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Microscopical evaluation and physicochemical analysis of *Madhuca indica* (JF Gmel) and *Spondias mangifera* Willd stem bark

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Abstract
Numerous pharmacologically active ingredients from the plants *Madhuca indica* and *Spondias mangifera* have Hepatoprotective, analgesic, anti-inflammatory, and antioxidant properties. It is frequently used in several conventional formulas used to treat stomach issues. The need for high-quality herbal medications is rising daily in the modern world. The goal of the current study was to examine *M. indica* and *S. mangifera* stem bark according to microscopical, phytochemical, and physicochemical criteria. Phytochemicals such as reducing sugar, triterpenoids saponin, and phenolic compounds were found in the methanol extract of both plants. These microscopical and phytochemical criteria will aid future studies in pharmacological exploration, standardization, and pharmaceutical formulations.

Keywords: *Madhuca indica*, *Spondias mangifera*, Wild mango, stem bark, microscopical studies, phytochemical exploration

Introduction
Madhuca indica JF Gmel. Synonym *M. longifolia* (Koen.) Macb. var. *latifolia* (Roxb.) Cheval. *Bassia latifolia* Roxb. Family Sapotaceae is an Indian-origin plant with incredible curative potential for the treatment of several diseases [1]. The plant is an evergreen or semi-evergreen tree with a height of around 20 meters. The plant is commonly appreciated for its oil-bearing seeds in great quantities, blooms that are widely employed in the creation of alcoholic drinks, and timbers used in the manufacture of furniture. Mahua flowers are fermented and used as animal feed. India produces around 0.12 million tons of mahua seeds and blossoms from various parts of the nation in structured sectors that are utilized for oil extraction [2-3]. Different parts of the plant *viz*. entire young plant, leaves, stem, bark, root, fruit, flower, and seeds are used in folklore medicines and are used in different conditions such as in tonsillitis, influenza, piles, arthritic pain, helminthiasis, tuberculosis, rheumatoid arthritis, cholera, paralysis, snake-bite, debility, low semen count, headache, flatulency, and infections; further it is also used as a blood purifier and poison antidote [4].
Spondias mangifera Willd. (F. Anacardiaceae) is a fast-growing tree, commonly known as hog-plum or bile-tree and amrata in Ayurveda, widely distributed in the tropics and abundantly in the eastern and northeast regions of India [5]. All parts of the plant have a turpentine-like odor when broken or crushed [6]. It is a tree with a deep heritage in the traditional health system of Ayurveda and North-East people to control rheumatism [7]. It is given to prevent vomiting and in the treatment of dysentery and diarrhea [8]. The juice of ripe fruit is the most vitamin-rich and extremely acidic fruit in Assam, with potential use in nutraceuticals [9]. In nations where the tree is natively found, the green fruit is pickled in brine and frequently used in culinary dishes such as curries, sauces, jams, and sherbet. Both plants' barks are rich in phenolic chemicals and have long been used to treat illnesses. They are also components of several herbal remedies [10]. Because of their medicinal importance, the bark of both plants microscopical and physicochemical including macro-microscopic descriptions, physico-chemical characters, and preliminary phytochemical investigations have been carried out.

Material and Methods
Plant material: The bark of the plant *M. indica* was collected in May 2021 from a local area of District Shahjahanpur, India, and authenticated from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India with reference no. CIMAP/Bot. Pharm/2021/06.

The bark of the plant *S. mangifera* was collected in month December 2021 from a local area of district Chandouli (India) and validated by the Dept. of Life Science, Dibrugarh University, Assam, to find out the reference number (DLS/MS/2021/14).

Morphology and microscopy

After being chopped into smaller pieces using a sharp, clean knife, the bark was allowed to dry in the shade. The physicochemical parameters and macroscopic and microscopic characteristics were examined using the dried bark. The samples were examined with the unaided eye to confirm the morphological characteristics. For the microscopic evaluation of the *M. indica* and *S. mangifera* barks the transverse sections were cut with the free hand sectioning. The TS was put on a glass slide mounted with glycerin and observed under the binocular microscope. A camera was attached to the microscope and pictures of the transverse sections were taken [11].

