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Pharmacognostic Investigation and Authentication of Potentially Utilized Fruit *Spondias mangifera* (willd)

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ABSTRACT
Spondias mangifera Willd (Hog-plum) a native plant of Indo-Malaysia is best known for its fruits, commonly used in culinary preparations such as curries, condiments, jams, sherbet in countries where the tree grows naturally. Green fruit is pickled in brine which is rich of minerals, acids, phenolic compounds, flavonoids, vitamins, peptides and reducing sugars. The fruit is aromatic, astringent, refrigerant traditionally used to give tone and treatment of rheumatic articular and muscular pain. It is given in preventing vomiting and in the treatment of dysentery and diarrhoea. This study deals with the pharmacognostical evaluation and some potential application of its fresh/dried fruits which includes macro and microscopic studies, determination of physicochemical parameters of the extract using TLC fingerprinting.

Keywords: *Spondias mangifera,* Hog plum, Wild mango, Amrata, Pharmacognostic evaluation, Neutraceutical potential.

INTRODUCTION

Spondias mangifera Willd. (F. Anacardiaceae) is a fast growing tree allied to Mangifera, commonly known as Hog-plum or Bile-tree and Amrata in Ayurveda, widely distributed in the tropics and abundantly in the eastern and in north-east region of India. [1] All parts of the plant have foeted turpentine like odour when broken or brushed. [2] It is the tree with rich tradition in the ancient health system of Ayurveda and North-East people for the management of rheumatism. The fruit is aromatic, astringent, refrigerant used to give tone and treatment of rheumatic articular and muscular pain. [3] It is given in preventing vomiting and in the treatment of dysentery and diarrhoea. [4] About 10 g of tender fruit juice mixed with 50 g of sugar candy and 0.6-0.8 g of black pepper powder is popular home remedy for biliousness. [5] The ripe fruit juice is highly acidic richest source of vitamins and has nutraceutical potentiality of a minor fruit of Assam. [6] The green fruit is pickled in brine and it is commonly used in culinary preparations such as curries, condiments, jams, sherbet in countries where the tree grows naturally.

In view of the medicinal importance, the drug was studied for different Pharmacognostic parameters including macro-microscopic descriptions, physico-chemical characters, TLC profile and preliminary phytochemical investigation of the plant fruit and results are presented that can be helpful in authentification and standardisation of plant materials.

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MATERIAL AND METHODS

Plant material

The fruit of the plant was collected in the month of November 2008 from the campus of Dibrugarh University, Assam and identified by Dr. Prof. Muhibul Islam from the Department of Life Sciences, Dibrugarh University, Assam. A voucher specimen No DULS-32/08 was retained in this laboratory for further reference.

The size of fruits was reduce by cutting with clean and sharp knife and dried in shade. The fresh fruit was used for the examination of macroscopic and microscopic characters whereas the dried fruit powder was used for determination of physico-chemical parameters. After drying the fruit, percentage of moisture content of fresh fruit was determined. The microscopic sections were cut by free hand sectioning and various parts of the fruit were examined and draw with help of camera Lucida. [7] Histochemical colour reactions were examined for the presence of lignin, suberin, tannins/phenolics, mucilage, starch grains, Ca-oxalate crystal, alkaloids, flavonoids, proteins, saponins by standard methods. [8]

Fig. 1a: Fresh fruit of *Spondias mangifera*

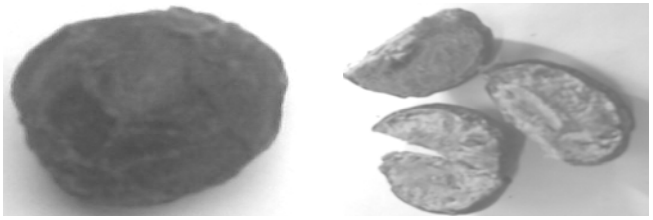

Fig. 1b: Dried fruit of *Spondias mangifera*

Fig. 1c: Seed of *Spondias mangifera*

Physicochemical parameters

For quantitative analysis, viz. Loss on drying, total ash, acid insoluble ash, water soluble ash, crude fibre content, extractive value were assayed, according to standard Indian Pharmacopoeia methods. [9] Successive soxhlet extractives of the drug were carried out with various solvents like petroleum ether, chloroform, ethyl acetate and methanol and weight, colour/consistency of the extractives were observed. [10]

Preliminary phytochemical screening

The different extractives were subjected to preliminary phytochemical investigation for the presence of various phytoconstituents. [11]

The fruit powder was incinerated and treated with dilute HCl and subjected for the detection of different inorganic elements also.

