



## Macro-microscopy and HPTLC Fingerprinting of Stem Bark, Leaf and Seed of *Swietenia macrophylla* King

Radha P<sup>1</sup>, Divya KG<sup>2</sup>, Rubeena M<sup>3</sup>, Nagaraj R<sup>4</sup>, Sunil Kumar KN<sup>5</sup>

<sup>1,4</sup> Department of Botany, Siddha Clinical Research Unit (Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India), Palayamkottai, Tirunelveli, Tamil Nadu, India

<sup>2,3,5</sup> Department of Pharmacognosy, Siddha Central Research Institute (Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India), Arumbakkam, Chennai, Tamil Nadu, India

<sup>1,4</sup> Siddha Medicinal Plants Garden (Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India), Mettur Dam, Salem, Tamil Nadu, India

### Abstract

**Background:** In Siddha System of Medicine, Meliaceae members such as *Azadirachta indica* A. Juss., *Melia azedarach* L., *Aglaiia odorata* Lour. etc., have been used as an ingredient in several formulations owing to their high potential therapeutic values. In recent decades, another member of this family *Swietenia macrophylla* King (broad leaved mahogany) has drawn a great attention by its commercial and medicinal values. The medicinal properties of mahoganies have been explored thoroughly by the distinguished researchers worldwide that urges to make the identification key to differentiate them from the adulterants. Since the micro-morphological characters are the key in the identification of herbal drugs, the present study was intended to explore macro-micro and HPTLC/TLC characteristic features of leaves, stem bark and fruits stem bark and fruits seeds of broad-leaved mahogany (*S. macrophylla*) and a comparison has been carried out with respect to the earlier studies of Indian mahogany (*S. mahagoni*).

**Materials and Methods:** Plant parts such as leaves, stem bark and fruits were collected from Periyaputhur, Salem District, Tamil Nadu. The macro-micro observations were made using standard protocols. Total ethanolic extracts of the plant parts were subjected to HPTLC studies too.

**Results:** Microscopic observation of transverse section and powder microscopy of leaves showed the presence of anomocytic stomata, prismatic crystals, crystal fibers, starch granules, spiral vessels etc. The transverse section of the stem bark showed the cork, lenticels, stone cells, cortex with plenty of starch etc. Microscopic observation of the seed wing and cotyledons shows the presence of starchy mesotesta, pitted fibres, oil globules, crystal fibres and stone cells.

**Conclusion:** In the present study, micro-macroscopic characteristic features of stem bark, leaves and seeds of *S. macrophylla* King (broad leaved mahogany) has been carried out and a comparative account with *S. mahagoni* (L.) Jacq., another Meliaceae members used in the traditional medicinal system of Siddha is been discussed briefly. HPTLC fingerprint obtained in the study will help in chemical identification of the plant materials.

**Keywords:** broad-leaved mahogany, Indian mahogany, micro-macroscopic characters, medicinal uses, pharmacopoeial standardization, quality standards

### 1. Introduction

Meliaceae, the mahogany family, is economically important owing to its quality of timbers and potential medicinal values. Some of the important tree species of this family are *Azadirachta indica* A. Juss., *Melia azedarach* L., *Aglaiia odorata* Lour, *Swietenia macrophylla* King, *Swietenia mahagoni* (L.) Jacq. *Walsura* sp etc. <sup>[1]</sup>. Amongst them, Mahogany considered as an important plant in the Agro-biology, Silviculture as well as potentially important drug for many ailments. Two species of Mahogany well known world-wide are *Swietenia mahagoni* (L.) Jacq. *S. macrophylla* King which are native to West Indies, Central America and introduced into Kerala, Tamil Nadu, Karnataka etc. *S. mahagoni* (Small leave mahogany) commonly known an Indian mahogany and *S. macrophylla* (Broad leaved mahogany) is known as Sky fruit or Madeira mahogany. The broad-leaved mahogany (*S. macrophylla*) is a very large tree, reaching up to a height of 30 to 40 m and a

girth 3 to 4 m. Trunk cylindrical, with a buttressed base; it is distributed in the deciduous and the rain forest in Brazil, Mexico and other Central American countries. Different parts of the *Swietenia* are used for distinguished purposes and it has been reported for its medicinal uses in the treatment of hypertension, inflammation, HIV, diabetes and malaria <sup>[2]</sup>. The timber is highly useful in the manufacture of door pan, cupboards, flooring items etc <sup>[3, 4]</sup>. Meliaceae plants shows vast diversity of the phytochemical compounds viz., limonoids and their derivatives, tri-terpenoids and flavonoids (quercetin and myricetin) medicinal properties like antimicrobial and anti-oxidant activities are attributed to them. Numbers of medicinally used phytochemicals are reported from the seed and stem bark of *S. macrophylla* (eg. Catechin, epi-catechin, limonoids, tetranortriterpenoids etc). Phytochemical 8, 30-epoxy-siwietenia acetate reported from bark and seed shows high anti-oxidant, anti-diabetic and anti-microbial activities. Seeds of *S. macrophylla* are

medicinally important as well as a good alternative source for omega-3 fatty acid - an important nutraceutical recommended globally as it contains favorable content of fatty acids - palmitic acid, arachidic acid, oleic acids and high amount of PUFAs-linoleic and linoleic acids.<sup>5-7</sup> Apart from seeds/bark, many phyto-constituents which are essential for human welfare are isolated and reported from other parts of the tree such as tender shoots, mature leaves and even senescence leaves. Pyro-oil (Bio -oil) an alternative fuel produced from the hard wood chips has also been reported<sup>[8]</sup>.

A number of researches have been done to explore the medicinal value, timber value, antibacterial activities etc. [1, 3, 6, 7, 9-11]. However, the morpho-anatomical work which is key for identification of medicinal herbs on the broad-leaved mahogany has not yet done. Hence, the present study aimed to characterize the morpho-micro and phytochemical profiling of broad-leaved Mahogany. Detailed pharmacognostic studies on the leaves, stem bark and fruits were carried out and the key characters are compared with *Swietenia mahagoni*, a medicinal plant of the same family which is used in the Siddha traditional system.

## 2. Materials and Methods

### 2.1 Macro-microscopic analysis

Fresh leaves, stem bark and fruits of *S. macrophylla* were collected from Periyaputhur, Salem District, Tamil Nadu, India and cleaned by running water to remove dust and adhering particles. Morphological characters were documented and photographed using DSLR Nikon Camera. The specific epithet confirmed using vegetative and reproductive characters were compared with regional flora<sup>[12]</sup>. The parts were dried under shade and used for further studies. For microscopic studies the plant materials (leaves, stem bark, winged seed) were processed by fixing in FAA (Formalin - 5ml + Acetic acid - 5ml + 70% Ethyl alcohol - 90ml) for 2 days. A serial hand section was taken using a sharp blade and stained with safranin. Selected transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss Axio Cam Erc5s digital camera under bright field light. Magnifications of the figures were indicated by the scale-bars. For powder microscopic studies, the samples were cleaned; shade dried for 12 days and finely powdered. A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerin. 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 embedded with pre-calibrated scale bars and magnification was indicated by the scale<sup>[13]</sup>.

