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Phytochemical and pharmacognostical studies of leaves of *Clerodendrum inerme*

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ABSTRACT

Clerodendrum inerme belonging to family Verbenaceae is an important traditional medicinal plant used in the treatment of various ailments like antibacterial, hepatoprotective, anticarcinogenic, uterine and intestine stimulating properties. However, detailed scientific information is not available to identify the plant material, in order to ascertain its quality and purity. In this paper, we report the pharmacognostic, macroscopy, microscopy, physicochemical parameters such as moisture content, ash values, extractive values, and preliminary phytochemical analysis of the leaf were investigated. Each extract was subjected to qualitative tests for identification of various constituents like alkaloids, carbohydrates, glycosides, anthraquinone glycosides, steroids, saponins, flavonoids, tannins and phenolic compounds and proteins. The present study will be useful for its identification prior to carrying out further research work. The findings of this study will facilitate pharmacognostic standardization of the plant material and aid in the preparation of a herbal monograph for the species.

Keywords: Macroscopy, Microscopy, Physicochemical parameters, Preliminary phytochemical analysis.

INTRODUCTION

During last decade there has been an exponential growth in use of herbal products for treatment of various types of diseases [1]. In general, treatment involving herbal drugs spans a long duration of time. In contrast to general old age myth that herbal drugs are safe and do not have toxic effects, These drugs may cause some moderate to severe side effects due to complex nature of their chemical compositions. Hence, there is a need to establish safety to herbal drugs. *Clerodendrum inerme* belonging to family Verbenaceae has been used as antidiabetic agent in folklore medicinal system of India. It is reported to have antibacterial, hepatoprotective, anticarcinogenic, uterine and intestine stimulating properties [2]. The various constituents characterised in its leaves include phenylethanoid glycoside, neo-clerodane diterpenoids antiviral proteins (CIP-29 and CIP-34) and three iridoid glucoside (Inerminoside A1, C and D[3,4]. However, its medicinal uses are not reported widely and its constituents are being investigated for herbicidal properties and for potential in human medicine.

MATERIALS AND METHODS

Plant material, chemicals and solvents:

The leaves of *Clerodendrum inerme* were collected from healthy plants in Sonipat during July 2009. The leaves were authenticated by Dr. H.B. Singh (Scientist F and Head Raw Materials Herbarium and Museum, NISCAIR, Delhi) with a Voucher number- Niscair/RHMD/Consult/-2009-10/1241/45.

The chemicals and solvents required for chemical evaluation and extracts were procured commercially from s.d. fine chemicals (Mumbai, India) and C.D.H. Private Limited, (New Delhi, India). Soxhlet extraction assembly of 2L

capacity (Borosil, New Delhi, India) was used for extraction of plant material. The extracts were concentrated on rotary vacuum evaporator (Hi - Con, New Delhi, India)

Pharmacognostical Evaluation:**Macroscopic Examinations:**

The fresh leaves were examined for appearance, odour, taste, color, surface characteristics, texture and fracture using standards methods. To study colour, the fresh leaves were examined under diffused day light. To study texture and surface fracture characteristics, untreated sample was examined under a magnifying lens (10x). Wetting with water or reagents was necessary to observe the characteristics of a cut surface. Material was touched to determine whether it is soft or hard. It was bent and ruptured to obtain information on its brittleness and the appearance of fracture plane, whether it was fibrous, smooth, rough, granular, etc. To identify odour a small portion of sample was placed in palm and rubbed slowly and repeatedly. The air present above the material was inhaled. [5].

Microscopic Examinations:

The fresh leaf of the plant was examined under microscope to establish its microscopic characteristics. The leaf was cut into thin sections with the help of a sharp blade. The sections were cleared with chloral hydrate solution and stained with various reagents, (phloroglucinol and HCl) and mounted in 158lycerine. The photographs of sections were taken under varied magnifications (10x, 45x and 100x) for the identification of various regions [6].

Physico chemical parameters:

Various physicochemical parameters like ash values (total ash value, water-soluble ash value, acid insoluble ash value), moisture content, crude fibre content, volatile oil content and foaming index were evaluated using standard methods [7].

Extraction of plant material:

The leaves were dried at room temperature under well-ventilated shade by spreading them uniformly. The dried leaves were sorted, powdered, weighed and subjected to successive solvent extraction with different solvents viz. Petroleum ether (60-80°C), chloroform, ethyl acetate, ethanol and water in soxhlet apparatus. Each extract was dried under vacuum and percentage yield was calculated as % w/w w.r.t. total weight of dried leaves taken for extraction [8].

