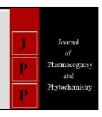


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Pharmacognostical and phytochemical study on stem bark of *Shigru* (*Moringa oleifera* Lam.)

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Abstract

Ayurveda, the science of life, had so many medicinal plants, which have been used since ancient time. They all have potential to help humanity to live healthy life. Shigru is one of them having vast medicinal and nutritional properties. Shigru (Moringa oleifera Lamk.) is popular from the ancient times because of its traditional use in ancient medicine. In this study Botanical description, chemical constituents, Ayurvedic properties including karma and dosage, pharmacological properties and uses and different chemical constituents of Shigru (Moringa oleifera Lamk.) are described. The present work deals with the preliminary pharmacognostical studies on the stem bark Shigru (Moringa oleifera Lamk.). Macroscopical and Microscopical Characters, physio-chemical constants, quantitative microscopy parameters, extractive values, TLC (Thin Layer Chromatography) and HPTLC were studied. Preliminary Phytochemical screening of stem bark of Shigru (Moringa oleifera Lamk.) was studied. The purpose of all these footprints will assist future researchers in their Phytochemical as well as Pharmacological analysis of this species.

Keywords: Shigru, Pharmacognostic study, phytochemical study, Moringa oleifera Lamk

Introduction

Moringa oleifera is a small or medium sized tree of about 10 meter height, found wild in the Sub-Himalayan tract from Chenab eastwards to Sarda and cultivated all over the plains of India [1]. It is a fast growing drought-resistant tree belonging to the family Moringaceae [2]. It is one of the medicinal plant having diseases curing and nutritional properties which is described in *Charak Samhita*³. Synonyms of *Shigru* in Sanskrit are *Shobhanjana*, *Krishnagandha*, *Tikshnagandhak*, *Akshiva*, *Mochaka* [3-4], while in hindi- it is called as *Sahijana*, *Saijan*, *Mungna*. English name of this plant is Horse Radish tree, Drum stick tree [4]. *Shigru* (*Moringa*) is known as the miracle tree because of its beneficial features, e.g., 10 times more vitamins than carrots, 7 times more vitamin C than oranges, 17 times more calcium than milk, and 15 times more potassium than bananas [2].

Botanical Description

Syn.: Moringa pterygosperma Gaertn.

Moringa oleifera Lamk.is a small or medium sized tree. its Bark - thick, soft, corky, deeply fissured; young parts tomentose; Leaves Usually tri-pinnate; leaflets elliptic; flowers white, fragrant, in large panicles; pods pendulous, greenish, 22.5-50.0 cm. or more in length, triangular, ribbed; seeds trigonous with wings on angles. It is often cultivated in hedge and homeyards. The tree can be propagated by seeds or from cutting; cutting are preferred. The tree sheds its leaves in December-January and new leaves appear in February-March. They are followed by flowers and long whip-like tender fruits, which ripen during summer.

The tree is valued mainly for the tender pods which are esteemed as vegetable. They are cut into slices and used in culinary preparations. Seeds are consumed after frying; they are reported to taste like peanuts [1].

Ayurvedic Properties of Shigru (Moringa oleifera Lam.)

Rasa - Katu

Guna – Ruksha, Tikshna, Laghu

Virya- Ushna

Vipaka - Katu

Dosaghnata –Vatakaphashamaka [4, 6].

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Karma— Deepana, rochana, vidahakara, sangrahi, shukral, hridya, chaksusya, krimighna, vishaghna, medohara, raktapittakara and sulahara.

Rogaghnata - Vidradhi, shotha, krimiroga, medoroga, apachi, visha, pleeha, Gulma, galganda roga, agnimandya, aruchi and netra roga [4,7].

Prayojyanga-mula (root), *tvak* (bark), *phala* (Fruit), *patra* (leaves) and *beeja* (seed) ^[6].

Dosage - seed powder: 1-3gm⁷, Decoction: 50 to 100 ml ^[5].

Pharmacological actions and uses of useful parts Antioxidant, anti-inflammatory, antispasmodic, stimulant, expectorant, diuretic, emmanagogue [6].

