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Research Article

PHARMACOGNOSTICAL AND ANALYTICAL STUDY OF TRIPARNIKAA (*Naregamia alata* Wight. & Arn.)

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Abstract

Triparnikaa (*Naregamia alata* Wight. & Arn.) is a small under shrub growing in rainy season. Flowers are seen in May andfruits are in October season. It grows throughout South India in all districts up to 900m, Western ghats and Konkan. It is widely used for wound healing as a folklore medicine. In Ayurvedic literatures the drug Triparnika ais mentioned in Raja Nighantu, moolakadi varga. Hence in this article a preliminary studies on Triparnikaa (*Naregamia alata* Wight. & Arn.) was carried out and results are documented which leads a base for further studies. The results showed the drug possess Tikta pradhana rasa and Kashaya Anurasa. In the present study whole plant extract was taken for the preliminary phytochemical analysis. It showed the presence of chemical constituents like Alkaloids, Carbohydrate, Carboxylic acid, Resin, Tannin and Flavonoid. HPTLC photo documentation, R_f values and densitometric scan at 254 nm, 366 nm and after derivatisation has been developed.

Key words: Triparnikaa; *Naregamia alata*; Phytochemical analysis; HPTLC.

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INTRODUCTION

Triparnikaa (*Naregamia alata* Wight. & Arn.) is a plant widely used as a folklore medicine. The whole plant is having wound healing property. It has been mentioned in Raja Nighantu, moolakadi varga,[1] Flora of Udupi and Indian Medicinal Plants etc. The *Naregamia alata* Wight. & Arn. is one among such folk medicine, which grows abundantly throughout in Southern India and used for the treatment of diseases like Kasa, Swaasa, Visha and Vrana hara. It is also known by the local names such as Nelakanchi, Nelagulabi, Nelanaariga etc. This shrub is not of classical origin; description about the plants has not been found in the Vedas and Laghutrayees. But references regarding this shrub are available in Raaja Nighantu and books of Modern era like Indian Materia Medica, Indian Medicinal Plants, and Recent Floras. It is useful in wounds, ulcers, vitiated condition of pitta and vata, cough, asthma, bronchitis, splenomegaly, scabies, pruritus, dysentery, dyspepsia, catarrh, anaemia and malarial fevers. In the present study an attempt has been made to study the pharmacognostical and analytical study of the drug, which forms a base for various studies in future.

MATERIAL AND METHODS

Collection and Identification of drug

Botanically identified authentic sample of Triparnikaa (*Naregamia alata* Wight. & Arn.) were collected locally from Manipal, under the authentication of Mr. Gopalakrishna Bhat, (Retd.) Professor, Dept. of Botany, Poornaprajna College, Udupi.

Pharmacognostical study

Macroscopic study

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.

Microscopic study

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Phytochemical study

Organoleptic study; Physico-chemical parameters such as (i) Total ash, (ii) Acid insoluble ash, (iii) Extractive values (Both alcohol and water); Qualitative tests for primary and secondary phyto constituents and HPTLC were carried out.

a) Organoleptic study

Determination of Taste: Taste with Tongue method

AcharyaCharaka says “Rasonipatadravyanam” felt when the substance comes in contact with Rasanendriya (Tongue) “Taste with Tongue” is the criteria for determining the Rasa or Anurasa of a drug. The taste determined by voluntary trial method is as follows. (Dr. S.C in Rasapanchaka)

30 healthy volunteers who were studying Ayurveda were selected. They were asked to wash and clean their mouth. After five minutes 5g of powder made into paste with water was served these volunteers and asked to taste the sample and to record the Rasa and Anurasa

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they feel. The method followed was blind method in which volunteers were not told about identity of drug.

OBSERVATION

Opinion of 30 volunteers was recorded for assesment of Rasa. On observation 29 volunteer's recorded Tiktapradhana rasa. 26 volunteer's recorded Kashaya as Anurasa and 3 volunteers opinioned that Madhura as Anurasa.

b) Physico-chemical parameters as:

Loss on drying at 105°C

10 g of sample was placed in tarred evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccators. Percentage of moisture was calculated with reference to weight of the sample.

(i) Total ash: 2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

(ii) Acid insoluble ash: To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

(iii) Water soluble ash: Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash with reference to the air-dried sample.

(iv) Extractive values (Both alcohol and water)

Alcohol soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccators for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask.

Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

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c) Qualitative tests for primary and secondary phyto constituents

Preliminary phytochemical tests

Tests for alkaloids

Dragendroff's test: To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

Wagners's test: To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.

Mayer's test: To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

Hager's test: To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

Tests for carbohydrates

Molisch's test: To the extract, 1 ml of α-naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Fehling's test: A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's test: To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

Test for steroids

Libermann-Burchard test: To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

Salkowski test: The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for saponins: To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

Test for tannins: To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

Test for flavonoids: Shinoda's test: To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

Test for phenol: To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for coumarins: To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

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Test for triterpenoids: The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for carboxylic acid: Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Test for resin: Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of turbidity.

Test for quinine: A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinine

d) HPTLC

1g of *Naregamia alata* Wight. & Arn. bark powder was extracted with 10 ml of alcohol. 4, 8 and 12µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 2.0). The developed plates were visualized in UV 254, 366, and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. Rf, colour of the spots and densitometry scan were recorded.

OBSERVATION AND RESULTS

Macroscopic study

The Macroscopical study reveals that leaves were alternate trifoliate with winged petiole, the leaflets were small, cuneate-obovate. The flowers were pentamerous, with 5 lobed sepals and 5 free, white petals. Stamens were 10, fused into a column with free aather which were appendage. Ovary was superior, three celled with two ovules in each cell. Fruit was loculicidal capsule, seeds were curved and truncate at both ends. (Figure 1and Figure 2)

Microscopic study

T.S of root showed cork; cortex; fibres; medullary rays; parenchyma; inner phloem; pith; starch grains; vessels; xylem; xylem fibres; xylem rays.

T.S of stem showed cork; cortex; fibres; parenchyma; inner phloem; pith; vessels; xylem; xylem fibres; xylem rays. T.S of leaf showed upper epidermis; lamina; lower epidermis; mesophyll; midrib; palisade; pericyclic fibres; phloem; pith; rosette crystal; spongy parenchyma; vascular bundle; xylem. (Figure 3 to Figure 9)

a) Organoleptic study

Determination of Taste: Taste with Tongue method

From the above experiment, the result shows that Rasa of *Naregamia alata* Wight. & Arn. is Tiktapradhana and Kashaya Anurasa. (Table 1) As per general rule the guna of *Triparnikaa* (*Naregamia alata* Wight. & Arn.) Laghu and Ruksha.

