

Pharmacognostical Evaluation of *Cedrela Toona* Roxb. Leaves and Fruits.

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ABSTRACT: Transverse section of leaf shows upper and lower epidermis with thin cuticle, cortex differentiated into collenchyma and cortical parenchyma, bi-collateral vascular bundle in midrib region; dorsiventral structure with palisade tissue lying towards upper surface, upper and lower epidermis with thin cuticle and spongy parenchyma in lamina portion with unicellular covering trichomes and glandular trichome at lower surface of lamina. Surface preparation of leaf shows epidermal cells with wavy margin of lower epidermal cells and straight margin of upper epidermal cells, anomocytic stomata. Transverse section of fruit shows Outer coat with Epidermis Single layered, polygonal tabular cells with thin anticlinal walls filled with mucilage, Sub-epidermis, Inner coat containing Sclerenchymatous layer, Parenchymatous layer, Pigment layer and also contains endosperm and cotyledon. The quantitative microscopy of leaf shows average stomatal index ~ 12.23-16.18 and 20.14-23.98 in upper and lower surfaces respectively, vein-islet number ~ 41-44/sq.mm, vein termination number ~ 74-77/sq.mm, palisade ratio ~ 8-10. Phytochemical observations including physical constant values like ash values, extractive values, moisture content and behaviour of powder drug on treatment with different chemical reagents were carried out.

Keywords: *Cedrelatoona*, Transverse section, Leaves, Fruit, Phytochemistry.

I. INTRODUCTION

The tree: Large canopy tree may attain a height of 120 ft with a clear bole to 80 ft; trunk diameters up to 60 in; sometimes buttressed and fluted. Spines, aerial roots and stilt roots are absent. The bark: Bark white, grey, or grayish brown, rough, scaly or flaky or fissured; Subrhytidome (under-bark) pink or red; less than 25 mm thick, 10.0 – 12.0; bark blaze consisting of one layer; faintly to non aromatic; cinnamon-like or pleasant; outer blaze red or brown, markings absent, fibrous, inner blaze red or brown, markings absent, fibrous; bark exudates (sap) present, yellow, not readily flowing (spotty), colour not changing on exposure to air, sticky, terminal buds not enclosed by leaves. The wood: Heartwood light brick red when first exposed, aging to a rich reddish brown; sapwood pinkish, grayish white, or yellow brown, rather sharply defined. Texture rather coarse and uneven; lustrous; grain generally straight to somewhat interlocked; fragrant cedary odor, pronounced when fresh, characteristic acrid taste. Indumentum: Complex hairs absent; stinging hairs absent; mature twig indumentum (hairs) present, hairs sparse. Leaves: Leaves spaced along branches, spiral (leaves occurring singly at a node and arranged spirally up the branch let), compound (a leaf made up from two or more leaflets); petiole present, not winged, attached to base of leaf blade, not swollen; rachis absent; leaves without a terminal leaflet (the number of leaflets even – paripinnate), broadest below middle, (7.0-)9.0-12.0(-16.0) cm, (2.2-)3.0-5.0(-6.0) cm, leaflets opposite, symmetric; venation pinnate, secondary veins open, prominent intramarginal veins absent; leaves lower surface green, upper surface dull dark green, indumentums (hairs) present; stipules absent. Flowers: Inflorescence terminal, flowers on a branched axis, cones absent; unisexual with male and female flowers on the same plant, stalked, 3.5 -5.0 mm long, 4-5 mm diameter, perianth present with distinct sepals and petals whorls, inner perianth white, pale yellow or cream colored; 5 stamens (male flowers) and 5 sterile anthers (female flowers) present, free of each other, free of perianth; ovary superior, carpels joined (when more than one), locules 5; styles solitary, 1. Fruits: In frutescence arranged on branched axis, 15.0 – 20.0 mm long, red or brown, not spiny, non – fleshy, simple, dehiscent, capsules; seeds many 100 to about 5 mm long, winged, diameter 1-10 mm.

II. HABITAT

Sub- Himalayan tract, Assam and throughout hilly regions of central and South India.

1. Part Used

Bark, Gum, and Flowers part of the plant mainly used in the formulation

2. Ayurvedic Properties

Rasa : Madhura, Tikta, Kasaya

Guna : Laghu

Virya : Sita
Vipaka : Katu
Karma : Grahi, Kaphahara, Medohara, Pittahara.