Root bark – aphrodisiac, alexeteric, anthelmintic, analgesic, biliousness; improves appetite; useful in heart complaints, eye diseases, fever, inflammation, enlargement of spleen, tuberculous gland in neck, tumours and ulcers. The bark removes all kind of pains.

Leaves- removes all kind of excessive pains, fattening, aphrodisiac, anthelmintic, useful in eye diseases, biliousness; cure hallucinations, dry tumours, hiccough, asthama.

Flower- anthelmintic, cure inflammations, muscle diseases, tumour, enlargement of spleen.

Fruits- cure kapha, biliousness, pain, leucoderma and tumours.

Seed – alexipharmac; cure eye diseases, head complaints. Oil is useful in leprous ulcer [13].

Chemical constituents

Shigru-Root bark contains two alkaloids (total main alkaloids-0.1%)- Moringine and Spirochin. Shigru barks yield coarse fibers. The roots contain an active antibiotic principle, pterygospermin, which is obtained as a low melting unstable substance with a characteristic odour, soluble in organic solvents but sparingly soluble in water. It readily decomposes to benzyl isothiocyanate.

Gum- The stem of the tree exclude a gum which is initially white in colour but changes to reddish brown or brownish black on exposure. It is a polyuronide consisting of arabinose, galactose and glucuronic acid in the proportion of 10:7:2 moles; rhamnose is present in traces. The seeds yield 38% of yellowish fatty oil with a mild pleasant odour ^[1,5].

Picture of Shigru (Moringa oleifera Lam.)



Fig 1 & 2: Shigru- Tripinnate leaves and White color panicle flowers



Fig 3 & 4: Shigru- Bark deep fissured and fresh bark

Material & Method

Stem bark of *Shigru* (*Moringa oleifera* Lam.) was collected from Ayurvedika Dravyaguna garden, B.H.U. Varanasi. Raw

drug sample was identified by the teacher of Dravyaguna department in Faculty of Ayurveda IMS, B.H.U Varanasi. Sample of collected raw drug was kept in the museum of the department of Dravyaguna, Faculty of Ayurveda, IMS, BHU, Varanasi as with Voucher specimen no- DG/17-18/167.

Preliminary Pharmacognostic characteristics Macroscopic characters of drug

In this study the coarse Powder of Stem bark of *Shigru* (*Moringa oleifera* Lamk.) was examined for its macroscopic characteristics.

Materials

- 1. Coarse powder of *Moringa oleifera* (stem bark)
- 2. Petri dish.

Method

5 gm. coarse powder of sample was taken in a Petri dish and examined with naked eye. Results shown in table no. 1

Table 1: Macroscopic Characteristic of stem bark of *Moringa* oleifera

S. No.	Parameters	Observation of stem bark powder			
1	Nature	Coarse powder with fragments			
2	Colour	Reddish brown			
3	Odour	Pungent			
4	Taste	Pungent and Bitter			
5	Texture	Rough & fibrous			
6	Size	Uneven sized coarse particles			





Fig 5& 6: Shigru stembark -dry form & coarse powder

Microscopic characteristic of sample drug

The coarse powder of stem bark of *Moringa oleifera* was pulverized in to fine powder. The powder sample was investigated for their microscopic characteristics [11].

Materials

- 1. Fine powder of stem bark of *Moringa oleifera* Lamk.
- 2. Chloral hydrate
- 3. Plain water
- 4. Microscope
- 5. Slide and Cover slip
- 6. Watch glass

Method

5 gm powder of stem bark of *Moringa oleifera* Lamk was boiled separately with chloral hydrate solution in small quantity. Cleaved powder was removed in three separate watch glasses respectively and stained with one drop each of Phloroglucinol and conc. HCl (1:1). A little of the treated powder was mounted in 60% Sulphuric Acid and the slides were observed under microscope at low power. This process was repeated with Picric acid solution in alcohol and after mounting, the slides were observed. Results are tabulated in table no. 2

Table 2: Microscopic characteristics of powdered sample of stem bark of Moringa oleifera

S. No.	Reagents	Observations	Characteristics
1.	Phloroglucinol + Conc. Hydrochloric Acid (1:1)	Pink	Lignified cells: Phloem fibers, cork cells
2.	60% H ₂ SO ₄	Insoluble	Needle shape Calcium oxalate crystals
3.	Picric acid solution in alcohol	Yellow	Aleurone grains

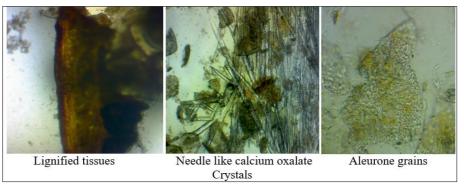


Fig 7, 8 & 9: Lignified tissue, Needle like Calcium oxalate crystal & Aleurone grains

Standardization of stem bark of Shigru (Moringa oleifera Lam.)

