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PHYTOCHEMICAL AND PHARMACOGNOSTIC STUDIES ON LEAF OF CHLOROXYLON SWIETENIA DC. AN ETHNOMEDICINALLY IMPORTANT MEDICINAL TREE

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ABSTRACT

Chloroxylon swietenia DC. belongs to the family Meliaceae is an important medicinal tree of dry deciduous forests with several medicinal uses in both folk and traditional system of medicine. Traditionally different parts of the plant are used in treating wounds, cuts, burns and skin diseases. Its pharmacognostic data for authentication of the leaf crude drug is available, but it is incomplete, hence, in the present study detailed macroscopical, microscopical, physicochemical and preliminary phytochemical studies of leaf are undertaken. Powder microscopy revealed the presence of xylem vessels with spiral and reticulate thickenings, phloem fibers, raphide crystals and unicellular trichomes. Anatomical studies showed the presence of raphide crystals, collateral vascular bundle, resin ducts and anomocytic stomata. The qualitative chemical tests of petroleum ether, chloroform, acetone, ethanol and water extracts of leaf indicated the presence of carbohydrates, alkaloids, glycosides, flavonoids, amino acids, phenolic compounds and tannins.

Key words: *Chloroxylon swietenia*, Ethnomedicine, Leaf, Pharmacognosy, Phytochemical study.

INTRODUCTION

Chloroxylon swietenia DC. (Fig.1) is a member of Meliaceae family, which is a tropical aromatic tree of dry deciduous forests [1, 2]. It is a moderate sized and deciduous tree of about 9-15 m in height and 1.0-1.2 m in girth with a spreading crown and clean bole up to 3 m. The tree is popularly known as Yellow wood, East Indian satin wood, Ceylon satin wood and is native to India and Sri Lanka [2, 3].

Various parts of this tree has long been used in the indigenous system of medicine such as the bark is used as an astringent [4], leaves are applied to worm infested wound of animals, fungal infection of skin and for the treatment of inflammation related disorders like pain and rheumatism [5]. A paste of the leaves and root in equal parts is taken internally to relieve headache or applied externally to the forehead as a balm by tribal inhabitants of Bastar District (Madhya Pradesh) [6]. The decoction of the leaves is used as a lotion for ulcers and for healing abrasions of the skin and the smoke from burning leaves is used to drive ticks out of stables [7]. Leaves have remarkable mosquito larvicidal activity and are also used as insect repellent [2, 7].

Owing to its heavy demand not only for medicinal use, but also for timber purpose, the tree now has become endangered. The tree has been cited under Red List category

under IUCN Red List of Threatened Species, as per the assessment of Asian Regional Workshop (Conservation and Sustainable Management of Trees, Viet Nam, August 1996) 1998 [8, 9].

Although the earlier work[10] on leaf of *Chloroxylon swietenia* DC. available, it is incomplete, hence, the present study is undertaken to establish detailed pharmacognostic profile of leaf crude drug which will help in the authentication of leaf crude drug without errors.

MATERIALS AND METHODS

Plant material: Fresh mature leaves of *Chloroxylon swietenia* DC. were collected from Karnatak University Campus Dharwad, Karnataka and The sample was authenticated for its botanical identity by one of the authors Dr. M. Jayaraj. A voucher specimen has been deposited in the P. G. Department of Botany, Karnatak University, Dharwad for future reference. After collection the fresh leaves of the plant were preserved in F.A.A solution for the subsequent study.

Drying of plant material: After authentication, the leaves were dried at room temperature until they were free from the moisture and then powdered with a mechanical grinder. The powder was passed through sieve and stored in an air tight container for further studies. The Pharmacognosy was carried out using standard methodology [11, 12, 13, 14].

Macroscopic and microscopic studies*:* The macroscopy and microscopy of the leaf are studied according to the methods [14, 15, 16]. For microscopic studies, free hand sections of leaf were taken and stained with safranine O as per the procedure [16]. Powder microscopy is performed according to the prescribed procedure [14, 15]. Powder microscopic analysis was carried out with small amount of leaf powder, which was mixed with phloroglucinol : HCl (1:1) and then placed on microscopic slides. The slides are mounted in the glycerin and are observed under light microscope. Photomicrography of the selected sections were taken using Axio star plus (Carl zeiss) Bright field /fluorescent modular microscope and Cannon's power shot G2 digital camera with required magnifications.

Organoleptic evaluation: Various sensory parameters such as color, taste, odour and texture of the powdered leaf were studied by the organoleptic evaluation [14, 15].

