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PHARMACOGNOSTIC AND PHYSIOCHEMICAL STANDARDISATION OF LEAVES OF CERIOPS DECANDRA (GRIFF.) DING HOU

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ABSTRACT

Ceriops decandra (Griff.) Ding Hou (Family: Rhizophoraceae) is an evergreen mangrove tree commonly known as Chiru kandal in Tamil language. It is traditionally used in the treatment of haemorrhage, hepatitis and ulcers. Towards authentication and quality assurance of medicinal plants, pharmacognostical studies of the leaves of Ceriops decandra (Griff.) Ding Hou were carried out. Macroscopical characters and microscopical characters of leaves were perceived. The Physiochemical properties (total ash, acid insoluble ash, water soluble ash, water soluble extractive value and alcohol soluble extractive value and pH) of leaf powder were studied. Information obtained from these studies can be used as markers in the identification and standardization of this plant and also towards monograph development on the plant.

KEYWORDS: Ceriops decandra, Leaves, Pharmacognostic profile, Physiochemical properties.

INTRODUCTION

Ceriops decandra (Griff.) Ding Hou belonging to the family Rhizophoraceae, commonly known as Chiru kandal (Tamil), Gatharu (Telugu), Goran (Hindi), Bara goran (Bengali), Gartah (Oriya)[1] is a straight columnar tree, usually of small to medium size, but under favourable conditions attaining a height of 35 m and a diameter of the trunk of 35 cm, with a relatively narrow crown and short basal buttresses which appear to develop from the fusion of

clusters of stilt roots. The roots are superficial, spreading radially, with small knobby or looping pneumatophores in wet sites. Bark whitish or pale grey, smooth but slightly fissured towards the base, peeling around the buttresses; branches conspicuously jointed with swollen nodes.[2] The leaves are coriaceous, opposite and clustered at the end of the twigs. The flowers are head-like with deeply lobed calyx, white petals, stamens twice the number of calyx lobes. Fruits are ovoid-conical berry.[4] The hypocotyl is characteristically green, 9–17 cm long, strongly ridged, sulcate, generally held erect or upright. Germination is epigeal and viviparous. Flowering during winter and fruiting during summer and monsoon months.[3]

However, no pharmacognostic study has been carried out on this plant and hence the objective of the present study is to evaluate various pharmacognostic properties including macroscpoic and microscopic and physicochemical characterization of the leaves of Ceriops decandra.

MATERIALS AND METHODS

Collection and authentification of plant

The leaves of Ceriops decandra (Griff.) Ding Hou were collected from Pichavaram Mangrove Forest near Chidambaram, South India. The specimen was authenticated by Prof. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai by specimen no. PARC/2015/3169 and has been deposited in the herbarium of the Department of Pharmacognosy, SRM College of Pharmacy, SRM University, Kattankulathur, Tamil Nadu, India.

PHARMACOGNOSTIC STUDIES

Macroscopy

Morphological characters were studied by observing the leaves of Ceriops decandra and colour, odour, taste, apex, base, size, shape and surface of the leaves were noted.

MICROSCOPY

Preparation of the specimens

Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol.[5] Infiltration of the specimens was carried by gradual

addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

SECTIONING

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure.[6] The sections were stained with Toluidine blue.[7] Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and IKI(for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid[5] were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with Naoh and mounted in glycerine medium after staining. Different cell component were studied and measured.

PHOTOMICROGRAPHS

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books.[8]

PHYSIO-CHEMICAL INVESTIGATIONS[9]

Loss on drying

Loss on drying determines both water and volatile matter in the crude drug. An accurately weighed quantity of about 1.5 g of powdered drug was taken in a tared porcelain dish. The powdered drug was distributed evenly. The porcelain dish kept in oven and the sample was

dried at temperature 110^0 C for 2 hrs until a constant weight was recorded. Then it was cooled in desiccator to room temperature, weighed and recorded. % Loss on drying was calculated using the formula.

ASH VALUES

Ash values are helpful in determining the quality and purity of crude drugs and to establish the identity of it.

TOTAL ASH VALUE

About 2g of the air dried powdered drug was accurately weighed and taken in a previously tarred crucible. The sample was ignited in a incinerator by gradually increasing the temperature until all the carbon was burnt off (the ash will be white). It is then cooled in a desiccator and weighed. The percentage of total ash value with reference to the air dried sample was calculated by using the formula.

