

# ANATOMICAL AND PHYTOCHEMICAL STUDIES ON *CALOPHYLLUM INOPHYLLUM* FRUIT SEED

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## ABSTRACT

*Calophyllum inophyllum* Linn. is a medium sized to large tree belongs to the family Calophyllaceae. Is the most abundant species, widely distributed throughout the tropical regions worldwide and they are mostly trees and shrubs, with milky sap and fruits or capsules for seeds. It is commonly called as tamanu, beach or beauty leaf. In Tamil, it is known as Punnai. All parts of this plant, such as leaves, root, and fruit, can be useful for humans. This paper is to discuss the anatomical phytochemical studies on *C. inophyllum* seed. The anatomical analysis was done by the freehand using with compound microscope. There are various kinds of phytochemicals contained in *C. inophyllum* fruit seed extract, such as carbohydrate, alkaloids, flavonoids, anthraquinone, phenolic compounds, tannin, cardiac glycosides, lignin and volatile oil compounds and they have benefits to human medicinal needs.

**Keywords:** *C. inophyllum*, beauty leaf, anatomicals, phytochemicals

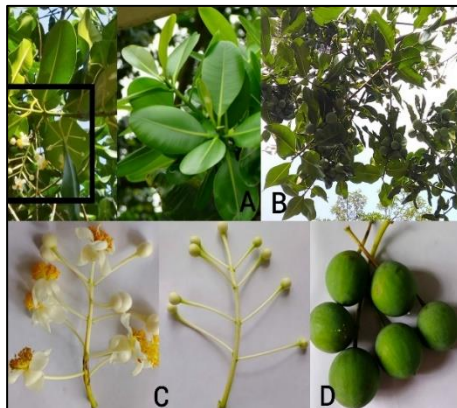
## 1. Introduction

*Calophyllum inophyllum* is a medium sized to large tree belongs to the family Calophyllaceae. The genus *Calophyllum* was previously included in the Clusiaceae family. Now, the APG III (Angiosperm Phylogeny Group) system of flowering plant classification classifies it under Calophyllaceae family<sup>[1]</sup>. It comprises of 180 to 200 species and they are mainly distributed in Asia, with some species in Africa, the Americas, Australasia, and the Pacific Island. The tree is a special ornamental in the Pacific. On the West Coast, it is found from Mumbai Southwards to Southern Kerala and along the East Coast, from Orissa southwards. It cannot withstand indefinite water logging. It is sensitive to frost and fire.

## CALOPHYLLUM INOPHYLLUM

*C. inophyllum* is upright, pyramidal, densely foliated evergreen tree can reach 60 feet in height in the forest with a broad spreading crown of irregular branches, but is generally much smaller because it grows slowly. The species is widely distributed throughout the tropical regions worldwide. It grows in areas with periodic downfall ranging from about 1000 to 5000 mm. The tree grows in a wide variety of soil, from nearly pure littoral beach to complexion, and is able of grown on degraded and inadequately drained spots. This tree has stick, latex clear or opaque and white, cream or yellow and bole usually twisted or leaning, without buttresses. Stems transude a milky white latex when cut. The leaves of *C. inophyllum* are dark green, shiny, and hairless with broadly elliptic, 10-20 cm long and 6-9 cm wide. The tip of the leaves is rounded, base acute and petioles 1 to 1.6 cm long stout, flat. The white flowers are about 25 mm wide and occur in

axillary, racemose or paniculate inflorescences consisting of 4 to 15 flowers and oblong petals and ovate sepals (Figure 1). The fruit (a drupe) is green, round, and typically 2 to 4 cm in diameter including a thin (3 to 5 mm) layer of pulp, the shell, and the single large seed with nutlike kernel that may be poisonous and fully mature fruits are yellow- or red-brown and wrinkled. Fruits are usually borne twice a year<sup>[2]</sup>.



**Figure-1 A – Branch of Leaf with Flowers, B – Branch containing Fruits, C – Inflorescence, D – Fruits**

## 2. MATERIALS AND METHODS

### 2.1 Source of Plant materials

The Fruit of *Calophyllum inophyllum* L. was collected in the month of December 2021, from campus of Nirmala College for Women (Autonomous), Coimbatore District, Tamil Nadu, India. Debris were removed and cleaned before the process of air drying.

### 2.2 Preparation of plant material

*C. inophyllum* fresh immatured fruit were chopped into two pieces then were separated into fruit peel, fruit shell, fruit kernel and nut are taken away for before finely cut and nut is used for further process. Direct sun drying is not recommended, because it may vaporize phytochemical in the plant materials, so only shade drying is preferred. Nut are grinded separately by using blender. It is grinded well more than 10 times until we get fine powder of these species. The powdery texture is most important for further testing.