Acharya Charaka states that the gunas Laghu, Ruksha are the qualities of Tikta and Kashaya Rasa. As per Acharya Triparnikaa (*Naregamia alata* Wight. & Arn.) is having sheeta veerya. Vipaka of Tikta and Kashaya Rasa should be Katuvipaka.

b) Physico-chemical parameters as: The Physico-chemical parameters are shown in Table 2.

c) Qualitative tests for primary and secondary phyto constituents: Results of preliminary phytochemical screening of *Naregamia alata*are shown in Table 3.

d) HPTLC: The R_f values of samples (*Naregamia alata*) are shown in Table 4. (Figure 10 and Figure 11)

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Figure 1: Macroscopy of *Naregamia alata*


Figure 2: Fruit of *Naregamia alata*


Table 1: Taste determination Pradhana Rasa and Anurasa of Triparnikaa (*Naregamia alata*)

Rasa	Pradhana	Anurasa
Tikta	29	1
Kashaya	1	26
Madhura	-	3

Table 2: Results of standardization parameters of stem of *Naregamia alata*

Parameter	Results n = 3 %w/w
Loss on drying	11.36
Total Ash	6.46
Acid Insoluble Ash	2.39
Water soluble Ash	0.0
Alcohol soluble extractive value	4.60
Water soluble extractive value	16.38

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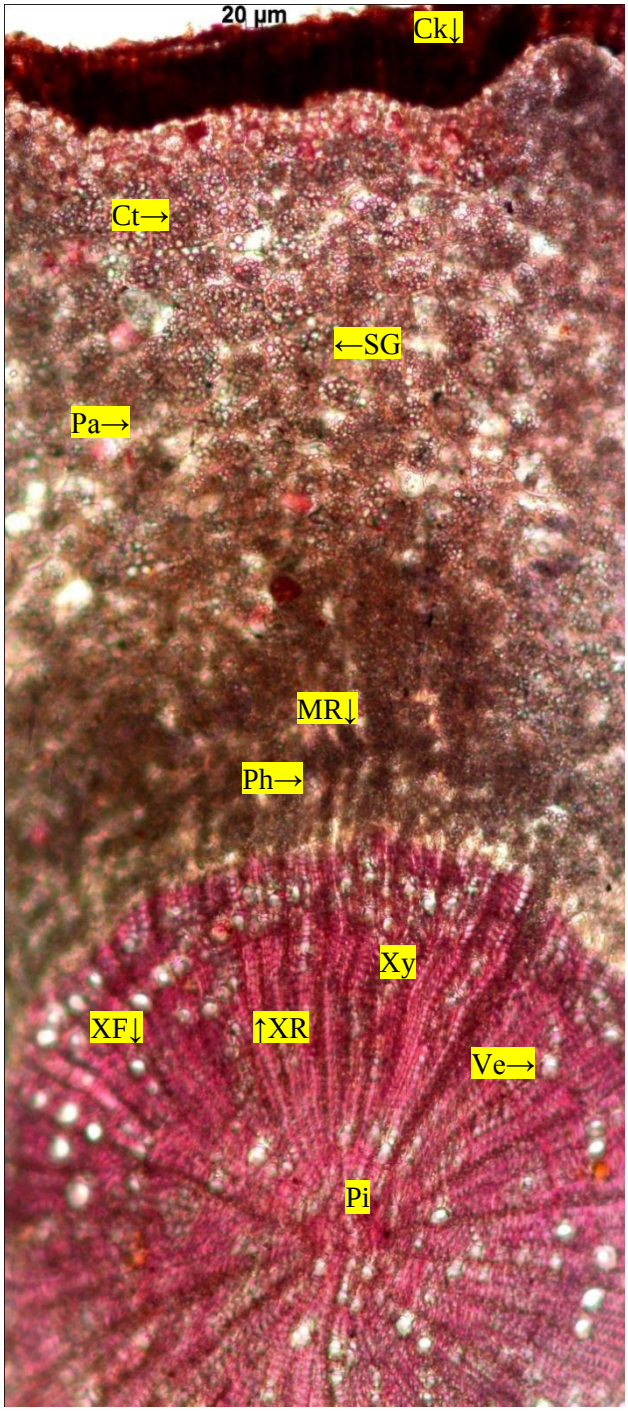
Figure 3: Microscopy (TS) of Root of *Naregamia alata* Wight. and Arn.



Ck – cork; Ct – cortex; F – fibres; MR – medullary rays;Pa – parenchyma; Ph – inner phloem; Pi – pith; SG – starch grains; Ve – vessels; Xy –xylem; XF– xylem fibres; XR – xylem rays

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Figure 4: Microscopy (TS) of Root of *Naregamia alata* Wight. and Arn.

