ANATOMICAL PROTOCOL OF BARK FOR IDENTIFICATION OF MANGROVE TREES, AVICENNIA MARIAN (Forssk.) Vierh. ACANTHACEAE

Haridass. B[*1] and Dr. V. Krishnan [2]

[1]*Department of Plant Biology and Plant Biotechnology, Presidency College, Chennai - 600005*
[2]*Assistant Professor, Department of Plant Biology and Plant Biotechnology, Presidency College (Autonomous), Chennai – 05*

Abstract

The bark of the plant serves as the main food conducting channel, realm of many storage of medicinal compounds and also serves as protective barrier of the internal part of the stem. Different aspects of bark particularly, the structural aspect, lags much behind the wood. This is true with respects the *Avicennia marina (Forssk.) Vierh*. Because study on the bark of this plant is a totally lacking. The present study deals with the surface feature, structure of outer bark (periderm) and inner bark of (Secondary phloem). The study brings to light the structure and organisation of the different cell types and tissues of the bark and present a microscopic protocol for taxonomic identify of the taxon.

Keywords: Anatomical Protocol of Bark for Identification of Mangrove *Avicennia marina*

INTRODUCTION

The term "mangrove" refers to an assemblage of tropical trees and shrubs that grow in the intertidal zone. These zones are frequently inundated with salt water due to tidal activity of gulfs, seas and oceans. Mangroves play a key role in near shore habitats of the tropical zone, Eman et al., (2012).[1] Today, it is important to use of ecological indicators, such as biomass for recognizing the special status of ecosystems, such as mangrove forests and also monitoring and evaluating changes through a specific period Akbar Ghasemi et. al., (2016). [2] *Avicenna marina* is small evergreen tree, up to 10m high, stem erect with fine pale gray scales. Leaves simple leathery, opposite, ovate, petiolate with entire margin and acute tip, dark glossy green on the upper surface, dull greyish on the lower surface with excreted salt crystals. Inflorescence cymose, in small terminal or axillary clusters on short stalks, flowers bracteate, scented;calyx lobes 2-4µm long, obtusish, fine fimbricate marginated; corolla dark yellow, exceeding the calyx with 4 unequal spreading lobes exceeding the tube. Fruit 2-valved capsule, globose, pale green, 1.5-2.5cm long; seeds 2-4 large. As *Avicennia* is growing in a specialized habitat, which is poorly aerated, it is adapted to life in this habitat by the presence of erect leafless outgrowths of the roots called pneumatophores or breathing roots wide up to 50cm long, they stick out above water and absorb air, which oxygenates the roots.

Rheumatoid arthritis is a common disease in today's society.mangrove (Avicennia marina) has long been used to reduce arthritic pain. Vitamin C as an antioxidant is effective in reducing symptoms of rheumatoid arthritis Shafie et al., (2013).[3] Heavy even-textured wood used for

poles and ribs of boats. Bark yields a brown dye. Leaves are used for camel fodder around the Red Sea. Branches are lopped and fed to cattle in India and Australia. The tree possesses a bitter aromatic juice, used as an abortive in tropical Africa and Asia. Root and bark are used as aphrodisiac, the wood for snakebite, the aqueous extract of the seed for sores.

Distribution of *Avicennia marina* is disjunctive in Western Australia; the population of the Abrolhos Islands is 300 kilometres further south than the nearest population of Shark Bay. Another mangrove system is found even further south (500 km) at Bunbury. This colonisation of southerly climes may have occurred relatively recently, perhaps several thousand years ago, when they were transferred by the Leeuwin Current. The most inland occurrence of mangroves in Australia is a stand of grey mangroves in the Mandora Marsh, some 60km from the coast. In South Australia along the Barker Inlet and Port River in Gulf St Vincent, as well as in sheltered bays in Spencer Gulf and the west coast of Eyre Peninsula, *A. marina* forests form hatcheries for much of the state's fish and shellfish commercial and recreational fisheries.

Material methods

Collection of specimens

As mentioned elsewhere, the studies, on bark anatomy is meagre and fragmentary. This is particularly true with respect to mangroves tree. The mangroves are unique compound of other ecological spectrum. Considering the unique habitat and other economic importance of the mangroves. The mangroves *Avicennia marina* was taken for the studying. The present investigation will provide set an anatomical features of the bark which may be used taxonomic identify.

Avicennia marina (Forssk.) Vierh. Is a common mangrove species on tropical and subtropical sea shores, swamps and stream banks In mature tree, grey mangrove or white mangrove, is a species of mangrove tree classified in the plant family Acanthaceae (formerly in the Verbenaceae or Avicenniaceae). As with other mangroves, it occurs in the intertidal zones of estuarine areas. This species is naturally growing in abundance in the salt marshes of Pichavaram 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 was identified and deposited in the herbaria of Madras Presidency College Chennai, and Prof. P. Jayaraman, Ph.D. Plant Anatomy Research Centre (PARC) West Tambaram, Chennai.

Infiltration:

The bark cut in the small pieces to enable to preparation of 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 24hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by Sass, 1940.[4-14] Infiltration of the specimens was carried by gradual addition of

paraffin wax (melting point 58-60C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure (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 colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein.

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).

OBSERVATION

***Avicennia marina (Forssk.) Vierh*. Acanthaceae , (Avicenniaceae)**

The surface of the bark is somewhat rough with thin irregular periderm peelings. As a result of irregular peeling of thin outer periderm, the bark surface assumes shallow ridges and furrows (Fig: 1:1, 2; 2:2). The periderm very thick measuring 2.5µm in radial plane. The periderm cells are horizontally rectangular fairly thick walled with narrow cell lumen and supervised walls. The periderm cells are homogeneous; the distinct phellum and phelloderm regions could be recognised there is also less sharp line of demarcation between the periderm and cortex. The phellem cells are thick walled and small in size. The phellogen is fairly visible and inner to the phellogen is the phelloderm layers which are quite thick comprising several layers of large thick walled cells (Fig 3:1,2). The phelloderm cells have cellulose walls and also possess some cell inclusion. The phellam cells are dead cells with suberised walls (Fig 3 & 4:1).

