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# EXPLORING DETAIL STUDY AND PHYTOCHEMICAL ANALYSIS OF *CLERODENDRUM INERME*(L) GAERTN

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**Abstract:** Garden quinine is an evergreen plant which belongs to the family Verbenaceae (Lamiaceae). It is distributed in tropical and subtropical regions of the countries. More than five hundred species of the genus are identified till now, which includes small trees, shrubs and herbs.It is as a versatile plant and can be grown as a topiary or as a bonsai in India. Ethno-medical importance of various species of Clerodendrum genus has been reported in various indigenous systems of medicines and as folk medicines. The genus is being used as medicines specifically in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of various life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension The researches on *Clerodendrum inerme* provide the proof that it contains chemical constituents like Triterpenoids, Tannins, diterpenoids, Alkaloids, glycosides, phenols, flavonoids, and, volatile oils and steroids. However, the researchers also prove that it is used as anti-diabetic, anti–microbial anti-inflammatory, anti-hepatotoxic activity, anti-malarial, anti-oxidant. These plant mainly used for the traditional purpose like febrifugal and uterine stimulant.Ethno-medical importance of various species of Clerodendrum genus has been reported in various indigenous systems of medicines and as folk medicines. Along with biological studies, isolation and identification studies of chemical constituents and its correlation with the biological activities of the genus has also been studied. Also phytochemical investigation of plant done.

**Keywords:** *Clerodendrum inerme*, Phytochemical, Pharmacological activities, Traditional uses, Microscopy systems.

## INTRODUCTION

The *Clerodendrum inerme (L) Gaertn*., is considered to a source of Aranika or KshudraAgnimantha. [1] It is a straggling shrub found throughout India, very common along the sea coast, often cultivated as a hedge plant or as garden plant whose flowering is seen more or less throughout the year. It has a wide pharmacological activity matching with qualities of Agnimantha, a plant included in Dashamoola group, thus being claimed as one of its botanical sources.[2]

*Clerodendrum inerme (L) Gaertn* belonging to family Verbenaceae is very widely distributed in tropical and subtropical regions of the world and is comprised of small trees, shrubs and herbs. Ethno-medicinal importance of various species of Clerodendron genus has been reported in various indigenous systems of medicine and as folk medicines .[3]*Clerodendrum inerme (L) Gaertn*is a sun loving plant and a sunny spot should be chosen for it. The plant produces suckers and seeds. The plant has medicinal properties. People who are familiar with this plant use a poultice made of its leaves to suppress buboes and the leaf juice as an alternative. Leaves and roots of the plant are used in rheumatism and skin diseases[4]

The genus *Clerodendrum* includes over 452 species of tropical regions. These plants widely distributed tropical and subtropical plant, it is mainly found in Bangladesh, Nepal, India, and Srilanka and Southest Asia. It is a versatile plant mainly grown as small trees, sprawlingshrubs and herbs in coastal India. Sometimes it can be grown as a topiary or as a bonsai in India[5,6].