### 2.2 High Performance thin layer chromatography

#### 2.2.1 Sample preparation

The extracts for HPTLC studies were prepared by taking 1 g of coarse powder and extracting with 10 ml of ethanol in a conical flask by cold percolation overnight. The following day it was boiled for 10 minutes and filtered. The filtrate was made up to 10 ml in standard flask.

#### 2.2.2 Developing solvent system (mobile phase)

A number of solvent systems were tried to obtain an appropriate mobile phase. The solvent system toluene: ethyl acetate: formic acid (5: 2: 0.1 v/v/v) gave a satisfactory

separation.

#### 2.2.3 Sample application

Sample application is performed on silica gel 60 F<sub>254</sub> pre-coated aluminium sheet. The extract is applied as bands using CAMAG Hamilton syringe using CAMAG Automatic TLC Sampler 4 (ATS4).

#### 2.2.4 Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG twin-trough development glass chamber (10 x 10 cm) pre-saturated with the mobile phase at room temperature.

#### 2.2.5 Documentation

The developed chromatogram was air dried to evaporate solvents from the plate and kept in CAMAG visualizer and the images were captured under short UV and long UV.

#### 2.2.6 Densitometry

The plate was scanned at 254 nm and 366 nm. The R<sub>f</sub> values and the finger print profiles are recorded with the CAMAG TLC scanner 4 synced with CAMAG winCATS software.

#### 2.2.7 Post chromatographic derivatisation

The plate was derivatized using vanillin-sulphuric acid spray reagent and heated at 105°C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and scanned at 575 nm and the densitometric chromatogram was recorded.

## 3. Results

### 3.1 Morphology

#### 3.1.1 Bark

Surface of the bark is grayish reddish brown, rough, cork is flaky in patches, corky warts present; fracture splintery; odour characteristic and tastes bitter.

#### 3.1.2 Leaves

Up to 20 cm, paripinnate; leaflets 4 to 6, pairs ovate to lanceolate, acuminate at apex, slightly oblique at base, light green or reddish when young, dark green and shining when mature, greenish brown in colour and bitter in taste.

#### 3.1.3 Seed

Light brown in color, 6 to 11 cm long, compressed and extended into a wing (Figure 1), bitter in taste.

### 3.2 Microscopic

#### 3.2.1 Stem bark

TS of bark shows the presence of 3 to 5 layers of rectangular cork cells, the cork is discontinuous and rhytidome and lenticels are present occasionally; underlying phelloderm cells of 5 to 7 layers containing a few starch grains and prismatic crystals; thin walled parenchymatous cortex containing abundant simple round to oval starch grains is present towards inner side; radially compressed cells containing a reddish orange substance are seen in the cortex. Phloem shows thick walled fibers, ray cells mostly biseriate and occasionally uniseriate, contain starch grains and crystals. Powder shows fragments of cork, phelloderm and cortical cells, parenchyma cells with reddish brown content, crystal fibres, fibre bundles with abundant starch grains and isolated prismatic crystals (Figure 2), pale brown in colour,

odor characteristic, and taste bitter.

### 3.2.2 Leaflet

TS is dorsi-ventral in structure, both upper and lower epidermis are barrel to oval shaped covered by cuticle; the secondary veins are represented by small vascular bundles. The midrib consists of the ground tissue composed of 2 to 3 layers of collenchyma cells, vascular bundles and mechanical tissue comprising of sclerenchyma cells surrounding the vascular bundle. Vascular bundle is crescent shaped with xylem surrounded by phloem. Tannin cells and few prismatic crystals are sporadically observed. Fibers are more in the abaxial side of the midrib. In lamina, the upper epidermis is followed by single layered palisade cells in the adaxial side and spongy tissue is found in the abaxial side.

Powder shows epidermal cells with anomocytic stomata, palisade cells, crystal fibers, spiral vessels and prismatic crystals (Figure 3), pale green in colour, odor characteristic, taste bitter.

### 3.2.3 Seed

The transverse section through wing of the seed shows a thin epidermis covered by cuticle followed by parenchymatous cortical tissue filled with starch grains and a small vascular bundle suspended in the middle. TS through cotyledons show a multilayered testa followed by tegmen, the mesotesta contains large amount of starch grains. The ground tissue of two cotyledons is separated by hilum; presence of prismatic crystals, cystoliths and mucilage cells are observed (Figure 4). The powder microscopy of the seed shows the cells of testa with brownish contents, longitudinally elongated epicarp cells,

pitted fibers, stone cells, oil globules and starch grains (Figure 5). There are characteristic differences in the morphology, microscopy and the alkaloid contents present in the two species of *Swietenia* viz, *macrophylla* and *mahagoni* (Table 1).

### 3.3 HPTLC analysis

Photo-documentation of 2 different concentrations (4 and 8 µl) of ethanol extract of plant materials were performed using toluene: ethyl acetate: formic acid – 5:2:0.1 as mobile phase and the plates were visualized/scanned at 254 nm, 366 nm and after derivatisation using vanillin-sulphuric acid at 575 nm (Table 2, Figure 6.1-6.3). Densitometric scan and  $R_f$  value of ethanol extract were also recorded (Figure 7, 8, 9). The same bands seen in all the three samples indicated the presence of similar compounds in all the plant parts. The  $R_f$  values and colour of the chromatogram of the three samples under white light after derivatisation were also compared. Many bands with same  $R_f$  values are present in all the three samples. But several bands are having different  $R_f$  values and colour indicating that the presence of different compounds in different plant parts. The  $R_f$  values reflect the phytoconstituents of the plant which may establish the identification of the genuine source. It is to be noted that the peak areas of the bands also vary to a great extent. The area of each peak is in proportion to the amount of the particular component present in the samples. The peaks with greater area were more prominent and the concentrations of the corresponding components in the samples were high. These results provide quantitative information about the main constituents present in the herbal drug (Table 2).