Cortical cells (Fig 4:2). The cortical cells are spherical and are less compact having narrow inter cellular spaces. The cells densely packed with calcium oxalate crystals. The crystals are unique in shape; they are double triangular spindle in shape with narrow pointed ends on the opposite sides (Fig 5: 1, 2). The crystal is 10µm long and 4µm thick. The secondary phloem consists of outer zone of collapsed phloem and inner noncollapsed phloem which is very nexit outer to the secondary xylem (Fig 1.2, 2.1). (The noncollapsed phloem layers have thick continuous cylinders of brachys sclereids and discontinuous isolated clusters of fibbers located cell around the phloem cylinders) (Fig 6.1, 2). The barchy sclereids are squarish in shape with thick secondary wall and reduced lumen, they have canal like simple pits of to the walls. The phloem fibres are small, angular in shaped, thick lignified

walls and wide lumen (Fig 6.2). The phloem consists of angular wide sieve elements and companion cells located at the corners of the sieve elements. The sieve elements do not posses any cell inclusions, Collapsed Phloem

Tangential longitudinal section (TLS)

Phloem in TLS view shows the phloem rays in vertical spindle shaped phloem rays, the rays are nonstoried, the rays are heterocellular, one, two, or multiseriate (Fig 7.1, 2, 3). The cells of thin rays are spindle shaped and thick walled. The cells in the middle part of this ray are squarish or circular. These cells are called procumbent cells, the cells; at the upper and lower ends of the rays are vertically oblong and conical, these cells are called upright cells (Fig 7.1, 3). The rays having two types of cells are called heterocellular rays. The height of the rays is 600µm for the uniseriate ray's, 350µm biseriate rays and 220µm for the multiseriate rays.

Radial longitudinal section (RLS).

In RLS view, the phloem rays appear in horizontal ribbon shape and the cells are in the form of bricks (Fig 8.1). The rays are hetrocellular, having two types of cells. The cells in the middle part of the rays are horizontally oriented and are rectangular and squarish. Those cells on the upper and lower parts of the rays are vertically elongated. The horizontal cells are procumbent cells; the vertically elongated cells are called upright cells (Fig 8.3). Phloem parenchyma cells are in vertical series of strand. The phloem fibbers are in compact vertical series.

THE MEDICINAL AND OTHER USE OF THE *Avicennia marina*

Bark astringent and used as aphrodisiac, for scabies, antifertlity agent and has tanning properties. Flowers for perfumes. Leaves are aphrodisiac and used for toothache, Leaves and seeds forage for camels and animals. Wood was used as fuel and in traditional buildings. The plant is known for the quality of its honey and the charcoal has special uses.

DISCUSSION

Bark has no as much commercial value as wood. However, bark serves as important as any other organ of a plant. It is the depository of many highly potential medicinal compounds, storage of food materials and above all it is the main pathway of food materials to entire area of the plant it also function as protective barrier for the internal tissue of the plant. Some plants have barks that are highly poisonous and irritant to the skin. *Avicennia marina* is said the possess poisonous and irritating liquid in the bark. One has to be highly precautious to identify such bark fragments while study the phyotochemical and pharmacological aspects such plants sample. Anatomical studies of the bark of *Avicennia marina* warrants microscopic determination of the structural aspects of the fragmentary bark in the absence of any other floral parts. The surface feature of the bark offers some clues for the determination of name of the plant in *Avicennia marina*, the periderm barks along the surface having rough shallow fissures. The phelloderm is homogenous and suberised and phelloderm and phellum regions could be recognised. This is little distinction between the cortex and phloem zone. The suberised phellem and cellulosed walled living phellum are well defined both in cell structure and cell wall composition a unique type of calcium oxalate crystal are abundant in the cortical cells, they spindle shaped with narrow opposite ends. Such crystals are not shaped any other species of *Avicennia marina* and so it offers a helping for diagnosis of *Avicennia marina.*

Secondary phloem which is major part of bark differentiated in to outer wider collapsed phloem and inner narrow noncollapsed phloem. In the collapsed phloem, the sieve elements and the companion cells are crushed in to dark thin streaks. Which on are beyond the level of reorganisation. The phloem parenchyma and phloem rays are dilated the collapse of the sieve elements in due to increase in the thickens of the bark and presence exerted on the sieve elements by dilating rays cells and parenchyma cells. The characteristic feature of diagnostic value of the bark is the presence of the phloem sclereids and phloem fibres. In the phloem zone are successive continues rings 2-5 layers of rectangular bark shaped scleried in between the circular rings of sclereids occur the discontinues ring which consists of small angular thick walled fibres which have wide lumen figure (6.1,2) this characteristic arrangement of sclereids and fibres worthy considering as a diagnostic feature for diagnostic of *Avicennia marina*.

Phloem rays as seen in TLS view offers certain features diagnostic feature. Rays storied non storied, uniseriate, multiseriate , homocellular, heterocellular and arrangement lenth, breadth of the rays are relaiable diagnostic feature for taxonomic identify of the taxa. In *Avicennia marina* the phloem rays are uni to multiseriate, heterocellular and nonstoried. In combination of other anatomical features, the phloem rays may be useful for identify of the plants.

CONCLUSION

Fragmentary bark sample procured from the market may some time being adulternant. In the bark sample is rare and not easely available, the market people may be tempted to supply duplicate bark sample. Organolettic charecters are not sufficient to detect the adulteration of plant sample, especialy bark smaple. Total microscopic features only help for showing adultaeration of the herbal druks.

