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# Macro and Microscopical, Histochemical and Fluorescence Evaluation of *Tectona grandis* L., Leaves Dye Extract

Amutha K*[1] and Sagaya Giri R[2]

[1-2] Department of Botany, Kunthavai Naachiyaar Government Arts College for Women (A), (Affiliation to Bharathidasan University, Trichy), Thanjavur - 613 007, Tamil Nadu, India

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## Abstract

Natural dyes have, therefore, always played a significant role in human life. Although indigenous knowledge system has been practiced over the years in the past, the use of natural dyes has diminished over generations due to lack of documentation. *Tectona grandis* L., belongs to the family Lamiaceae is an important dye yielding plant in ancient times. Teak leaves can also 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. The term macroscopic refers to substances that can be seen with the naked eye, whereas the term microscopic refers to substances that cannot be seen without the use of a magnifying device. It is possible to identify and localize biomolecules and organelles at the cellular level in various types of cells and tissues using histochemical techniques. Fluorescence is the emission of light by a substance that emits electromagnetic radiation. The results shows that the *Tectona grandis* leaves have dorsal and ventral surfaces that are 30-60cm long and 20-30cm wide. Teak leaf powder was treated with diluted ammonia and $H_2SO_4$ to produce a yellow colour, indicating the presence of flavonoids. A few drops of $FeCl_3$ produced a black colour, indicating the presence of tannin. Blue, green or red coloration indicates the presence of polyphenol, while dinitrophenol hydrazine indicates the presence of terpenoids. Under both regular and UV light, the fluorescence behaviour of teak powder under various situations was investigated.

***Key words*:** *Tectona grandis*, Natural dye, Collection and authentication, Macro & microscopic estimation, Histochemical and Fluroscence study

*Tectona grandis* L., is a large deciduous tree, Family Lamiaceae. Various genera formerly included in the family Verbenaceae are now treated under other families. GRIN genera sometimes placed in Verbenaceae. *Germplasm Resources Information Network*. United States Department of Agriculture. Archived from the original on 2004-11-18. Keeping these points of view the *Tectona grandis* L., should become a Lamiaceae family and subfamily Chloanthoideae. The genus *Tectona* comprises 3 species viz. *T. grandis*, *T. hamiltoniana* and *T. philippinensis*. *Tectona grandis* is native to India, Pakistan, Bangladesh, China, Sri Lanka, Indonesia, Myanmar, Northern Thailand and Northwestern Laos. *Tectona hamiltoniana* (Dahat teak) is an endangered local endemic species confined to Burma. *Tectona philippinensis* (Philippine teak) is also endangered endemic to the Philippines. *T. grandis* was sometimes known as the "Burmese teak" [1]. The leaves are 6 – 75 cm long, 8 - 45 cm wide and hairless on the upper surface. When mature, with many star-shaped hairs below. Typically, through annual rainfall in areas where teak grows averages 1,250-1,650 mm with a 3-5 month dry season. *T. grandis* Leaves is used in the Treatment of Skin diseases, Diarrhoea, Anti- inflammatory, Antipyretic, Antidiabetic, Anti-ulcerogenic, Antibacterial, Analgesic and Antioxidant. It also carries Anti-diuretic, Hypoglycemic, Antiasthmatic, Antifungal and dyeing activities. The macroscopic of a drug includes its visual appearance by the naked eye. For the anatomical studies Fresh leaves were collected from the plant and investigated in different organoleptic features by repeated observation. Microscopic identity of a medicinal plant material is based on shape, size, colour, taste, apex, surface, base, margin, venation, texture, fracture, and odour [2].

A number of different bases are used for morphological studies and a natural variation in these characteristics play an important role for preliminary evaluation of crude drugs. The basis of analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as for as the cell characteristics are concerned. Standardization profiles of herbal drugs are not available for most drugs [3]. The macroscopic and microscopic evaluation is a crucial step in the preliminary identification of plants as well as for detection of small fragments of crude or powder drugs and detection of adulterants like insects, animal's faces, moles, fungi etc., by identifying characteristic tissue features [4].

When physical and chemical parameters are inadequate as it often happens with the powdered drugs and the plant material may be identified from their adulterants on basis of fluorescence study [5-6]. Behaviour of leaves of some

***Correspondence to:** Amutha K, E-mail: amuthakarunanithi14@gmail.com; Tel: +91 8525983102

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medicinal plants with different chemical reagents such as Alumium chloride, sulphuric acid, hydrochloric acid, Ammonia, chloroform, Sodium hydroxide and nitric acid was performed to detect the occurrence of phyto constituents along with colour changes. The powders were observed in normal daylight and under short (254nm) and long U.V. light (365 nm), where the colours observed by application of different reagents. Fluorescence depends on the chemical nature of the molecular environment providing information such as acidity or lipophilicity. Quantitative microscopy based on fluorescence remains a challenge, however, because it is not easy to control the chemical environment [7].

