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Research Article

Stem Bark

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Comprehensive Evaluation of the Pharmacognostic Study and Analytical Parameter of Shigru Kanda Twaka (Stem Bark of Moringa Oleifera Lam.)

Gabani A[1*], Singh S[2], Jani D[3]

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[1*] Abhishek Gabani, Second Year Post Graduate Scholar, Upgraded PG Department of Dravyaguna, Government Ayurveda College, Vadodara, Gujarat, India.

[2] Suman Singh, Assisstant Professor, Upgraded PG Department of Dravyaguna, Government Ayurved College, Vadodara, Gujarat, India.

[3] Dharmendra Jani, Asossiate Professor, Upgraded PG Department of Dravyaguna, Government Ayurved College, Vadodara, Gujarat, India.

Shigru (Moringa oleifera Lam..) is one of the most important plants having nutritional as well as medicinal value in Ayurveda. This present study has been carried out to establish the stem bark of plant for its morphological, microscopical and physicochemical characters with different phytochemical qualitative tests as per API and Sophisticated analysis such as High-Performance Thin Layer Chromatography (HPTLC). Microscopy of stem bark showed Phloem, Rosette crystal, Resin duct, Stone cell, Medullary rays, Gum secretion, Crystal, Stratified cork. Powder microscopy of Shigru (Moringa oleifera Lam.) stem bark shows starch grain, Crystal fibres, Prismatic crystal, reddish brown tears of gum mass, Rosette crystal. Physicochemical parameters showed pH (6.28), loss on drying (6.28 %w/w), ash value (7.12 %w/w), Acid insoluble ash (1.05 %w/w), water soluble extractive (37.28 %w/v) and alcohol soluble extractive (19.10 %w/w). Preliminary phytochemical analysis for the presence of various functional groups such as Alkaloids, Flavonoids, Tannin, Saponin, Steroids were also studied. These observations can be helpful for identification and standardization of Shigru (Moringa oleifera Lam.).

Keywords: Shigru, Moringa oleifera Lam., Stem bark, Pharmacognosy, Physicochemical, Phytochemical, HPTLC

Corresponding Author

Abhishek Gabani, Second Year Post Graduate Scholar, Upgraded PG Department of Dravyaguna, Government Ayurveda College, Vadodara, Gujarat, India.

Email: gabaniabhishek1998@gmail.com

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Introduction

Shigru, botanically identified as *Moringa oleifera* Lam. is a tree growing all over the tropical places of World.**[1]** *Shigru* (*Moringa oleifera*), often referred to as the "drumstick tree" or "miracle tree," holds significant importance due to its exceptional nutritional, medicinal, and ecological benefits. Its wide range of uses in traditional medicine, modern healthcare, agriculture, and nutrition makes it an invaluable resource across different cultures. It's native to Pakistan, Afghanistan, India, and Bangladesh.**[2]**

The *Shigru* tree typically grows to about 10-12 meters in height but can reach up to 15 meters under ideal conditions. It has a fast growth rate, especially in tropical and subtropical climates. The leaves of the Moringa tree are compound and pinnate, with small, oval leaflets arranged alternately along the leaf stalk. The tree produces clusters of small, fragrant white or creamy-yellow flowers. The roots are long and taproot-like, and they store water in dry environments. The bark of *Moringa oleifera* is typically light brown to greyish-brown. It has a relatively smooth texture, though it can become slightly rougher as the tree matures. Many studies have been conducted related to Pharmacognosy on different parts of *Shigru* such as leaves, flowers, roots etc. but Pharmacognosy of stem bark of *Shigru* very few studies were conducted. *Shigru* is considered a versatile and powerful herb that promotes overall health and well-being. Its ability to balance the *Doshas*, support digestion, enhance skin health, reduce inflammation, and detoxify the body makes it a key component in *Ayurvedic* healing practices. Whether used in traditional formulations or as a dietary supplement, *Shigru* is valued for its numerous benefits across a wide range of health concerns.

Table 1: Taxonomical classification of *Moringa oleifera* Lam.[3]

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Mangoliophyta
Class	Mangoliospida
Sub class	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	Moringa
Species	Oleifera

Table 2: Vernacular names of *Shigru*[4]

English	Drumstick tree
Hindi	Sahijana
Gujrati	Saragvo
Bengali	Sajina, Sajne
Marathi	Shewga
Kannada	Nugge
Tamil	Murungai
Telugu	Sajana
Punjabi	Sohajana
Urdu	Sahajana

Aim and Objectives

Aim:

To perform Pharmacognostical, Physicochemical, Phytochemical and HPTLC study of *Shigru* (*Moringa oleifera* Lam.) stem bark.

Objectives:

1. To study the macroscopic features of *Shigru* (*Moringa oleifera*) stem bark.
2. To study the microscopic features of *Shigru* (*Moringa oleifera*) stem bark.
3. To study the powder microscopic features of *Shigru* (*Moringa oleifera*) stem bark powder.
4. To determine physicochemical parameter of *Shigru* (*Moringa oleifera*) stem bark.
5. To determine preliminary phytochemical parameters of *Shigru* (*Moringa oleifera*) stem bark.
6. To determine High Performance of Thin Layer Chromatography (HPTLC) of *Shigru* (*Moringa oleifera*) stem bark.