Avicennia marina **Growth habit**

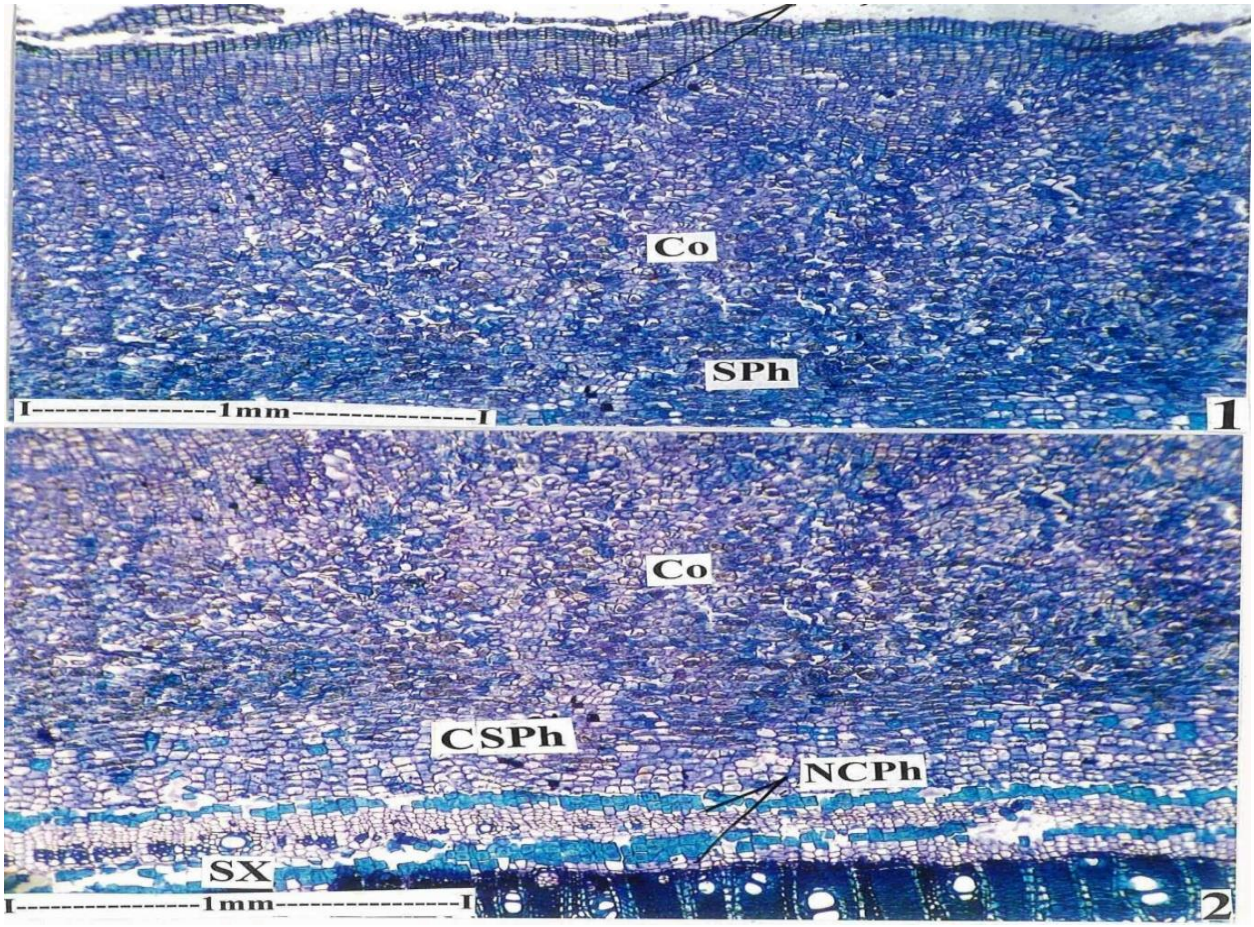

Figure 1.1,2. Avicennia marina T.S of outer Bark
Co – Cortex; CSPh – Collapsed Scondary Phloem; NCPh – Non collapsed Phloem; SPh – Scondary Phloem; Pe – Periderm.

