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Pharmacognostical Investigation and Physicochemical Analysis of *Celastrus paniculatus* Willd. Leaves

Kalaskar Mohan G*, Saner Sachin Y., Pawar Manohar V., Rokade Dipak L and Surana Sanjay J.

Department of Pharmacognosy, R C Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule, MS, India.

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ABSTRACT

Objective: *Celastrus paniculatus* Willd. is an important Indian medicinal plant and widely used in the treatment of the variety of disease and well explored scientifically for their pharmacological properties. The current study was therefore carried out to provide requisite pharmacognostic details about the plant. **Methods:** Pharmacognostic investigation of the fresh, powdered and anatomical sections of the leaves of *Celastrus paniculatus* Willd. was carried out to determine its morphological, anatomical, and phytochemical diagnostic features. Quantitative diagnostic characteristics, physicochemical properties and quantitative phytochemical measures were established. **Results:** The morphology of leaf revealed, leaves are alternate, acute, acuminate or obtuse, rounded with cuncate base, margin is finely crenate, venation is reticulate. The microscopy reveals the dorsiventral type of leaf, with anomocytic stomata, covering (lower epidermis) and glandular (upper epidermis) trichomes. The collateral vascular bundle crowned with sclerenchymatous fiber, ideoblast of calcium oxalate. The Quantitative diagnostic characteristics such as leaf constant were measured. Physicochemical properties such as ash, extractive values and fluorescence analysis were established. Quantitative phytochemical revealed presence of carbohydrates, fixed oil, glycosides, coumarins, tannins, flavonoids, saponins, steroids and triterpenoids. **Conclusion:** The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

1. Introduction

Celastrus paniculatus Willd. (CP) belongs to family Celastraceae is commonly known as *Malkangani*, (*Jyotishmati*), is an important medicinal large, woody, climbing shrub, distributed almost all over India up to an altitude of 2000 m. In an Indian traditional system of medicine, the plant has used for prevention and treatment of various diseases as cough, asthma, leprosy, paralysis, Leucoderma, rheumatism, gout and headache. The leaves are emmenagogue and the leaf sap is a good antidote for opium poisoning. The bark is reported to have abortifacient activity [1].

The sesquiterpene ester called malkangunin, sesquiterpene alkaloids named celapanin, celapanigin and celapagin have been isolated from the plant [2]. The triterpenoid lupeol was isolated from leaves and reported to be responsible for

wound healing activity [3]. CP Seed oil has been reported to improve memory and it is one of the components of the formulation 'Mentat Syrup' recommended for memory enhancing and mental disorders. The methanolic extract exhibits free-radical-scavenging properties and anti-oxidant effects in human non-immortalized fibroblasts. The seed of this plant has been found to enhance cognition and the mechanism by which it improves cognition is partly attributed to the antioxidant properties [2]. The seeds also experimentally showed sedative action, as an anti-rheumatic agent, alleviation of intestinal spasms, analgesic and anti-inflammatory and anti-diarrhoeal activities [4–6]. The seeds were also reported as sedative, anxiolytic and anticonvulsant properties. The powdered root and root bark considered to be useful in the treatment of malaria and cancerous [7].

Establishment of the pharmacognostic profile of the leaves of *C. paniculatus* will assist in standardization, which can guarantee quality, purity and identification of samples.

*Corresponding author: Kalaskar Mohan G. Department of Pharmacognosy, R C Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule, MS, India.

E-mail: kalaskar.mohan@gmail.com

Tel.: +918149448119

2. Materials and Methods

Fresh leaves of CP were collected from medicinal garden and Identified and confirmed from head of the department, Department of Pharmacognosy R C Patel Institute of Pharmaceutical education and Research, Shirpur. Dhule, the voucher specimens were preserved in the institute herbarium library (RCP–35).

2.1 Macroscopy

The leaves part was separated from other parts, washed, cleaned and dried for further use. The following macroscopic characters of the fresh leaves were noted: color, odor, taste, size and shape, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture [8].

2.2 Microscopy

The free hand thin transverse sections of the fresh leaves through the lamina and the midrib were treated with different staining agent and observed for the general and specific microscopic characteristic. The quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein–islet number and vein let termination number were carried out. Furthermore, small quantity of the powdered leaves was cleared, mounted and observed for diagnostic powder characteristics [8].

2.3 Physicochemical investigations

The dried leaf powder material was used for the determination of ash values, extractive values, and preliminary phytochemical investigation. The chemomicroscopic examination and behavior of powder with chemical reagents were also studied [9].