Fluorescence analysis

Fluorescence analysis of the powder sample was carried out by treating with different chemical reagents to observe various colour instances. [12]

TLC fingerprint profile

For the TLC fingerprint the methanolic extract was analysed. The mobile phase solvent system Methanol: Ethyl acetate: Acetic acid: Formic acid was used in ratio of (40: 20: 2: 2). Anisaldehyde-suphuric acid reagent was used as detecting agent. The R_f values are compared with standard drug Gallic acid and colours are recorded. [13]

RESULT AND DISCUSSION

Organoleptic characters

The fruit is simple, succulent, fibrous and drupe type of fruit. The Epicarp is thin, greenish yellow when ripe. Mesocarp is soft acidic, juicy when ripe, aromatic, 6-8 celled and Endocarp is tough, fibrous and woody Fig. 1a, 1b.

Shape & size : Ovoid or oblong, up to 4-5 cm in diameter.

Colour : Fresh fruit is yellowish green and dried fruit is externally dark brown and internally yellowish brown.

Taste : Astringent.

Odour : Aromatic pleasant

Texture : Hard, stone semi-woody, fibrous with many cavities outside.
Epicarp and mesocarp is very brittle, easily fragmented from fibrous endocarp after drying.

Microscopy: The detailed TS of mature fruit passing through the centre are circular in outline with Epicarp, mesocarp and fibrous endocarp Fig. 2.

Table 1: Histochemical colour reactions of powdered fruit of *S. mangifera* with different chemical reagents

Reagents+ powdered fruit	Constituents	Colour	Degree of Intensity
Phloroglucinol + Conc. HCl	Lignin	Pink	+++
Aniline hydrochloride	Lignin	Bright yellow	++
Chlor-zinc-Iodine Solution	Suberin	Yellowish brown	--
Ruthenium red solution	Mucilage	--	--
Iodine solution	Starch	Blue	+
Millon's reagent	Protein	Brick red	++
Dragendorff's reagent	Alkaloids	--	--
Conce. NaOH (Aq)	Flavonoids	Golden yellow	+++
Frothing test	Saponins	--	--
Aqs Ferric Chloride	Phenolics / Tannins	Smoke colour	++
Keddy reagent	Sterol glycoside	Pink colour	++
Salvoski's test	Steroids	Orange color at the junction of two layer	++
5% KOH (Aq)	Anthraquinone glycoside	--	--
Spot test	Fixed oil	--	--

+++ High, ++ Moderate, + Slight, -- Negative.

Table 2: Quantitative Standards for Powdered fruits of *S. mangifera* (Values in %)

Parameters	Values of 3 Replicates (%) w/w	Mean ± SEM
1) Moisture content	69.81% 13.69%	--
2) Loss on Drying	14.20% 13.42%	13.77±0.228
3) Ash Value		
a) Total ash	5.24% 5.50% 5.74%	5.49±0.144
b) Acid insoluble ash	0.78% 0.85% 0.81%	0.81±0.020
c) Water soluble ash	4.65% 4.52% 4.70%	4.62±0.053
d) Sulphated ash	1.39% 1.46% 1.32%	1.39±0.040
4) Crude Fiber Contents	42.90% 40.37% 42.18%	41.81±0.752

Table 3: Extractive values for powdered fruit of *S. mangifera* with different solvents

Type of solvent	Colour & consistency	Values of 3 replicates (%) w/w	Mean ± SEM
Petroleum ether	Oily greenish yellow	1.70% 1.63% 1.67%	1.66±0.020
Chloroform	Dark green	0.43% 0.50% 0.47%	0.46±0.020
Ethyl-acetate	Brown	0.70% 0.64% 0.76%	0.70±0.034
Methanol	Reddish brown	10.14% 9.58% 10.23%	9.98±0.203
Water	Dark brown	8.53% 8.10% 8.46%	8.50±0.220

Table 4: Fluorescence analysis of the powdered fruit of *S. mangifera*

S. No.	Treatment	Colour in Day light	Colour in Short UV (254nm)	Colour in Long UV (365nm)
1.	Dry powder	Brown yellow	Yellow	Fine particles gives orange colour
2.	Powder + 1M-NaOH alcoholic	Light brown	Flesh	Italic
3.	Powder + 1M-NaOH aqueous	Light pink	Light yellow	Coffee brown
4.	Powder + 1 M-HCl aqueous	Straw colour	Coffee brown	Dark brown
5.	Powder + 50% H_2SO_4	Light yellow	Yellow	Chocolate brown

Table 5: Qualitative phytochemical analysis of various extractives of fruit of *S. mangifera*

