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STANDARDIZATION OF A MEDICINAL PLANT - CLERODENDRUM INERME

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ABSTRACT

From the ages plants have been used for health and medical purposes for treating various diseases which affects the human population. A survey conducted by World Health Organization (WHO) indicated that about 70-80% of the world's population rely on non-conventional medicine, mainly of herbal sources, in their primary health-care as their side effects are less or they are at minimum risk due to their active components obtained from the source. The plant *Clerodenrum inerme* (Verbenaceae) has been used in Traditional System of Medicine to treat various ailments like malaria, anti-inflammatory, hepatoprotective, antiviral etc. Thus to explore the plant standardization of plant becomes an utmost need for its scientific validation. This paper will emphasize on the various standardization parameters required for its authentication and validation by various methods like Pharmacognostical studies, physicochemical parameters, extractive values, Ash values swelling factor, fluorescence analysis and phytochemical studies. Thus these findings will be beneficial in identifying the genuine drug and adulterated drug.

KEYWORDS

Extraction, Macroscopic, Microscopical Features, Phytochemical screening

INTRODUCTION

Plants have been used for health and medical purposes for several thousands of years. The number of higher plant species on earth is about 250,000. It is estimated that 35,000-70,000 species have, at one time or another, been used in some cultures for medicinal purposes. Plants are the essential and integral part in complementary and alternative medicine due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases(Harvey et al., 2000). The plant *Clerodenrum inerme* (Verbenaceae) has been used in folklore for treating various diseases like inflammation, antiviral, hepatoprotective, antimalarial febrifuge and many more. As per the literature survey it has been observed that no systemic approach has been made to study the Standardization, preliminary phytochemical and its biological activity on the leaf and the stem part of the plant. Moreover for sporadic references not much work has been done on stem of this plant to establish its biological activity. Therefore, it was thought worthwhile to explore the plant on the above aspects with the help of modern analysis techniques as per WHO guidelines. Thus in the present investigation our main aim was to standardize the drug on the basis of pharmacognostical and phytochemical evaluation which includes morphological and microscopical studies,

quantitative determination of leaf constants, Fluorescence Analysis, various physiochemical parameters and phytochemical analysis.

Materials and Methods

Plant Materials:

The fresh leaves and stem of plant specimens for the purposed study were collected from wild source of Greater Noida, Uttar Pradesh, in month of February. The plant *Clerodendrum inerme* Linn. (Verbenaceae) was identified and authenticated from PUSA, New Delhi bearing the authentication number :NHCP/NBPGR/2012-40. The fresh dried fine powder of the plant part was used for the preparation of extracts.

A.Pharmacognostical Evaluation of the Crude Drug:

a) Macroscopical Evaluation of Leaf.

Macroscopical studies were done by using simple visualizing agent (naked eye) to determine the color, odour, margin, veinneation, apex and taste of the leaf.

The macroscopically studies of leaf revealed certain characters:

Color: Emerald green (upper surface), Light green (lower surface)

Shape: Lanceolate

Surface: Smooth on both surface

Taste: Characteristic

Odour: Characteristic to odourless

Size: 4.2 cm(length), 2.2 cm(breadth)