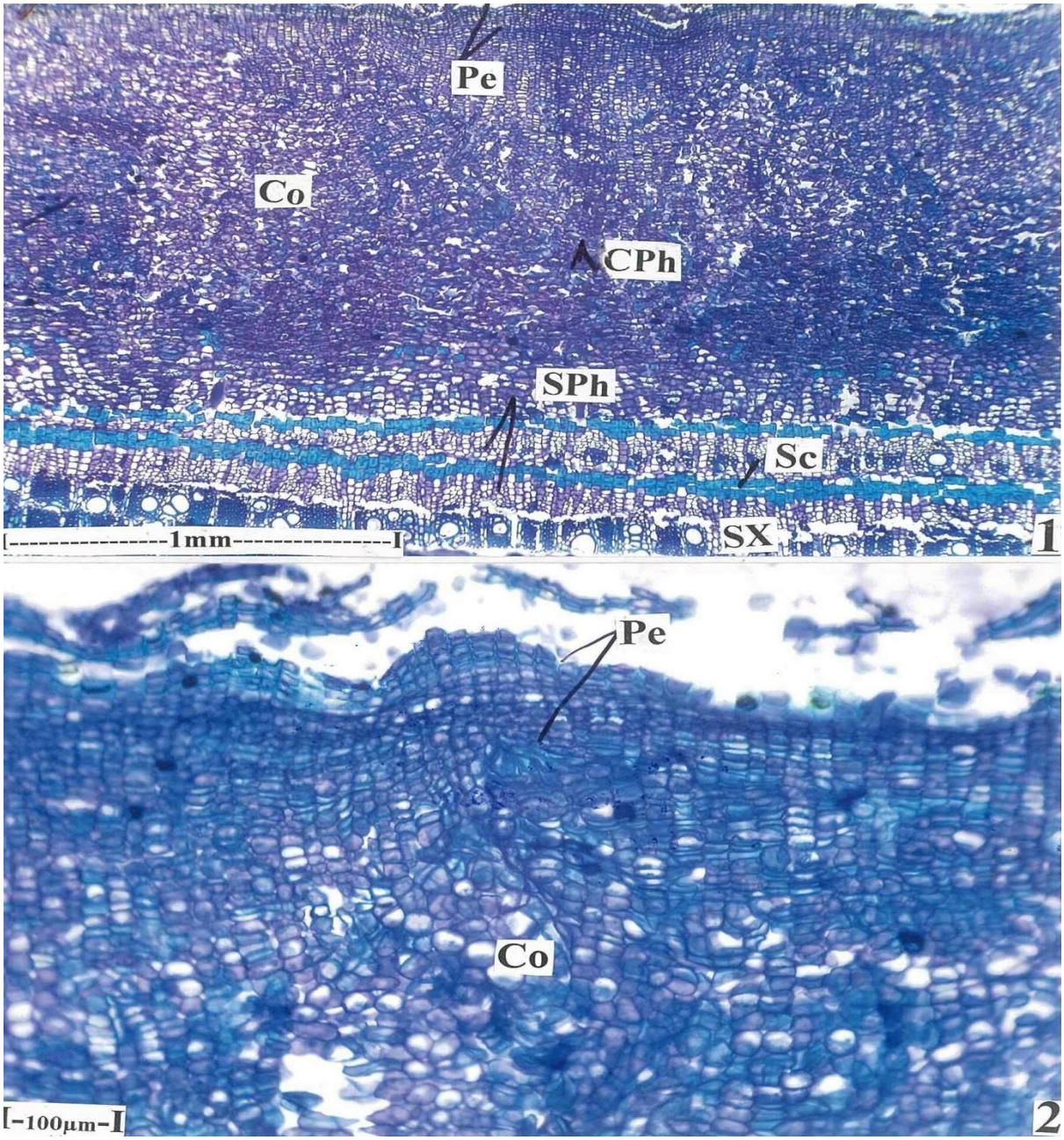

Figure : 2.1, *Avicennia marina Bark T.S showing outer collapsed phloemand inner non collapsed phloem. (2.1) T.S of Periderm portion.*

Co – Cortex; NCPh – Non Collapsed Phloem; Pe – Periderm; SPh – Scondary Phloem; Sc – Sclerenchyma.

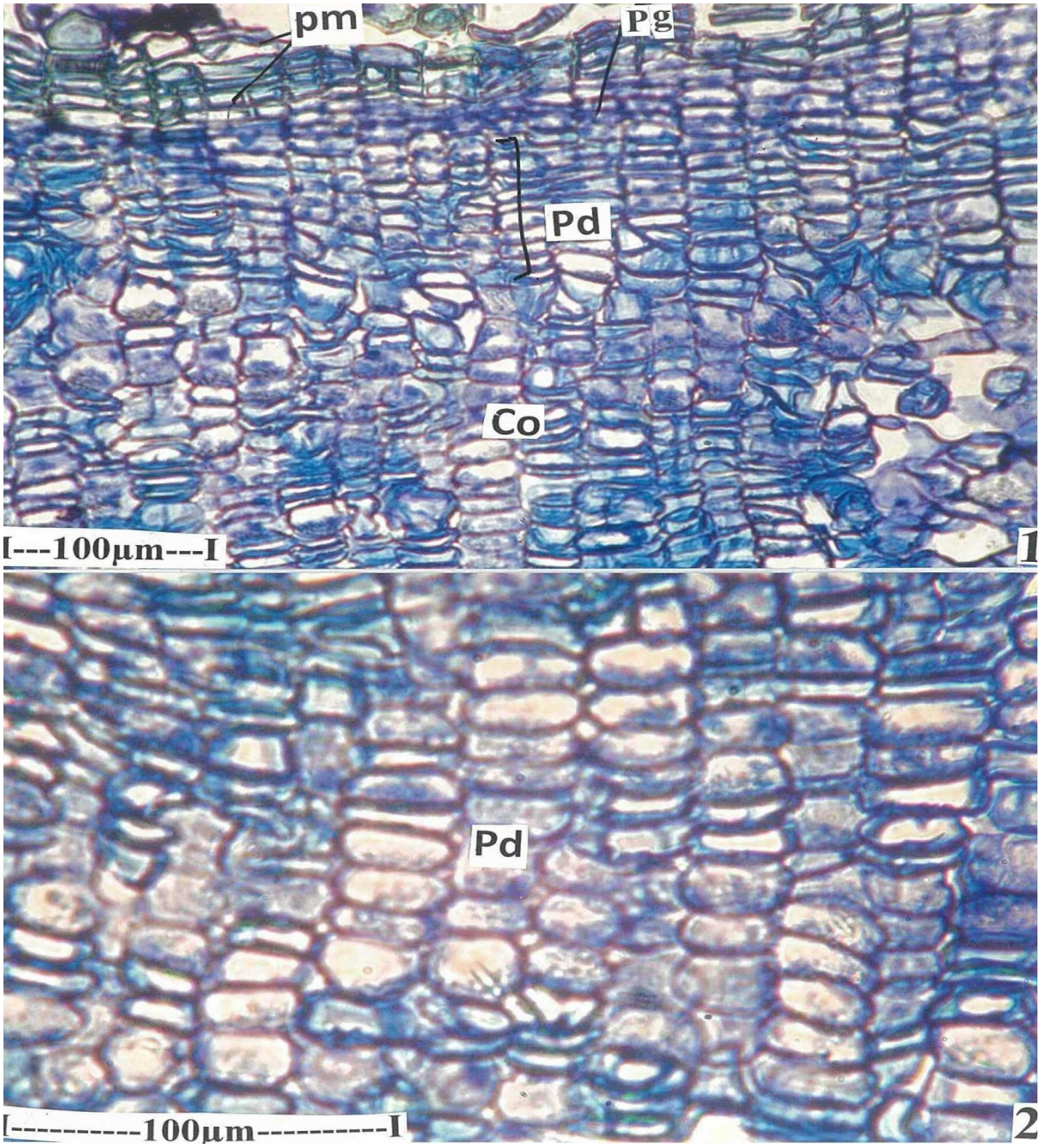

Figure : 3.1, *Avicennia marina T.S of Periderm, T.S of Periderm showing outer Phellen.*

Co – Cortex; Pd – Phelloderm; Pg – Phellogen; Pm – Phellem.