Type of constituents	Pet-ether	Chloroform	Ethyl-acetate	Methanol	Water
1. Alkaloids	–	–	–	–	–
2. Reducing sugar	–	–	–	+	+
3. Glycoside					
a) Cardiac glycoside					
i) Legal test	–	+	–	–	–
ii) Deoxy-sugar	–	+	–	+	+
iii) Keddy reagent	–	+	–	–	–
b) Saponin glycoside	–	–	–	–	–
c) Anthraquinone	–	–	–	–	–
d) Flavonoids glycosides	–	–	+	+	+
4. Tannins/Phenolic compounds	–	–	+	+	+
5. Phytosterol	–	+	–	–	–
6.Terpenoids	–	–	+	–	–
7. Protein	–	–	–	+	+
8. Oils / Resins	+	–	–	–	–
9. Ascorbic acid	–	–	–	+	+

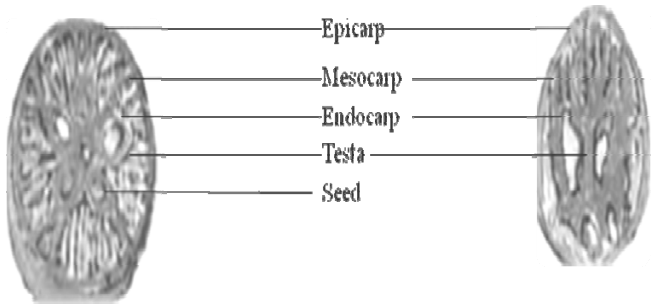

Fig. 2: TS & LS of fruit *S. mangifera* (diagrammatic)

Epicarp is consisting of closely packed, radially elongated cells; each cells in its outer portion highly thickened. Hypodermis is composed of tangentially elongated pentagonal parenchymatous cells filled with green colour chloroplast. Mesocarp is composed of 6-8 layers of tangentially elongated thick-walled cells containing oil globules. Some oil cells of the mesocarp consisting of a single layer of large, polygonal to rectangular cells with slight thickened walls and containing globules of volatile oil. Endocarp is very wide fibrous, stony, highly lacunated, transverse with groups of stone cells and sclerides of various size and thickness. The cells of layer is not differentiated but the outermost 6-8 rows are composed of thick and thin walled pitted stone cells. There are a number of small and big size lacunae are present in outer region of endocarp. Underneath this lies radially thin walled pitted sclerides embedded with groups of highly thickened stone cells and fibres. Testa is stony multilayered consisting of various size (62.4 × 18.5 µm) of stone cells and spindle shape sclerides followed by small sized, thick walled stone cells. [14] Endosperm is thin walled colourless, parenchymatous cells containing masses of protein Fig. 3a, 3b.

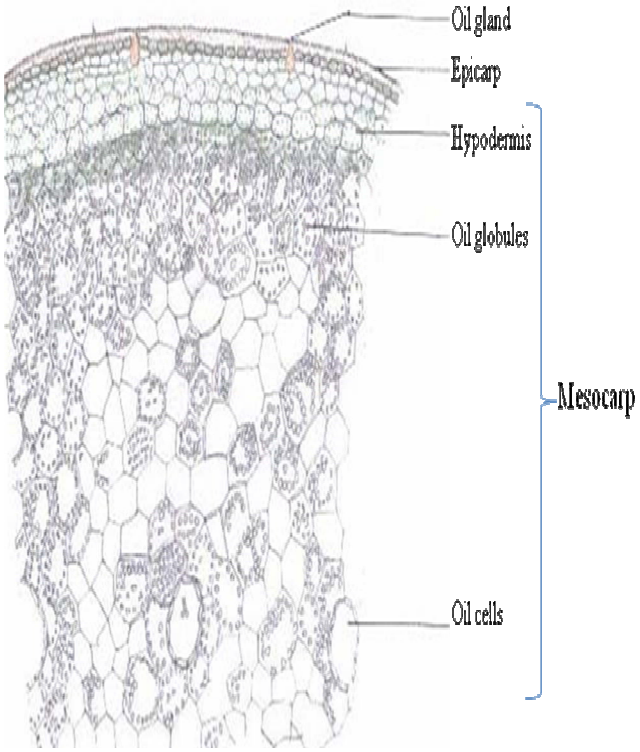

Fig. 3a: TS of fruit *S. mangifera* (Portion of pericarp)