Materials and Methods

Stem Bark of *Shigru* (*Moringa oleifera* Lam.) was collected from Netranga, located in Rajpipala, Gujarat, is positioned at latitude of 21.8653°N & an altitude of approximately 73.5001°E meters above sea level, after proper identification from Ayuvedic Pharmacopeia of India. After proper drying stem bark was converted into 80 mash powder form in controlled environ. at Pharmacognosy Laboratory of Upgraded Dept. of Dravyaguna, GAC, Vadodara.

1. Macroscopic Study

Macroscopic characters of dried stem bark were achieved by visual observations, following standard procedure of taxonomy. Observations are mentioned in table no. 3.

Table 3: Macroscopic characters of *Shigru* (*Moringa oleifera* Lam.) stem bark

SN	Characteristic of Bark	Observation	Fig. No.
1.	Shape	Corky	Fig 1a, 1b
2.	Size	Cut pieces of varying size, up to 5 to 8 cm wide and 10-20 cm length	Fig. 1c
3.	Colour	External: Grey or dark green Internally: Light brown or cream	Fig. 1b
4.	Surface	1) External bark rough 2) Inner bark smooth	Fig. 1a, 1b
5.	Fracture	Splintery and deep fissured	Fig 1a, 1b

2. Microscopic Study

The bark was soaked into hot water for about 4-5 hours and free hand transverse section was taken. The section was stained with safranin.**[5]**

Cork region was very wide, composed of 7-10 layers, thin walled, rectangular cells within coloured contents, thin-walled parenchymatous cells containing a few rosette crystals, several groups of thick walled, lignified, elongated to polygonal stone cells, a few small, simple, round to oval, secondary phloem consists of thin walled, resin duct, medullary rays, gum secretion, stratified cork were observed in transverse section of stem bark of *Moringa oleifera* Lam.

Prepared sections were examined under microscope for various identification characters, observations are mentioned in table no. 4 and plate no. 1.

Table 4: Microscopic identifications of *Shigru* (*Moringa oleifera* Lam.) stem bark

SN	Observation	Fig. No.
1.	Phloem	Fig. 3a
2.	Rosette crystal	Fig. 3b
3.	Stone cell	Fig. 3c
4.	Resin duct	Fig. 3d
5.	Medullary Rays	Fig. 3e
6.	Gum secretion	Fig. 3f
7.	Crystal	Fig. 3g
8.	Stratified cork	Fig. 3h
9.	Cork	Fig. 3i

3. Powder Microscopical Study

Powder of drug was spread on slide. 1-2 water drop was added on the glass slide. Then properly mounted powder slide is studied microscopically for the characters using microscope.

Glycerine was used as a mounting agent.

Starch grains measuring 5 to 14 micros in diameter which is oval to round, a few rhomboidal, rosette crystals of calcium oxalate, crystal fibres, reddish brown tear of gum resin and prismatic crystals were observed in powder microscopy of stem bark of *Moringa oleifera* Lam.

Observations are mentioned in table no. 5 and plate no. 2.

Table 5: Microscopic identifications of *Shigru* (*Moringa oleifera* Lam.) stem bark powder

SN	Microscopic features of Shigru (Moringa oleifera Lam.) stem bark powder	Fig. No.
1.	Starch grains	Fig. 4a
2.	Crystal fibres	Fig. 4b
3.	Prismatic crystals	Fig. 4d
4.	Reddish brown tear of gum resin	Fig. 4c
5.	Rosette crystals	Fig 4d

4. Physicochemical Parameters

Assessment of the parameter such as pH, loss on drying, ash value, acid insoluble ash, water soluble extractive and alcohol soluble extractive was carried out by following standard procedure recommended by Ayurvedic Pharmacopoeia of India**[6]** and other standard texts. Detailed outcome of physicochemical parameters is given in table no. 6.

Determination of Moisture (Loss on drying):

In this method, 5 g powder of stem bark *Shigru* was taken in evaporating dish then put into the hot air ovum at 105° Celsius temperature for 1 hour than evaporating dish put into desiccator for cooling and then weigh. And continues dry and weighing until the difference between two successive weighing correspond more than 0.5 percent.

$$\text{The \% of loss on drying} = \frac{\text{Difference in weight after heating}}{\text{Weight of sample taken}}\ x\ 100$$

Determination of total ash:

2 g powder of *Shigru* stem bark was taken in crucible than it put in electric muffle furnace at 450° Celsius temperature until free from carbon and cool in desiccator and weight. Then calculate the percentage of ash with reference to the air-dried drug.

Wt. of Silica crucible = A_1 gm, Wt. of Sample(X) = X gm, Wt. of Silica crucible with Ash = A_2 gm.

$$\text{Percentage of Total ash} = \frac{A2 - A1}{X}\ x\ 100$$

Determination of Acid insoluble ash:

Ash of *Shigru* was taken than 25 ml diluted HCL add and insoluble matter collect on Ash less filter paper and it wash with hot water until the filtrate is neutral. Transfer the filter paper containing insoluble matter to the crucible and dry on hot plate. Ignite to constant weight in muffle furnace. Allow the residue to cool in suitable desiccator for half hour and count the weight. And calculate the content of acid insoluble ash with reference to the air-dried drug.

$$\text{Acid insoluble Ash value of the sample} = \frac{100}{\text{y}}\ x\ \text{a}\ \%$$

A = weight of the residue, y = weight of drug taken

Determination Water soluble extractive:

5 g powder of *Shigru* stem bark and 100 ml distilled water mixed in glass beaker than shaking frequently during 6 hours and allowing standing for 18 hours. Filter rapidly, taking precaution against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and was evaporated to dryness on water bath. It was followed by drying at 105^{0}C for 6 hours. Cooled in a desiccator for 30 minutes and was weighed without delay. Calculate the percentage of Water extractive value with reference to air dried drug.