Margin: Entire

Vennation: Alternate to opposite

Base: Symmetrical

Apex: Acute

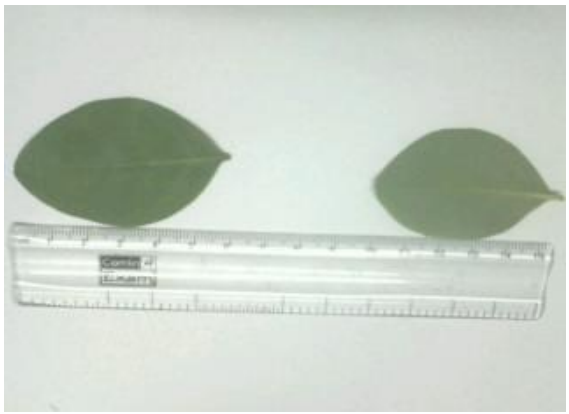


Fig 1: Leaves of *Clerodendrum inerme*

b. Microscopical Evaluation of leaf

Microscopic studies were done by preparing a thin handmade section of the plant part (leaf) region of *C. inerme*. The section was cleared with chloral hydrate solution and was stained as per the protocol. Histochemical reactions were applied with concentrated Hydrochloric acid and phloroglucinol and were mounted in glycerin for the identification of starch grains, ruthenium red for mucilage, 60% sulphuric acid for calcium oxalate crystals and ferric chloride for the phenolic compounds.

The Transverse section of leaf was distinguished in to two regions lamina and midrib.

Lamina region: The leaf represents a typical dorsi-ventral leaf. The lamina region consists of upper and lower epidermis with the presence of cuticle. It consists of palisade cells which are long, elongated and columnar. The mesophyll region consists of spongy parenchyma cells and presence of calcium oxalate crystals. It also showed the presence of "covering glandular trichomes" which were uniseriate, multicellular in nature. Stomata were anisocytic in nature.

Midrib region: this region consists of xylem and phloem with a arc depression at the centre. It consists of collenchyma and parenchyma cells which are located at the top and bottom of the vascular tissues.

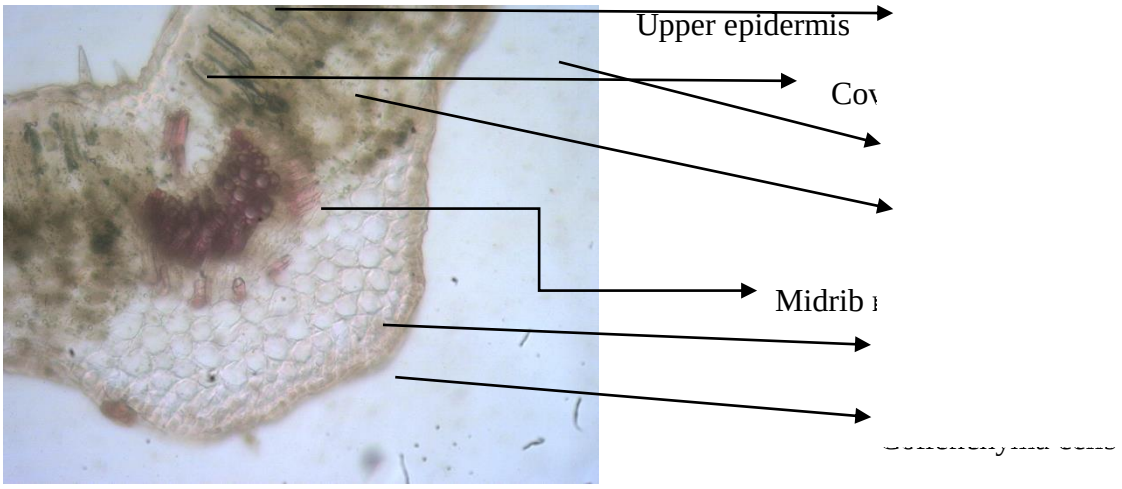


Fig 6: Transverse section of leaf of *Clerodendrum inerme*

Determination of Stomatal Number and Stomatal Index

Small pieces from the middle of lamina were soaked in ethanol for overnight. Peeling of upper and lower were done separately. The mount was prepared by the addition of glycerin. Squares of known dimension was drawn with the help of stage micrometer and camera Lucida. After obtaining square of 0.5 mm it was replaced by the specimen preparation. Stomata were drawn on a black chart paper with the help of camera lucida by a superimposed image. The number of stomata and epithelial cells were counted and calculated by the formula and results are tabulated in Table 1:

$$S.I = \frac{S}{E + S} * 100$$

S.I - Stomatal Index

S - Number of stomata

E – Number of epidermal cells

Table 1: Stomatal number and Stomatal Index.

