



**PHARMACOGNOSTICAL EVALUATION AND THERAPEUTIC POTENTIAL OF
SIMAROUBA GLAUCA DC: A PROMISING MEDICINAL PLANT**

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ABSTRACT

Simarouba glauca DC., commonly known as the "Paradise Tree," is a versatile evergreen species with significant medicinal and pharmacological potential. This study focuses on the pharmacognostical evaluation of *S. glauca* leaves and stem bark, which have shown promise in treating a variety of ailments, including cancer and infectious diseases. The tree is rich in bioactive compounds such as Quassinoids, alkaloids, flavonoids, and phenolic compounds, which contribute to its antimicrobial, antifungal, antioxidant, and anticancer properties. The research involved a comprehensive analysis, including morphological, anatomical, and histochemical studies. Techniques like powder microscopy and High-Performance Thin Layer Chromatography (HPTLC) were employed for qualitative and quantitative characterization of phytochemicals. Histochemical tests revealed the presence of alkaloids, lignin, tannins, and fatty oils in various plant parts. HPTLC profiles confirmed the diversity and concentration of bioactive components in chloroform and methanol extracts of leaves and stem bark. The findings underscore the potential of *S. glauca* as a natural therapeutic agent, particularly for enhancing quality of life in cancer patients through its immune-boosting and tumour-reducing properties. The study also highlights the significance of using pharmacognostical methods for authentication and standardization of crude drugs in the herbal market. This work supports the inclusion of *S. glauca* in future pharmacopeia and underscores its potential as a sustainable resource for pharmaceutical applications.

KEYWORDS: Histochemical, pharmacognostical, Quassinoids.

INTRODUCTION

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. Some of them are also used for prophylactic purposes. An increasing interest in herbal remedies has been observed in several parts of the world and many of these remedies have been incorporated into orthodox medicinal plant practice. Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (essential and fixed) (Guru Priya *et al.*, 2017).



Figure 1: Tree of *Simarouba glauca DC.*

India possesses a rich biodiversity of medicinal plants that are still not explored completely. Medicinal plants have been a valuable source of natural products which

are of great importance in maintaining human health. The need for natural products of pharmaceutical purposes of the plant has attained a great interest in the present research world due to the cost and the higher side effects that is associated with the chemically manufactured drugs. According to WHO (World Health Organization), 80% of the people rely primarily on traditional health care system and mostly on herbal medicines. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, and volatile oils. It is necessary to identify the bioactive constituent of medicinal plants usually employed by herbalists in the treatment of infectious diseases (Guru Priya *et al.*, 2017).

Simarouba glauca DC. is commonly known as 'Laxmitaru' or 'paradise tree' and belongs to the family

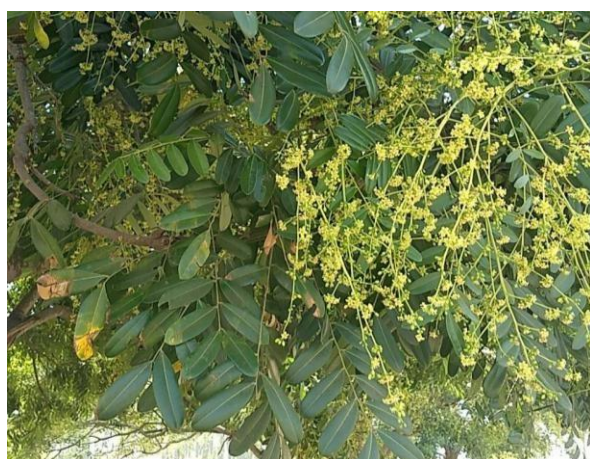


Figure 2: Flowering twig of *S. glauca*.

S. glauca is a good source of carbohydrates, fatty acids, lipids and proteins. *Simarouba glauca* decoction is used in traditional medicine in the treatment of cancer and tumors. *Simarouba* leaf decoction when taken in limited amounts can raise the immunity, reduce appetite loss and increase quality of life in cancer patients. It can also be used to complement modern cancer treatment. *S. glauca* has very good anti-bacterial, anti-tumorous properties. Hence, *S. glauca* is very effective in reducing the size of tumors and secondary infections in cancer patients. It is very effective in curing cancer of first/second stages, whereas in later stages it can considerably increase the quality of life. In the present study, we have concentrated on the morphology, anatomy, pharmacognosy, HPTLC, powder chemistry and maceration of leaf and stem bark. (Sneha Kochath Santhosh *et al.*, 2016).

AIMS AND OBJECTIVES

Aim

To develop quality standards for *Simarouba glauca* DC. leaves and stem bark in Pharmacognostical aspects.

Simaroubaceae (Fig. 1). This evergreen tree grows to a height of 12-15m with large circular crown. *S. glauca* occurs as an understory shade tolerant tree, commonly grown under the canopy of large fruit trees. The species name *glauca* is derived from Greek word, it means covered with the bloom which refers to the bluish green foliage. This species of flowering tree is native to Florida in the United States, southern Florida, South America, and the Lesser Antilles. Common names include Paradise Tree, Aceituno, and Bitter wood. Its seeds produce edible oil. The tree is well suited for warm, humid, tropical regions. Its cultivation depends on rainfall distribution, water holding capacity of the soil and sub-soil moisture. It is suited for temperature range of 10 to 50 °C. It can grow at elevations from sea level to 1,000 m (3,300 ft) and grows 40 to 50 ft (12 to 15 m) tall. (Sneha Kochath Santhosh *et al.*, 2016).



Figure 3: Fruiting twig of *S. glauca*.

OBJECTIVES

- To carry out detailed literature survey and collect authentic leaves and stem bark of the selected medicinal plant *Simarouba glauca* DC.
- To collect documentary evidences with the use of photography, photomicrography for the selected drug.
- To carry out detailed morphological, micro-morphological and anatomical studies of the selected drug through microscopy.
- To carry out detailed Histochemistry and Powder microscopy studies of the selected drug through microscopy.
- To carry out TLC/HPTLC studies for characterization of constituents.
- To establish distinguishing diagnostic characteristics for leaves and stem bark of *Simarouba glauca* DC.

REVIEW OF LITERATURE

Globally India has a rich biodiversity in medicinal plants and that are not fully explored. Nowadays, the need for natural products of pharmaceutical purposes of the plant has attained a great interest in the present research world

due to the cost and the higher side effects that is associated with the chemically manufactured drugs. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, and volatile oils (Osagie-Eweka *et al.*, 2016).

Simarouba is an evergreen, multi-purpose edible oil tree, also known as Aceituno, bitterwood, dysentery bark, Palo Amargo, paradise tree, pitomba, robeceillo, simaba and Shorgum Maram (Osagie-Eweka *et al.*, 2016). Inflorescence is terminal panicle with ultimate branches producing dichasial cyme (Fig. 2). Flowers are bisexual, inconspicuous, calyx is green in color and dome shaped united with variable number of sepals. Petals are present in single whorl creamy greenish or yellowish creamy in color. The staminate flowers are with gynophores but without carpel with single ovule. It bears yellow flowers and oval elongated purple-colored fleshy fruits (Fig. 3). In India it was first introduced in 1966 in Amaravati Research station Maharashtra by National Bureau of Plant Genetic Resources (NBPGR). *S. glauca* is also spread to semi-arid dry and saline land areas of Gujarat, Tamil Nadu, Karnataka and Andhra Pradesh. *S. glauca* tree has an ability to grow well even in marginal wastelands or dry lands with degraded soil. *Simarouba* cultivation came to the University of Agricultural Sciences, Bangalore during the year 1986. However, systematic research and developmental activities of *S. glauca* began only from the year 1992 (Joshi and Hiremath, *et. al.*, 2000).

The use of higher plants and their extracts to treat infections is an age-old practice. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavanoids and phenolic compounds. The main active group of chemicals in *Simarouba* is called quassinoids, which belong to the triterpine chemical family. *S. glauca* has several uses, mainly as herbal medicine. All parts of the plant namely seed, shell, fruit pulp, leaf, leaf litter, unwanted branches, stem, bark, and root generate products that are useful in the production of food, fuel, manure, timber, medicine. A chemical constituent of *Simarouba* extract exhibits antimicrobial, antifungal, antiprotozoal and antiviral activity. Components with antimalarial, and antileukemic activities have been also isolated from *Simarouba* sp. The plant is considered to have antiseptic, analgesic, astringent and vermifuge properties (Biba Vikas, Sujathan Kunjiraman *et al.*, 2021).

Infusion of the leaves or bark is considered to be astringent and used as a digestion and menstrual stimulant and an antiparasitic remedy. It is taken internally for diarrhea, dysentery, malaria and colitis. The leaves (and occasionally the bark) are boiled in water to yield a powerful astringent tonic used to wash skin sores and to treat stomach and bowel disorders,

hemorrhages and internal bleeding. It is used externally to heal wounds and sores. *Simarouba* leaf extract is used for reducing patchy skin pigmentation (Manasi and Gaikwad, 2011). *Simarouba* water extract was found to increase skin keratinocyte differentiation and to improve skin hydration and moisturization. The seeds extracted in alcohol are used against snake bites. It was reported that the leaf, fruit, pulp and seed of *S. glauca* possess medicinal properties such as analgesic, antimicrobial, antiviral, astringent, emmenagogue, stomachic, tonic and vermifuge (Joshi and Joshi, 2002). *S. glauca* leaf has 11 medicinally important quassinoids namely, glaucarubin, quassinoids, ailanthinone, benzoquinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide and sitosterol. *Simarouba glauca* extract has been reported for the presence of alkaloids, flavonoids, cardenolides, glycosides, phenolic compounds, saponins and fixed oils (Manasi and Gaikwad *et al.*, 2011).

The extract of *S. glauca* has been used in Guatemala for the treatment of gastrointestinal disorders. Glaucarubin, a crystalline glycoside isolated from *S. glauca* leaf has amoebicidal properties. Several quassinoids from *S. glauca* leaf and seed have exhibited cytotoxic activity *in vitro* against KB cells (human oral epidermoid carcinoma), including glaucarubin, glaucarubinone, glaucarubol and glaucarubolone. The esters of glaucarubolone, ailanthinone and glaucarubinone exhibited significant activity *in vivo* in the P388 lymphocytic leukemia model. Chloroform-soluble extract of *S. glauca* exhibited significant cytotoxicity against several human cancer cell lines (Manasi and Gaikwad *et al.*, 2011). *S. glauca* is very effective in reducing the size of tumors and secondary infections in cancer patients. It is very effective in curing cancer of first/second stages, whereas in later stages it can considerably increase the quality of life. In the present study, we have concentrated on the preliminary screening of proximate compounds and secondary metabolites from *S. glauca* leaves. (SD.E. OsagiEweka, 2021).

The bark and leaf extract of *Simarouba* is well known for its different types of pharmacological properties such as haemostatic, antihelmenthic, antiparasitic, antidysentric, antipyretic and anticancerous. The bark is used to cure fever, malaria, stomach and bowel disorders, haemorrhages, ameobiasis as well as leaf, fruit pulp and seeds are possessing medicinal properties such as analgesic, antimicrobial, antiviral, astringent emmenagogue, stomachic tonic and vermifuge. The crushed seeds are used against snake bites. The crude drug contents and active principles such as glaucarubin, quassinoids, ailanthinone, benzoquinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sistosterolare mainly involved in pharmacological activities of this plant. The bark and leaf extracts of *Simarouba* is well known for its different types of pharmacological properties such as

haemostatic, anthelmintic, antiparasitic, antidysentric, antipyretic, anticancerous, antimicrobial, antiherpetic, antiprotozoal, antiamoebic, antimalarial, antifungal, antioxidant and antiulcer activities along with hepatoprotective property. The main group of chemicals in *Simarouba glauca* is quassinoids, which belong to the triterpene family. This includes: ailanthinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, glaucarubinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol, tirucalla etc. After the initial discovery of the antileukemic activity of bruceantin, a quassinoid, these molecules gained much attention. Based on the chemical structures and biological properties nearly 150 quassinoids have been isolated and classified. A wide range of inhibitory effects has been shown by quassinoids which includes anti-inflammatory, antiproliferative effects on tumor cells types. Considering the future in generating anticancer agents with more active and less toxic compounds, natural quassinoids represent a promising source of small molecules (Ramya *et al.*, 2018). The present review summarizes pharmacological, Ethnobotanical Phytochemical aspects as well as nursery practices of this medicinal plant.