**Table 1:** A comparison of characteristic features of *Swietenia macrophylla* King and *Swietenia mahagoni* (L.) Jacq.

Particulars	<i>Swietenia macrophylla</i> King	<i>Swietenia mahagoni</i> (L.) Jacq.	References
Native	Central America, South America	Cuba, Hispaniola Jamaica and South Florida	[14]
Morphology	Habit	Tree	Tree
	Leaves arrangement	Alternate	Alternate
	Leaves type and size	Even-pinnately compound	Even-pinnately compound
	Leaves length × width (cm)	5.5 to 12 × 3 to 4.5	4.5 to 7 × 1 to 2
	Leaflets	4 to 6 pairs	4 or 5 pairs
	Leaves shape	Ovate-lanceolate, falcate	Oblong-lanceolate, falcate.
	Bark	Grayish-brown, smooth, flakes into patches	Rugose, gray-black or dark brown flaked
	Inflorescence/Flower	Supra-axillary panicles /greenish yellow	Axillary pendulous panicles /greenish yellow
	Fruit type	Woody capsule	Woody capsule
	Fruit shape	Ovoid	Ovoid
	Fruit length (cm)	8 to 15	5 to 10
	Number of seed	50 to 60	30 to 40
Seed length (cm)	6 to 11	4 to 6	
Anatomy	Leaves	Presence of crystal fibers, starch granules and spiral vessels	-
	Stem Bark	Presence of cork cell, lenticels, stone cells and starchy inner cortex	Presence of cork cell, crystal, starch grains and cortex.
	Seed wing and cotyledons	Presence of starchymesotesta, fibrous with pits, oil globules along with starch grains, fibrous crystals and stone cells.	-
Phytochemical	Leaves	Phragmalinortho esters, swietephragmin H-J and swietemacrophine.	Phargmalinlimonoids swietephragmins A-G.
	Seed	Tetranortriterpenoids	6-Desoxyswietenine

Table 2 R<sub>f</sub> values and colour of major bands obtained by TLC of ethanolic extract of *Swietenia macrophylla*

Table 2.1: R<sub>f</sub> values and colour observed at 254 nm

Seed		Leaf		Stem bark	
R <sub>f</sub>	Colour	R <sub>f</sub>	Colour	R <sub>f</sub>	Colour
0.03	Dark green	0.03	Dark green	0.03	Dark green
0.12	Light green	0.07	Light green	0.17	Dark green
0.19	Light green	0.12	Light green	0.25	Light green
0.24	Light green	0.17	Dark green	0.29	Light green
0.39	Light green	0.25	Light green	-	-
0.48	Light green	0.28	Light green	-	-
-	-	0.32	Light green	-	-
-	-	0.74	Dark green	-	-
-	-	0.86	Dark green	-	-
-	-	0.96	Light green	-	-

Table 2.2: R<sub>f</sub> Values and colour observed at 366 nm

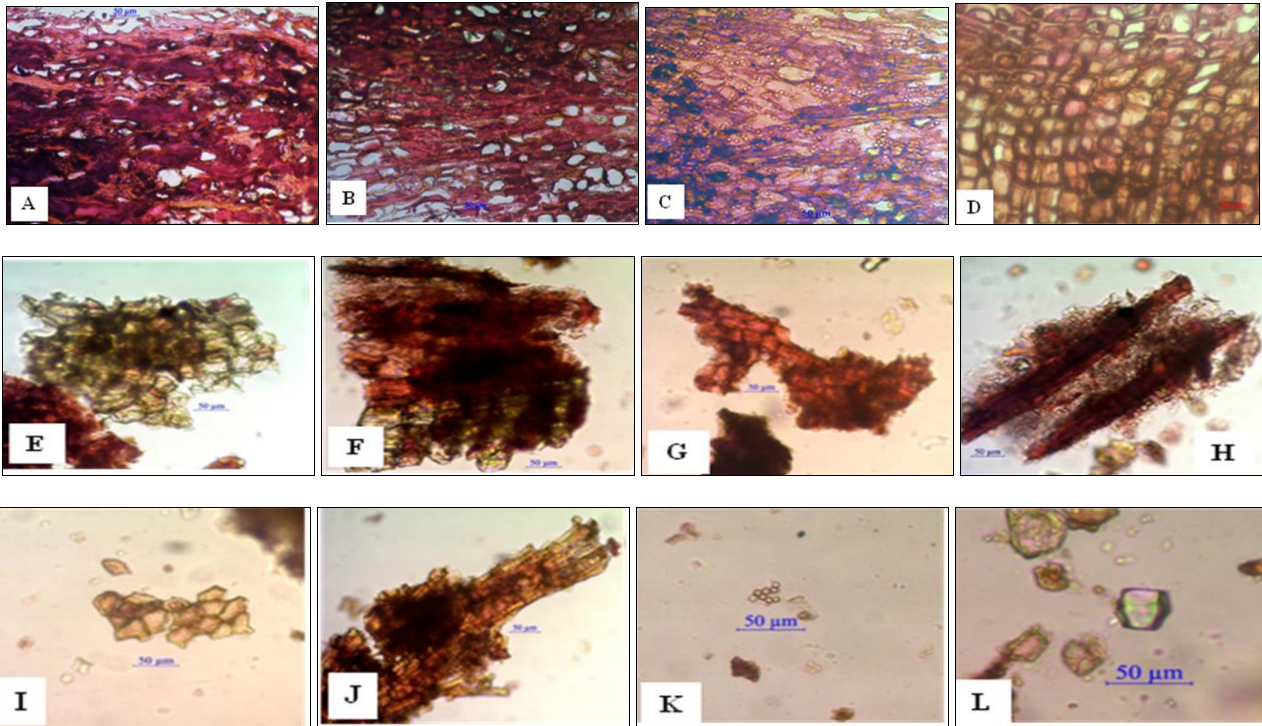
Seed		Leaf		Stem bark	
R <sub>f</sub>	Colour	R <sub>f</sub>	Colour	R <sub>f</sub>	Colour
0.06	Blue	0.04	Violet	0.05	Blue
0.12	Blue	0.17	Pink	0.14	Light blue
0.17	Blue	0.20	Brown	0.25	Blue
0.25	Blue	0.25	Red	0.72	Light green
0.71	Light green	0.31	Brown	-	-
-	-	0.38	Brown	-	-
-	-	0.58	Light red	-	-
-	-	0.76	Dark red	-	-
-	-	0.88	Dark red	-	-

