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Identification of *Salacia chinensis* (Celastreaceae) using Leaf Anatomical Characters.

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Abstract

The objective of the present study was to observe the anatomical features of *Salacia chinensis,* to study the unique characters in the family, to observe the shape, structure and arrangement of cells which will help us to find out the adulterated drugs. *Salacia chinensis* of Celastraceae family was a promising medicinal plant with great economic potential. The plant used in traditional system of medicine for diabetics. The leaf and petiole of the plant were cast into paraffin blocks and sectioned with the help of rotary microtome. Microscopic investigation of the leaf and petiole showed few promising features like Phloem elements in the leaf are located with in U-shaped tanninferous cells. The adaxial epidermis of the lamina consist of square wide cells with thick cuticle. The vascular cylinder of the petiole is deeply curved with incurved margins. The present study can assist the diagnostic characters of the leaf and petiole of *Salacia chinensis* which may be taken for pharmacognostical standards for the identification of plant drug.

Keywords: Celastraceae, *Salacia chinensis* ,Microscopy, Microtome, Anti-diabetic

Introduction

Herbal medicines has become a topic of global importance, making an impact on both world health and international trade. Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. one such herbal plant is *Salacia chinensis*. This wonderful plant has anti-diabetic and anti-obesic healing properties (Ramakrishna et al., 2015)[1]. *Salacia chinensis* is a versatile plant used in treating variety of diseases like respiratory disorder, chronic fever, cold, cough, malaria, dysentery, diarrohoea, arthritis, skin diseases, trauma, convolusions, diabetes treatment of internal organs, hepatic vessel and immunologic disorders (Nadkarni and Nadkarni 1976) [2]. According to Sumalatha *et al*., (2012) [3] aqueous root extract of *Salacia chinensis* can boost the immune system.

The present study is an attempt to study the anatomical character of the plant. This research is based on the anatomical studies of herbal drugs for the benefit of man and is likely to decrease the dependence on synthetic drugs. To observe the anatomical features of *Salacia chinensis,* to study the unique characters in the family, to observe the shape, structure and arrangement of cells which will help us to find out the adulterated drugs. This study will help

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to explore the anatomical characters of *Salacia chinensis* leaf and petiole which may serve as a marker to differentiate the authentic and adulterated plant.

Materials and methods

Collection of plant material

The plant specimen for the proposed study were collected from various places in Karnataka namely Hubili, Karikan, Udipi, Jogimat and Bandal.

Preparation of permanent slides

For detailed anatomical studies, care was taken to select healthy plants. The leaves, petiole, stem and fruit were cut and fixed in FAA (Formalin+Acetic acid +70% Ethyl alcohol-1:1:18v/v) for 24 hours. After fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol (TBA) as per the schedule given by Sass, (1940)[(4)]. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60ºC) until TBA solution attained super saturation. The specimen were cast into Paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickenss of the sections was 10-12µm. Dewaxing of the sections was done by Customary procedure (Johansen, 1940)[(5)] .The sections were stained with (TB) Toluidine Blue (O'Brien *et al.*, 1964) [(6)]. The sections were dehydrated employing graded series of ethyl alcohol and xylol and mounted with DPX mountant.

For studying the midrib of the leaf, lamina, stomatal morphology, vascular system of the petiole and stem, crystal types and venation pattern temporary preparations were made. Small pieces of Lamina (About 1mm ^{2}) were immersed in hot ethanol for 10 min. The specimens were thin and kept in 10% sodium hydroxide (NaOH) for several hours. Epidermal peeling by partial maceration employing Jeffrey's maceration fluid, was done. Powdered materials (stem) were cleared with NaOH and mounted in Glycerine medium after staining.

Photomicrographs

Photographs were taken using Nikon labphoto-2 microscopic Unit. For normal observations Bright Field was used. For the study of crystals, Starch grains and lignified cells, Polarised light was employed. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy book (Esau, 1964) [(7)].

Results and Discussion

Transverse section of Leaf

The leaf is thick with prominent midrib. The midrib has adaxial narrow conical growth and thick and wide abaxial part (Fig. 1. 1). The midrib is 1mm thick and basal part 1mm wide.