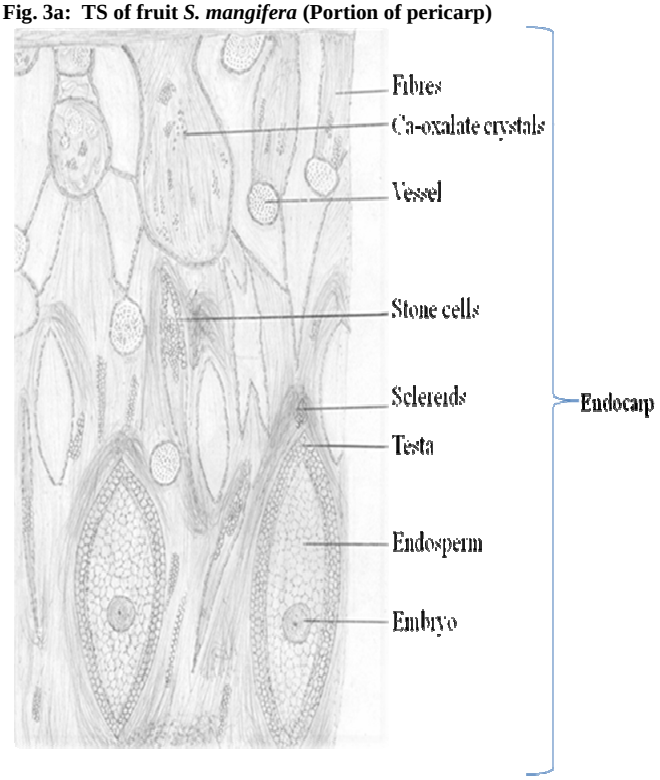

Fig. 3b: TS of fruit *S. mangifera* (Portion of endocarp, testa and cotyledon)

Histochemical colour reactions: Histochemical colour reactions of powdered fruit were carried out by reported methods. Behaviour of the powder with different chemical reagents is shown in Table 1.

