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Pharmacognostic and HPTLC study of Saṁvartikā (Tender leaves) of Kamala (*Nelumbo nucifera* Gaertn.)

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Abstract
Objective: To investigate Macroscopic, Microscopic structures, Powder analysis and HPTLC study of Kamala Saṁvarikā (Tender leaves). Which is a well mentioned plant in all the Ayurvedic classics. Methods: The Pharmacognostic and Hptlc study was done based on the principles mentioned in Pharmacognosy of Kokate. Results: Macroscopic and Microscopic study showed distinct characteristics in the leaves of Kamala like Anomocytic type of stomata, Collenchymatous hypodermis, Vascular bundle covered with bundle sheath etc. Powder analysis revealed the presence of Rosette crystals of Calcium oxalate, few spherical to oval shaped starch grains etc. HPTLC Densitometric scan at 254nm shows 7spots with R_f 0.03 (has maximum area of 36.46%), at 366nm densitometric scan shows 8 spots with Rf 0.55 (which had maximum area 24.56%). Conclusion: Pharmacognostic study of leaves is helpful in sample identification and to ensure quality and purity standards. HPTLC is one of most important instrumental technique for the identification and Quantitative determination of the main Phytochemical constituents present in the leaves of *Nelumbo nucifera* Gaertn.

Keywords: *Nelumbo nucifera*, Pharmacognostic, powder analysis, HPTLC, Saṁvartikā (Tender leaves)

Introduction
Nelumbo nucifera Gaertn. a very small genus of aquatic herbs distributed in Asia, Australia and America. One species occurs in India, it is called as Sacred Lotus, Indian Lotus, and Chinese Water Lily. To this day, the lotus is an *iconic flower* stands as a symbol related not only to Hinduism, but also to numerous other religions, historical and Modern alike. While there are many species of Lotus flowers across Asia, The Hindu's Sacred Lotus is scientifically known as *Nelumbo nucifera* Gaertn. It is a perennial aquatic herb bearing the famous Red Lotus flower. It is found in the fresh water ponds and lakes in India [1]. The genus is derived from the Tamil word 'Nelum', which means Blue and the specific Epithet '*nucifera*' derived from the Latin words 'nux' (nut) and 'fera' (bearing) for nut bearing.

Pharmacognostic Study
Aims and Objectives
To study the Macroscopic, Microscopic study and Powder Analysis of *Nelumbo nucifera* Gaertn. Tender leaves.

Materials and Methods
Drug Collection and Authentication
The fresh leaves of Kamala (*Nelumbo nucifera* Gaertn.) were collected from S.V.University, Tirupati, Andhra Pradesh. Authenticated by Botanist S. Koteswara Rao, Post graduate teacher in Botany, A.P.Model School and Junior College, Madanapalli, Chitoor Dist. Tirupati.

Observation and Results
Pharmacognosy of Tender leaves of *Nelumbo nucifera* Gaertn.

Name of the Sample	: Kamala
Scientific Name	: *Nelumbo nucifera* Gaertn.
Family	: Nymphaeceae
Plant part	: Leaf
Drug description	: Freshly collected Green colour raw leaves, Simple Orbiculate or Peltate in shape.
Powder	: Light green in colour, fine and Aromatic

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Macroscopic Properties

Size	: Leaf blade around 6 to 24 inches depending upon age.
Shape	: Orbiculate or Peltate
Colour	: Dark Green
Odour	: Not specific
Taste	: Not specific

The leaves are Simple, Entire with Smooth margins, Orbiculate or Peltate, Round, Upper surface Smooth and Waxy with water repellant property and exhibit multi costate divergent reticulate venation.

Microscopic Properties

Transverse section of Leaf: Transverse Section of Leaf is done by Free hand Section cutting and simple staining procedure and findings are as mentioned below.