Table 2.3: R<sub>f</sub> Values and colour observed at 575 nm

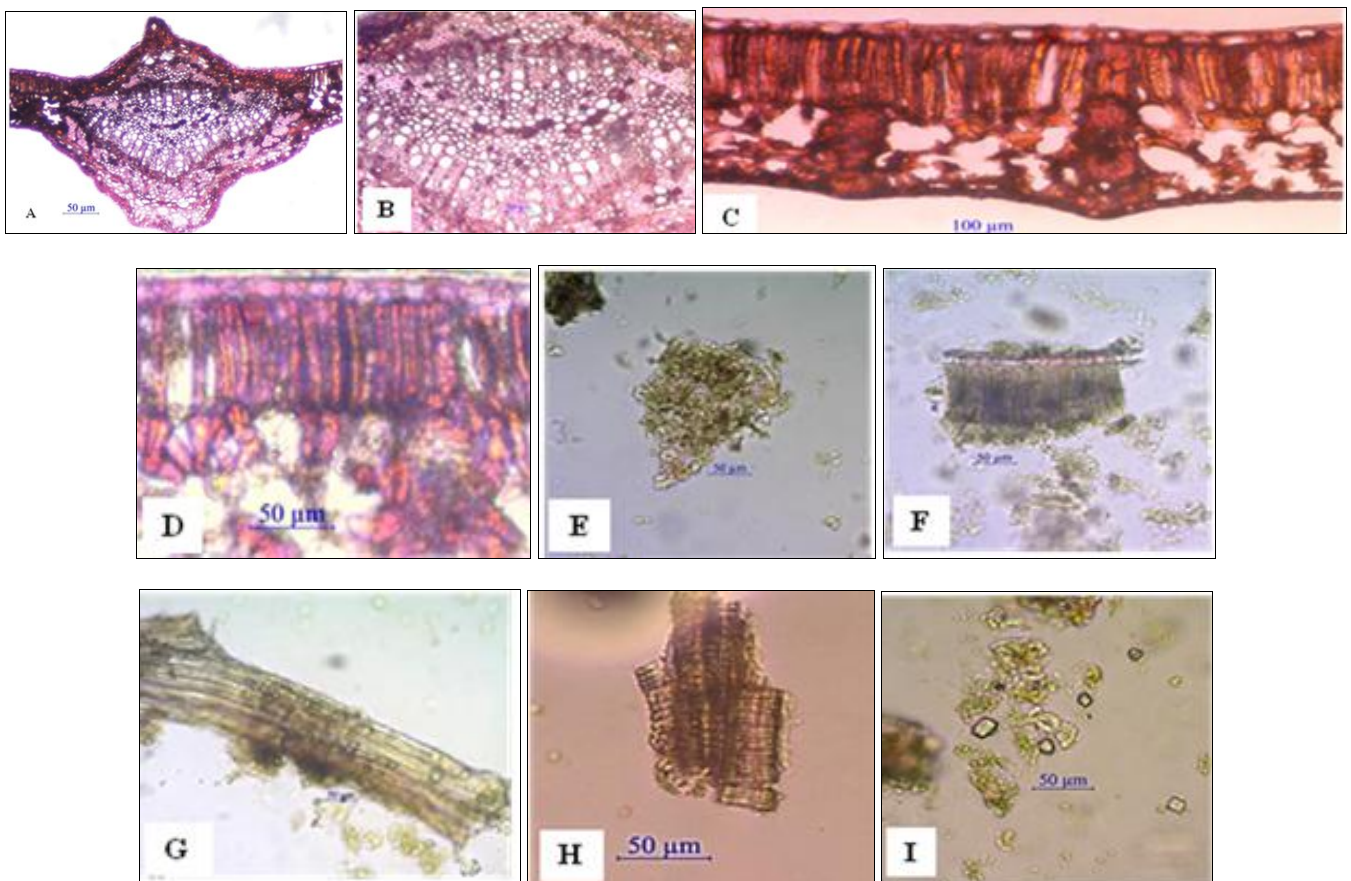
Seed		Leaf		Stem bark	
R <sub>f</sub>	Colour	R <sub>f</sub>	colour	R <sub>f</sub>	Colour
0.20	Purple	0.10	Purple	0.03	Light brown
0.25	Purple	0.14	Light purple	0.25	Light yellow
0.33	Light brown	0.19	Light yellow	0.60	Light purple
0.37	Light brown	0.58	Purple	0.67	Light purple
0.40	Light brown	0.66	Purple	0.97	Light purple
0.48	Light brown	0.77	Light green	-	-
0.58	Purple	0.84	Purple	-	-
0.67	Purple	0.94	Purple	-	-
0.76	Purple	0.96	Purple	-	-
0.93	Purple	-	-	-	-



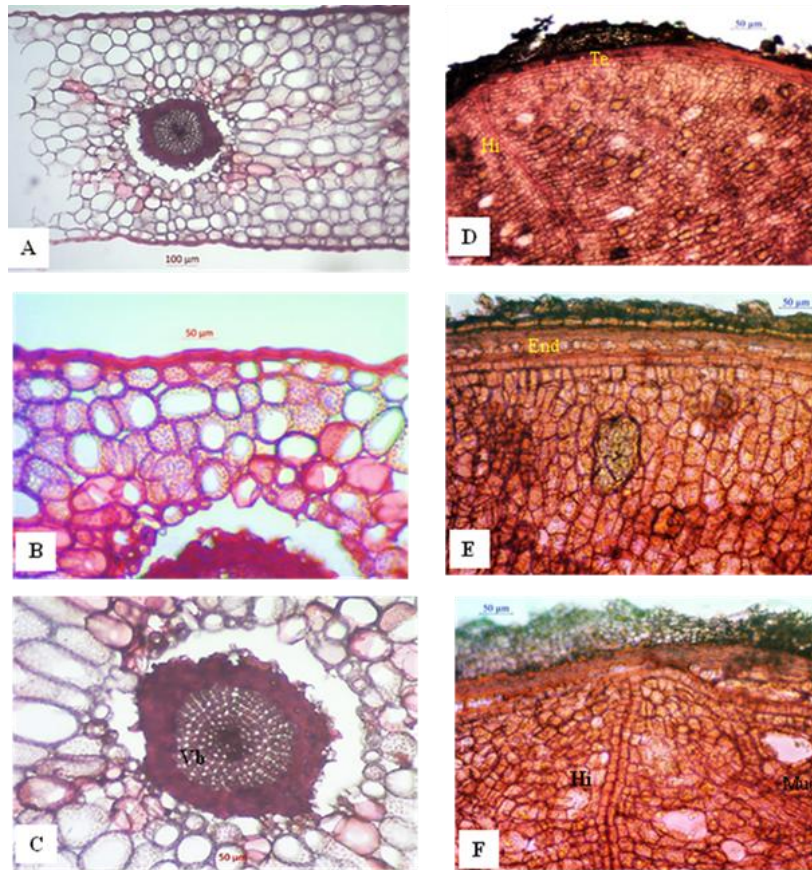
Fig 1: Details of *Swietenia macrophylla* King **A**. Twig with leaves and fruits; **B**. Opened fruit shows seed arrangement; **C**. Bark; **D**. Winged seeds.