Simarouba extract is treated for patchy skin and pigmented skin. An infusion of the bark is used against malaria, rheumatism, shingles and fever. *S. glauca* was found to have amoebicidal properties *in vitro* and experimental animal amoebiasis were evaluated in the treatment of human infection. The chloroform soluble extract of *S. glauca* exhibited significant cytotoxicity against several human cancer cell lines. *Simarouba* forms an important source of edible oil for various South and Central American Countries and is widely grown in countries like Costa Rica, El-Salvador, Honduras Cuba, Nicaragua, Mexico, Haiti, Jamaica from 1950 onwards, in El-Salvador and other Central American Countries the oil marketed for edible purposes under the trade name manteca vegetal "nieve" and oil is well accepted. The use of *S. glauca* as a vegetable oil crop was first considered near the end 1939. The oil is extracted from seeds in existing oil mills and processed by adopting conventional methods (Joshi and Hiremath *et al.*, 2011). *S. glauca* is rich in edible oil, that has been used for cooking in tropical countries. The solid fraction rich in steric acid and palmitic acids can be used as coco-butter substitutes (CBS) or coco-butter extenders in confectionary and bakery industries. The leaves contained good amounts of phytochemicals. Ailanthinone, glaucarubinone, and holacanthone are considered some of the main active quassinoids in *Simarouba*. Other chemicals include benzoquinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol, and tirucalla. Some of these active compounds have demonstrated *in vitro* anti-tumour, anti-viral, anti-amoebic, anti-bacterial and *in vivo* anti-malaria activity among and others (N. Thangamani, Joshi and Hiremath *et al.*, 2001).

Globally cancer is the one of the major disease causes of death. In 2018 the death rate was approximately 9.6 million. Breast cancer is considered a leading cause of death worldwide which can be attributed to the metastasis of primary cancer to different sites, including organs such as the bones, brain, liver, lymph nodes and lungs. Moreover, an increased drug resistance pattern towards existing antibiotics by pathogens is a serious threat. The presence of antimicrobial compounds and natural antioxidants in plants that remove harmful free radicals is one of the major reasons for using plant extracts as a potential tool for treating drug-resistant pathogens and cancer. (Manasa M, Dr. V Palanimuthu *et al.*, 2019).

Simarouba glauca is used as anti-inflammatory, anti-allergic, and anti-carcinogen. The plant also shows antiviral, antioxidant, and myocardial activities. The main active group of phytochemicals in *Simarouba* are the quassinoids, which belong to the triterpene chemical family. Ailanthinone, glaucarubinone and holacanthone are considered some of the main active quassinoids in *Simarouba*.

Pharmacognostic characters of herbal drugs play an important role since macro- microscopic features are unique for each plant. The macroscopic and microscopic studies of the herbs should be the first and fundamental step to authenticate the botanical source. Morphology is the study of the description of the plant material. The plant anatomical studies deal with the structure of organisms, structure gross and minute, external and internal. As part of standardization adulterants are generally tested on the herbal drug, depending on the facilities, comparatively cheaper and easier technique is adapted for detection of adulteration. Plants from different genera, families, species, cultivars (cultivated variety) and sibling plants can be distinguished by anatomical and powder microscopic methods. (Manasi Shirish Patil Sadguru *et al.*, 2023).

High-performance Thin Layer chromatography (HPTLC) is a sophisticated technique for analyzing plants and herbal drugs. It's used in herbal drug standardization to confirm and establish identity and Screen for adulterations. HPTLC can combine and consequently be used for different modes of evaluation, allowing identification of compounds having different light absorption characteristics or different colors.

High Performance Thin Layer Chromatography (HPTLC) is the most potent and sophisticated type of Thin Layer Chromatography (TLC). It uses chromatographic layers with the highest levels of separation, efficiency and employs high-tech equipment for every step of the process, including accurate sample application, standardised reproducible chromatogram development, and software-controlled evaluation. HPTLC is a concept that incorporates both the use of established methodologies for qualitative and

quantitative analysis and a widely standardized methodology founded on scientific facts. The resolution can be increased and more exact quantitative measurements, which satisfies all quality standards for today's analytical needs. Development of an analytical technique based on HPTLC and parameter validation in line with practical assessment. It complies with standards while reducing mistakes and inquiries. Quality Control and Quality Assurance of raw materials of Plant Origin can easily and effectively be done for qualitative characterization and quantitative determination of mixtures of substances, Chemical Fingerprinting by High Performance Liquid Chromatography. (Shanmuga Priya Ramasamy *et al.*, 2022)

MATERIAL AND METHODS

Collection and authentication of plant material

The plant sample of *Simarouba glauca* DC. leaves and stem bark were collected from Chennai. The collected samples were authenticated by the Department of Pharmacognosy, Siddha Central Research Institute (SCRI), Chennai, Tamil Nadu, India.

Macroscopy

External feature of test sample was documented using Nikon D-5600 Digital camera.

Size: Morphological or Macroscopical details of the respective plant part are studied by observing with naked eye or magnifying with lens. A ruler graduated in millimeters for the measurement of length, width and thickness of crude materials was used (Mean of 10 number samples).

- **Colour:** natural colour of the sample observed under daylight with naked eye.
- **Odour:** A small portion of sample was placed in plate and repeatedly inhaling the air in the material instead of make it as a powder and identified.
- **Taste:** A pinch of powder (all particles passed through 80 sieve meshes) by the tip of moist glass rod and applying it on different place of tongue.
- **Texture & Fracture:** Touching the material to determine soft or hard, bending and rupturing it to obtain information on brittleness and to ascertain the appearance of the fractured plane

Microscopy

Sample was preserved in fixative FAA for more than 48 h. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with 0.8% Safranin and 0.5% Astra Blue. Transverse sections were photographed using Axiolab5 trinocular microscope attached with Zeiss Axiocam208 color digital camera under bright field light. Magnifications were indicated by scale bar.

Powder microscopy

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate.

Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.

Histochemical tests

Plant sections were treated following the standard procedures.

1. **Crystals:** The section was mounted in water and one end of the cover slip was irrigated with acetic acid. While looking through the microscope, the water within the cover slip was replaced using a piece of filter paper at the opposite end of the cover slip.
 - Formation of air bubbles indicated Calcium carbonate crystals
 - If no air bubbles were formed, the experiment was repeated with conc. HCl, wherein dissolution of crystal and formation of needles of Calcium sulphate indicated the presence of Calcium oxalate crystals.
2. **Fats, Fatty oils volatile oils and resins:** About 1 to 2 drops of Sudan-IV was added to the section and allowed to stand for a few minutes. Presence of fatty oil substances were indicated by orange red/pink/red colored globules; while red-coloured irregular contents indicated resin.
3. **Starch:** A drop of 2% iodine water solution was added - blue colour indicated starch.
4. **Tannin:** A drop of alcoholic ferric chloride was added - bluish black-coloured contents indicated tannin.
5. **Mucilage:** A drop of ruthenium red was added - pink to red colored contents indicated mucilage.
6. **Lignified cell walls:** A drop of phloroglucinol was added to the section and allowed to stand for about 2 min or until almost dry. A drop of 50% HCl was added and observed over a cover-glass - cell walls-stained pink to cherry red indicating presence of lignin.
7. **Suberized or cuticular cell walls:** A drop of Sudan red III was added and allowed to stand for a few minutes, warmed gently if necessary - cell walls-stained orange-red or red indicated suberin or cutin deposition over cell wall.
8. **Alkaloids:** A drop of Wagner's reagent was added - the presence of yellow to reddish brown colored contents confirmed alkaloids.

Chemicals, Solvents and Reagents

The chemicals and solvents used were AR grade, for visualizing the developed spots in TLC reagent containing vanillin (1 g) sulphuric acid (5%) in ethanol (VSA) was used; stationary phase - silica gel 60 F254; mobile phase - toluene: ethyl acetate: formic acid: methanol (7.5:2.5:0.5:0.5 v/v/v/v) was used. For HPTLC, aluminium plate (Merck) pre coated with Silica gel 60 F254 of 0.2 mm thickness was used.

Instrument for HPTLC

Automatic sampler ATS4 was used for application of extracts on TLC plate; twin trough chamber (10 × 10 cm) was used for plate development; Scanner 4 with win CATS software was used for obtaining densitograms; TLC plate heater was used for derivatization (all from CAMAG, Switzerland).

TLC/HPTLC Procedure

Simarouba glauca leaves and stem bark Methanol and Chloroform (10 µl) extracts were applied as 8 mm bands on silica gel 60F254 coated aluminium plate (8 x 10 cm) using ATS4 applicator from 10 mm from left side and 10 mm from bottom of the plate. The plates were developed in the mobile phases after pre-saturation of the twin trough chamber (10×10cm). The plates were developed

up to 90 mm from the bottom. The developed plates were air dried, viewed under UV 254 nm, 366 nm and the images were documented using visualizer followed by dual wavelength scanning using scanner 4 at λ 254 nm (D2 lamp, absorption mode) and λ 366 nm (Hg lamp, fluorescence mode) with a slit dimension of 6 × 0.45 mm and scanning speed of 20 mm/s. Then, the TLC plates were dipped in a dip tank containing VSA reagent and heated at 100 °C or till the appearance of coloured spots. The derivatized TLC plates were photo documented at white light followed by scanning at λ 540 (W lamp, absorption mode) for finger prints.

RESULTS AND DISCUSSION

Macroscopy



Figure 4: Dried stem bark of *Simarouba glauca*.



Figure 5: Dried leaves of *Simarouba glauca*.

Stem bark

Dried cut pieces of stem barks are varying in sizes, externally rough with longitudinally fissured surface in greyish brown color. Fracture hard and fibrous, internal surface yellowish- brown colour with fibrous (Fig.4).

Leaf

Leaves are dark green coloured on upper surface, dull green on lower surface, alternate; pinnately; rachis long, 20 to 28 cm long; leaflets 5 to 6 pairs, elliptic to oblong, alternately arranged, margin entire, petiole very short, pinnately reticulate convergent venation(Fig.5).

Microscopy

Stem bark

TS of stem bark shows outer 14 to 20 layers of thick-walled cork cells followed by 10 to 20 layers of parenchymatous secondary cortex region; parenchyma cells are filled with brownish contents and plenty of starch grains; few cortical fibers are seen in cortex; major part of the section is occupied by broad secondary phloem region; outer phloem consists of several layers of thin-walled phloem cells intermingled with thick phloem fibers; inner phloem appears like phloem plates as the radially running biseriate to multiseriate phloem rays separates it to distinct portions; plenty of starch grains and oil globules are distributed throughout the phloem;

numerous secretory cavities and some crystals are also found in inner phloem(Fig.6& 7).

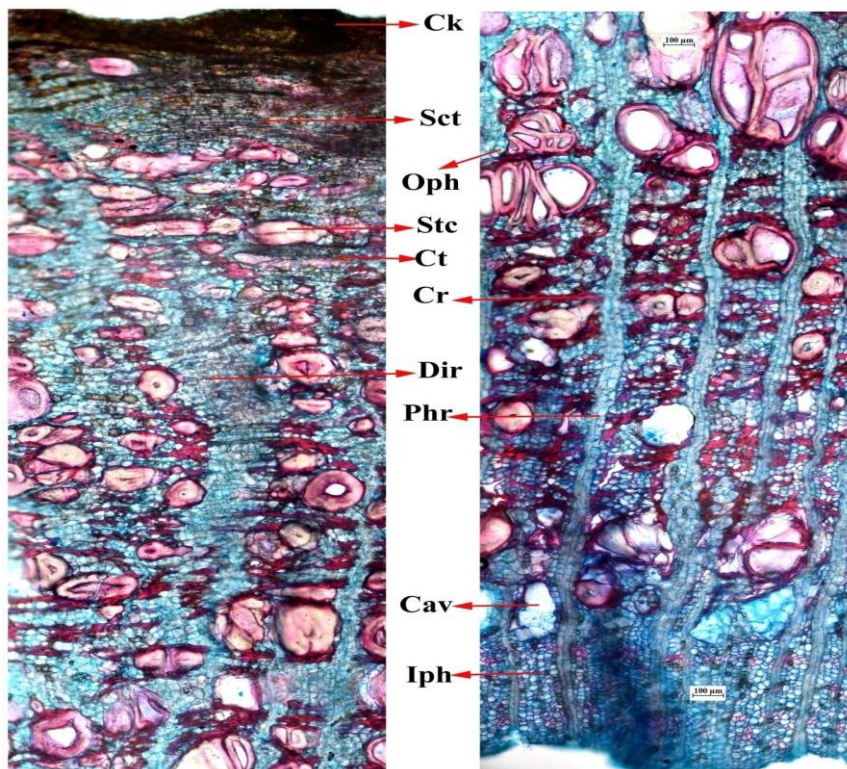


Figure 6: Detailed TS of stem bark of *Simarouba glauca* Ck: Cork; Ct: Cortex; Sct: Secondary cortex; Stc: Stone cell; Oph: Outer phloem; Cr: Ceretenchyma; Cav: Cavity;Dir: Dilated ray; Phr: Phloem rays; Iph: Inner phloem.

