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Pharmacognostical Studies on Stems of *Clerodendrum Paniculatum*

Ms. Pavithra T R[1], Mrs. Akila Sukumaran[2], Dr. Ajith Babu T K[3]

[1]M Pharm, Department of Pharmacognosy, Malik Deenar College of Pharmacy, Kasaragod
[2]Associate Professor, Department of Pharmacognosy, Malik Deenar College of Pharmacy, Kasaragod
[3] Principal, Department of Pharmaceutical Chemistry, Malik Deenar College of Pharmacy, Kasaragod

ABSTRACT

Clerodendrum paniculatum is a lamiaceae family member and a plant with significant medicinal and pharmacological properties. The genus consists of about 500 species; among them, *Clerodendrum paniculatum* L. is one of the most important species. This species is commonly grown for ornamental purposes but has been found to have numerous medicinal benefits. The plant has been used in traditional medicine for centuries and has been reported to have ethnomedicinal properties that can treat various ailments. Some conditions that can be treated using this plant include wounds, typhoid, snakebites, jaundice, giddiness, malaria, anemia, and hemorrhoids. This work has been an approach to carry out pharmacognostic studies on stems of *C. paniculatum*. The pharmacognostic properties were comprehensively examined to determine the foreign matter and assess the moisture content, ash value, extractive value, and macroscopic and microscopic evaluations. Phytochemical screening has revealed the presence of various constituents such as alkaloids, flavonoids, glycosides, phenols, phytosterols, saponins, and terpenoids in *Clerodendrum paniculatum*. These constituents have been found to exhibit multiple biological activities. The findings of the study could be valuable for the identification and preparation of a monograph of the plant.

Keywords: *C. paniculatum*, Lamiaceae, Macroscopy, Microscopy, Physico-chemical

INTRODUCTION

Clerodendrum L. is a fascinating and diverse genus of plants. This expansive group contains over 500 different species that exhibit a wide range of characteristics, making them a favourite among botanists and garden enthusiasts alike. Previously classified in the Verbenaceae family, it is now categorized in the Lamiaceae family.

The biennial herb, *C. paniculatum* is known for its unique and attractive features. It can grow up to 1.5 meters in height, making it a remarkable addition to any garden. The plant's most remarkable feature is its beautiful and often fragrant flowers. These flowers come in a variety of colors, including pink, white, red, and blue, and can be seen throughout the year. *Clerodendrum* L. is also known for its medicinal properties. The plant has been used in traditional medicine for centuries to treat a variety of ailments, from fever and headaches to skin problems and respiratory issues. Stems are hollow, slightly pubescent, obtusely quadrangular, nodes annulate, and slightly hairy. Leaves are opposite, palmately lobed outline, ovate-cordate to ovate-suborbicular, Dark green, slightly pubescent above, slightly paler below. Lateral veins are 3-7, veins impressed above and densely pubescent and prominent beneath. The petiole is stout, slightly pubescent, canaliculated, about 8-36 cm long. Plant flowers have both male and female parts, with reddish-orange petals and a bell-shaped calyx. The slender corolla tube is curved and covered with tiny hairs outside. There are four stamens with long, slender filaments and oblong anthers. The ovary has two parts. The style is purple and thin, and the stigma has two parts. Fruit drupaceous, globose, about 1 cm in diameter with 4 pyrenes, fleshy, purplish black when ripe, fruiting calyx persistent. A literature review indicates the absence of comprehensive pharmacognostic studies on the stems of *Clerodendrum paniculatum*. Consequently, our current initiative aims to conduct in-depth pharmacognostic and phytochemical investigations on the stems of *C. paniculatum*. The resulting data will establish a standard reference for the identification and authentication of the plant, as well as facilitate the differentiation of the species from potential adulterants.