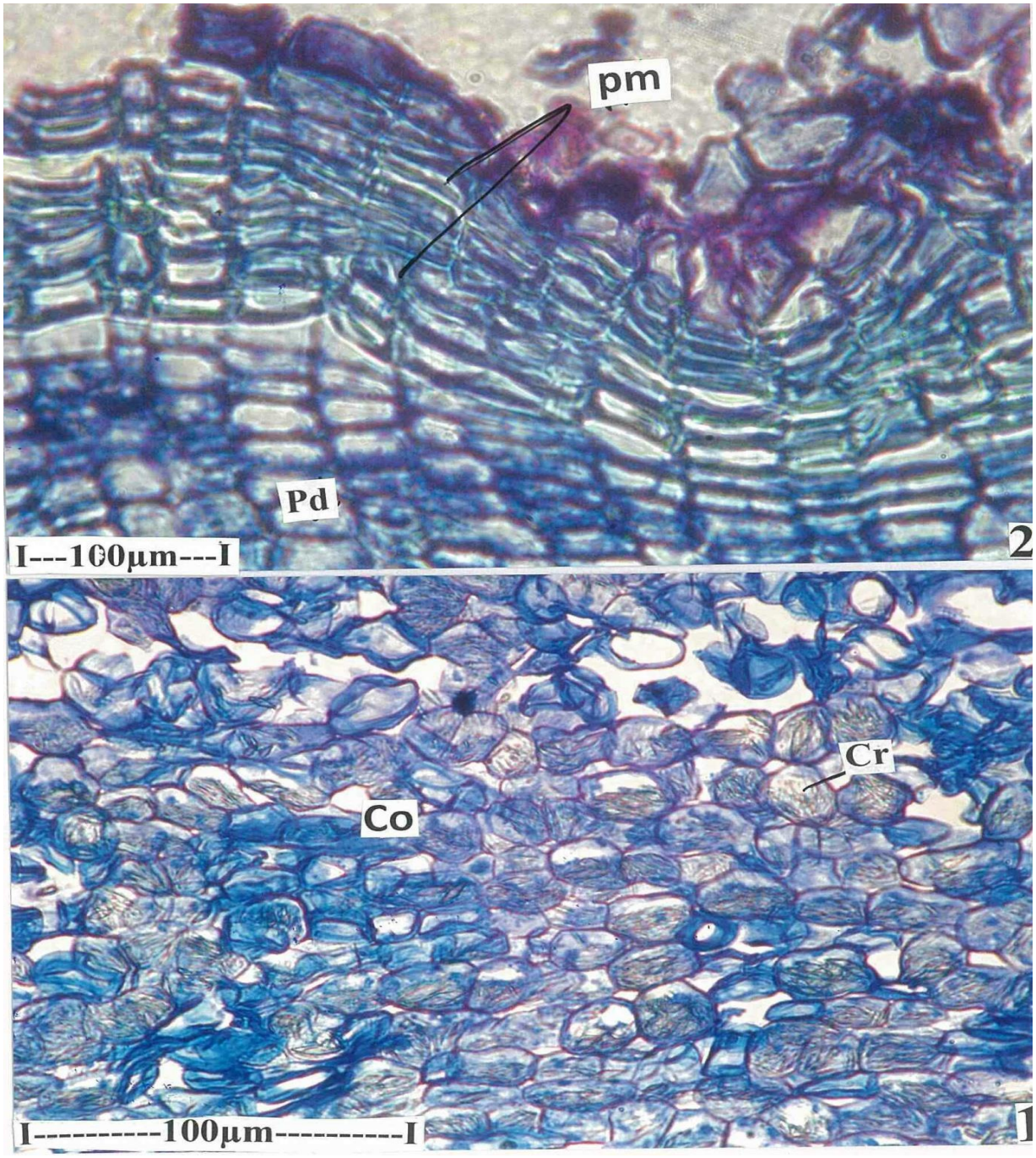

Figure : 4.1,2. *Avicennia marina T.S of Bark showing Phellem and Phelloderm, (4.2) T.S of Bark showing cortical Paranchyma with crystals.*

Cr – Crystal; Co – Cortex; Pm – Phellem; Pd – Phelloderm.

