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Research Article

Microscopical, histochemical and fluorescence evaluation of *Tectona grandis* L. leaves dye extract

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ABSTRACT

Teak (*Tectona grandis* L.) leaves can aid in the treatment of inflammations, leprosy, skin diseases, and indolent ulcers. Dyes are important in human life because they are both safe and environmentally friendly. In the present investigation teak leaves were evaluated macro, microscopical and fluorescence methods. The results showed that the teak leaves have dorsal and ventral surfaces that are 30-60 cm long and 20-30 cm wide. Teak leaf powder was given a yellow tint after being treated with diluted ammonia and H_2SO_4 to reveal the presence of flavonoids. $FeCl_3$ generated a black coloration after a few drops, indicating the presence of tannin. While dinitrophenol hydrazine showed the presence of terpenoids, blue, green, or red coloring indicates the presence of polyphenol. Under both regular and UV light, the fluorescence behavior of teak powder under various situations were investigated.

Keywords:

Tectona grandis, natural dye, histochemical, fluorescence study

INTRODUCTION

Tectona grandis L. is a huge deciduous tree in the Lamiaceae family and was formerly classified under the family Verbenaceae, some genera now fall under other families as well. The genus *Tectona* comprises 3 species viz *T. grandis*, *T. hamiltoniana* and *T. philippinensis. T. grandis* is native to India, Pakistan, Bangladesh, China, Sri Lanka, Indonesia, Myanmar, Northern Thailand and Northwestern Laos. *T. hamiltoniana* (Dahat teak) is an endangered local endemic species confined to Burma. *T. philippinensis* (Philippine teak) is also endangered endemic to the Philippines. *T. grandis* was sometimes known as the "Burmese teak" [1]. The leaves have a top surface that is hairless and measure 6 to 75 cm long by 8 to 45 cm broad and has numerous below star-shaped hairs when mature. Teak-growing regions typically receive 1,250–1,650 mm of yearly rainfall, with a 3-5 months dry season. *T. grandis* leaves are used as an anti-inflammatory, antipyretic, antidiabetic, anti-ulcerogenic, antibacterial, analgesic and antioxidant in the treatment of skin illnesses, diarrhoea and other conditions. Additionally, it has hypoglycemic, anti-asthmatic, antifungal, anti-diuretic and dyeing properties.

Fresh leaves from the plant were taken for anatomical research, and they were repeatedly observed for various organoleptic properties. A medicinal plant material's microscopic identity is determined by its size, colour, taste, apex, surface, base, margin, venation, texture, fracture, and fragrance [2]. For morphological research, many steps are employed, and the inherent diversity in these properties is crucial for the initial assessment of crude pharmaceuticals. The premise for analysis by microscopic characters evaluation is that, as far as cell characteristics are concerned, there are always enough variances between plants of the same kind or other types. For the majority of medications, standardization profiles for herbal pharmaceuticals are not available [3]. Microscopic analysis is a critical step in the early identification of plants, as well as in the detection of minute fragments of raw or powder pharmaceuticals and the detection of adulterants like insects, animal faces, moles, fungi, etc. [4].

Fluorescence analysis can be used to distinguish plant material from its adulterants when physical and chemical criteria are insufficient, as is frequently the case with powdered pharmaceuticals [5,6]. Fluorescence is dependent on the molecular environment's chemical makeup, which can provide information like acidity or lipophilicity. However, because it is difficult to manage the chemical environment, quantitative microscopy based on fluorescence continues to be difficult [7].

In order to examine the chemical makeup of cells and tissues, science uses the methods of biochemistry and histology. Differentiating cell structures is made possible by the numerous stains that histologists have created. It's vital to keep in mind that the colors of stains are not necessarily representative of the true colour of a given tissue, and that a structure that seems one colour when stained with one colour may actually be quite another colour when stained with a different colour [8].

Hence the present study, leaves of *Tectona grandis* were studied using macro and microscopic techniques, as well as fluorescence and histochemical analysis and also phytocompound.

