

Original Article: Pharmacognostic Standardization and Physicochemical Analysis of *Clerodendrum Wallichii* (Merr.) Leaves

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ABSTRACT

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Background: Presently, the use of herbal medicines is expanding rapidly across the world. While considering source materials, authentication and standardization are prerequisites for herbal formulation in any system of medicine. The plant *Clerodendrum Wallichii* Merr. (Family: Lamiaceae) has been used for various ailments in traditional systems of medicines, particularly in the treatment of diarrhea, skin infection, inflammation and fever.

Objectives: The present study was designed to establish the pharmacognostic standards and perform the physicochemical analysis of *C. wallichii* leaves. Macroscopic and microscopic studies were performed using the simple and trinocular microscope, respectively.

Methods: The World Health Organization guidelines were followed for the physicochemical analysis of the plant. Fluorescence analysis was observed at daylight, short UV light, and long UV light. The leaves of *C. wallichii* were found dark green on the upper surface and light green in the lower surface which is odorless and bitter. The leaves are oblong to oblong-lanceolate with a smooth surface. The size of leaves varies from 11 to 18 cm in length and 2.5 to 4 cm in diameter.

Results: Powdered microscopy showed the various characters like rare multicellular covering trichome, xylem vessels (reticulate), fiber, trichome base, stellate trichome, adaxial epidermal cell (rectangular), abaxial epidermal cell (irregular), vessels, stomata (anisocytic), calcium oxalate crystals (square and cubic). Physicochemical parameters like moisture content of dry powder of the plant was determined 9.3% W/W. The total ash, acid-insoluble, and water-soluble ash values were calculated as 10.48%, 1.08%, and 8.17%, respectively. The loss on drying was calculated as 9.3% W/W.

Conclusion: Extractive values by cold and hot maceration method were also determined. Our obtained data help to authenticate the plant and establish its pharmacopoeial standards.

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Introduction

This genus *Clerodendrum* comprises more than 400 species and widely spread in Asia, Africa, and America [1, 2]. The plant *Clerodendrum Wallichii* Merr. (Family: Lamiaceae) commonly-known as Sampulis is native to Southern Asia from the Himalayas to Southern China, the Nicobar Islands, and northeast into Pakistan [3, 4]. In India, it is found in Sikkim, Tripura, Mizoram, Meghalaya, Assam, Maharashtra, and Uttarakhand [5, 6]. Ethnopharmacological reports on this plant revealed that its leaves are used for the treatment of skin infection and inflammation in the Mao Naga tribe in India. It is used as a vegetable and traditional medicine among Khasi and Jentia tribes in Meghalaya in India [7].

The popularity of herbal drugs is increasing rapidly all over the world, particularly in developed countries, but one of the obstacles in their widespread use is the lack of standardization/quality control of herbal drugs. The World Health Organization (WHO) emphasizes macroscopic and microscopic examination, physicochemical, and phytochemical evaluation of crude drug materials for developing a standardized quality control profile of herbal medicine [8, 9].

WHO encourages countries to provide safe and effective traditional remedies and use them in public and private health sectors. Preparations of monographs are primarily intended to promote harmonization in the use of herbal medicines concerning levels of safety, efficacy, and quality control [10]. The leaves of *Clerodendrum wallichii* have never been subjected to pharmacognostic standardization studies. Thus, it is worthwhile to study the pharmacognostic and physicochemical properties of *Clerodendrum wallichii* leaves.

Materials and Methods

Collection and authentication of plant

The plant material *C. Wallichii* was collected from Forest Research Institute (FRI), Dehradun, India. The plant *C. wallichii* was identified and authenticated by the Department of Botany, Botanical Survey of India, Dehradun, India vide reference No. 118175. The voucher specimen was maintained in the Botanical Survey of India, Dehradun laboratory, for further reference.

Pharmacognostic evaluation

Macroscopy

The macroscopic evaluation was done by identifying the color, odor, taste, shape, surface characteristics, texture, and

fracture characteristics [11]. In the present study, the macroscopic study of leaves of the plant was based on color, odor, taste, surface characteristics, and so on [12, 13].

Microscopy

Preparing the slides: First, a clean glass slide was selected, and the fine powder of the drug was placed on the glass slide, and the slide was tapped such that uniform powder was distributed on the surface of the slide. Then, the glass slide was observed by using different reagents for the internal study of powdered drugs with a microscope [12, 13]. Transverse Section (TS) of leaf: The leaf was cut into thin sections through the midrib portion of the leaf with a sharp blade and observed under a microscope by using different reagents [14].