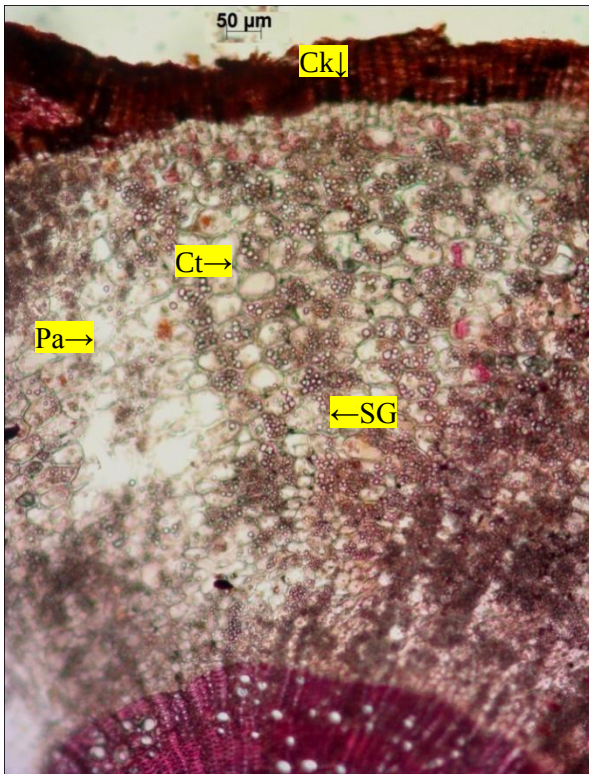

Figure 4a. Cork and cortex

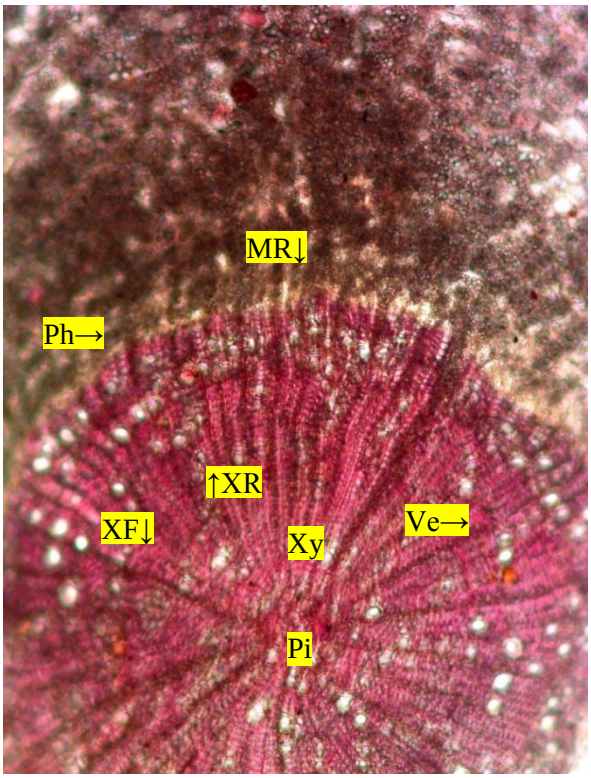

Figure 4b. Phloem and pith

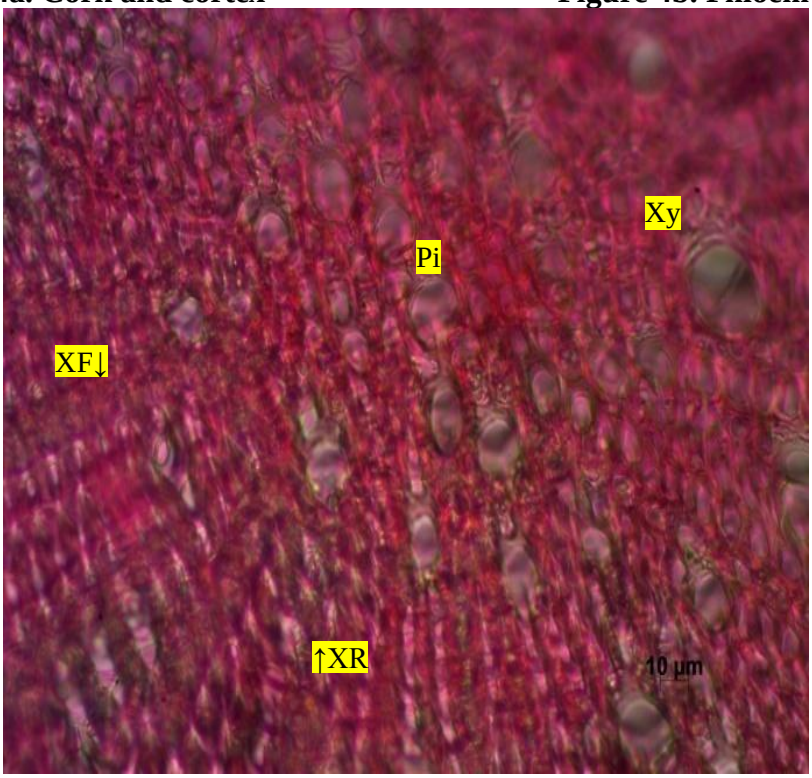

Figure 4c. Pith

Ck – cork; Ct – cortex; F – fibres; MR – medullary rays;Pa – parenchyma; Ph – inner phloem; Pi – pith; SG – starch grains; Ve – vessels; Xy –xylem; XF– xylem fibres; XR – xylem rays

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Table 3: Results of preliminary phytochemical screening of *Naregamia alata*

Tests		Colour if positive	Alcoholic extract	Inference
Alkaloids	Dragendroff's test	Orange red precipitate	Orange red precipitate	+
	Wagners test	Reddish brown precipitate	Reddish brown precipitate	
	Mayers test	Dull white precipitate	Dull white precipitate	
	Hagers test	Yellow precipitate	Yellow precipitate	
	Liebermann- buchard test	Bluish green colour	No bluish green colour	
Steroids	Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	No bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	-
	Liebermann- buchard test	Bluish green colour	No bluish green colour	
Carbohydrate	Molish test	Violet ring	Violet ring	+
	Fehlings test	Brick red precipitate	Brick red precipitate	
	Benedicts test	Red precipitate	Red precipitate	
Tannin	With $FeCl_3$	Dark blue or green or brown	Green color	+
Flavanoids	Shinoda's test	Red or pink	Red color	+
Saponins	With $NaHCO_3$	Stable froth	No stable froth	-
Triterpenoids	Tin and thionyl chloride test	Pink	Green color	-
Coumarins	With 2 N NaOH	Yellow	Green color	-
Phenols	With alcoholic ferric chloride	Blue to blue black	Green color	-
Carboxylic acid	With water and $NaHCO_3$	Brisk effervescence	Brisk effervescence	+
Amino acid	With ninhydrine reagent	Purple colour	Green color	-
Resin	With aqueous acetone	Turbidity	Turbidity	+
Quinone	Sulphuric acid	Pink/purple/red	Green color	-

(+): present; (-): negative

Table 4: R_f values of samples

Short UV	Long UV	Under white light (after derivatisation)
-	-	0.06 (D. purple)
-	0.11 (F. red)	0.11 (D. purple)
-	-	0.16 (D. purple)
-	0.19 (F. purple)	-
-	-	0.23 (D. purple)
-	0.25 (F. red)	0.25 (D. purple)
-	-	0.31 (D. purple)
-	-	0.38 (D. purple)
-	-	0.43 (D. purple)
0.45 (L. green)	-	-
-	0.47 (F. red)	0.47 (D. purple)
0.51 (L. green)	-	-
-	-	0.54 (D. purple)
-	-	0.63 (D. purple)
-	0.65 (F. red)	-
0.68 (D. green)	0.68 (F. purple)	-
0.72 (D. green)	0.72 (F. red)	0.72 (D. purple)
-	0.76 (FD. red)	0.76 (D. purple)
-	0.83 (F. red)	0.83 (D. purple)
0.85 (L. green)	-	-
-	0.94 (F. red)	0.94 (D. purple)
-	-	0.97 (D. purple)

***F: Fluorescent; L: Light; D: Dark**

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Figure 5: Microscopy of Stem of *Naregamia alata*

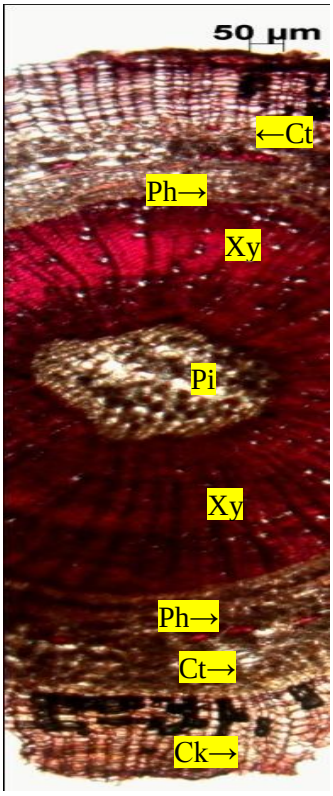

Fig 5. T.S of stem

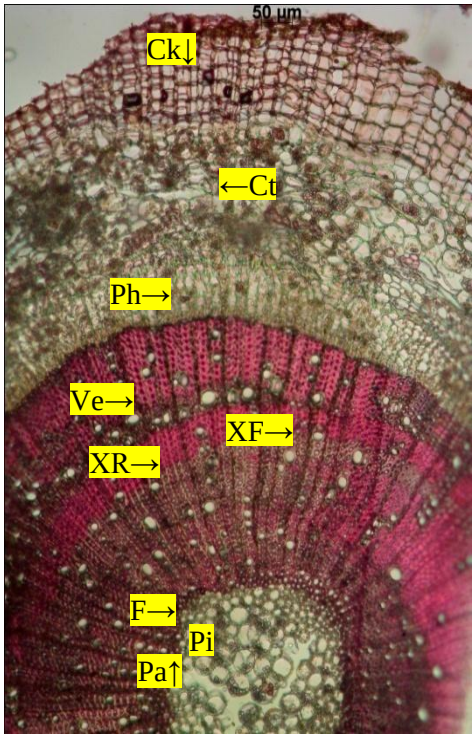

Fig 5. T.S of stem enlarged

DISCUSSION

Discussion on macroscopic study

The Macroscopical study reveals that leaves were alternate, trifoliate with winged petiole, the leaflets were small, cuneate-obovate. The flowers were pentamerous, with 5 lobed sepals and 5 free, white petals. Stamens were 10, fused into a column with free anther which was appendage.

Ovary is superior, three celled with two ovules in each cell. Fruit is a loculicidal capsule, seeds are curved and truncate at both ends.