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The apical cone is 10 µm in height and 10 µm wide. The midrib has thin epidermal layer of squarish, thick walled and lignified cells. The cuticle is very thick (Fig. 1.2 and 2). The epidermis is 20 µm thick. The outer and central ground tissue consists of angular, thin walled compact parenchyma cells. Most of the cells have tannin content (Fig. 1.2). The Vascular system includes almost closed circular-adaxial-abaxially compressed vascular cylinder. The cylinder consists of several long and short radial rows of xylem elements. The xylem elements are wide, circular and thick walled. The xylem elements on the adaxial part of the cylinder are only few. Phloem occurs along the outer periphery of the xylem cylinder. Phloem elements are located with in U-shaped tanniniferous cells. The vascular cylinder enclosed with a thick closed cylinder of fibres. The fibre cylinder is thin on the lower part and becomes thicker on the upper part. The vascular cylinder is 600µm in horizontal plane and 450µm in vertical plane. The metaxylem elements are 35µm wide.

The lamina is 180µm thick, distinctly dorsiventral. The adaxial epidermis consists of squarish wide cells with thick cuticle. The abaxial epidermis is comparatively very thin with small cells and thick cuticle. The adaxial epidermis is 20µm thick and the abaxial epidermis is 15µm thick. There is single horizontal layer of short cylindrical palisade cells which are less compact. (Fig.3.1). The spongy mesophyll cells are small, lobed and loosely arranged forming wide air-chambers. Small lateral vascular bundles are seen in the median part of the leaf. Calcium oxalate crystals are abundant in the leaf, especially along the sclerenchyma elements of the vascular bundles (Fig.3. 2). The crystals are druses, which are spherical bodies with surface. The druses are solitary and are 30µm thick.

Transverse section of Petiole

The petiole is semi-circular in sectional view with flat adaxial side with short wings and semicircular abaxial side (Fig 4.1). The petiole is 1.6mm wide and 1.5mm thick. The petiole has parenchymatous ground tissue and wedge shaped vascular strand with incurved margins (Fig.4.1). Almost all ground cells possess accumulation of tannin. Calcium oxalate crystals are fairly abundant in the phloem parenchyma and central core of ground tissue (Fig.4.2). The crystals are druses, which are spherical bodies with spiny surface. The crystals are scattered without definite distribution pattern.

The vascular cylinder of the petiole is deeply curved with incurved margins. The concavity of the vascular bundle is directed towards adaxial sides. (Fig.5.). The vascular cylinder consists of several radial lines of xylem elements which are circular, narrow and thin walled. Phloem is located in thin layer all along the lower side of the xylem cylinder (Fig.5). The vascular cylinder is 950µm wide and 200µm thick.

Discussion

Medicinal plants have formed the basis for traditional medicine and believed to be an important source of new chemical substances with potential therapeutic effects. The natural ingredients of traditional medicine are believed to be more acceptable to the human body when

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Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

compared to the synthetic drugs (Ghani ,1990)[8]. *Salacia* holds a place as an effective antidiabetic herb and many species of this plant are in use as anti-diabetic. *Salacia chinensis* is used worldwide mainly for diabetes and obesity because of its high antioxidant activity and phytocompounds.

The macroscopic and microscopic features will be useful in the proper identification of the plant. Microscopic analysis allows more detailed examination of a drug, to identify the organised drugs by their known histological characters. It is mostly used for qualitative evaluation of organised crude drugs in entire and powdered form(Mohammed *et al.*, 2004)[9]. In the present study, the microscopic examination of leaf and petiole of *Salacia chinensis* was done to facilitate its appropriate identification.

The leaf shows a prominent midrib consist of thin epidermal layer of squarish, thick walled and lignified cells. Phloem occurs at the outer periphery of the xylem cylinder. Phloem elements are located within U-shaped tanninferous cells. The mesophyll consists of compact single horizontal layer of cylindrical palisade cells and spongy cells of small, lobed and loosely arranged with wide air-chambers. Small lateral vascular bundles are seen in the median part of the leaf. Abundant calcium oxalate crystals are present in the leaf. The crystals are spherical bodies with echinate surface, the druses are solitary with 30µm thick. Similar results were obtained on *Salacia* species with stomatal number,vein islet number, number of spongy parenchyma cells contribution for more than other characters for species separation (Senevirathna *et al.*,)[10] .

The petiole has parenchymatous ground tissue with accumulation of tannin and wedge shaped vascular strand with incurved margins. Calcium oxalate crystals are scattered and abundant in the phloem parenchyma and central core of ground tissue. The crystals are druses, which are spherical bodies with spiny surface.