Determination of foreign matters

One hundred grams of drug was weighed and spread on a white tile without overlapping. The sample was inspected with naked eyes or by a magnifying lens (10x or above), and the foreign organic matter was separated. After the complete separation, the matter was weighed, and the percentage W/W present in the sample was calculated [15].

Determination of ash values

Determination of total ash value

A crucible was weighed and ignited in an oven up to reach a constant weight. Then, 3 g powdered drug was put into it and covered with a lid and put into muffle furnace at 500-600°C for 6 h. Next, it was cooled in a desiccator. Finally, the obtained ash was weighed, and the total ash was calculated with reference to the air-dried sample of the drug [11].

Determination of acid-insoluble ash value

Twenty five milliliters of dilute hydrochloric acid was added to the crucible containing total ash and boiled gently for 5 minutes. The mixture was filtered, and the residue was washed with hot water twice. The filter paper was put into the crucible, which was placed into a muffle furnace at 500-600°C for 6 h. Then it was cooled in a desiccator. Next, the obtained residue was weighed, and the acid-insoluble ash calculated with reference to the air-dried sample of the drug.

Determination of water-soluble ash value

Twenty five milliliters of water was added to the crucible containing total ash and boiled gently for 5 minutes. The mixture was filtered, and the residue was washed with hot water twice. The filter paper was put into the crucible and placed into a muffle furnace at 500-600°C

for 6 h. Then, it was cooled in desiccators. The obtained residue was weighed, and the water-soluble ash was calculated with reference to the air-dried sample of the drug.

Determination of extractive values

Determination of alcohol-soluble extractive values

Ten grams of powdered drug was weighed and put into a 250-mL conical flask. It was filled with 90% alcohol up to 100 mL mark and was corked. The conical flask was kept aside for 24 h and frequently shook during this time. The mixture was filtered, and 25 mL filtrate was collected into a porcelain dish. It was allowed to evaporate up to dryness on a water bath and wholly dried on the oven at 100°C. Then it was cooled in desiccators. The percentage W/W of extractive value was calculated with reference to the air-dried drug [11].

Determination of water-soluble extractive values

Ten grams of powdered drug was weighed and poured into a 250-mL conical flask. Then, it was filled with water up to 100 mL mark and was corked. Next, it was kept aside for 24 h and frequently shook during this time. The mixture was filtered, and the 25 mL filtrate was collected into a porcelain dish. It was allowed to evaporate up to dryness on a water bath and completely dried on the oven at 100°C. Then it was cooled in desiccators. The percentage W/W of extractive value was calculated with reference to the air-dried drug.

Determination of loss on drying

Two grams of powdered drug was weighed and poured into a porcelain dish. Then, it was dried in the oven at 105°C. After cooling in desiccators, the loss in weight was recorded. This procedure was repeated until a constant weight was obtained. The percentage loss on drying was calculated with reference to the initial weight of the crude drug.

Fluorescence analysis

The powdered drugs were mixed with the different solvents to make pasty materials and then put on the glass slides. Then the glass slides were further analyzed under the three regions of light (daylight, long UV light, short UV light) for the investigation of the fluorescence produced by the drug after treatment with various inorganic/organic reagents [15].

Results

Macroscopic characters of *Clerodendrum wallichii* leaf

The leaves of *C. wallichii* were dark green on the upper surface and light green on the lower surface. They are odorless and bitter. The leaves are oblong to oblong-lanceolate with a smooth surface. The size of leaves varies from 11 to 18 cm in length and from 2.5 to 4 cm in diameter. The apex of the leaf is acuminate to acute and entire margin, with a narrowly cuneate base with 7-8 pairs venation. Table 1 presents the macroscopic features of the plant leaf.

Microscopic characters of *Clerodendrum Wallichii* leaf

Powder microscopy showed the various characters like rare multicellular covering trichome, xylem vessel (reticulate type), trichome base, stellate trichome with unicellular branches, adaxial epidermal cell (rectangular type), abaxial epidermal cell (irregular type), stomata (anisocytic type) and calcium oxalate crystals (square and cubic type). Figure 1 shows the results for powder microscopy of leaves. The transverse section of the leaf showed various characters like collenchyma, spongy parenchyma, cystolith, glandular trichome, xylem, phloem, etc. Figure 2 shows the results of transverse sections.

Foreign matter

The foreign matter was calculated as 0.89% W/W. The results are depleted in triplicate.

