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Pharmacognostical study and Phytochemical evaluation of the *Toona ciliata* M. Roem. leaf

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ABSTRACT

Objective: To evaluate the pharmacognostical study of leaf of *Toona ciliate* M. Roem. **Methods:** The qualitative and quantitative microscopy, phytochemical screening, physicochemical evaluation and fluorescence analysis of the plant were done according to the standard procedure recommended in the WHO guidelines. **Results:** Macroscopic study shows that leaves are compound type, 30-50 cm length, shape: lanceolate, apex: acute and having the entire margin. Stomata present in the leaf are anomocytic type of stomata. Microscopic evaluation of the leaves powder shows the presence of trichomes (multicelluar), lignified vascular bundles and stomata. The transverse section of the leaf shows the presence of epidermis layer followed by cuticle layer, lignified vascular bundles, trichomes, collenchyma, and palisade cells. **Conclusion:** Various pharmacognostical parameters evaluated in his study help in the identification and standardization of the *Toona ciliata* M. Roem.

1. Introduction

Toona ciliata (Synonyms: Cedrela australis, Cedrela velutina DC, Cedrela australis R. Muell) belonging to the family Meliaceae commonly known as Toonee and Tuni in Hindi; Red ceder in English and Nandi in Sanskrit [1]. It is a large, evergreen plant, that grow up to height of 25-35 m [2]. It is mainly distributed in tropical Himalayas from Indus Eastward and throughout the hills of central and southern India. Traditionally the flowers used as emmenagogue, and 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 acrid, bitter and used as anthelmintic, antiperiodic, aphrodisiac, astringent, expectorant and tonic [3]. It is also useful in blood complaints, chronic dysentery, ulcer, fever, headache, leprosy(Ayurveda), anthelmentic, aphrodisiac, cardio tonic and expectorant (Yunani) [4]. The bark may be used to tanning leather and has been traditionally used to make twine and string bags [5]. Wood of the plant used for furniture, cigar-cases and tea- boxes. The phytochemistry of the plant reported the presence of cedrelone in the heartwood, 5- methylcoumarins in the stem bark and limonoids (toonaciliatins) and siderin in the leaves of the plant [6-10]. The leaf extract of the plant has been reported to have antioxidant activity, whereas the heartwood extract of the plant showed the antiulcer, analgesic and antifeedant activity [11-14]. Establishment of the pharmacognostic profile of the leaf of *Toona ciliata* will assist in standardization which can guarantee quality, purity and identification of samples.

2. Material and methods

2.1 Chemicals

All the chemicals used were of analytical grade and purchased from the Himedia Lab. Pvt. Ltd. Mumbai, India and Sd Fine Chem. Limited Mumbai, India.

2.2 Plant collection and identification

The leaves of the plant were collected from the Himachal Pradesh in the month of October 2011. The plant was identified and authentified as *Toona ciliata* M. Roem. (Family: Meliaceae) by Dr. H.B. Singh, Scientist In charge & Head, Raw Materials Herbarium & Museum,

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National Institute of Science Communication & Information Resources, New Delhi, India where a voucher specimen (NISCAIR/RHMD/Consult/-2011-12/1849/149) has been deposited.

2.3 Processing of collected plant sample

The collected plant material was air-dried for two weeks and then powdered using mortar and pestle. The powder obtained was stored in air tight for use in phytochemical analysis and determination of pharmacopoeia standards.

2.4 Macroscopic analysis

The macroscopical study includes the evaluation of organoleptic characters and external features of the various parts of selected plant material. The following macroscopic characters for the fresh leaves were noted size, shape, colour, surfaces, venation, margin, base, lamina, texture, odour and taste [15, 16].

2.5 Microscopic analysis

In microscopic evaluation were conducted on both grounds qualitatively and quantitatively studies of *Toona ciliata* Leaf [17, 18].

2.5.1 Qualitative microscopy

In this study transverse section and powder microscopy of leaf was carried out. Staining procedure was used as per standard procedures. The staining reagents used for staining procedure were phloroglucinol and conc. hydrochloric acid (1:1). The various characters were identified and studied.

2.5.1.1 Leaf microscopy

In this study, first of all leaf was dipped in the chloral hydrate solution for the removal of the color and pigments. The cubical portion of the pith obtained from the potato was selected, vertically cut, leaf inserted in the pith and the fine section of the leaf obtained. The fine section mounted on the slide with the help of glycerin with or without the staining reagent placed under the microscope. Various identified characters were recorded.

2.5.1.2 Powder microscopy

In this study the dry leaves were powdered. The cleared powder mounted on slide with the help of glycerin. Then stain the cleared powder with the staining reagents such as phloroglucinol and conc. hydrochloric acid (1:1). Various identified characters were observed.

