Pharmacognostical studies on stem bark of *Madhuca longifolia* (Koen.) Macbr. var. *latifolia* (Roxb.) A. Cheval.

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*Madhuca longifolia* (Koen.) Macbr. var. *latifolia* (Roxb.) A. Cheval. of Sapotaceae family is commonly known as *Mahuwa*. The stem bark is used by the aboriginals of Maharashtra in curing itch, bleeding gums, cyst, ulcers, madhumeha, etc. Although the bark is in use, deliberate attempt to study them has lacked. Pharmacognosy is the first step in deciding the status of a plant organ as a crude medicine; hence the current study was done. The present study comprises macroscopy, microscopy, histochemistry, physicochemical parameters, fluorescence analysis and preliminary phytochemistry. TLC of saponin present in the drug is carried out to establish the biomarker compound. These studies will help in establishing the pharmacopoeial standards for the said drug.

**Keywords:** *Madhuca longifolia* var. *latifolia*, *Mahuwa*, Pharmacognosy, Sapotaceae, Stem bark.

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**Introduction**

*Madhuca longifolia* (Koen.) Macbr. var. *latifolia* (Roxb.) A. Cheval. commonly known as *Mahuwa* belongs to family Sapotaceae. This tree is well known among the aboriginals (*Mahadeo-Koli, Agaree* and *Warli* tribes) of Maharashtra for its economic as well as medicinal value. The bark is recommended for phlegm and in rheumatism bark flakes are mildly heated and tied on the joints. For bleeding gums stem bark is powdered and used as tooth powder for strengthening the gums. In diarrhoea a cup of infusion of bark is taken orally twice a day by the tribals. Besides the stem bark is used in chronic tonsillitis, fever, leprosy, etc. Although this plant part is in use very little work is done on its pharmacognostical studies, hence the present investigation was carried out.

It is a tall deciduous tree with milky latex. Leaves are clustered near the ends of the branches, stipulate, elliptic to obovate, tomentose, when young deeply pink in colour, glabrous, base rounded or acute. Flowers in dense fascicles near the ends of the branches. Calyx with 4 sepals arranged in 2 whorls. Corolla cream coloured, twice as many as sepals. Stamens 20-24, staminodes zero. Ovary hirsute, hairy at the base, style 1. Fruit a berry (Plate I.1).

**Material and Methods**

The bark was collected during summer season from wild plant growing in Murbad district in Maharashtra. The sample was authenticated for its botanical identity from the standard herbaria at Blatter herbarium (Mumbai). A voucher specimen has been deposited in Botany Research Laboratory of K.V. Pendharkar College, Thane, India (Acc. No. 36504). After collection some of the bark pieces were preserved in FAA solution. Remaining stem bark pieces were dried and made into powder. Pharmacognosy of the bark was carried out using standard methodology.

**Macroscopy:** The barks were studied for its morphological characters using the appropriate techniques.

**Microscopy:** Transverse hand cut sections were taken and made permanent with suitable stains. Quantification and photomicrographs were taken of the permanent preparations. The cell contents were measured using stage and ocular micrometer.
Histochemistry: The histochemical studies for the cell contents were performed using standard methodology.14

Powder study: The powdered drug was soaked in aqueous solution of chloral hydrate and mounted in 50% glycerin for microscopical studies.15

Proximate analysis: The physico-chemical parameters like ash values and extractive values were done.16

Fluorescence analysis: The fluorescence response of powdered drugs exposed to UV radiations (365 nm wavelength) was studied using the standard procedure.17,18
Preliminary phytochemical screening: A known quantity of dried powder was extracted with chloroform, alcohol and water. These extracts were tested for different constituents.

Thin Layer Chromatography (TLC):

**Extraction of Saponins:** Powdered sample (5 g) was refluxed with 25 ml methanol (90% v/v) for an hour. The residue was extracted 2 more times with 25 ml methanol and solvent was evaporated; the soft extract left after evaporation was treated with 25 ml petroleum ether at 60-80°C and refluxed for half an hour. After cooling the solvent was removed by decantation. The soft extracts obtained were treated with 25 ml chloroform and 25 ml ethyl acetate and were again refluxed. Further the solvents were removed after cooling. The soft extracts obtained were dissolved in 25 ml methanol (90% v/v) and then filtered and concentrated to 5 ml. The concentrated extracts were dropped into 25 ml acetone with constant stirring to precipitate the saponins. The precipitate obtained was filtered, collected and dried at 105°C.

For TLC dried residue was dissolved in methanol and the standard saponin was also dissolved in methanol (2mg/ml). Both the samples were loaded on silica gel 60F (E Merck). The plate was developed using, chloroform : glacial acetic acid : methanol : water (6 : 3.2 : 1.2 : 0.8). The plate was sprayed with Anisaldehyde sulphuric acid.

Results and Discussion

Macroscopical characters of stem bark

Stem bark is quilled, 3-4 cm in thickness. Outer surface is grayish brown with deep vertical cracks exfoliating in scales. Inner bark when fresh is deep pinkish red in colour, on drying it turns brown with striations of milky latex. It has fibrous fracture, without any odour and with astringent taste (Plate I.2).

Microscopical characters

Transverse section of stem bark exhibits following:

**Cork** shows two types of cork cells, thin walled radially widened and few thick walled radially flattened cells (26.6 - 43.29 μm in breadth and 15.6-26.6 μm in length). These cells alternate in radial rows. Suberized tannin filled cells and scattered starch grains in various layers are observed.

**Cork cambium** is thin walled and few thick walled radially flattened cells (33.3-66.6 μm in breadth and 16.6-39.96 μm in length); usually 2-3 layered, tangentially elongated.

