Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 5, 2015

Original Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF *TREMA ORIENTALIS* LEAF

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Received: 23 Apr 2013 Revised and Accepted: 22 Mar 2014

ABSTRACT

Trema orientalis (Ulmaceae) is native to India. This tree species has been of interest to researchers because it is a medicinal plant employed in the Indian indigenous system of medicine. Pharmacognostic standardization, physic-chemical evaluation of the leafs of *Trema orientalis* was carried out to determine its macro-and micro-scopical characters and also some insoluble ash and sulphated ash values, alcohol-and water-soluble extractive values were determined for phytochemical evaluations. Preliminary phytochemical screening was also done to detect different phytoconstituents. Microscopically, Leaf showed trichomes, Lamina, midrib regions, stomata and calcium oxalate crystals. Powder microscopy showed mesophyll region, abundant xylem vessels with annular thickenings and xylem vessels, Unicellular, multiseriate covering trichomes and glandular trichomes, Rosette and prism shape calcium oxalate crystals, Anomocytic stomata. Total ash was approximately two times and four times more than acid insoluble and water soluble as, respectively Ethanol soluble extractive was approximately two times higher than water soluble extractive. TLC of petroleum ether and ethanol extract showed five spots using Hexane: Ethyl acetate (12:4) and four spot using Choloroform: Ethyl acetate (5:4). Phytochemically, root exhibited phytosterols, Flavanoids, Tannin and phenolic compounds.

Keywords: *Trema orientalis*, Macroscopy, Microscopy, Phytoconstituents, R_f (retention factors).

INTRODUCTION

Trema orientalis which is popularly known as Gol in Ayurveda is distributed more or less throughout (except Kutch) in deciduous forests of India, Ceylon, China-Malay Island, Singapore, Australia, tropical and subtropical regions of Pakistan [1,2]. Leaf, Stem and Root of the plant is reported for treatment of diarrhea [3], passing of blood in the urine, epilepsy and muscular pain [4].

Literature revealed that pharmacognostic studies have not been reported for the leaf of this plant. Therefore the main aim of the present work is to study the macro, microscopic and some other pharmacognostic characters and physic-chemical standards of leaf of *Trema orientalis* which could be used to explore this plant.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves were collected from the Idar, Gujarat during November, 2008. Botanical identification was carried out using local floras and authenticated by Dr. Reddy, Prof. and Head of Botany Dept., Sardar Patel University, Vallabh Vidyanagar. Voucher herbarium specimen [HSP/TO-6/37] is preserved along with crude drug sample at the herbarium of A. R. College of Pharmacy, Vallabh Vidyanagar.

Pharmacognostic evaluation

Macroscopy

Macroscopical studies of leaf were done by naked eye and shape, colour, taste and odour of leafs were determined and reported.

Microscopy

Pharmacognostical evaluation including histochemical study was carried out by taking free-hand sections according to Wallis and powder studies according to Evans. The section was stained with toludiene blue solution and mounted in glycerin [5, 6]. A separate section was prepared and stained with iodine solution for the identification of starch grains. Powder (Sieve mesh 60 of the dried leaf was used for the observation of powder microscopical character [7]. The powdered drug was separately treated with pholoroglucinol-hydrochloric (1:1) solution.

Photomicrographs were obtained by observing free-hand sections of drug under compound Trinocular microscope (Labomed-Lx-004).

Physico-chemical evaluations

Physicochemical parameters of *T. orientalis* leaf powder was determined and reported as total ash, water-soluble ash, acid-insoluble ash, and sulphated ash values. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components [8]. The moisture content was also determined. As a part of quantitative microscopy, stomatal number, stomatal index, vein islet number, vein termination number and palisde ratio were determined by using fresh leaves of the plant [9].

Phytochemical analysis

Preliminary phytochemcial screening

The dried powdered plant material was successively extracted with the solvents of increasing polarity in a Soxhlet apparatus utilizing petroleum ether (60-80), Toluene, acetone, chloroform, methanol and water. The liquid extracts obtained with different solvents were collected and the consistency, color, appearance of the dried extracts and their percentage yield were noted. The extracts obtained from powder by successive solvent extraction were subjected to qualitative examination for the phytoconstituents like alkaloids, glycosides, carbohydrates, phytosterols, fixed oils, saponins, phenolic compounds, tannins and flavonoids, proteins and amino acids by the reported methods [10].