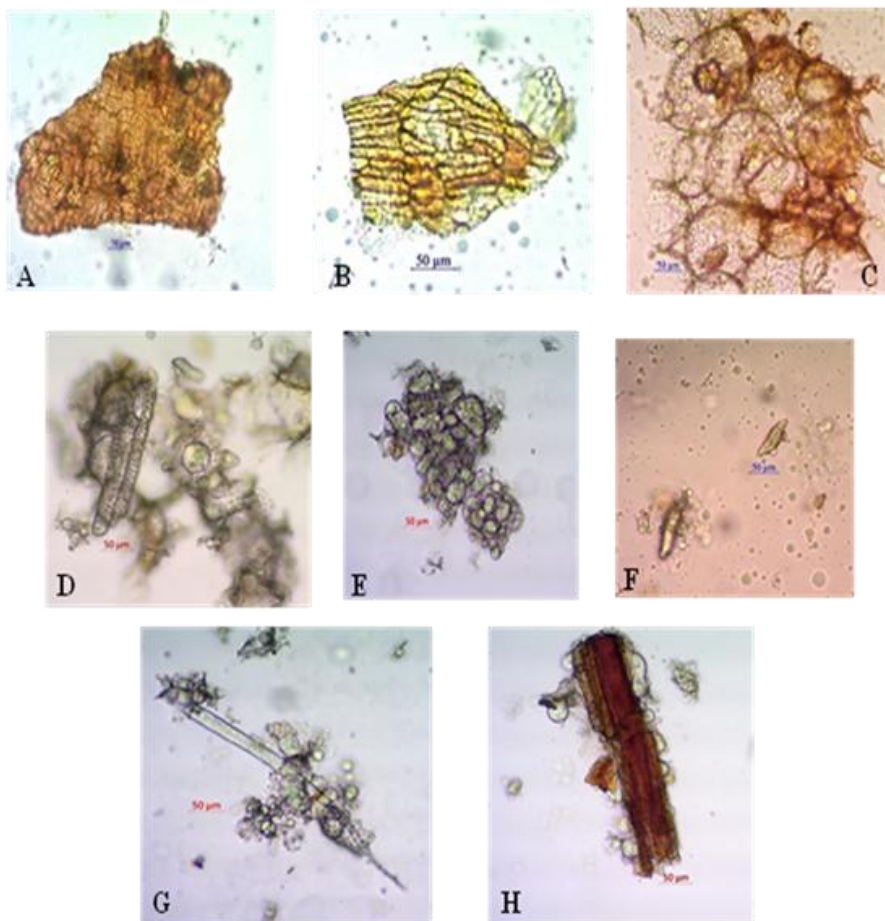
**Fig 2:** A-D. Microscopy of bark of *Swietenia macrophylla* King A. Periderm; B. Outer cortex; C. Inner cortex with starch grains; D. Phloem rays; E-L. Powder characteristics. E. Cork cells; F. Cortex cells; G. Cells with brown contents; H. Crystal fibre; I. Cork cells surface view; J. Fibre bundle; K. Starch grains; L. Prismatic crystals.



**Fig 3:** A-D. Microscopy of leaflet of *Swietenia macrophylla* King A. Midrib; B. Vascular bundle; C. Lamina; D. Palisade layer. E-I. Powder characteristics. E. Epidermal cells with stomata; F. Palisade layer; G. Crystal fibres; H. Spiral vessels; I. Prismatic crystals



**Fig 4:** A-C. Microscopic details of wing of seed of *Swietenia macrophylla* King. D-F. TS of cotyledon. Vb - Vascular bundle; Hi - Hilum; Muc - Mucilage cells; End - Endotesta; Te – Testa.



**Fig 5:** A-H. Powder microscopy of *Swietenia macrophylla* King seed. A. Cells of testa; B. Exotesta; C. Cells of Mesotesta; D. Pitted vessels; E. Oil globules with starch grains; F. Stone cells; G, H. Fibres.

Figure 6. TLC photo-documentation of ethanolic extract of *Swietenia macrophylla*

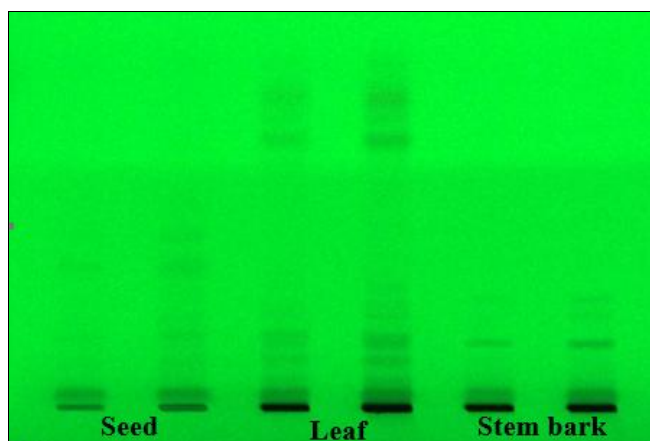


Fig 6.1: Under short UV 254 nm

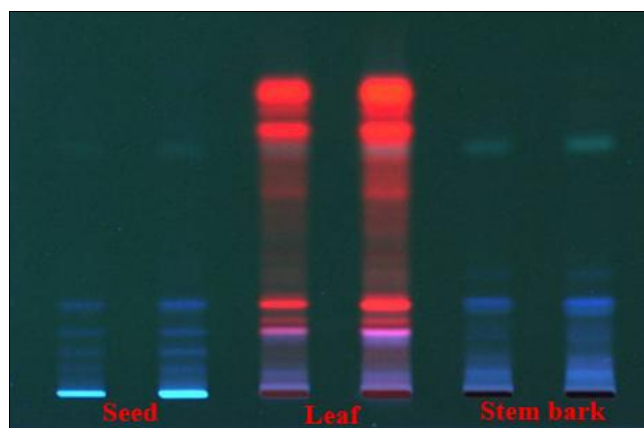


Fig 6.2: Under long UV 366 nm

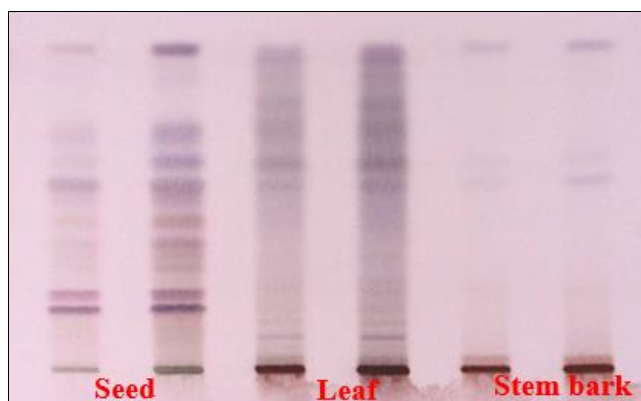


Fig 6.3: Under white light after derivatisation using vanillin-sulphuric acid reagent at 575 nm Toluene: Ethyl acetate: Formic acid 5:2:0.1