ACID INSOLUBLE ASH VALUE

25ml of the dilute hydrochloric acid was taken in crucible containing the total ash. The ash is washed with acid and transferred to a 100ml beaker. It is then boiled in a water bath for 5 minutes, cooled and then filtered through an ash less filter paper. The filter paper along with the ash was ignited in a silica crucible. First it has to be heated gently till all the vapours has been evolved, then strongly until all carbon has been removed. The crucible was then cooled in a desiccator and weighed. The acid-insoluble ash of the crude drug with reference to the air dried sample of the crude drug was calculated by using the formula.

WATER SOLUBLE ASH VALUE

The total ash was boiled with 25ml of water for 5 minutes and it was filtered through an ash less filter paper. The filter paper along with the ash was ignited in a silica crucible, cooled and the water insoluble ash was weighed. The water soluble ash of the crude drug with reference to the air dried sample of the crude drug was calculated by using the formula.

EXTRACTIVE VALUES

Extractive values are used for the evaluation of crude drug, to predict the nature of the chemical constituents present in a crude drug and also for the estimation of constituents soluble in that particular solvent used for extraction.

ALCOHOL SOLUBLE EXTRACTIVE VALUE

About 5g of the powdered drug was accurately weighed and transferred to a conical flask containing 100ml of 95% alcohol, a flask was corked and set aside for 24hrs with frequent shaking. Then it was filtered and 25ml of the filtrate was transferred to a previously weighed porcelain dish. The filtrate was evaporated to dryness in a water bath and then dried in an oven at 100 degree Celsius then cooled in a desiccator and weighed. The percentage W/W of extractive value with reference to the air dried drug was calculated.

WATER SOLUBLE EXTRACTIVE VALUE

About 5g of the powdered drug was accurately weighed and transferred to a conical flask containing 100ml of chloroform water (chloroform act as a preservative), a flask was corked and kept aside for 24hrs with frequent shaking. Then it was filtered and 25ml of the filtrate was transferred to a previously weighed porcelain dish. The filtrate was evaporated to dryness in a water bath and then dried in an oven at 100 degree Celsius, cooled in a desiccator and weighed. The percentage W/W of extractive value with reference to the air dried drug was calculated.

DETERMINATION OF pH RANGE[10]

The pH of 1% w/v (1g: 100ml) of water soluble portions of leaf powder was determined using standard simple glass electrode pH meter.

RESULTS AND DISCUSSION

PHARMACOGNOSTIC STUDIES

Macroscopic characters

The morphological characters are useful in quick identification of plant material and also serve as an important tool in standardization parameter. The results are tabulated in TABLE-01.

LEAF OF CERIOPS DECANDRA (UPPER SURFACE)

LEAF OF CERIOPS DECANDRA (LOWER SURFACE)

TABLE: 01 Morphological characters of Ceriops decandra (Griff.) Ding Hou leaves

MORPHOLOGICAL FEATURES	OBSERVATION
Colour (Upper surface)	Dark Green
Colour (Lower surface)	Light Green
Odour	Aromatic
Taste	Bitter
Apex	Emarginate
Base	Cuneate
Size	3.0-9.8 × 3.3-4.3 cm
Shape	Elliptic-Oblong
Surface	Smooth and Glabrous

MICROSCOPIC CHARACTERS

ANATOMY OF THE LEAF

The leaf is bifacial having distinct adaxial and abaxial sides (FIGURE-1.1). The midrib is flat on the adaxial side and prominently conical on the abaxial side. It is 800µm thick and 450µm wide. It consists of central group of vascular bundles (FIGURE-1.2).

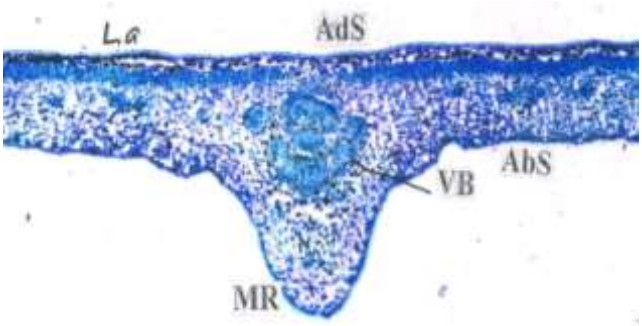

FIGURE-1.1 TS OF LEAF THROUGH MIDRIB

The epidermal layer of the midrib is thin and the epidermal cells are small with thick cuticle. The ground tissue of the midrib is parenchymatous; the cells are small, angular and thin walled. Some of the cells have dark inclusion of tannin.