### 2.3 Preparation of extraction for phytochemicals:

#### Maceration

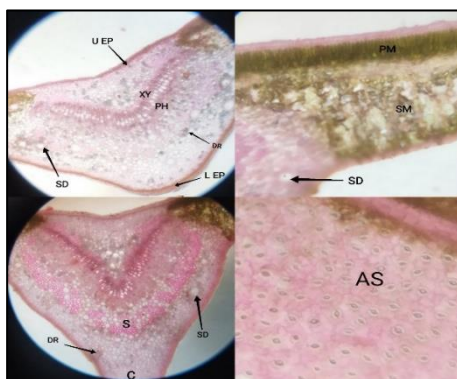
Maceration was done by using shaker, 50 gm of *C.inophyllum* seed powder was macerated with ethanol is taken in the conical flask and placed in the shaker for 4 to 5 days. The contents of conical flask were stirred intermittently to ensure the efficiency of the extraction. After 5 days, contents in the conical flask were filtered and the ethanolic extract of nut was subjected to various chemical tests to know the nature of the compounds present.

## 3. RESULTS

### 3.1 Anatomical studies

An anatomical character of *Calophyllum inophyllum* show some unique feature which helps us to identify the plant species. Upper and lower epidermis of T.S of leaf, enclosing a dorsiventral mesophyll. Both Epidermis are covered with cuticles and except for the absence of trichomes. Its upper (adaxial) epidermis composed of parenchyma cells is flat while the lower (abaxial) surface forms a convex shape mesophyll has two layers of closely arranged palisade parenchymatous cells with chlorophyll which perform photosynthesis

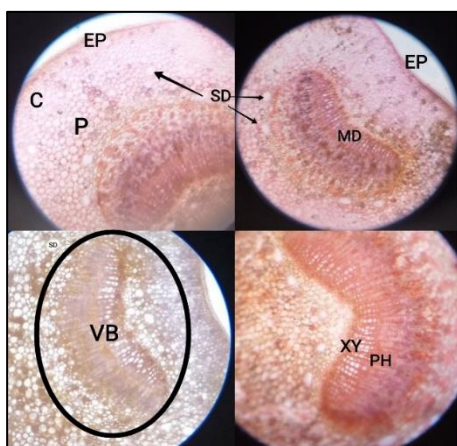
(Figure-2). The third layer of mesophyll consist of loosely arranged spongy parenchymatous cells with air chambers.



**Figure-2** EP – Epidermis, PM – Palisade mesophyll, SM – Spongy mesophyll, C – Collenchyma, P – Parenchyma, XY – Xylem, PH – Phloem, SD – Secretory ducts, M – Midrib, DR – Druse, PS – Anomocytic Stomata.

Endodermis and pericycle is indistinct which is a unique feature in the leaf of *C. inophyllum*. The vascular bundle has V format where xylem surrounded by phloem. The condition of the xylem is endarch. The vascular bundles are surrounded by parenchymatous cells and the position of its xylem is adjacent to the upper epidermis while the phloem is near towards the lower epidermis. It has anomocytic stomata and secretory ducts presented in lower (abaxial) surface. Anomocytic stomata lack subsidiary cells. These glands are responsible for the production of viscous exudates that can be toxic, deterrent or repellent to herbivores, in addition to acting against microorganisms and sealing wounds. The druse which is indicating present of calcium oxalate crystals are scattered.

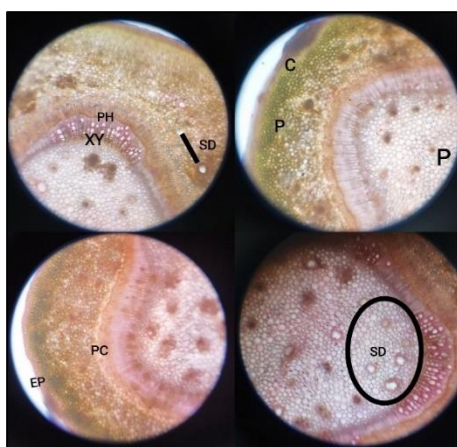
T.S of petiole with epidermis, glabrous, with thick cuticle layer. Under the epidermis occurs parenchyma and collenchyma, both with secretory ducts. Throughout the petiole there is only one wide collateral vascular bundle in the U form it composed of phloem and xylem and crossed by medullary rays (Figure-3). The medullary rays are elongated thick, lignified walled cells. Leaf lamina presents in epidermis central vein, collenchyma in small amounts on both sides, parenchyma, secretory ducts and a single collateral vascular bundle, also in the U form.



**Figure-3** EP – Epidermis, VB – Vascular bundle, XY – Xylem, PH – Phloem, MD – Medullary rays, C – Collenchyma, P – Parenchyma, SD – Secretory ducts.

It consists of 2-3 rows of small, thick walled collenchymatous cells with no intercellular spaces followed by 4- 6 rows of large thin walled parenchymatous cells with small intercellular spaces. Endodermis is indistinct and the pericycle is formed of parenchymatous cells interrupted by small fibre. In lateral and upper (adaxial) epidermis there is laminar vascularized expansion composed of collenchymatous tissue with secretory ducts. The pith is formed of large thin walled parenchyma cells and some clusters of calcium oxalate (Druse) are scattered in the pith region. Secretory ducts and calcium oxalate crystals (druse) is unique feature which is found in this genus *Calophyllum*.

