ABSTRACT

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Pterocarpus marsupium commonly known as Indian Kino tree, Asana or Vijayasar is a large deciduous tree found in the subtropical regions of the world. It is highly enriched with an array of phytoconstituents including pterosupin, pterostilbene, liquiritigenin, isoliquiritigenin, epicatechin, kinoin, kinotannic acid, kino-red beta-eudesmol, carsupin, marsupol, marsupinol and so on. Many of these constituents have been explored for numerous biological actions like analgesic, anti-bacterial, anti-cancer, anti-cataract, anti-diabetic, anti-fungal, anti-hyperlipidemic, anti-inflammatory, anti-oxidant, aphrodisiac, cardiotonic, hepatoprotective etc. The present study was carried out to investigate macroscopical, microscopical and physico-chemical analysis of Pterocarpus marsupium wood. Some of the diagnostic features of the wood were studied. Proximate analysis and phytochemical analysis of the crude drug were carried out according to WHO guidelines. The pharmacognostic and phsico-chemical studies will help researchers in authentication and standardization of the crude drug and the further studies.

KEYWORDS: Pterocarpus marsupium, microscopy, Proximate analysis, standardization.

INTRODUCTION

Pterocarpus marsupium Roxb.-Fabaceae known as Indian Kino Tree or Malabar Tree in English; Vijayasar or Bija in Hindi and Asana in Sanskrit is indigenous to India, Nepal and Sri Lanka. *Pterocarpus marsupium*(PM) is a moderate to large deciduous tree up to 30m or more high, common in Central and peninsular India; found at 3000 ft in Gujarat, Madhya Pradesh and sub Himalayan tracts. *PM* is a versatile plant with a broad spectrum of
pharmacological actions. It has been mentioned in various traditional systems of medicine like Ayurvedic, Unani and Homeopathic systems of medicine. \textit{PM} find its place in the Rasayans group of Ayurveda. Due to the exploitation of the tree for its timber and medicinal bark, its population is decreasing in the wild and thus, it has been mentioned in the red data book. \textit{PM} is a medium to large sized deciduous tree, with dark brown to grey bark having superficial fissures; leaves compound and imparipinnate; flowers yellow in terminal panicles; fruit circular, flat, winged pod; seed convex & bony. Flowering and fruiting duration of the tree is from March to June. The major phytoconstituents of \textit{PM} are pterostilbene and marsupin. Others being liquiritigenin, isoliquiritigenin, pterosupin, p-hydroxybenzaldehyde, 7, 4’-dihydroxyflavone, propterol, marsupol, carsupin, epicatechin and so on.\cite{2} Different plant parts of \textit{PM} have been used for various diseases like leaves for boils, sores, skin diseases and stomach pain; flowers for fever; Gum-Kino for diarrhoea, dysentery, leucorrhoea etc. and bark as astringent & for toothache.\cite{4} Decoctions of bark and resin have been used traditionally for the treatment of tumours of the gland, urethral discharges and as abortifacient.\cite{5,6}

The heartwood possesses astringent, anti-inflammatory, antihyperlipidemic, antimicrobial anti-diabetic, antiallergic, antioxidant, wound healing and anticattract activity.\cite{7-15}

**Vernacular names:**\cite{16,17}

Eng. - Indian Malabar Kino, Indian Kino  
Guj. - Biyo  
Hindi - Bija, Bijasal, Vijayasara  
Kan. - Bijasara, Asana  
Kash. - Lal. Chandeur  
Mal. - Venga  
Mar. - Biyala lakda, Bibala  
Punj. – Chandan Lal, Channnlal  
Sans. - Pitasala, Asana, Sarfaka, Pijaka  
Tam. - Vegaimaram chakkal, Nengai  
Tel. - Paiddagi Chekka, Yegi, Vegisa  
Urdu – Bijasar

Habitat: A moderate to large deciduous tree about 30ft or more high, common in Central and peninsular India; found in Gujarat, Madhya Pradesh and sub Himalayan tracts. It grows
on a variety of formation provided the drainage is good. It prefers a soil with a fair proportion of sand though it is often found on red loam with a certain amount of clay. The normal rainfall in its natural habitat ranges from 75 to 200cm. but it attains its largest size in parts of Mysore and kerela, where the rainfall is even higher. It is a moderate light demander and the young seedlings are frost-tender.

![Fig 1: External surface of wood.](image)

**MATERIAL AND METHODS**

**Procurement and authentication**
The wood of *Pterocarpus marsupium* was collected from local market of Dadar, Mumbai. The wood was dried in sunlight, and authentied by Dr. Harshad Pandit from Guru Nanak Khalasa college of pharmacy, Matunga, Mumbai. The voucher specimen (arp# 1030731) was deposited in the laboratory.