Determination Alcohol soluble extractive:

This process is same like to the Water-soluble extractive but in this procedure methanol solution used instead of distilled water.

Determination pH value:

Take 2 types of Buffer solution no 4 & 7 & digital pH meter then take buffer solution in beaker & deep electrode in it. Carry out same exercise for another buffer solution also, after washing electrode thoroughly with distilled water, then take sample & dip electrode in it & then calculate value.

Table 6: Values of Physicochemical Parameters of Stem Bark of *Shigru*

Physicochemical Parameter	Results	Limit as per API[7]
Loss on drying (%w/w)	6.28	-
Total Ash value (%w/w)	7.12	Not more than 16%
Acid insoluble ash (%w/w)	1.05	Not more than 4%
Water soluble extractive value (%w/w)	37.28	Not less than 22%
Alcohol soluble extractive value (%w/w)	19.10	Not less than 8%
pH (1% Solution)	6.28	-

5. Preliminary Phytochemical Analysis of Stem Bark:

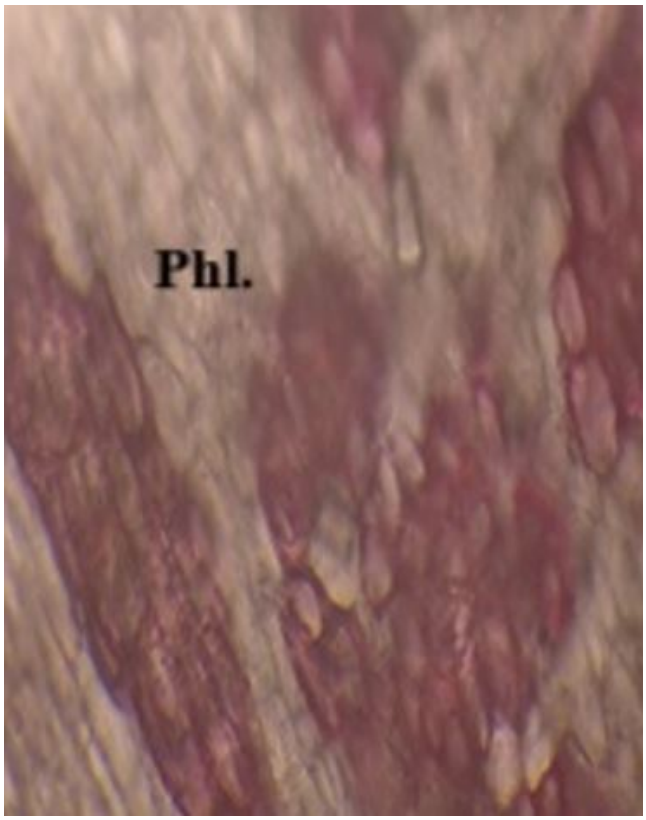
By performing various qualitative tests, one can get idea about the type of Phyto constituents present in the sample. Hence the powder of samples subjected for following tests. Preparation of plant extracts is as per shown in below table no. 7.

Table 7: Steps for preparation of Plant extracts[8]

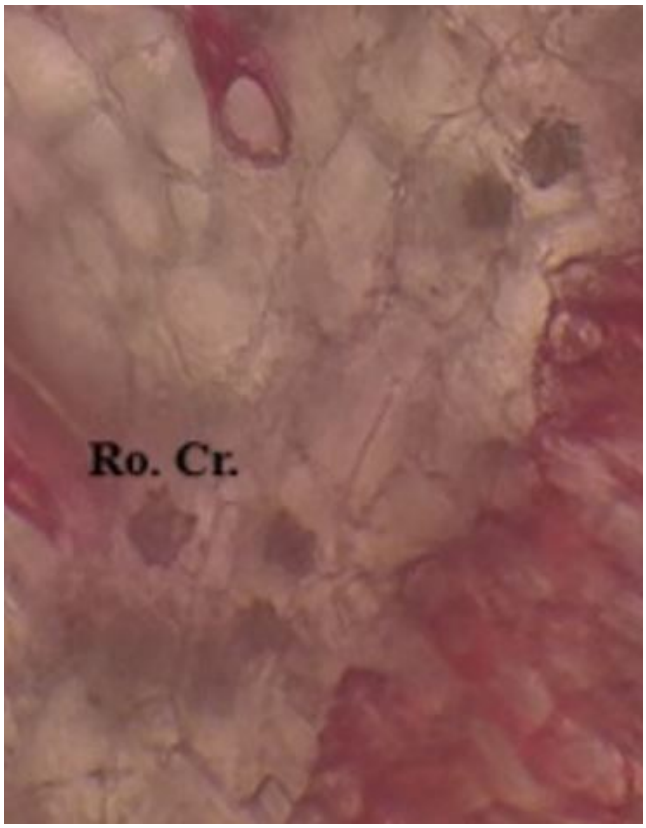
Step 1	Samples of powder taken 5 gm and was extracted with methanol (100ml).
Step 2	Kept it for overnight. Initially occasional shaking up to 6 hours was performed and then kept aside for settle down.
Step 3	After 24 hours, it was filtered and alcoholic extract was collected
Step 4	Similarly, water extracts of sample were prepared and collected
Step 5	After that, qualitative tests were done by using appropriate extracts