T.S of stem is cylindrical shape, epidermis covered with thin cuticle and hairs are absent. The cortex tissue consists of an outer collenchymatous zone and an inner parenchymatous one (Figure-4). The collenchymatous layer is formed of 3-4 rows of small, rounded thick walled cells with no intercellular spaces. The parenchymatous layer is formed of 4-7 rows, large thin walled cells with intercellular spaces. Some of the phloem parenchyma contains clusters of calcium oxalate. The endodermis is parenchymatous and indistinguishable.



**Figure-4** EP – Epidermis, XY – Xylem, PH – Phloem, P - Pericycle, C – Collenchyma, P – Parenchyma, SD – Secretory ducts.

The pericycle is formed of parenchymatous cells interrupted by scattered groups of lignified pericyclic fibres. The cambium is formed of 4-6 rows of radially arranged, tangentially elongated thin walled. The cortex, followed by the vascular tissue consisting of phloem and xylem extending to the centre. The pith consists of large, thin walled, more or less rounded parenchymatous cells showing clusters of calcium oxalate and secretory ducts. Central cylinder shows on the periphery groups of not lignified fibres, primary phloem and scarce secondary, cambium, reduced amount of secondary xylem, and primary xylem. There is pith of parenchymatic nature with secretory ducts.

### 3.2 Phytochemical Analysis

The preliminary phytochemical analysis of *C. inophyllum* immature seed was carried out in present study. There are tested 17 different chemical constituents such as Carbohydrate, Protein, Amino acid, Alkaloids, Flavonoids, Sterols, Terpenoids, Anthraquinone, Phenolic compounds, Quinones, Tannin, Saponins, Cardiac glycosides, Glycosides, Lignin, Coumarins and Volatile oils. It was observed that seeds in ethanol extract possess high content of carbohydrate and also traces number of alkaloids, flavonoids, anthraquinone, phenolic compounds, tannin, cardiac glycosides, lignin and volatile oils were also present. Whereas the content of protein, amino acid, sterols, terpenoids, quinones, saponins, glycosides and coumarins were absent in seed extract of *C. inophyllum*.

Our results were argument with ethanol extract of seed which also shows the presence of all the reported chemical constituents. Except protein, all other secondary metabolites such as alkaloids, flavonoids, anthraquinone, phenolic compounds, tannin, cardiac glycosides, lignin and volatile oil present in *C. inophyllum*.

Carbohydrates constitute a group of chemically defined substances with a range of physical and physiological properties and health benefits for consumers. Volatile oils are made up of a mixture of compounds that give a characteristic flavour and odour. Cardiac glycosides are chemical compounds responsible for the poisoning of livestock and the treatment of congestive heart failure. Phenolic compounds responsible for several bioactivities such as antioxidant, cytotoxic, antimicrobial, among others. Anthraquinone used as anticancer agents. Flavonoids are main class of secondary metabolites and occur in different tissues and organs in diverse plant species. The higher accumulation of flavonoids in a wide variety of fruits and vegetables increases their economic value because flavonoids are good for human health. Alkaloids are secondary metabolites originally defined as pharmacologically active compounds, primarily composed of nitrogen. Thus, the seed of *C. inophyllum* high content of phytochemical which utilize them for the production of medicinal compound.

**Table 1. PHYTOCHEMICAL CONSTITUENT PRESENT IN SEED (NUT)  
EXTRACT OF *C. INOPHYLLUM***

| S. No | Test for phytochemical | Ethanol |
|-------|------------------------|---------|
| 1.    | Carbohydrate           | +++     |
| 2.    | Protein                | –       |
| 3.    | Amino acid             | –       |
| 4.    | Alkaloids              | +++     |
| 5.    | Flavonoids             | ++      |
| 6.    | Sterols                | –       |
| 7.    | Terpenoids             | –       |
| 8.    | Anthraquinone          | ++      |
| 9.    | Phenolic compounds     | +++     |
| 10.   | Quinones               | –       |
| 11.   | Tannin                 | +++     |
| 12.   | Saponins               | –       |
| 13.   | Cardiac glycosides     | ++      |
| 14.   | Glycosides             | –       |
| 15.   | Lignin                 | +++     |
| 16.   | Coumarins              | –       |
| 17.   | Volatile oils          | +       |

#### 4. CONCLUSION

Anatomical studies, phytochemical analysis and cytotoxicity studies of *C. inophyllum* was attempted in the present study. From in this study we have conclude that possible phytochemicals of *C. inophyllum* was explored and also the anatomical features which we can identify from the other genus of the same species. The unique characteristic

features help to identify this plant species. Further studies are needed to explore the possible biological active constituents from immature fruit seed of *C. inophyllum*.

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