A. Epidermis
▪ Leaf on either side covered with Epidermal layers i.e., Upper Epidermis and Lower Epidermis.
▪ Epidermal Layers composed of a single row of tangentially elongated barrel shaped cells.
▪ Externally upper epidermal layer is covered by a very thin cuticle. Cuticle absent on lower epidermis.
▪ Anomocytic type of stomata are distributed on the upper epidermal layer only (Epistomatous) as it a hydrophytic plant with floating leaves.
▪ Upper Epidermal layer covered by several unicellular minute hairs.

B. Mesophyll
▪ In between upper and Lower epidermal layers mesophyll region is present

▪ Mesophyll is distinguished into Palisade parenchyma and Spongy parenchyma.
▪ Palisade parenchyma cells are arranged in 1 to 3 layers compactly without any intercellular spaces and filled with dense chlorophyll pigment.
▪ Cells of spongy parenchyma are irregular in shape, lossely arranged with big size air spaces in between them, which help the leaves to float over the surface of water.
▪ The cells lining the air spaces are having star shaped astrosclereids that are protruding into the air spaces.
▪ Some of the parenchymatous cells below the palisade parenchyma are having rosette crystals of calcium oxalate. And some other cells are filled with oval to spherical shaped starch grains.
▪ At the region of prominent middle vein both the epidermal layers are followed by several layers of collenchymatous hypodermis.

C. Vascular Strand
▪ In the region of veins epidermal layers are followed by several layers of collenchymatous hypodermis.
▪ Vascular bundle covered with the cells of bundle sheath.
▪ Vascular strands composed of Xylem with prominent small size proto xylem and large size meta xylem elements and closely arranged cells of Phloem.
▪ As the leaf is showing multi costate divergent reticulate venation several vascular bundles of different sizes, surrounded by bundle sheath are dispersed in lamina region here and there.
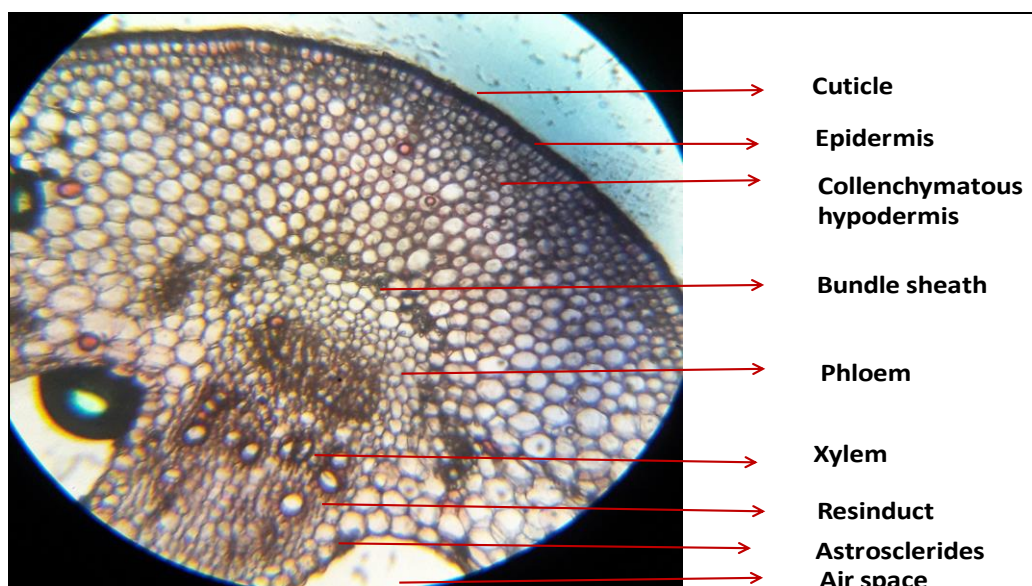▪ Vascular bundles are conjoint, collateral and open type.