Science combines the techniques of biochemistry and histology in the study of the chemical constitution of cells and tissues. Histologists have developed many stains which are suited to particular purpose, allowing cell structures to be differentiated. It is important to remember that the colours of stains are not the real colour of a particular tissue and that a structure that appears as one colour using one stain, may be a quite different colour using another stain [8]. Hence the present study, leaves of *Tectona grandis* were studied using macro and microscopic techniques, as well as fluorescence and histochemical analysis. Overall, based on the above concept, this article will evaluate whether or not there are phytocompounds.

## MATERIALS AND METHODS

*Chemicals*

Chloral hydrate [$C_2H_3Cl_3O_2$], Phloroglucinol [$C_6H_6O_3$], Hydrochloric acid [HCl], Aluminum chloride [$AlCl_3$], Sulphuric acid [$H_2SO_4$], Ammonia [$NH_3$], Chloroform [$CHCl_3$], Sodium hydroxide [NaOH], Nitric acid [$HNO_3$], Ferric chloride [$FeCl_3$], Toludine [$C_7H_9N$], Dinitrophenol hydrazine [$C_7H_9N$].

*Collection of Tectona grandis L., leaves*

The leaves of the plant, *Tectona grandis* L. were collected wildly from the Orathanadu, Thanjavur (Dt), Tamil Nadu, India, during the month of May 2020.

*Macro and microscopic evaluation*

*Macroscopic study*

The macroscopic of a drug includes its visual appearance by the naked eye. For the anatomical studies Fresh leaves of *Tectona grandis* were collected from the plant and investigated in different organoleptic features by repeated observation. Macroscopic identity of a medicinal plant material is based on shape, size, colour, taste, apex, surface, base, margin, venation, texture and odour [9].

*Microscopic study*

The microscopic of transverse section of *Tectona grandis* L., leaves was carried out to study the anatomical characters. The fresh leaves of *T. grandis* were collected and free hand sections were taken to obtain a thin section. The thickness of the section was 10-12 micrometers. Anatomical study invariably slides were prepared. The transverse sections of leaf was taken on a glass slide to which are added a few drops of chloral hydrate and was heated for 1-2 min, After placing a cover slip, care should be taken to avoid air bubbles and to see that there is sufficient chloral hydrate under the cover slip. Excess of chloral hydrate outside the cover slip is to be withdrawn using a blotting paper (Chloral hydrate is used to clear the tissues and to bring in clarity of the view) Lignified tissue is to be confirmed by staining. To the powder a few drops of mixture of 1:1 Phloroglucinol +Conc HCl was added and after 3 to 4 minutes it was observed under microscope. The well-known identifying characters were taken Photomicrographs by Sony digital camera under the microscope [10].

*Preparation of leaves powder*

The *Tectona grandis* L. leaves were cleaned and dried under shade for 7 days. These dried leaves were mechanically powdered and stored in an airtight container because it could not be denatured the colour. These powdered materials were used for further analysis.

*Fluorescence behavior* [5-6]

When physical and chemical parameters are in adequate as it often happens with the powdered drugs the plant material may be identified from their adulterants on basis of fluorescence study. Behaviour 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 observed in normal daylight and under short (254nm) and long U.V. light (365 nm), where the colours observed by application of different reagents in different radiations were recorded.

*Histochemical analysis* [11-12]

A small quantity of dried and finely powdered *T. grandis* leaves sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemical was indicated by the appearance of the appropriate colour change after application of the reagent. Using a light microscope to observe and record any colour changes. The powder of *T. grandis* leaves powder was treated with specific chemicals and reagents. The treated plant powder further analyzed in light microscope. The *T. grandis* leaves powder treated with few drops of $Fecl_3$ gave black color indicates the presence of tannin. The *T. grandis* leaves powder treated with diluted ammonia and $H_2SO_4$ gave yellow colour indicates flavonoids. Plant powder treated with Toludine blue to give blue green/red colour indicates the presence of polyphenol. Plant powder treated with dinitrophenol hydrazine (few drops) to give orange colour indicates the presence of terpenoids.

## RESULTS AN DISCUSSION

*Identification and authentication of Tectona grandis L., leaves*

The plant is identified, authenticated and registered under the number, RK 3007 of selected plant *Tectona grandis* L., (Fig 1). Teak by professional, Dr. S. Soosairaj, Director, The Rapinet Herbarium and Centre for Molecular Systematic, St. Joseph's College (Campus), Tiruchirappalli – 602 002, India, for future reference.