Phytochemical analysis was performed with following the standard protocol given in API.**[9]** As like - Alkaloid - Dragendorff's reagent, Saponin - Foam test, Flavonoids, Tannin, Steroids. Observations are mentioned in table no. 8.

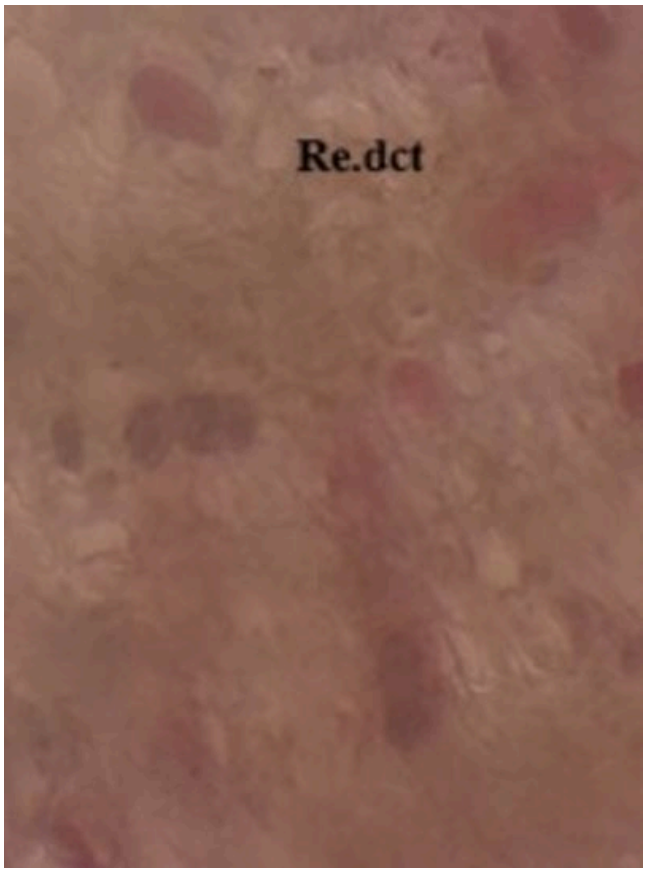
Plate 1: Microscopic structure



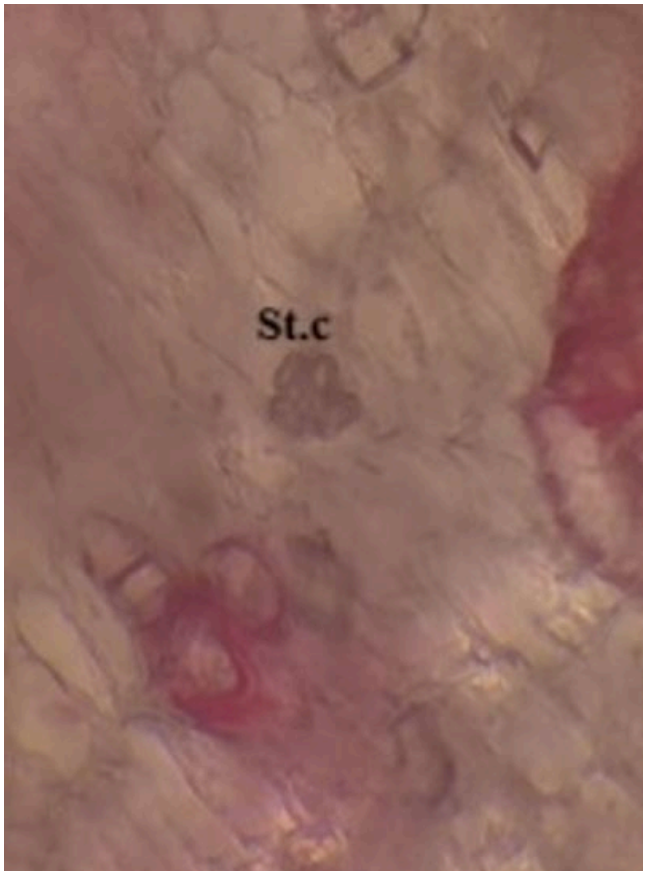
Phloem



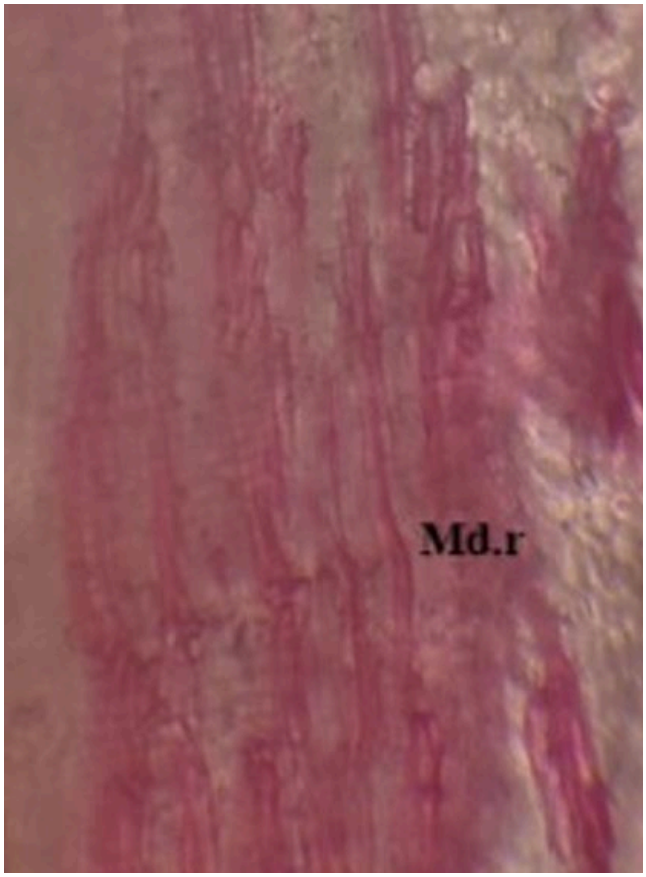
Rosette crystal



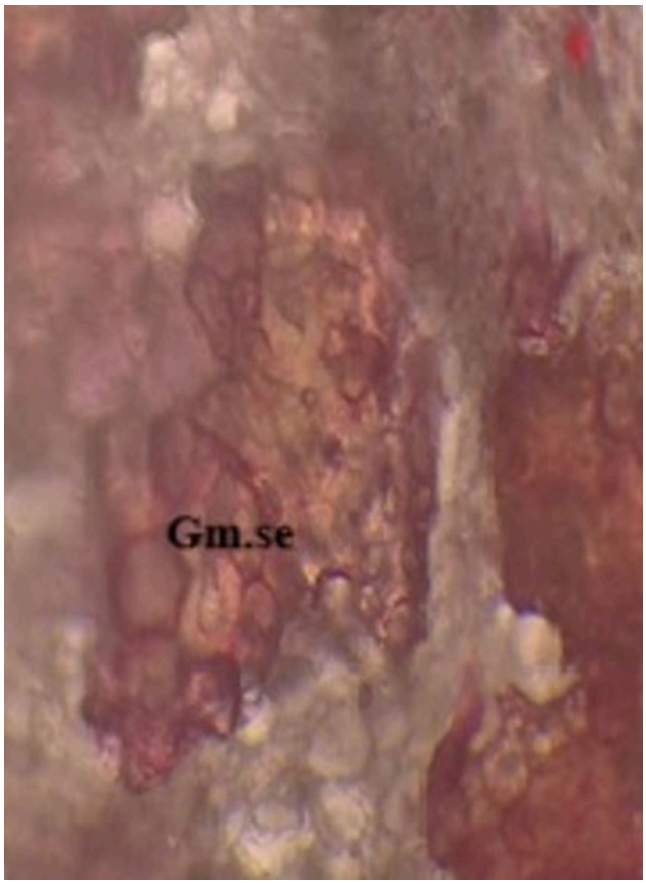
Resin duct



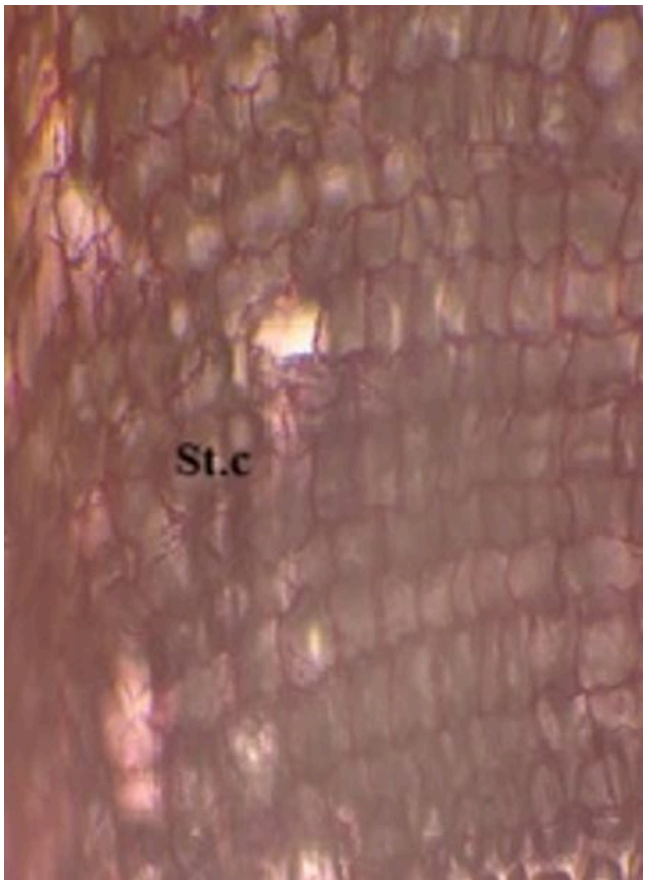
Stone cell



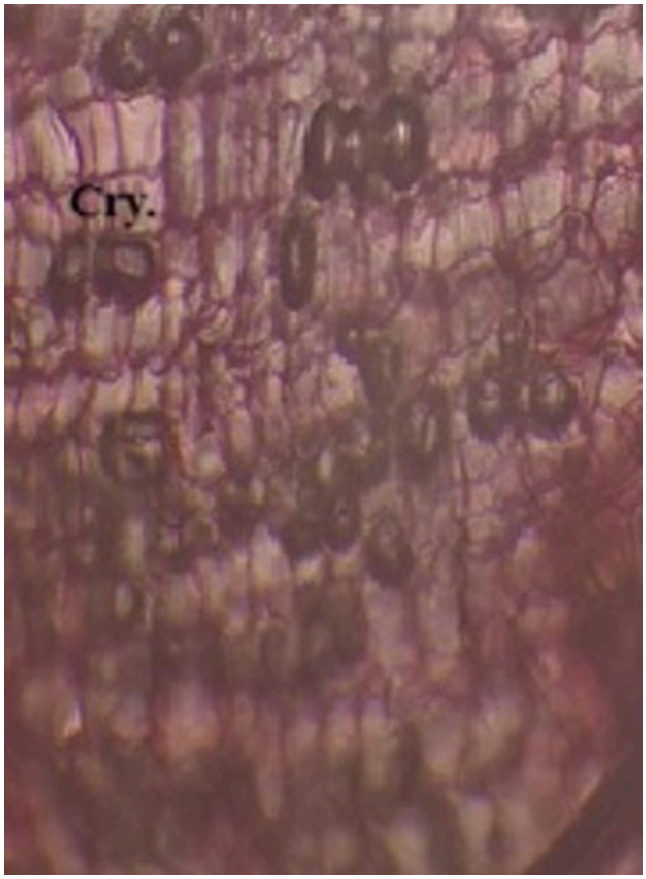
Medullary Rays



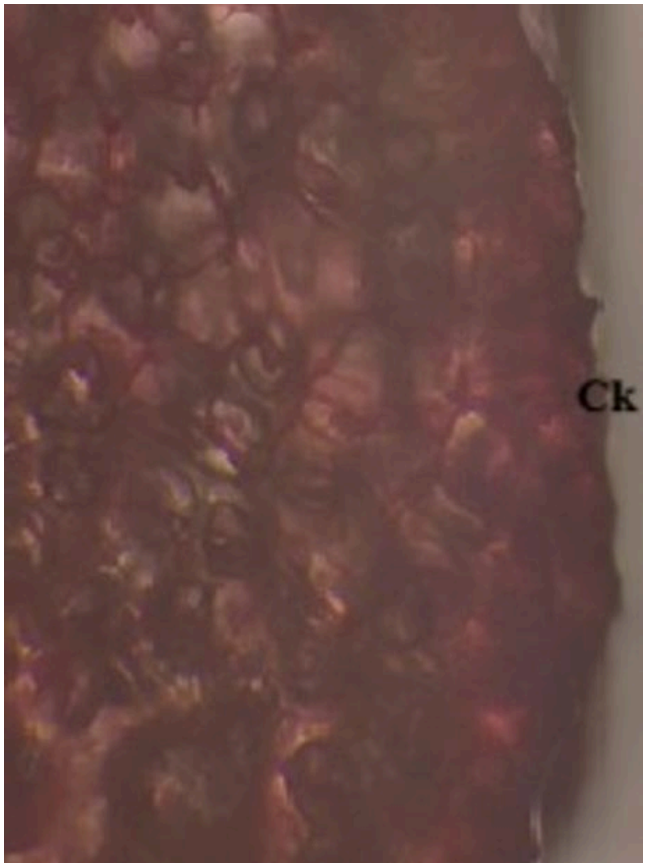
Gum secretion



Stratified cork

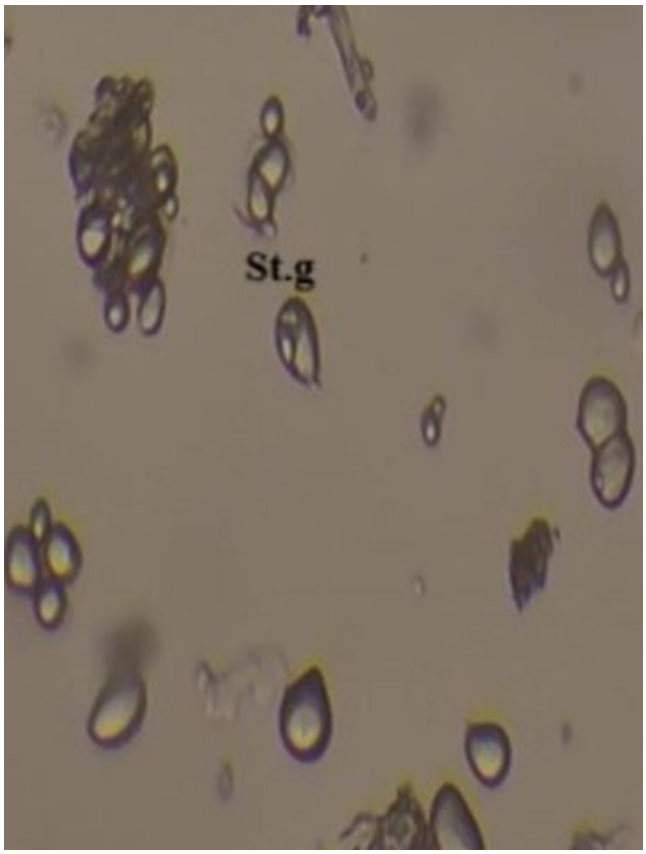


Crystal

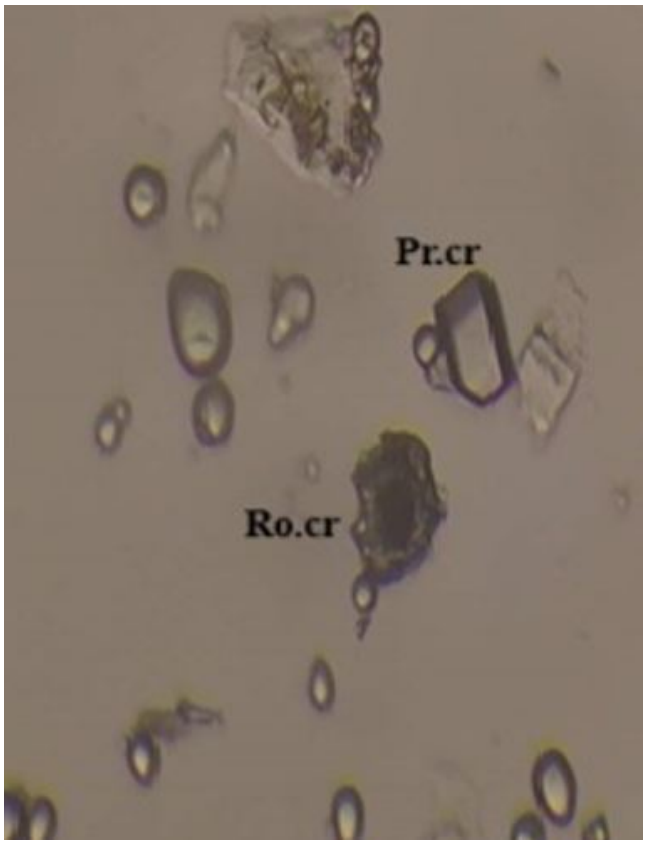


Cork cells

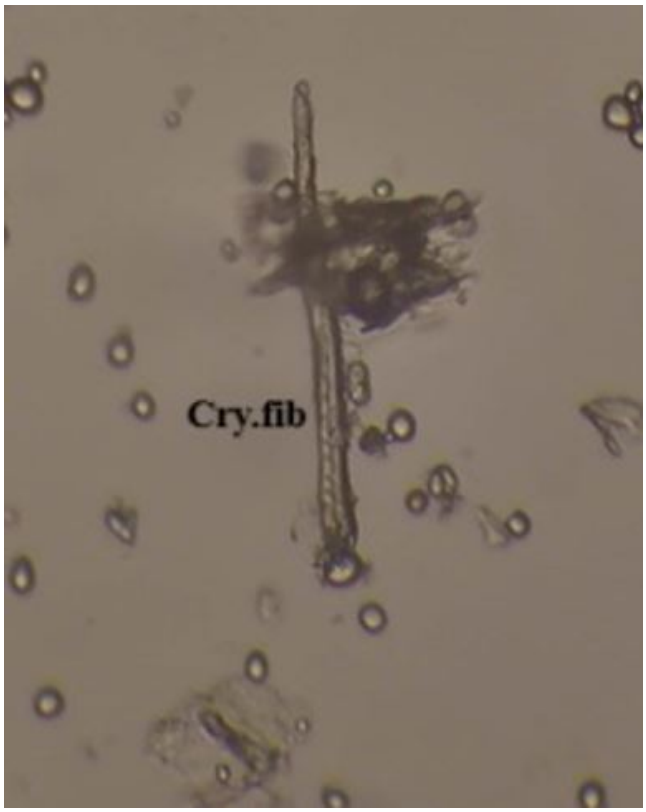
Plate 2: Powder microscopy structure



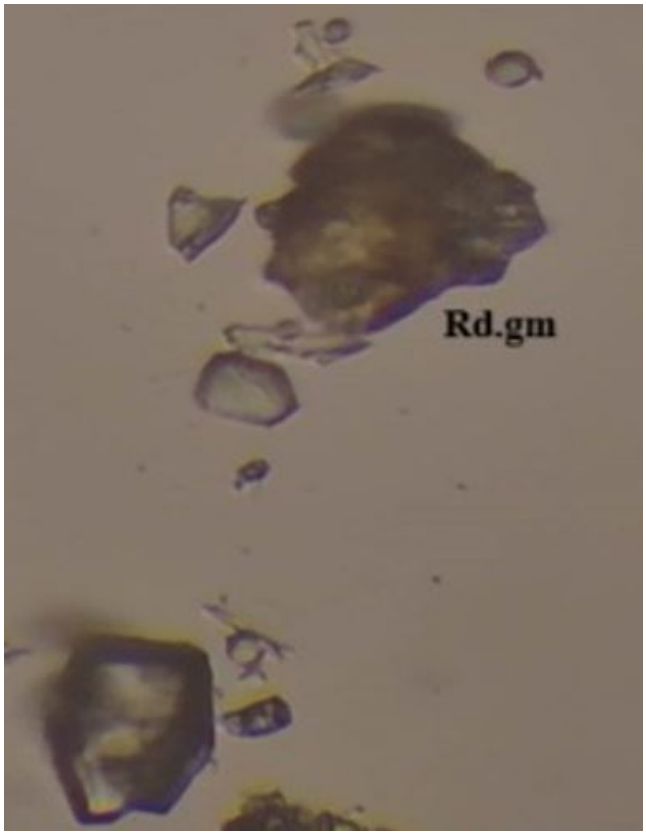
Starch grain



Prismatic Crystal, Rosette Crystal



Crystal Fibres



Reddish brown tears of gum mass

Table 8: Result of Preliminary Qualitative tests of Stem bark of *Shigru* (*Moringa oleifera* Lam.)

Plant Metabolites	Results
Alkaloid	+ve
Saponin	+ve
Flavonoids	+ve
Tannin	+ve
