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PHARMACOGNOSTICAL AND PHYTOCHEMICAL SCREENING OF ROOT EXTRACTS OF CLERODENDRON SERRATUM (Linn.)

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ABSTRACT

The present research work is mainly based on pharmacognostical investigation and phytochemical screening of root extracts of *Clerodendron serratum*. The pharmacognostical and phytochemical screening is done by preparing different extracts of the following roots of the plant with the help of different solvents such as chloroform, aqueous, ethanol, acetone, petroleum ether, benzene and ethyl acetate. Analysis of the root is done according to their sensory, microscopic and macroscopic characteristics. Majority of the information is drawn such as purity and quality by these observations. The phytochemical screening implicates the presence of different constituents as secondary metabolites. Secondary metabolites present in the root are identified by general chemical tests. From the above research work carried out it can be implicated that the flora should be explored still further for different secondary metabolites and non adulterated forms of the roots and other potential compounds. The natural system of medicine is gaining passion due to their fewer side effects than that of traditional medicines. Different species of *Clerodendron serratum* should be explored for different versatile pharmacological activities which improve the health care system of mankind.

KEYWORDS: *Clerodendron serratum*, Phytochemical Screening, extracts, microscopic, macroscopic characterization.

INTRODUCTION

Clerodendrum serratum (L.) Moon. belongs to the family Verbenaceae[1] is an important traditional plant with lots of medicinal values growing in the tropical and temperate parts of the world such as Asia, Malaysia, Africa, India, Nepal and Sri Lanka.[2] It is extensively distributed in many parts of India. There are many species varying about 450 species.[3] It is featured for many pharmacological values in the treatment of pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever in India with a long history.[4] Scientific studies on extracts and formulations revealed anti-asthmatic, mast cell stabilization and anti-allergic effects of roots of *C. serratum.*[5] *C. serratum* shows many traditional and ethno medicinal uses, phytochemistry and biological activities. Clinical and toxicity data were also present which makes the future researcher to concentrate on this wonder plant.

According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs.[6] About 80% of individuals from developed countries use traditional medicine, which has compound derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicinal compound; have continued to play a dominant role in the maintenance of human health, since ancient times. Over 50% of all modern clinical drugs are of natural product origin and it also plays an important role in drug development programs in the pharmaceutical industry.[7] Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This made worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural product in health care. Most of the drugs derived from plants were developed because of their use in traditional medicine. India's use of plants for health care dates back close to 5000 years.

Preliminary chemical examination of the water soluble constituents of the root bark of Clerodendron serratum(Linn) moon(family: verbenaceae), commonly known as bharangi, was reported.[8] The aqueous was found to be containing free glucose and glycosidic principle in addition to a considerable amount of manitol.[9] Isolation at mannitol from the root bark of *Clerodendrun serratum* and reported that, it can be used as new source of mannitol having 10% of yield.[10,11] Examination of petroleum ether extract of the root bark of C. serratum(Linn) Moon the pet ether extract was found be contain gamma a-sitosterol which

was established by mixing melting point determination with an authentic specimen.[12,13] Ethanol extract of C serratum showing antinociceptive, antiinflammatory and antipyretic effects Ant allergic the activity of C serrarum herbal formulation.[14] Formulations of *Ceredendron serratum* manufactured are more than 10 polyherbal formulations.

PLAN AND OBJECTIVE

The design and objective of the present research work concentrates on the identification of pharmacognostical and phytochemical constituents of different extracts of *Clerodendron serratum*.

PHARMACOGNOSTICAL STUDY

MATERIALS AND METHODS

Materials Used

Compound microscope, Camera lucida, Black drawing sheet, Leica DMLS microscope attached with Leitz MPS 32 camera, Whatmann filter paper 44, UV apparatus, Phloroglucinol, hydrochloric acid and different solvents such as petroleum ether, Benzene, Chloroform, Acetone, Ethanol and water for extraction.

Collection and authentication of plant material

The roots of the plant, *Clerodendron serratum* growing in the local areas of manipal university of Karnataka state were collected during the month of September-October. It was identified and authenticated by Dr. S.B.Padal, Dept. of Botany, Andhra University and Sample specimen was kept in our laboratory for future reference. Plant material was freed first to remove all the dust particles and unwanted material then it was washed thoroughly, initially with tap water and then with distilled water and then allowed to dry in shade. The dried plant material was made into fine powder and stored at room temperature in air tight container until used further. Iodine water was used to locate the starch grains that gave blue black color.