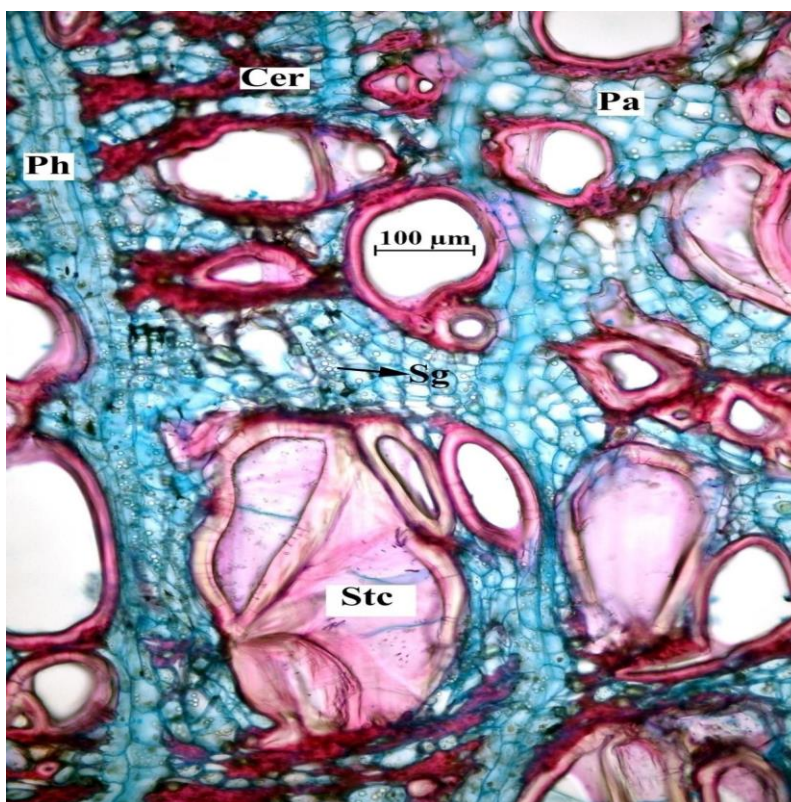


Figure 7: TS of bark enlarged view Cer: Ceretenchyma; Ph: Phloem; Stc: Stone cell; Sg: Starch grains; Pa: Parenchyma.

Leaves

Rachis

TS of rachis is nearly oval shaped in outline with wing like projections; epidermis is single layered and formed of round to oval shaped small cells and covered by thick cuticle; cortex is narrow and consists of 5 to 6 layers of collenchyma cells followed by 8 to 9 layers of parenchyma; several secretory canals are found distributed in cortical region; most of the cortical cells contains reddish brown pigments; major portion of the section is occupied by wide secondary vascular elements made up of a ring of conjoint, collateral vascular bundle surrounded by 3 to 4 thick discontinuous patches of pericyclic fibres; phloem is found towards outside and xylem towards inner side; phloem is formed of 3 to five layers of thin walled cells; xylem consists of isolated or group of vessels, tracheids, parenchyma, fibres and radially running uniseriate xylem rays; several secretory canal are scattered in between secondary vascular elements and primary vascular elements; 4 primary vascular bundles arranged nearly as cordate shaped at the center; inter xylary phloem central small pith region is present made up of parenchymatous cells; few oil globules are distributed in this region (Fig. 8 - 12).

Midrib

Midrib transverse section shows slightly convex shaped lower surface and flat upper surface with lateral laminar

extensions; epidermis is single layered and formed of tangentially elongated barrel shaped cells covered by thick cuticle; mucilage and few reddish brown contents are present in epidermal cells; 3 to 4 layers of collenchyma cells forms the hypodermal layer followed by parenchymatous ground tissue; several reddish brown pigmented cells are scattered in hypodermal and ground tissue; central vascular bundle consists of secondary elements followed by primary vascular bundles arranged same as that of petiole; prismatic crystals are scattered in phloem region (Fig. 13-16).

Lamina

Transverse section of lamina shows single layered upper and lower epidermis covered by cuticle and followed by mesophyll cells; mesophyll cells are differentiated into upper single layered compactly arranged columnar cells of palisade and lower loosely arranged 3 to 4 layers of spongy parenchyma with intercellular spaces; several astrosclereids are scattered in the mesophyll region; collateral vascular bundles can be seen traversing through the mesophyll tissue; phloem is found towards upper side and xylem to lower side; several reddish brown contents are present throughout the lamina (Fig. 17).



Figure 8: Detailed TS of rachis of *Simarouba glauca*.

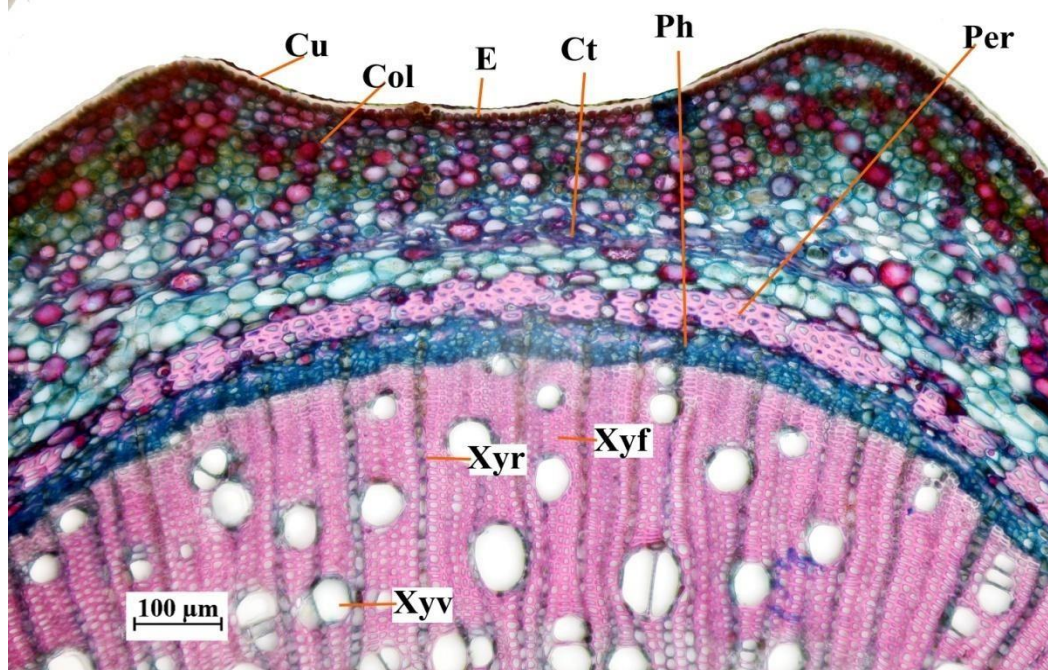


Figure 9: TS of rachis upper portion enlarged view Cu: Cuticle; Col: Collenchyma; Ct: Cortex; Per: Periderm; Ph:Phloem; Xyf: Xylem fibre; Xyr: Xylem ray; Xyv:Xylem vessels.

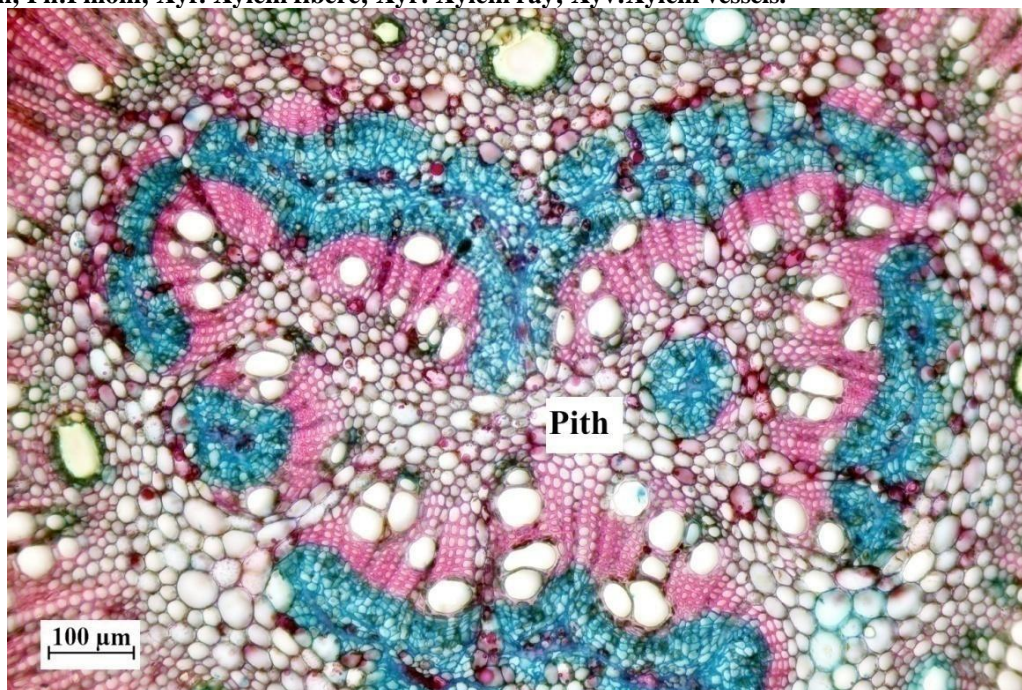


Figure 10: TS of rachis middle vascular portion enlarged view.

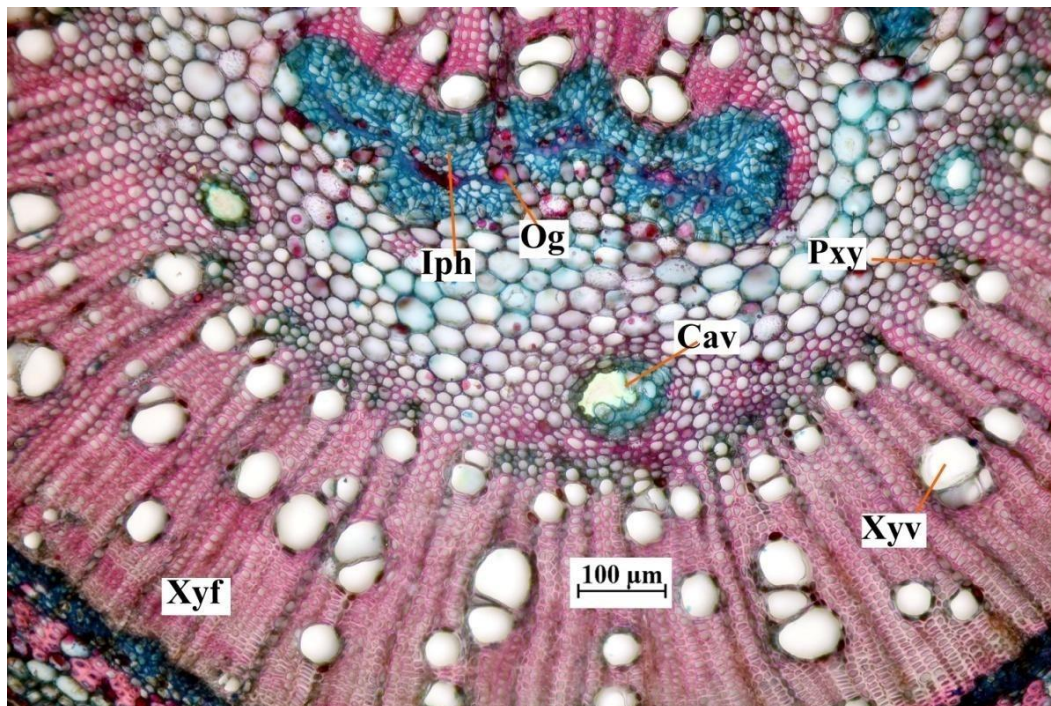


Figure 11: TS of rachis lower middle portion enlarged view Cav: Cavity; Iph: Inner phloem; Og: Oil globules Pxy:Primary xylem; Xyv: Xylem vessels; Xyf: Xylem fibers. Per: Pericycle.

