Pharmacognostic studies of the stem bark of *Chloroxylon swietenia* DC.

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**ABSTRACT**

*Chloroxylon swietenia* DC. (Family: Rutaceae) is an important traditional medicinal plant used in the treatment of various ailments like fungal infection of skin, rheumatism, common cold, cough, ophthalmic infection and cataract, wounds and as an astringent. However, detailed scientific information is not available to identify the plant material, in order to ascertain its quality and purity. In this paper, we report the pharmacognostic evaluation of the bark for the purpose of its identification and differentiation from related species. The macroscopy, microscopy, physicochemical parameters such as moisture content, ash values, extractive values, fluorescence analysis and preliminary phytochemical analysis of the bark were investigated. Transverse section of the bark shows presence of cork, cortex, medullary rays, and stone cells. Presence of cork, stone cells, phloem fibres and parenchyma cells which were also observed in the powdered sample of the bark. The result of preliminary phytochemical screening indicated presence of alkaloids, steroids, carbohydrates, proteins, phenolic compounds, tannins flavonoids and triterpenoids. The present study will be useful for its identification prior to carrying out further research work. The findings of this study will facilitate pharmacognostic standardization of the plant material and aid in the preparation of a herbal monograph for the species.

**Introduction**

The nature has provided a complete store house of remedies to use for all ailments for mankind. The knowledge on drugs has accumulated over thousands of years as a result of man's inquisitive nature [1]. For centuries, plants with medicinal properties have been utilized successfully in the treatment of ailments of varying degrees of severity [2]. Therefore screening of medicinal herbs has become a potential source of biodynamic compounds of therapeutic value. The standardization of crude drugs is an integral part of establishing its correct identity. Pharmacognostical study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopic and physical characteristics of the crude drugs that are necessary for their proper identification [3].

*Chloroxylon swietenia* DC., a member of Rutaceae family is a medium sized deciduous tree with height upto 9 -15 m and 1.0 - 1.2 m girth having a spreading crown. The tree is native to India and Sri Lanka and commonly known as Ceylon Satinwood or East Indian Satinwood. *C. swietenia* is considered as a folklore medicinal plant having several medicinal uses in the folklore remedies [4]. The Malasar tribes inhabiting the forest areas in Coimbatore district of Tamil Nadu, South India apply the leaf paste on wounds, cuts, burns and skin diseases for quick recovery [5]. The leaf paste is also applied to treat worm infested wound of animals, fungal infection of skin and rheumatism [6]. Various parts of the plant are traditionally used in snakebites [7]. The stem bark is credited for its effectiveness in the treatment of common cold and cough [8], ophthalmic infection and cataract [9], wounds [10, 11] and as an astringent [12]. The dried stem barks alone or in combination with sesame oil (*Sesamum indicum*) in the form of a paste is applied externally to treat itches [13, 14]. A paste of the leaves and roots is taken orally and also applied as a balm to treat headache [15]. In the present paper, we report the pharmacognostical studies of the bark of *C. swietenia* since there are no such reports available in the literature.
Materials and Methods

Plant Material
The fresh bark was collected from well grown and matured trees from Tirumala, Andhra Pradesh during March 2013 and authenticated by the Botanist Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Part of the fresh bark was shade dried, followed by milling into coarse powder. The remaining fresh bark was used for the study of macroscopical and microscopical characters while the dried bark powder was used for powder microscopy, physicochemical analysis and preliminary phytochemical studies.

Chemicals
The chemicals used in the study were of standard analytical grade obtained from S.D Fine Chem Ltd., Mumbai and Loba Chemie, Mumbai.

Macroscopy
The colour, odour, taste, size, shape, fracture and texture were studied [16].

Microscopy
The microscopic sections of the bark were cut by free hand sectioning and thinnest possible transverse sections were obtained. The selected sections were heated with chloral hydrate solution for 5 min, followed by staining with few drops of phloroglucinol and conc. HCl in the ratio 1:1. The sections were mounted in glycerin and studied under the binocular research microscope (Model BD 10, BD Instrumentation, India) fitted with Canon Photoshot (A3200IS) camera. [17-20]. Photomicrographs were taken during observation from several fields.

Powder Microscopy
A small quantity of the bark powder was heated for 10 min in chloral hydrate solution followed by staining with phloroglucinol and conc. HCl. A pinch of the powder was taken on a slide and observed under the microscope [21].

Physicochemical Constants
Various physicochemical parameters like moisture content, ash values and extractive values were evaluated in triplicate according to standard procedures mentioned in Indian Pharmacopoeia [22, 23].

Fluorescence Analysis
The fluorescence characteristics of the bark powder was studied in both daylight and UV light (254 nm and 365 nm) after treatment with different reagents [24].

Preliminary Phytochemical Studies
The powdered bark (100 g) was extracted successively with petroleum ether (60-800°C), chloroform, methanol and distilled water using a soxhlet extractor. The extracts were then subjected to qualitative chemical tests using standard procedures in order to identify presence of different class of phytoconstituents in the bark sample [17, 20].

Results

Macroscopy
The bark is externally dark brown and internally light brown in colour having characteristic odour with slightly bitter in taste. Pieces of the bark are curved or occur in the form of flat pieces about 7-10 cm long and about 3-5 mm in thickness. The surface of the bark is rough.