Physicochemical analysis of *Clerodendrum wallichii* leaves

The ash value such as total ash, acid-insoluble, and water-soluble ash value were calculated as 10.48%, 1.08%, and 8.17%, respectively (Table 2). In our study, ethanol, chloroform, petroleum ether, acetone, ethyl acetate, and water were used to evaluate the extractable constituent in the leaves of *C. wallichii*. Alcohol soluble, water soluble, petroleum ether, chloroform, acetone, ethyl acetate and hexane extractive values were calculated as 1.31%, 2.19%, 0.36%, 1.08%, 0.44%, 0.29%, and 0.48, respectively by cold maceration method, and 2.15%, 3.17%, 1.85%, 2.2%, 1.83%, 2018%, 2.11%, respectively by hot maceration method as shown in Table 3.

Loss on drying

The loss on drying was calculated as 9.3% W/W.

Table 1. Macroscopic characters of *Clerodendrum wallichii* leaf

No.	Characters	Observations
1	Color	Upper surface: Dark green Lower surface: Light green
2	Taste	Bitter
3	Odor	Odorless
4	Shape	Oblong to oblong-lanceolate
5	Size	11-18 cm in length
6	Diameter	2.5-4 cm in width
7	Surface	Smooth
8	Nature	Dicot
9	Margin	Entire
10	Apex	Acuminate to acute
11	Base	Narrowly cuneate
12	Venation	7 to 8 pairs

PBR**Table 2.** Ash values of *Clerodendrum wallichii* leaves

No.	Ash Value	Mean±SD
		Yield (%W/W)
1	Total ash value	10.48±0.027
2	Acid-insoluble ash value	1.08±0.063
3	Water-soluble ash value	8.17±0.011

PBR**Table 3.** Extractive values of *Clerodendrum wallichii* leaves

No.	Extractive Value	Methods (Maceration)	
		Cold	Hot
		Mean±SD	
		Yield (%W/W)	
1	Alcohol soluble	1.31±0.013	2.15±0.020
2	Water soluble	2.19±0.006	3.17±0.018
3	Petroleum ether	0.36±0.006	1.85±0.031
4	Chloroform	1.08±0.017	2.2±0.040
5	Acetone	0.44±0.032	1.83±0.009
6	Ethyl acetate	0.29±0.035	2.18±0.009
7	Hexane	0.48±0.237	2.11±0.011

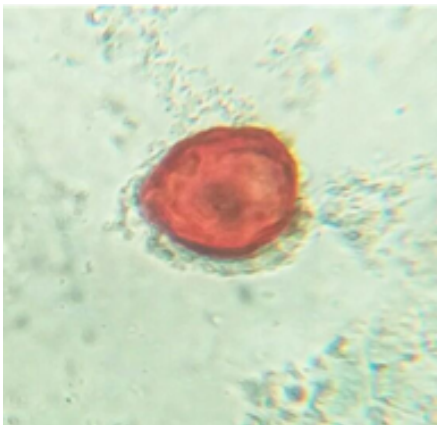
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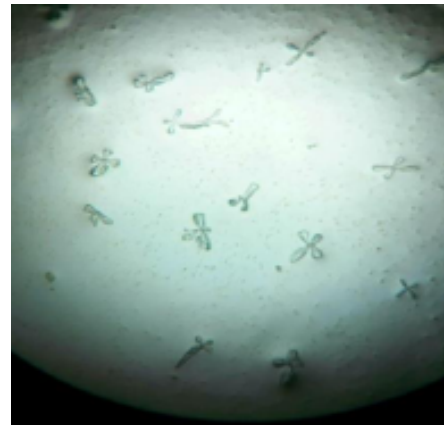
Multicellular covering trichome (10X×40X)



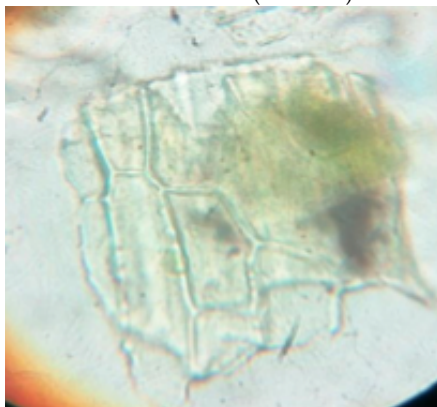
Xylem vessel (reticulate type) (10X×40X)



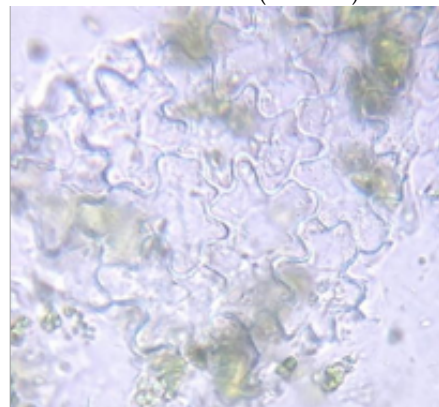
Trichome base (10X×40X)