Physicochemical parameters

The extractive value was measured for quantitative analysis, including loss on drying, total ash, acid insoluble ash, water soluble ash, and crude fiber content, following the official Indian Pharmacopoeia book. Using a variety of solvents, including water, methanol, petroleum ether, and chloroform, the extractive value of the bark powder was determined. The extractives' weight, color, and consistency were also determined. A preliminary phytochemical research was conducted on the various extractives to see if they contained distinct phytoconstituents [12-14].

Fluorescence analysis

Fluorescence analysis of the powder sample was carried out by treating it with different chemical reagents to observe various color instances [15].

Results

Morphology

Fig. 1a displays the morphological characteristics of the bark of *M. indica.* The inside surface is bricking brown and devoid of striations, while the exterior is even, smooth, and dark brown. The cracked surface is shattered and the fracture is splintery. Its powder has an astringent, mucilaginous flavor and is odorless.

Fig. 1b displays the morphological characteristics of the bark of *S. mangifera*. The bark has two surfaces: an inner chocolate brown tint with longitudinal striations, and an outside smooth, uniform, white-brown surface. Tiny concave depressions that create a thick colony of mosses are visible on the outside. It has a fibrous texture, a splintery cracked surface, a faint turpentine smell, and a mucilaginous, astringent taste.

Fig 1: (A) *Madhuca indica* stem bark and (B) *Spondias mangifera* stem bark

Microscopy

The bark of *M. indica* is divided into outer bark (periderm) and inner bark (secondary phloem) by a transverse slice of 2.2 mm. Simple superficial homogeneous cork cells make up the periderm, which also includes phellogen with three to five-layer thicknesses. The phelloderm, which has five to seven layers arranged in a radial plane, is large and conspicuous. The phelloderm cells lack a defined cell composition and have thin walls. The axial parenchyma of the inner bark has the most noticeable characteristic of the collapsed phloem zone: Random distribution. The phloem ray cells contain a large number of tannins (Fig 2).