Steroids	-ve

6. Sophisticated Analysis:

HPTLC is a sophisticated and automated technique, which is useful in separation of compounds. Precoated plates and auto sampler are used for precision and to achieve significant separation. UV, visible and fluorescence scanner are used for qualitative and quantitative estimation.

Preparation of Test Solution: Weight approximately 1 g of sample in a reflux flask. To it add 20 ml of chloroform and reflux it for 30 min on water bath. On completion of time, remove the flask from the water bath and filter with the help of whatmann No. 1. Use the test solution thus obtained for HPTLC fingerprinting.

Preparation of Spray reagent [Anisaldehyde – Sulphuric acid reagent]: 0.5 ml Anisaldehyde is mixed with 10 ml Glacial acetic acid followed by 85 ml methanol and 5 ml sulphuric acid (98 %).

HPTLC of *Madhushigru* was scanned under 254nm, 366 nm and 540nm. Observation of Rf value and number of spots at different visualization tabulated in table no. 9.

Table 9: Observation of Rf value and number of spots at different visualization

Visualization	Rf Value	Number of Spots
254 nm	0.19, 0.31, 0.55, 0.63, 0.88	5
366 nm	0.45, 0.84, 0.90	3
540 nm	0.14, 0.19, 0.31, 0.40, 0.45, 0.55, 0.68, 0.77, 0.88	9

Results and Discussion

In the present study Pharmacognostical standardization of stem bark of *Moringa oleifera* Lam. was done which included macroscopical, microscopical, powder microscopic, physicochemical and phytochemical analysis. This provides the easy, speedy and economical means to establish the identity and purity of drug and also acts as a reliable implement detection of adulteration.

Macroscopic characters likes corky shape of stem bark externally grey to dark green colour and internally light brown or cream colour, externally rough surface and internally smooth surface of stem bark and splintery and deep fissured fracture identified the *Shigru*(*Moringa oleifera* Lam.).**[10]**

In microscopic identification, transvers sections of *Shigru* stem barks shows the structures like phloem, rosette crystals, stone cell, resin duct, medullary rays, gum secretion, crystals and stratified cork was found**[11]** whereas in powder microscopic identification structures like starch grains, crystals fibres, prismatic crystals, reddish brown tear of gum resin and rosette crystals was found.**[12]** Which shows the authenticity of the sample.