MATERIALS AND METHODS

Collection of leaves

Tectona grandis L. leaves were widely collected from Orathanadu, Thanjavur (Dt), Tamil Nadu, India. The plant was recognized, authenticated, and registered under the number RK 3007. For future use, the information (Fig. 1) was created by a qualified individual, Dr. S. Soosairaj, Director, The Rapinet Herbarium and Centre for

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Molecular Systematic Botany, St. Joseph's College (Campus), Tiruchirappalli - 602 002, India.

Fig. 1. Collection and authentication of *Tectona grandis* leaves.

Macro and microscopic evaluation

Macroscopic study

For the macroscopic studies, fresh *Tectona grandis* leaves were taken from the plant and repeatedly observed for various organoleptic properties. Shape, size, colour, taste, apex, surface, base, margin, venation, texture, and odour are used to determine the macroscopic identity [9].

Microscopic study

To examine the anatomical characteristics, a transverse section of *Tectona grandis* L. leaves was done. To get a thin section, free hand sections of young leaves were taken. The slice was 10 to 12 mm thick prepared in slides for anatomical studies. Transverse leaf sections were placed on a glass slide to which a few drops of chloral hydrate were added and heated for 1-2 minutes. Chloral hydrate is used to clean the tissues and improve visibility. A few drops of a 1:1 mixture of phloroglucinol and concentrated hydrochloric acid were applied and after 3 to 4 minutes, it was examined under a microscope. Under a microscope, photomicrographs of the well-known identifying features were shot using a Sony digital camera [10].

Preparation of leaf powder

The leaves of *Tectona grandis* L. were cleaned and allowed to dry for a week in the shade. Because the colour could not be denatured, the dried leaves were manually pulverised and kept in an airtight container. These powdered ingredients for used for more investigation.

Fluorescence behavior [5,6].

The plant material can be distinguished from their adulterants on the basis of a fluorescence research. When physical and chemical parameters are appropriate, which is frequently the case with powdered medications. Behavior of leaves of *Tectona grandis* L. with different chemical reagents such as aluminum chloride, sulphuric acid, hydrochloric acid, ammonia, chloroform, sodium hydroxide and nitric acid was performed to detect the occurrence of phytoconstituents along with colour changes. The powders were examined in natural daylight as well as under short (254 nm) and long (365 nm) UV light, where the colors seen by applying various reagents in various radiations were noted.

Histochemical analysis [11,12].

Dried and finely powdered *T. grandis* leaves were placed on a microscopic slide free of grease, subjected to a series of chemicals, and allowed to react for 1-2 minutes. The presence of the suitable colour shift following the administration of the reagent served as a sign of a successful histochemical test observing any colour changes using a light microscope. *T. grandis* leaf powder underwent a series of chemical and reagent treatments. When the powdered leaves were treated with a few drops of $FeCl_3$, the result was black, indicating the presence of tannin. When leaf powder was treated with diluted ammonia and H_2SO_4, flavonoids were detected by the yellow coloration. Toludine blue treatment on plant powder resulted in a Blue green/Red colour, which denotes the presence of polyphenol. Plant powder that has been dyed orange with a few drops of dinitrophenol hydrazine contains terpenoids.

RESULT AND DISCUSSION

Macroscopic study

Tectona grandis leaves are green in colour with distinct dorsal and ventral surfaces as shown in Fig. 2. Size varied from 30-60cm in length and 20-30cm in width. These have a rough upper surface, acute, and elliptic or obovate in shape. However, they are typically glabrous, with the lower portion covered in petiolate, opposing, or whorled, big, complete dense stellate-grey or tawny colour. Macroscopic characteristics are given in Table 1. According to Vyas et al. [13], evergreen sprawling shrubs between 1 and 1.8 m tall are the primary characteristics of *Tectona grandis* leaves. Smooth, woody stems. Leaves are opposite, simple, and oblong to elliptical in shape (5–10 cm long, sharp to acuminate tip). They are green, smooth with a little shining upper surface. A cyme or umbel typically consists of three flowers connected at the base; the corolla is white, fused, and has five lobes; the stamens are four, scarlet to purple, and they are bent upward. Fruit is oblong and 1 to 1.5 cm long before becoming black.