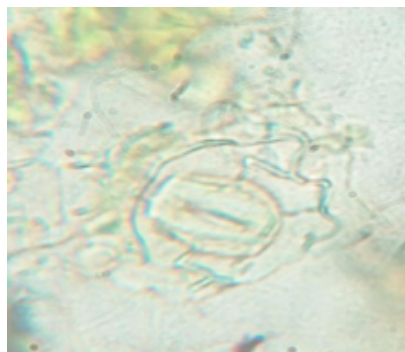
Stellate trichome (10X×10X)



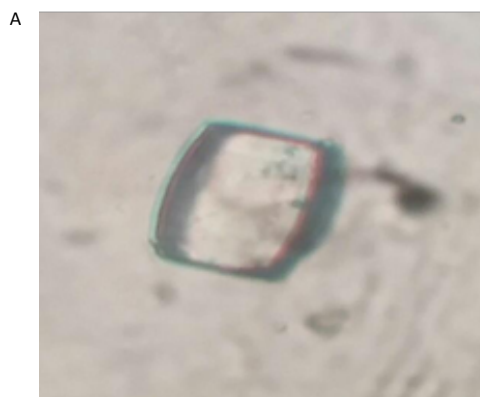
Adaxial epidermal cell (Rectangular) (10X×10X)



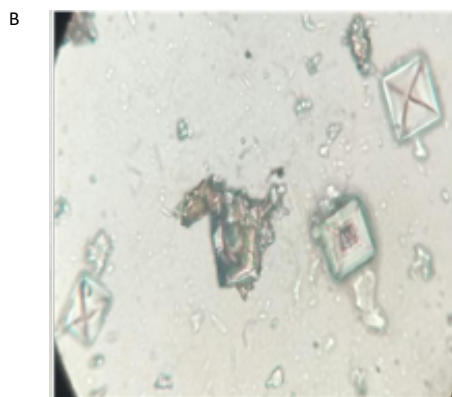
Abaxial epidermal cell (Irregular) (10X×10X)



Stomata (Anisocytic type) (10X×10X)



Calcium oxalate crystal (cubic type) (10X×40X)



Calcium oxalate crystal (square type) (10X×10X)

Figure 1. Powder microscopy of *Clerodendrum Wallichii* leaves

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Fluorescence analysis

It is one of the critical parameters for the evaluation of selected plant material’s quality, strength, and purity. The results are tabulated in [Table 4](#).

Discussion

In the current scenario, the use of herbal medicines continues to expand rapidly across the world. Many countries now turn towards herbal medicines and products and use them in national health-care settings. Authentication and standardization are prerequisites while considering source materials for herbal formulation in any system of medicine [16]. The plant *Clerodendrum Wallichii* (Family: Lamiaceae), commonly known as Sampul, is one of the popular folkloric medicine used in North East India, especially among Garo and Khasi tribes in Arunachal Pradesh, Manipur, and Meghalaya

for the treatment of skin infection, inflammation, and diarrhea. Despite a long history of traditional use, the plant has never been subjected to pharmacognostic evaluation studies so far. Thus, it was considered worthwhile to evaluate *C. Wallichii* for various pharmacognostic standardization studies and physicochemical analysis.

The current study aims at macroscopic and microscopic evaluation and physicochemical analysis of *Clerodendrum Wallichii* leaves.

Macroscopic studies help to determine the identity and degree of purity of herbal materials. *C. Wallichii* was evaluated for the study of macroscopic characters. The leaves of *C. Wallichii* were found dark green on the upper surface and light green on the lower surface. They were odorless and bitter. The leaves are oblong to oblong-lanceolate with a smooth surface. The size of leaves varies from 11 to 18 cm in length and 2.5 to 4 cm

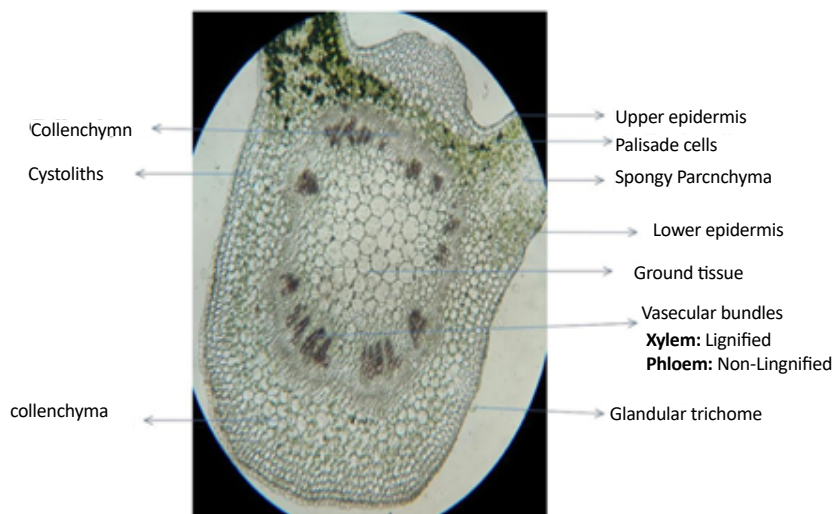


Figure 2. Transverse section of *Clerodendrum wallichii* leaf (10X×10X)