Plant Description

Plant	*Clerodendron serratum*
Family	Verbenaceae
Synonyms	
Sanskrit	Bharangi, Angaravallari, Bhargi, Kasaghi
Hindi	Barangi
Kannada	Gantubarangi, Kiriteggi
Tamil	Angarangi, Kandubarangi, Sirudekku
Telugu	Barangi,Chiruteka, Panja

Formulations of *Clerodendron serratum*

1. Bharangirasayanam
2. Nochitailam
3. Kaphasurakkudineer
4. Vasthasurakkudineer
5. Rasaganthimeahugu
6. Sarabungavilvathiezhagam
7. Sirutekkukudinner
8. Katankatthirikudinner
9. Thontasurakiyazham
10. Thalisapatthirichoornam.

Description of the root

S.No	Description part	Identifications
1	Shape	Woody, cylindrical. The crown is knotty and much more border than the immediate lower
2	Size	30cm long, 2-3 cm wide at the upper end
3	Colour	Earthy brown in colour
4	Surface	Longitudinally wrinkled, furrowed and ridged. T.S showd dark round bark. Yellowish brown wood with wavy striations.
5	Fracture	Root breaks with difficulty and fracture is short and starchy
6	Odour and taste	Hardly any perceptible odour and slightly acrid taste.

Histology

T.S of the root shows the following

Peiderm	Phellum	Stratified, alternate bands of large and small bands. Large cells are radially arranged and thin walled, unlignified with brown content
	Phellogen	Indistinct, narrow layer of thin walled cells.
	Phelloderm	Consist of 5-6 layers of thick parenchyma with scattered sclereids with isolated or in groups. Parenchymatous cells consists of acicular raphides and starch grains.
Secondary Phloem	It is widening of primary rays, consists of phloem parenchyma. Phloem parenchyma consists of numerous starch grains and acicular crystals of calcium oxalate.	
Secondary Xylem	Well developed medullary rays of 1-4 cells width. Xylem vessels are round and radially elongated, occurs either in single or in pairs. Starch grains are present in xylem parenchyma and xylem ray cells.	

Powder Analysis

The roots of the plant were examined for their macroscopic characters. The roots are boiled with chloral hydrate to remove the coloring matter and mounted on glass plate and covered with cover slip, then the powder is stained with phloroglucinol in the presence of hydrochloric acid for identification of lignified structures.

Wood elements	Vessels are lignified, with numerous bordered pits.
Cork cells	Cork is of several layers of parenchymatous cells which apper like benzene rings
Stone cells	They appear oval to quadriatic, evenly thickened lignified cellswith simple pits.
Fibres	Thin, narrow and isolated bast fibres
Brown matter	Abundent yellowish brown masses
Starch grains	Consist of granules large in size, Hilum though indistinct, may be seen as cleft
Parenchyma	Starch grains and acicular raphides are seen . Xylem rays and Xylem parenchyma with lignified walls showing simple pits.

Ash value

Total Ash: About two grams of the powder was weighed and spread as a fine layer at the bottom f the tare weight calculated silica crucible. The crucible was incinerated at temperature not exceeding 600^0C until free from carbon. The crucible was cooled and weighed. Entire procedure was repeated until constant weight observed. The percentage of the total ash was calculated with reference to the weight of the air dried.

Acid insoluble ash: The ash obtained in total ash was boiled with 25ml of HCl for 5 minutes. The insoluble ash was collected on filter paper and washed with hot water. The insoluble ash was then transferred to a tare weight calculated silica cruicible and it was incinerated at temperature not exceeding 600^0C until free from carbon. The crucible was cooled and weighed. Entire procedure was repeated until constant weight observed. The percentage of the total ash was calculated with reference to the weight of the air dried.

Water soluble ash: The ash obtained from the total ash was boiled with 25 ml hot water for 5 minutes and filtered on ashless filter paper, washed with hot water. The insoluble ash was then transferred to a tare weight calculated silica cruicible and it was incinerated at temperature not exceeding 600^0C until free from carbon. The crucible was cooled and weighed. Entire procedure was repeated until constant weight observed. The weight of the

insoluble matter was subtracted from the weight of the total ash. The difference in the weight is considered as water soluble ash.

Total Ash	3.47%(w/w)
Acid insoluble ash	1.123%(w/w)
Water soluble ash	1.03 %(w/w)

Extractive Values

Hot percolation: 5g of the drug was taken in a conical flask and added with 100 ml of water. The mixture was gentle stirred and warmed in he water bath for 30 minutes. The solution was shaked gently at regular intervals. The solution was taken out from the water bath, cooled and filtered with cotton plug. 25ml of the filtrate was taken and evaporated o dryness. The amount of the extract was weighed and multiplied by 4 to get the actual weight.

Cold maceration: Cold maceration a Ethanol soluble extraction: 5 g of previously weighed air dried drug was taken in a stoppered flask and 100 ml of 95 ethyl alcohol was added to it. It was shaken continuously for on an electric shaker. It was then filtered rapidly 4 hours taking precautions against loss of solvent. 50 ml of the filtrate was evaporated to dryness in a tared flat bottomed petridish, dried at 105o c and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug.