Conclusion

It is concluded from the present study of anatomoical characters of Leaf and petiole of *Salacia chinensis* , Phloem elements in the leaf are located with in U-shaped tanninferous cells. The adaxial epidermis of the lamina consist of square wide cells with thick cuticle. The vascular cylinder of the petiole is deeply curved with incurved margins. This study will be helpful to identify and select the authentic plant of *Salacia chinensis* for traditional system of medicine for preparation of Anti-diabetic drugs.

Acknowledgement

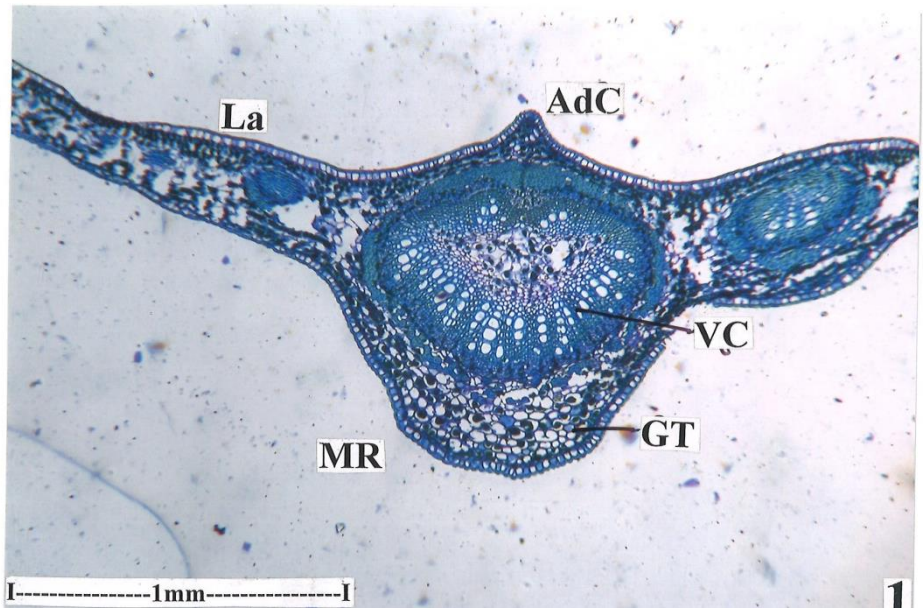
Our heartfelt thanks to **Dr.Jayaraman**, Plant Anatomy and Research Centre (PARC) for identifying the plants and support and guidance throughout the study.