3. Results

3.1 Macroscopy

Fresh leaves are green in colour, odourless with a slightly acrid taste. The leaves are simple, alternate in arrangement, apex is acute, acuminate or obtuse and base is cuncate, obtuse or rounded; margin is finely crenate, venation is reticulate, shape is very variable, elliptic, ovate, broadly. The leaves are glabrous; sometime pubescent, average leaf size is 11 cm length and 6 cm breadth.

3.2 Microscopy

In microscopy of leaf lamina, the two to three palisade cells were observed below upper epidermis, confirms the

dorsiventral type of leaf (Figure 1A).

Micro–morphological features revealed that the cells of the epidermis were cuticularized. The upper epidermal cells are comparatively larger than lower one, while the lower epidermis have a thick cuticle compare to upper epidermis. The polygonal epidermal cells observed with anticlinal walls. The leaf shown the presence of anomocytic type of stomata, ranging from 18 to 20 mm in length and 14 to 15 mm in width, they were in abundance on the lower epidermis while upper epidermis showed comparatively less and mostly observed along the midrib region of the lamina.

The multicellular uniseriate covering trichomes with round and thick apex were observed only on lower epidermis, while very few numbers of glandular sessile trichomes were observed especially along the midrib region of the upper and lower epidermis along with small papillae (Figure 1D).

The midrib (Fig. 1A and B), in transverse section, is biconvex. Upper and lower epidermis layers continuous over the midrib, the epidermal cells shown similar features as seen in the lamina region. Adjacent to the epidermis, angular collenchyma occur, comprising approximately three to five rows on the ventral side and two to four on the dorsal side one embedded in the ground parenchyma. The collateral vascular bundles arranged nearly as a closed arc showed lignified spiral xylem vessels measuring 22 – 41 micron in diameter and non lignified phloem as sieve tubes. These exhibits an evident cambial zone and a sclerenchymatic cap adjoining the phloem (Figure 1B). Idioblasts with calcium oxalate prisms are found in the ground paremchymatous tissue (Figure 1C).

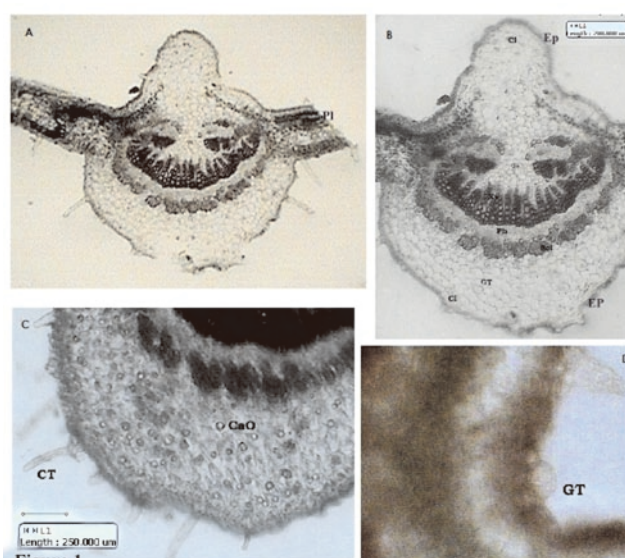


Figure 1. Transaction of the midrib of *C. paniculatus* showing Ep: epidermis, Cl: collenchyma; Gt: ground parenchyma, Ph: phloem, Xy: xylem, Scl: sclerenchymatic cap (Plate A); Ct: covering trichome, CaO: Calcium oxalate (Plate C); Gt: glandular trichomes (Plate D); Pl: palisade cells (Plate A).

Table 1

Chemomicroscopic color reactions of CP leaf powder.

Reagents	Constituent	Color	Histological zone	Degree of intensity
Aniline So ₄ + H ₂ SO ₄	Lignin	Yellow	Xylem, Sclerenchyma	++
Phloroglucinol + HCl	Lignin	Pink	Xylem, Sclerenchyma	+++
Conc. H ₂ SO ₄	Cellulose	Green	Ground parenchyma Collenchyma	+ ++
Weak Iodine solution	Starch	---	---	---
Millons reagent	Proteins	-	-	-
Dragendorff's reagent	Alkaloids	---	---	---
H ₂ SO ₄	Ca. Oxalate	Needles	Mesophyll, and Ground parenchyma	++
SbCl ₃	Steroids/Triterpenoids	Reddish pink	Mesophyll	+++

+++ High, ++ Moderate, + Slight, - Negative.