Stomatal type, number and index: Stomatal types were determined by the classification of stomata on the grounds of nature and number of subsidiary cells [14, 15, 17]. Stomatal number is determined by taking average number of stomata per square mm of epidermis of leaf. Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stomata being counted as one cell.

Physicochemical Evaluation: Various physicochemical parameters such as ash values (total ash, water soluble ash and acid-insoluble ash) and extractive values (water, alcohol and ether soluble extractives) were determined as per standard procedures [18, 19, 20].

Fluorescence analysis: The fluorescence behavior of the leaf powder in the visible light and ultraviolet light were carried out by using leaf powder and freshly prepared reagent solution. A small quantity of dried and finely powdered leaf were placed on clean microscopic slide and added 1-2 drops of the freshly prepared reagent solutions

(Table-3), mixed by gentle tilting the slide and allowed for 1-2 minutes. The colors observed by application of different chemical reagents in different radiations were recorded [21, 22, 23].

Preliminary phytochemical screening: Dried, coarsely powdered leaves were extracted successively with petroleum ether, chloroform, acetone, ethanol and water using Soxhlet apparatus. All the extracts were tested for the presence of phytoconstituents. Preliminary phytochemical tests for various extracts were carried out according to the standard procedures [24, 25, 26].

RESULTS AND DISCUSSION

Macroscopic studies

Leaf : Pinnately compound.
Leaf size: 12-24 cm in length and 3.5-4.8 cm in breadth.
Leaves : Alternate.
Leaflets size: 1.5-3.0 cm long and 0.6-1.5 cm wide.
Leaflets: 10-12 pairs.
Leaflet margin : Entire.
Leaflet lamina : Unequal-sided.
Color :Dark green adaxially and light green abaxially (Fig-2).

Organoleptic characters of leaf powder

Color	Odour	Taste	Touch
Light green	Characteristic	Tasteless	Fibrous, smooth

Microscopic studies

Anatomy of leaf petiole: In the cross section, the leaf peliole is more or less triangular in shape. The adaxial side is concave and the abaxial part is hemispherical. It is surrounded by single layered epidermis, which is thin and continuous and is made up of small quadrangular parenchyma cells with thin cuticle. The epidermis is covered by simple unicellular trichomes. The ground tissue (cortex) is homogenous, parenchymatous, thin walled and compactly arranged without any intercellular space. The cells are lignified and consist of resin duct. After the ground tissue (cortex) is the 5-6 layers of compactly arranged thick walled cells, which are sclerenchymatous in nature. The entire vascular system and the pith are surrounded by this sclerenchymatous sheath. The vascular bundle is collateral. Here xylem and phloem remain side by side arranged on the same radius, phloem on the outer side and xylem towards the pith. Some of the xylem vessels showed presence of raphide crystals (Fig-4C). Inner to this vascular system is the pith, which is made up of compactly arranged parenchymatous cells. Many cells in this region are also lignified (Fig-4A, B).

Anatomy of leaf let-midrib: The cross sectional view of midrib shows that, the adaxial side it is more or less flat, on the abaxial side it is hemispherical in shape. Upper epidermis is continuous with broad and prominent parenchyma cells. But the lower epidermal cells are discontinuous due to the presence of stomata. The adaxial region of the leaf is composed of two layers of elongated columnar cells known as palisade parenchyma. These cells remain arranged more or less at right angles to the upper epidermis. The abaxial region of the leaf is composed of loosely arranged isodiametric cells known as spongy parenchyma. The vascular strand is single, broadly shaped, collateral and closed. It consists of radial files of thick walled angular xylem elements lying on the upper side and the phloem on the lower side. Some of xylem vessels showed presence of raphide crystals. Phloem consists of thick walled darkly staining elements forming a

dense layer beneath the xylem tissue. Vascular bundle is surrounded by parenchymatous cells called bundle sheath (Fig-5A).

Anatomy of leaf let-lamina: Leaf let-lamina is bifacial and mesomorphic, it consists of broad adaxial and abaxial epidermal layers. The mesophyll tissue is differentiated into adaxial layer of cylindrical palisade cells and abaxial zone of spongy mesophyll tissue. Resin ducts are present at palisade mesophyll zone. The abaxial epidermis is discontinuous and stomatiferous (Fig- 5B).

Stomatal type, number and index: Anomocytic type of stomata are present only on lower epidermis, where as stomata are absent on upper epidermis. The stomata surrounded by 4-5 subsidiary cells and these subsidiary cells are in no way differing from epidermal cells (Fig-3). Stomatal number of leaf let is 72.2* and stomatal index of leaf let is 17.32* (Table-1).