Figure 7. HPTLC finger print profile of ethanolic extract of *Swietenia macrophylla* at 254 nm

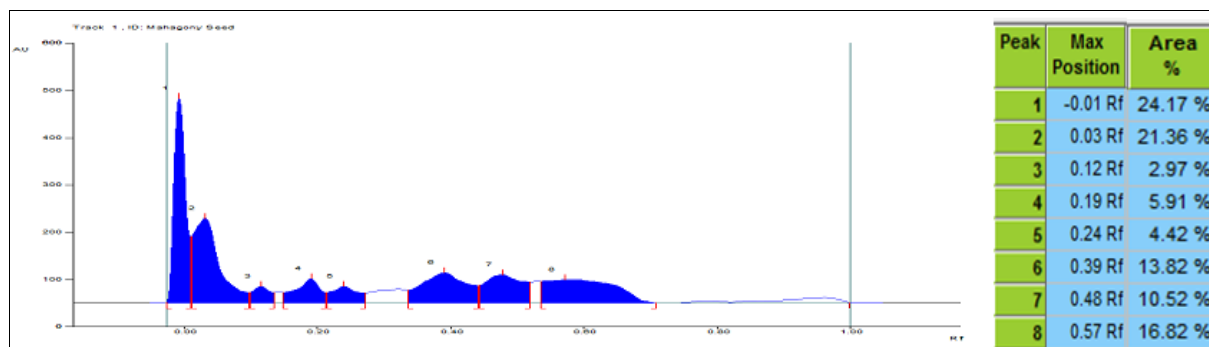


Fig 7.1: Seed

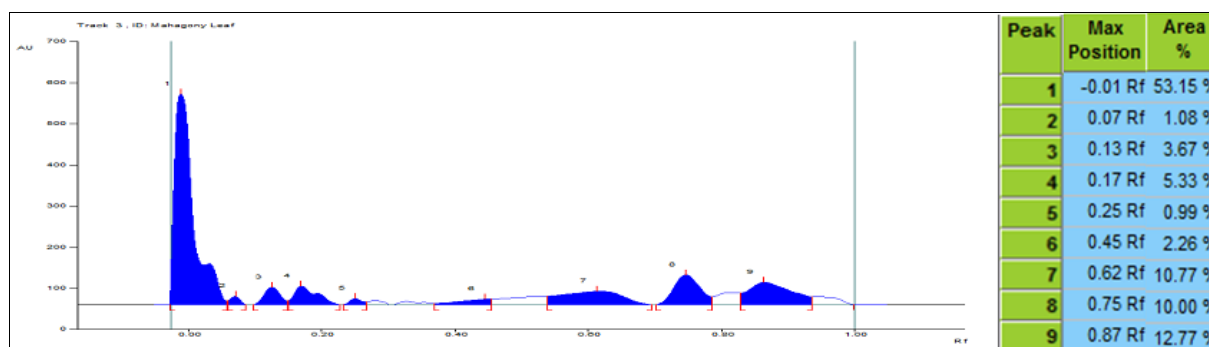


Fig 7.2: Leaf

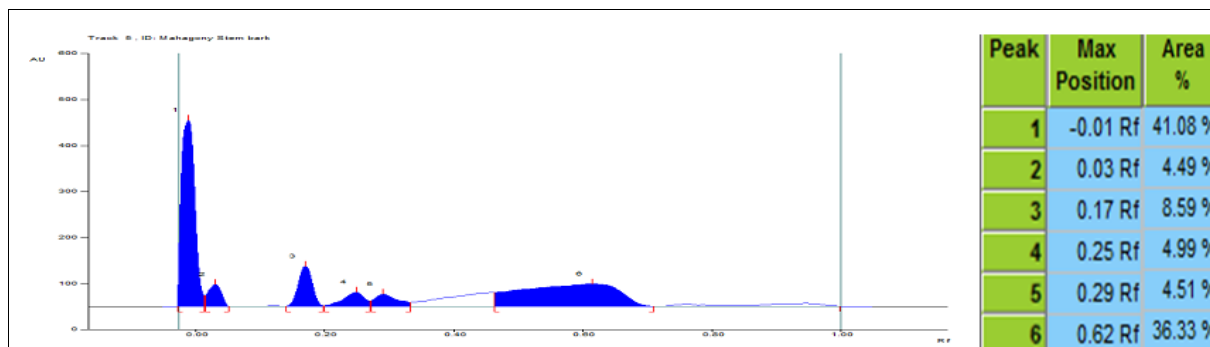


Fig 7.3: Stem bark

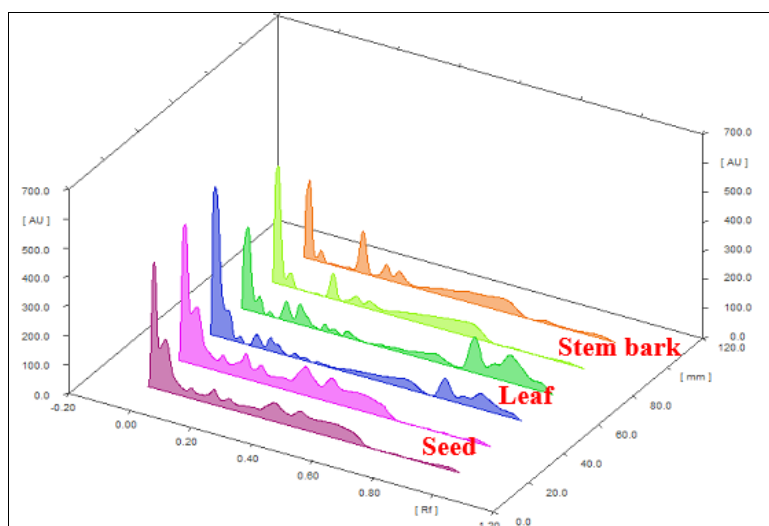


Fig 7.4: 3D of denistograms

Figure 8. HPTLC finger print profile of ethanolic extract of *Swietenia macrophylla* at 366 nm