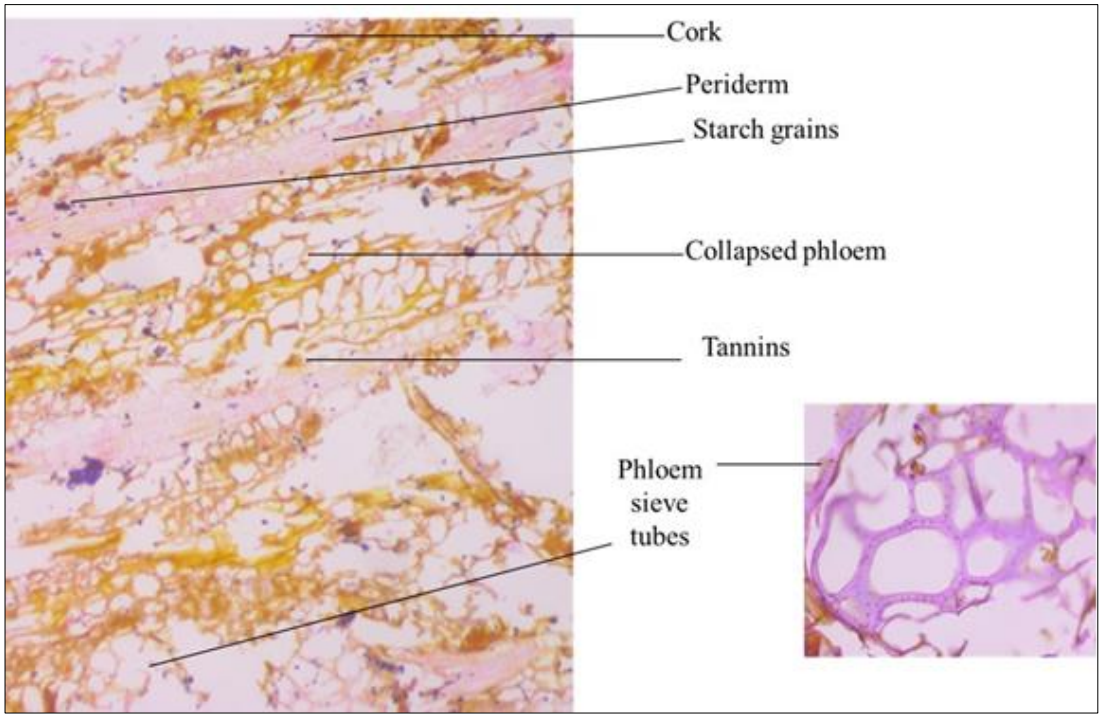

Fig 2: Microscopical features of *Madhuca indica* stem bark 40X views of microscope showed xylem, phloem vessels, and tannins

The transverse section of *S. mangifera* bark has a thickness of 2.4 mm and is differentiated into outer bark (periderm) and inner bark (secondary phloem). Simple superficial homogeneous cork cells, or phellogen-a portion of the periderm with thicknesses ranging from three to four layers-make up the outer bark. The walls of the suberized cells are homogeneous and tangentially oblong. The phelloderm, which has five to seven layers arranged in a radial plane, is large and conspicuous. The phelloderm cells lack a defined cell composition and have thin walls. The axial parenchyma of the inner bark has the most noticeable characteristic of the collapsed phloem zone: random distribution. Cubical crystals may be seen in the cross-sectional view, but they take on an extended rectangular form in the longitudinal section. The majority of the fusiform fibers in the phloem sclerenchyma have thick lignified walls and a small lumen16. The phloem ray cells contain a large number of tannins (Fig 3).