Microscopic study of *Nelumbo nucifera* Gaertn

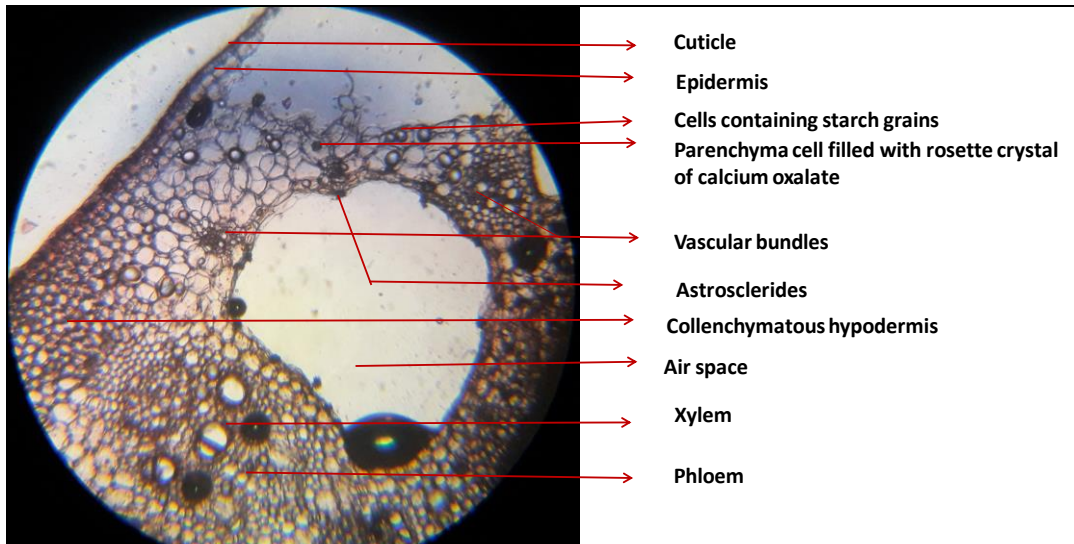**Image 1:** Tranverse Section of Tender Leaf (Midrib region)

Image 2: Transverse Section of Leaf Tender Leaf of Kamala
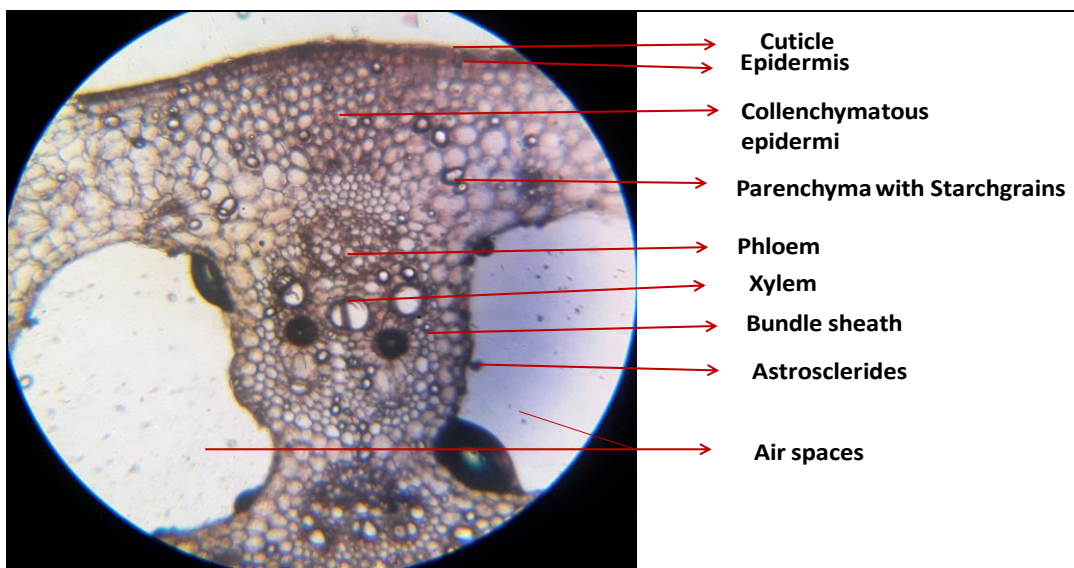