Fig.1.Salacia chinensis

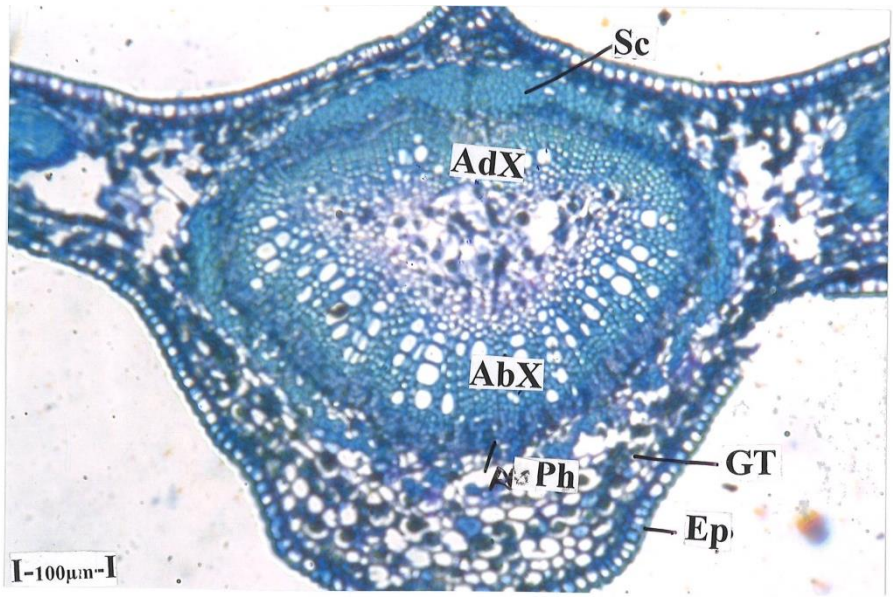
1.T.S.of leaf through Midrib

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023



1.2.T.S.of Midrib



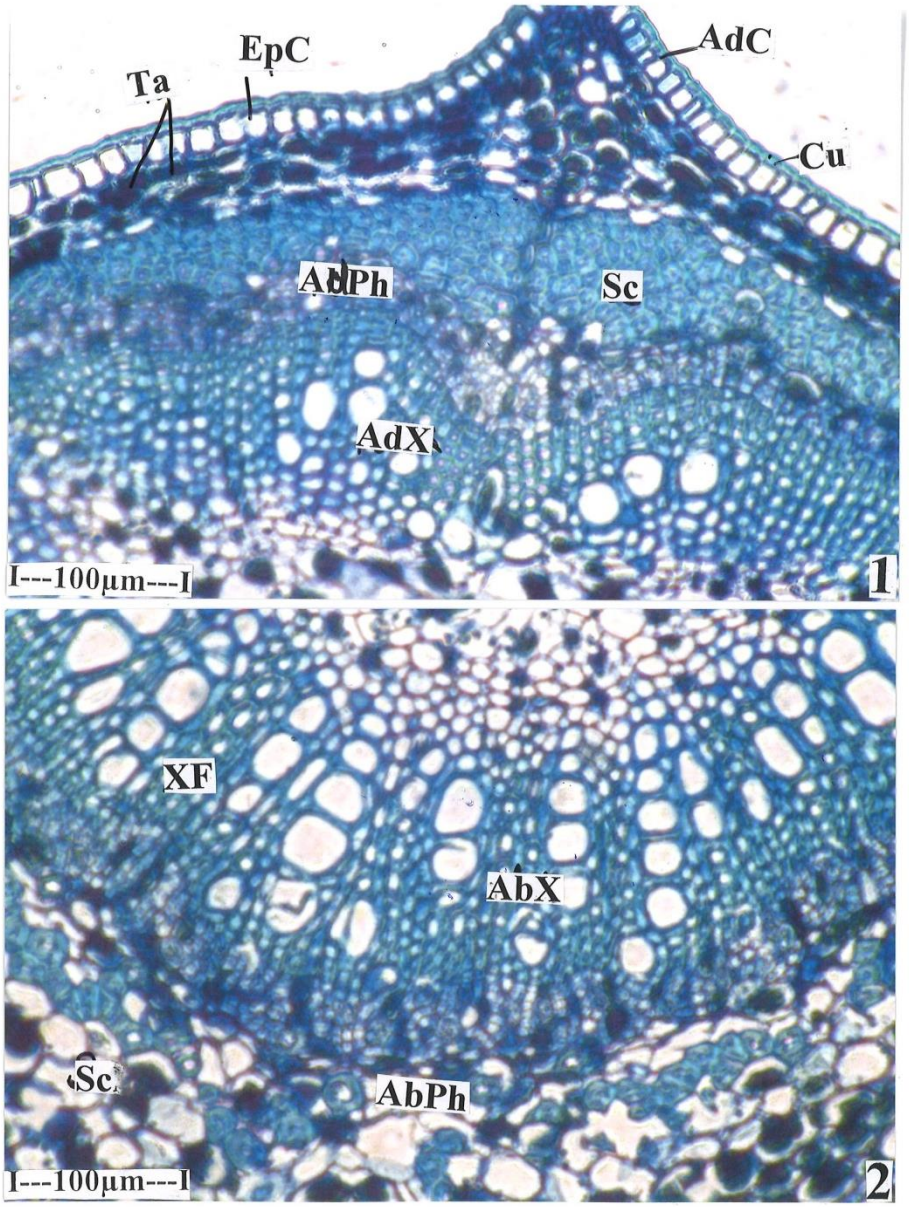
AbPh-Abaxial Phloem; AbX-Abaxial Xylem; AdC-Adaxial Cone;
AdX-Adaxial Xylem; Ep-Epidermis; GT-Ground Tissue; La-Lamina;
MR-Midrib; Sc-Sclerenchyma; VC-Vascular Cylinder