In this study, after authentication of drug sample it is dried at room temperature until they were free from the moisture and subjected to physical evaluation. There are different physical parameters which used for evaluation of nature, odour, colour, taste and texture, moisture content, Determination of hydroalcoholic extractive value, Determination of total ash, acid insoluble ash, water soluble as hand determination of foreign matter by different procedures.(Ayurvedic Pharmacopoeia of India, Part I, Volume V &IX).

Determination of hydro-alcoholic extractive value

Macerate 50 gm of the air dried drug sample, coarsely powdered, with ethanol and distilled water (50:50) in a closed flask (Soxhlet apparatus). Shaking frequently during 6 hours and allowing standing for 18 hours. Filter rapidly, taking precautions 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 a water-bath. It was followed by drying at 105 °C for 6 hours, cooled in a desiccators for 30 minutes and was weighed without delay. Calculate the percentage of hydro-alcohol extractive with reference to the air-dried drug. This extract was used for subsequent experiments [11].

Determination of Moisture Content (Loss on Drying)

Dry the evaporating dish for 30 min under the same conditions to be employed in the determination. Place about 5 to 10 g of powder/drug accurately weighed in a tared evaporating dish. Place the loaded bottle in the drying chamber. Dry the test specimen at 105 °C for 5 hours and weigh. Continue the drying and weighing at an hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 min and cooling for 30 min in a desiccators, show not more than 0.01 g difference [12].

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

Determination of total ash

Incinerate about 2 to 3 g, accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° until free from carbon, cool in desiccators for 30 min and weigh without delay. 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 Crucible with $Ash = A_2$ gm

Percentage of Total Ash = $[A_2 - A_1/X] * 100$

Determination of Acid-insoluble ash

To the crucible containing total ash, add dropwise25 ml of dilute hydrochloric acid. Collect the insoluble matter in a Gooch crucible or on an ashless filter paper (Whatman 41) and wash with hot water. 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 desiccators for 30 min and weigh. Calculate the content of acid-insoluble ash with reference to the air-dried drug.

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

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

Determination of Water-soluble Ash

Boil the obtained ash for 5 min with 25 ml of *water*; collect insoluble matter in a Gooch crucible or on an ash less filter paper (Whatman 41), wash with hot *water*, and ignite for 15 min at a temperature not exceeding 450°. Subtract the weight of the insoluble matter from the weight of the ash; Difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

Water-soluble ash value of the sample =
$$\frac{100 \times a}{y}$$
%

a = weight of the residue, y = weight of the drug taken.

Determination of Foreign matter

Take 100 g of drug sample and spread in a thin layer on a suitable platform. Examine in daylight with naked eye or using 6X or 10X magnifying glass and separate the foreign matter. Dust regarded as mineral admixture is separated by sifting the sample through a 250 no. sieve. Weigh the sorted foreign matter and calculate the foreign matter content in percent with reference to drug sample [12].

Observation

Table 3: Percentage yield of Extract of sample drug

S. No.	Extracts	Nature of Extract	Weight (gm)	% Yield w/w
1.	Hydro-alcohol	Viscous	30.061	10.372

Table 4: Certificate of analysis of stem bark of *Moringa oleifera*Lam

S. No.	Parameters	Observation			
I.	Physical tests				
	Nature	Coarse powder			
	Color	Reddish brown			
	Odour	Pungent			
	Taste	Bitter pungent			
II.	Moisture content (w/w %)	7.32			
III.	Ash values				
	Total ash (w/w %)	13.5			
	Acid insoluble	5.9			
	Water soluble ash	2.55			
IV.	Foreign matter %	0.5			

Preliminary Screening of Phytochemicals

The air-dried powdered sample of stem bark was subjected to preliminary phytochemical screening analysis for qualitative determination of phytoconstituents. General phytochemical screening of sample drug extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them [12, 14, 15]. Results of phytochemical screening are shown in table no. 5.