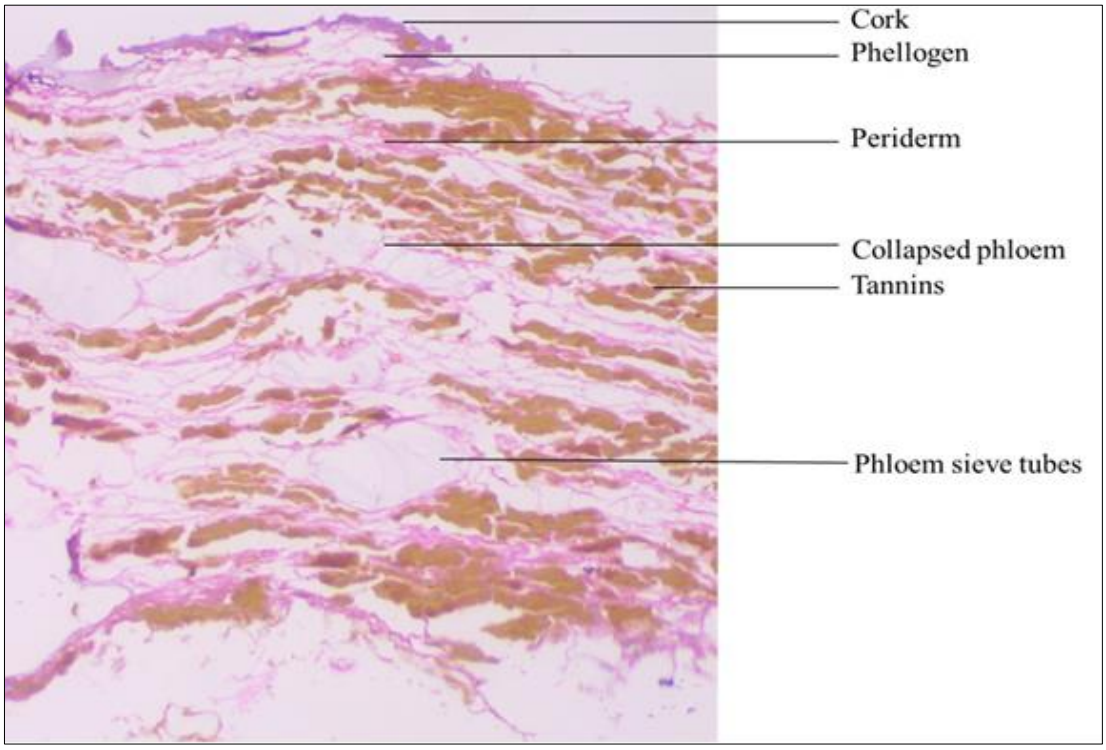

Fig 3: Microscopical features of *Spondias mangifera* stem bark 40X views of microscope showed xylem, phloem vessels, and tannins

Physicochemical evaluation

Physicochemical evaluation of the bark of both plants like ash values, loss on drying, and crude fiber contents of the dried sample was determined as per Indian Pharmacopoeia and results are shown in Table 1.

Table 1: Physicochemical analysis for powdered bark *M. indica* and *S. mangifera*

Parameters	Values in (% w/w)
Loss on Drying	
M. indica	6.5±0.171
S. mangifera	11.27±0.228
Ash Value	
Total ash	
M. indica	6.95±0.115
S. mangifera	4.89±0.164
Acid insoluble ash	
M. indica	0.81±0.060
S. mangifera	0.72±0.050
Water soluble ash	
M. indica	4.49±0.040
S. mangifera	3.62±0.073
Crude fiber contents	
M. indica	21.27±0.153
S. mangifera	18.83±0.252

Extractive values

By assessing the grade and purity of the crude pharmaceuticals, the extractive values aid in the evaluation of their nature. Adulterants can also be identified using it. It was ascertained utilizing a cold successive solvent extraction technique using methanol and water. Using the air-dried drug as a guide, the color, consistency, percentage, and mean values±SEM of the extractive values in triplicate were determined Table 2.

Table 2: Cold extractive values for powdered stem bark of *M. indica* and *S. mangifera*

Parameters	Colour of dried extract	Values in (%)
Pet. Ether *M. indica* *S. mangifera*	 Colorless Colorless	 0.68±0.172 0.86±0.120
Methanol *M. indica* *S. mangifera*	 Reddish brown Dark brown	 7.43±0.671 8.98±0.203
Water *M. indica* *S. mangifera*	 Italic brown Chocolate brown	 8.46±0.482 9.50±0.220

Fluorescence analysis

The fluorescence analysis of the powdered bark treated with different reagents in daylight, short UV, and long UV was examined by reported methods. The observations are given in Tables 3 and 4.

Table 3: Fluorescence analysis of *Madhuca indica* bark powder

S.N.	Treatment	Day Light	Short UV Light (254 nm)	Long UV Light (365nm)
1	Drug Powder + 1N NaOH	Reddish Brown	Greenish black	Bluish Black
2	Drug Powder + 1N NaOH (Alc.)	Reddish Brown	Slight Black	Dark Black
3	Drug Powder + Ammonia Solution	Reddish Brown	Greenish black	Dark Black
4	Drug Powder + Picric Acid	Yellow	Light Green	Greenish Yellow
5	Drug Powder + HCl (50%)	Brown	Greenish Black	Bluish Black
6	Drug Powder + H_2SO_4	Brown	Greenish Black	Black
7	Drug Powder + Pet. ether	Brown	Greenish Black	Black
8	Drug Powder + Methanol	Brown	Greenish Brown	Black