Microscopic study

T.S of Root: Root has wide undulate, fissured prominent periderm wide homogenous cortex, wide zone of secondary phloem, solid circular cylinder of secondary xylem.

T.S of Stem: Circular in cross sectional outline with their superficial periderm. The cortex fairly wide comprises 4 or 5 layers of dilated thin walled parenchyma cells. The secondary phloem is narrow continuous sheath around the xylem.

T.S of Leaf: Upper epidermis single layered with more or less rectangular cells, trichomes absent, xylem includes narrow, angular or circular vessels, phloem was continuous sheath around the xylem, centrally pith present.

Seed: Epidermis single layered cells with irregular wall and has a thick cuticle.

Fruit: Thin outer layer of cuticle with trichomes, single layered epidermis.

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Figure 6: Microscopy of Stem of *Naregamia alata*

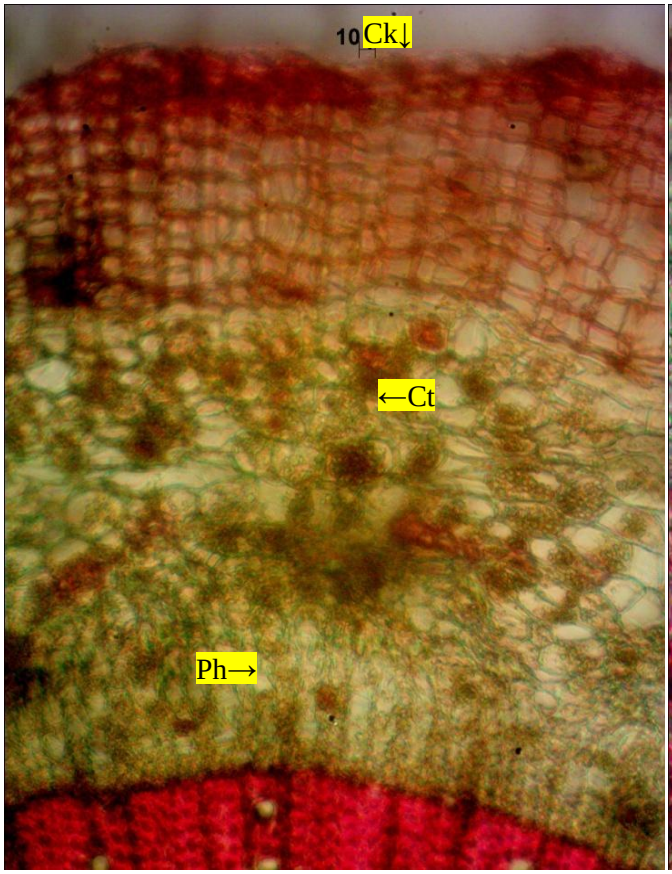

Figure 6a. Cork and cortex

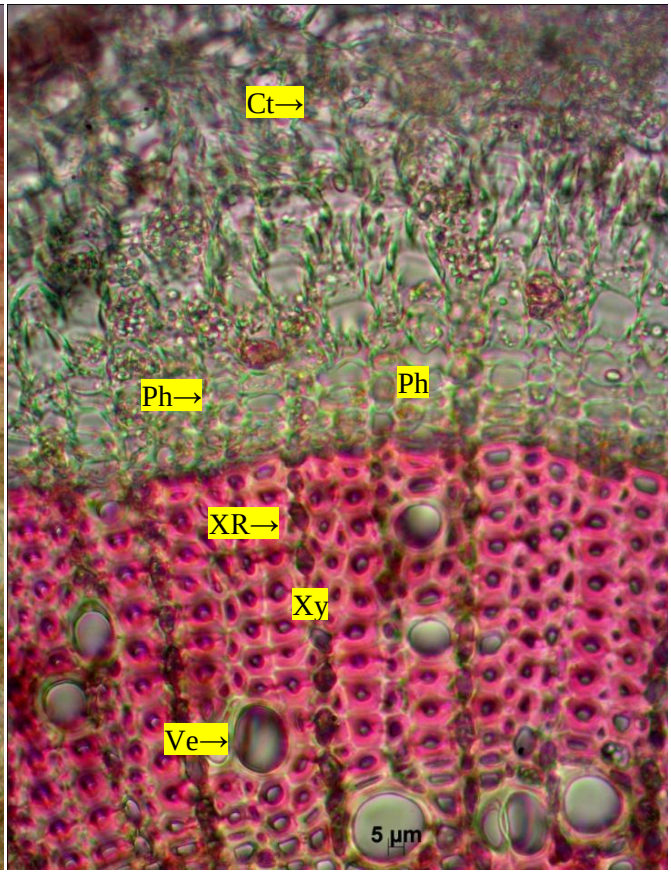

Figure 6b. Phloem and xylem

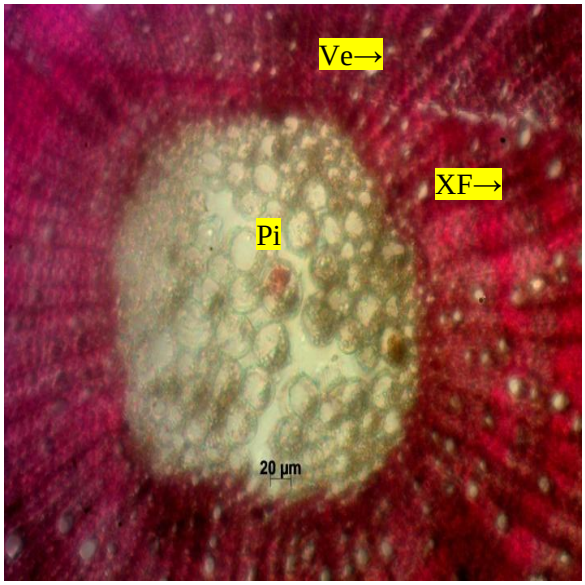

Figure 6c. Xylem and pith parenchyma

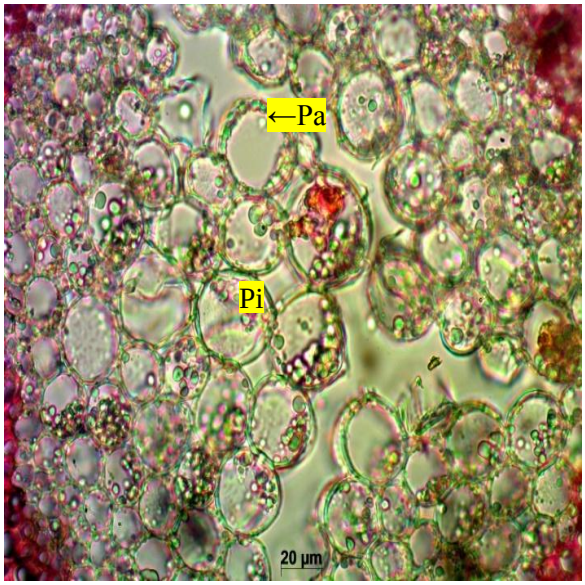

Figure 6d. Pith enlarged

Ck – cork; Ct – cortex; F – fibres; Pa – parenchyma; Ph – inner phloem; Pi – pith; Ve – vessels; Xy–xylem; XF– xylem fibres; XR – xylem rays.