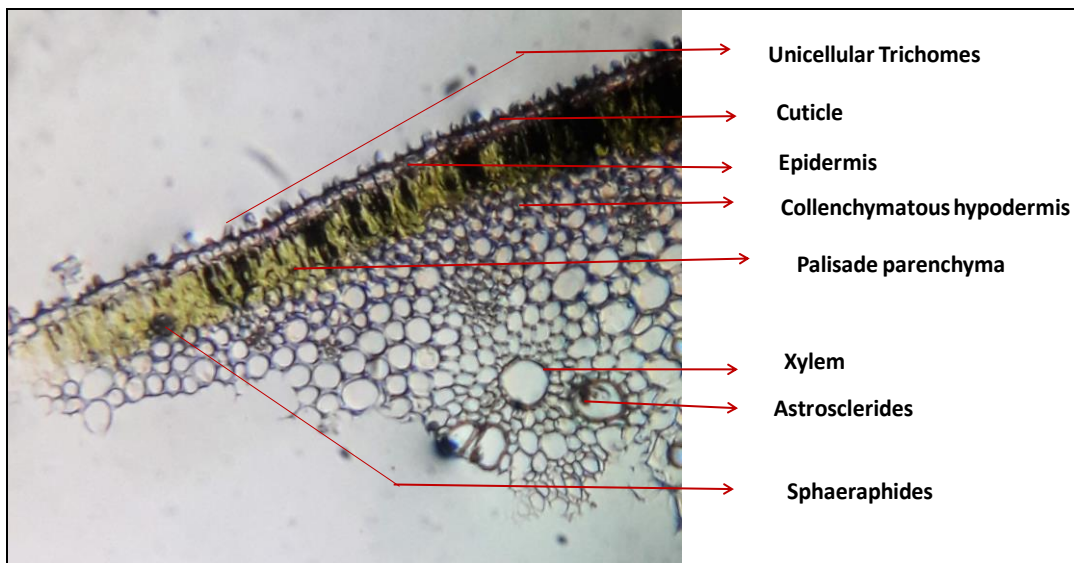**Image 3:** Transverse Section of Tender Leaf

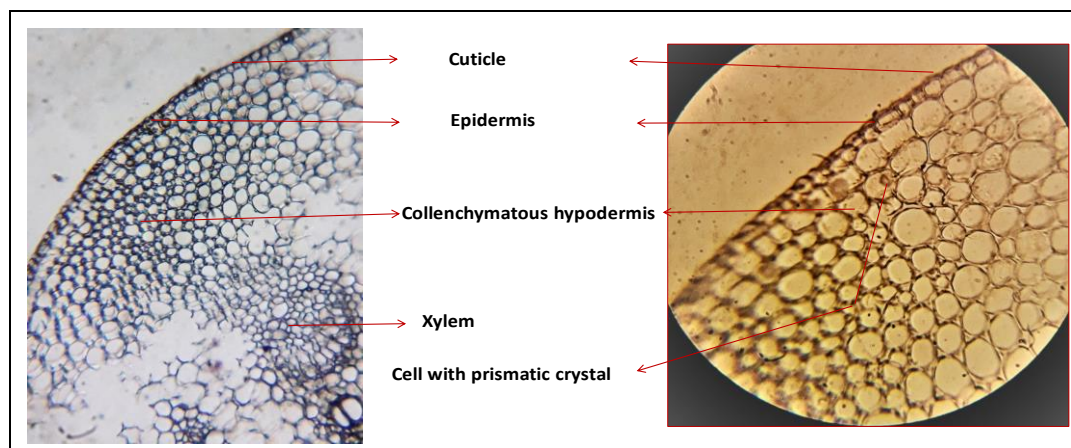**Image 4:** Transverse section of Tender Leaf (Lamina region)

Image 5: Transverse section of Tender Leaf Lamina region

Powder Analysis: Powder Analysis is carried out by clarifying the powder in chloral hydrate solution and prepared Glycerin mount, Iodine solution mount and Saffranin solution mount and the following characters are identified.