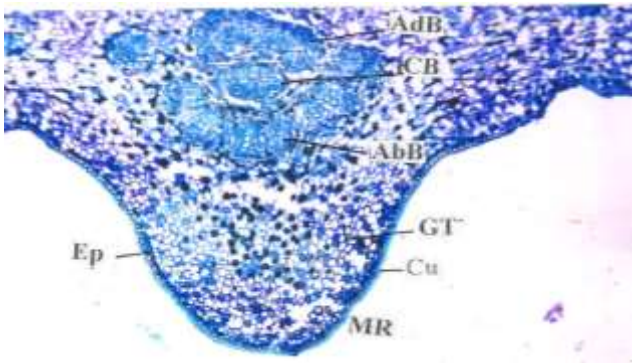

FIGURE-1.2 TS OF MIDRIB-ENLARGED

The vascular system of the midrib is multi-stranded (FIGURE-1.2; 2.1). There is a wide bowl shaped abaxial vascular strand, small curve shaped adaxial strand and a small circular vascular strand. There are two small vascular strands, one on either side if the central group of vascular bundles (FIGURE-2.1). All the vascular bundles are Collateral, having inner xylem elements and outer phloem elements. The xylem elements are small, angular and thick walled. The cells are in short parallel vertical lines. Phloem occurs in thick bands on the outer part of the xylem strands (FIGURE-2.1).

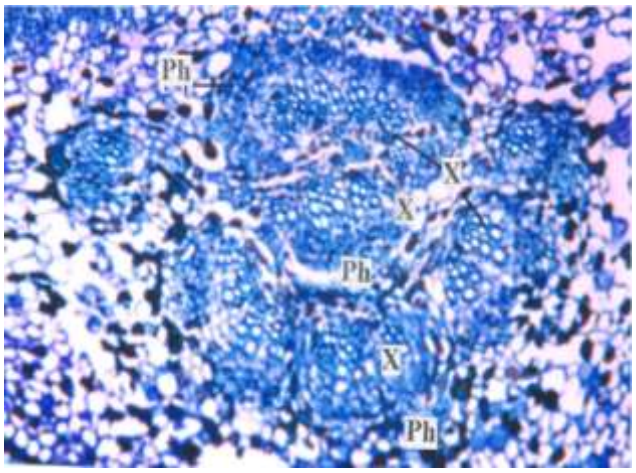

FIGURE-2.1 VASCULAR SYSTEM OF THE MIDRIB

LAMINA (FIGURE-2.2)

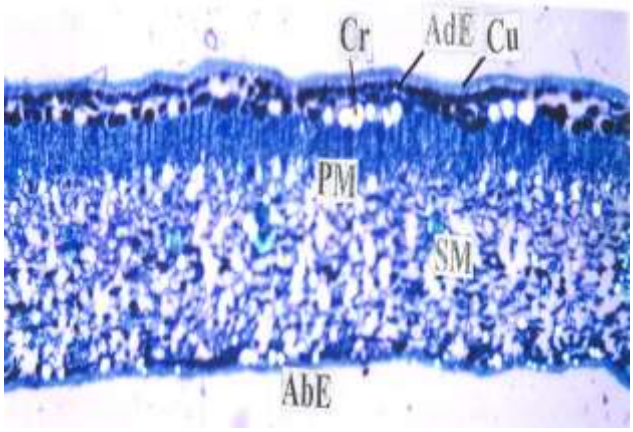

FIGURE-2.2 TS OF LAMINA

The lamina is smooth and even on both sides. It is 260µm thick. The epidermal cells are small, square shaped and thin walled. The cuticle is thick and smooth. One or two hypodermal layers on the adaxial side of the leaf are slightly dilated and possess dark coloured tannin (FIGURE-3.1). The abaxial epidermis has slightly larger cells and thicker cuticle.