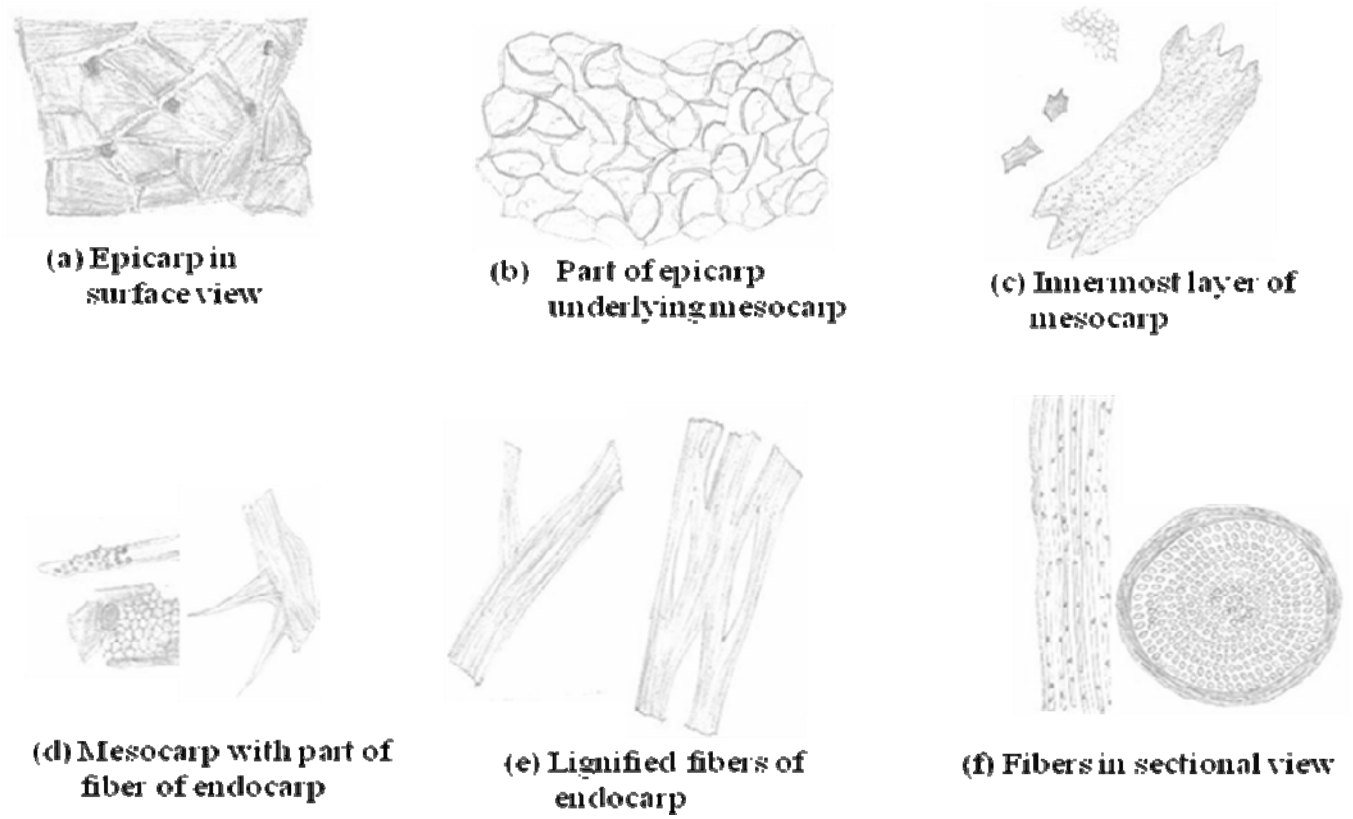

Fig. 4 Identifying characters of powdered fruit *Spondias mangifera*

Fig. 5 TLC fingerprints of methanolic extract of *Spondias mangifera* fruit (a: Methanolic extract of fruit, b: Standard Gallic acid in methanol)

Powder characteristics

Colour : Yellowish brown
Odour : Acidic, aromatic
Taste : Acidic astringent
Texture : Rough

After pressing a little amount of powder between filter paper, no greasy stain was found; indicate the absence of fatty oil, after shaking powder with water in test-tube persistent froth was not formed, which indicate the absence of Saponins.

Microscopical characters of powder

Powdered fruit were pass through # 60 and generally mounted in chloral hydrate, iodine, phloroglucinol + HCl, ruthenium red solution etc. [15] Majority of the tissues were studied and drawn directly from the microscope Fig. 4.

a. The numerous dark brown colour fragments of epicarp in surface view composed of a single layered of polygonal to slightly elongated cells.
b. The abundant of parenchymatous epicarp underlying mesocarp, usually contain orange colour oil globule.
c. The layer of the mesocarp is composed of yellowish regularly arranged elongated cells.
d. The innermost layer of mesocarp attached with fibres of endocarp occasionally containing orange colour of oil globules.
e. The fibres of endocarp have thick, lignified, lacunated, occasional pitted and associated with sclereids.
f. Sectional view of fibbers.

Quantitative Standards for Powdered fruits

Quantitative standards of fruit like moisture content of fresh sample and crude fiber contents of fruits were determined as usual method. Ash values and Loss on drying of dried sample were determined as per Indian Pharmacopoeia and results are shown in Table 2. Percentage of moisture content of fresh fruit was determined as follows:

$$\%MC = \frac{(\text{fresh mass} - \text{dry mass})}{\text{fresh mass}} \times 100.$$

Ash values: Total ash, acid insoluble ash, water soluble ash and sulphated ash values of fruit powder were done as per Indian Pharmacopoeia. The results are shown in Table 2.

Extractive values: Successive extractive values with different solvents of powdered fruit were done. The colour, consistency and percentage of extractive values in triplicate and their mean values ± SEM was calculated with reference to the air dried drug Table 3.

Fluorescence analysis: The fluorescence analysis of powdered drug in day light, short UV and long UV were examined by reported methods. The observations are given in Table 4.

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 usual prescribed methods and results are given in Table 5. The incinerated drug was detected for the presence of inorganic elements which are Potassium, Calcium, Magnesium, Aluminium, Iron, Phosphorus, Iodine and Sulphur.

TLC fingerprints: A greenish brown spot (R_f 0.81) corresponding to gallic acid was visualised. Another black brown spot (R_f 0.96) was also observed. The number of spots of TLC fingerprints is shown in Fig. 5.

CONCLUSION

Phytoconstituents of the fruit have potential of minerals, organic acids, phytosterols, terpenoids, flavonoids, phenolic compounds, tannins, peptides and reducing sugars which has to found possesses antioxidant, mast cells stabilising, can act against allergies, ulcers, tumors, platelet aggregation, and controlling hypertension and immunomodulatory effects. The constituents of this plant have tremendous impact on the health care system and may provide medical health benefits including the prevention and or treatment of diseases. The incinerated drug was tested for the presence of various inorganic elements such as Potassium, Calcium, Magnesium, Aluminium, Iron, Phosphorus, Iodine and Sulphur which have good neutraceuticals potentiality and can be used as food supplement, preventive medicine and the growing evidence points in the direction that certain foods fight and or prevent against diseases. There is a need to identify and scientific screening for various activities of such useful trees for their utilization in food, beverages and in preparation of phyto-pharmaceuticals.

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