2.5.2 Quantitative microscopy

This is the one of the important histological aspects for the evaluation of the crude drugs.

2.5.2.1 Determination of stomatal number and stomatal index

Stomata is a minute epidermal opening covered by two kidney shaped guard cells. These guard cells, in turn, are surrounded by the epidermal cells. Stomata perform the function of gaseous exchange and transpiration in plants. Stomatal number is defined as the average number of stomata per sq mm of epidermis of the leaf. Stomatal index is the percentage which the numbers of stomata form to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following equation [17, 18].

$$S.I = (S/E+S) \times 100$$

Where, S.I = Stomatal index
S = Number of stomata per unit area
E = Number of epidermal cells in the same unit area.

2.5.2.2 Determination of vein-islets and vein termination number

Vein- islet is the small area of green tissue surrounded by the veinlets. 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 sq. mm of the central part of the leaf between the midrib and the margin.

Veinlet termination number is defined as the veinlet termination per square millimeter of the leaf surface, midway between midrib of the leaf and its margin. A vein termination is the ultimate free termination of veinlet [17, 18].

2.6 Physicochemical analysis

Physicochemical analysis of the leaves powder of the selected plant material was determined according to the WHO guidelines and the official methods [19]. In the physicochemical analysis various parameters such as ash values, extractive values, loss on drying and foaming index were calculated.

2.7 Preliminary phytochemical screening

The preliminary phytochemical screening of the leaf extract mainly done for the evaluation of the various phytoconstituents such as steroids, tannin and glycosides etc. present in the plant [17,18,19].

2.8 Fluorescence analysis

Fluorescence analysis is the one of the most important parameter for the evaluation of the quality, strength and purity of the selected plant material. The powdered leaf material was analysed under the three region of light like

visible, short U.V region and long U.V region after the treatment with various inorganic/organic reagents.[20]

3. Results

3.1 Macroscopic characteristics (Morphology)

The macroscopical characters such as colour, odour, taste, shape, margin, apex, base, surface and size of *Toona ciliata* leaf were observed and shown in figure 1 and table 1.

Figure 1: Toona ciliata leaf

Table 1

Macroscopical characters of Toona ciliata leaf

S.No.	Macroscopical characters	Observation
1.	Condition	Fresh
2.	Type	Compound
3.	Size: Length	30–50 cm
	Width	13–18 cm
4.	Shape	Lanceolate or oblong
5.	Apex	Acute
6.	Margin	Entire
7.	Venation	On both surfaces
8.	Base	Oblique
9.	Petiole	Present
10.	Surface	Smooth
11.	Color: Outer	Dull dark green
	Inner	Green
12.	Odour	Characteristics
13.	Taste	Bitter

3.2 Microscopic characteristics

3.2.1 Leaf microscopy

The leaf surface showed the presence of stomata covered with guard cells followed by epidermal cells. The stomata were found to be anomocytic shown in figure 2. The transverse section of the leaf was found to be dorsiventral showed the presence of cuticle, lower and upper epidermis, trichomes, palisade cells and vascular bundles shown in figure 3. Trichomes were found to be multicelluar and vascular bundles was found to be lignified when treated with phloroglucinol and conc. hydrochloric acid.

Figure 2: Stomata present in leaf 100X

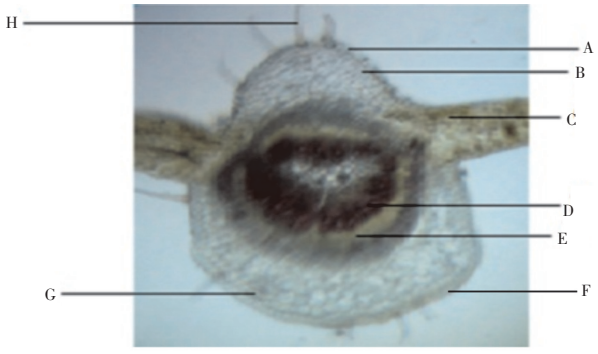

Figure 3: T.S. of Toona ciliata leaf 100X
A– Cuticle, B– Upper epidermis, C– Palisade cells, D– Lignified xylem, E– phloem, F– Lower epidermis, G– Collenchyma, H– Tichomes

3.2.2 Powder microscopy

The powder microscopy of the leaves of *Toona ciliata* showed the presence of the stomata and epidermal cells shown in figures 4 & 5. The trichomes were found to be multicelluar and uniseriate shown in figure 6. The vascular bundles were found to be lignified when stained with phloroglucinol and conc. hydrochloric acid (1:1) shown in figures 7A & 7B.

