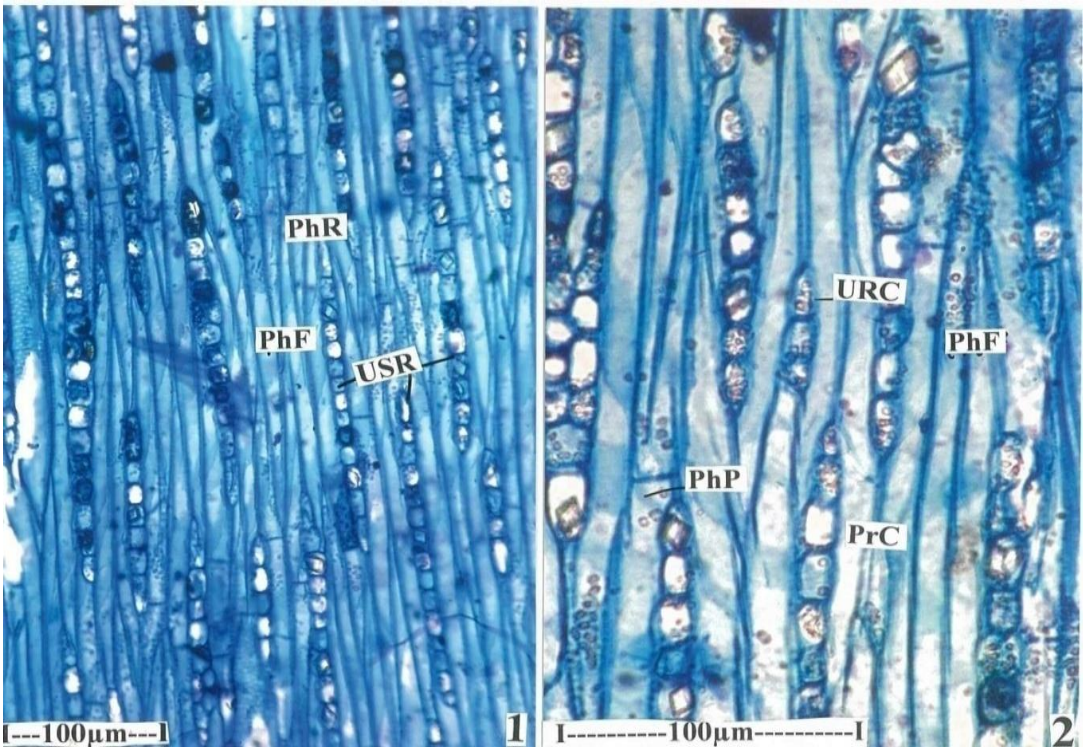
Figure 5. 2. *Excoecaria agallocha* cortical vascular bundle in the Bark.



Cr – Crystal; EPC – Epithelial cell; SC – Secretary Cavity; Scl – Sclereids.