Table 5: Phytochemicals screening: hydro-alcoholic extract of stem bark of *Moringa oleifera* Lamk

S. No.	Chemical Test	Present/absent		
1.	Carbohydrates	+		
2.	Proteins	+		
3.	Amino acids	+		
4.	Glycosides	+		
5.	Flavanoids	-		
6.	Alkaloids	+		
7.	Tannins and Phenolic Compounds	+		
8.	Steroids	-		

(+ve): Presence of constituent (-ve): absence of constituent

TLC (Thin layer chromatography) of hydroalcoholic extract of stem bark of *Moringa oleifera* Lamk. (Ayurvedic Pharmacopoeia of India, Part I, Volume IX).

TLC is a semi quantitative method of analysis and its sophisticated version or highly precise quantitative version is high performance thin layer chromatography (HPTLC). It is based on adsorption chromatography, in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved. Once separation occurs, individual components are visualized as spots at respective level of travel on the plate. After development of chromatogram, the resolved spot are revealed by spraying suitable detecting agent⁸.

Material

- 1. Extract of sample
- 2. Solvent system-A mixture of Toluene, Ethyl acetate& Methanol(8:2:0.5)
- 3. TLC plate (Silica gel 60 F₂₅₄Merck KGaA, 64271)
- 4. Developing tanks
- 5. Spraying agent:10% H₂SO₄
- 6. Heating oven
- 7. UV lamp Detector.

Procedure

- The stationary phase is applied onto the plate uniformly and then allowed to dry and stabilize.
- 2. A thin mark is made at the bottom of the plate with a pencil to apply the sample spots.
- 3. Then samples solutions are applied on the spots marked on the line at equal distances.
- 4. The mobile phase mixture of Toluene, Ethyl acetate & Methanol (8:2:0.5) poured into the TLC tanks to a level few centimeters above the tanks bottom.
- 5. Then the plate prepared with sample spotting is placed in TLC tanks such that the side of the plate with sample line is towards the mobile phase. Then, the chamber is closed with a lid
- 6. The plate is immersed such that sample spots are well above the level of mobile phase but not immersed in the

solvent for development. Sufficient time is allowed for development of spots. Then the plates are removed and allowed to dry. The sample spots are visualized in suitable UV light chamber (Fig. 8).

Heat

Heat at 110 °C for 10 minutes and examines the plate under day light.

The sample spots are visualized in suitable UV light chamber. TLC plate was labeled, calculated the Rf Value for each spot using the following formula and took the image of it.

 $Rf value = \frac{Distance travelled by the substance}{Distance travelled by the solvent}$

Results

Rf Value: Spot 1- 0.22

Spot 2- 0.26

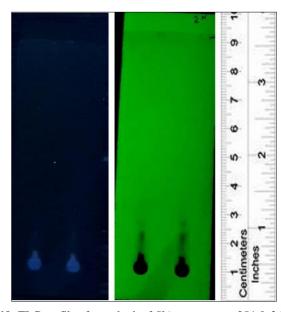


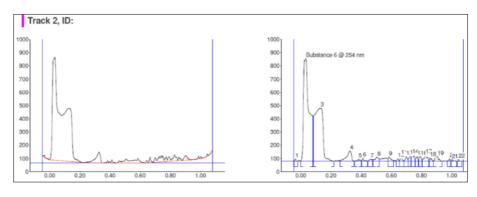
Fig 10: TLC profile of stem bark of *Shigru* extract at 254 & 366 nm

HPTLC of hydro - alcoholic extract of stem bark of *Moringa oleifera* Lamk

High performance thin-layer chromatography (HPTLC) is a major advancement of TLC (thin layer chromatography) principle requiring shorter time and better resolution. It is very useful in qualitative and quantitative analysis of pharmaceuticals. The basic difference between TLC and HPTLC is only in particle and pore size of the sorbents [9].