3. Ayurvedic formulation

NyagrodhadiKvathaCurna

4. Traditional uses

Traditionally the flowers used as emmenagogue, used in menstrual disorders. The flowers also yielded a reddish or yellowish dye, which has been used in tropical Asia to colour silk. Bark is bitter, acrid, powerful astringent, tonic, expectorant, anthelmintic, approdisiac and antiperiodic¹. It is useful in chronic dysentery, ulcer, leprosy, cures fever, headache, blood complaints (Ayurveda), cardiogenic, aphrodisiac, anthelmintic, good for scabies and expectorant (Yunani)². The stem bark of the tree is used as antiulcer, antileprotic and had been used traditionally to heal wounds.^{3,4,5} The leaves have hypoglycaemic, spasmolytic and anti-protozoal activity. Gum obtained from the bark is useful in fever.⁶ Garasia tribe of Rajasthan applies the leaves as bandage tied on the stomach to reduce swelling occurs during pregnancy, where they called it locally as 'Bhurla'.⁷ The inhabitants of Abbotabad district of Pakistan uses the dried leaf powder along with table salt and water orally for treating diabetes, skin allergy, wounds and as blood purifier, where they pronounced the plant locally as 'Nem'.⁸

III. AUTHENTICATION AND COLLECTION OF FRESH PLANT

The fresh parts of *CedrelatoonaRoxb.* were collected in March 2010, from botanical garden of Dang, Gujarat, India. Dried Samples of Bark and fruit of *CedrelatoonaRoxb.* were collected from Paritosh Herbals, Dehradun. The plant was identified by comparing its morphological and microscopical with description given in different standard texts, floras and Aayurvedic Pharmacopoeia of India¹. Besides these, the plant was then identified and authenticated by Dr. M. S. Jangid, Botany Department, Sir P. T. Science College, Modasa, Gujarat, India and a voucher specimen was deposited. For further confirmation, the microscopic characters of this plant was studied and compared with available literature as mentioned above. The leaves were dried in shade and stored at 27°C. It was powdered, passed through 40# and stored in air tight containers.

IV. PREPARATION OF FRESH SAMPLE

Fresh parts of *CedrelatoonaRoxb.* was used for pharmacognostical studies. The leaves and aerial parts were separated out and dried under shade. They were then powdered to 40# separately, stored in airtight containers and used for phytochemical and pharmacological studies.

V. PHARMACOGNOSTICAL STUDIES

5.1 Macroscopical study

The morphology or macroscopical description of *CedrelatoonaRoxb.* Including size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc were studied and compared with available literature.

5.2 Microscopical Study

5.2.1 Transverse sections of leaf and fruit of CedrelatoonaRoxb.

Microscopic evaluation of leaf and fruit of *CedrelatoonaRoxb.* was carried out. The material to be sectioned was held between the thumb and four finger of the left hand. Using sharp razor blade held in the right hand, thin section was made the razor blade across the object in quick successions. Transferred the sections in to watch glass containing water, added chloral hydrate to these sections, boiled, filtered and the sections were stained with phloroglucinol and hydrochloric acid (1:1) and the same mounted in glycerin and observed under low power and high power of the microscope.

5.2.2 Surface preparation of leaf of CedrelatoonaRoxb.

Stomata, trichomes and epidermal cells are important identifying characteristics of leaf drug. In transverse sections their exact nature can't be studied hence exposure of surface/epidermis becomes must for the detail microscopical study. Type of stomata present, nature of epidermal cell wall, type of trichomes and their details can be studied only after exposing the epidermis. This technique has significance in the determination of leaf constants, identification of crude drug and detection of adulterants. The leaf of *CedrelatoonaRoxb.* was placed on a glass slide and tissues were scrapped off with the sharp edge of razor with care. Water was slowly and continuously added and scrapping was done till transparent and colourless epidermis was exposed. The scrapped material was then placed on a glass slide, mounted in glycerin and examined microscopically.

5.3 Quantitative microscopy of leaf

Quantitative microscopy of leaf of *CedrelatoonaRoxb.* was done and stomatal index, vein-islet number, vein termination number and palisade ratio were determined.