Phytochemical evaluation:

Each extract was subjected to qualitative tests for identification of various constituents like alkaloids, carbohydrates, glycosides, anthraquinone glycosides, steroids, saponins, flavonoids, tannins and phenolic compounds and proteins.

Detection of alkaloids:

To a small portion of extracts, few drops of dilute HCl were added separately and filtered. The filtrates were tested with various reagents such as Mayer's, Dragandroff's, Hager's, and Wagner's to detect the presence of alkaloids.

Detection of carbohydrates:

Small quantities of extracts were subjected to Molish's test, Fehling's test, Benedict's test and Iodine test.

Detection of glycosides:

To the extracts few drops of dilute HCl were added and heated on water-bath for hydrolysis and extracts were separately subjected to Legal's tests, Borntrager test to detect the presence of glycosides.

Detection of anthraquinone glycosides:

To the extracts few drops of ferric chloride solution and dilute HCl were added, heated on water-bath for hydrolysis. The content were filtered, few ml of chloroform were added to the filtrate and shaken well. The organic layer was separated and a few drops of ammonia solution was added to it and shaken slightly. The test tube was kept aside, lower organic layer showing pink colour indicated the presence of anthraquinone glycosides [9].

Detection of steroids:

Small amounts of extracts were subjected to Salkowaski test. For this, extract was mixed with equal amount of chloroform and conc. sulphuric acid. Chloroform layer appear red while acid layer showed greenish yellow fluorescence.

Detection of saponins:

Small amount of extracts were shaken with water to check foam formation and its time of stability.

Detection of flavonoids:

Extract was subjected to Shinoda test. Treatment of extract with conc. sulphuric acid gives yellow orange colour and ferric chloride test, colour changes from green to black.

Detection of tannins and phenolic compounds:

Small amount of extract was mixed with few drops of freshly prepared 5% FeCl₃ solution; deep blue-black color indicated the presence of phenolic compounds. Small amount of extracts mixed with lead acetate solution, formation of white ppt. indicated the presence of phenolic compounds [10,11].

Detection of proteins:

Small amount of extract was treated with Biuret reagent for the protein detection [12].

RESULTS AND DISCUSSION**Pharmacognostical evaluation:****Macroscopic examination:**

The leaves were 5-6 cm long, 1.5-2.5 cm wide, green in colour, bitter in taste with characteristically pleasant odour, Shape was elliptical, apex rounded, margin entire, base rounded, lamina dark green, veins reticulate and texture smooth and shining.

Physicochemical parameters:**Determination of Ash values:**

The total ash, water soluble ash and acid insoluble ash values of the dried leaves were found to be 11.3, 5.21 and 4.36 %w/w, respectively. Crude fibre content and moisture content of dried leaves were found to be 16.5 and 4.9 % w/w respectively. Extractive values of dried powdered leaves were determined in different solvents. The ethanol soluble and water soluble components were found to be 26.4 and 10.8 %w/w respectively. Foaming index was upto 1000 while no volatile oil was obtained from fresh leaves.

Phytochemical Investigations:

The yield and characteristic appearance of different successive extracts of dried powdered leaves are given in Table 1.

Table 1 : Successive extracts of leaves of *Clerodendrum inerme*

S. No.	Extract	Yield(%w/w)	Characteristic
1.	Pet. ether	2.96	Yellowish brown
2.	Chloroform	3.81	Greenish black
3.	Ethyl acetate	2.1	Slightly greenish black
4.	Ethanol	22.8	Dark brown
5.	Aqueous	10.12	Dark brown

The qualitative chemical examination showed the presence of alkaloids, carbohydrates, glycoside, anthraquinone glycoside, steroids, flavonoids and saponins. The main active compound glycosides and different flavanoids were present in ethanol and aqueous extract. (Table 2)

Table 2 : Phytochemical screening of the extracts

S. No.	Chemical Constituents	Petroleum ether extract	Chloroform Extract	Ethyl acetate extract	ethanol extract	Aqueous Extract
1.	Alkaloids	-ve	+ve	-ve	+ve	-ve
2.	Carbohydrates	-ve	-ve	-ve	+ve	+ve
3.	Glycosides	-ve	-ve	-ve	+ve	+ve
4.	Anthraquinone Glycosides	-ve	-ve	-ve	+ve	+ve
5.	Steroids	-ve	+ve	+ve	+ve	+ve
6.	Flavonoids	+ve	+ve	+ve	+ve	+ve
7.	Saponins	-ve	-ve	-ve	+ve	+ve
8.	Free amino acids	-ve	-ve	-ve	-ve	-ve
9.	Phenolics & Tannins	-ve	-ve	-ve	-ve	-ve
10.	Starch	-ve	-ve	-ve	-ve	-ve