Powder microscopy: Powder microscopic study revealed the presence of xylem vessel with spiral and reticulate thickenings (Fig-6A & B), Phloem fiber composed of elongated cell with pointed ends and thick walls (Fig-6C), upper epidermal cells in surface view appear hexagonal to polygonal in shape (Fig-6D), epidermal hairs were observed as unicellular trichomes on petiole (Fig-6E & F) and raphide crystals were observed and are needle-like structures in form of bundles (Fig-6G).

Physicochemical Evaluation: The physicochemical characterization including extractive values and ash values were measured and shown in Table-2. The pH of the sample was 6.42.

Fluorescence analysis: The fluorescence characteristics of leaf powder with different chemical reagents are summarized in Table-3.

Preliminary phytochemical screening: Preliminary phytochemical screening of the *Chloroxylon swietenia* DC. leaf powder is done and the results are presented in Table-4.

Phytochemical test shows the presence of carbohydrates in all the extracts. Phenolic compounds and tannins were observed in Petroleum Ether, Acetone, ethanol and water Extract. Alkaloids observed only in chloroform extract. Glycosides observed only in petroleum ether. Flavonoids were observed in ethanol extract and amino acids in water extract, whereas steroids were absent in all the extracts.

CONCLUSION

The present study provides in-depth macroscopical, microscopical features and showed presence of carbohydrates, phenolic compounds, tannins, alkaloids, glycosides, amino acids and flavonoids. It also provides pharmacognostic data of *Chloroxylon swietenia* DC. leaf, which could be used for determining correct identity and purity of leaf and for the detection of adulteration.

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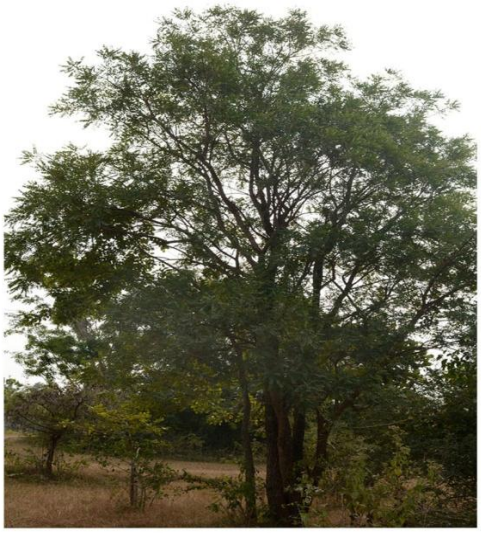