Fig. 2. *Tectona grandis* leaves showing dorsal and ventral surfaces.

Table 1. Macroscopic characters of *Tectona grandis* L. (Leaves).

S.no	Characters	Leaves
1.	Shape	Elliptic or Obavate
2.	Size	Length 30-60 cm x Width 20-30cm
3.	Colour	Green
4.	Taste	Astringent, Sweet, Acrid
5.	Apex	Subacute
6.	Surface	Rough
7.	Base	Usually cuneate
8.	Margin	Entire or Crenulate
9.	Venation	Pinnate
10.	Texture	Scaber
11.	Odour	Characteristic, Mild bitter

Microscopic evaluation

Stains and microscopic examination of a transverse section assist distinguish the anatomy in adulterants. With the aid of anatomical features of *Tectona grandis*, the major components of the plant's transverse section were correctly recognised. The transverse section of leaves was examined to determine its internal features. *Tectona grandis* leaves cross-section showing single-layered epidermis and thick, striated cuticle. There are both adaxial and abaxial epidermal cells. Adaxial has a surface appearance that is pentagonal in shape with a very modest wavy contour and is made up of bigger cells. Apostomatic. It has a thick cuticle covering it. Abaxial epidermis has a wavy appearance and is composed of smaller cells. It appears that cell rosettes that have silicified contain cystolith-like structures. Epidermal cells that stretch to create covering trichomes on the ventral surfaces primarily have pointy ends. Collenchymas cells are arranged in three to six rows in the hypodermis. Vascular bundles take up the majority of the midrib, are large, and are positioned in the core. It has a closed xylem cylinder inside of it, and fibres and totally closed pholem cylinders surround it. The adaxial and abaxial elongated and rectangular cells are visible in the lamina's T.S. Palisade and spongy parenchyma formed from distinct mesophyll. A single layer of densely packed columnar parenchyma makes up a palisade. Parenchyma cells are organised in two or three layers of spongy mesophyll (Figures 3-5). According to Gupta et al. [14], the diagnostic macroscopic and microscopic characteristics as well as the numerical criteria given in this work may be helpful for the creation of an appropriate monograph for its accurate identification.

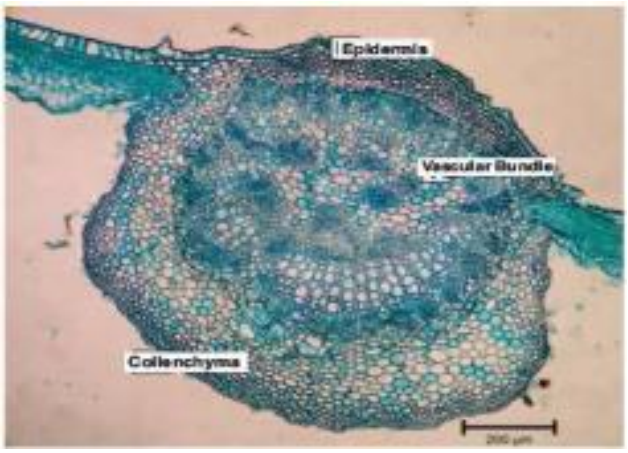


Fig. 3. Transverse Section of *Tectona grandis* L. leaves.