5.3.1. Determination of stomatal number

Stomatal number is the average number of stomata per square mm of the epidermis of the leaf. Cleared the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peeled

out upper and lower epidermis separately by means of forceps. Kept it on slide and mounted in glycerin water. Arranged a camera lucida and drawing board for making the drawings to scale and drew a square of 1 mm by means of stage micrometer. Put the slide with cleared leaf (epidermis) on the stage. Traced the epidermal cells and stomata. Counted the number of stomata present in the area of 1 sq. mm. Include the cell if at least half of its area lies within the square. Found out the result for each of the ten fields and calculated the average number of stomata per sq. mm. Stomatal number is affected by various factors like age of the plant, size of the leaf, environmental conditions etc. Stomatal index is not much affected by these factors. It is relatively constant. Hence it is more significant in the evaluation of a leaf drug.

5.3.2. Determination of Stomatal Index

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. Stomatal index can be calculated by using following equation.

$$I = \frac{S}{E + S} \times 100$$

Where,

I = Stomatal index

S = No. of stomata per unit area

E = No. of epidermal cells in the same unit area

Cleared the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peeled out upper and lower epidermis separately by means of forceps. Kept it on slide and mounted in glycerin water. Arranged a camera lucida and drawing board for making the drawings to scale and drew a square of 1 mm by means of stage micrometer. Put the slide with cleared leaf (epidermis) on the stage. Traced the epidermal cells and stomata. Counted the number of stomata, also the number of epidermal cells in each field. Calculated the stomatal index using the above formula. Found out the values for upper and lower surface (epidermis) separately.

5.3.3. Determination of Palisade Ratio

Palisade ratio is the average number of palisade cells beneath one epidermal cell of a leaf. It is determined by counting the palisade cells beneath four continuous epidermal cells. Cleared the piece of the leaf by boiling with chloral hydrate solution. Arranged the camera lucida and drawing board for making drawings. Using the 4 mm objective, traced off the outlines of four cells of the epidermis. Then, focused down to palisade layer and traced off sufficient cells to cover the tracings of the epidermal cells. Completed the outlines of those palisade cells, which were intersected by the epidermal walls. Counted the palisade cells under the four epidermal cells. (Included the palisade cell in the count when more than half was within the area of epidermal cells and excluded it when less than half was within the area of epidermal cells.) Calculated the average number of cells beneath a single epidermal cell, this figure was the 'palisade ratio'. Repeated the determination for five groups of four epidermal cells from different parts of the leaf and found the average of the results for the five groups. This average was the 'palisade ratio' of the leaf.

5.3.4. Determination of Vein-islet Number

Vein-islet is the small area of green tissue surrounded by the vein-lets. The vein-islet number is the average number of vein-islets per square millimeter of a leaf surface. It is determined by counting the number of vein-islets in an area of 4 sq. mm. of the central part of the leaf between the midrib and the margin. Cleared the piece of the leaf by boiling with chloral hydrate solution for about thirty minutes. Arranged the camera lucida and drawing board for making drawings to scale. Put stage micrometer on the microscope and using 16 mm objective, drew a line equivalent to 1 mm as seen through the microscope and constructed a square on this line. Moved the paper so that the square was seen in the eyepiece, in the centre of the field. Put the slide with the cleared leaf (epidermis on the stage). Traced off the veins, which were included within the square, completing the outlines of those islets, which overlapped two adjacent sides of the square. Counted the number of vein islets in the square millimeter. Where the islets were intersected by the sides of the square, included those on two adjacent sides and excluded those islets on the other sides. (To obtain a critical result for a leaf, 4 sq. mm. should be used, preferably in one large area of 4 sq. mm). Found the average number of vein islets from the four adjoining squares, to get the value for one sq. mm.

5.3.5. Determination of Vein-let Termination Number

Vein-let termination number is defined as the number of vein-let termination per sq. mm of the leaf surface, midway between midrib of the leaf and its margin. A vein termination is the ultimate free termination of vein-let. Cleared the piece of the leaf by boiling with chloral hydrate solution for about thirty minutes. Arranged the camera lucida and drawing board for making drawings to scale. Put stage micrometer on the microscope and using 16 mm objective, drew a line equivalent to 1 mm as seen through the microscope. Constructed a square on

this line. Moved the paper so that the square was seen in the eye piece, in the centre of the field. Put the slide with the cleared leaf (epidermis on the stage). Traced off the veins, which were included within the square, completing the outlines of those islets, which overlapped two adjacent sides of the square. Counted the number of vein-let terminations present within the square. Found the average number of vein-let termination number from the four adjoining squares to get the value for one sq.mm.