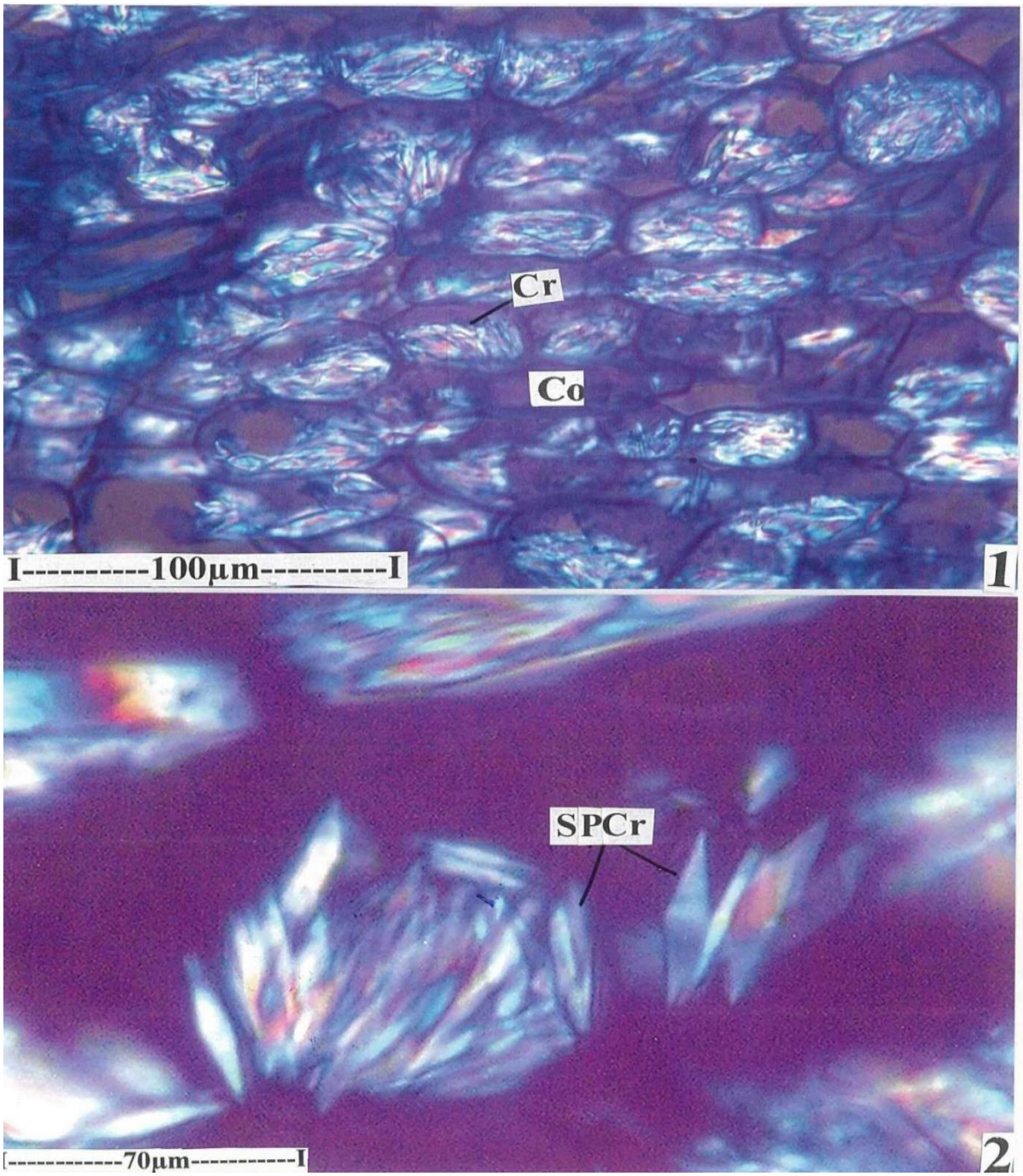

Figure : 5.1,2. *Avicennia marina T.S of Bark - Cortical Paranchyma cell with crystals (5.2) Paranchyma cells heaving spindle shaped crystals.*

Co – Cortex; Cr – Crystals;SPer – Spindle shaped crystals.

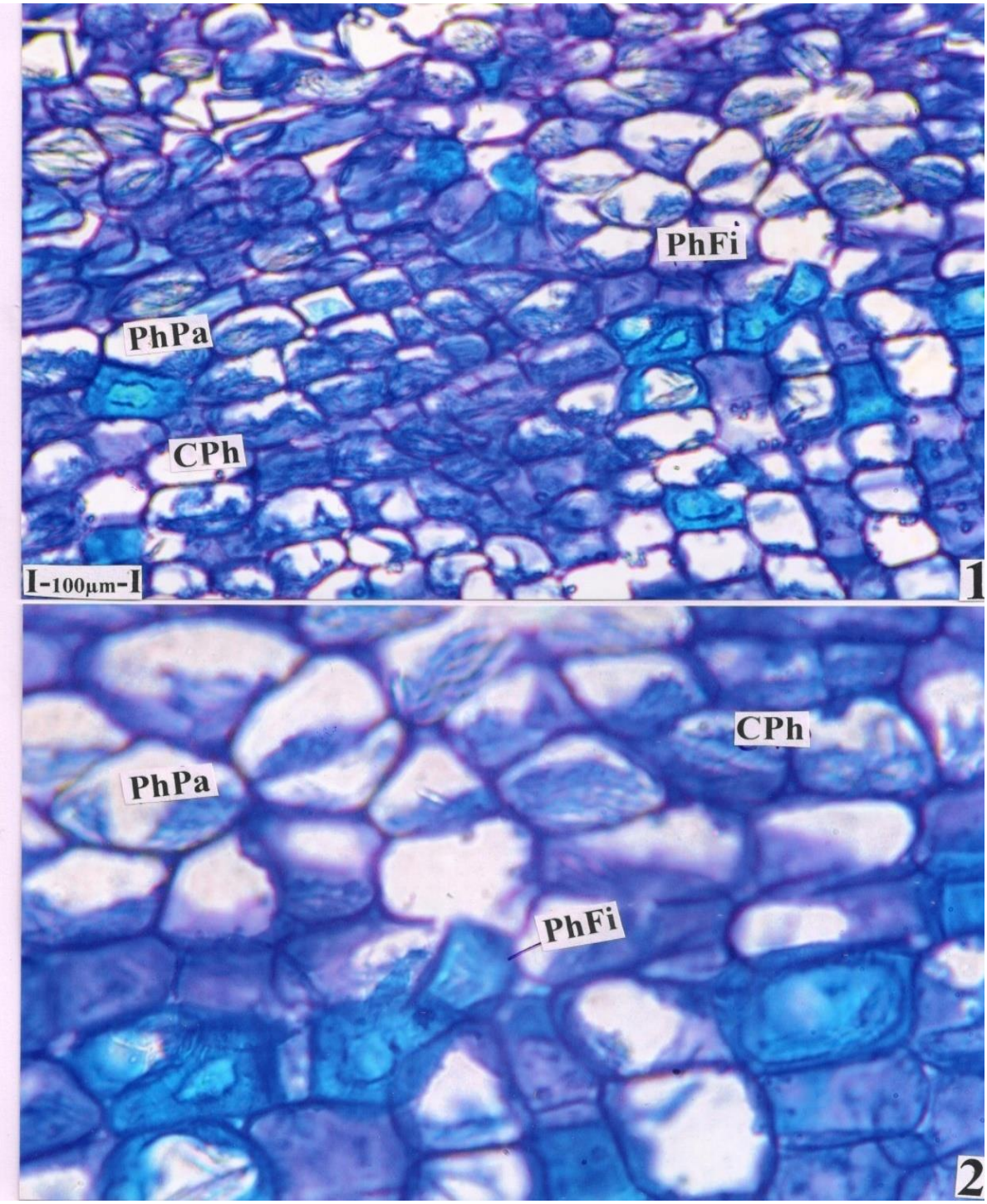

Figure : 6.1,2. *Avicennia marina,* T.S *of collapsed phloem showing sclereids fibbers*

CPh – Collapsed Phloem; PhFi – Phloem Fibbers; PhPa – Phloem Parenchyma.