Water soluble extractive: 5 g of previously weighed air dried drug was taken in a stopper flask and 100 ml of chloroform water was added to it was shaken continuously for 4 hours on an electric shaker. It was then filtered rapidly taking precautions against loss of solvent. 50 ml of the filtrate was evaporated to dryness in a tared flat bottomed petri dish, dried at 105 C and weighed The percentage of water soluble extractive was calculated with reference to the air dried drug.

Non volaille Ether soluble extractive: 5 g of previously weighed air dried drug was taken in a stoppered flask and 100 ml of ether was added to it ft was shaken continuously for 4 hours on an electric shaker was then filtered rapidly taking precautions against loss of solvent, 50 ml of the filtrate was evaporated to dryness in a tared flat bottomed petri dish, dried at 105"Cand weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Hot maceration	27.92%(w/w)	
Cold maceration	Water soluble extractive	21.12%(w/w)
	Ethanol soluble extractive	18.32%(w/w)
	Non volatile ether soluble extractive	3.28%(w/w)

Foreign Organic Matter (FOM): It is the material consisting of any or all of the following

a) Parts of the organ or organs from which the drug is derived other than dry parts named in the definition and description or for which the limit is prescribed in the individual monograph

b) Any organs other than those named in the definition and description,

c) Matter not coming from the source plant and

d) Moulds, insects or other animal contamination.

Method: 500 g of the original sample was spread in a thin layer The sample was inspected with the unaided eye or with the use of a 6X lens and the foreign organic matter separated manually as completely as possible, The percentage of FOM from the weight of the drug taken was weighed and determined.

Foreign organic matter of 100g leaves: 0.090%(w/w)

Volatile matter/ Moisture content or water content: 14.085%(w/w)

Bitterness value: NIL

Foaming Index : Less than 100

Swelling index of leaf powder : 11.0

Fluorescence Analysis

The powder is treated with different solvents in different test tubes. Then they were subjected to fluorescence analysis in the daylight and in UV light.

S.No	Treatement	White light	UV Light (254nm)
1	Powder	Greyish green	Greenish black
2	Powder + 1N NaOH(aqueous)	Light brown	Fluorescent yellow
3	Powder + 1N NaOH(alcoholic)	Fluorescent yellow	Fluorescent green
4	Powder + 1N HCl	Dark brown	Brownish black
5	Powder + 50% H_2SO_4	Greenish yellow	Fluorescent green

Preparation of Plant extracts

To 1Kg of *Clerodendron serratum* root powder, solvents such as petroleum ether, Benzene, Chloroform, Acetone, Ethanol and water are used in about 2 liters in capacity for extraction

of different chemical constituents from the plant material in increasing solvent polarity. (flow chart-1). Extraction with the solvent was done for one day at 27^0C, after maceration the supernatant of each solvent was recovered by filtering through whatmann filter paper. This process was repeated thrice and the respective solvent from the supernatant was distilled off to obtain crude extracts which are to be stored at 4^0c until used for evaluation. Preparation of the extract is well illustrated in the Fig I. Phytochemical investigation tests are well illustrated in the table I.