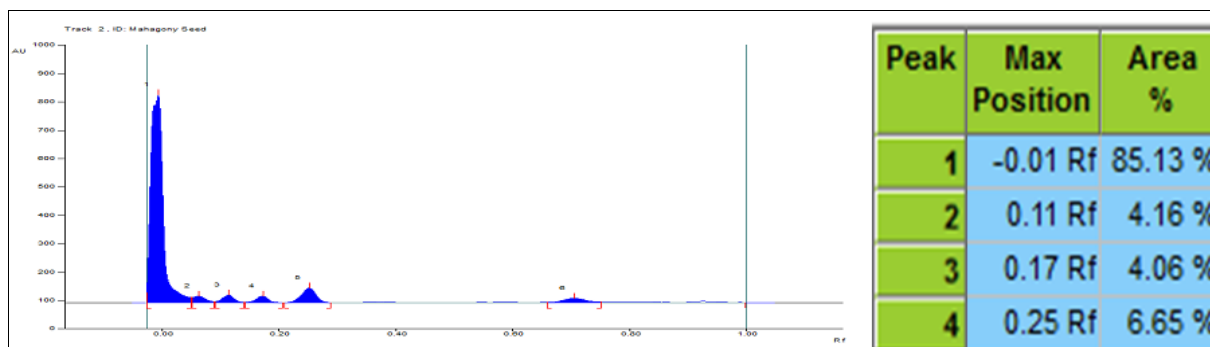


Fig 8.1: Seed

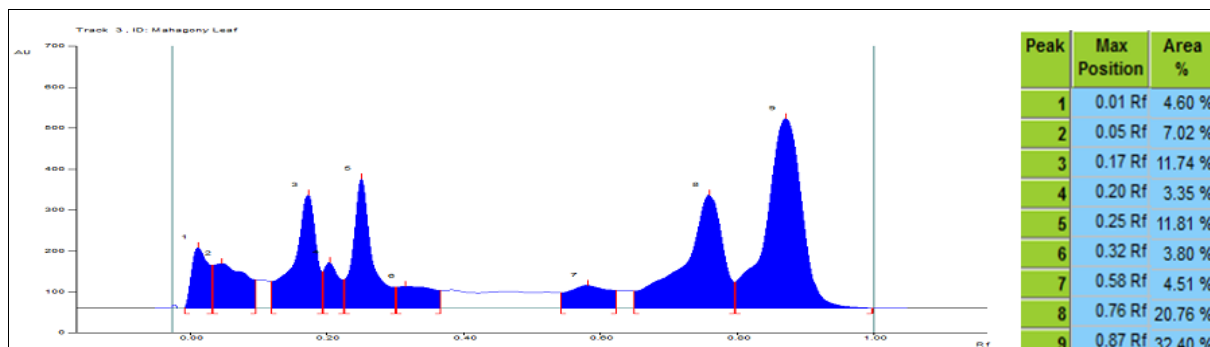


Fig 8.2: Leaf

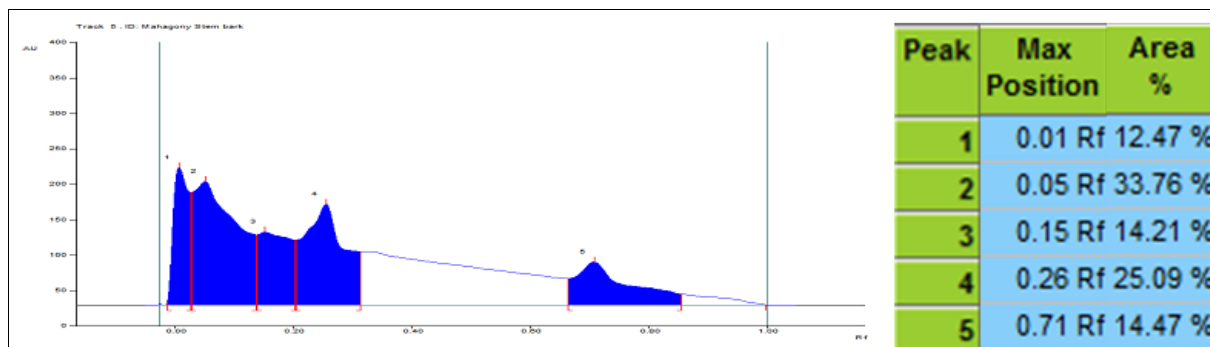


Fig 8.3: Stem bark

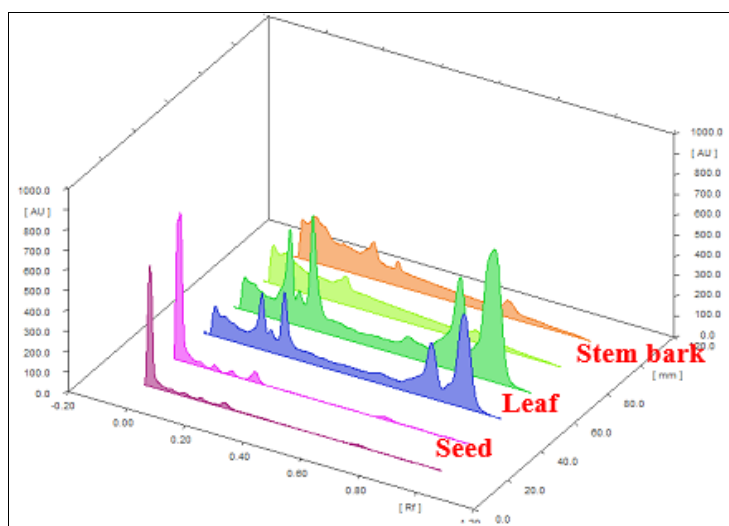


Fig 8.4: 3D of denistograms

Figure 9. HPTLC finger print profile of ethanolic extract of *Swietenia macrophylla* at 575 nm post derivatisation