Part	Stomatal Index (per sq mm)
Leaf	7.69

c. Powder Study of Leaf Part

The powder was characterized on its morphological features as

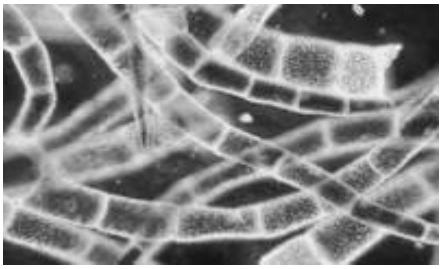
Color : Emerald Green

Odour : Characteristic to odourless

Taste : Characteristic

The powder was stained with chloral hydrate to detect the presence of calcium oxalate crystals. When stained with phloroglucinol and conc. hydrochloric acid vascular bundles, spiral vessels, parenchyma cells, lignified fibers and non-lignified fibers were observed. When mounted with glycerin covering trichomes were observed. All the results are figured from fig7- 12.

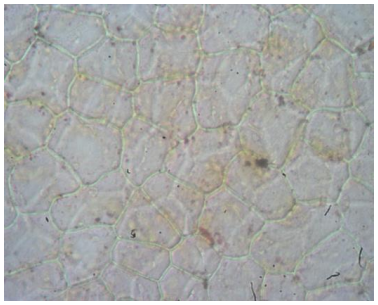
7. Unicellular covering trichomes



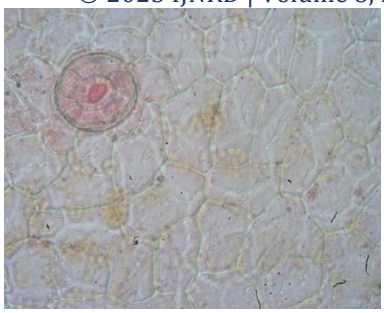
8.Multicellular Trichomes

9. Calcium oxalate crystals

10. Annular vessels



11. Epidermal cells



12. Glandular Trichomes

Fluorescence analysis of the plant part:

Leaf powder: The powder drug (dried leaf) was treated with different chemicals as given in Table 2. Then, it was visualizing under ultra violet radiation to see the effect in terms of color change under visible; short ultra violet and long ultra violet light.

Table 2 Effect of different chemicals on powdered drug (Leaf)

DRUG	VISIBLE ULTRA VIOLET (400 nm)	SHORT ULTRA VIOLET(256nm)
Powder	Dark Green	Green
Powder + Water	Light Brown	Light Green
Powder + Conc. HCl	Black	Black
Powder + Conc. H_2SO_4	Blackish Brown	Greenish Brown
Powder + NaOH	Dark Brown	Light Bluish Green
Powder + Methanol	Emerald Green	Light Green
Powder + Acetone	Emerald Green	Orange
Powder + Acetic Anhydride	Dark Brown	Dark Brown

e) Quantitative Study of Leaf Powder.

The quantitative analysis of plant part (Leaf) was carried out by Stage and Eye Piece Micrometer. The eyepiece from microscope was removed and eye piece micrometer was placed on the ridges inside the cavity. The stage micrometer was placed on the stage of microscope and it was focused on high power with eye piece scale superimposed. The Calibration factor was calculated from the superimposed images.

Preparation of Powder

The powdered sample (Leaf) was prepared by staining with phloroglucinol and conc. hydrochloric acid and was mounted with glycerin. The specimen was covered with a coverslip and the characters were identified and calculated.

For leaf the length and width of trichomes was calculated. The data is tabulated in Table 3.

Table 3. Quantitative Analysis of Powdered Drug for Determination of Trichomes (Leaf).

Part	Parameters	Minimum (µm)	Average (µm)	Maximum (µm)
Leaf	Length of trichomes	**4**	**77.96**	**17**
	Width of trichomes	**0.5 – 2.5**		

PHYSIOCHEMICAL EVALUATION OF CRUDE DRUG:

Ash Value Determination (Indian Pharmacopoeia, 1996**)**

Total Ash:

The air-dried crude drug (Leaf) was weighed in silica dish and was incinerated at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed. The percentage of ash was calculated with reference to air-dried drug. The results were tabulated in Table 4.