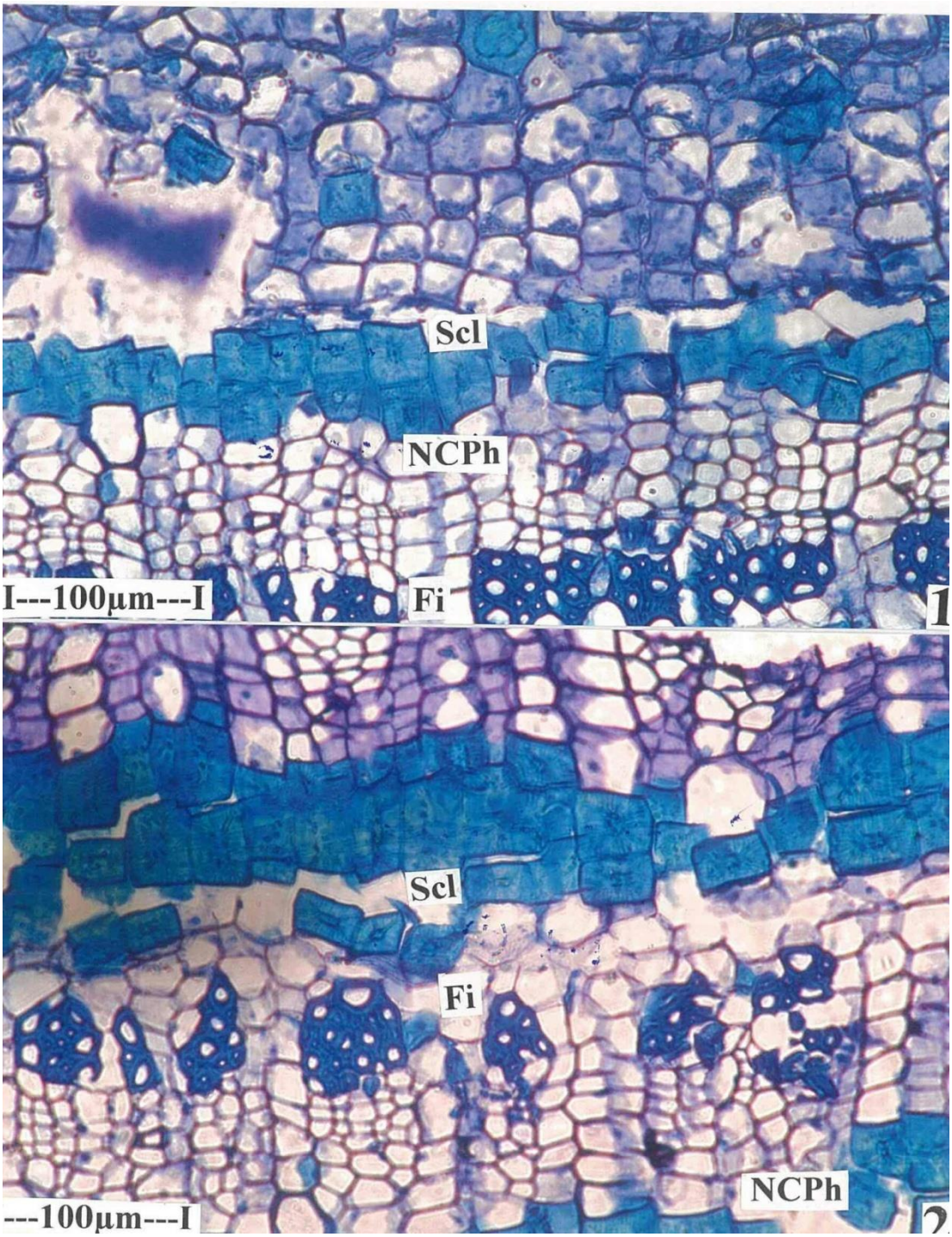

Figure : 7.1,2. *Avicennia marina Non Collapsed Phloem with Sclereids and Fibers.*

Fi – Fibers; NcPh – Non Collapsed Phloem; Scl – Sclereids.