In physicochemical analysis parameters like total ash value, acid insoluble ash and extractive values were estimated which serves reliable aid in identification of adulteration and identification of plant material. Ash value gives an idea about inorganic composition and other impurities present with drug.

Extractive values are the values which gives the knowledge of chemical constituents of crude drugs that are soluble in particular solvent, which are helpful to determine exhausted and adulterated drugs. Loss on drying should be at minimum level so the bacteria, fungi etc. will not grow during the time of storage. Physicochemical parameters of *Shigru* sample were compliance with API value that shows the purity of sample.**[13]**

The chemical constituents of plants/herbs contribute to their physiological properties and consist of primary metabolites, viz., sugars, amino acids, and proteins, along with secondary metabolites such as alkaloids, flavonoids, tannins, saponin, steroids etc. In the present study, different qualitative tests were carried out with the methanol extracts of the *Moringa oleifera* stem bark powder. The results of the phytochemical screening revealed the presence of alkaloids, saponins, tannins, and flavonoids but absence of steroids in the extracts of this the sample.

In HPTLC profile, each and every metabolite has played specific role and function in harmony with other metabolites within the organization framework of the cells in the defence mechanism of the plants. Here in this HPTLC study different peaks are observed at different Rf.

Total 5 peaks are observed at 254nm, and 3 peaks are observed at 366nm and 9 peaks are observed at 540nm of UV light out of which 5 peaks resembling each other at Rf 0.19, 0.31, 0.55, 0.88, and 0.45. The number of observed peaks shows the presence of numerous active constituents in the given sample of the stem bark of *Shigru*.

Conclusion

Shigru (*Moringa oleifera* Lam.) is an important plant which is used in many diseases mentioned in Ayurvedic literature. In this study results of Pharmacognostical, Physicochemical and Phytochemical studies are compared with the API which proves that the results are as per API and the drug is authentic.

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