Pharmacognostical studies and evaluation of total phenolic contents of trunk bark of *Spondias mangifera* Willd.

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Received 15 May 2008; Accepted 1 January 2009

Abstract

*Spondias mangifera* Willd., commonly known as Wild mango or Hog plum (Hindi-*Amara*) is an important medicinal plant. The dry bark is silver brown coloured with mucilaginous astringent characters. It is traditionally used in North-East regions of India as refrigerant, aromatic, tonic and for the treatment of dysentery, diarrhoea and rheumatism. This study deals with the pharmacognostical evaluation of its dried trunk bark which includes macro and microscopic studies, determination of physicochemical parameters of the extract using TLC fingerprinting.

Keywords: *Spondias mangifera*, Wild mango, Hog plum, *Amara*, Phenolic contents, Macroscopic, Microscopic.

IPC code; Int. cl.—A61K 36/22, A61P 1/12, A01G 17/00

Material and Methods

The bark material was collected in the month of November 2006 from the campus of Dibrugarh University, Assam and identified from the Department of Life Sciences, Dibrugarh University, Assam. A voucher specimen No L-32/06 was retained in this laboratory for further reference.

For microscopic studies, a manual section was prepared transversely and longitudinally stained with safranin and fast green. Histological studies were performed for the presence of lignin, suberin, tannins, mucilage, starch grains and types of crystal present. For quantitative analysis, viz. total ash, acid insoluble ash, water soluble ash, total cold and hot water soluble extractives and successive soxhlet extractives were assayed, according to standard Indian Pharmacopoeia methods. For the TLC fingerprint different polar and non-polar solvent extractives were analysed. The mobile phase solvent system Toluene: Ethyl acetate: Water: Acetic acid were used in ratio of (7: 3: 1: 0.1). The Rf values and colours are recorded.

For fluorescence analysis of the powder sample it was treated with different chemical reagents to observe various

Introduction

*Spondias mangifera* Willd. (Family-Anacardiaceae) is a glabrous tree with characteristic pleasant smell of wood. There are about twelve species of the genus *Spondias* being native of Indo-Malaysia, South Eastern Asia and tropical America. In India it is cultivated in Punjab, Maharashatra, Bengal and Assam for the edible fruits. All parts of the plant have foetid, turpentine like odour when broken or brushed; the smell varies from species to species and is characteristic. Perusal of literature reveals that information on the bark of this species is minimal. Ethnomedicinally, the trunk bark is used as refrigerant, tonic and for the treatment of articular and muscular rheumatism and in dysentery and diarrhoea of the tree is used as a demulcent and also for fumigation. The leaves are aromatic, acidic and astringent used for flavouring while its juice is applied in ear ache. Bark paste with three bulb of garlic given twice a day for three days in stomach pain in *majidi* area of Hazaribag district, Jharkhand. The root bark powders have been recommended for regulation of menstruation. About 10g of tender fruit juice mixed with 50g of sugar candy and 8-10 grain of black pepper powder is popular home remedy for biliousness. The ripe plant fruit has nutraceutical potentiality of a minor fruit of Assam. In view of the medicinal importance, the pharmacognostical examination of the plant was carried out and results are presented.
colour reactions which may help to ascertain the purity of the drugs\textsuperscript{13}.

The total phenolic content of different extracts/fractions of the bark was determined according to the method described by Taga \textit{et al}\textsuperscript{15}. Suitable aliquots of different extracts/fractions was taken in a test tube and made up to the volume of 1ml with distilled water. Then, 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20\%) were added sequentially in each tube, tubes were vortexed, placed in dark for 40 min and the absorbance was recorded at 725nm. The amount of total phenolics was calculated as gallic acid equivalents /mg from the extract.

\section*{Result and Discussion}

\subsection*{Macro & microscopic characters}

The surface of the bark is smooth, even, silver brown in colour. At certain places there are small concave depressions forming a dense colony. It is fibrous in texture and cut surface is smooth with slight turpentine like odour and mucilaginous astringent taste (Fig. 1). The bark has total thickness of 2.6 mm and differentiated into outer bark (periderm) and inner bark (secondary phloem). The outer bark consists of simple superficial homogenous cork cells, phellogen as the part of periderm with four to six layer thicknesses. The cells are uniform tangentially oblong and walls are suberized\textsuperscript{12} (Fig. 2a). The phelloderm is broad and prominent consisting of seven to ten layers in radial plane. The phelloderm cells are thin walled with no specific cell contents. The most striking feature of the collapsed phloem zone is
the abundance of calcium oxalate crystals, randomly distributed in the axial parenchyma of the inner bark. The crystals are basically of rhomboidal and prism type. The cross sectional view reveals cubical crystals while in longitudinal section, they appear in elongated rectangular shape. The phloem sclerenchyma is mostly of fusiform fibres with thick lignified walls and narrow lumen\(^6\). The phloem ray cells are heavily loaded with tannins (Fig. 2b).