Methodology

The HPTLC fingerprinting profile of sample drug developed on Silica gel 60 F 254 as stationary phase. The plate was developed in Toluene: Chloroform: Ethanol: methanol (4:3:2:1) as mobile phase. The developed plates were visualized in UV 254 and 366 and scanned under UV 254 and 366 nm. Rf value of the spots and densitometry scan were recorded.



Peak	Start	Start Height	Max Rf	Max Height	Max %	End	End Height	Area	Area %
1	-0.04	3.1	-0.04	17.0	0.93	-0.02	0.0	86.7	0.19
2	0.00	2.2	0.03	777.6	42.75	0.08	342.4	21035.6	46.60
3	0.08	342.6	0.13	401.9	22.09	0.22	0.0	16707.3	37.01
4	0.26	4.5	0.33	80.6	4.43	0.35	0.9	1733.6	3.84
5	0.36	0.2	0.38	17.8	0.98	0.40	5.3	161.9	0.36
6	0.40	5.6	0.41	23.9	1.31	0.44	0.5	177.4	0.39
7	0.45	0.3	0.46	17.1	0.94	0.47	11.1	180.2	0.40
8	0.48	12.4	0.51	29.8	1.64	0.52	16.1	507.7	1.12
9	0.58	20.0	0.59	33.4	1.84	0.62	4.4	409.1	0.91
10	0.63	1.1	0.65	19.8	1.09	0.65	3.3	135.9	0.30
11	0.66	4.6	0.67	42.5	2.34	0.68	4.2	313.8	0.70
12	0.69	4.6	0.70	35.5	1.95	0.71	10.9	325.1	0.72
13	0.71	11.6	0.73	42.8	2.35	0.73	29.1	367.8	0.81
14	0.73	30.6	0.74	48.5	2.67	0.76	9.8	590.1	1.31
15	0.76	12.0	0.77	37.2	2.05	0.78	10.5	309.7	0.69
16	0.78	11.9	0.80	35.6	1.96	0.80	11.8	366.7	0.81
17	0.81	10.4	0.83	47.6	2.62	0.85	5.2	681.6	1.51
18	0.85	8.1	0.86	27.3	1.50	0.88	2.5	231.6	0.51
19	0.88	2.1	0.91	35.5	1.95	0.94	0.3	567.0	1.26
20	0.97	0.4	0.99	20.7	1.14	1.00	0.7	115.9	0.26
21	1.00	1.5	1.00	13.0	0.72	1.04	0.1	72.4	0.16
22	1.04	0.0	1.05	14.2	0.78	1.07	0.0	61.0	0.14

Fig 11: Presenting Rf peaks and area % of HPTLC (High Performance Thin Layer Chromatography)

Discussion & Conclusion

Pharmacognosy may be defined as a branch of bioscience which treats in detail medicinal and related products of crude or primary type obtained from plant, animal and mineral origins. It is an objective study of crude drugs from natural sources treated scientifically and it encompasses the knowledge of history, distribution, cultivation, collection, processing for market and preservation, the study of sensory, physical, chemical and structural characters and the uses of crude drugs [10]. In the present study, pharmacognostical standards have been established with regards to stem bark of *Shigru* (*Moringa oleifera* Lamk.).

In Powder microscopy examination: showed the presence of lignified cells: phloem fibres, cork cells, needle shape calcium oxalate crystals and aleurone grains. Percentage yield of hydro-alcoholic extract of *Shigru* is 10.37%.

On physio-chemical analysis of stem bark of *Shigru* (*Moringa oleifera* Lamk.) the moisture content was found 7.32%. The Total ash was found 13.5%; Acid insoluble ash was 5.9%; water soluble ash was 2.55% and foreign matter 0.5%.

The phyto-chemical analysis showed the presence of Carbohydrate, Protein, Amino acids, Glycosides, Alkaloids, tannins and phenolic Compounds in the stem bark of *Shigru* (*Moringa oleifera* Lamk.).

The TLC (Thin layer chromatography) and HPTLC (High performance thin layer chromatography) of stem bark of *Shigru* (*Moringa oleifera* Lamk.) –Hydro-alcoholic extract was performed and the developed plates were visualized in UV 254nm, 366nm. In TLC, two peaks were present with Rf value 0.22& 0.26 and HPTLC chromatogram with area % presented in previous picture (fig.-10&11).

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Conflict of interest

None

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