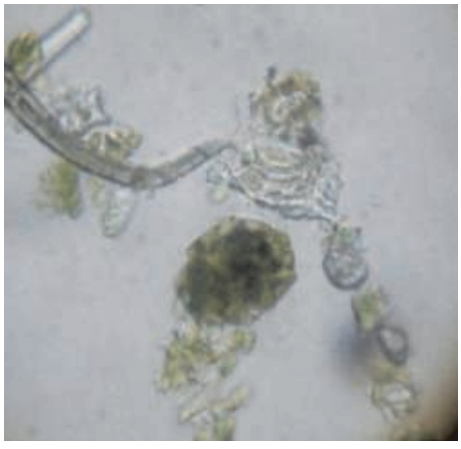

Figure 4: Stomata 400X

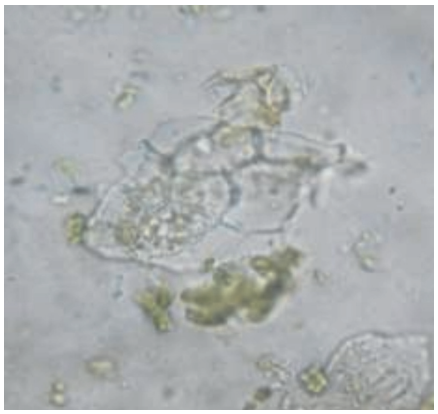

Figure 5: Epidermal cells 400X

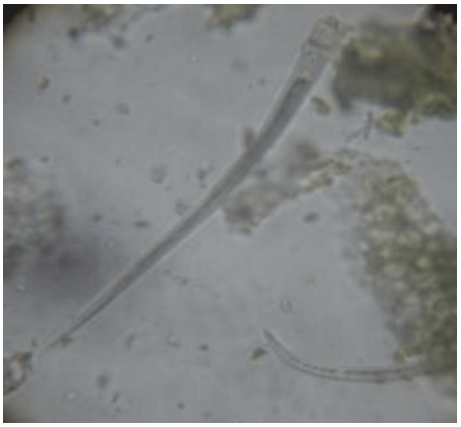

Figure 6: Trichomes 400X

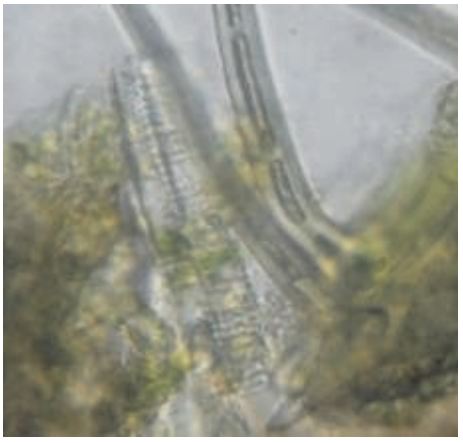

Figure 7A: Vascular bundles 400X

3.3 Quantitative microscopy

In the quantitative microscopy the stomatal index, vein-islets number and veinlet termination ware determined and shown in table 2.

Table 2

Quantitative microscopic of Toona ciliata leaf

Parameters	Values
Stomatal number	43
Stomatal index	18.53
Veinislet number	8
Veinlet termination number	9

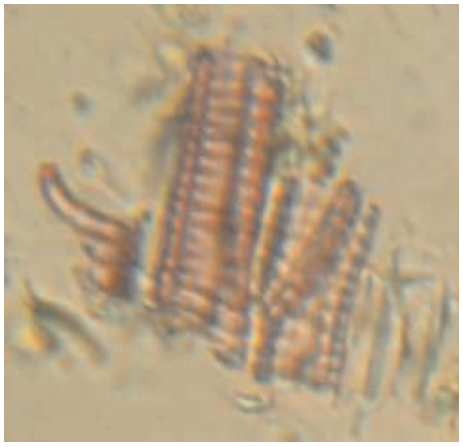

Figure 7B: Lignified vascular bundles 400X

3.4 Physicochemical analysis

The results of the analysis discussed in the table 3 and figures 8, 9.