The powdered bark reveals fibrous flesh, brown in colour with slight turpentinic odour and mucilaginous astringent taste. Simple hexagonal two types of cork cells are found. Typically thick walled dark brown colour periderm and thin walled light brown coloured phellem cells are observed. Cork cells are stratified, appears like benzene ring in surface view. Stone cells present in the groups are barrel shaped having elongated walls with pitted thickening. Phloem fibres with linear fusiform arrangement are visible as highly pitted, yellowish green in colour, 101.25\(\mu\)m thick. Calcium oxalate crystals are also present as prisms scattered all over the powder with a diameter of 10.8\(\mu\)m. Prominent medullary rays are also observed in tangential longitudinal view. Black blue colour starch grains are also seen inside the cells that are scattered over the slide when treated with iodine\(^7\) (Fig. 3). The results of fluorescence analysis\(^8\) of powdered trunk are tabulated in the Table 1.

**Phytochemical studies**

Different successive extractives were subjected to qualitative phytochemical screening for the presence of phenolics, flavonoids, free sugars, alkaloids, terpenoids, steroids, saponins, tannins and xanthoproteins. The samples showed positive tests for mainly flavonoids, phenolics, tannins, xanthoproteins and free sugars. The percentages of total ash, acid insoluble ash and water soluble ash were determined (Table 2). Cold and hot water soluble extractives and successive solvent extractive values of crude bark in petroleum ether, chloroform, acetone and methanol were also determined. The experiments were carried out in triplicate and their mean values ± SD calculated. The results are tabulated in Table 3. The number of spots of TLC fingerprints is shown in (Figs. 4a & 4b) and Rf values of colours are tabulated in Table 4.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>Day light</th>
<th>Short UV light 254nm</th>
<th>Long UV light 365nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dry powder</td>
<td>Straw colour</td>
<td>Deep dark brown</td>
<td>Yellowish colour</td>
</tr>
<tr>
<td>2.</td>
<td>Power + 1M NaOH alcoholic</td>
<td>Straw colour</td>
<td>Dark colour</td>
<td>Yellowish fluorescence</td>
</tr>
<tr>
<td>3.</td>
<td>Powder + 1M HCl aqueous</td>
<td>Straw colour</td>
<td>Chocolate brown</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>4.</td>
<td>Powder + 1 M HCl alcoholic</td>
<td>Straw colour</td>
<td>Coffee brown colour</td>
<td>Greenish colour</td>
</tr>
<tr>
<td>5.</td>
<td>Powder+ 50% H(_2)SO(_4)</td>
<td>Straw colour</td>
<td>Dark brown</td>
<td>Greenish colour</td>
</tr>
</tbody>
</table>

**Table 2: Quantitative standards for the *Spondias mangifera* bark\(^{13,18}\)**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Values of 3 Replicates (%) w/w</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Ash Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Total ash</td>
<td>12.23 14.70 11.70</td>
<td>12.87 ± 1.60</td>
<td></td>
</tr>
<tr>
<td>b) Acid insoluble ash</td>
<td>2.55 2.06 2.78</td>
<td>2.46 ± 0.367</td>
<td></td>
</tr>
<tr>
<td>c) Water soluble ash</td>
<td>7.09 7.60 7.32</td>
<td>7.33 ± 0.255</td>
<td></td>
</tr>
</tbody>
</table>
Phenolic contents

These extracts and fractions were found to have various phenolic levels ranging from 83 to 138 (µg/ml) (Table 5). The highest concentration of total phenolics was present in ethyl acetate and butanol fractions. The total content of phenolic compounds in ethyl acetate fraction was 117µg/ml and butanol fraction was 138µg/ml.

Conclusion

From the ongoing studies, it can be concluded that the above pharmacognostical characteristics, phytochemical parameters, TLC fingerprint profiles and microscopic characters, together, may be utilized for the identification of *S. mangifera* trunk bark and differentiating it from other species. Due to the presence of phenolic compounds the species is recommended as potential anti-inflammatory, immunomodulatory, mast cells stabilising, blood pressure and cholesterol lowering natural resource.

Acknowledgement

We are thankful to the All India Council for Technical Education (AICTE) for providing financial Assistance for this project work. We also express our thanks to Prof. P. K. Gogoi, Deptt of Chemistry, Dibrugarh University, Assam for assistance in chemical analysis.
References