3.2.1 Powder analysis and quantitative microscopy

I. Numerous anomocytic or ranunculaceous stomata meaning thereby that the cells surrounding the stomatal pores are irregularly arranged and cannot be differentiated from other epidermal cells (Figure 2A).

II. Numerous bi-cellular to multi-cellular covering trichomes measure 440 – 530 microns in length (Figure 2B).

III. Fibers are few, lignified well developed sclerenchymatous fibers from the vascular bundle region, thin, and isolated fibers measure 450 – 800 microns in length and 12 – 21 microns in breadth (Figure 2C).

IV. Fragments of mesophyll tissue containing vascular strands i.e. Spiral xylem vessel are seen in a good many in number (Figure 3D).

V. Numerous prismatic calcium oxalate crystals as individual or in fragments of spongy parenchyma measure 37–51 microns in dimensions (Figure 2E).

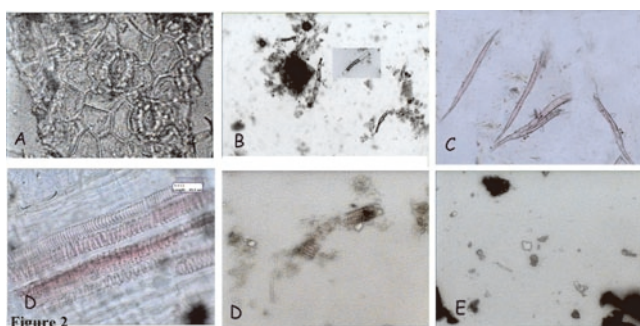


Figure 2. Diagnostic features for the powder microscopy of the leaf of *C. paniculatus*. (A) Anomocytic stomata, (B) Covering trichomes, (C) Sclerenchymatous fiber, (D and E) Spiral Xylem vessels, (F) Simple prisms of calcium oxalate.

3.2.2 Quantitative microscopy

The results of chemomicroscopic examination and vital quantitative microscopic were determined and results were tabulated in table 1 and 2.

3.3 Physicochemical investigations

The behaviors of leaf powder with different chemical reagents and fluorescence analysis were shown in table 3, 4. The physicochemical standards are important to check the quality, purity and adulteration of given crude drug. The different types of ash and extractive values were determined and summarized in table 5. Furthermore the phytochemical analysis of extracts of leaves showed the medicinally potential constituents includes, carbohydrates, tannins, flavonoids, steroids and triterpenoids.

Table 2

Vital quantitative microscopy of CP leaf

Leaf constant	Values
Stomatal number	Upper epidermis – 85.66±5.13 Lower epidermis – 127.67±7.51
Stomatal index	Upper epidermis – 14.41±0.91 Lower epidermis – 11.91±1.43
Vein islet number	37.67±3.51
Vein termination number	23.33±1.52

Values are mean±SD, n = 3

Table 3

Behavior of CP leaf powder with different chemical reagents.

Reagents	Color/ppt	Constituents
Picric acid	No precipitations	Alkaloids absent
Conc. H ₂ SO ₄	Reddish brown	Steroids/triterpenoids present
Aq. FeCl ₃	Blackish brown	Tannins present
Iodine solution	No change	Starch absent
5% Aq. KOH	No change	Anthraquinone glycosides absent
Mayer's reagent	No perception	Alkaloids absent
Spot test	Stains observed	Fixed oils present
Aq. AgNO ₃	No precipitation	Proteins absent
Aq. NaOH	Yellow	Flavonoids present
Mg – Hcl	Magenta	Flavonoids present
Dragendorff's reagent	No precipitation	Alkaloids absent

Table 4

Fluorescence analysis of CP leaf.

Color reaction	Day light	Uv light 365nm
Powder + NaOH	Green brown	Greenish fluorescence
Powder + Methanol + nitrocellulose	Green	Dark green fluorescence
Powder + nitrocellulose	Grayish green	Strong yellow fluorescence
Powder + NaOH in water	Green	Faint green fluorescence
Powder + nitrocellulose +Hcl	Grayish green	Faint green color
Powder + Hcl	Yellowish green	Dark brown with faint yellow fluorescence
Powder + H ₂ SO ₄	Blackish	Black
Powder + HNO ₃	Brown	Green
Powder	Green	greenish florescence

Table 5

Physicochemical analysis of CP leaf.