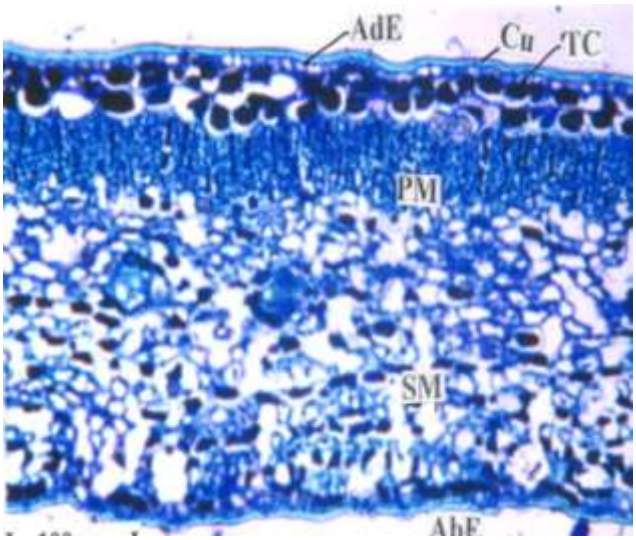

FIGURE-3.1 TS OF LAMINA-ENLARGED VIEW

The mesophyll tissue is differentiated into adaxial band of palisade cells and abaxial zone of spongy mesophyll. Above the palisade layer and beneath tannin containing hypodermal cells, there is a layer of fairly wide circular cells containing calcium oxalate crystals. The crystals are druses which are spherical bodies with minute spines on the surface (FIGURE-3.2).

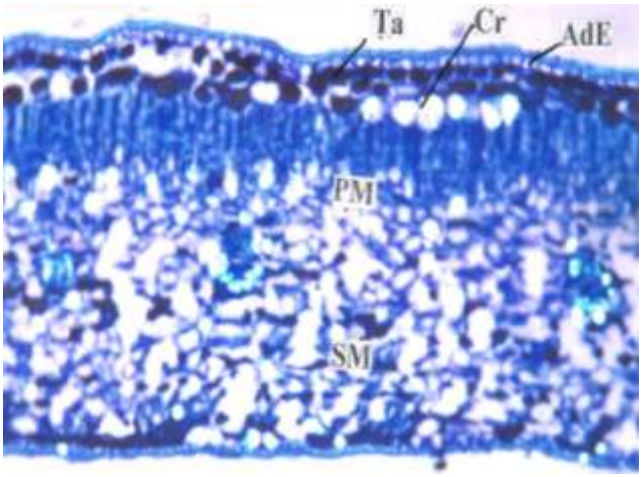

FIGURE-3.2 TS OF LAMINA SHOWING TANNIN CONTAINING

HYPODERMAL CELLS AND CALCIUM OXALATE CRYSTALS IN THE CELLS IN BETWEEN PALISADE AND HYPODERMAL CELLS

The palisade layer includes one or two thin, compact columnar cells. The palisade layer is 40-60µm in height. The spongy mesophyll tissue includes about 12 layers of lobed or spherical cells which are loosely arranged forming wide air chambers (FIGURE-4.1). In the spongy mesophyll tissue occurs circular cluster of sclereids. The sclereids are brachy sclereid type. They are isodiametric cells with thick lignified walls and wide lumen.

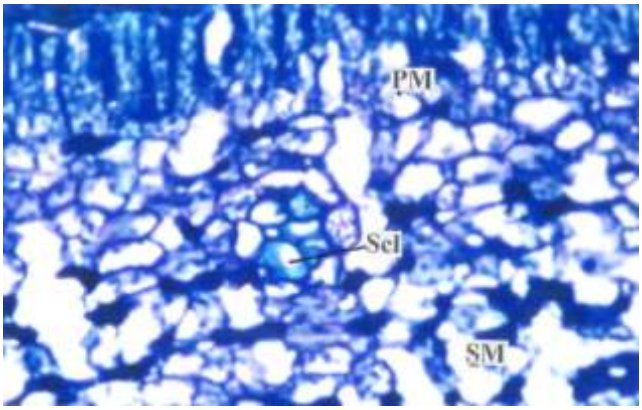

FIGURE-4.1 TS OF LAMINA SHOWING A CLUSTER OF BRACHY SCLEREIDS IN THE MESOPHYLL TISSUE

LEAF MARGINAL PART (FIGURE-4.2)