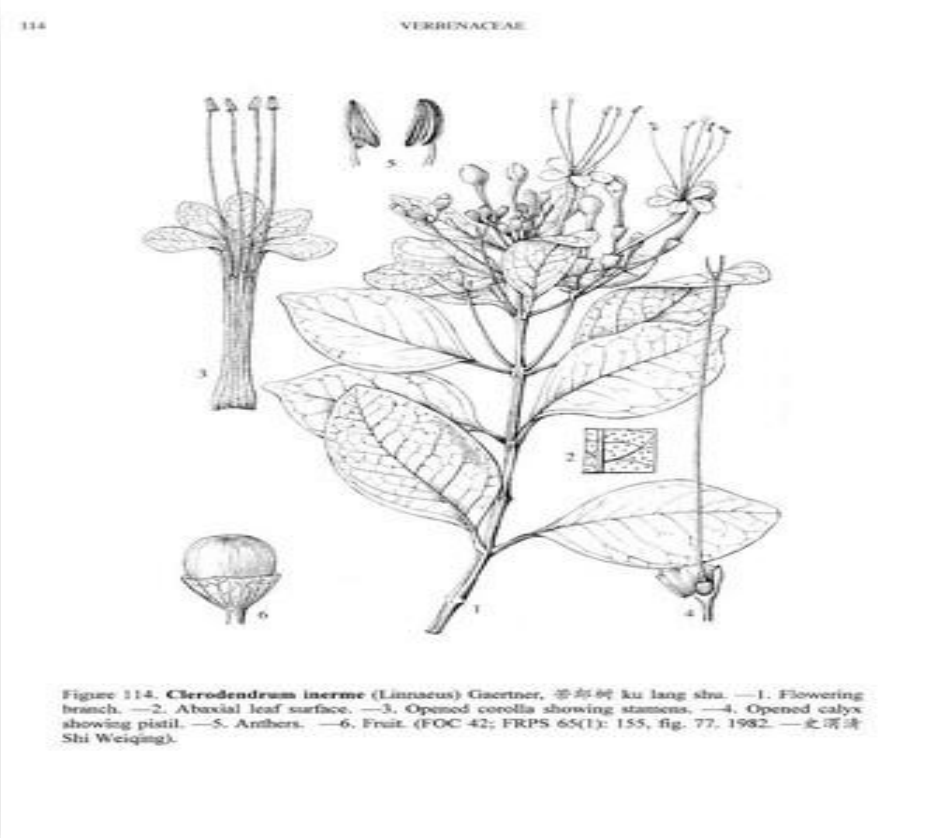


**Fig. 1- Herbarium sheet of *Clerodendrum inerme (L) Gaertn***

**TAXONOMICAL DETAILS OF *CLERODENDRUM INERME (L) GAERTN*.**

Kingdom: Plantae

Division: Spermatophyta

Sub-Division: Angiosperm

Class: Dicotyledonae

Sub-Class: Gematopetalae

Series: Bicarpellatae

Order: Lamiales

Family: Lamiaceae (Verbenaceae)

Genus: Clerodendrum

Species: inerme

Scientific Name: *Clerodendrum inerme (L) Gaertn*[7]

**Vernacular Names:**

English Name: Garden quinine

Hindi Name: Lanjai, Sang kupi, Binjoam, Chhotiarani

Kannada name: Kundali, Nayitakkali, Naitakkilay

Tamil Name: Anjali, Pinarichanganguppi, Pinasangamkoppi

Telugu Name: Takkolarkamu, Etipisinika, Pishinita, Eruppichha

Malayalam name: Nirnochi, Nirnotijil

Marathi name: Vanajari, Koivel, Lahankharinarval

Gujarati name: Dariajai

Bengali name: Benjuen, Banjai, Batraj, Bonjoi, Ganibhari, Ganiyari, Ganira[8]

**DISTRIBUTION:** [9,10]

Global Distribution:

Coastal India, Sri Lanka; now naturalized on the shores of Myanmar, Australia, China

Indian distribution:

In Kerala, Kottayam, Alappuzha, Kasaragode, Kollam, Palakkad, Kannur, Thiruvananthapuram, Malappuram, Kozhikode, Wayanad, Thrissur, Ernakulam.

It ordinarily develops in nearness to the ocean and is regularly found close edges or on the edges of shoreline woodland. Additionally, happens in Asia, Malesia and the Pacific islands.

Climate:

*Clerodendrum* requires clammy tropical and subtropical atmosphere, which ought to be free from ice amid winters and dry warmth in summers. It is additionally conceivable to develop the species in the dry areas under incomplete shade. The plant is influenced by ice in northern India, which causes consuming of leaves, defoliation, and going away of youthful shoots, and the plants at last pass on.[11]

BOTANICAL DESCRIPTION:

*Clerodendrum inerme (L) Gaertn*is evergreen mangrove sprawling and much expanded bush, it becomes here and there scandentupto 1-1.8 m tall. Leaves are inverse once in a while interchange upto 5*3.8 cm, elliptic or obovate, Green, smooth, slight sparkling upper surface whole, acute or emarginated at optimum and glabrous. Flowers are joined at basic base point, Corolla white combined with five lobes.

Inflorescence usually terminal, sepals usually connate, often colored, usually a crescent. Corolla red to yellow, pink, or white and corolla tube 5-lobed the lobes are usually unequal. Stamens 4 (rarely 5), usually in 2 pairs of unequal length and projecting well beyond the mouth of the corolla. Ovary incompletely 4-locular and 4 Ovules. Style terminal on the ovary, bifid.[12] Fruits are drupes,obovoid with 4-lobed separating in to 4 pyrenes. The stems are smooth and are without thistles. Seeds are Cotyledons thick and beefy, around 12-20 x 6-9 mm, bit by bit decreasing into the petioles. Bark grayish dark coloured, branches and branchlets slim, harshly quadrangular, lenticellate, marginally pubescent, terminal branches frequently twining.