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

Fig.2.Salacia chinensis Linn

2. T.S.of Midrib –A Sector enlarged



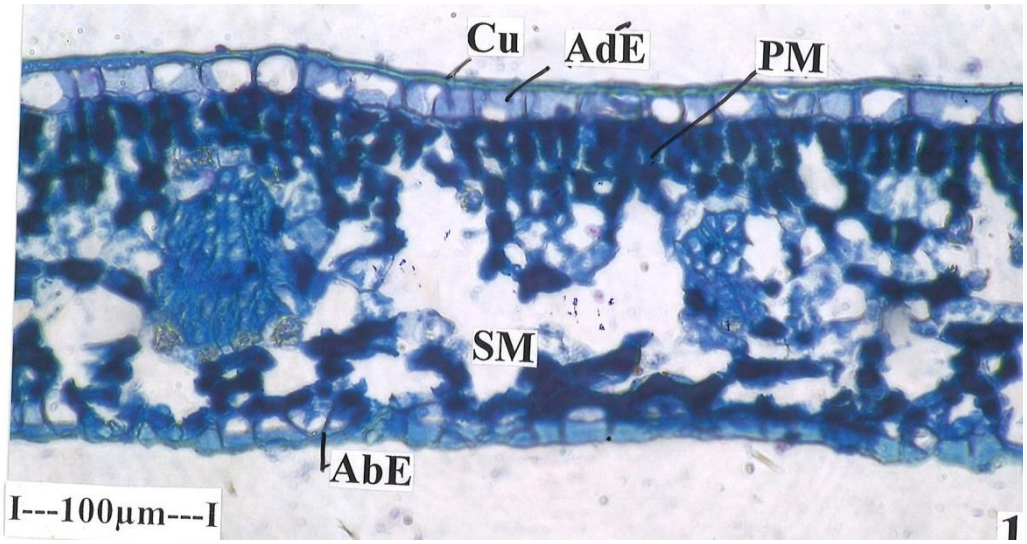
AbPh-Abaxial Phloem; AbX-Abaxial Xylem; AdC-Adaxial Cone;
AdPh-Adaxial Phloem; AdX-Adaxial Xylem; Cu-Cuticle; EpC-Epidermal Cells;
Sc-Sclerenchyma; Ta-Tannin ;XF-Xylem Fibres)

ISSN PRINT 2319 1775 Online 2320 7876

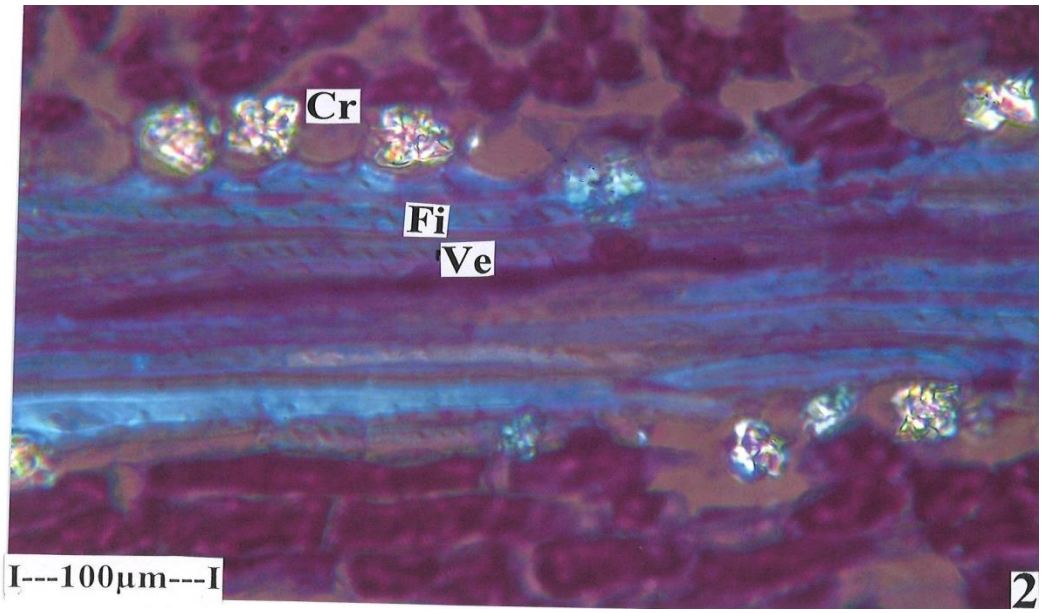
Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