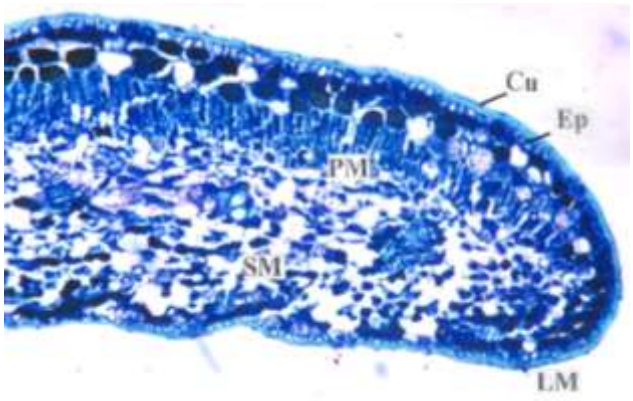

FIGURE-4.2 TS OF LEAF – MARGIN

The marginal part of the lamina becomes gradually thin ending in wide blunt semi-circular end. The marginal part is 150µm thick. The structure of the leaf margin is not much different from the mid-part. The margin includes the palisade layer and spongy mesophyll.

ABBREVIATIONS

FIGURE 1.1 &1.2	**FIGURE 2.1 & 2.2**	**FIGURE 3.1 & 3.2**	**FIGURE 4.1 & 4.2**
AbS - Abaxial side AdB - Adaxial side AbB - Abaxial bundle AdB - Adaxial bundle Cu - Cuticle CB - Central bundle Ep - Epidermis GT - Ground tissue La - Lamina	AbE - Abaxial epidermis AdE - Adaxial epidermis Cr - Crystals Cu - Cuticle Ph - Phloem PM - Palisade mesophyll	AbE - Abaxial epidermis AdE - Adaxial epidermis Cr - Crystals Cu - Cuticle PM - Palisade mesophyll SM - Spongy	Cu - Cuticle EP - Epidermis LM - Leaf margin PM - Palisade mesophyll Scl - Sclereids SM - Spongy mesophyll

MR - Midrib VB - Vascular bundle	SM - Spongy mesophyll X - Xylem	mesophyll TC - Tannin containing cells	

PHYSIO-CHEMICAL INVESTIGATIONS

The physiochemical parameters of the leaves of Ceriops decandra are tabulated in TABLE-02.

TABLE: 02 Physiochemical analysis of Ceriops decandra leaves

PHYSIO-CHEMICAL PARAMETERS	VALUE
LOSS ON DRYING	**14 % w/w**
ASH VALUES	
Total ash value	9.035 % w/w
Water soluble ash value	3.95 % w/w
Acid insoluble ash value	8.035 % w/w
EXTRACTIVE VALUES	
Alcohol insoluble extractive value	22.48 % w/w
Water soluble extractive value	25.6 % w/w
pH	6.46

The deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to contamination by fungal colonies. The loss on drying at 110 °C was found to be 14 % w/w. The percentage of total ash, acid insoluble ash and water soluble ash are carried out and the analytical results showed that total ash value, water soluble and acid insoluble ash values were 9.035 % w/w, 3.95 % w/w, 8.035 % w/w respectively observed. Extractive values were determined which are primarily useful for the determination of exhausted or adulterated drugs. The water soluble and alcohol soluble extractive values were also determined. The water soluble extractive value was indicating the presence of polar sugar, acid and inorganic compounds and the alcohol soluble extractive value indicating the presence of polar constituents like phenols, steroids, glycosides and flavonoids. The water soluble and alcohol soluble extractive value of the leaves of Ceriops decandra were 25.6 % w/w and 22.48 % w/w respectively. The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent. The pH of 1% w/v solution was found to be 6.46.

CONCLUSION

The evaluation of a crude drug is an integral part of establishing the correct identification of a precious indigenous plant material. The pharmacognostic standard for the leaves of

Ceriops decandra (Griff.) Ding Hou is laid down for the first time in this study. To conclude, this study could be used as a diagnostic tool for the standardization of this medicinal plant towards quality assurance and also for preparation of a monograph on the plant.

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