(1) Organoleptic properties [2]:
Colour   : Brownish dark green
Odour   : Not characteristic
Taste   : Not specific, slightly astringent
Texture : Fine Powder

(2) Microscopic Characters
- Fragments of wavy thin walled epidermal cells in surface view.
- **Numerous rosette crystals of Calcium oxalate**
- **Few simple spherical to oval shape starch grains**
- Fragments of Xylem elements
- Bundles of lignified xylem fibers
- Fragments of epidermal hairs.

Microscopic Study of Powder of *Nelumbo nucifera* Gaertn.
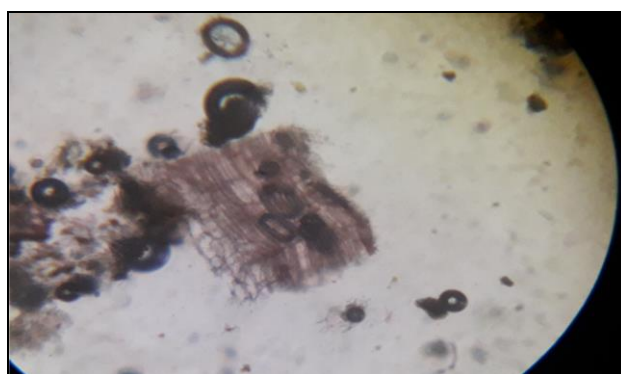
Image 6: Xylem components and fibers

Image 7: Epidermis and Trichome
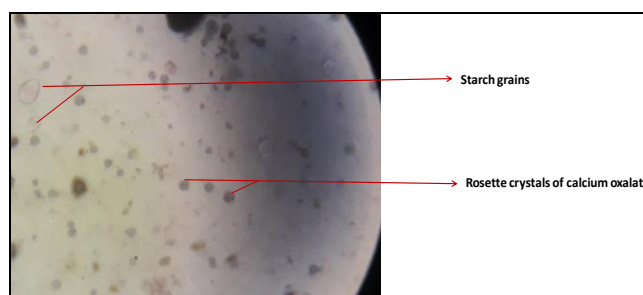
Image 8: Calcium oxalate and Strach grains
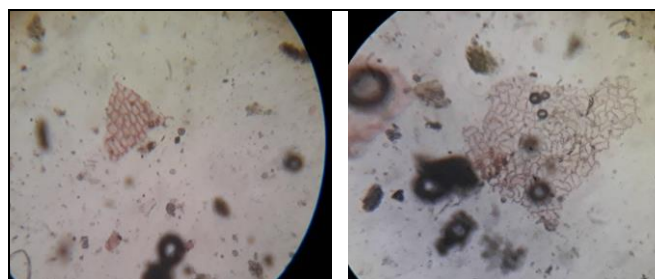
Image 9: Fragments of epidermis
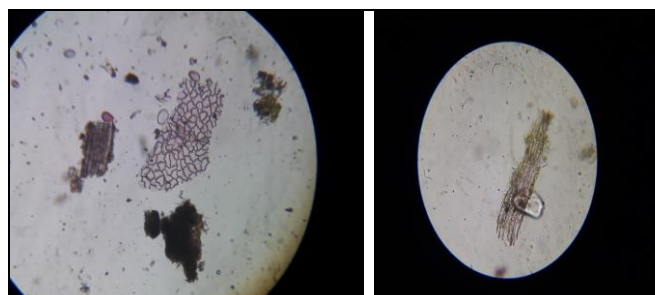
Image 10: Epidermis and Fibres

High Performance Thin Layer Chromatography
Aim: To study the identification and Quantitative determination of Phytochemical constituents of Tender leaves of Kamala

The HPTLC study of the powdered drug of *Kamala Saṁvartikā Cūrṇa* was executed at S.D.M Centre for Research in Ayurveda and allied sciences, UDIPI (Karnataka).

Particulars of sample submitted: *Kamala Saṁvartika Patra cūrṇa* for the given sample of Kamala patra *(Nelumbo nucifera)*, HPTLC was performed photodocumentation, R_f

values and densitograms are presented in respective tables and figures.