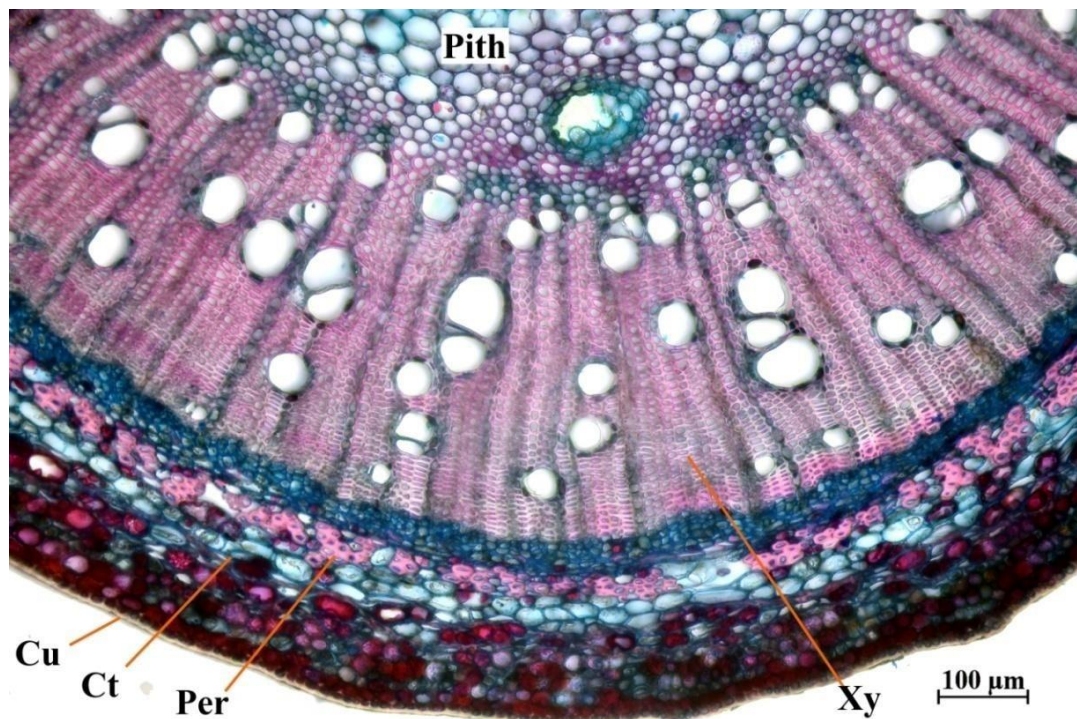


Figure 12: TS of rachis lower portion enlarged view. Cu: Cuticle; Ct: Cortex; Per: Pericycle Xy: Xylem.

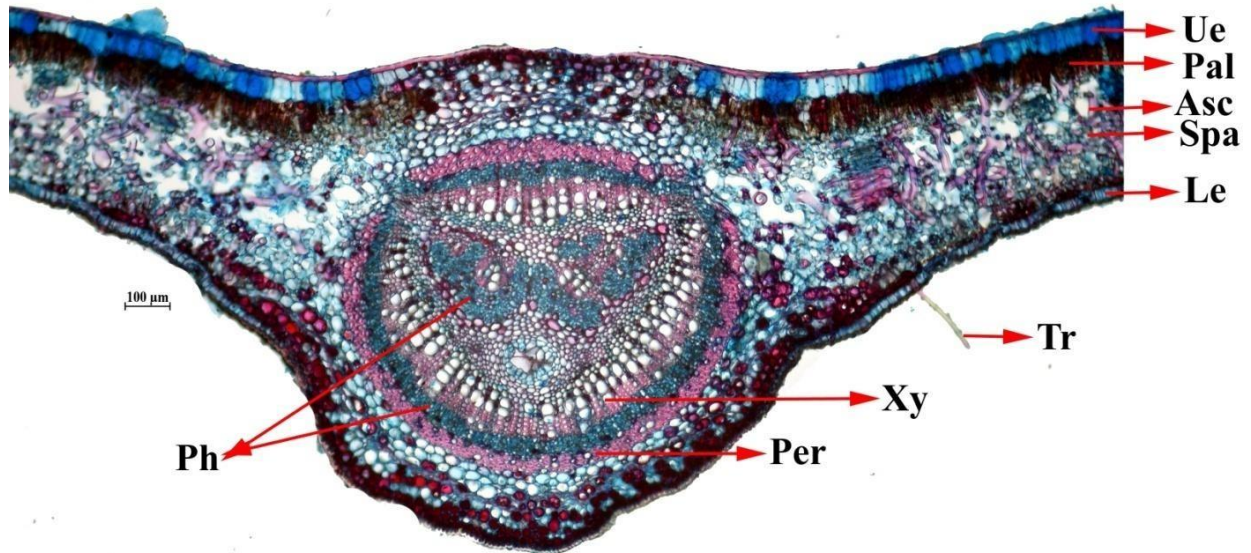


Figure 13: Detailed TS of leaf midrib *Simarouba glauca*. Asc: Asterosclerides; Pal: Palisade parenchyma; Per: Pericycle; Ph: Phloem; Spa: Spongy parenchyma; Le: lower epidermis Tr: Tracheid; Ue: Upper epidermis; Xy: Xylem;j.

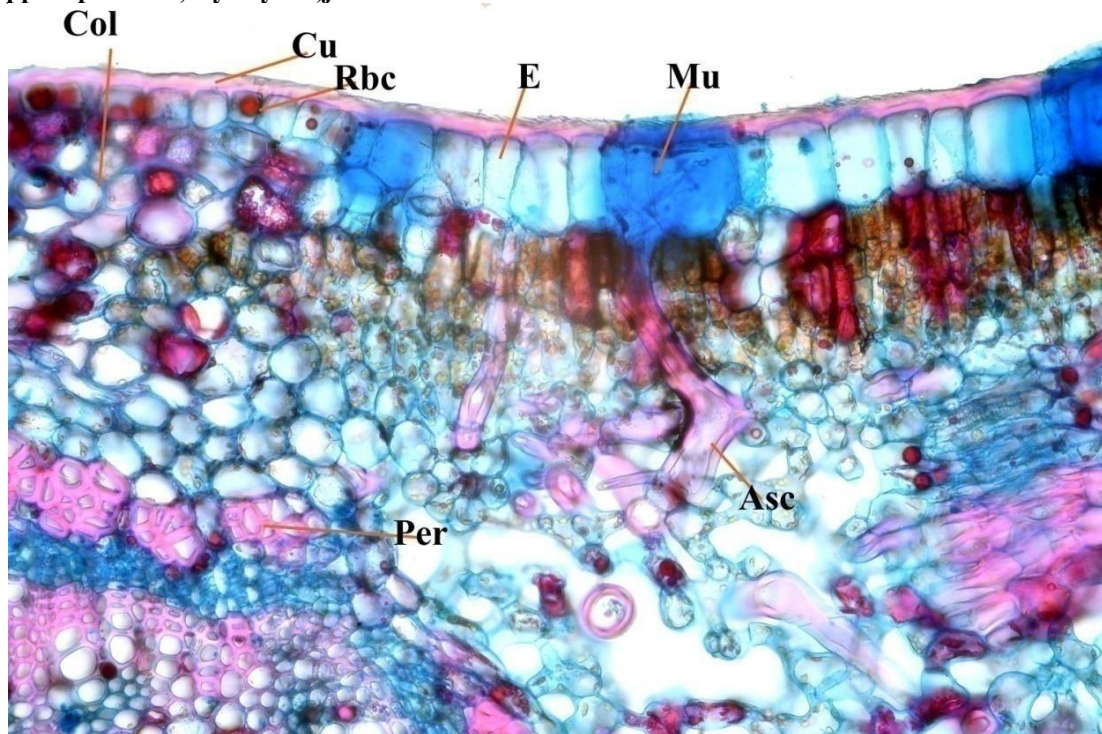


Figure 14: TS of leaf midrib upper portion enlarged view. Asc: Asterosclerides; Col: Collenchyma; Cu: Cuticle; Rbc: Reddish brown; E: Epidermis; Mu: Mucilage; Per: Pericycle.

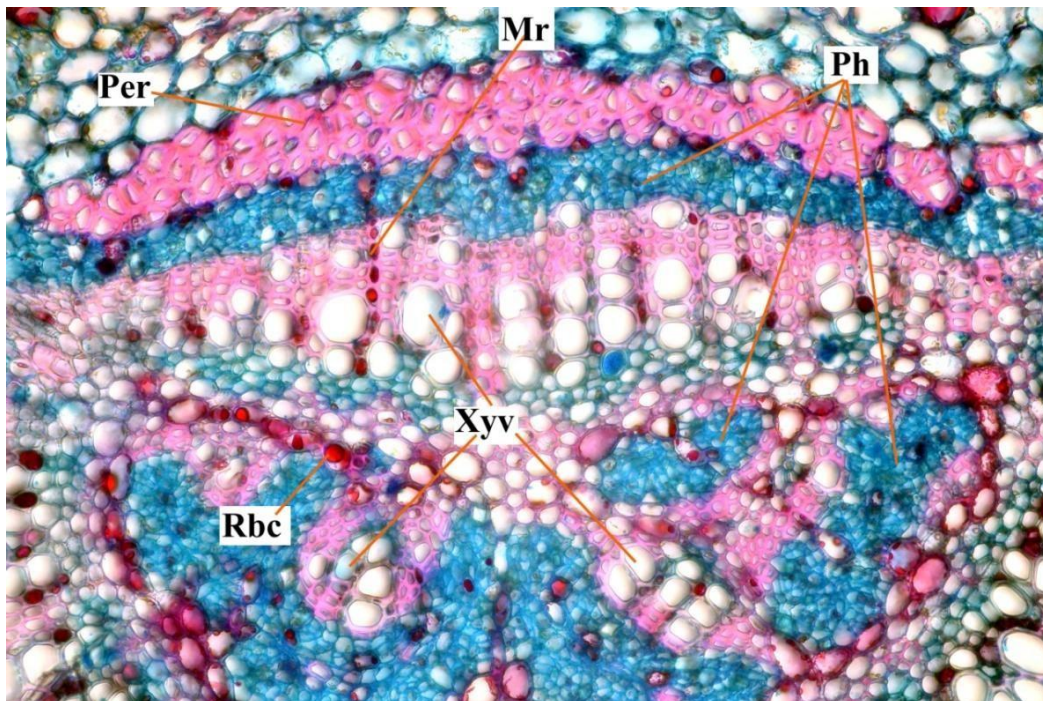


Figure 15: TS of leaf midrib middle upper portion enlarged view. Mr: Medullary ray; Ph: Phloem; Per: Pericycle; Rbc: Reddish brown content; Xyf: Xylem fibre; Xyv: Xylem vessels.

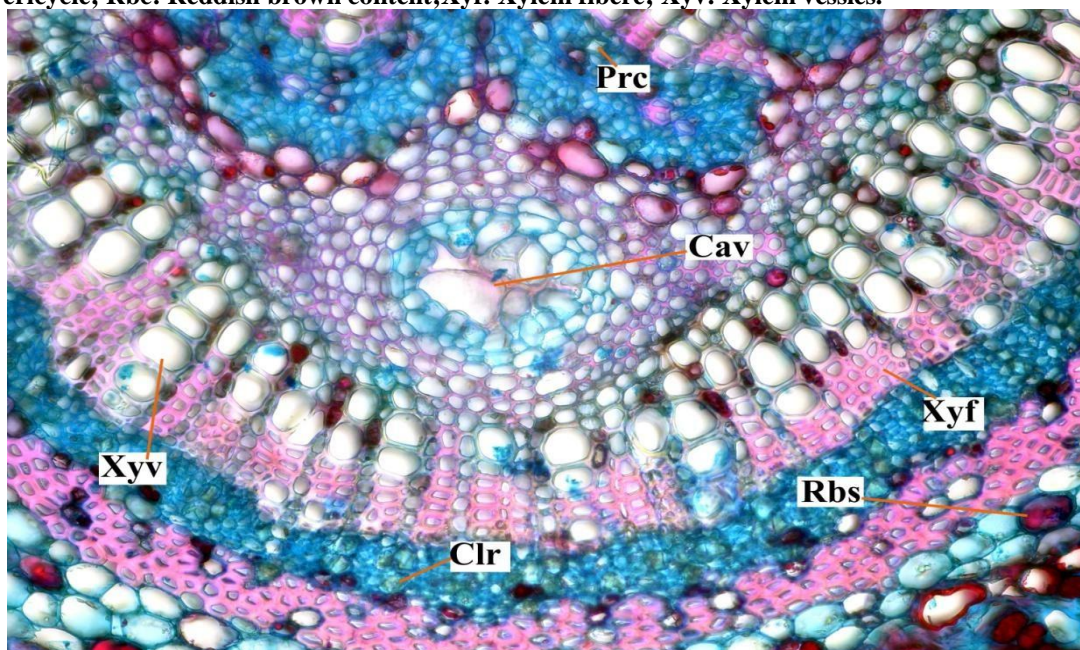


Figure 16: TS of leaf midrib middle lower portion enlarged view. Cav: Cavity; Clr: Cluster crystal; Per: Pericycle; Rbc: Reddish brown content; Xyf: Xylem fibre; Xyv: Xylem vessels.