Table 3

Physicochemical constant of Toona ciliata leaf

Parameters	Values % w/w
Total ash values	Not more than 11.5
Acid-insoluble ash values	Not more than 1.4
Water-soluble ash values	Not more than 4.2
Sulphated ash	Not more than 15.4
Extractive values	
Petroleum ether-soluble	14.08
Chloroform-soluble	5.28
Alcohol-soluble	8.72
Hydroalcoholic-soluble	23.12
Water-soluble	16.0
Loss on drying	10
Foreign organic matter	0.01
Foaming index	Less than 100

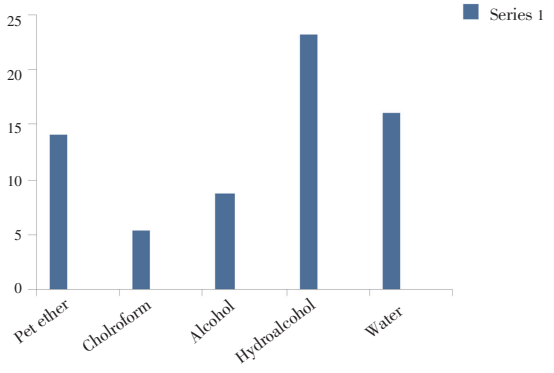

Figure 8: Extractive value of Toona ciliata leaf

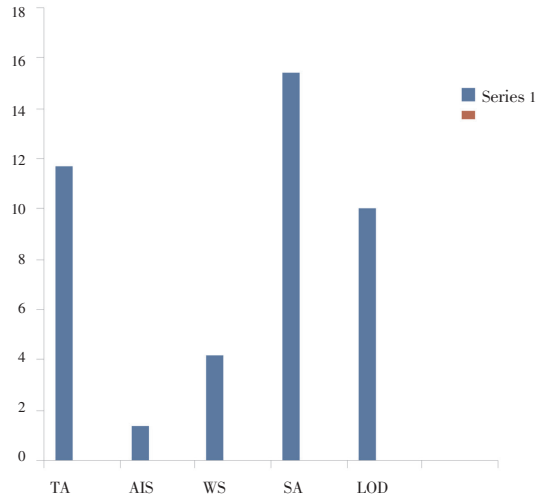

Figure 9: Physicochemical parameter of Toona ciliata leaf (TA- Total ash, AIS- Acid insoluble ash, WS- Water soluble ash, SA- Sulphated ash, LOD- Loss on drying.)

3.5 Phytochemical screening

Preliminary phytochemical screening of the hydroalcoholic extract mainly revealed the presence of the phytoconstituents such as carbohydrates, tannins, flavonoids and steroids. The petroleum ether extract showed only the presence of steroids shown in table 4.

3.6 Fluorescence analysis

The results of fluorescence analysis discussed in table 5.

4. Conclusion

To ensure reproducible quality of herbal medicines proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentification followed by creating numerical values of standards for comparison. Pharmacognostical parameters

Table 4
Phytochemical screening of Toona ciliata leaf

S.No.	Class of Compound		Hydroalcohlic extract	Petroleum ether extract	Chloroform extract
1	Carbohydrates	Molish test	+	-	+
		Reducing sugars	+	-	+
		Monosaccharides	+	-	-
		Hexose sugar	+	-	-
2	Glycosides	Keller killiani	-	-	-
3	Protiens	Biuret test	-	-	-
		Million's test	-	-	-
4	Steroids	Salkowiski test	+	+	-
5	Tannin	Extract + Iodine solution	+	-	-
		Extract + Acetic acid	+	-	-
		Extract + 5% ferric chloride	+	-	-
8	Flavonoids	Extract + Lead acetate	+	-	-
		Shinoda test	+	-	-
7	Amino Acids	Ninhydrin test	-	-	-
8	Alkaloids	Dragendorff's test	+	-	-
		Mayer's test	+	-	-
		Hager's test	+	-	-
9	Saponins	Foam test	-	-	-

Table 5
Fluorescence analysis of Toona ciliata leaf

S.No.	Treatment	Visible light	Short UV light (254 nm)	Long UV light (366 nm)
1.	Powder as such	Brownish green	Light green	Dark green
2.	Powder + Picric acid	Yellow	Light green	Dark green
3.	Powder + 1 N NaOH in alcohol	Green	Dark green	Yellowish brown
4.	Powder + 1 N NaOH in water	Yellow	Green	Dark green
5.	Powder + Chloroform	Dark green	Brownish green	Orange
6.	Powder + Petroleum ether	Yellow	Light green	Orange
7.	Powder + 50% H2SO4	Greenish black	Dark black	Dark green
8.	Powder + 50% HCl	Yellowish brown	Brownish green	Dark green
9.	Powder + Ammonia solution Green	Yellow	Green	Yellowish
10.	Powder + Alcohol	Green	Dark green	Orange

for easy identification like leaf constants, microscopy & physicochemical analyses are few of the basic protocol for standardization of herbals [21]. The information obtained from the preliminary phytochemical screening will reveal the useful finding about the chemical nature of the drug. The total ash value, florescence analysis, and extractive value will be helpful in identification and authentification of the plant material [22,23]. The pharmacognostical and phytochemical evaluation of *Toona ciliata* leaves can provide useful information for the identification and authentification of the plant.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgement

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