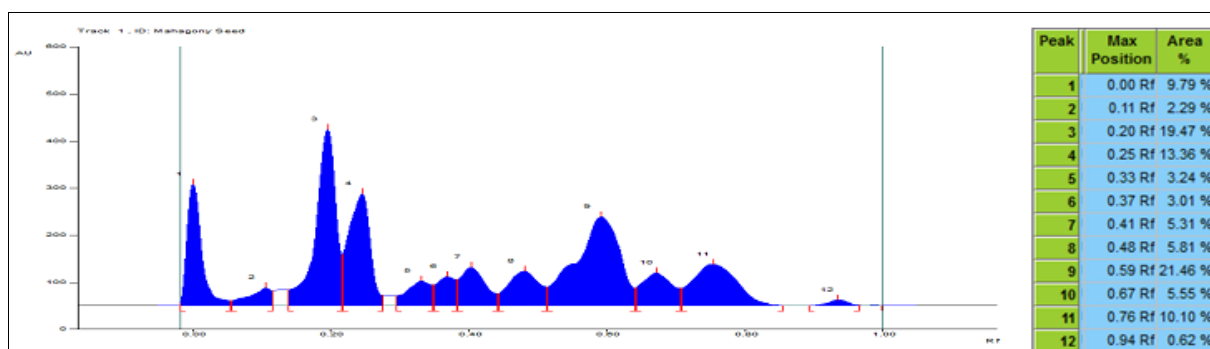


Fig 9.1: Seed

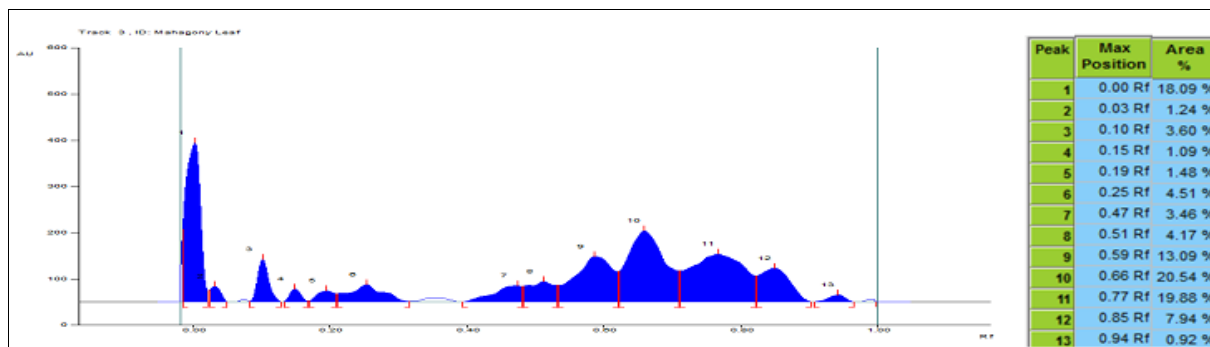


Fig 9.2: Leaf

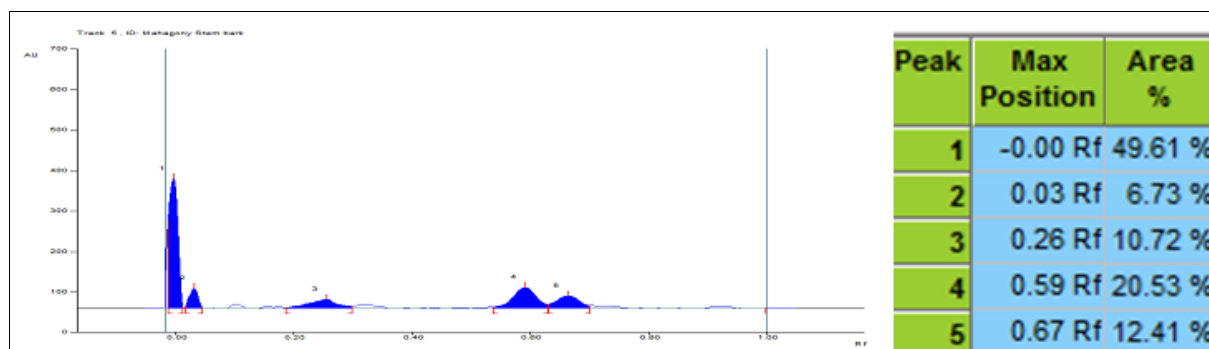


Fig 9.3: Stem bark

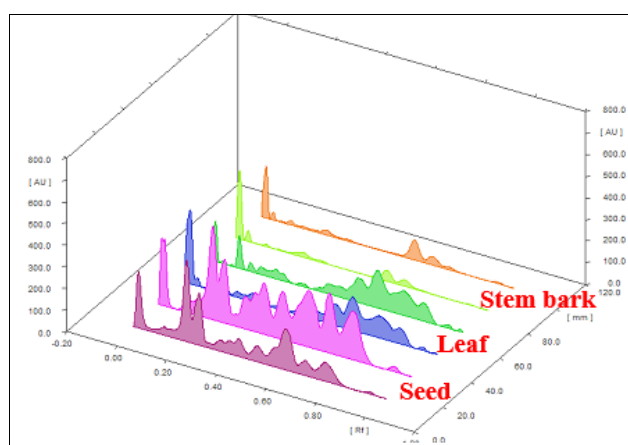


Fig 9.4: 3D of denistograms

#### 4. Discussion

*Swietenia macrophylla* King is well renowned for its timber values, the wood of this tree is considered as good choice for carpentry work. However, *S. mahagoni* (L.) Jacq with its centre of origin in Central America was introduced into the various parts of the World during 18<sup>th</sup> Century. In India, it was introduced by British [3, 10, 14] which is known as “Mahogany”, “Small leaved mahogany” and “Indian Mahogany”. The broad-leaved mahogany is also origin from Central America differs from Indian mahogany by bark and other vegetative characters.

Very few studies have been published on the bark, fruit and leaves characterization of this two species *S. macrophylla* and *S. mahagoni*. The bark of *S. macrophylla* was studied here for the first time with a comprehensive characterization of their structural and anatomical features. The phytochemical, pharmacognostical, timber values, medicinal properties of mahogany were reported by many workers.<sup>3</sup> Like other medicinal plants of Meliaceae, mahogany also having active phytochemicals such as tri-terpenoid, steroids, catechin etc. However, the external morphology of the stem bark is characteristic; the phytochemical and pharmacological activities of stem bark and seeds are more or less comparable with *Azadirachta indica* A. Juss., *Melia azedarach* L. *Toona ciliata* M. Roem. etc., as they show bitter, expectorant, anthelmintic, aphrodisiac, fever and antidiabetic properties [15-18]. Various parts of Mahogany have been used as medicine by inhabitants of different parts of the world. It has been reported that, the barks are sold as an adulterant for Cinchona bark [20]. The fruits and seeds are used in the improvement of blood circulation, diabetes, high blood pressure, hypertension, pain, diarrhoea, leishmaniasis, inflammation, hyperglycaemia etc [9, 10, 21, 22]. Owing to the presence of number of important phytochemicals such as

limonoids, phenolic compounds etc in leaves, seed and bark the medicinal activities, anti-microbial activities of this plants has been studied extensively by various workers worldwide [5, 11, 22, 23]. Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) profile of seed and leaves extract were reported in *S. macrophylla* [24]. High Performance Thin Layer Chromatography (HPTLC) profile was done by other Meliaceae plants like *Azadirachta indica* A. Juss. *Aglaia elaeagnoidea* (A.Juss.) Benth. *Naregamia alata* Wight & Arn. etc [25-27]. The medicinal properties of mahoganies has drawn a great attention of herbal medicine industries in the last few decades, at this scenario, the present work may help to identify the market sample to avoid the usage of adulterants. However, phyto-pharma-pharmacognostical studies are in need to explore the various therapeutic application of this plant which may be useful for mankind in future.

#### 5. Acknowledgements

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