PLANT **PROFILE**

- **Synonym:** Clerodendron, Orange Tower Flower, Pagoda Flower, Krishnakireedam, Hanumankireedam.
- **Distribution:** India, Sri Lanka, Malaysia, and much of southeastern Asia, Southern China including Taiwan, Indochina, Bangladesh, Andaman & Nicobar Islands, Borneo, Sulawesi, Sumatra, Philippines, Bismarck Archipelago.
- **Taxonomy:**

Domain: Eukaryote

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledons

Order: Lamiales

Superorder: Asteranae

Family: Verbenaceae

Genus: *Clerodendrum*

Species: *Clerodendrum paniculatum*

Fig No. 1: Whole plant of *C. paniculatum*

Fig No.2: Leaf of *C. paniculatum* **Fig No.3: Flower of *C. paniculatum***

MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTICATION

The stems of *Clerodendrum paniculatum* were collected from Kannur. The plant material was taxonomically identified by the botanist, Dr. Biju P, Department of Botany, Government College, Kasaragod. The stems of plant materials were dried under shade for a few days, powdered with a mechanical grinder, and stored in an air-tight container.

PHARMACOGNOSTIC STUDIES

1. Determination of foreign matter

About 50g of powdered stems of the plant to be examined were weighed and spread out in a thin layer. The foreign matter was detected. It was separated and weighed and the percentage of foreign matter was calculated.

2. Determination of moisture content

Five grams of the powdered stems of the plant were placed in an evaporating dish. Drying was carried out at 105˚C for five hours. The drying was continued with intermittent weighing at a one-hour time interval until the difference between two successive weights was not more than 0.25%. Constant weight was reached when the two-consecutive weighing after drying for 30 minutes and cooling for 30 minutes in desiccator, showed not more than 0.01gm difference.

Loss on drying = Initial weight – Final weight

$$\% \text{ Loss on drying} = \frac{\text{Loss on drying}}{\text{Weight of powdered drug taken}} \times 100$$

3. Determination of ash value

The ash value is an important parameter for evaluating crude drugs, due to variation of values within wide limits. The ash value of any organic material is composed of inorganic materials like metallic salt and silica.

The following three methods were developed;

- Total ash
- Acid insoluble ash
- Water soluble ash

Ashing involves an oxidation of the component of the product and a high ash value involves contamination, substitution, adulteration or carelessness in the preparation of crude for marketing.

- **Total Ash**

Two grams of ground air-dried stem powder were accurately weighed in a crucible previously ignited for 30 minutes. The material was spread in an even layer and ignited at a temperature not more than 450˚C until it indicated the absence of carbon, cooled in the desiccator, and weighed. Calculate the content of total ash per gram of air-dried material.

$$\% \text{ Total ash} = \frac{\text{Weight of total ash}}{\text{Weight of sample}} \times 100$$

- **Acid insoluble ash**

25 ml of 2N HCl was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and added to the crucible. Collected the insoluble matter on an ash-less filter paper and washed it with hot water until the filtrate was neutral. Filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator for 30 minutes, then weighed, and calculated the content of acid-insoluble ash per gram of air-dried material.

$$\% \text{ Acid insoluble ash} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of sample}} \times 100$$

- **Water soluble ash**

25 ml of water was added to the crucible containing the total ash and boiled for 5 minutes. The insoluble matter was collected in sintered glass crucibles. Washed with hot water and ignited in a crucible for minutes at a temperature not exceeding 450˚C. The weight of those residues in mg was subtracted from the weight of the total ash. The content of water-soluble ash was calculated per gram of air-dried material.

$$\% \text{ Water soluble ash} = \frac{\text{Weight of water-soluble ash}}{\text{Weight of sample}} \times 100$$

4. Determination of extractive value

This method determines the number of active constituents in each amount of plant material when extracted with solvent. The extractive value is used as a means of evaluating crude drugs that are not readily estimated by other means. For example, lowering the prescribed values indicates the addition of exhausted or unwanted material with the original drug or incorrect processing of the drug.

$$\% \text{ Extractive value} = \frac{\text{Weight of extract obtained}}{\text{Weight of sample}} \times 100$$

- **Alcohol soluble extractive value**

Macerated 5 grams of coarsely powdered air-dried stems of *Clerodendrum paniculatum* with 100 ml ethanol in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours, and allowed to stand undisturbed for another 18 hours. Filtered rapidly by taking precautions against the loss of alcohol. The 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W ethanol-soluble extractive regarding air-dried material.