Fig.1 Habite

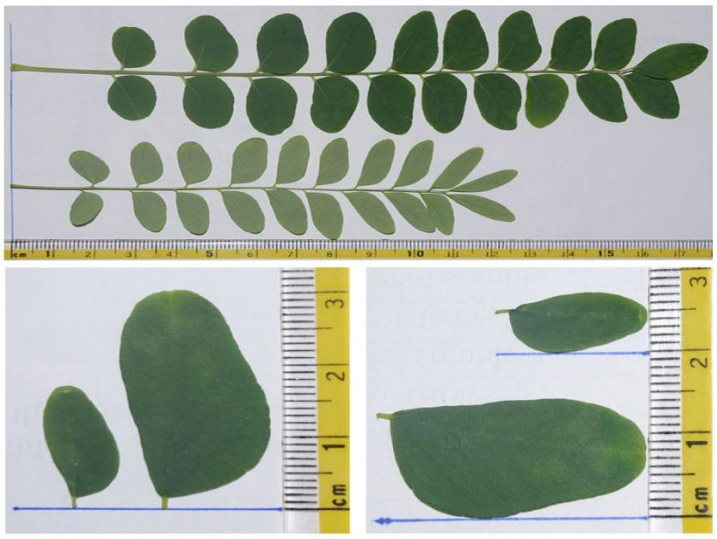


Fig. 2 Leaf morphology

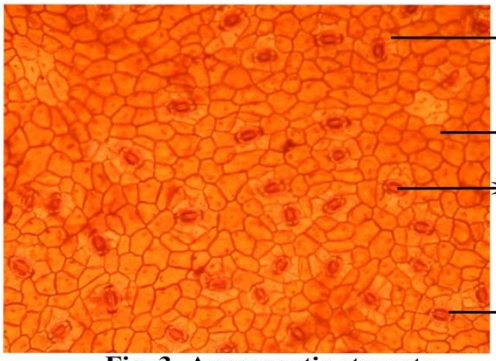
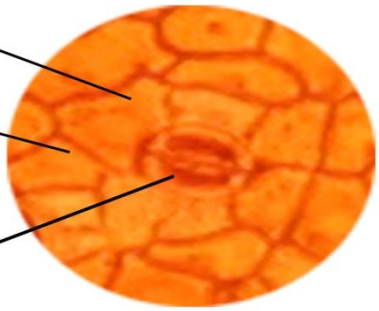
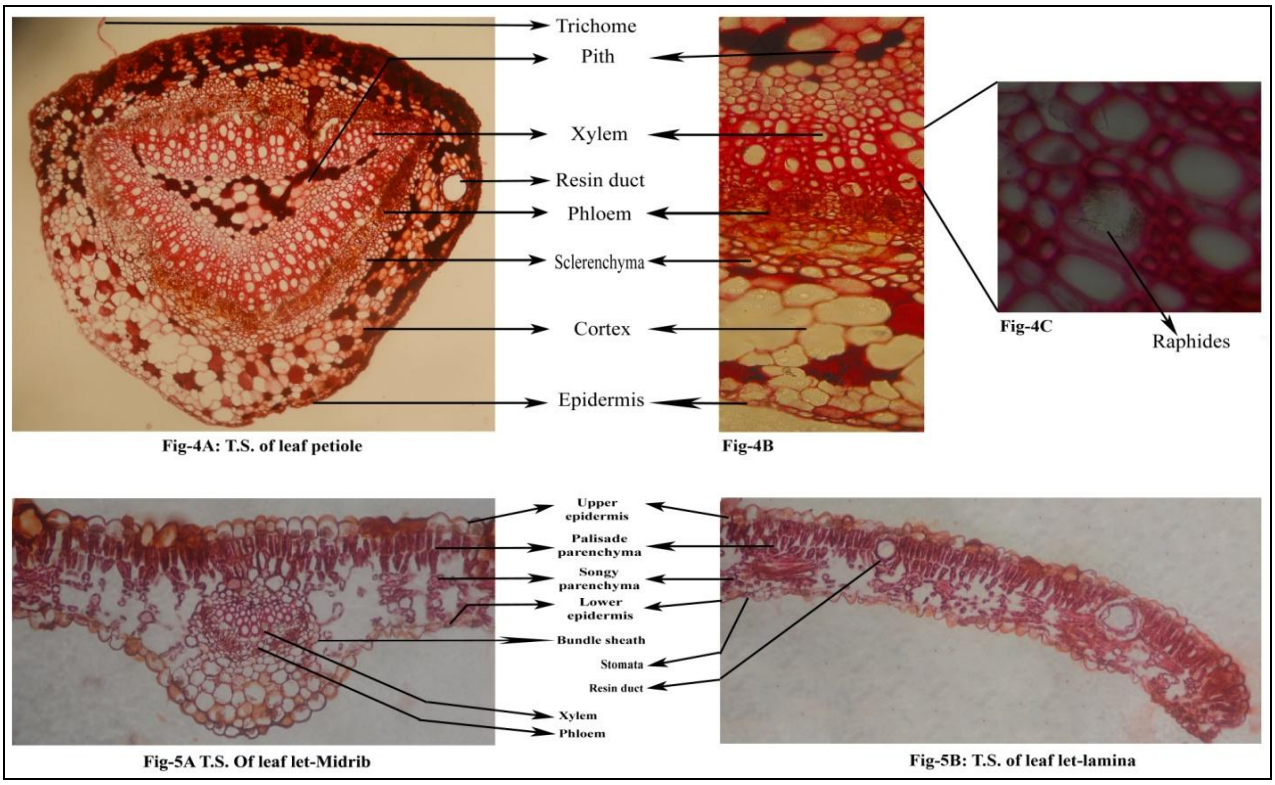