Fig.3.Salacia chinensis Linn

3.1. T.S.of Lamina



3.2.Crystals in the Lamina



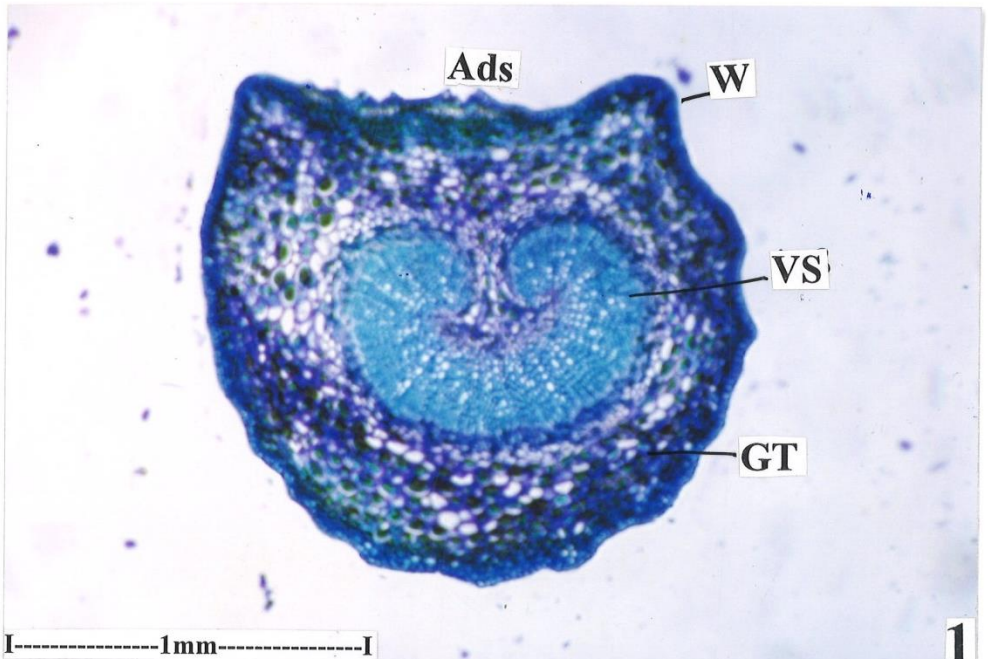
AbE-Abaxial Epidermis; AdE-Adaxial Epidermis; Cr-Crystals; Cu-Cuticle; Fi-Fibres; PM- Palisade Mesophyll ; SM-Spongy Mesophyll ;Ve-Vein

ISSN PRINT 2319 1775 Online 2320 7876

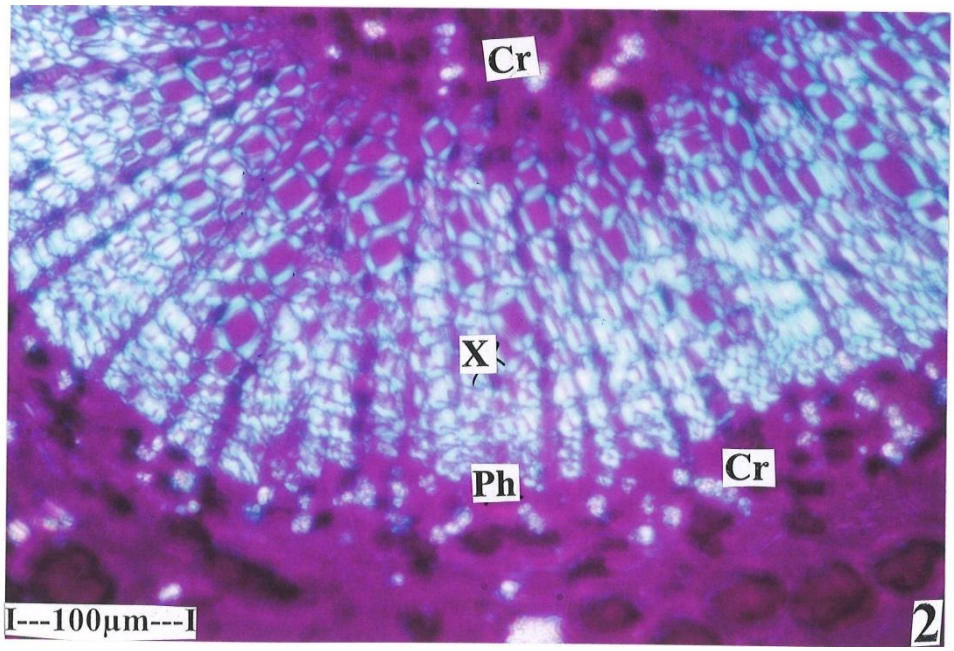
Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

Fig.4.Salacia chinensis Linn

4.1. T.S.of Petiole-Entire View



4. 2.Vascular strand of the petiole as seen under Polarised Light.