Acid Insoluble ash:

The total ash obtained as above was boiled with 25ml of 2M hydrochloric acid for 5min, the insoluble matter was collected on as ash less filter paper, and it was then washed with hot water, ignited, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to air-dried drug and the result were tabulated in table 4.

Water Soluble ash:

The total ash obtained was boiled with 25ml of water. The insoluble matter was collected on an ash less paper. It was then washed with hot water and ignited for 15min for a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference of weight represented the water soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug and the result were tabulated in Table 4.

2) Extractive values determination:

a) Alcohol Soluble Extractive:

5 gm of dried coarse powdered drugs (leaf and Stem) was macerated with 100 ml of 90% ethanol in a closed flask for 24hr, shaking frequently during first 6hr, and allow standing for 18hr. It was then filtered immediately taking precaution against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in tarred flat bottomed shallow dish. It was dried at 105°C and weighed. The percentage of alcohol soluble extractive value was calculated with reference to air-dried drug and the results were tabulated in table 4.

b)Water Soluble Extractive:

Similar method was followed for water soluble extractive as directed for the determination of alcohol soluble extractive. The percentage of water soluble extractive value was calculated with reference to air-dried drug and the results were tabulated in table 4.

3) Determination of loss on drying:

The powdered drug sample (10g) was placed on a tarred evaporated dish. It was then dried at 105°C for 6hr and weighed. The drying procedure was repeated until two successive reading matched or the difference between the two successive weighing was not more than loss on drying is 0.25% of the constant weight. The results were recorded in table 4.

Table 4: Physicochemical Evaluation of the Crude Drug *C. inerme*

Standardization	Leaf
Total ash	9.66 % W/W
Acid Soluble ash	0.01 % W/V
Water Soluble Ash	0.13 % W/V
Loss on Drying	7.5 % W/W
Extractive value	
Alcohol Soluble	0.52 % W/V
Water Soluble	1.32 % W/V

4. Foaming Index (WHO, 1998**):** The foaming ability of an aqueous decoction of plant material and extract was measured in terms of a foaming index as prescribed in WHO guidelines for standardization of herbal drugs. Crude drug (1gm) reduced to coarse powder was transferred to 500ml flask containing 100ml of boiling water. The mixture was moderately boiled. It was then cooled and filtered into a 100ml volumetric flask and sufficient water was added through the filter paper to make up the volume. The decoction was poured into 10 test tubes in a successive portion of 1ml to 10 ml and the volume of liquid in each tube was adjusted with water to 10ml. The tubes were shaken in lengthwise motion for min, two shakes per sec. It results were assessed as follows:
If the height of the foam in every tube was less than 1cm, the foaming index was less than 100.
If the height of the foam was measured 1cm in any test tube, the volume of the plant material decoction in this tube (a) was used to determine the index. If this tube was he first or the second tube in series, an intermediate dilution in a similar manner was prepared to obtain a more precise result.
If the heights of the foam more than 1cm in any test tube, the foaming index was over 1000. In this case the determination using a new series of diluting the decoction was repeated in order to obtain the result. The foaming index was calculated using the formula:

$$\frac{1000}{a}$$

Where, a= the volume of decoction used for preparing the dilution in test were foaming height of 1cm is observed. Standards were also carried out using a saponins containing drug (*Glycyrrhiza glabra*) in the same manner as that of the test sample. The results were recorded in table 5.

Table 5: Foaming index of *Clerodendrum inerme* (Leaf).

Treatments	**Sample number of test tubes**									
	01	02	03	04	05	06	07	08	09	10
Dilution (Drug extract + Water)	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1	10:0
Height of foam in standard (mm)	4	5	5	8	9	10	12	13	16	19
Height of foam in leaf decoction (mm)	<10	<10	<10	<10	<10	=10	>10	>10	>10	>10

(Drug Extract = Leaf and Stem of *Clerodendrum inerme* and *Glycyrrhiza glabra* aqueous extracts).