RESULTS AND DISCUSSION

Macroscopy

Leaf blade 10-18(-22)×5-9(-11) cm, leathery and fragile, obliquely ovate, crenate-serrulate; unequal sided at the base; alternate charactaceous somewhat rough above; clothed beneath with soft often white pubescent, and surface completely hidden by hairs, apex acuminate to acute; basally 3-veined; secondary veins 4-6 on each side of midvein. Petiole is 6-10 mm long; slender; pubescent. The leaf is green in color; obliquely ovate in shape; Surface is completed hidden by hairs; Margin is crenate-serrulate; basally 3-veined; (fig. 1) Taste: slightly bitter; Odour: without characteristic.

Microscopy

Transverse section of leaf

The Transverse Section of the leaf showed a lamina and midrib portion. Lamina is of dorsiventral type, differentiated into palisade and spongy parenchyma. Upper and lower epidermis (Ep) shows the

presence of unicellular multiseriate covering and glandular trichomes. Upper epidermis is the single layer, polygonal and covered with cuticle. The palisade cells are present upto the midrib in 2 to 3 layer with rosettes type of calcium oxalate crystals present.

Fig. 1: External morphology of *T. orientalis* leaf

Below palisade cells 2 to 3 layer of spongy parenchymatous cells are present having xylem vessels and calcium oxalate crystals. In midrib portion collenchyma (Co) is well developed, which is present below the upper epidermis and above the lower epidermis. In the middle region presence of vascular bundle and rest of the area of midrib region contain the presence of parenchymatous cells. Rosettes shaped calcium oxalate crystals are seen in the parenchymatous cells of midrib portion (fig. 2).

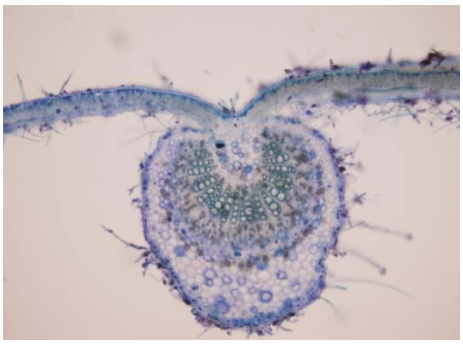

Fig. 2: Microscopical view of. *T. orientalis* leaf

Surface preparation of leaf

In the surface view of *Trema orientalis* leaf, the lower epidermal cells (fig. 3a) show the presence of irregular epidermal cells, anomocytic stomata and simple, unicellular covering trichomes and rosettes of calcium oxalate crystals. In the surface view of upper epidermis (fig. 3b), characteristics noted were absence of stomata (abaxial) and simple covering unicellular trichomes with only epidermal cells.

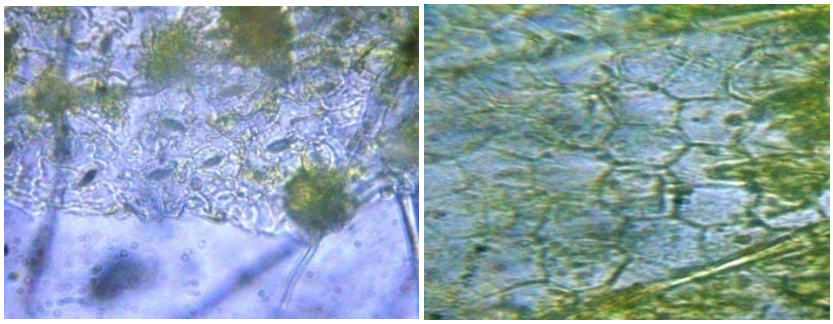

Fig. 3: (a) Lower epidermis (b) Upper epidermis

Powder microscopy of dried powder of leaf

The powder of *T. orientalis* was greenish yellow, without characteristic odour and with slightly bitter taste. When powder was mounted with chloral hydrate, phloroglucinol and HCl the following elements were observed: Mesophyll region which contain palisade and spongy parenchyma with epidermis.

Lignified xylem vessel, annular thickening and spiral vessel Unicellular, multiseriate covering trichomes and glandular trichomes

Rosette and prism shape calcium oxalate crystals Anomocytic stomata (fig. 4)

Phytochemical analysis

Preliminary profiles of Successive solvent extracts

T. orientalis leaf showed presence of moisture content-40% w/w; total ash, acid insoluble ash and water-soluble ash determined were 15.85, 8.35 and 2.55% w/w, respectively.