+ve- Present, -ve- Absent

Macroscopic examination of leaves of *Clerodendrum inerme*

S.No.	Parameters	Inference
1.	Color	Green
2.	Taste	Bitter
3.	Odors	Characteristics
4.	Shape	Elliptical
5.	Apex	Acute
6.	Margin	Entire
7.	Base	Rounded
8.	Lamina	Dark green
9.	Veins	Reticulate
10.	Texture	Smooth and shining
11.	Length	4-5cm
12.	Breadth	0.5-1cm

Determination of Physicochemical constants**Crude fibre and moisture content**

S. No.	Particulars	Leaf(%w/w)
1.	Crude fibre content	16.5
2.	Moisture content	4.9

Determination of Ash Values

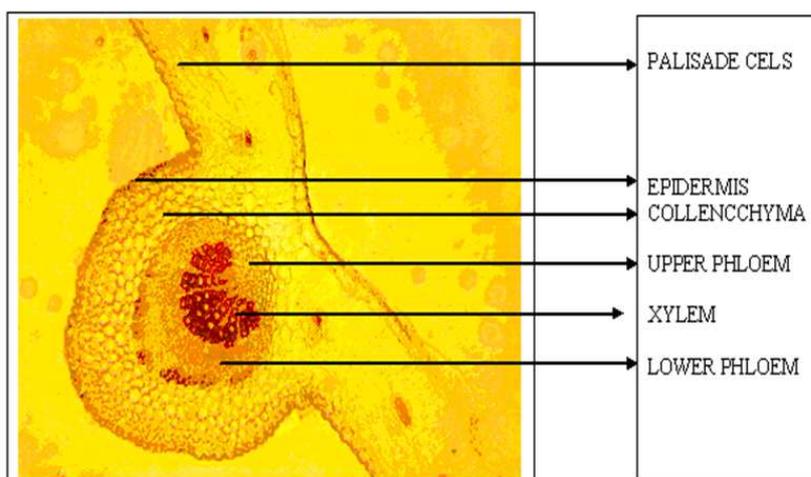
S.No.	Particulars	Ash values of Leaf(%w/w)
1.	Total ash	11.3
2.	Water soluble ash	5.21
3.	Acid insoluble ash	4.36

Extractive Values

S.No.	Solvents	Extractive values of Leaf(%w/w)
1.	Ethanol	26.4
2.	Aqueous	10.8

Foaming Index and Volatile oil content

S.No.	Particulars	Result of Leaf
1.	Foaming index	Upto 1000
2.	Volatile oil content	Nil

Microscopic Examination of Leaf of *Clerodendrum inerme*(10X)**Fig.1 TS at 10X**

CONCLUSION

With the tremendous expansion in the use of traditional medicine worldwide, safety and efficacy as well as quality control of herbal medicines has become a matter of most importance. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and degree of purity and should be carried out before any other tests are undertaken. In this context the study was undertaken to set the standardized parameters for the establishment of standard quality of the leaves of *C. inerme*. Evaluation of physicochemical parameters is an important part in the preparation of modern monograph. Thus ash, extractive values, moisture content and fluorescence study determined here signifies standard parameters to ensure the quality and purity of the crude drug. The phytochemical analysis of different extracts revealed the presence of alkaloids, steroids, tannins and phenolic compounds, flavonoids and terpenoids.

The phytochemical findings of the study confirm the presence of plant phenolics, flavonoids and other secondary metabolites which are currently of growing interest owing to their functional properties in promoting human health [13]. Flavonoids and other plant phenolics act as remedies in the treatment of stress-related ailments and as dressings for wounds, cuts, rheumatism etc. [14]. The diagnostic features established here will help in quality control and authentication of the drug. Further, this investigation will be beneficial enough firstly, to identify the plant in its crude form and secondly to arouse research interest among the researchers to elucidate the pharmacological activities supported with possible mode of action.

The pharmacognostical, microscopical and physicochemical parameters of leaves of *Clerodendrum inerme* were studied. The leaves were successively extracted and phytochemical investigation on each extract were performed.

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