5.4 Proximate analysis

Proximate analysis aids to set up certain standards for dried crude drugs in order to avoid batch-to-batch variation and also to judge their quality. Their studies also give an idea regarding the nature of phytoconstituents present. Proximate analysis of powders of leaves, stems and fruits of *CedrelatoonaRoxb* was carried out using methods prescribed in the Ayurvedic pharmacopoeia of India by subjecting them to various determinations like, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value, moisture content.

5.5 Determination of ash values

The determination of ash values help to determine quality and purity of a crude drug. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects 'Total ash value'. Such variables are then removed by treating with acid and then acid insoluble ash value is determined. Ash values of powders of leaves, stems and fruits of *CedrelatoonaRoxb*. were determined by following methods :

5.5.1 Determination of total ash

Each of 2 g of accurately weighed powder of leaves, stems and fruits of *CedrelatoonaRoxb*. was incinerated in a crucible at a temperature 500-600°C in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

5.5.2 Determination of acid insoluble ash

Each of total ash of powder of leaves, stems and fruits of *CedrelatoonaRoxb*. obtained by the above procedure was subjected separately for the estimation of acid insoluble ash using the following procedure. The total ash obtained above was boiled for 5min with 25 ml of 70 g/l hydrochloric acid and filtered using an ash less filter paper to collect insoluble matter. The ash obtained was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the air-dried powdered drug (60#).

5.5.3 Determination of Water soluble ash

Each of total ash of powder of leaves, stems and fruits of *CedrelatoonaRoxb*. obtained by the above procedure was subjected separately for the estimation of water soluble ash using the following procedure. The total ash was boiled for 5min with 25 ml of water and insoluble matter collected on an ash-less filter paper washed with hot water and ignited for 15 min at a temperature not exceeded 450°C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powdered drug (60#).

5.6 Determination of Extractive Values

The determination of Extractive values help to determine the amount of soluble constituents in a given amount of medicinal plant material, when extracted with solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of drug and solvent used. The use of single solvent can also be used by means of providing preliminary information of quality of a particular drug sample. Extractive values of powders of leaves, stems and fruits of *CedrelatoonaRoxb*. were determined by the following methods:

5.6.1. Determination of alcohol soluble extractive value

Each of 4g of the air-dried powdered material (60#) of leaves, stems and fruits of *CedrelatoonaRoxb*. was macerated with 100 ml of alcohol in a closed flask for 24 h, shaking frequently at an interval of 6 h. It was then allowed to stand for 18h and filtered rapidly to prevent any loss during evaporation. 25 ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105° to a constant weight. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

5.6.2. Determination of water soluble extractive value

Each of 4g of the air-dried powdered material (60#) of leaves, stems and fruits of *CedrelatoonaRoxb.* was soaked in 100ml of water in a closed flask for 1h with frequently shaking. It was then boiled gently for 1 h on water bath; cooled and weighed and readjusted the weight. 25 ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105° to a constant weight. The percentage of water-soluble extractive was calculated with reference to the air-dried powdered drug (60#).

5.7 Determination of moisture content

Placed about 100gm each of leaves, stems and fruits (without preliminary drying) of *CedrelatoonaRoxb.* after accurately weighing in a tarred evaporating dish. After placing the above said amount of the drug in the tarred evaporating dish, dried at 105°c for 5 hours, and weighed. Continued the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 50mins. and cooling for 30 minutes in a desiccator, showed not more than 0.01gm difference.

VI. RESULT AND DISCUSSION

6.1 Macroscopic evaluation

Table 1 :Morphology of *CedrelatoonaRoxb.* Leaves

No.	Features	Observations
1	Upper Surface	Dull Dark Green
2	Lower surface	Green
3	Odour	Aromatic when crushed
4	Shape	Variable in shapes like ovate or suborbicular, broadly cordate to ovate with oblique base
5	Size	15–45 cm long
6	Texture	Hairy
7	Color Of Leaf	Green

Table 2: Botanical evaluation of *CedrelatoonaRoxb.* Leaves

No.	Features	Observations
1	Apex	Acute
2	Margin	Entire
3	Arrangement	Spiral
4	Leaf type	Compound
5	Venation	Pinnate
6	Mid rib	prominent intramarginal veins absent
7	Surface	Hairy
8	Petiole	4-11 cm long
9	Lamina	Ovate
10	Base	Asymmetric