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Figure 7: Microscopy of Leaf of *Naregamia alata*

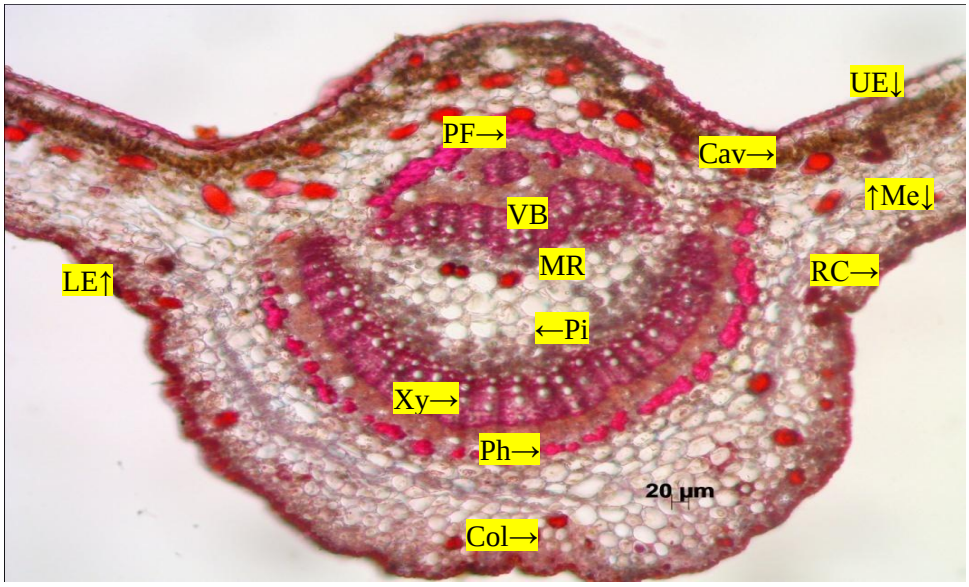

Figure 7a. T.S of leaf

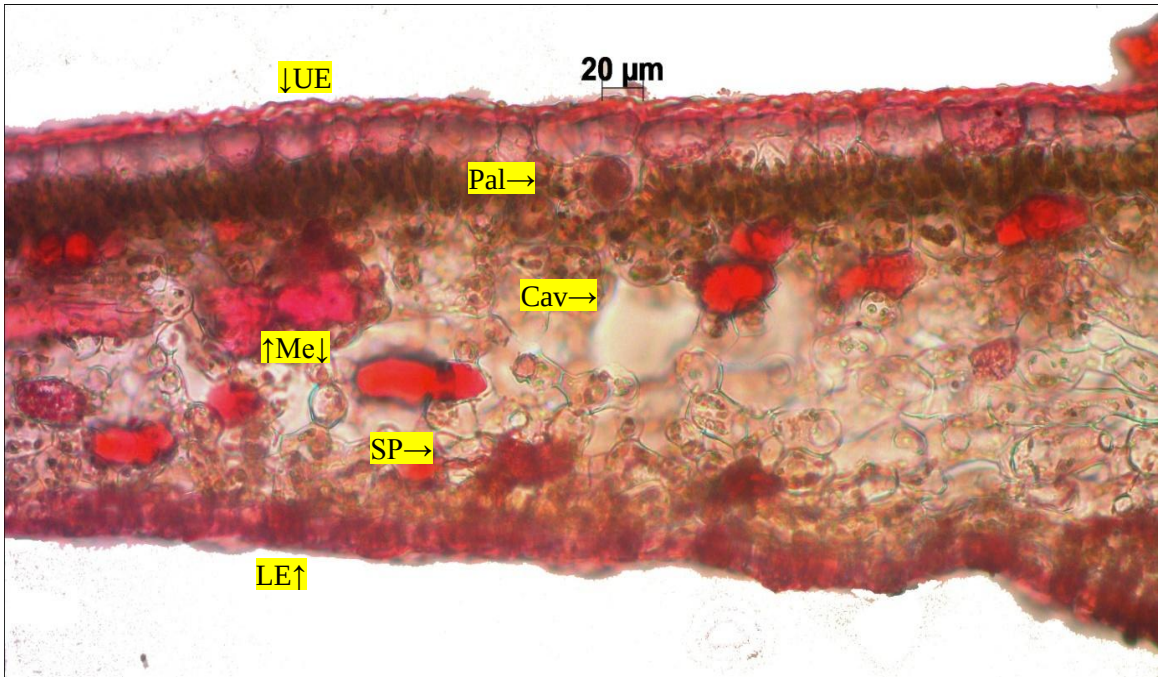

Figure 7b. A portion of Lamina enlarged

UE – uppr epidermis; LAM – lamina; LE – lower epidermis;Me – mesophyll; MR – midrib; Pal – palisade; PF – pericyclic fibres; Ph – phloem; Pi – pith; RC – rosette crystal; SP – spongy parenchyma; VB–vascular bundle; Xy – xylem.

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Figure 8: Microscopy of seed of *Naregamia alata*