Methodology
HPTLC Analysis of *Nelumbo nucifera* Gaertn. Tender Leaves Sample

A. Sample Details
1g of Kamala Saṁvartikā patra Cūrṇa

B. Test Solution
1g of Kamala Saṁvartikā Patra Cūrṇa (*Nelumbo nucifera* Gaertn.) was suspended in about10.0ml of 99.9% Ethanol [3].

C. Stationary Phase
Precoated silica gel F_{254} on aluminum plates to a band width of 7mm using Linomat 5 TLC applicator.

D. Mobile Phase
The plate was developed in Toluene: Ethyl acetae (9.0:1.0).

E. Development
The developed plates were visualized under short UV 254nm, long UV 366nm

F. HPTLC Instrumentation [4]
Densitometric sacn

G. Derivatization
Derivatised with vanillin sulphuric acid

Results

Table 2: Rf values of sample of *Nelumbo nucifera* Gaertn.

Short UV	Long UV	After derivatisation
-	0.22(F.red)	-
-	-	0.28(Purple)
-	0.39(F.red)	-
0.46(Green)	0.46(F.red)	0.46(Purple)

* F- Fluroscent

Interpretation of Sample *Kamala patra Cūrṇam*
At short UV (254nm) there were one bands observed with Rf of 0.46 intensities of (Green), at long UV (366nm) 3 spots were observed with Rf values of 0.22,0.39, 0.46, (with different fluorescent intensities of Red). After derivatisation with vanillin sulphuric acid there were 2 spots observed (with various color intensities of purple) with Rf values of 0.28, 0.46. Densitometric scan at 254nm shows 7spots with Rf 0.03 (has maximum area of 36.46%), at 366nm densitometric scan shows 8 spots with Rf 0.55(which had maximum area 24.56%), was more prominent.

Results

Short UV	Long UV	After derivatisation
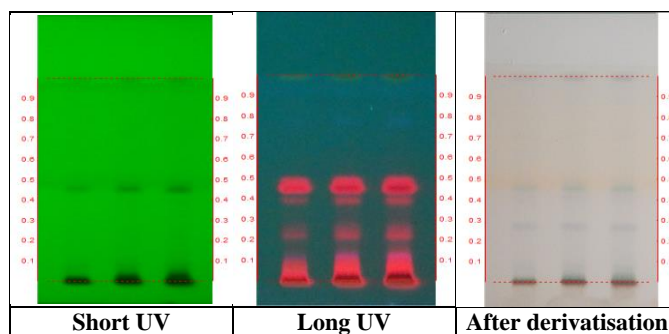
Track 1: Kamala patra *(Nelumbo nucifera)*- 3µl
Track 2: Kamala patra *(Nelumbo nucifera)*- 6µl
Track 3: Kamala patra *(Nelumbo nucifera)*- 9µl

Fig 1: HPTLC Photodocumentation of sample of Kamala Saṁvartikā patra

Solvent system-Toluene: Ethyl acetate (9.0: 1.0)

Table 3: Rf values of sample

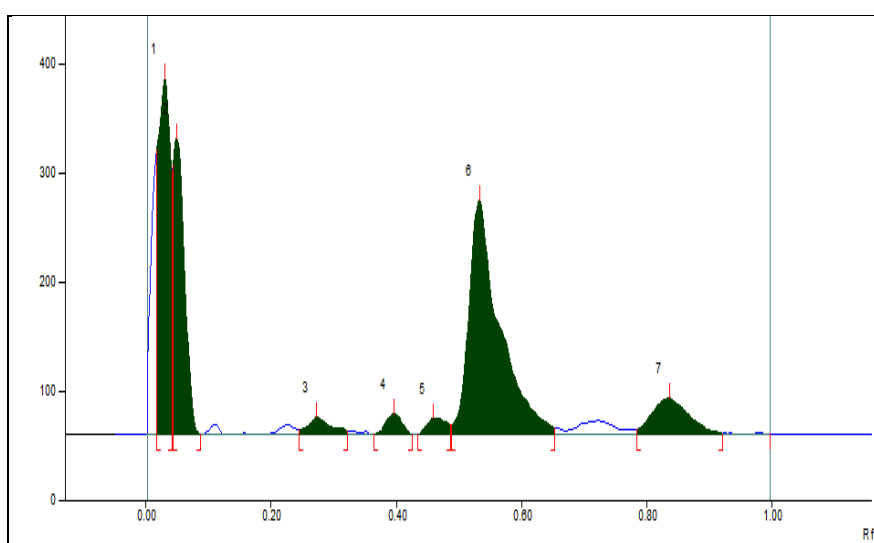| Short UV	Long UV	After derivatisation
-	0.22 (F. red)	-
-	-	0.28 (Purple)
-	0.39 (F. red)	-
0.46 (Green)	0.46 (F. red)	0.46 (Purple)