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

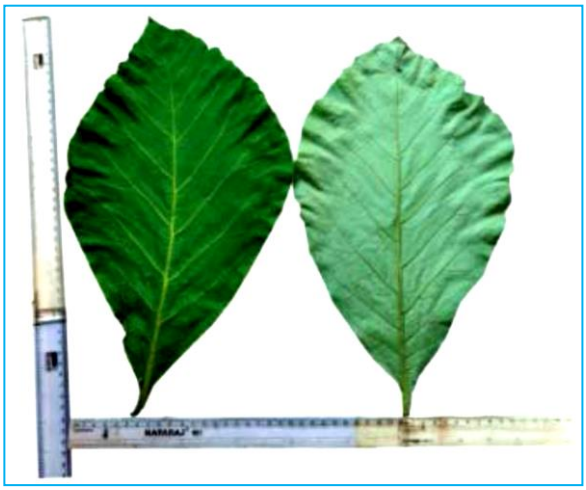


Fig 2 Dorsal and ventral leaves of *Tectona grandis* L.

*Macroscopic study*

*Tectona grandis* leaves are green in colour, Dorsal and ventral surface of the leaves had showed in (Fig 2) 30-60cm in length and 20-30cm in width. These are Elliptic or Obovate, Acute and the upper surface rough but usually glabrous, the lower clothed with dence stellategrey or tawny tomentum, petiolate, opposite or whorled, large, entire. Macroscopic features were represented in (Table 1). Dineshkumar *et al*. [13] reported that the main diagnostic features of *Tectona grandis* leaves are evergreen sprawling shrub 1-1.8 m tall. Stems woody, smooth. Leaves ovate to elliptical (5-10 cm) long, acute to acuminate tip, green, smooth, slightly shiny upper 2 surface, pinnate venation, margins entire, leaves opposite, simple. Cyme or umbel usually comprised of 3 flowers joined at a common base point; corolla white, fused, with 5 lobes; stamens 4, reddish to purple and upwardly curved. Fruit green turning black, 1 – 1.5 cm long, obovoid.

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

| Characters | Leaves |
|---|---|
| Shape | Elliptic or Obavate |
| Size | Length 30-60 cm × 20-30 cm |
| Colour | Green |
| Taste | Astringent, sweet, acrid |
| Apex | Subacute |
| Surface | Rough |
| Base | Usually, cuneate |
| Margin | Entire or crenulate |
| Venation | Pinnate |
| Texture | Scaber |
| Odour | Characteristic, mild bitter |

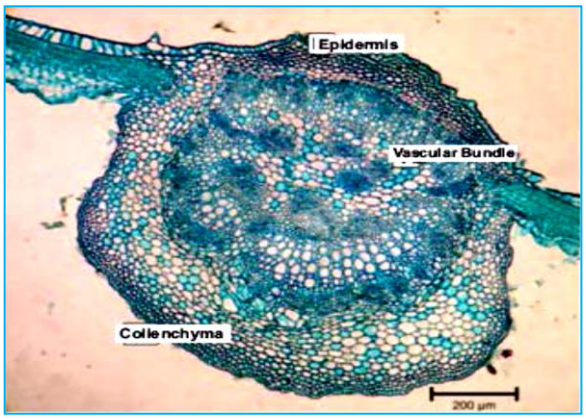
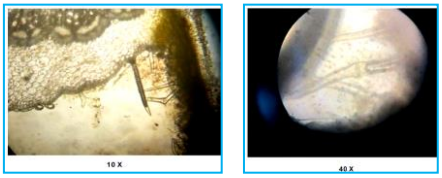
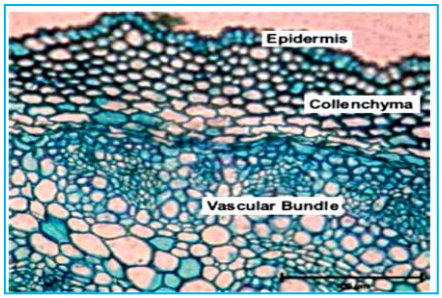