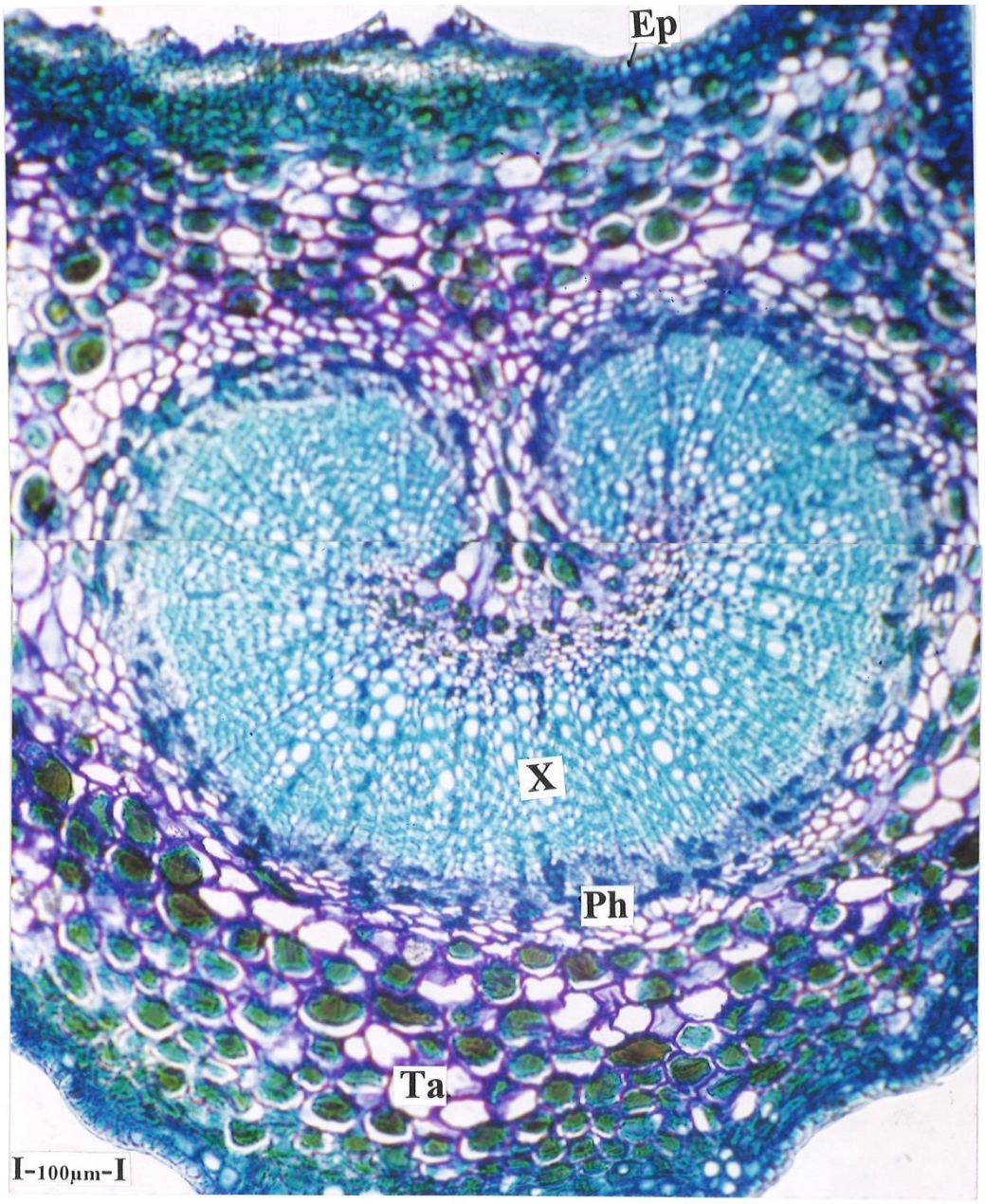
AdS-Adaxial Side; Cr-Crystals ; GT-Ground Tissue; Ph-Phloem;
VS-Vascular strand; W-Wing ;X-Xylem)

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

Fig.5.Salacia chinensis Linn

5.T.S.of Petiole-Vascular system enlarged



Ep-Epidermis ;Ph-Phloem ;Ta-Tannin ;X-Xylem

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

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