Water-soluble extractive value was 5; alcohol-soluble extractive value was 11; the color consistency and % yield of successive extractive values of powder were petroleum ether (60-80℃) (yellowish brown, sticky mass, 4.7), toluene (Brownish black, sticky mass, 2.53), chloroform (Brownish black, sticky mass, 1.49), ethanol (Brownish black, sticky mass, 13.23), water (Dark brown, solid mass, 4.82) (table 2).

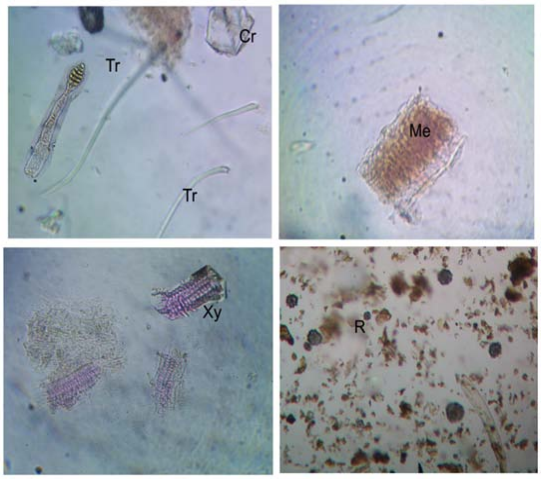

Fig. 4: M: Mesophyll region; Tr: Trichomes; Xy: Xylem vessels; R: Rosette crystals Physico-chemical evaluations

Table 1: Physico-chemical evaluations of leaf of *Trema orientalis*

S. No.	Parameter	Result
1.	Stomatal number	Lower epidermis: 6-9
2.	Stomatal index	Lower epidermis: 11-15
3.	Vein islet number	14.75
4.	Vein termination number	31.75
5.	Palisade Ratio	23.87
6.	Total ash	15.85% w/w
7.	Water-soluble ash	2.55% w/w
8.	Acid-insoluble ash	8.35% w/w
9.	Water-soluble extractive value	5% w/w
10.	Alcohol-soluble extractive value	11% w/w
11.	Moisture content	40% w/w

w/w-weight/weight; Total ash is approximately two times and four times, more than acid insoluble and water soluble ash, respectively. Ethanol soluble extractive is approximately two times higher than water soluble extractive.

Table 2: Preliminary profile of successive solvent extracts of leafs of *Trema orientalis*

S. No.	Solvent	Color and consistency after drying	Average value (%w/w)
1.	Petroleum ether (60-80ºC)	Yellowish brown sticky mass	4.7%
2.	Toluene	Brownish black sticky mass	2.53%
3.	Chloroform	Brownish black sticky mass	1.49%
4.	Methanol	Brownish black sticky mass	13.23%
5.	Water	Dark Brown solid mass	4.82%

Table 3: Phytochemical screening

S. No.	Constituents	Pet. Ether	Toluene	Chloroform	Acetone	Methanol	Water
1.	Phytosterol	+	-	-	-	-	-
2.	Triterpenoids	+	-	-	-	-	-
3.	Saponins	-	-	-	-	-	-
4.	Tannins and phenolics	-	-	-	-	+	+
5.	Carbohydrates	-	-	-	-	+	+
6.	Fixed oils	+	+	-	-	-	-
7.	Mucilage	-	-	-	-	-	+
8.	Flavonoids	-	-	-	-	+	+
9.	Glycosides	-	-	-	-	-	-
10.	Alkaloids	-	-	-	-	-	-

Preliminary phytochemical screening

All the above extracts were tested with various reagents and the results for the same are reported in table no.3. The various extracts showed the presence of phytosterols, carbohydrates, phenolic compounds, tannins, fixed oils and mucilage.

CONCLUSION

As there is no pharmacognostical anatomical work on records for this traditionally much valued shrub, present work is taken up in the view to lay down the macroscopic and microscopic standards, which could be used in deciding the genuineness of the herb, irrespective of their collection from different sources. The colored photographs of the Leaf of the above mentioned plant might facilitate the researcher for identification. The results of the phytochemical screening, histological tests can be considered as distinguishing parameters to identify and decide the authenticity of *T. orientalis* and thus can be used as standards for reference purpose also. The outcome of the quantitative parameters described on the above-mentioned plant parts(Leaf) might be useful in determining the authenticity of the drugs. HPTLC profile helps in standarization and also for undertaking work on isolating and identifying the bioactive compounds.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, A. R. College of Pharmacy for evincing interest in this work. They are also thankful to Dr. Reddy, Prof. and Head of Botany Dept., Sardar Patel University, for help in providing authentic plant material.

CONFLICT OF INTERESTS

Declared None

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