The codes are as follows

CE – Chloroform extract of *Clerodendron serratum*

PE- Petroleum ether extract of *Clerodendron serratum*

BE- Benzene extract of *Clerodendron serratum*

WE- Water extract of *Clerodendron serratum*

AE- Acetone extract of *Clerodendron serratum*

EE- Ethanolic extract of *Clerodendron serratum*

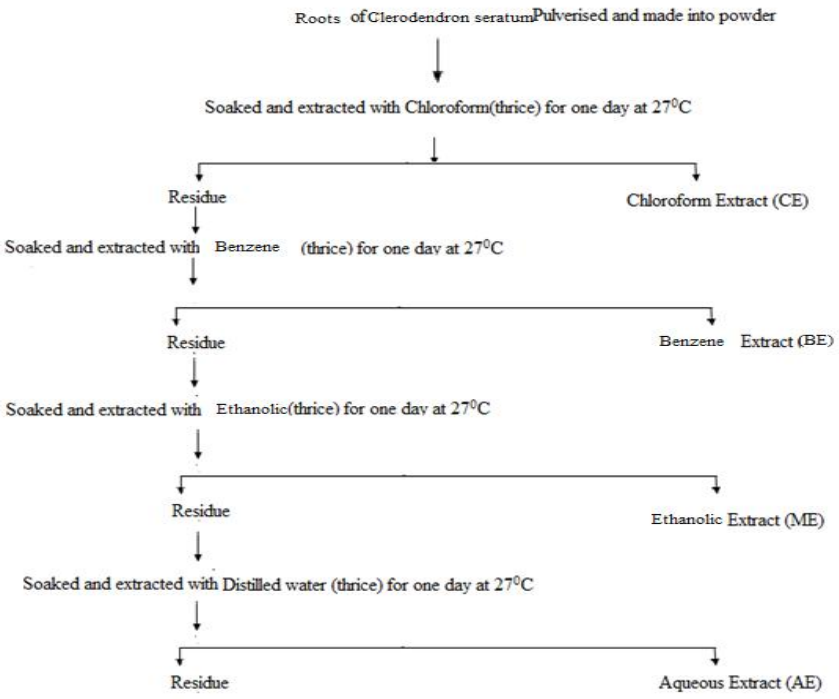

Fig I Schematic representation of showing extraction procedure from roots of *Clerodendron serratum*.

Table I: Tests carried out for Preliminary Phytochemical Screening of the extracts

S.No	Name of the test	Procedure
1	Mayer's test (for Alkaloids)	2ml of plant extract was taken and to it 2ml of concentrated HCl and Mayer's reagent were added. Green color or white precipitate indicates presence of Alkaloids.
2	Keller-killiani test (for Cardiac glycosides)	0.5 g of extract was added with 5 ml of water, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.
3	Ferric chloride test (for Flavonoids)	About 0.5g of each extract was boiled with 5 ml of distilled water and then filtered. To 2 ml of this filtrate, a few drops of 10% ferric chloride solution was added. A green-blue or violet coloration indicated the presence of a phenolic hydroxyl group.
4	Xanthoproteic test (for Proteins)	The extract (few mg) was dissolved in 2 ml water and then 0.5 ml of conc. HNO3 was added in it. Yellow color indicated the presence of proteins.
5	Ferric chloride reagent test (for Tannins)	The test sample of each extract was taken separately in water, warmed and filtered. To a small volume of this filtrate, a few drops of 5 % w/v solution of ferric chloride prepared in 90 % alcohol were added. Appearance of a dark green or deep blue color indicated the presence of tannins.
6	Salkowaski test (for Sterols and Phenols)	A few milligrams of the plant extract was dissolved in 2 ml chloroform and then 2 ml of conc. H_2SO_4 was added from the sides of the test tube. The test tube was shaken for a few minutes. Red colour development in the chloroform layer indicated the presence of sterols.
7	Foam test (for Saponins)	0.5 gram of each extract was boiled with 5 ml of distilled water and filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins.
8	Salkowaski test (for terpenoids)	To 0.5 g of each extract, 2 ml of chloroform was added, followed by a further addition of 3ml of concentrated H_2SO_4 to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids
9	Fehling's solution test (for Reducing sugars)	About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.
10	Anthraquinone	An aliquot of 0.5 g of the extract was boiled with 10 ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

S.No	Phytochemical constituent	Petroleum ether	Chloroform	Benzene	Methanol	Ethanol	Aqueous
1	Alkaloids	-	-	-	-	+	+
2	Cardiac glycosides	-	+	-	-	-	-
3	Flavonoids	-		-	+	+	+
4	Proteins	-		-	-	-	+
5	Tannins	-	-	-	-	-	-
6	Terpenoids	-	-	+	-	-	-
7	Saponins	-	-	+	+	+	+
8	Sterols	+	+	-	+	+	+
9	Sugars	-	-	-	-	-	+
10	Anthraquinones	+	-	-	-	+	+

Table III: Results of Preliminary Phytochemical analysis of root extract of *Clerodendron serratum***.**

+ = slightly Presence.

++= moderately present.

+++ = Significantly present.

RESULTS AND DISCUSSIONS

Different macroscopic and microscopic investigations are carried out on the plant *Clerodendron serratum***.** The above results show different pharmacognostical exploration and phytochemical exploration of the plant. The standard values of the plant help the future researchers to identify the adulterated forms of the plant. From the above results one can appraise that the compounds which are having high lipid solubility may be responsible for their pharmacological action. The secondary metabolites mainly the phenols, sterols, flavonoids, reducing sugars which are identified in the phytochemical investigation may be responsible for the activity against bacterial or mycobacterium. The potency may be attributed to the penetration coefficient of the secondary metabolites and the possible mechanism yet to be implicated.

CONCLUSIONS

From the present study it is clearly shows that the use of this plant from the supporting data of phytochemical investigation. The study is responsible for determining the adulterants and differenciation of different species. The secondary metabolites which are investigated may be responsible for pharmacological activity. Further the extract should be processed and different compounds are to be isolated and carried out treatment of different hereditary diseases.

FUTURE SCOPE

Traditional systems of medicines are mostly preferred due to their fewer side effects and compatibility to the biological system. So future researcher has to take necessary steps to explore the flora in different parts of the nation and produce comparatively potent drugs such as conventional drugs.

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