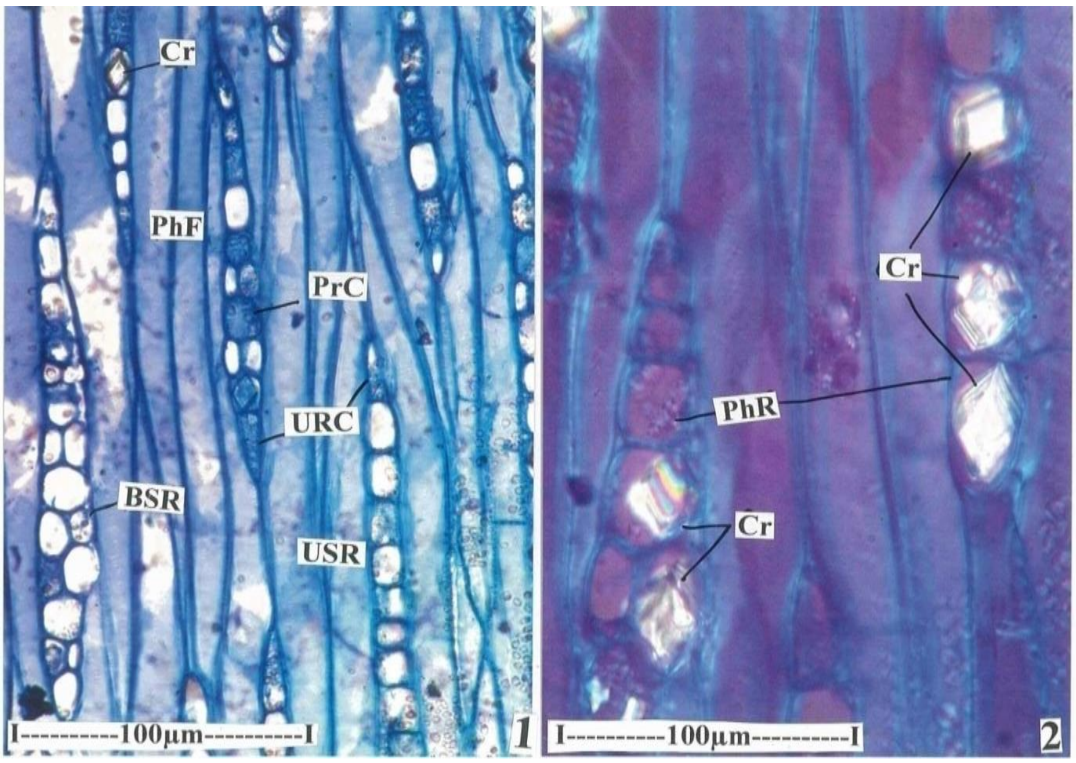
Figure 6.1. *Excoecaria agallocha* , Crystals associated in Phloem fibres,

Figure 6:2. Secretary Canal enlarged.



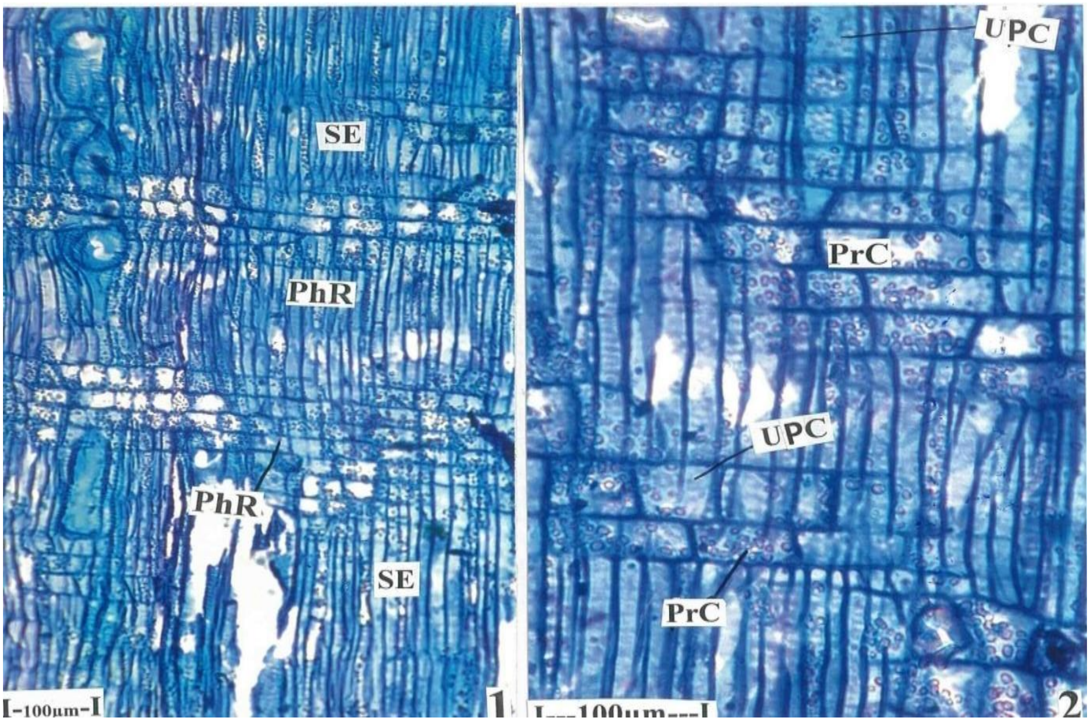
PhF – Phloem fibre; PrC – Procumbent cell; PhP – Phloem; PhR – Phloem ray;USR – Uniseriate ray; URC – Upright cell.