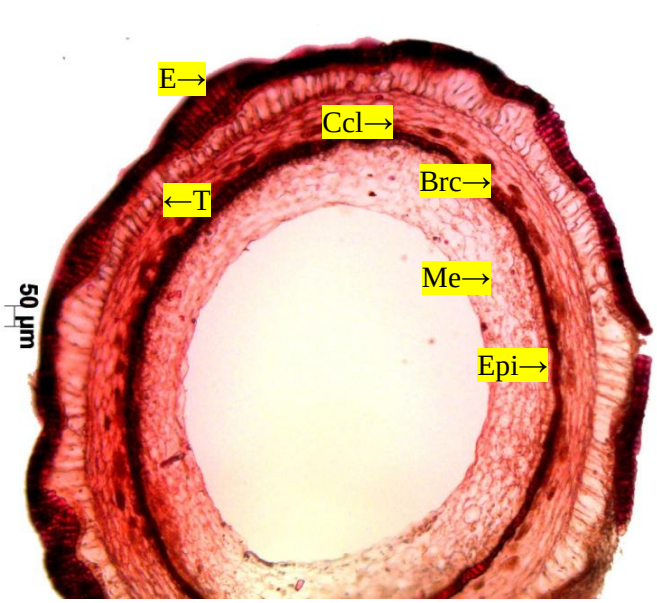

Figure 8a. Seed

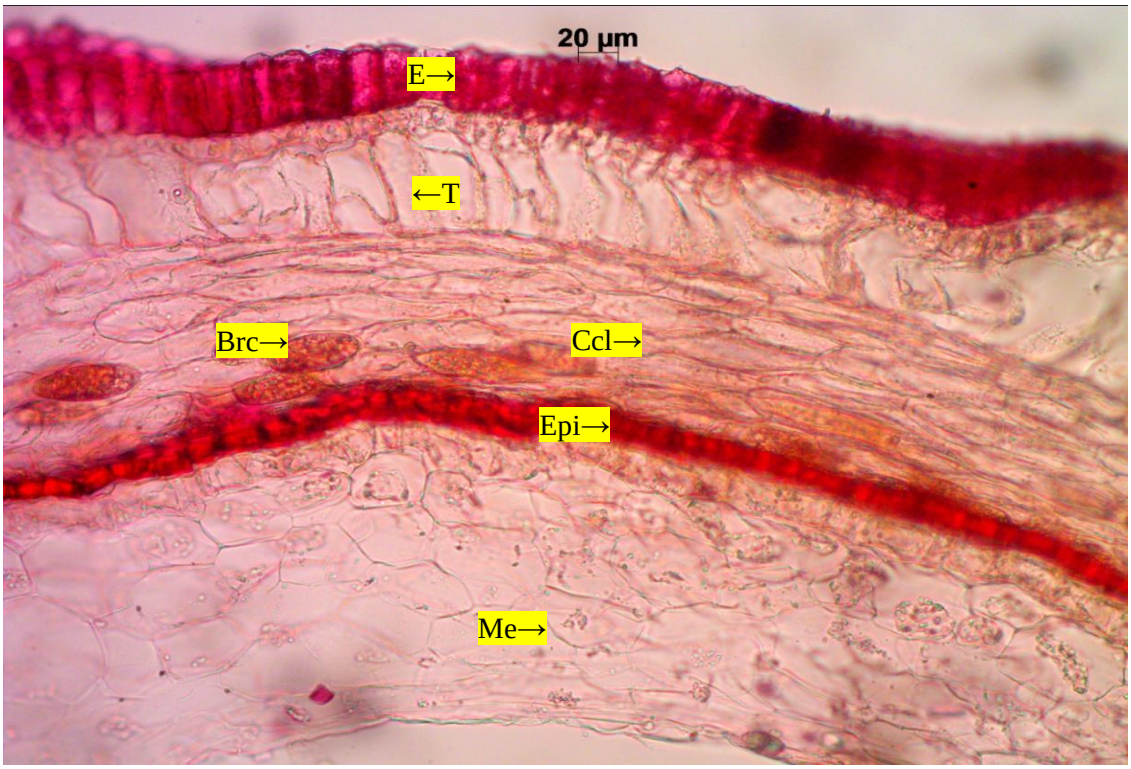

Figure 8b. A portion of seed enlarged

Cu – cuticle; Ccl – collapsed cells;E – epidermis;Epi – epidermis; Me – mesophyll; SP – spongy parenchyma; T – trichomes; VB – vascular bundle

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Figure 9: Microscopy of fruit of *Naregamia alata*