Types of ash value/extractive values	% w/w
Ash values	
Total ash	6.24±0.35
Acid insoluble ash	1.15±0.11
Water soluble ash	2.58±0.43
Sulphated ash	5.13±0.28
Extractive values	
Petroleum ether 60–80 °C	3.52±0.42
Ethyl acetate	2.61±0.56
Alcohol	8.27±0.63
Water	6.53±0.56

Values are mean±SD, n = 3

4. Discussion

Celastrus paniculatus “Malkangani” is currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in Herbal Pharmacopoeia, pharmacognostic parameters and standards must be established. CP is a plant that has been confused with other species due to their relative similarities. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant.

Regarding the leaf venation, the reticulate type observed in CP represents a characteristic of dicotyledonous plants. Besides being involved with the transport of substances, the venation system is also related to the leaf mechanical stabilization, owing to the presence of the lignified xylem and sclerified elements. Pant and Kidwai have mentioned for the CP the occurrence of anomocytic type of stomata, the stomata were found in abundance at lower surface than upper one [10]. We confirmed the similar pattern which revealed from stomatal number and the stomatal index (Table 2). From Woodward’s point of view, although the presence of stomata increases photosynthetic potential, protects

xylem from cavitation favoring water flow and promotes heat dissipation by water loss, herbaceous species maintain low stomatal densities and hydraulic conductances, maximizing the control on loss of water to a dry atmosphere [11]. This control is optimized by a well-developed cuticle, a barrier which contributes to the maintenance of plant water status [12].

The calcium oxalate crystals in higher plant, is most common as mineral deposits, they are formed from environmentally derived calcium and from biologically synthesized oxalate and typically deposited within intra-vacuolar membrane chambers of specialized cells in any organ or tissue [13, 14]. According to Francheschi and Horner Jr, the differentiation of a cell into a crystal idioblast is surely under genetic control and the shape of the crystal formed may also be genetically determined [15]. The functions assigned to calcium oxalate crystals are varied, since they can be related to ionic balance and osmoregulation, storing form of calcium or oxalate, mechanical support and protection against foraging animals. In present investigation, the simple prisms of calcium oxalate were found scattered in ground parenchymatous cells of midrib, thus it can become one of the important parameter for identification and standardization of CP.

Trichomes are epidermal outgrowths of considerable value for taxonomic purposes. Most of the plant species are completely devoid of trichomes while other shows in abundance. The environmental conditions influence more the length, size and density than the types of trichomes. These outgrowths play a role in plant defense especially with regard to phytophagous insects, avoiding insect feeding and ovi position responses, and the nutrition of larvae [16]. They may be also involved in the regulation of temperature and water repellency as well [17]. The upper epidermises of CP devoid of covering trichomes, but sessile unicellular uniseriate glandular trichomes were frequently observed over midrib region. The lower epidermis showed presence of only multicellular uniseriate covering trichomes with round apex.

Preliminary phytochemical analysis indicated presence of carbohydrates, fixed oil, glycosides, coumarins, tannins, flavonoids, saponins, steroids and triterpenoids. The information obtained from the preliminary phytochemical screening will be helpful in finding out the genuity of the drug. The ash values of a drug give an idea of the earthy matter or inorganic composition and other impurities present along with the drug. Extractive values are preliminary useful for determination of exhausted or adulterated drug [18]. Thus ash, extractive values, fluorescence analysis will be helpful in identification and authentication of plant material.

5. Conclusion

The morphological and microscopical characteristics of CP correspond to the general features of celastraceae family. Although they have to be evaluated altogether for the species identification, some characters may be pointed out, such as alternate leaf showing multicellular uniseriate covering trichomes with round thick-walled apex and sessile glandular trichomes, the presence of anomocytic stomata largely confined to the lower epidermal side, large prismatic calcium oxalate crystals, dorsiventral mesophyll, collateral vascular bundles in the midrib with sclerenchymatous cap. The macro and micro morphological features of the leaf described, distinguishes it from other members of the genera. Chemomicroscopy, numerical data and quantitative leaf microscopy are parameters that are unique to the plant and are required in its standardization. Phytochemical evaluation revealed the presence of carbohydrates, fixed oil, glycosides, coumarins, tannins, flavonoids, saponins, steroids and triterpenoids, which have been claimed to be responsible for therapeutic uses.

Conflict of interest statement

We declare that we have no conflict of interest.

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