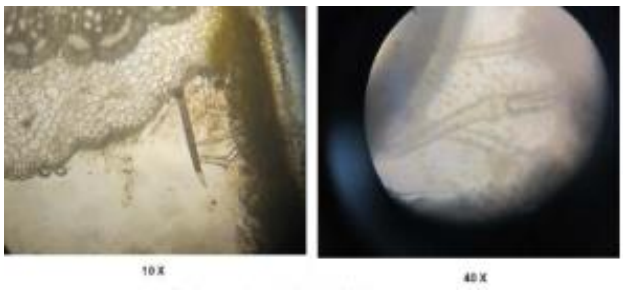
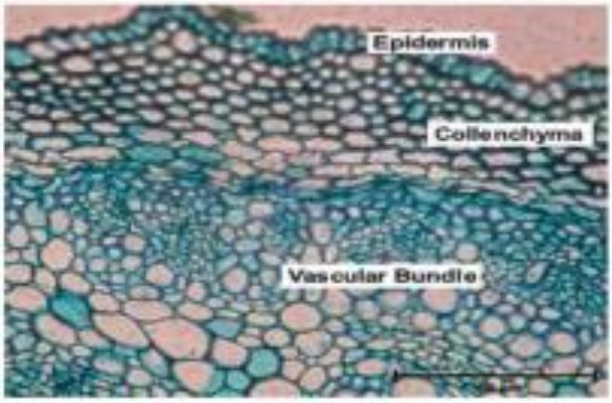
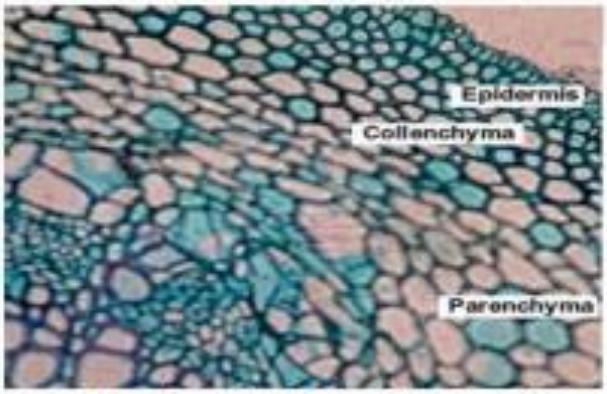


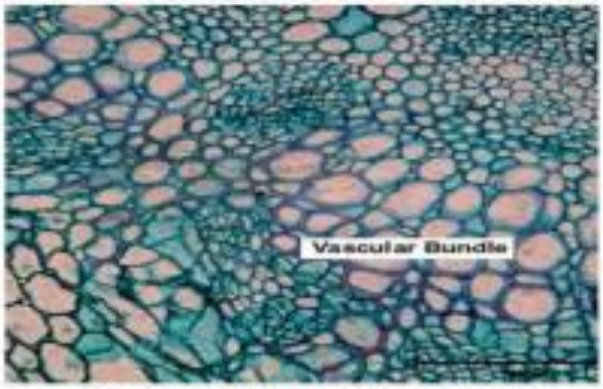
Fig. 4. Enlarged portion of trichomes from *Tectona grandis* L. leaves.



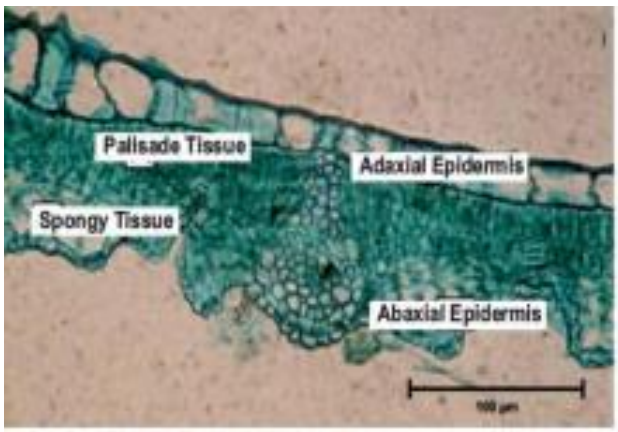
Enlarged portion of adaxial epidermis



Enlarged portion of abaxial epidermis



Enlarged portion of vascular bundles



Enlarged portion of T.S lamina

Fig. 5. Enlarged portions of *Tectona grandis* L. leaves.