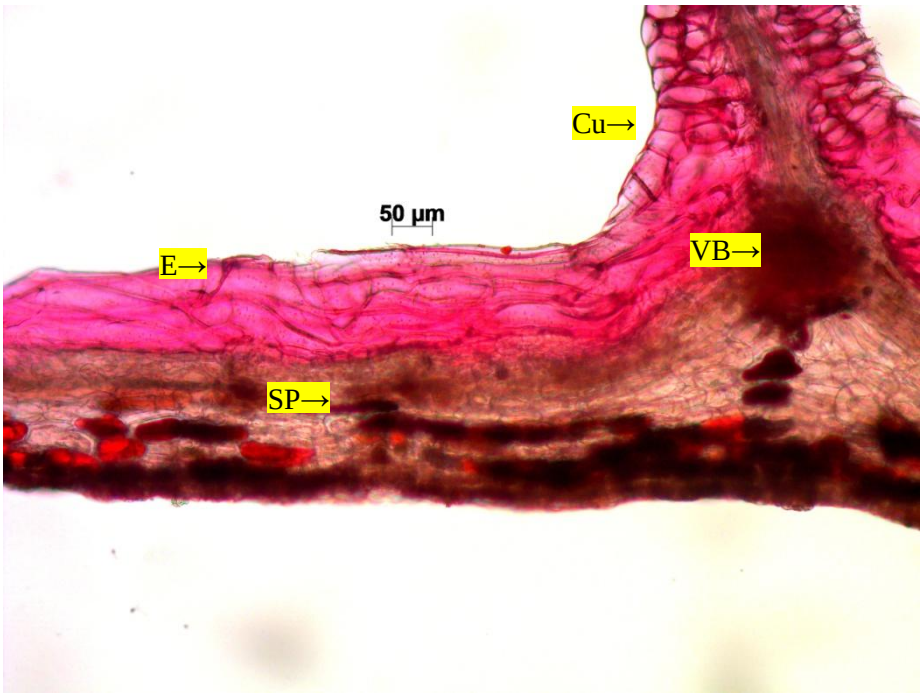

Figure 9a. Fruit

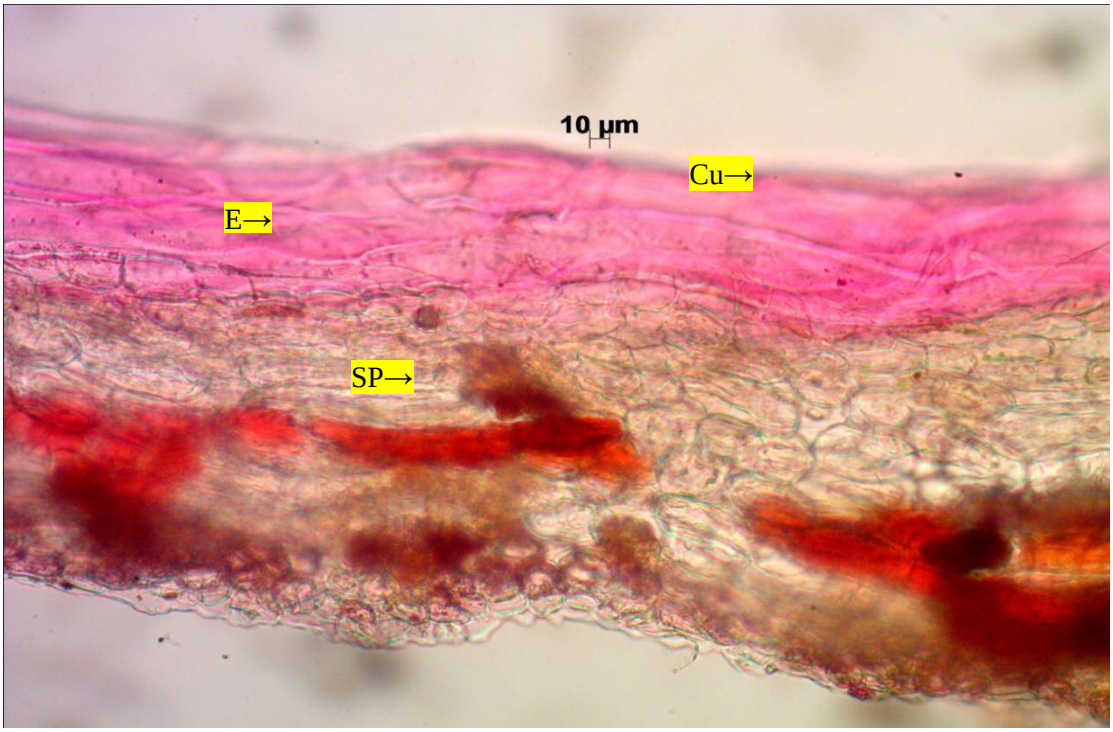

Figure 9b. A portion of fruit enlarged

Cu – cuticle; E – epidermis; SP – spongy parenchyma; VB – vascular bundle

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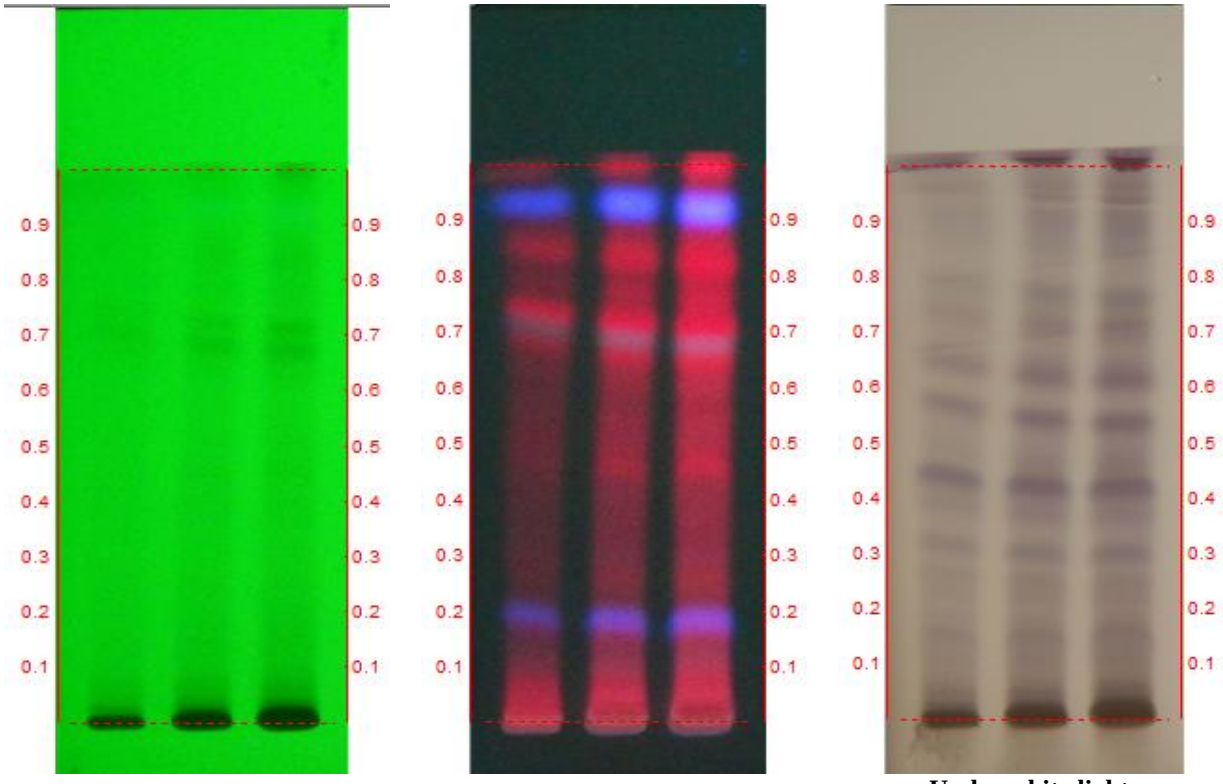
Figure 10: .HPTLC photo documentation of ethanolic extract of *Naregamia alata*



Short UV **Long UV** **Under white light (after derivatisation)**

Track 1 – *Naregamia alata* – 4µl
Track 2 – *Naregamia alata* – 8µl
Track 3 – *Naregamia alata* – 12µl
Solvent system – Toluene: Ethyl Acetate (7.0: 2.0)

Figure 11: Densitometry scan with data

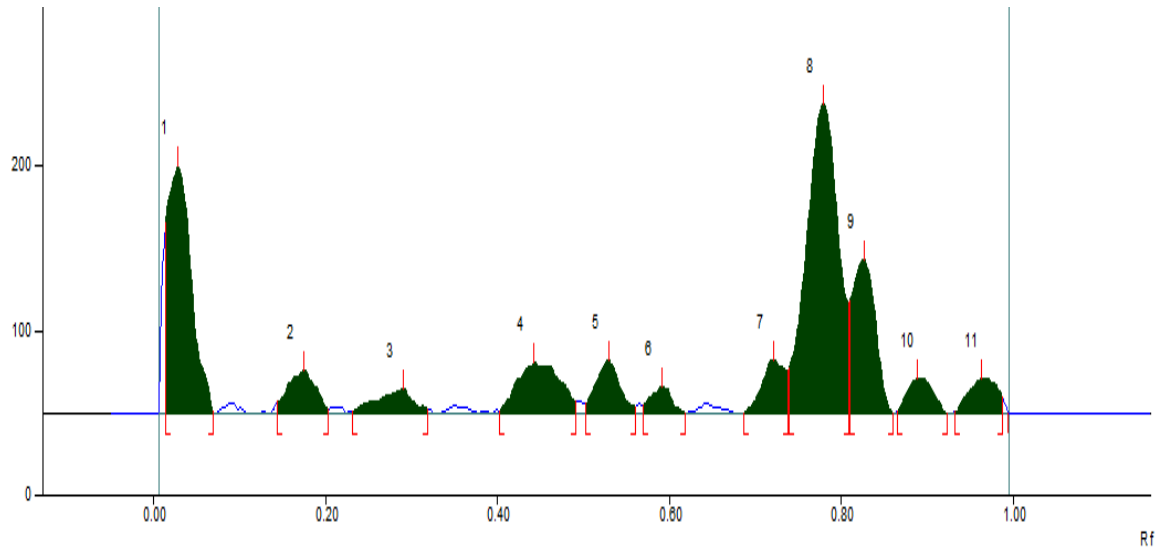


Figure 11a. Densitometric scan at 254nm

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Track 3, ID: Naregamia alata

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	114.8 AU	0.03 Rf	149.5 AU	23.86 %	0.07 Rf	1.8 AU	3005.3 AU	20.82 %
2	0.15 Rf	7.5 AU	0.18 Rf	26.2 AU	4.18 %	0.20 Rf	2.8 AU	600.0 AU	4.16 %
3	0.23 Rf	0.8 AU	0.29 Rf	15.4 AU	2.45 %	0.32 Rf	3.1 AU	443.8 AU	3.07 %
4	0.40 Rf	1.7 AU	0.44 Rf	30.6 AU	4.88 %	0.49 Rf	7.0 AU	1108.0 AU	7.68 %
5	0.50 Rf	6.6 AU	0.53 Rf	32.8 AU	5.24 %	0.56 Rf	4.0 AU	641.9 AU	4.45 %
6	0.57 Rf	5.0 AU	0.59 Rf	16.7 AU	2.66 %	0.62 Rf	0.2 AU	288.6 AU	2.00 %
7	0.69 Rf	0.6 AU	0.72 Rf	32.2 AU	5.14 %	0.74 Rf	26.2 AU	625.6 AU	4.33 %
8	0.74 Rf	27.3 AU	0.78 Rf	187.7 AU	29.94 %	0.81 Rf	67.4 AU	4954.2 AU	34.32 %
9	0.81 Rf	68.5 AU	0.83 Rf	93.3 AU	14.88 %	0.86 Rf	0.4 AU	1797.1 AU	12.45 %
10	0.87 Rf	0.7 AU	0.89 Rf	21.5 AU	3.42 %	0.92 Rf	0.3 AU	459.7 AU	3.18 %
11	0.93 Rf	1.6 AU	0.96 Rf	21.1 AU	3.36 %	0.99 Rf	10.4 AU	511.8 AU	3.55 %

Naregamia alata (12µl)