**Secondary phloem** extend into **Secondary cortex** hence cannot be distinguished from one another. This broad layer consists of polygonal cells (38.3-69.93 μm in diam.). It shows radial parenchymatous rays in zigzag pattern. Rays are uni-biseriate (33.3-43.29 μm in breadth and 36.36-47.3 μm in length) containing simple and compound type of starch grains with few prism shaped calcium oxalate crystals (2.14-4.9 μm in breadth and 5.64-8.76 μm in length). The phloem tissue is associated with thick patches of pitted fibers (73.26-79.26 μm in breadth and 39.9-49.95-54.6 μm in length). Tannin filled cells, patches of stone cells and non septate laticiferous tissues are also observed in this region. This layer is followed by thin layer zone which is continuous with secondary phloem.

**Innermost secondary phloem** consists of polygonal compactly arranged cortical cells (29.97-35.6-39.8 μm in diam). Most of the cortical cells are filled with abundant simple and compound starch grains (2.6-4.5 μm in diam.). This region is interrupted by uni-biserriate phloem rays (14.5-18.6-25.6 μm in breadth and 32.4-46.5-60.7 μm in length). Broad patches of stone cells alternate with phloem rays at regular intervals (23.43-35.6 μm in diam.). Rectangular cells of compactly arranged sclerenchymatous patches with pits are observed (74.6-80.4 μm in breadth and 40.42-47.2-50.34 μm in length). Prismatic calcium oxalate crystals, tannin filled cells and laticiferous cells are observed frequently (Plate I.3, 4, 5 & 6).

Powder study

Powdered bark is brown, non aromatic, astringent and coarse in texture. Microscopically, the powder shows: cork cells, cortical cells, stone cells, crystal fibers, tannin filled cells, fibers, starch grains of simple and compound types, calcium oxalate crystals are prism shaped, phloem fibers and sclerenchymatous patches are thick walled, internally simple pitted (Plate I.7-I.12).

Histochemical tests

The sections of stem bark treated with different reagents showed the presence of primary chemical constituents such as starch, lipids, proteins, tannins, saponins, glucosides, calcium oxalate crystals and mucilage.

Physicochemical studies

The physicochemical constants such as ash values showed total ash 7.05%, water soluble ash 5% and
acid insoluble ash 1%. Thus the acid insoluble ash value states the presence of least amount of silica in the bark powder. The extractive value of water is 11.4%, ethanol 23.04% and chloroform 2.60%. The above extractive values determine that more chemical constituents are soluble in the solvent ethanol.

**Fluorescence analysis**

The bark powder treated with different chemicals exhibited various colours in the UV light. The predominant colour was brown in most of the test. The results are depicted in Table 1.

**Preliminary phytochemical analysis**

The preliminary phytochemical studies revealed the presence of diverse types of primary and secondary metabolites like starch, proteins, amino acids, mucilage, terpenoids, antraquinone glycosides, cardiac glycosides, saponins and tannins in water, alcohol and chloroform extracts (Table 2).

**TLC:** The sample of plant extract gave R_f value of 0.2 and the standard saponin also revealed R_f value at 0.2 (Plate I.13)

**Diagnostic characters**

*Madhuca longifolia* var. *latifolia* is identified by its elliptic to obovate, tomentose leaves and stamens. The stem bark is identified by presence of uni-biseriate rays, sclerenchymatous cells with pits, simple and compound starch grains, prismatic calcium oxalate crystals, stone cells and non septate laticiferous tissues.

**Conclusion**

The macroscopical and microscopical studies will be useful in identifying the plant drug in crude form. The above microscopical powder study, physicochemical analysis and fluorescence test will prove the authenticity of the drugs in powder form. Histochemical and preliminary phytochemical screening will provide the general idea regarding the presence of primary and secondary metabolites. TLC detected the presence of saponin, a biomarker compound. Thus, the pharmacognostical standards put forth can add valuable information about the said plant and can be a potential source of saponin yielding herbal drug.

**References**


**Table 1—Fluorescence analysis (UV 365 nm)**

<table>
<thead>
<tr>
<th>Test</th>
<th>i</th>
<th>ii</th>
<th>iii</th>
<th>iv</th>
<th>v</th>
<th>vi</th>
<th>vii</th>
<th>viii</th>
<th>ix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence</td>
<td>2F</td>
<td>3yF</td>
<td>1F</td>
<td>2yF</td>
<td>3P</td>
<td>Bf</td>
<td>yF</td>
<td>2yF</td>
<td>yF</td>
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</tbody>
</table>

Keys to the letters and numbers used-

- Predominant colours: Modifying colours: Quality of colours:
  - F- Brown y- Yellowish 1 Very light
  - B- Blue g- greenish 2 Light
  - P- Purple 3 Dark

**Table 2—Preliminary phytochemical analysis of stem bark**

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Water extract</th>
<th>Chloroform extract</th>
<th>Ethanol extract</th>
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<tbody>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
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<tr>
<td>Proteins</td>
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<td>+</td>
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<tr>
<td>Amino acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Mucilage</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Alkaloids</td>
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<td></td>
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<tr>
<td>Antraquinone glycoside</td>
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<td>+</td>
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<tr>
<td>Cardiac glycoside</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Saponin</td>
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<td>Tannins</td>
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<tr>
<td>Steroids</td>
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<td>Flavonoids</td>
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</tbody>
</table>

+ Present, - Absent
17 Chase C R and Pratt R, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *J Amer Pharm Assoc*, 1949, **38**, 324-333.


19 Brain K R and Turner T D, The practical evaluation of phyto-pharmaceuticals, Wright Scientechnika, Bristol, 1975, pp. 4-17, 36-58, 81-90.