- **Water soluble extractive value**

Macerated 5 grams of coarsely powdered air-dried stems of *Clerodendrum paniculatum* with 100 ml water in a stoppered flask for 24 hours, with occasional shaking during the 1st 6 hours, and allowed to stand undisturbed for another 18 hours. Filtered rapidly, then 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105˚C, and weighed. Calculated W/W water soluble extractive regarding air-dried material.

5. Organoleptic evaluation

Organoleptic evaluation can be done using organs of sense. This refers to the evaluation of a drug by colour, odour, size, shape, taste, and special features including touch, texture, etc. For this purpose, an authentic specimen of the material under study and a sample of pharmacopeial quality should be available to serve as a reference. However, the judgment based on sensory characteristics like odour, taste, etc. may vary from person to time based on the individual's nature. No preliminary treatment is necessary for evaluating the sample in this manner.

- **Colour**

The untreated samples were examined under diffused sunlight or an artificial light source with a wavelength like daylight.

- **Odour and Taste**

Samples were crushed by gentle pressure and examined by repeated inhalation of air over the material.

- **Size**

Size was measured using a graduated ruler in millimeters.

- **Texture and Fracture**

The texture was examined by taking a small quantity of material and rubbing it between the thumb and forefinger. Bent and rupture caused to the sample provided information on the brittleness and appearance of the fractured plane as fibrous, smooth, rough, granular, etc.

6. Microscopic evaluation

- **Histology of stem**

A small piece of the stem was selected. The transverse section was prepared with the help of a sharp razor blade at the right angle to the longitudinal axis of the material and they were put in water and taken in a watch glass. Transparent and floating sections were selected. The selected sections were then cleared by warming with a few drops of 5%w/v potassium hydroxide solution. The sections were washed with tap water and stained with phloroglucinol: concentrated HCl (1:1). The sections were then mounted in glycerin with a coverslip on a clean glass micro slide and observed under a digital microscope.

- **Powder analysis**

For powder analysis, the plant was collected and washed thoroughly with water to remove the unwanted matter. This was further dried in the shade. After complete drying, the plant was powdered and passed through sieve no. 60. A small quantity of the powder was treated with phloroglucinol and conc. HCl (1:1) solution for the detection of various microscopic characters proving the authenticity of the drug. Another sample was mounted in water to see whether it contained calcium oxalate and yet another sample in an iodine solution to detect the presence of starch grains.

SUCCESSIVE SOLVENT EXTRACTION

Successive solvent extraction of the dried powder of stems of *Clerodendrum paniculatum* was carried out by using solvents of increasing polarity viz. petroleum ether, chloroform, ethyl acetate, methanol, and water. Around 20g of dried powder was weighed, moistened with the respective solvent, and packed in the soxhlet apparatus and was then extracted with 500 ml each of the petroleum ether, chloroform, ethyl acetate, methanol, and water. After each extraction, the same dried marc was used for the subsequent extraction. Each extract was then filtered, the solvent distilled off and finally, the dried extract was obtained. The percentage yield of each extract was calculated.

PHYTOCHEMICAL STUDIES

1. PRELIMINARY PHYTOCHEMICAL SCREENING OF STEM EXTRACTS

I. Chemical tests for alkaloids

A small portion of the dried alcoholic extract was shaken (acidified) with dilute hydrochloric acid and filtered. The acidified filtrate was tested with the following reagents, to detect the presence of alkaloids.

a. Mayer's Test

The acidified extract (two ml) was treated with 1 ml of Mayer's reagent (potassium mercuric iodide), shaken, and noted for the presence of a creamy precipitate.

b. Hager's Test

The acidified extract (two ml) was treated with 1 ml of Hager's reagent (saturated picric acid solution) and observed for the presence of yellow precipitate.

c. Wagner's Test

The acidified extract (two ml) was treated with a few ml of Wagner's reagent (solution of iodine in potassium iodide) and observed for the presence of a reddish-brown precipitate.

d. Dragendorff's Test

The acidified extract (two ml) was treated with a few ml of Dragendorff's reagent (Potassium bismuth iodide) and observed for the presence of orange-red precipitate.