The height of the foam in test tube till dilution 5:5 was less than 1cm but from dilution 7:3 height of foam was more than 1cm, after 15 min in leaf and stem of *C. inerme*.

5. Swelling Factor: (WHO, 1998)

The plant part (Leaf) was evaluated for swelling factor. Accurately weighed 1gm of the powdered sample (Leaf powder) was placed on a stopper cylinder. The cylinder was filled up to 20ml mark with water. Further it was agitated gently and occasionally during 24hrs. and was allowed to stand. The volume occupied was measured for the swollen drug. The results are tabulated in table

Table 6: Determination of Swelling Factor of Plants Parts (Leaf)

Part of Plant	Parameter	Volume occupied (cm)
Leaf	Swelling factor	21

6. P^H Determination:

The P^H of the powdered plant part (Leaf and Stem) was determined to find out the acidity or basicity of the drug solution. Accurately weighed 1gm of the powdered sample was taken and to it 100ml of double distilled water was added and boiled. The extract was filtered and the filtrate was determined for P^H by Ph meter. The results are tabulated in table 7.

Table 7: Determination of P^H values of powdered drug (Leaf)

Part of Plant	Parameter	Value
Leaf	**P^H**	**6-7**

C. PHYTOCHEMICAL ANALYSIS:

Qualitative chemical test for the presence/absence of primary/secondary metabolites.

Depending upon the type of natural drug under examination, test solutions were chosen as per their polarity. For the study Alcoholic and Aqueous was taken up.

Purification of solvents.

Methanol: The methanol was lime distilled and used for study.

Aqueous: The water was double distilled and was used for the study.

1. Preparation of Reagents (Harbone, 1998, kokate, 2005)

Dragondroff's Reagents:

14g of Sodium Iodide was boiled with 5.2g of basic bismuth carbonate in 50ml glacial acetic acid for few minutes. It was to stand overnight and filtered. To this 40ml of red-brown filtrate 160mln of ethyl acetate and 1ml of water was added. The stock solution were preserved in amber colored bottle and used whenever necessary. For using 20ml of acetic acid was added to 10ml of the stock solution and the volume was made up to 100ml with water.

Fehling' Solution:

34.66gm of Copper Sulphate is dissolve in distilled water and the volume is made up to 500ml. this stock solution is named as A. 173gm of Potassium Sodium Tartarate and 50gm of Sodium Hydroxide are mixed with distilled water and the volume is made up to 500ml and named as B. Mixture of A and B in equal volume is noticed when it is used.

Ferric chloride solution:

5 gm of ferric chloride is dissolved in 90% alcohol.

Hager's Solution:

Saturated aqueous solution of picric acid.

Lead Acetate Solution:

25gm of basic lead acetate is dissolved in1000ml of distilled water.

Liebermann Burchard Solution:

10ml of Acetate Anhydride is mixed with 10 ml of concentration Sulphuric acid under cooling process. To this 100ml of absolute alcohol is also added under cooling process to get the desired mixture of components.

Mayer's Solution:

1.36gm of Mercuric Chloride is dissolved in 60ml of distilled water (A). 5g of potassium iodide is dissolved in 20ml distilled water (B). Mixture of A and B is done and the volume is made up to 100ml with distilled water for use.

2. Preliminary phytochemical analysis:

Procedure for preparation of extract:

About 10gm of powdered sample (Leaf and Stem) of Clerodendrum inerme were separately subjected to soxhelet extraction for 36 hrs, using solvents in the increasing order of their polarity (Petroleum ether, Ethyl acetate, Chloroform, Methanol, Hydro-alcoholic and Aqueous) to check the presence/absence of various phytochemical constituents.