Fig. 3- Anomocytic stomata



Magnified view of single stomata



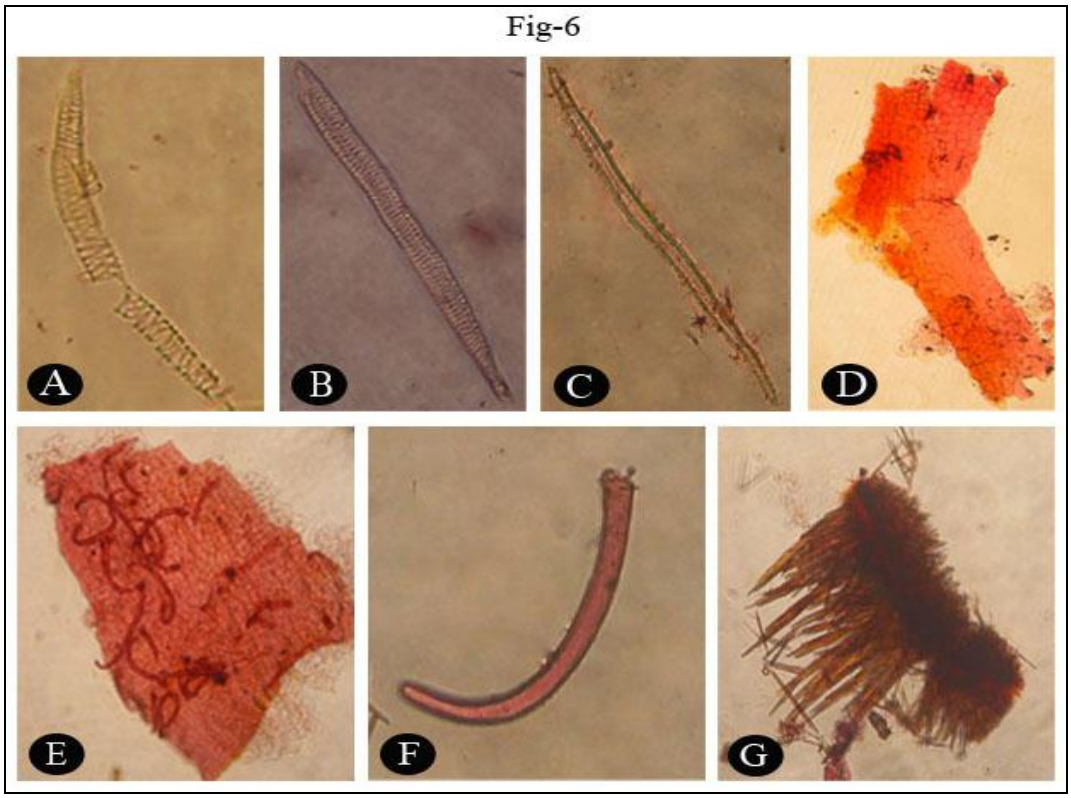


Fig-6: A-xylem vessel with spiral thickening; B-xylem vessel with reticulate thickening; C-Phloem fiber; D-upper epidermal cells; E-unicellular trichomes on petiole; F-magnified view of single trichome; G-raphide crystals.

Table-1: Leaf constants

Parameters		Observations
Stomata type		Anomocytic
Trichome type	Leaf let	Absent
	Petiole	Unicellular
Stomatal number of leaf let	Upper epidermis	00
	Lower epidermis	72.2*
Stomatal index of leaf let	Upper epidermis	00
	Lower epidermis	17.32*

*: Average five readings per microscopic view (40X).

Table-2: Physicochemical analysis

Parameters		Values (w/w %)
Ash values *	Total ash	5.39 ± 0.13
	Sulphated ash	23.49 ± 0.28
Extractive values*	Ether soluble	6.42 ± 0.14
	Alcohol soluble	8.16 ± 0.25
	Water soluble	18.4 ± 0.51
Moisture content		7%

* Average of three readings. Standard Deviation. Calculated based on dry weight of the sample

Table-3: Fluorescence analysis

Treatment	Visible/Day light	UV light Short	UV light Long
Powder(P) as such	Light Green	Dark algal green	Brownish black
P+ phlouroglucinol : HCl (1:1)	Green	Dark green	Black
P+ methanol	Light green	Green	Dark brown
P+ 50% H_2SO_4	Brown	Dark green	Ink blue
P+ 50% HNO_3	Dark saffron	Dark green	Black
P+ 50% HCl	Algal green	Dark green	Brownish black
P+ 10% NaOH	Dark brown	Dark green	Brownish black
P+ ammonia	Green	Black	Distemper green
P+ glacial acetic acid	Muddy black	Black	Black
P+ 1% Picric acid	Yellowish green	Green	Green
P+ 5% FeCl3	Light green	Dark green	Black
P+ 10% potassium dichromate	Yellowish brown	Dark green	Brownish back

Table-4: Phytochemical analysis

Plant constituents	Test/Reagent Used	NAMES OF EXTRACT				
		Petroleum Ether Extract	Chloroform Extract	Acetone extract	Ethanol extract	Water extract
Carbohydrates	Benedict's test	-	+	+	+	+
	Molisch's test	+	+	+	-	-
	Fehling's test	-	+	-	+	+
Phenolic compounds and Tannins	Ferric chloride test	-	-	+	+	+
	Dilute HNO3	+	-	+	-	-
Alkaloids	Mayer's test	-	+	-	-	-
Glycosides	Molisch's test	+	-	-	-	-
Flavonoids	Shinoda's test	-	-	-	+	-
Amino acids	Ninhydrin test	-	-	-	-	+
Steroids	Salkowski test	-	-	-	-	-

+ : Presence, - : Absent

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