II. Chemical test for glycosides

A small portion of the extract was hydrolyzed with dilute hydrochloric acid for a few hours in a water bath and the hydrolysate was later subjected to the following tests to detect the presence of glycosides.

a. Legal's Test

The residue (dry extract) left after evaporation was dissolved in a few milliliters of pyridine. Two milliliters of freshly prepared sodium nitro prusside solution was added to it and then made alkaline with sodium hydroxide solution. It was observed for the formation of pink red colour.

b. Baljet's Test

The few ml of the extract was treated with 1ml sodium picrate solution and a yellow to orange color reveals the presence of cardiac glycosides.

c. Liebermann Burchard's Test

The five ml of the hydrolysate taken in a test tube was evaporated, the residue taken in dry chloroform (one ml) and then it was mixed with two ml of specially distilled acetic anhydride followed by a few drops of concentrated sulphuric acid through the sides of the test tube. It was then observed for the development of a deep red color in the lower portion and green color in the upper portion which changed to blue and violet.

d. Bontrager's Test

A little of the residue obtained from the hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated to which dilute ammonia solution was added and shaken well and noted whether any pink color was present in the ammoniacal layer.

e. Modified Bontrager's Test

The residue obtained was treated with ferric chloride and dilute HCl, for the oxidative hydrolysis of C-glycoside. Then it was extracted with chloroform. The chloroform layer was separated, and dilute ammonia solution was added and shaken. The ammoniacal layer was observed for pink in colour.

III. Chemical test for phenolic compounds and tannins

a. Ferric chloride Test

A small quantity of the extract diluted with water was treated with dilute ferric chloride solution (5%) and observed for the presence of blue color.

b. Gelatin Test

The extract dissolved in water was filtered. To the filtrate, 2% solution of gelatin containing 10% sodium chloride was added. Noted for the presence of milky white precipitate.

c. Lead acetate Test

The extract dissolved in water was treated with a 10% lead acetate solution. Noted for the presence of bulky white precipitate.

d. Decolorization Test

The extract dissolved in water was treated with a dilute potassium permanganate solution. Noted for the decolorization of potassium permanganate.

IV. Chemical test for flavanones and flavonoids

a. Aqueous sodium hydroxide Test

Aqueous sodium hydroxide solution was added to the few ml of the extract and the presence of yellow coloration of the solution was noted.

b. The filter paper was wetted with a small quantity of alcoholic solution of the extract. That filter paper was exposed to ammonia vapors and noted the yellow color.

V. Chemical test for carbohydrates

A small quantity of ethanolic extract was mixed with water or alcohol and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

a. Molisch's Test.

The filtrate (two ml) was treated with a few drops of Molisch's reagent and two ml of concentrated sulphuric acid was added through the sides of the test tube without shaking. Observed for the presence of a violet ring at the junction of two solutions.

b. Benedict's Test

The filtrate (a few drops) was treated with two ml of Benedict's reagent. Then the mixture was heated in a boiling water bath for two min and the presence of red precipitate was noted.

c. Fehling's Test

The filtrate (one ml) was treated with 1 ml each of Fehling's solutions A and B and boiled in a water bath for half an hour, then observed for the presence of red residue at the bottom of the test tube.

VI. Chemical test for proteins and amino acids

a. Millon's Test

The extract (two ml) was treated with a few drops of Millon's reagent (1gm of mercury+ 9ml of fuming nitric acid) and observed for the presence of white precipitate, which on warming turned into a red-colored solution.

b. **Biuret Test**

The extract (two ml) was treated with one drop of 2% copper sulphate solution. To this 1ml of 95% ethanol was added followed by an excess of potassium hydroxide solution and Observed for the presence of violet-colored solution.

c. Ninhydrin Test

The extract (a few ml) was treated with two drops of ninhydrin solution and heated in a water bath and then the presence of violet colour was noted.