Fig 3a Transverse section of *Tectona grandis* L. leaves

Enlarged portion of trichomes

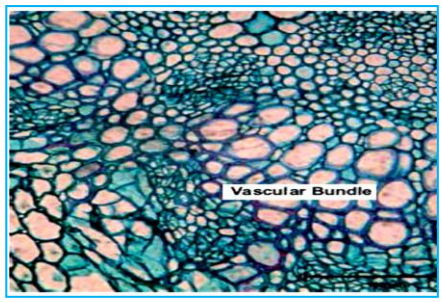


Enlarged portion of adaxial epidermis

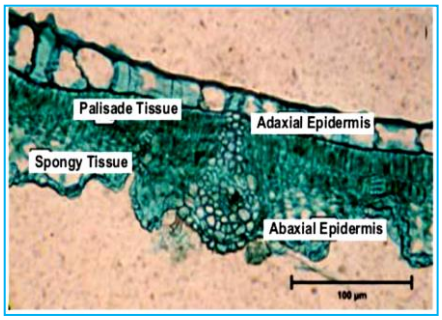



Enlarged portion of abaxial epidermis


Enlarged portion of vascular bundles




Enlarged portion of T.S lamina




*Microscopic evaluation*

Microscopic examination of transverse section aided by stains help in distinction of anatomy in adulterants. Main characters of the transverse section of plant were properly identified with the help of anatomical characters in *Tectona grandis* L. leaves transverse section was studied to identify its internal characters. Transverse section of *Tectona grandis* L. leaves covered with single layered epidermis with thick striated cuticle. Adaxial and Abaxial Epidermal cells are present. Adaxial composed of larger cells and pentagonal in surface view with very slightly wavy contour. Apostomatic. It is covered by thick cuticle. Abaxial epidermis made up of smaller cells with wavy outline. Silicified rosettes of cells contain cystolith like bodies are seen. Epidermal cells on the ventral sides elongate to form covering trichomes show mostly pointed end. Hypodermis region composed of 3-6 rows of collenchymas cells. Vascular bundles are Large, suited in the centre and occupies the major portion of the midrib. It contains closed cylinder of Xylem and surrounded by completely closed

cylinder of Pholem and fibres. T.S of lamina shows the Adaxial and Abaxial Elongated and Rectangular cells. Mesophyll differentiated into palisade and spongy parenchyma. Palisade composed of single layer of closely packed columnar parenchyma. Spongy mesophyll is composed of 2 or 3 layers of loosely arranged parenchyma cells (Fig 3a,b). Gupta *et al*. [14] reported that the diagnostic macroscopic and microscopic features and the numerical standards were reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

Table 2 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. | Plant powder (pp) | Green | Green | Black |
| 2. | Plant powder + $H_2O$ | Green | Light yellow | Black |
| 3. | Plant powder + $C_6H_{14}$ | Green | Green | Black |
| 4. | Plant powder + $CHCl_3$ | Green | Light yellow | Black |
| 5. | Plant powder + $CH_3OH$ | Black | Black | Black |
| 6. | Plant powder + $CH_3COCH_3$ | Dark green | Light yellow | Black |
| 7. | Plant 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 |

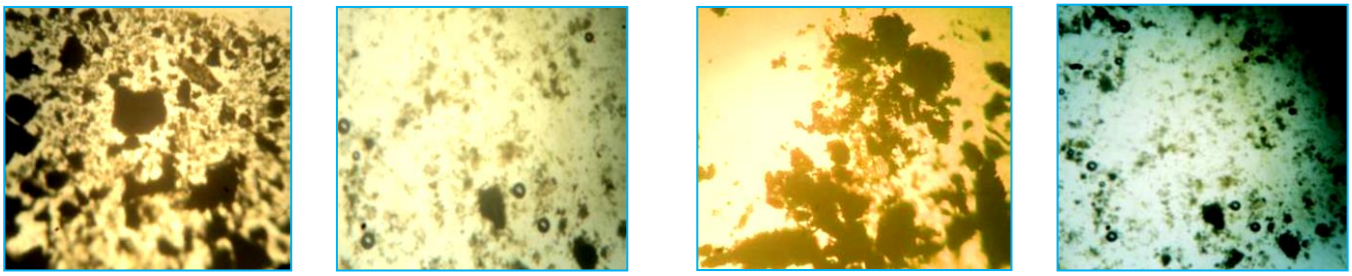
Table 3 Histochemical analysis of *Tectona grandis* leaves powder – phytochemical result

| Phytochemical | Result |
|---|---|
| Tannin | + |
| Flavonoids | ++ |
| Terpenoids | ++ |
| Polyphenol | + |

Single plus (+) represents presence and double plus (++) represents high concentrations

*Fluorescence behaviour*

Fluorescence behaviour of the powdered leaf of *Tectona grandis* L., in various chemical reagents was performed under normal and Ultra Violet (UV) light. The results were presented in (Table 2). The total ash is particularly important in the evaluation of purity and quality of drugs, extractive of the polar or non-polar extractable compounds in a plant material. Fluorescence behavior is useful to study the important biological studies such as anti- inflammatory, anticancer and antioxidant activities. Dinesh Kumar *et al*. [13] stated that the Various pharmacognostic parameters evaluated in this study helps in botanical identification and standardization of *Tectona grandis* L. leaf part in crude form and provide the authentic data for the researchers and scientists involved in carrying out further research on this plant part. The significance of total ash content in evaluating the purity and quality of drugs, as well as the extractability of polar or non-polar compounds from plant materials.