*F - fluuorescent

Track 11, ID: Kamala patra

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	259.9 AU	0.03 Rf	325.9 AU	36.46 %	0.04 Rf	40.5 AU	4602.1 AU	25.28 %
2	0.04 Rf	245.9 AU	0.05 Rf	270.4 AU	30.25 %	0.09 Rf	0.2 AU	3529.5 AU	19.39 %
3	0.25 Rf	3.8 AU	0.27 Rf	15.9 AU	1.78 %	0.32 Rf	3.4 AU	406.7 AU	2.23 %
4	0.36 Rf	0.1 AU	0.40 Rf	19.2 AU	2.15 %	0.43 Rf	0.1 AU	349.0 AU	1.92 %
5	0.44 Rf	0.2 AU	0.46 Rf	14.9 AU	1.67 %	0.49 Rf	8.7 AU	331.5 AU	1.82 %
6	0.49 Rf	8.8 AU	0.53 Rf	214.3 AU	23.98 %	0.65 Rf	6.0 AU	7588.6 AU	41.69 %
7	0.79 Rf	4.8 AU	0.84 Rf	33.2 AU	3.72 %	0.92 Rf	0.7 AU	1393.4 AU	7.66 %

Fig 2a: At 254nm

Track 11, ID: Kamala patra

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.8 AU	0.02 Rf	114.6 AU	3.78 %	0.02 Rf	84.6 AU	545.9 AU	0.61 %
2	0.03 Rf	32.8 AU	0.04 Rf	374.9 AU	12.37 %	0.05 Rf	12.9 AU	2523.6 AU	2.82 %
3	0.05 Rf	6.9 AU	0.10 Rf	671.6 AU	22.16 %	0.22 Rf	27.2 AU	33929.5 AU	37.86 %
4	0.22 Rf	27.4 AU	0.27 Rf	323.3 AU	10.67 %	0.37 Rf	4.7 AU	12008.6 AU	13.40 %
5	0.40 Rf	4.9 AU	0.46 Rf	101.2 AU	3.34 %	0.48 Rf	77.9 AU	2579.9 AU	2.88 %
6	0.49 Rf	79.0 AU	0.52 Rf	686.8 AU	22.66 %	0.53 Rf	97.8 AU	13240.1 AU	14.77 %
7	0.53 Rf	600.9 AU	0.55 Rf	744.3 AU	24.56 %	0.64 Rf	0.2 AU	24265.6 AU	27.07 %
8	0.81 Rf	0.4 AU	0.90 Rf	14.1 AU	0.47 %	0.97 Rf	0.9 AU	536.5 AU	0.60 %

Fig 2b: At 366nm

Graph 1: Densitometric scan of the sample of Kamala Saṁvartikā patra

Table 4: R$_f$ Value and % area of *Nelumbo nucifera* Gaertn. Tender leaves Sample at 254nm

Peak No.	R$_f$ Value	Area (AU)	% Area (AU)
1	0.04	4602.1	25.28
2	0.09	3529.5	19.39
3	0.32	406.7	2.23
4	0.43	349.0	1.92
5	0.49	331.5	1.82
6	0.65	7588.6	41.69
7	0.92	1393.4	7.66