1. Test for Alkaloids

The methanolic extract was treated with dil. Hydrochloric acid and filtered. The filtrate was treated with various alkaloid reagents.

a) Few drops of **Mayer's reagent** were added in each extract and observed for formation of white or cream color precipitate.

b) Few drops of **Hager's reagent** were added in each extract and observed for formation of yellow color precipitate.

c) Few drops of **Wagner's reagent** were added in each extract and observed for formation of precipitate.

d) Few drops of Dragondroff's reagent were added in each extract and observed for formation of orange yellow precipitate .

2. Test for Carbohydrates

a) Molisch Test:

Small quantity of methanolic and aqueous extract were dissolved separately in 5ml of DW and filtered. To this solution 2-3 drops of α-naphthol were added. To this about 1ml of conc. sulphuric acid was added along the sides of inclined test tube so as to form two layers and observed for formation of violet colored ring at the interface.

b) Fehling's Reagent:

Few drops of Fehling's A and B in equal volume were added in dil. methanolic and aqueous extracts and heated for 30 min. and observed for the formation of brick red colored precipitate.

c) Benedict's Test:

Few drops of Benedict's reagent was added in dil. methanolic and aqueous extracts and observed for the formation of reddish orange precipitate.

3. Test for Steroids and Sterols:

The alcoholic extract was evaporated to dryness ant the residue was extracted with petroleum ether and acetone. The insoluble residue left after extraction with petroleum ether and acetone was tested for above.

a) Libermann Burchard:

The insoluble residue was dissolved in chloroform and few drops of acetic anhydride were added along with few drops of conc. sulphuric acid from the sides of the test tube and observed for the formation of blood red color.

b) Salkowski Reaction:

To the chloroform extract 2ml of conc. sulphuric acid was added and observed for the formation of yellow ring at the junction, which turns red after a minute.

c) Herche's Reaction:

To the residue 2-3ml of tri-chloroacetic acid was added, heated and observed for the formation of red to violet color.

4. Test for Proteins and Amino acid:

a) Biuret Test:

To the ammoniated alkaline filtrate of the extract 2-4 drops of 0.02% copper sulphate were added and observed for the formation of violet color.

b) Millon's Reagent:

To 2ml of this filtrate 5-6 drops of Millon's reagent were added and observed for the formation of red precipitate.

c) Ninhydrine Test:

To the methanolic extract, lead acetate solution was added to precipitate tannins. The precipitate was spotted on a paper chromatogram, sprayed with ninhydrine reagent and heated at 110^{o} for 5 min and observed for the formation of red or violet color.

d) Xanthoprotein Test:

To the methanolic extract 2ml nitric acid was added by the sides of the test tube and observed for the formation of yellow color.

5. Test for Phenolic compound:

a) Ferric chloride solution:

The aqueous extract was taken and warmed. To this 2ml ferric chloride was added and observed for the formation of green or blue color.

b) Sodium chloride Solution:

The aqueous extracts were taken and to this 10% sodium chloride was added and observed for the formation of cream color.

6. Test for Tannins:

a) Lead acetate Test:

To the aqueous extract 2ml of 10% lead acetate solution was added and observed for the formation of white precipitate.

b) S odium chloride Solution:

The aqueous extract was taken and to this 10% sodium chloride was added and observed for the formation of white color.

c) Bromine Solution:

The extract was taken in bromine solution added and observed for the formation of white precipitate.

7. Test for Flavonoids:

When the methanolic extract was treated with amyl alcohol followed by the addition of sodium acetate and ferric chloride no characteristic color was seen which confirms the absence of Flavonoids.

8. Test for Gums and Mucilage:

When the aqueous extracts were treated with 25ml absolute alcohol, the solution was filtered and filtrate was examined for its swelling properties.

9. Test for Glycosides:

A pinch of the substance was dissolved in glacial acetic acid and few drops of ferric chloride was added followed by the addition of conc. sulphuric acid and observed for the formation of red color ring at the junction of two liquids.