Table 3 : Morphology of *CedrelatoonaRoxb.* Fruit

No.	Features	Observations
1	Surface	Hairy smooth
2	Type	not spiny, non – fleshy, simple, dehiscent, capsules; seeds many 100 to about 5 mm long, winged, diameter 1-10 mm.
3	Size	15.0 – 20.0 mm long
4	Colour	red or brown
5	Taste	Sour to sweet

6.2 Microscopic evaluation



Fig. 1 : Stain T.S. of CedrelatoonaRoxb. leaf

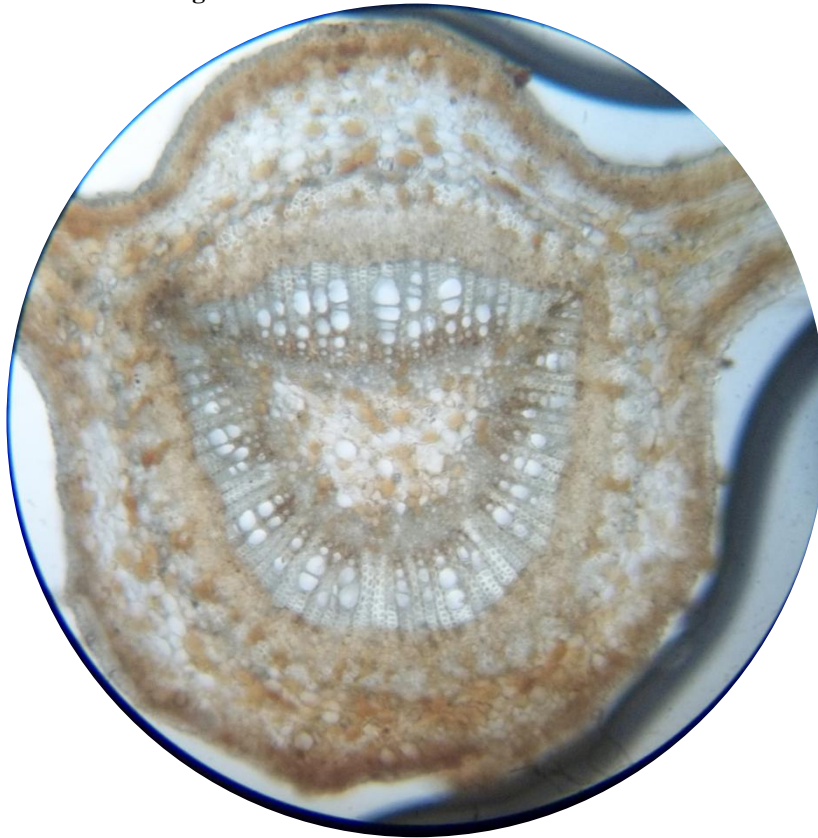


Fig. 2 :Unstain T.S. of CedrelatoonaRoxb. leaf

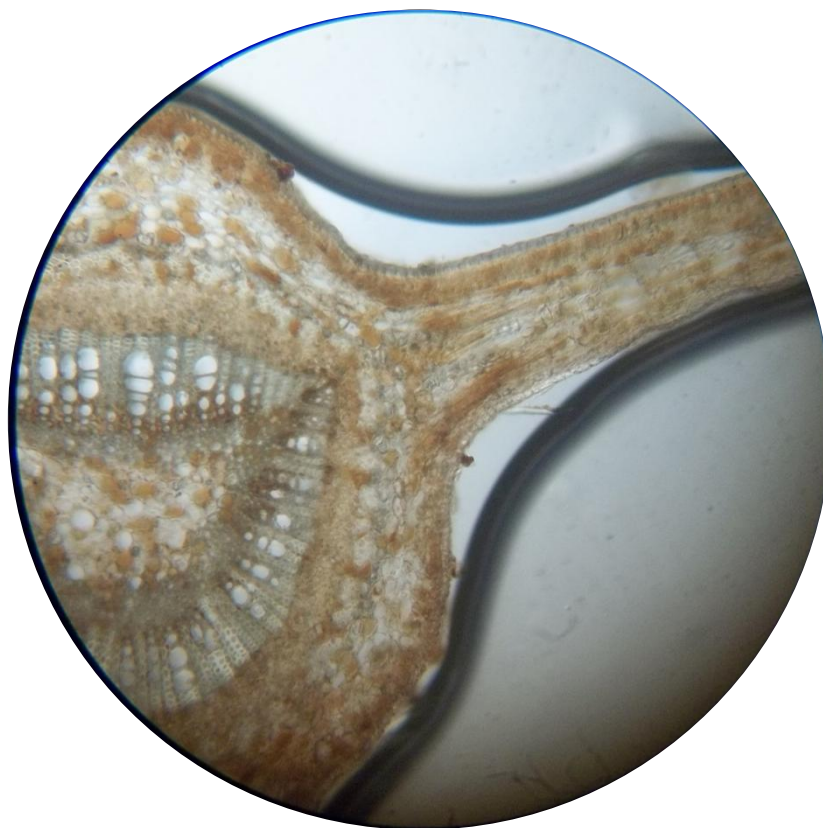


Fig. 3 :Unstain T.S. of CedrelatoonaRoxb. leaf with lamina portion.

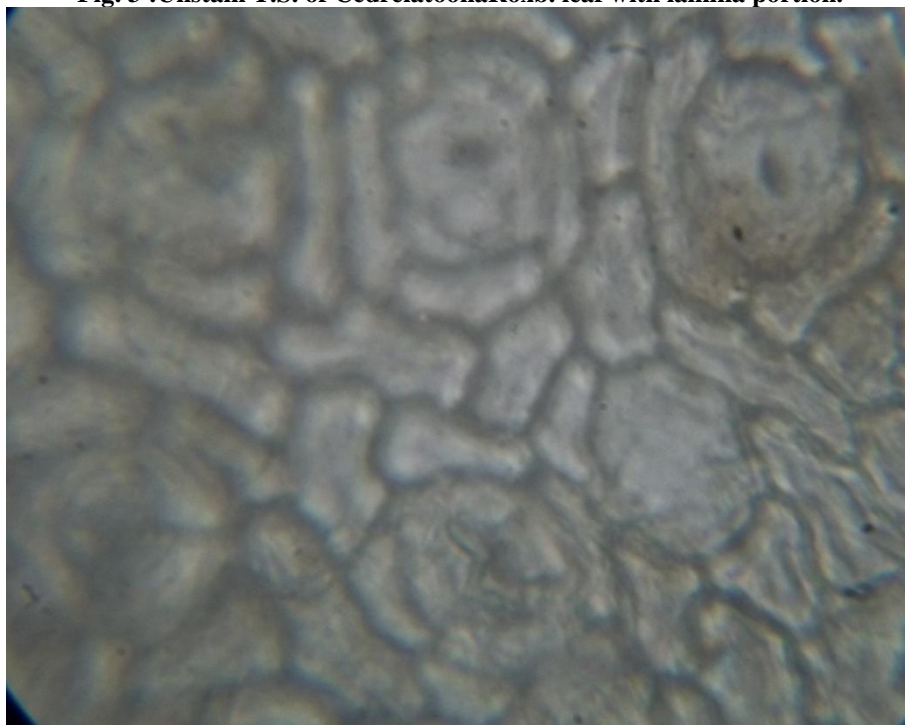


Fig. 4 : Surface preparation of CedrelatoonaRoxb. leaf.

Free hand transverse sections (T.S) of fresh leaf samples of *CedrelatoonaRoxb* were taken and studied for their histological characters T.S of leaf shows midrib and lamina portion. Lamina is dorsiventral in nature. Upper Epidermis: Straight walls, 600 to 1100 cells/sq. mm, single layered, rectangular. Trichomes : Covering , uniseriate, waxy, blunt tip, Glandular Trichomes: stalk unicellular, head 2-4 celled. Stomata, Palisade: Single layered, compact celled, radially elongated, covering $\frac{2}{5}^{th}$ of lamina. Spongy parenchyma: 6-8

layers, loosely arranged, intercellular spaces, cluster crystals (sphaeraphides), Micro-spheroidal crystals and vascular strands are found in upper layers. Lower Epidermis: Similar to upper epidermis, stomata and numerous trichomes are seen; wavy walls and cuticle. Mid Rib: Epidermis: Epidermal layers are continuous. Collenchyma: Below upper and above lower epidermis, Thick walled cellulosic cells. Cortical Parenchyma containing prisms of calcium oxalate and micro – phenoidal crystals. Vascular Bundle: Bicollateral vascular bundle present, xylem is lignified, Phloem non lignified. Surface preparation: Anomocytic stomata present.