Total No. of Peaks - 7
Total Area – 18200.8

Table 5: R$_f$ Value and % area of *Nelumbo nucifera* Gaertn. Tender leaves Sample at 366 nm

Peak No.	R$_f$ Value	Area (AU)	% Area (AU)
1	0.02	545.9	0.61
2	0.05	2523.6	2.82
3	0.2	33929.5	37.88
4	0.37	12008.6	13.40
5	0.48	2579.9	2.88
6	0.53	13240.1	14.77
7	0.64	24265.6	27.07
8	0.97	536.5	0.60

Total No. of Peaks - 8
Total Area – 89629.7

Discussion

Pharmacognostic study of Kamala Saṁvartikā showed following description Macroscopic study reveals leaves as Simple, Entire with Smooth margins, Orbiculate or Peltate, Round, Upper surface Smooth and Waxy with Water repellant property and exhibit multi costate divergent reticulate venation. Microscopic study shows Cuticle, Epidermis, Mesophyll, Vascular strands, Collenchymatous hypodermis, cells with Prismatic Crystals, Astrosclereids, Starch Grains, Rosette crystals of Calcium oxalate cells, Sphaerphides, Palisade parenchyma, Xylem, Phloem, Vascular bundles, Air spaces and Collenchymatous Epidermis. Anomocytic type of stomata are distributed on the upper epidermal layer only (Epistomatous) as it a hydrophytic plant with floating leaves. Upper Epidermal layer covered by several Unicellular minute hairs. Cells of spongy parenchyma are irregular in shape, lossely arranged with big size air spaces in between them, which help the leaves to float over the surface of water. The cells lining the air spaces are having star shaped Astrosclereids that are protruding into the air spaces. Some of the parenchymatous cells below the palisade parenchyma are having Rosette crystals of calcium oxalate. And some other cells are filled with oval to spherical shaped starch grains. Vascular bundle covered with the cells of bundle sheath. Vascular strands composed of Xylem with prominent small

size proto xylem and large size meta xylem elements and closely arranged cells of Phloem.

Powder Analysis of Kamala Saṁvartikā Patra Cūrṇa shows the presence of Fragments of wavy thin walled epidermal cells in surface view, Numerous rosette crystals of Calcium oxalate, Few simple spherical to oval shape starch grains, Fragments of Xylem elements, Bundles of lignified xylem fibers, Fragments of epidermal hairs.

HPTLC studies of Kamal Saṁvartikā Patra Cūrṇa shows the following waves At short UV (254nm) there were one bands observed with Rf of 0.46 intensities of (Green), at long UV (366nm) 3 spots were observed with Rf values of 0.22,0.39, 0.46, (with different fluorescent intensities of Red). After derivatisation with vanillin sulphuric acid there were 2 spots observed (with various color intensities of purple) with R_f values of 0.28, 0.46.

Densitometric scan at 254nm shows 7spots with R_f 0.03 (has maximum area of 36.46%), at 366nm densitometric scan shows 8 spots with R_f 0.55(which had maximum area 24.56%) was more prominent.

As per the studies the major pharmacological active components of Lotus Tender leaves are Aporphine alkaloids such as Nuciferine, 2-hydroxy -1- methoxyaporphine, Pronuciferine and Roemerine. These alkaloids compounds have been reported to show biological activities including Antioxidant, Anti HIV, Antimicrobial, Antihyperlipidemic, Antiobesity, Antidiabetic. (Development and Validation of HPTLC method for Quantitative Estimation of Nuciferine from Lotus leaves.) [5].

Conclusion
The Pharmacognostic study provided the data helpful for the correct identification and authentication of raw drug. The HPTLC showed the presence of Alkaloid Nuciferin, in large amount an important Chemical composition present in *Nelumbo nucifera* leaves useful for treating different ailments. The number of peaks is proportionl to the amount of active principles. There were 7peaks at 254nm and 8 peaks at 366nm in the sample. The area of the peak indicates the concentration of active ingredients.

Acknowledgement

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