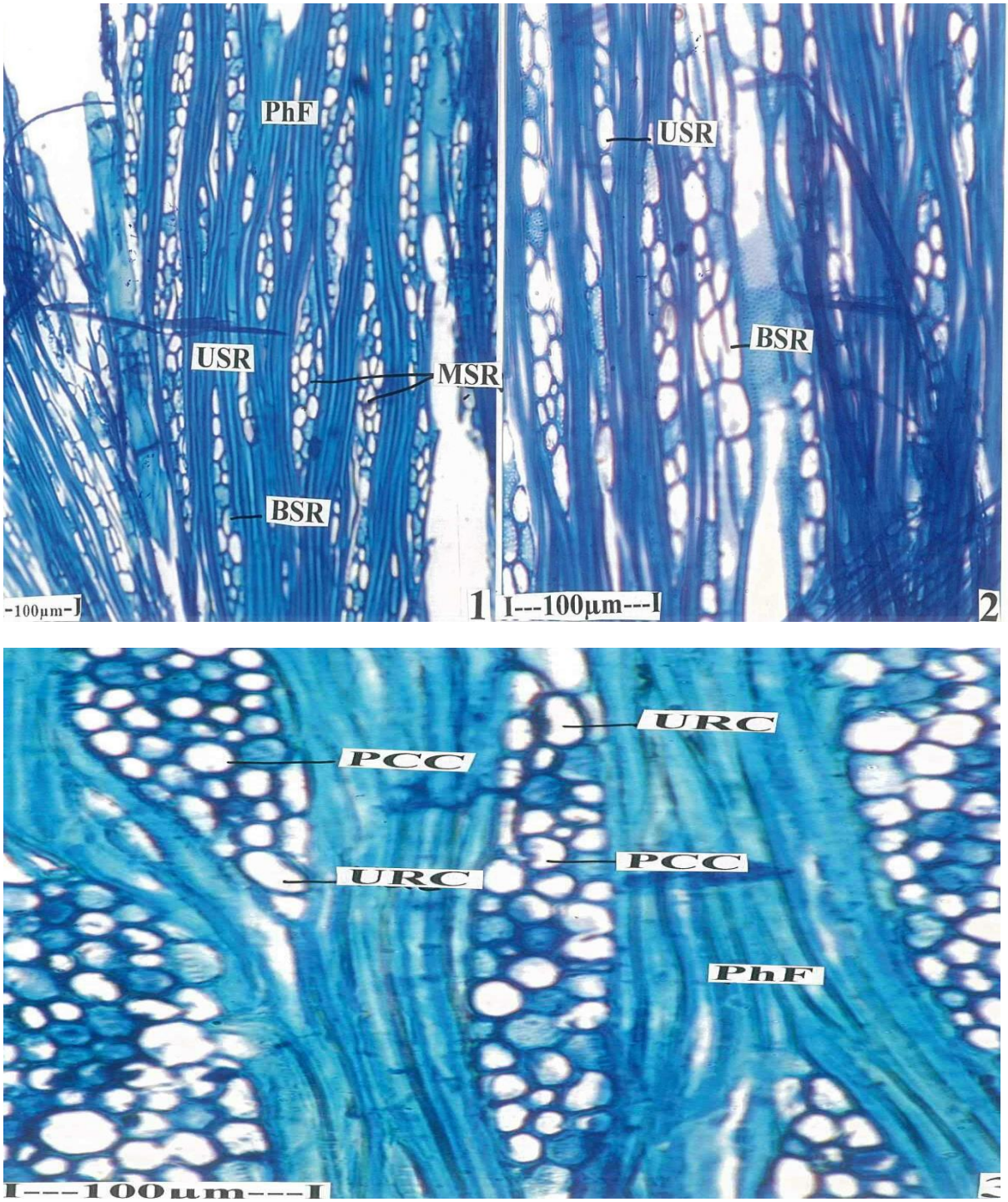

Figure : 8.1,2,3. *Avicennia marina Tangential* Longitudinal Section of Phloem showing. BSR – Biseriate Ray; MSR – Multi Seriate Ray; PhF – Phloem Fiber; PCC – Procumbent cells; USR – Uniseriate Ray; MSR – Multi Seriate Ray; URC – Up right cell.

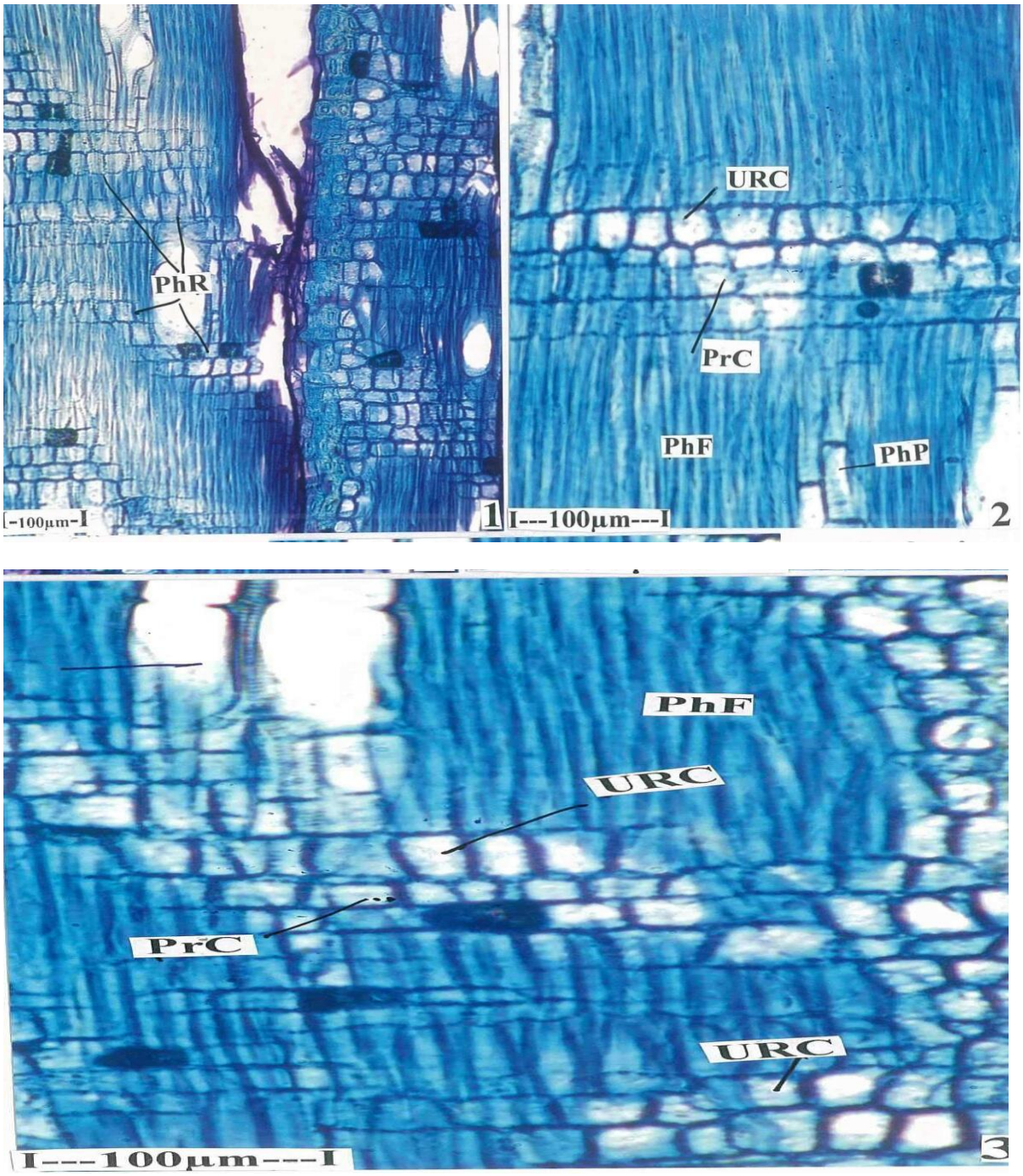

Figure : 9.1,2,3. *Avicennia marina Radial longitudinal Section of the Phloem rays.*
PhR – Phloem Ray; PrC – Procumbent cell; PhF – Phloem Fibre; PhP – Phloem Paranchyma; URC – Up righy cell.

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