**Macroscopic evaluation**
The morphological studies were carried out for shape, size, colour, odour and taste and fracture identification of the wood.\(^{[19,20]}\)

**Microscopic evaluation**
The anatomical preparations like Tranverse Section, Tangential Longitudinal Sections, Radial Longitudinal Section of wood were prepared and mounted under microscope after staining with Phloroglucinol HCl For study of powder characeristics the powdered crude drug was stained with phloroglucinol and Hydrochloric acid to detect the lignified tissues. The unstained powder was also mounted to detect the presence of calcium oxalate crystals and other non-lignified cells / tissues.\(^{[21]}\)
Proximate analysis
The various parameters for the proximate analysis included moisture content, ash values, extractive values, elemental analysis were determined according to the standard methods.\cite{22,23,24}

Moisture content
An accurately weighed quantity of about 1.5 gm of the powdered drug on electronic balance (Contech CA series) was transferred to a tare crucible and heated in oven at 110°C ± 5°C till the constant weight was obtained. The difference in initial and final weight after drying indicated the loss on drying.

Ash values
Inorganic content of the crude drugs was determined through the determination of various ash values.

Determination of total ash
Weighed accurately about 2 g of the air-dried substance and evenly distributed in the silica or platinum crucible and incinerated to constant weight in a muffle furnace at temperature not exceeding 450 ± 25°C. The crucible was allowed to cool in desiccator after each ignition. Ignition was done till constant weight was obtained. Calculated the percentage of ash with reference to air dried drug.

Determination of acid insoluble ash
Weighed accurately about 1 gm of total ash obtained by above process and boiled with 25 ml of 2N hydrochloric acid for 5 minutes. Insoluble matter was collected on an ash less filter paper and washed it with hot water, then the filter paper was dried, ignited in tared crucible at a temperature of 450 ± 5°C. The residue obtained was cooled in a desiccator and weighed. Content of the acid insoluble ash was calculated with reference to the air-dried drug.

Extractive values
Determination of water soluble, alcohol soluble, chloroform or petroleum ether soluble extractive values of the fresh and dried crude drug were determined as per the standard pharmacopoeial procedures described below.
Water soluble extractive
Weighed accurately about 5 g of air-dried coarsely powdered as well as fresh crude drug and were macerated separately with 100 ml of chloroform water in a closed flask for 24 hours. The flask was shaken vigorously for the first 6 hour and allowed to stand for 18 hours. At the end of 24 hours, it was filtered rapidly to prevent solvent loss. 25 ml of filtrate was evaporated and dried at 105°C in a tared shallow dish and weighed. The percentage of water-soluble extractive was calculated with respect to the air-dried drug and fresh drug.

Alcohol soluble extractive
5.0 g of air-dried coarsely powdered and fresh crude drug were macerated separately with 100 ml of alcohol in a closed flask for 24 hours. The flask was shaken vigorously for the first 6 hour and allowed to stand for 18 hours. At the end of 24 hours, it was filtered rapidly to prevent solvent loss. 25 ml of filtrate was evaporated and dried at 105°C in a tared shallow dish and weighed. The percentage of alcohol-soluble extractive was calculated with respect to the air-dried drug and fresh drug.

Petroleum ether soluble extractive value
5.0 g of the powder was accurately weighed into 250 ml stoppered conical flask. 100ml of petroleum ether was added and tightened firmly. The flask was shaken intermittently during first 6 hours, and then allowed to stand for 18 hours. The extract was filtered quickly (through filter paper). The weight of a clean, heated and cooled flat-bottomed evaporating dish was accurately determined. 25 ml of the filtrate was evaporated to dryness. The residue was dried to constant weight at 105°C in an oven and the final weight determined.

Elemental Analysis
The metals including Na, K, Pb, As, and Hg were determined using Atomic Absorption Spectroscopic standard method.

Preparation of Sample
2 g of dried powder of woods of Pterocarpus marsupium was weighed and subjected to dry-ash in a well cleaned porcelain crucible at 550 °C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO₃/ HCl /H₂O (1:2:3) and gently heated on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transformed into a 100 ml volumetric flask by filtration through a Whatman No
42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis and concentration of element in the sample was calculated as the percentage of dry matter.

**Preparation of Blank**

To 5 ml of HNO3/ HCl /H2O (1:2:3), 5 ml of de-ionized water was added and the volume was made up to 100 ml in a volumetric flask. This was used as blank.