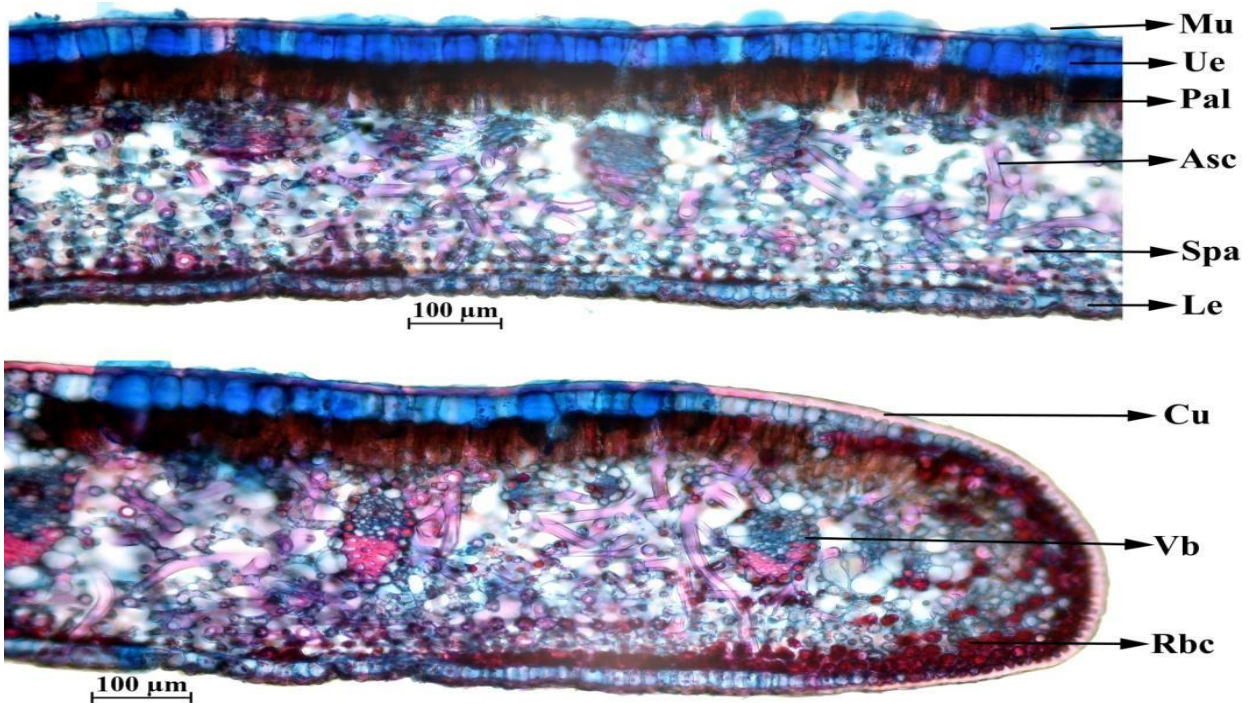


Figure 17: Detailed TS of leaf lamina. Asc: Asterosclerides; Cu: Cuticle; Mu: Mucilage; Pal: Palisade parenchyma; Rbc: Reddish brown content; Spa: Spongy parenchyma; Le: lower epidermis; Vb: Vascular bundle; Ue: Upper epidermis.

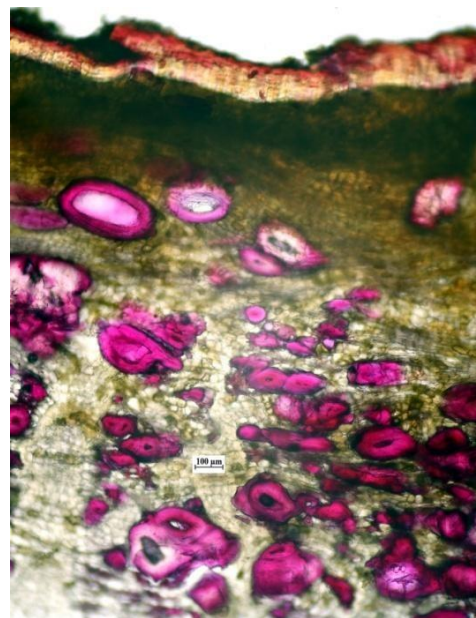
Histo-chemistry

Stem bark

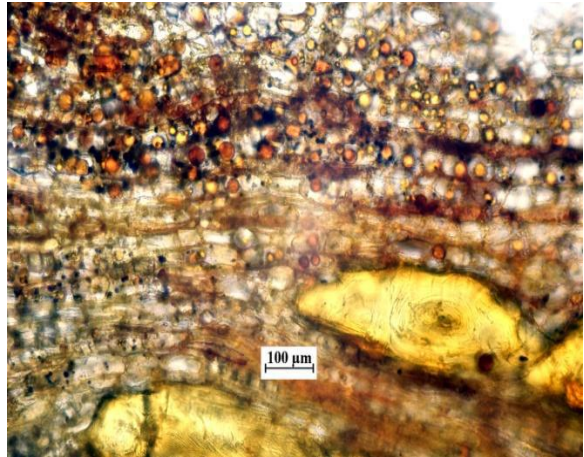
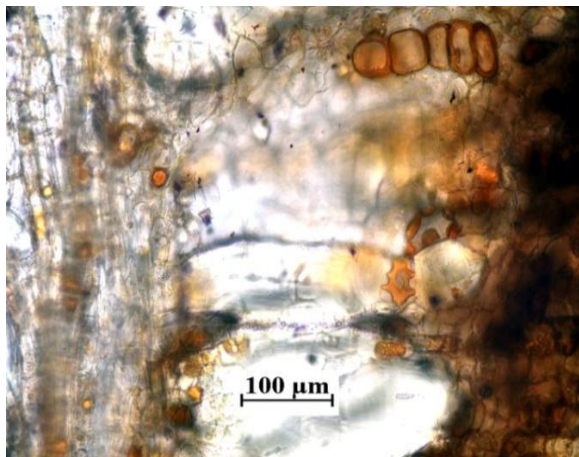
Alkaloids were observed in cortex; phenolic compounds, starch grains and oil globules detected in phloem; lignin present in phloem fibres and stone cells (Fig. 18).

Leaf

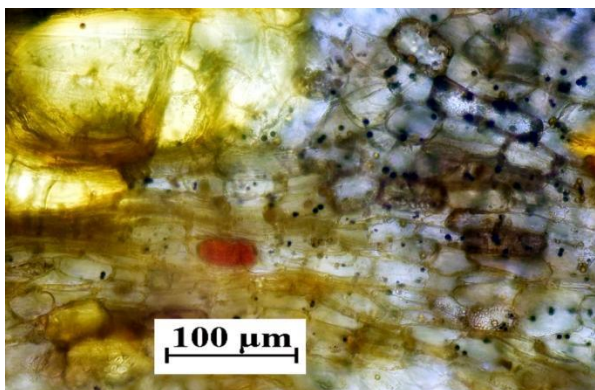
Alkaloids and phenolic compounds were present in lamina and midrib; oil globule was found in lamina; lignin observed in pericycle and xylem; cutin absent on epidermal wall (Fig.19).



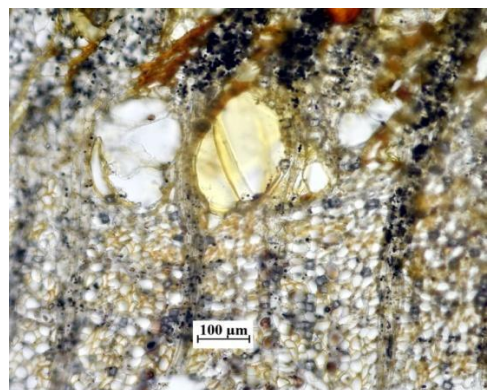
Lignin in phloem fibers



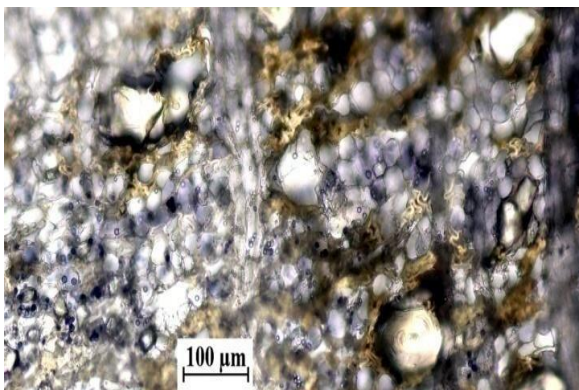
Oil globules in phloem



Alkaloid in cortex



Phenolic compounds in inner phloem



Starch grains in phloem

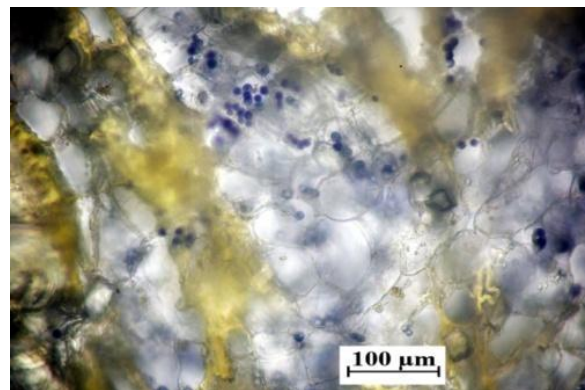
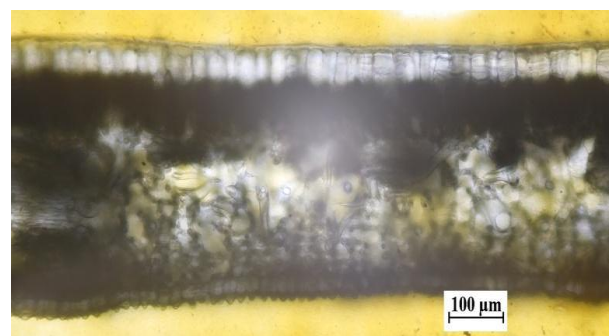


Figure 18: Histochemistry of bark.