VII. Chemical test for terpenoids

a. Salkowski's Test

The extract (a few ml) was dissolved in chloroform. An equal volume of concentrated sulphuric acid was added to it and noted for the appearance of red colour in the chloroform layer and greenish-yellow fluorescence in the acid layer.

VIII. Chemical test for sterols

A small amount of the alcoholic extract was refluxed with a solution of alcoholic potassium hydroxide until saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated, and the residue was subjected to Liebermann Burchard's and Salkowski's tests

a. Liebermann-Burchard Test

The residue was taken with dry chloroform (one ml) and then it was mixed with two ml of specially distilled acetic anhydride followed by a few drops of concentrated sulphuric acid through the sides of the test tube and observed for the development of a deep red colour in the lower portion and green colour in the upper portion which changes to blue and violet.

b. Salkowski's Test

The residue was dissolved in chloroform and an equal volume of concentrated sulphuric acid was added to it and observed for the red colour in the lower layer.

IX. Chemical test for saponins

a. Foam or Froth Test

A small quantity of extract was diluted with 20 ml of distilled water in a graduated cylinder. The suspension was shaken for 15 minutes and waited to see if any froth was formed.

X. Chemical test for gums or mucilage

To 10 ml aqueous extract of the plant, 25 ml of absolute alcohol was added with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

RESULTS AND DISCUSSION

PLANT COLLECTION AND AUTHENTICATION

The stems of the plant *Clerodendrum paniculatum* (Lamiaceae) were collected from Kannur. The plant material was taxonomically identified by the botanist, Dr Biju P, Dept. of Botany, Government College, Kasaragod, and dried under shade and powdered.

PHARMACOGNOSTIC STUDIES

1. Determination of foreign matter, moisture content, ash value, extractive value

CHARACTERS	**%w/w**
Foreign matter	1.82
Moisture content	9.33
Total ash value	7.33
Acid insoluble ash value	1.50
Water soluble ash value	3.00
Alcohol soluble extractive value	12.8
Water soluble extractive value	6.33

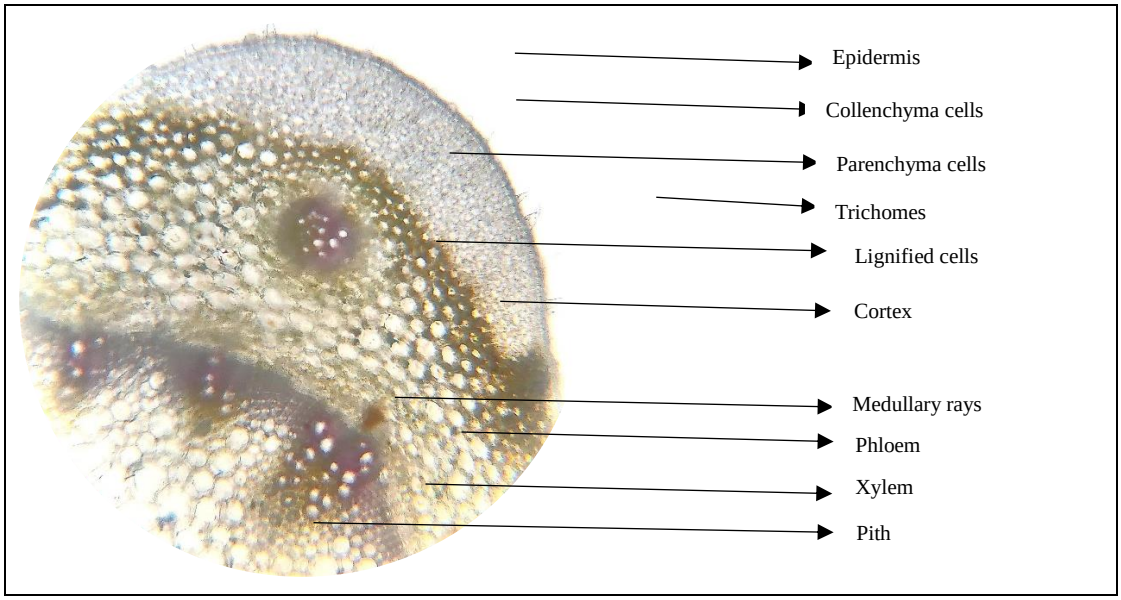
2. Organoleptic evaluation of stem

The organoleptic evaluation was done using organs of sense. This included evaluating the drug by colour, odour, size, shape, taste, and special features including touch, texture etc. The results are presented in the table below.