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Table 4. Fluorescence analysis of powder of *Clerodendrum Wallichii* leaves

No.	Reagents	Daylight	Short UV Light	Long UV Light
1	Powder	Black	Green	Green
2	50% H ₂ SO ₄	Dark Black	Dark black	Dark brown
3	95% Ethanol	Pale yellow	Dark green	Dark green
4	Conc. H ₂ SO ₄	Dark black	Dark black	Dark brown
5	5% KOH	Black	Pale green	Dark green
6	5% FeCl ₃	Dark black	Black	Dark green
7	50% HNO ₃	Black	Green	Brownish
8	5% NaOH	Black	Light green	Brownish
9	1N HCl	Dark black	Black	Greenish
10	Benzene	Light brown	Brown	Dark green
11	Acetic acid	Pale yellow	Dark green	Dark brown
12	Chloroform	Light green	Dirty green	Dark green
13	Methanol	Black	Green	Pale green
14	Petroleum ether	Black	Brown	Yellowish

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in diameter. The apex of the leaf is acuminate to acute and entire margin, with a narrowly cuneate base with 7-8 pairs venation (Table 1).

Powder microscopy showed the various characters like rare multicellular covering trichome, xylem vessel (reticulate type), trichome base, stellate trichome with unicellular branches), adaxial epidermal cell (rectangular type), abaxial epidermal cell (irregular type), stomata (anisocytic type), calcium oxalate crystals (square and cubic type). The results for powder microscopy of leaves are shown in Figure 1. The transverse section of the leaf shows the various characters like collenchyma, spongy parenchyma, cystolith, glandular trichome, xylem, phloem, etc. (Figure 2).

The physicochemical parameters help set standards for a crude drug as these parameters are mostly constant for a plant. Various physicochemical parameters were evaluated for the leaves, as mentioned in WHO guidelines. These parameters are important for the detection of drug adulteration or improper handling of raw materials. The ash value gives an idea of inorganic composition and other impurities in plant drug. The ash values such as total ash, acid-insoluble, and water soluble ash value were calculated as 10.48%, 1.08%, and 8.17%, respectively (Table 2).

The extractive value determines the amount of active constituent extracted with solvents from a given amount

of medicinal plant material. In the present study, ethanol, chloroform, pet ether, acetone, ethyl acetate, and water were used to evaluate the extractable constituent in the leaves of *C. Wallichii*. The extractive values were determined in a different solvent by using cold and hot maceration method. The alcohol soluble, water soluble, petroleum ether, chloroform, acetone, ethyl acetate, and hexane extractive value were calculated by cold and hot maceration method results (Table 3).

The percentage of active chemical constituents in any crude drugs is mentioned on an air-dried basis. Therefore, the loss on drying of plant materials should be determined, and the water content should be controlled. The moisture content of dry powder of the plant was determined 9.3% W/W. The foreign matters include insects, molds, animal excreta, and other contaminants like soil, stone, dust, metal parts, etc. According to WHO, it should be within prescribed limits [15]. In the present study, the content of foreign matter was found very negligible.

Fluorescence analysis shows the emission of light by a substance against absorbed light. This technique of observing plant material under fluorescence light has been used as a pharmacognostic tool to distinguish between plants and their species. The results are tabulated in Table 4.

In the present study, the pharmacognostic standards are established, which would be helpful for correct identi-

fication of this plant. Most of the diagnostic characters, like different types of calcium oxalate crystals, various types of trichomes, and so on, were identified the first time in this plant. Moreover, the physicochemical analysis of multiple parameters was also conducted. These sets of standards would be useful in the future to assess the quality and purity of *C. Wallichii*.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article. The participants were informed about the purpose of the research and its implementation stages; they were also assured about the confidentiality of their information; Moreover, They were allowed to leave the study whenever they wish, and if desired, the results of the research would be available to them.

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Authors' contributions

All authors contributed equally in preparing all parts of the research.

Conflict of interest

The authors declared no conflict of interest.

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