10. Test for Saponins:

a) Foam Test:

100mg of residue was taken in a test tube with a small quantity of water and shaken vigorously for a 1min added and observed for the formation of rich lather which was stable for more than 10min.

b) To the alcoholic extract few drops of sodium bicarbonate was added, shaken well and observed for the formation of honeycomb like frothing.

c) Haemolysis Test:

A little of the residue was dissolved in normal saline in such a way that 5ml of the solution represented 1gm of the crude drug. In a series of 5 test tubes, doses of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml were added and volume was made up to 1 ml each with normal saline. 1 ml diluted blood (0.5 ml rabbit's blood diluted to 25 ml with normal saline) was added to each tube and changes observed . if haemolysis of the blood occurs the saponins are present.

11. Test for Terpenes:

To the extract, tin and thionyl chloride were added and observed for the formation of pink color.

Preliminary Phytochemical Screening

Table 8: Phytochemical screening of Leaf and Stem powder extracts of *Clerodendrum inerme.*

Phytoconstituents	Alcoholic Extract	Aqueous Extract
Alkaloids	++	++
Carbohydrates	+	++

Steroids	+	+
Sterols	+	+
Proteins and Amino acid	–	–
Phenolic compound	+	+
Tannins	+	+
Flavonoids	+	+
Gums and Mucilage	–	–
Glycosides	--	--
Saponins	++	--
Triterpenes	--	--

+ = Present -- = Absent

RESULT AND DISCUSSION

The pharmacognostical evaluation was carried on the leaf part of standardization:

Following parameters were used as

Macroscopic Evaluation of crude drug

Microscopic Evaluation of crude drug

Macroscopic evaluation of the drug revealed the morphological characters of the plant:

Leaf color: emerald green , Odour: characteristic, Taste: characteristic, Margins: entire, Surface: smooth, Base: symmetry, veinneation: alternate to opposite (Fig:4)

Microscopic evaluation was carried out on transverse section of leaf and the characters were as follows: Epidermis, Midrib region(Fig:6), Covering trichomes(Fig:7), collenchyma cells, palisade cells, Calcium oxalate crystals, Vessels.

Powder microscopy of leaf was carried out which it revealed the presence of epidermal cells(Fig:11), spiral vessels(Fig:10), glandular trichomes(Fig:7) , anisocytic stomata and calcium oxalate crystals(Fig:9).

The powder of leaf was also treated with different reagents. Various color reactions were observed in ultra violet light in different wavelength (Table 2). Quantitative analysis were carried out on powdered leaf of the plant. The result are tabulated in (Table 3).

Physiochemical parameter like ash value, extractive value, loss on drying were carried out on leaf(Table4), rate of formation of foam, which indicates the presence of saponins(Table 5) the result indicate that foaming was within the limit as prescribed by WHO. The parameter were done in replication to standardize the drug with proper

authentification. Swelling factor an p^H were determined in leaf parts of the plant. They are tabulated in (Table 6 and Table 7).

Phytochemical Analysis

Leaf of *Clerodendrum inerme* was subjected to preliminary phytochemical studies as mentioned in (Table) and it revealed the presence of alkaloids, carbohydrates, sterols and steroids, phenolic compound, saponins, tannins and flavonoids in the aqueous extracts but in alcoholic extract saponins are absent from above mentioned.

REFERENCES

1. Harish S.K and Marungan K; Biochemical and Genetical Variation in the Mangrove Associate (L) Gaetrn. Under Different Habitats of Kerala.Asian J. Exp. Boil. Sci. 2011;2(4): 553-561.

2. Sher Alam; Antimicrobial Activity of Natural Products from Medicinal Plants, Gomal Journal of Medical Sciences. January-June 2009; 7(1):72-78.

3. Starr Forest, Starr Kim and Loope Lloyd; *Clerodendrum inerme* Scaside *Clerodendrum* Verbenaceae. United States GeologicalSurvey-Biological Resources Division Halcakala Field Station, Maui, Hawai'I January 2003;1-3.