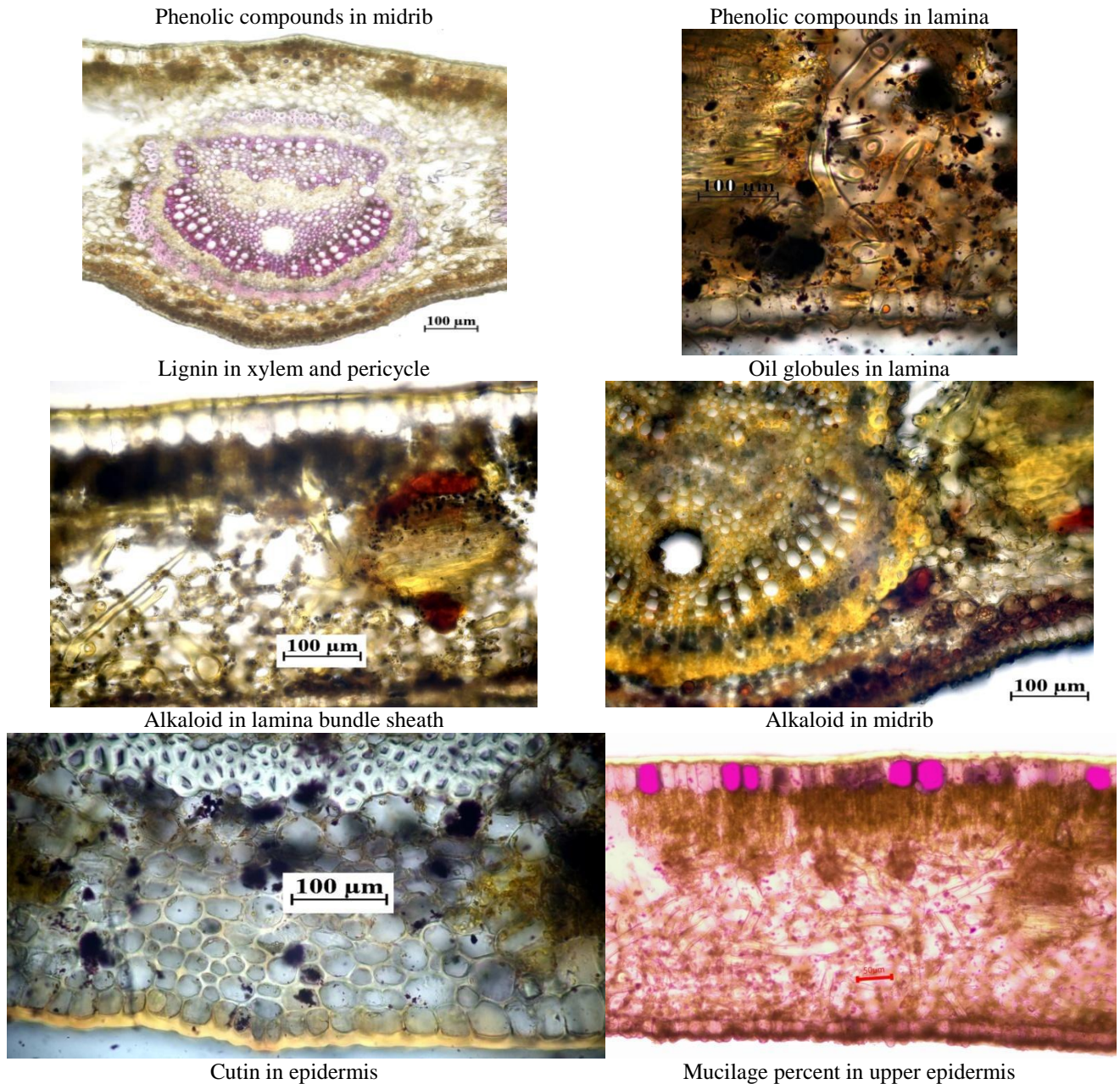


Figure 19: Histochemistry of leaf.

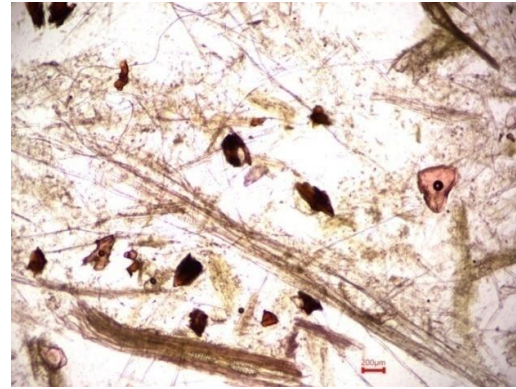
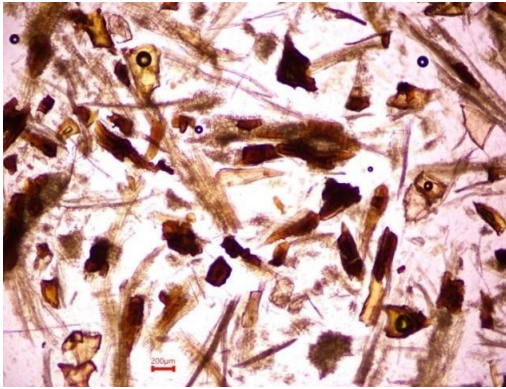
Powder microscopy

Bark

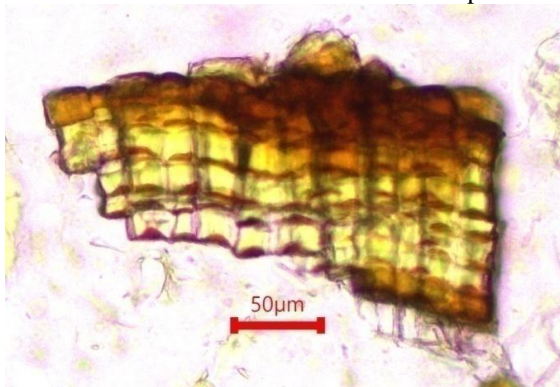
A pinch of powder under microscope shows thick walled, compressed cork cells in section view, thick walled, narrow and wide lumen varying in size stone cells and sclereides, thin-walled parenchyma cells with simple starch grains and brownish content, cork cells in surface view with brownish content, tangential longitudinally cut phloem rays associated with phloem fibers, radially cut phloem rays crossing with phloem fibres (Fig. 20).

Leaves

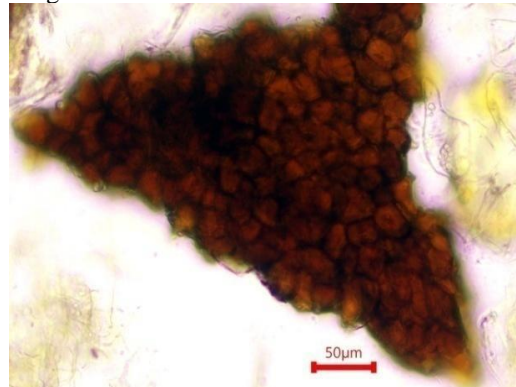
Leaf powder shows upper epidermis in surface view, lower epidermis in surface view with anomocytic stomata, thick-walled rectangular shape of epidermis in surface view from rachis, thin-walled wide lumen varying in shape astrosclereids, thin walled, wide lumen tapering end fibres, slightly wavy thin walled wide lumen simple pits fibre tracheids in shape vessels with spiral, pitted scalariform thickening vessels (Fig. 21).



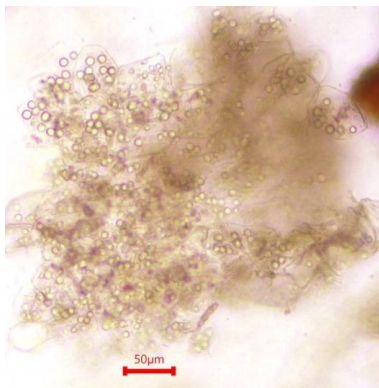
Stem bark powder under lower magnification



Cork cells in sectional view



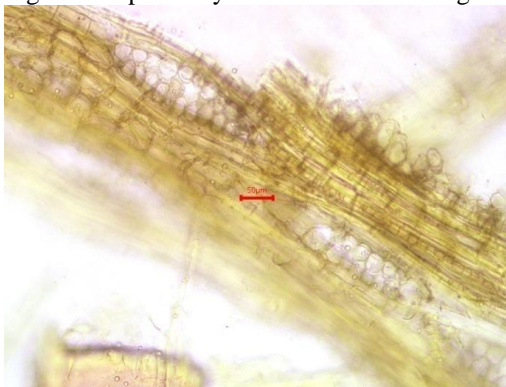
Cork cells in surface view



Fragment of parenchyma tissue with starch grains

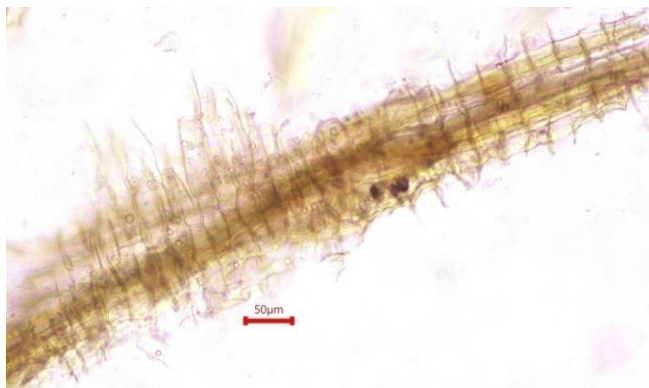


Stone cells



Tangential longitudinally cut phloem ray associated with phloem fiber

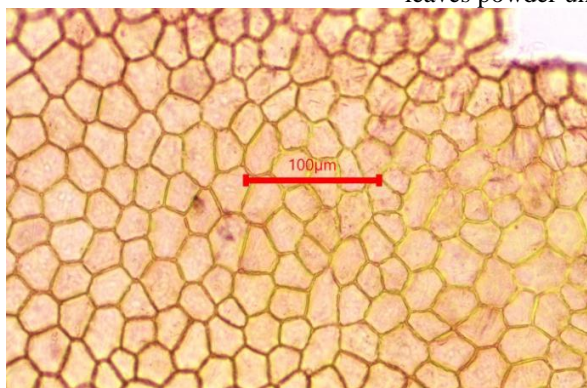




Radial longitudinally cut phloem ray crossing with phloem fiber
Figure 20: Powder microscopy of stem bark.



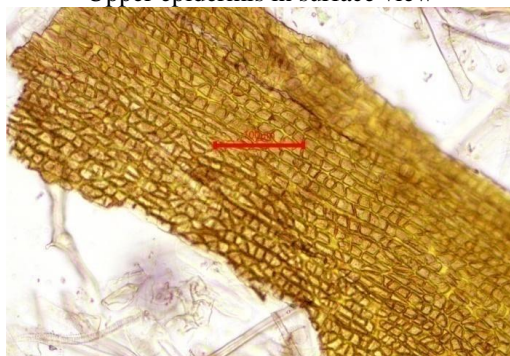
leaves powder under lower magnification



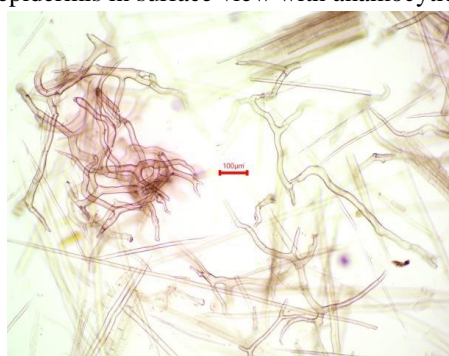
Upper epidermis in surface view



Lower epidermis in surface view with anamocytic stomata



Rachis epidermis in surface view



Fragment of astero sclereids

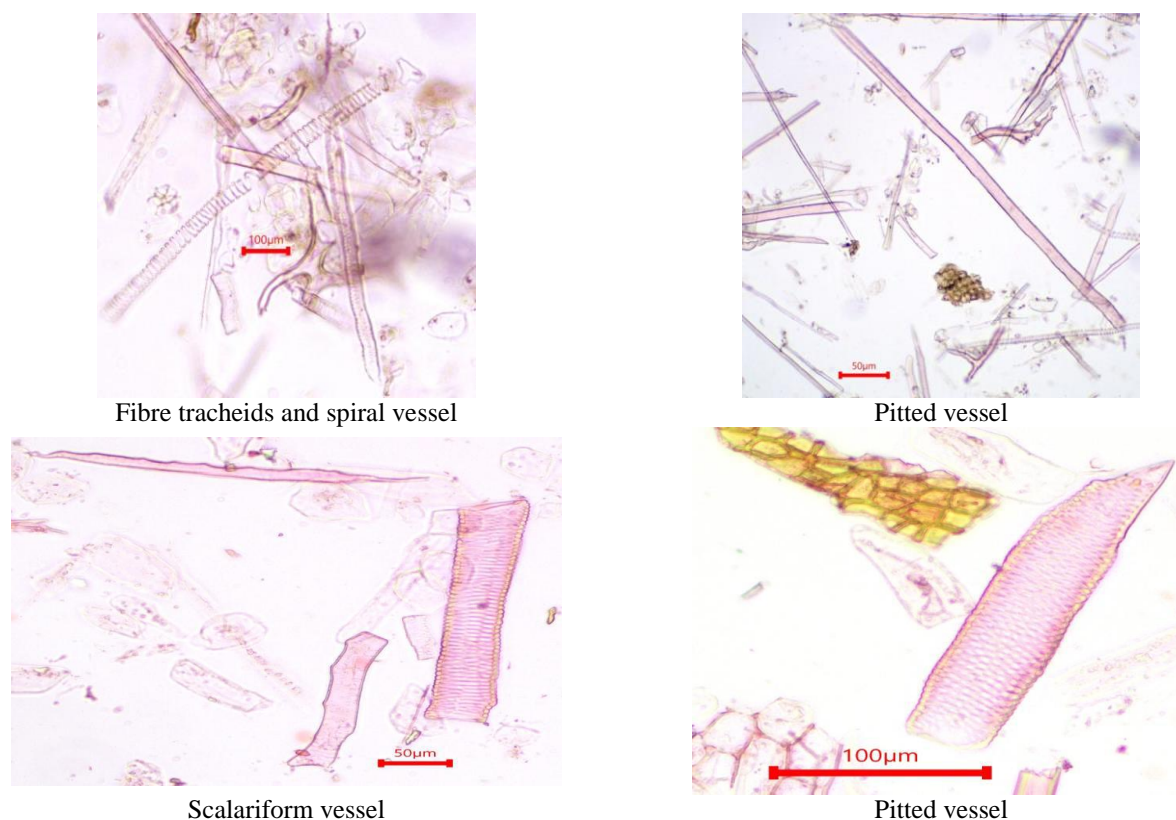


Figure 21: Powder microscopy of leaves.