Figure 11b. Densitometric data at 254nm

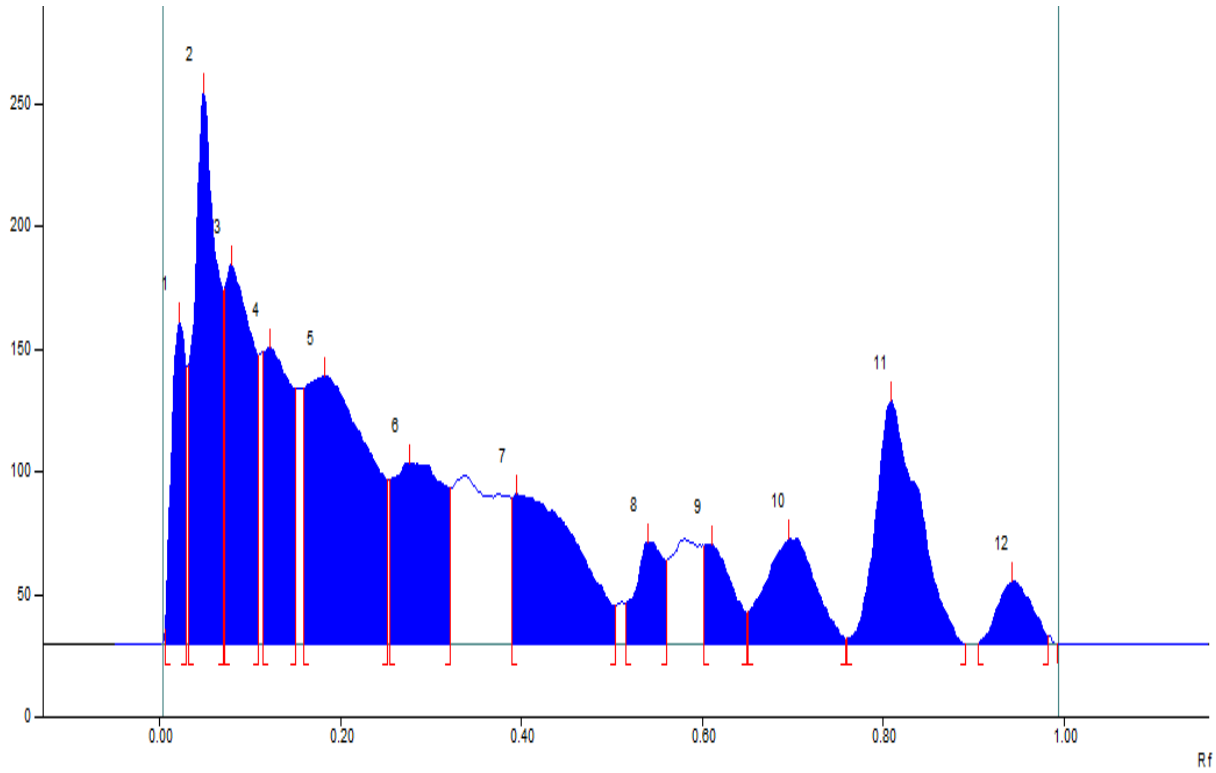

Figure 11c. Densitometric scan at 366nm

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Track 3, ID: Naregamia alata

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	5.5 AU	0.02 Rf	131.1 AU	11.66 %	0.03 Rf	12.4 AU	1473.9 AU	4.77 %
2	0.03 Rf	112.9 AU	0.05 Rf	224.4 AU	19.96 %	0.07 Rf	44.1 AU	4186.0 AU	13.54 %
3	0.07 Rf	144.6 AU	0.08 Rf	154.6 AU	13.75 %	0.11 Rf	17.5 AU	3325.7 AU	10.76 %
4	0.11 Rf	118.8 AU	0.12 Rf	120.6 AU	10.73 %	0.15 Rf	04.0 AU	2723.0 AU	8.81 %
5	0.16 Rf	103.8 AU	0.18 Rf	109.2 AU	9.71 %	0.25 Rf	66.8 AU	5498.2 AU	17.79 %
6	0.25 Rf	66.8 AU	0.28 Rf	73.6 AU	6.55 %	0.32 Rf	63.3 AU	2990.8 AU	9.68 %
7	0.39 Rf	59.3 AU	0.40 Rf	60.9 AU	5.42 %	0.50 Rf	15.7 AU	3173.7 AU	10.27 %
8	0.52 Rf	16.7 AU	0.54 Rf	41.7 AU	3.71 %	0.56 Rf	34.2 AU	918.1 AU	2.97 %
9	0.60 Rf	40.1 AU	0.61 Rf	40.7 AU	3.62 %	0.65 Rf	12.8 AU	879.4 AU	2.85 %
10	0.65 Rf	12.9 AU	0.70 Rf	42.8 AU	3.81 %	0.76 Rf	2.0 AU	1708.9 AU	5.53 %
11	0.76 Rf	2.2 AU	0.81 Rf	99.3 AU	8.83 %	0.89 Rf	0.1 AU	3340.7 AU	10.81 %
12	0.91 Rf	0.3 AU	0.94 Rf	25.5 AU	2.27 %	0.98 Rf	3.0 AU	686.2 AU	2.22 %

Naregamia alata (12µl)

Figure 11d. Densitometric data at 366nm

Discussion on organoleptic study

The result shows that Rasa of Triparnikka (*Naregamia alata* Wight. & Arn.*)* was Tikta pradhana and Kashaya Anurasa. As per general rule the guna of *Naregamia alata* Wight. & Arn. Laghu and Ruksha. AcharyaCharaka state that Laghu, Ruksha are the qualities of Tikta and Kashaya Rasa.

Discussion on Phyto-chemical study

In the present study whole plant extract was taken for the preliminary phytochemical analysis. It showed the presence of chemical constituents like Alkaloids, Carbohydrate, Carboxylic acid, Resin, Tannin and Flavonoid. Here alkaloids and Tannin responsible for anti-inflammatory, analgesic and anti-microbial activity.

Flavanoids documented to possess potent anti-oxidant activity.

The above results give support to the claim regarding the actions of the plant about its wound healing activity.

Discussion on Physio chemical constituents

Loss on drying: The loss on drying signifies the moisture content of the substance. Percentage of moisture content present in Triparni is 11.36 %. The Ash value indicates the presence of inorganic material in the sample; here we can note total Ash value is 6.46 %. Water soluble extract and alcohol soluble extractive value are indicative of the bioavailability of the plant. Water soluble extractive value is 16.38 % and Alcohol soluble extractive value is 4.60%

HPTLC: *Naregamia alata* Wight. & Arn. has been standardized as per pharmacopoeial testing protocol. HPTLC photo documentation, R_f values and densitometric scan at 254 nm, 366 nm and after derivatisation has been developed.

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On photo documentation there were 11 spots under 254 nm and 12 spots under 366 nm of post derivatisation.In Densitometric scan *Naregamia alata* Wight. & Arn. revealed 11 peaks at 254 nm and 12 peaks at 366 nm. One major peak at 366 nm.

CONCLUSION

The drugs selected were identified taxonomically based morphological characters. The samples were genuine and the preliminary photochemical were analyzed based on pharmacognostic and chromatographic assays. As the trial drugs were collected in person, there is no chance of adulteration in them. Organoleptic observations represents, the test drug Triparnikaa (*Naregamia alata* Wight. & Arn.) possesses Tikta and Kashaya Rasa. Phytochemical study revealed the presence of phyto constituents- Flavonoids, Tannins etc. Scope for further studies is literary study on identifying the drug with references in Dravyaguna sasthra is quite essential. Various studies should be conducted on primitive animal models (albino rats) for its pharmacological activities.

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