Microscopy
T.S of the bark shows periderm, a wide cortex and secondary phloem (Fig. 1).

Periderm
Cork: It consists of few layers of cells which are thin walled rectangular cells, some with yellowish matter.
Phellogen: It consists of 2-3 layers of rectangular cells with or without any cellular contents.
Phelloderm: It comprises of 6-10 layers of cells, at times arranged radially.

Cortex: It consists of several layers of thin walled and tangentially elongated cells where some cells containing yellowish brown matter. The cortex also contains groups of lignified, pitted stone cells of large lumen and are rectangular to elongated in shape. Non lignified pericyclic fibres are also seen in the cortex.

Secondary phloem: This region comprises of mostly phloem parenchyma, medullary rays which are not uniformly arranged. They appear to run in different directions. Stone cells are seen in groups which are arranged in tangential rows separated by medullary rays.

Fig 1: TS- Chloroxylon swietenia
CK= Cork, PG= phellogen PD= phelloderm, SC=Sclereid, CT=Cortex, PF=Phloem fibres, MR= Medullary rays, SP= Secondary phloem
Powder microscopy

The powder microscopy revealed presence of cork, parenchyma, stone cells and phloem fibres.

**Cork:** cork consists of thin walled cells are seen which are polygonal in shape containing occasional yellowish or brownish matter.

**Parenchyma:** Typical parenchymatous cells which are either rounded or elongated are found.

**Stone cells:** Present in the form of groups. These are rectangular to elongated in shape. The walls are striated and have pitted thickenings.

**Phloem fibres:** Occurs lengthwise in groups of 3-5. Brown masses are seen adhering to the fibres. Some of the parenchymatous cells surrounding the groups of fibres contain calcium oxalate crystals arranged in rows.

- Cork
- Parenchyma
- Stone cell
- Fibre

**Physicochemical Constants**

The percentage of total ash, acid-insoluble ash, water soluble ash, water soluble extractive, ethanol soluble extractive and moisture content are presented in Table 1.

**Fluorescence Analysis**

Many herbs show typical fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification [25,26]. The result of the fluorescence analysis of the drug powder is presented in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obtained values (%) (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>6.53 ± 0.76</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>1.14 ± 0.11</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>2.03 ± 0.34</td>
</tr>
<tr>
<td>Water soluble extractive</td>
<td>22.85 ± 0.33</td>
</tr>
<tr>
<td>Ethanol soluble extractive</td>
<td>12.96 ± 0.27</td>
</tr>
<tr>
<td>Moisture Content</td>
<td>10.85 ± 0.72</td>
</tr>
</tbody>
</table>

Results expressed as Mean ± SEM from three observations

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Reagents</th>
<th>Daylight</th>
<th>Observed colour under UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Short wavelength (254 nm)</strong></td>
</tr>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Reddish brown</td>
<td>Brown</td>
</tr>
<tr>
<td>2</td>
<td>Nitric acid</td>
<td>Yellowish brown</td>
<td>Light Brown</td>
</tr>
<tr>
<td></td>
<td>1N Hydrochloric acid</td>
<td>Reddish brown</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>4</td>
<td>1N Sulphuric acid</td>
<td>Reddish brown</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>5</td>
<td>Potassium hydroxide</td>
<td>Yellowish green</td>
<td>Green</td>
</tr>
<tr>
<td>6</td>
<td>Sodium hydroxide</td>
<td>Reddish brown</td>
<td>Brown</td>
</tr>
<tr>
<td>7</td>
<td>5% Ferric chloride</td>
<td>Deep brown</td>
<td>Brown</td>
</tr>
</tbody>
</table>
Preliminary Phytochemical Studies

The results of the preliminary phytochemical tests are depicted in Table 3. The different extracts of the bark were found to contain alkaloids, steroids, phenolic compounds, tannins, flavonoids and terpenoids.

Table 3: Preliminary phytochemical studies of different extracts of C. swietenia

<table>
<thead>
<tr>
<th>Test for</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Methanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins &amp; amino acids</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>Carbohydrates</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>Steroids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds</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>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = present, ‘-’ = absent

Discussion

According to the WHO reports, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and degree of purity and should be carried out before any other tests are undertaken. In this context, the study was undertaken to set the standardized parameters for establishment of standard quality of the bark of C. swietenia. Evaluation of physicochemical parameters is an important part in the preparation of herbal monograph. Thus ash, extractive values, moisture content and fluorescence study determined here signifies standard parameters to ensure the quality and purity of the crude drug. The total ash indicates the presence of inorganic matter present in a plant material. The extractive values are indicative of approximate measures of their chemical constituents extracted with specific solvents. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of evaluation [27]. The preliminary phytochemical tests are usually carried out to identify presence of various phytoconstituents in different extracts of crude drugs. Thus, all these parameters will augment in standardization of the plant material.

Conclusion

The phytochemical findings of the study confirm the presence of plant phenolics, flavonoids and other secondary metabolites which are currently of growing interest owing to their functional properties in promoting human health. The diagnostic features established here will help in quality control and authentication of the drug. Further, this investigation will be helpful to identify the plant and also provide valuable information to the researchers to establish the pharmacological activities supported with possible mode of action.

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Conflict of interest: We declare that we have no conflict of interest.

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