TLC/HPTLC

The TLC mobile phase was fixed based on literature and by trial and error. The chosen phase contained Toluene: Ethyl acetate: Formic acid: Methanol (75:2.5:0.5:0.5: v/v/v/v). The chromatogram profile of chloroform and methanol extract of stem bark shows at 254 nm 2 and 4 bands; at 366 nm shows 8 and 13, after derivatization at 366 nm shows 11 and 15 bands respectively. The TLC plate derived with vanillin-sulfuric acids seen under 540 nm shows 11 and 13 bands; Leaves of chloroform and methanol extract of TLC under 254 nm shows 6 and 9 bands; at 366 nm shows 11 and 11 respectively. After derivatization with vanillin-sulfuric acid in 366 nm shows 12 and 15 bands; in 540 nm visible lights shows 11 and 14 bands (Fig. 22 – 25) (Table 1 – 4).

The HPTLC finger print profile of chloroform extract of the stem bark densitogram shows 2 spots of R_f 0.27 (area 39.30%) and R_f 0.82 (area 60.70%) at 254 nm and 4 spots of R_f 0.44 (area 52.39%), R_f 0.51 (area 27.22%), R_f 0.64 (area 14.48%), R_f 0.92 (area 32.91%) at 366 nm having more than 10% of the of the R_f values; 540 nm densitogram shows 10 spots appeared 3 spots R_f 0.41 (area 14.36%), R_f 0.64 (area 27.98%), R_f 0.68 (area 24.61%) having more than 10% of the R_f values (Fig. 26).

Stem bark of methanol extract densitogram at 254 nm shows 3 spots R_f 0.22 (area 57.77%), R_f 0.43 (area 23.52%), R_f 0.50 (area 18.71%) appearing more than 10% of R_f values. Densitogram 366 nm shows 5 spots

appeared 2 spots R_f 0.21 (area 66.71%) and R_f 0.96 (area 15.6%) having more than 10% of R_f values. 540 densitogram shows 14 spots having R_f 0.024. (area 26.43%), R_f 0.63 (area 14.05%) and R_f 0.68 (area 18.64%) 3 spots appeared more than 10% of the R_f values (Fig. 26).

The HPTLC finger print profile of chloroform extract of the leaves densitogram shows 4 spots of R_f 0.2 (area 19.88%), R_f 0.49 (area 15.71%), R_f 0.77 (area 15.52%) and R_f 0.91 (area 48.89%) at 254 nm and 6 spots at 366 nm appeared R_f 0.49 (area 15.22%), R_f 0.78 (area 12.52%) and R_f 0.91 (area 60.78%) 3 spots having more than 10% of the of the R_f values; 540 nm densitogram shows 12 spots appeared 5 spots R_f 0.43 (area 11.64%), R_f 0.47 (area 11.44%), R_f 0.74 (area 17.53%), R_f 0.84 (area 23.70%), R_f 0.92 (area 10.28%) having more than 10% of the R_f values (Fig. 27).

Leaves methanol extract densitogram shows 6 spots at 254 nm appeared 2 spots R_f 0.012 (area 53.23%) and R_f 0.19 (area 32.29%) more than 10% of R_f values. densitogram 366 nm shows 8 spots appeared 3 spots R_f 0.015 (area 28.83%), R_f 0.19 (area 26.28%) and R_f 0.91 (area 26.68%) having more than 10% of R_f values. 540 densitogram shows 12 spots having R_f 0.021 (area 11.68%), R_f 0.43 (area 11.46%), R_f 0.74 (area 11.44%), R_f 0.84 (area 27.05%) and R_f 0.92 (area 11.32%) 5 spots appeared more than 10% of the R_f values (Fig. 27).

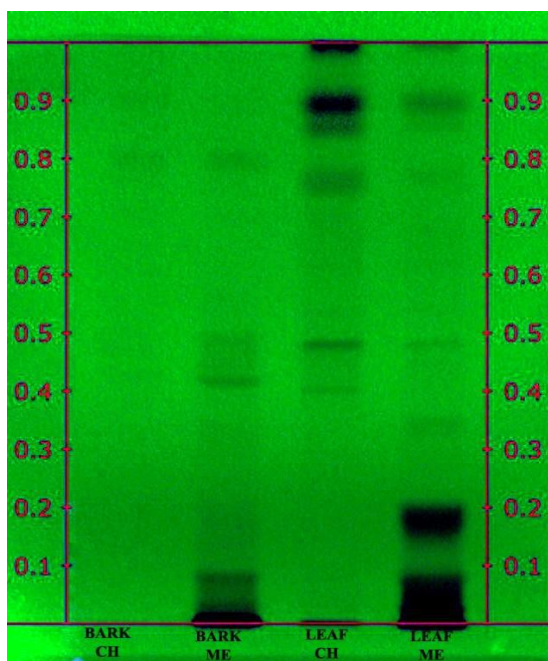


Figure 22: TLC plate at 254 nm.

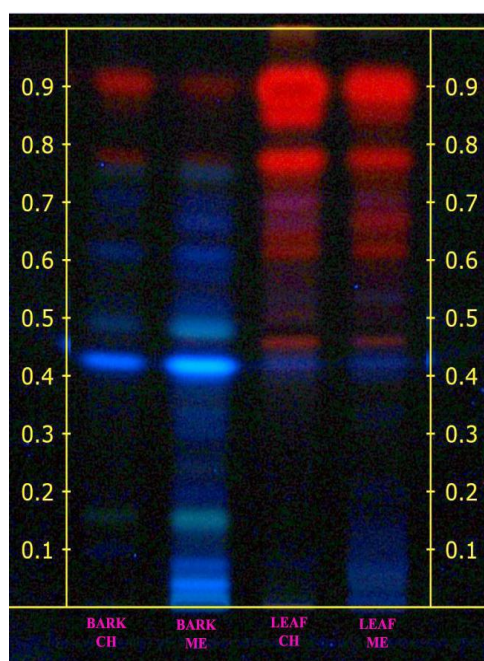


Figure 23: TLC plate at 366 nm.

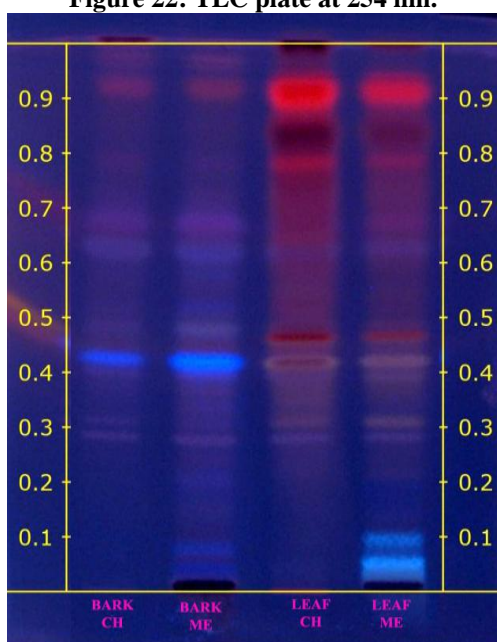


Figure 24: TLC plate at 366 nm (Derivatized)

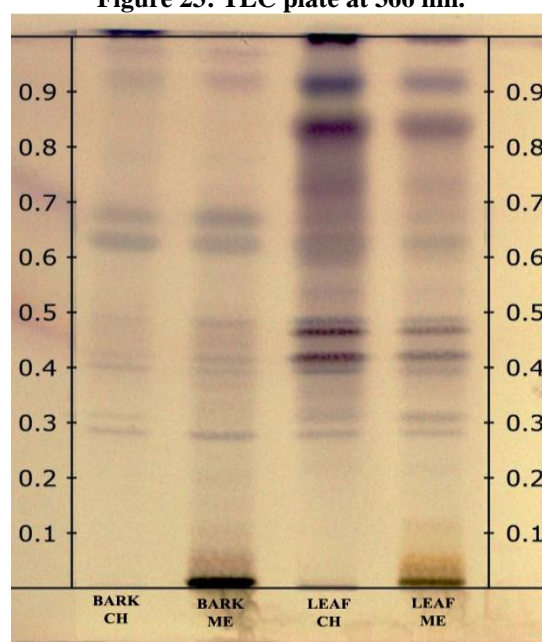


Figure 25: TLC plate at 540 nm (Derivatized)

Table 1: Rf values of TLC photo documentation at $\lambda = 254$ nm (chloroform and methanol extract of leaves and stem bark) of *Simarouba glauca*.

Stem bark				Leaves			
Chloroform		Methanol		Chloroform		Methanol	
Rf Value	Color	Rf Value	Color	Rf Value	Color	Rf Value	Color
-	-	-	-	-	-	0.4	Dark green
-	-	-	-	-	-	0.6	Dark green
-	-	0.10	Dark green	-	-	0.10	Dark green
-	-	-	-	-	-	0.20	Dark green
-	-	-	-	0.41	Light green	0.35	Light green
-	-	0.44	Dark green	-	-	-	-
-	-	-	-	0.48	Light	-	-

					green		
-	-	-	-	-	-	0.49	Light green
-	-	0.50	Light green	-	-	-	-
-	-	-	-	0.75	Light green	-	-
-	-	-	-	-	-	0.77	Light green
0.80	Light green	0.80	Light green	-	-	-	-
-	-	-	-	0.87	Light green	-	-
0.90	Light green	-	-	0.90	Dark green	0.90	Light green
-	-	-	-	0.99	Dark green	0.99	Light green

Table 2: Rf values of TLC photo documentation at $\lambda = 366$ nm (chloroform and methanol extract of leaves and stem bark) of *Simarouba glauca*.

Stem bark				Leaves			
Chloroform		Methanol		Chloroform		Methanol	
Rf Value	Color	Rf Value	Color	Rf Value	Color	Rf Value	Color
-	-	0.2	Blue	-	-	-	-
-	-	0.4	Blue	-	-	-	-
-	-	0.8	Blue	-	-	-	-
0.18	Light green	0.18	Blue	-	-	-	-
-	-	0.42	Dark blue	-	-	0.42	Blue
0.43	Light blue	-	-	-	-	-	-
-	-	-	-	0.44	Blue	0.44	Red
-	-	-	-	0.46	Red	-	-
-	-	0.48	Light blue	-	-	-	-
0.50	Light blue	-	-	-	-	-	-
-	-	-	-	0.51	pink	-	-
-	-	-	-	-	-	0.52	Pink
-	-	-	-	-	-	0.54	Blue
-	-	-	-	0.55	Purple	-	-
-	-	0.58	Blue	0.58	pink	0.58	Light pink
-	-	0.60	Blue	-	-	-	-
0.62	Light blue	-	-	0.62	Red	0.62	Red
-	-	-	-	0.64	red	-	-
-	-	-	-	-	-	0.66	Red
-	-	0.68	Blue	0.68	pink	-	-
-	-	0.70	Blue	-	-	0.70	Pink
0.71	Light blue	-	-	-	-	-	-
-	-	-	-	0.72	pink	-	-
-	-	-	-	-	-	0.75	Brown
0.76	Light green	-	-	-	-	-	-
-	-	0.77	Blue	-	-	-	-
0.78	Light red	-	-	0.78	Dark red	0.78	Red
-	-	0.80	Light red	-	-	-	-
-	-	-	-	0.85	Dark red	0.85	Light red
-	-	0.90	Red	0.90	Red	0.90	Dark red
0.91	Red	-	-	-	-	-	-
-	-	-	-	-	-	-	-

Table 3: Rf values of TLC photo documentation at $\lambda = 366$ nm (Derivatized) (chloroform and methanol extract of leaves and stem bark) of *Simarouba glauca*.