Table 4: Fluorescence analysis of *Spondias mangifera* bark powder

S.N.	Treatment	Daylight	Short UV light (254 nm)	Long UV light (365 nm)
1	Drug Powder + 1N NaOH	Straw color	Dark color	Yellowish color
2	Drug Powder + 1N NaOH (Alc.)	Straw color	Dark color	Yellowish fluorescence
3	Drug Powder + Ammonia Solution	Pink brown	Dark brown	Pink orange
4	Drug Powder + Picric Acid	Yellow	Brown	Greenish yellow
5	Drug Powder + HCl (50%)	Straw color	Chocolate brown color	Yellowish green
6	Drug Powder + H_2SO_4	Straw color	Dark brown	Greenish color
7	Drug Powder + Pet. ether	Greenish yellow	Brown yellow	White
8	Drug Powder + Methanol	Yellowish brown	Green	Dark green

Preliminary phytochemical screening

The presence or absence of different phytoconstituents *viz*. alkaloids, reducing sugar, glycosides, flavonoids, terpenoids, steroids, tannins, and phenolic compounds were detected by usually prescribed methods and results are given in Tables 5 and 6.

Table 5: Phytochemical analysis of bark powder of *Madhuca indica*

S.N.	Tests	Pet. ether	Chloroform	Methanol
1	Alkaloids	_	_	_
2	Reducing sugar	_	_	+
3	Cardiac glycoside	_	_	_
4	Saponins	_	_	+
5	Anthraquinones	_	_	_
6	Flavonoid	_	_	+
7	Tannins	_	_	+
8	Phenolics	_	_	+
9	Steroids	+	+	+
10	Triterpenoids	+	+	+
11	Proteins	_	_	+
(+) Present, (-) Absent				

Table 6: Phytochemical analysis of bark powder of *Spondias mangifera*

S.N.	Tests	Pet. ether	Chloroform	Methanol
1	Alkaloids	_	_	_
2	Reducing sugar	_	_	+
3	Cardiac glycoside	_	_	_
4	Saponins	_	_	+
5	Anthraquinones	_	_	_
6	Flavonoid	+	+	
7	Tannins	_	_	+
8	Phenolics	_	_	+
9	Sterols	+	+	+
10	Triterpenoids	+	+	+
11	Proteins	_	_	+
(+) Present, (-) Absent				

Discussion

The curious organoleptic and microscopic features of *Madhuca indica* and *Spondias mangifera* are essential in proving their authenticity and serve as analytical tools for the selection of these plants. Both plants have unique physical traits in their stem bark. The cork layer, phloem fibers, and secondary xylem are among the distinctive feathers that are indicative of these plants, as shown by the T.S. micrograph on the stem bark. The plant material's T.S. was carried out by free-hand sectioning and safranin staining. The drying result

indicates that the crude powder was dried appropriately and stored appropriately. When evaluating the purity of a medicine, the values of total ash, acid-insoluble ash, and water-soluble ash are mostly important. To find the existence of different phytoconstituents, a qualitative chemical analysis was performed on all the extractives of both crude medications. Both plants' phytochemical analyses revealed the presence of triterpenoids, phenolic compounds, steroids, flavonoids, and saponins in addition to reducing sugars. Triterpenoids and phytosterol are present in the Pet. ether and chloroform extracts. Methanol-based extract demonstrates the presence of polar components such as glycosides, flavonoids, phenols, and reducing sugar.

Conclusion

The morphological, microscopic, and physicochemical data gathered during the aforementioned analyses may prove beneficial in the creation of pharmaceutical formulations as well as in the standardization of the extracted medication. Potential acids, phytosterols, terpenoids, flavonoids, phenolic compounds, tannins, peptides, and reducing sugars are among the phytoconstituents found in bark. These substances also need to have antioxidant properties, stabilize mast cells, and have the ability to act against allergies, ulcers, tumors, platelet aggregation, control hypertension, and have immunomodulatory effects. The components of this plant have a significant effect on the healthcare system and may offer advantages for treating or preventing certain illnesses.

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