**Phytochemical screening**

The phytochemical evaluation of drug was carried out as per the method described.\(^\text{[22,24]}\)

Previously dried powdered wood were extracted in a Soxhlet apparatus with petroleum ether (60-80), chloroform, ethanol, and water successively. The extracts were evaporated to dryness under vacuum. These extracts were used for the analysis of different phytoconstituents viz. alkaloids, carbohydrate, phenolic, flavonoids, amino acids, saponins, steroids, etc.\(^\text{[19,20,25]}\)

**RESULTS AND DISCUSSION**

Phramacognostic, studies involved both macroscopic and microscopic evaluation. These parameters help in authentication of the crude drug to some extent. The results of the macroscopic characters of evaluation are given in Table 1

**Table 1: Macroscopical evaluation.**

<table>
<thead>
<tr>
<th>Macroscopical characters</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Golden yellowish brown</td>
</tr>
<tr>
<td>Odour</td>
<td>Nil</td>
</tr>
<tr>
<td>Size</td>
<td>Vary in size</td>
</tr>
<tr>
<td>Shape</td>
<td>Very hard, strong and tough</td>
</tr>
<tr>
<td>Surface</td>
<td>Longitudinally ridged and striated</td>
</tr>
<tr>
<td>Taste</td>
<td>Astringent</td>
</tr>
</tbody>
</table>

**Microscopic Characteristics:** The wood consists of vessels, tracheids, fibre tracheids and wood parenchyma all the elements being lignified and filled with tannin. Transverse section (TS) of wood shows vessels which are solitary or arranged in small radial group and often blocked with tyloses. wood parenchyma embedded with prismatic crystals of calcium oxalate, medullary rays are uniseriate rarely biseriate. In TS they run straight and parallel to each other, in Tangential longitudinal section (TLS) medullary rays are seen vertically running linear bands and in radial longitudinal section (RLS) medullary rays are horizontally running banda crossing the vessels and fibers.
**Powder:** Shows bordered pitted vessel, long thick acicular fibers, medullary rays tangentially and radially cut, prismatic calcium oxalate crystals.

Fig 2: T .S. of wood *P. marsupium*.

Fig 3: T. L. S. of wood *P.marsupium*.

Fig 4: R. L. S. of wood *P. Marsupium*. 
Fig 5: Xylem fibres.

Fig 6: Tangentially cut medullary rays.

Fig 7: Radially cut medullary rays.
Fig 8: Bordered pitted xylem vessel.

Table 2: Results of physiochemical evaluation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>2 %</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.35%</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>0.30%</td>
</tr>
<tr>
<td>Moisture content</td>
<td>0.25%</td>
</tr>
<tr>
<td>Ethanol soluble extractive value</td>
<td>4 %</td>
</tr>
<tr>
<td>Water soluble extractive value</td>
<td>7%</td>
</tr>
<tr>
<td>Pet. Ether (60-80 °C)</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Table 3: Elemental analysis using Atomic Absorption Spectroscopy.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Elements</th>
<th>Values (PPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pterocarpus marsupium</em> wood</td>
<td>Sodium Na</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Potassium K</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>Arsenic As</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Lead Pb</td>
<td>3.78</td>
</tr>
<tr>
<td></td>
<td>Mercury Hg</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND-Not detected

Table 4: Results of Preliminary phytochemical analysis.

<table>
<thead>
<tr>
<th>Test</th>
<th>Petroleum Ether</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tannins</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Amino acids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
DISCUSSION
Proper authentication and identification of plants play a significant role in the field of research and health care system. Detailed pharmacognostical study of plant drug is very necessary before its use in the field of research and also in pharmaceutical formulation. It helps to identify other allied species and adulterants from the authentic drug. The present study has been attempted to evaluate the macroscopic and microscopic characteristics, physicochemical parameters as well as phytochemical analysis of *Pterocarpus marsupium* wood. The macroscopic study of the wood indicated that its colour, odour, and taste may be an important characteristic feature for identifying the plant. The microscopic study of the powder revealed the presence of bordered pitted vessel, long thick acicular fibers, and medullary rays tangentially and radially cut. Proximate analysis of drugs is an important parameter in detecting adulteration. Total ash value, acid insoluble ash, and water soluble ash were determined and found to be 2%, 0.35%, and 0.30% respectively. Extractive values of ethanol, water and pet-ether (60-80 °C) was found to be 7%, 4% and 0.5 % respectively. Preliminary phytochemical analysis showed the presence of various phytoconstituents in the extract such as Condensed tannins, saponins, sterols and triterpenoids. The pharmacognostic constants for the wood, the diagnostic microscopic features, and the numerical standards reported in this study can be useful for the compilation of a suitable monograph of *Pterocarpus marsupium* for its proper identification and the extract showed the absence of heavy metals like arsenic, mercury a contamination and other metals like lead, sodium and potassium are in limits. Therefore, the drug is very effective and safe for making of any formulation.

CONCLUSION
The present study on pharmacognostical, physico-chemical and phytochemical analysis of *Pterocarpus marsupium* were investigated. This study showed botanical characteristics that can differentiate the plant from other plants. The macroscopic description will be helpful in identification of the plant. Microscopical study in entire and powdered form of the drug is one of the aspects of histological evaluation. The results of elemental analysis for elements Na, K, Pb, As, and Hg are in limits, so drug is safe for making formulation useful for human beings. These results will be useful with regard to its identification and standardization.
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CONFLICT OF INTEREST
Authors declare that there is no conflict of interests regarding the publication of this article.

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