Stem bark				Leaves			
Chloroform		Methanol		Chloroform		Methanol	
Rf Value	Color	Rf Value	Color	Rf Value	Color	Rf Value	Color
-	-	0.4	Bright blue	-	-	0.4	Sky blue
-	-	0.8	Bright blue	-	-	-	-
-	-	-	-	-	-	0.9	Light sky blue
-	-	0.12	Dark blue	-	-	-	-
-	-	0.20	Violet	-	-	-	-
-	-	0.25	Purple	-	-	-	-
-	-	-	-	0.28	Blue	0.28	Purple
0.29	Violet	0.29	Purple	-	-	-	-
-	-	0.31	Purple	-	-	0.31	Purple brown
0.32	Violet	-	-	0.32	Purple	-	-
-	-	0.38	Purple	-	-	0.38	Purple brown
0.40	Blue	-	-	-	-	-	-
0.42	Sky blue	0.42	Sky blue	0.42	Light brown	0.42	Dark Purple brown
0.45	Purple	-	-	-	-	-	-
-	-	0.46	Purple	-	-	-	-
0.48	Light purple	0.48	Purple	0.48	Reddish brown	0.48	Reddish brown
-	-	-	-	0.54	brown	-	-
-	-	-	-	0.60	red	0.60	Light red
-	-	0.62	Purple	0.62	Purple	0.62	Purple
0.63	Purple	-	-	-	-	-	-
-	-	0.67	Purple	-	-	-	-
0.68	Purple	-	-	-	-	0.68	Pink
-	-	-	-	0.70	red	0.70	Light reddish brown
-	-	0.78	Light pink	0.78	Purple	0.78	Reddish brown
0.79	Pink	-	-	-	-	-	-
-	-	-	-	0.84	Dark brown	0.84	Dark brown
-	-	-	-	0.90	Dark red	0.90	Dark red
0.92	Light red	0.92	pink	-	-	-	-
0.95	pink	0.95	Light pink	-	-	-	-
-	-	-	-	0.99	Blackish brown	0.99	Blackish brown

Table 4: Rf values of TLC photo documentation at $\lambda = 540$ nm (Derivatized) (chloroform and methanol extract of leaves and stem bark) of *Simarouba glauca*.

Stem bark				Leaves			
Chloroform		Methanol		Chloroform		Methanol	
Rf Value	Color	Rf Value	Color	Rf Value	Color	Rf Value	Color
-	-	-	-	-	-	0.4	Brown
-	-	0.5	Orange brown	-	-	-	-
-	-	-	-	-	-	0.12	Light brown
-	-	0.28	purple	0.28	purple	0.28	Purple
0.29	Purple grey	-	-	-	-	-	-
0.31	Purple grey	-	-	-	-	-	-
-	-	-	-	0.32	purple	0.32	purple
-	-	0.38	Light orange	-	-	0.38	Light purple
0.40	purple	0.40	purple	0.40	Dark grey	-	-
0.42	Light purple	0.42	purple	0.42	Dark brown	0.42	Dark purple
-	-	-	-	-	-	0.46	Dark purple
0.48	Light pink	0.48	Light pink	0.48	purple	0.48	Light purple
0.50	Light purple	0.50	Light purple	-	-	-	-
-	-	-	-	0.55	Pinkish grey	-	-
-	-	0.62	Grey purple	0.62	Grey	0.62	Grey

0.64	Grey	-	-	-	-	-	-
0.68	Grey	0.68	Grey purple	0.68	Pinkish grey	0.68	Pinkish grey
-	-	0.72	Light pink	-	-	-	-
-	-	-	-	0.74	Dark pink	0.74	Pinkish grey
-	-	0.78	Light green	-	-	-	-
0.80	Grey	-	-	-	-	-	-
-	-	-	-	0.82	Bright brown	-	-
-	-	-	-	-	-	0.84	purple
-	-	-	-	0.90	Dark blue	-	-
0.92	Grey	0.92	Light pink	-	-	0.92	purple
-	-	0.95	Light pink	-	-	-	-
0.98	Light pinkish grey	-	-	0.98	Blackish blue	0.98	purple

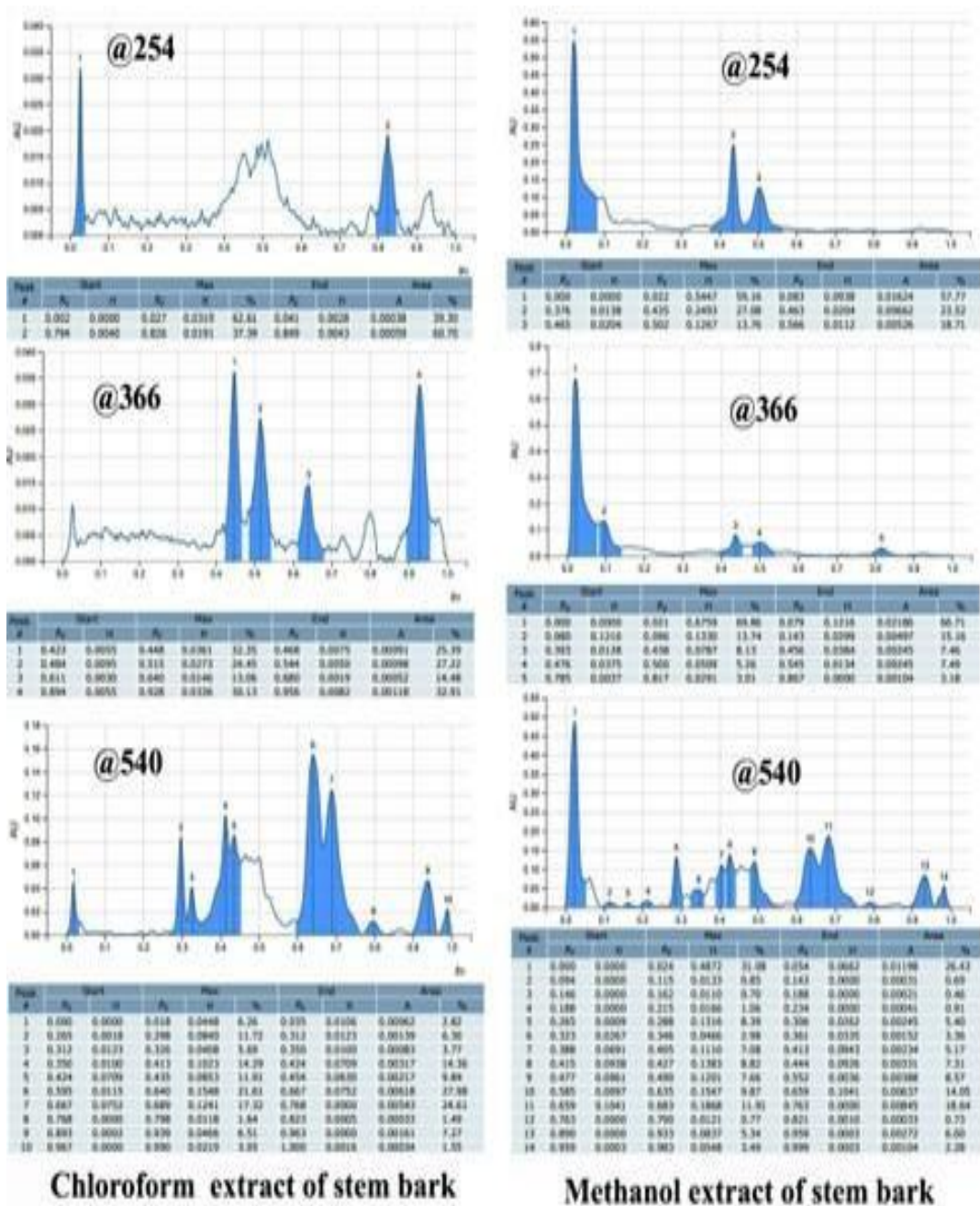


Figure 26: HPTLC densitogram of chloroform and methanol extract of stem bark of *Simarouba glauca*.

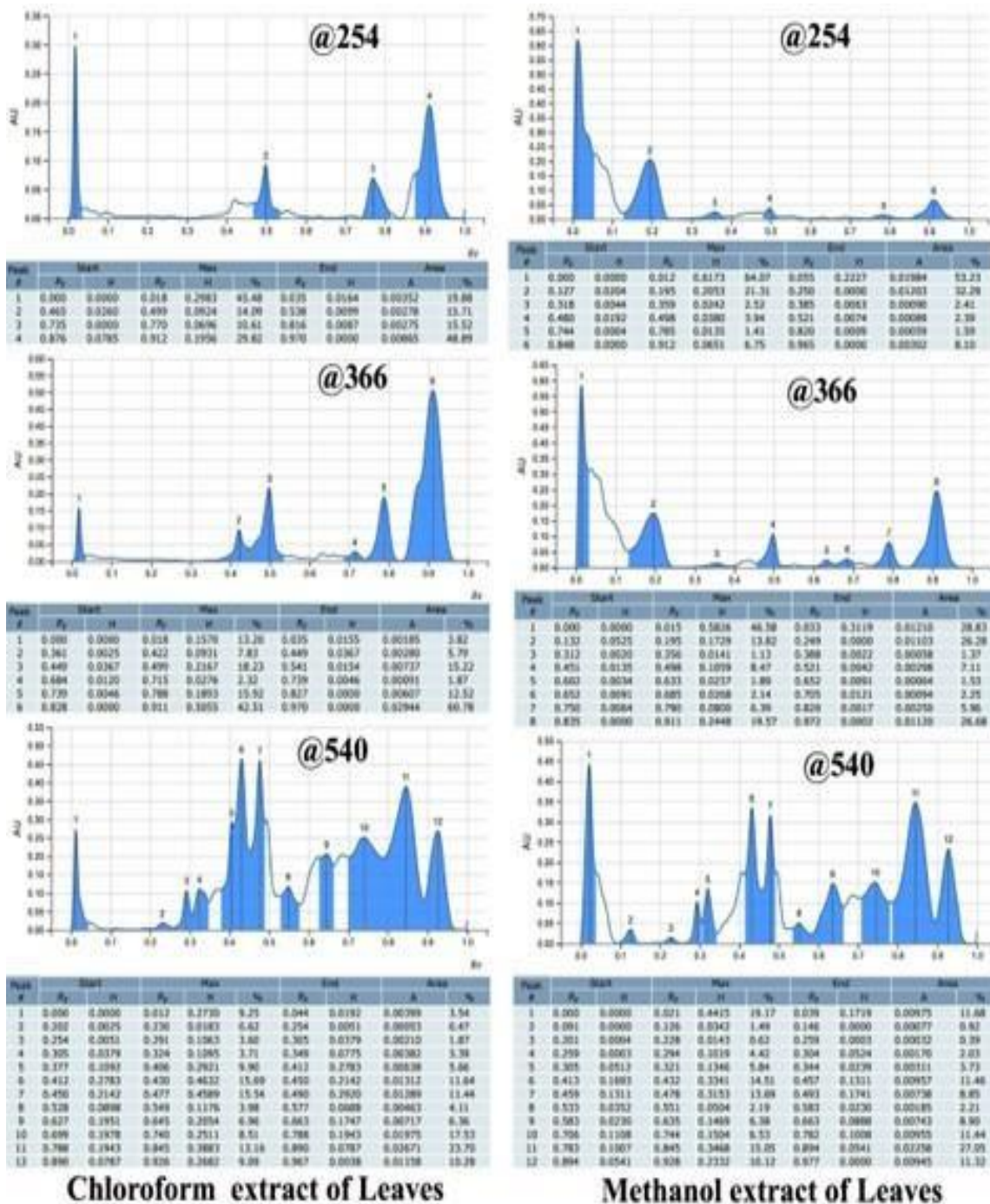


Figure 27: HPTLC densitogram of chloroform and methanol extract of Leaves of *Simarouba glauca*.

CONCLUSION

Simarouba glauca also known as ‘Paradise tree’ is a multipurpose evergreen tree receiving great interest as a promising medicinal plant. Its leaves and bark have been used as a natural medicine with good antimicrobial, antifungal and antioxidant activities. Nowadays leaf and stem bark decoction is used by many cancer patients for curing the disease. It has aroused great enthusiasm as a miraculous tree of solace for cancer patients the reason being the presence of good number of proximate compounds and phytochemicals of medicinal value. The marketing of *S. glauca* stem bark and leaves in the raw drug herbal market can be widely used by people in the

future. This research will be very useful for the identification and authentication of genuinely at various levels. It supports the benefits of *S. glauca* in future Indian pharmacopeia committee creating monograph of this plant. Anatomy, powder microscopy, histochemical and TLC/HPTLC studies are very useful in the authentication and identification of *S. glauca* leaf and stem bark available in the crude drug market and to check the adulterants with other species of *Simarouba* before being used in formulations.

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