Macroscopic features	
Colour	Green
Odour	Odourless
Taste	Bitter
Size	4-7cm in width 8-10 cm in length
Shape	Hollow, slightly pubescent, obtusely quadrangular, nodes annulate, slightly hairy

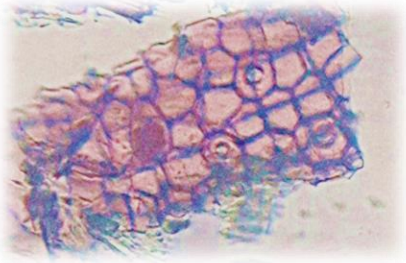
3. Microscopic evaluation

- **Transverse section of stem**

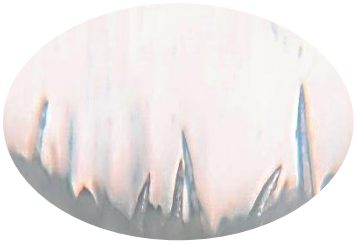


- **Powder microscopy of stem**

Collenchyma cells

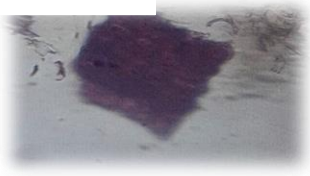


Xylem



Trichomes

Phloem



Lignified fibres

SUCCESSIVE SOLVENT EXTRACTION

The coarse powder of stems of the *Clerodendrum paniculatum* was subjected to successive solvent extraction using a soxhlet apparatus. After extraction, the percentage yield of each extract was calculated based on the air-dried drug used for the study.

Sl. no	Solvent used for extraction	Colour	Consistency	Percentage yield (%w/w)
1	Petroleum ether	Light green	Semisolid	2.8
2	Chloroform	Dark green	Semisolid	16.73
3	Ethyl acetate	Light green	Semisolid	1.73
4	Methanol	Dark green	Semisolid	13.6
5	Water	Brown	Powder	4.86

PHYTOCHEMICAL SCREENING

Phytoconstituents	Pet. ether	Chloroform	Ethyl acetate	Methanol	Water
Alkaloids	-	++	+	++	+
Glycosides	+	++	++	+	++
Phenols	+	++	++	++	++
Flavonoids	-	+	+	+	-
Carbohydrates	-	++	++	++	++
Proteins & amino acids	-	+	+	+	-
Terpenoids	-	-	-	-	+
Sterols	-	-	-	-	+
Saponins	-	-	-	-	++
Gums & mucilage	+	+	+	+	+

SUMMARY AND CONCLUSION

Clerodendrum paniculatum plays a significant role in traditional medicine across various countries, including China, India, Japan, Korea, and Thailand. The genus encompasses a wide variety of plants, comprising herbs, shrubs, and small trees, and is highly valued for its decorative purposes. With approximately 580 identified species distributed globally, Clerodendrum is known for its diversity. To accurately identify the plant, a comprehensive examination of its pharmacognostic properties is carried out, which involves the determination of foreign matter, assessment of moisture content, ash value, extractive value, and both macroscopic and microscopic evaluations. Notably, the phytochemical screening of *Clerodendrum paniculatum* has revealed the presence of various compounds such as alkaloids, glycosides, carbohydrates, flavonoids, saponins, tannins, proteins, and phenols in the stems. These findings highlight the potential of *Clerodendrum paniculatum* in the development of novel medicinal drugs.

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