4. Rajasekaran A and Ponnusamy K; Antifungal Activity of *Clerodendrum inerme*(L). and *Clerodendrum phlomidis* (L). Turk J Biol 2006; 30: 139-142.

5. Khare C.P (Ed.); Indian Medicinal Plants . an illustrated Dictionary.

New Delhi: Springler; 2007: 159-160.

6. Bandarnayake W. M; Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management 2002; 10: 421-452. http://dx.doi.org/10.1023/A:1021397624349

7. Avani Kothari, Harish Padh and Srivastava Neeta; Ex. Situ Coservation Method for *Clerodendrum inerme:* a medicinal plant of India. B. V. Patel Pharmaceutical Education & Research Development (PERD) Centre, Thaltej, Ahmedabad , 380 054, India. October, 2005;1-4.

8. Devi Gayatri, Vijayan Chithra, John Anitha and Gopakumar K; Pharmacognostic and Antioxidant Studies on *Clerodendrum inerme* and Identification of Ursolic Acid as Marker Compound. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(2):145-148.

9. Mohammed Rahamatulla, Rownak Jahan, FM Saful Azam , S. Hossa, MAH Mollik, Taufiq Rahman. Afr J Tradit Complement Altern Med. 2011;8(55): 53-65.

10. Dr. Nadkarni K.M; Indian Materia Medica. Popular Prakashan Private Ltd, Mumbai 2000; 1:352.

11. George M and Joseph L; Hepatoprotective Effect of *Clerodendrum inerme* Linn. Ethanolic Extract. East and Central African Journal of Pharmaceutical Sciences 2008; 11;49-51.

12.Harvey et al., 2000.

13. Bhakuni, Dhar OS; ML, Dhar; MM, Dhavan and Mehrotra, BN (1969); "Screening of Indian plants for biological activities Part II"; Indian Journal of Experimental Biology; 7, 250-262.

14. Chae, S Kim; JS, Kang; KA,, Bu; HD, Lee; Y, Hyun; JW and Kang, SS (2004); "Antioxidant activity of jionoside D from *Clerodendrum inerme*"; Biological and Pharmaceutical Bulletin; 27;1504-1508.

15. Gayar, R and Shazll, A (1968); “Toxicity of certain plants to Culex pipiens larvae”; Bulletin of the Society of Entomology; 52; 467.

16. George. M and Pandalai KM (1949); “Investigations on plant antibiotics, Part IV. Further search for antibiotic substances in Indian medicinal Plants”; Indian Journal of Medical Research, 37;169-181.

17. Kalyanasundaram M and Das PK (1985); “Larvicidal and synergistic activity of plant extracts for mosquito control”; Indian Journal of Medical Research; 82; 19-23.

18. Mehdi H Tan; GT Pezzuto, JM; Fong, HHS; Farnsworth, NR and EL-Feraly, FS (1997); “Cell culture assay system for the evaluation of natural product mediatedanti-hepatitis B virus activity”; Phytomedicine;369-377.

19. Nakata M (1999); “Evaluation of the antioxidant activity of environmental P. Verma et al. / Pharmacophore 2013, Vol. 4 (6), 230-232http://www.pharmacophorejournal.com 232 plants: activity of the leaf extracts from seashore plants”, Journal of Agriculture and Food Chemistry, 47, 1749-1754.

19. Sharaf, A Aboulezz; AF Abdul-Alim, MA and Goman, N (1969); “Pharmacological studies on the leaves of C. inerme”, Quality Plant Material Vegetation, 17, 293.

20. Sharma W and Verma HW (1991); “Antifungal activity of *Clerodendrum inerme* on fungal rotting fungi”, Fitoterapia, 62, 517-518.

21. Somasundram S and Sadique J (1986); “Anti-hemolytic effect of flavonoidal glycosides of C. inerme: An in vitro study”, Fitoterapia, 57, 103-110.

22. www.wikipedia.com Plant profile, accessed on 16 October 2013.