Tannin Flavonoids Terpenoids Polyphenol

Fig 4 Histochemical analysis of *Tectona grandis* leaves powder

*Histochemical analysis*

In the present study, *Tectona grandis* leaves were treated with specific chemicals and reagents. The *Tectona grandis* leaves powder treated with diluted ammonia and $H_2SO_4$ gave yellow colour indicates flavonoids. The *Tectona grandis* leaves powder treated with few drops of $Fecl_3$ gave black color indicates the presence of tannin. Plant histology dealing with the identification of chemical components of cells and tissues; it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques powder treated with Toludine blue gave Blue green/Red colour indicates the presence of polyphenol. Plant powder treated with Dinitrophenol hydrazine (few drops) to give Orange colour indicates the presence of Terpenoids, represented in (Fig 4, Table 3). Tectona grandis leaves contain flavonoids, tannins, and polyphenols. Histochemical techniques were employed to localize and identify these chemical components within the cells and tissues of the leaves. Butler *et al*. [15] reported that these compounds were identified in the mesophyll cells in the spongy and palisade parenchyma. Furthermore, the alkaloids, typical of Proteaceae, were also found in leaves of *Roupala montana*, in the same region where the flavonoids are present.

## 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.

***Acknowledgements***

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## LITERATURE CITED


1. Palanisamy K, Hegde M, Yi JS. 2009. Teak (*Tectona grandis* Linn. f.): A renowned commercial timber species. *Jr. Forage Science* 25(1): 1-24.
2. Joshi RA, Shukla E, Sairam RK. 2011. In vitro screening of rice genotypes for drought tolerance using polyethylene glycology. *Acta Physiology Plant* 33(6): 2209-2217.
3. Mukherjee G. 2002. Pharmacognostic strudies of Hybanthus enneasperm. *Phytochemistry* 27: 268-271.
4. Babalola KA, Victoria AA. 2009. Foliar epidermal morphology of two west African genera of Haloragaceae (Saxifragales). *Journal of Science Research Development* 11: 84-91.
5. Chase CR, Pratt RJ. 1949. Activity of pharmacology. *Asian Journal of plant Science Research* 38: 32-34.
6. Kokoski CJ, Kokoski RJ, Slama FJ. 1958. Fluorescence of powdered vegetable drugs under ultra violet radiation. *Journal of American Pharma Association* 2(10): 715-717.
7. Peter K, Satoshi N, Christopher GH, Sierin L, Ryo F. 2020. Direct fluorescence imaging of lignocellulosic and suberized cell walls in roots and stems, *AoB Plants* 12-4, plaa032, https://doi.org/10.1093/aobpla/plaa032
8. Gupta. 2001. Tissue culture process for producing a large number of variable cotton in vitro plants. *Patent*. 6: 242-257.
9. Kirtikar KR, Basu BD. 1984. *Indian Medicinal Plants*. Bishen Singh, Mahendra Pal Singh, Dehra Dun. 2(2): 1550-1552.
10. Evans WC, Trease. 2009. *Textbook of Pharmacognosy*. Saunders, London. pp 562.
11. John Peter Paul J. 2014. Histochemistry and fluorescence analysis of *Turbinaria Ornata* (Turner). J. Ag. –An Important Brown Seaweed (Phaeophyceae). *Indian Journal of Plant Sciences* 3(1): 40-44.
12. Gersbach SP, Wyllie VG, Sarafis V. 2001. A new histochemical method for localization of the site of monoterpene phenol accumulation in plant secretory structures. *Annals of Botany* 88(4): 521-525.
13. Dinesh K, Ajay K, Om P. 2014. Pharmacognostic investigation of *Clerodendrum phlomidis* L. root. *Journal of Tropical Life Science* 4(2): 96-100.
14. Gupta D, Chandrashekar Richard, Yogendra L, Gupta N. 2012. *In-vitro* antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. *Der Pharma Letters* 4: 614-661.
15. Butler MS, Katavic PL, Davis RA, Forster PI, Guymer GP, Quinn RJ. 2000. 10-Hydroxydarlingine, a new tropane alkaloid from the Australian proteaceous plant *Triunia erythrocarpa*. *Jr. Nat. Prod*. 63: 688-689.