6.3 Transverse section of fruit of *CedrelatoonaRoxb.*



Fig. 5 : T.S. of *CedrelatoonaRoxb.* fruit

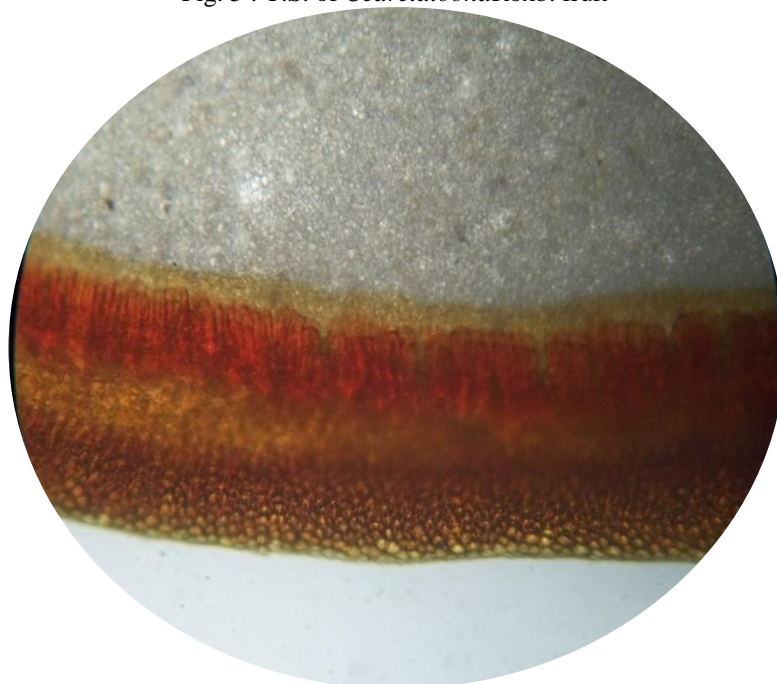


Fig. 6 : Stain T.S. of *CedrelatoonaRoxb.* Fruit

Testa: Outer coat: Epidemis: Single layered, polygonal tabular cells with thin anticlinal walls filled with mucilage. Sub – epidermis: contains cylindrical collenchymas. Inner coat: Sclerenchymatous layer: Longitudinally elongated, lignified sclerides, thick walled, pitted, very small lumen. Parenchymatous layer: One or two layers, thin, tangentially elongated, collapsed parenchymatous cells. Pigment layer: Single layer of

flattened polygonal pigment cells with reddish brown contents. Endosperm: Polyhedral, cellulosic parenchyma with oil globules and aleurone grains. Cotyledon: Cells and cell contents are similar to endosperm.

VII. QUANTITATIVE MICROSCOPY OF LEAF OF CEDRELA TOONAROXB.

Quantitative microscopy of leaf of *CedrelatoonaRoxb.* was done and stomatal index, vein-islet number, vein termination number and palisade ratio were determined which are given in Table 4.

Table 4: *Quantitative microscopy of leaf*

Sr. No.	Determination	Value (per sq. mm)
i)	Stomatal index Upper epidermis Lower epidermis	13.23-18.56 23.14-25.98
ii)	Vein-islet number	41-44
iii)	Vein-termination number	74-77
iv)	Palisade ratio	8-10

Proximate analysis

Proximate analysis of powder of leaves and fruits of *CedrelatoonaRoxb.* was done and the results obtained are given in Table 5.

Table 5: *Study of different parameters obtained from proximate analysis*

Sr. No.	Determination	Percentage w/w	
		Leaves	Fruits
i)	Total Ash	13.80	12.65
ii)	Acid insoluble ash	7.52	7.42
iii)	Water Soluble ash	6.21	5.25
iv)	Alcohol Soluble extractive	6.58	6.76
v)	Water Soluble extractive	7.69	7.15
iv)	Moisture content	82.65	80.65

VIII. CONCLUSION

Pharmacognostical studies of the *CedrelatoonaRoxb.* leaves and fruits are useful to researches for further study on plant.

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