Figure :7.1.2. *Excoecaria agallocha*, Tangential longitudinal sections of the Phloem.



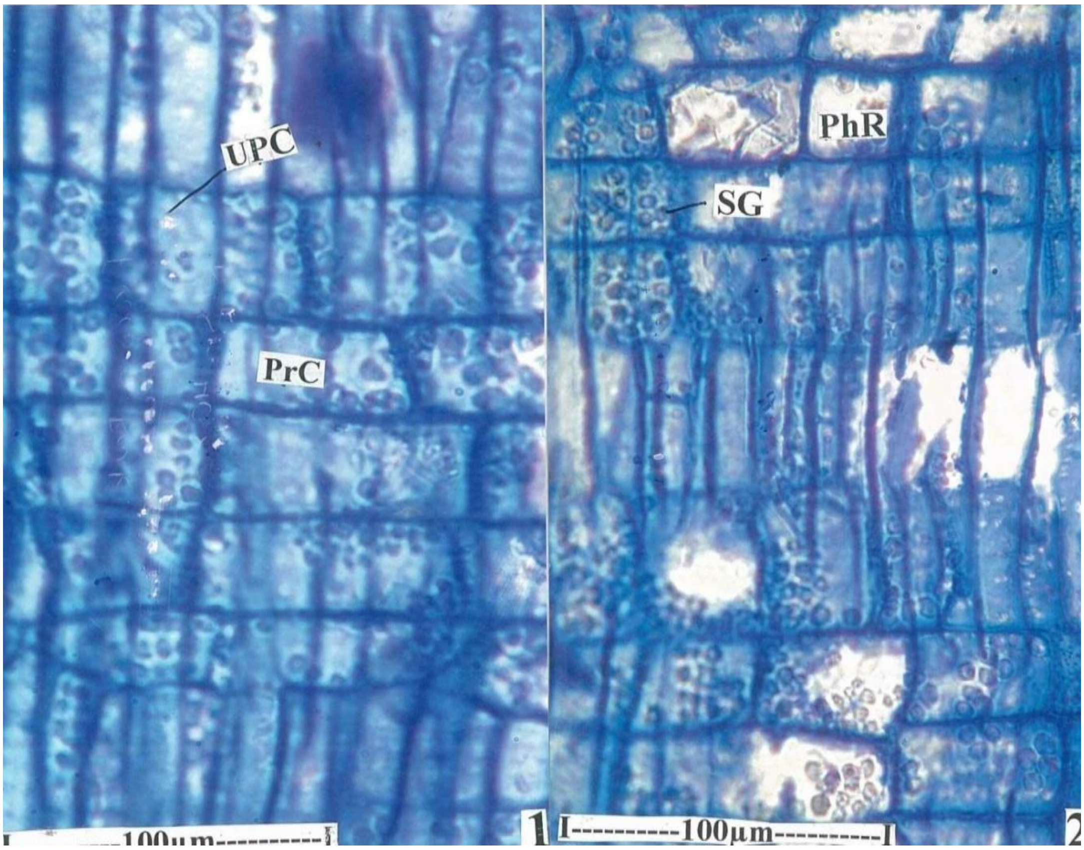
BSR – Biseriate Ray; Cr – Crystal; PhF - Phloem; PrC – Procumbent cell; PhR – Phloem Ray; URC – Upright cell; USR – Uniseriate Ray.

Figure :8.1.2. *Excoecaria agallocha*, TLS, view phloem rays, 8:2, Crystals in the phloem rays.



PrC – Procumbent cell; PhR – Phloem Ray; SE – Sieve element; URC – Upright cell.

Figure :9.1.2. *Excoecaria agallocha*, Radial longitudinal section 2. (RLS) of phloem rays.



Prc – Procumbent cell; PhR – Phloem Ray; .SG – Starch grain; URC - Upright cell.

Figure :10.1.2. *Excoecaria agallocha*, RLS view of phloem.

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