Histochemical analysis

Tectona grandis leaves were subjected to a variety of tests in the current investigation. When *T. grandis* leaves powder was treated with diluted ammonia and H_2SO_4, yellow tint suggests flavonoids. The *Tectona grandis* leaves powder treated with few drops of $FeCl_3$ gave black color indicates the presence of tannin. This way, plant histology is dealing with the identification of chemical components of cells and tissues; and it is considered as a powerful tool for localization of trace quantities of substances present in biological tissues. Histological methods toludine blue treatment of powder resulted in colours of blue, green, or red denote the presence of polyphenol. Several drops of Dinitrophenol hydrazine were used to treat plant powder to detect the presence of terpenoids and is indicated by the colour orange, represented in Fig. 6, Table 2. These substances were discovered in the mesophyll cells of the spongy and palisade parenchyma, according to Butler et al. [15]. Additionally, *Roupala montana* leaves were discovered to contain the alkaloids, typical of the Proteaceae family, in the same area as the flavonoids [16].

Table 2. Histochemical analysis of *Tectona grandis* leaves powder

Phytochemical	Result
Tannin	+
Flavonoids	++
Terpenoids	++
Polyphenol	+

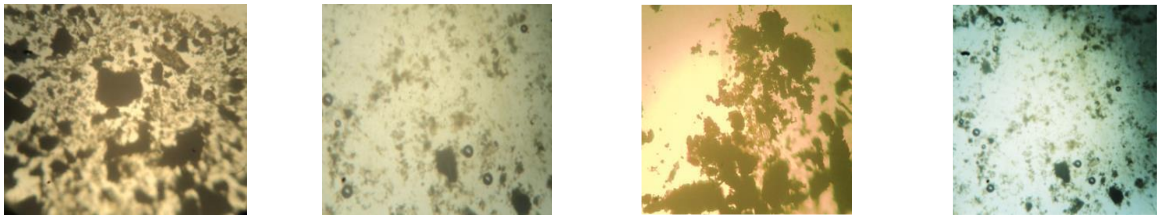
Single plus (+) represents presence and double plus (++) represents highconcentrations

Fluorescence behaviour

Fluorescence behaviour of the powdered leaf of *Tectona grandis* in various chemical reagents was performed under normal and ultra violet (UV) light. The results were presented in Table 3. In determining the quality and purity of medications, whether they are made from polar or non-polar plant materials, the total ash is crucial. Fluorescence behavior is useful to study the important biological studies such as anti- inflammatory, anticancer and antioxidant activities. According to Dinesh Kumar et al. [17], the various pharmacognostic parameters assessed in this study aid in the botanical identification and standardization of the crude leaf part of *Tectona grandis* and provide the researchers and scientists involved in conducting further research on this plant part with the accurate data.

Table 3. Fluorescence behavior of powder on treatment with different chemical reagents of *Tectona grandis* L. Leaves

S.No	Chemicals	Visible light	UV light Short –UV 245nm	Long –UV 365 nm
1.	Young leaf powder (ylp)	Green	Green	Black
2.	Plant leaf powder + H_2O	Green	Light yellow	Black
3.	Leaf powder + C_6H_{14}	Green	Green	Black
4.	Leaf powder + $CHCl_3$	Green	Light yellow	Black
5.	Leaf powder + CH_3OH	Black	Black	Black
6.	Leaf powder + CH_3COCH_3	Dark green	Light yellow	Black
7.	Leaf powder + 1N H_2SO_4 in water	Dark green	Green	Black
8.	Plant powder + 1N HCl	Light green	Light green	Black
9.	Plant powder + H_2SO_4 with equal amount of water	Light green	Green	Black
10.	Plant powder + HNO_3 diluted with equal amount of water	Light green	Light yellow	Black



Tannin **Flavonoids** **Terpenoids** **Polyphenol**

Fig. 6. Histochemical analysis of *Tectona grandis* leaves powder

CONCLUSION

Teak leaf was selected and subjected to formal recognition in this study, which included macro and microscopic, histochemical and fluorescence studies, and at the end of this study, all biomolecules were detected and determined that the plant contains natural dyes.

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