Fig. 2 Leaves


Fig. 3 Flowers

Fig. 4 Bark


Fig. 5 Fruits

PHYTOCHEMISTRY OF *Clerodendrum inerme (L) Gaertn*

Table 1. Different phytoconstituents classified on basis of main active constituent

| Sr. No. | Preliminary study | Phytoconstituents |
|---|---|---|
| **1** | Steroid | β-sitosterol, γsitosteroloctacosanol, clerosterol, bungein A, acteoside, betulinic acid, clerosterol 3-O- β-D glucopyranoside, colebrin A-E, campesterol, 4α-methylsterol, cholestanol and 24-β- 22-25- bis-dehydrocholesterol |
| **2** | Terpene | monoterpenes, diterpenes, triterpenes, iridoids and sesquiterpenes. Terpenes such as α-amyrin, β-amyrin, caryoptin, 3-epicaryoptin, 16-hydroxy epicaryoptin, clerodendrin A, B and C, clerodin, clerodermic acid, cleroinermin, gramisterol, iridoids (inerminoside A, B, C and sammangaoside, ugandoside, 8-O-acetylmioporoside), oleanolic acid, dehydroroylean-one, sesquiterpene (sammangaoside A, B) clerodendrin A, uncinatone, Misaponins-A, friedelanone and lupeol |
| **3** | Phenolic constituent | β-benzyl alcohol, β-benzyl alcohol-D-glucoside, neolignan, darendoside-B, phenyl propanoids, vanillic acid, anisic acid, para-hydroxy benzoic acid and gallic acid |
| **4** | Flavonoid | cynaroside, 5-hydroxy-4'-7-dimethoxy methyl flavone, kaempferol, salvigenin, 4-methyl scutellarein, 5,7,4 Otrihydroxyflavone, apigenin, luteolin, acacetin-7-O-glucuronide, hispudulin, 2'-4- 4'trihydroxy-6'methyl chalcone, 7-hydroxy flavone, luteolin, naringin-4'-O-αglucopyranoside, pectolinarigenin, cirsimaritin, cirsimaritin-4'-glucoside and quercetin-3- methyl ether |
| **5** | Carbohydrate | glucose, fructose and sucrose |
| **6** | Other constituents | ribosome-inactivating protein, salidroside, jinoside-D and acetoside |

Aerial parts of the plant contain clerosterol as major sterol components. Leaves possess clerodanediterpene, clerodermic acid along with known compounds friedelin, salvihenin, acacetin and apigenin. Stem afford two hydroxyl diterpenoidquinones and botulin.[13]

In preliminary studies of this plant, carbohydrates, steroids, flavonoids, volatile constituents, and terpenes have been isolated. Other constituents include ribosomeinactivating protein, salidroside, jinoside-D, acetoside; Steroids such as β-sitosterol, γsitosteroloctacosanol, clerosterol, bungein A, acteoside, betulinic acid, clerosterol 3-O- β-D glucopyranoside, colebrin A-E, campesterol, 4α-methylsterol, cholestanol and 24-β- 22-25- bis-dehydrocholesterol have been isolated[10-16].

Another class of constituents is terpenes, which include monoterpenes, diterpenes, triterpenes, iridoids and sesquiterpenes. Terpenes such as α-amyrin, β-amyrin, caryoptin, 3-epicaryoptin, 16-hydroxy epicaryoptin, clerodendrin A, B and C, clerodin, clerodermic acid, cleroinermin, gramisterol, iridoids (inerminoside A, B, C and sammangaoside, ugandoside, 8-O-acetylmioporoside), oleanolic acid, dehydroroylean-one, sesquiterpene (sammangaoside A, B) clerodendrin A, uncinatone, Misaponins-A, friedelanone and lupeol have been isolated.[14-23]

The phenolic profile of the plant revealed the presence of β-benzyl alcohol, β-benzyl alcohol-D-glucoside, neolignan, darendoside-B, phenyl propanoids, vanillic acid, anisic acid, para-hydroxy benzoic acid and gallic acid[27].

Flavonoids are another class of compounds, which are mainly present in Clerodendron species and they are also responsible for few biological activities. The major flavonoids present are cynaroside, 5-hydroxy-4'-7-dimethoxy methyl flavone, kaempferol, salvigenin, 4-methyl scutellarein, 5,7,4 Otrihydroxyflavone, apigenin, luteolin, acacetin-7-O-glucuronide, hispudulin, 2'-4- 4'trihydroxy-6'methyl chalcone, 7-hydroxy flavone, luteolin, naringin-4'-O-αglucopyranoside, pectolinarigenin, cirsimaritin, cirsimaritin-4'-glucoside and quercetin-3- methyl ether, which were isolated from C. inerme[24-28] .

Carbohydrates like glucose, fructose and sucrose are reported. Other constituents such as ribosome-inactivating protein, salidroside, jinoside-D and acetoside have also been isolated[29].

B-friedoolean-5-ene-3-β-ol (1),14 β-sitosterol (2),15 stigmasta-5,22,25-trien-3-β-ol (3),16, 17 betulinic acid (4),18, 19 and 5-hydroxy-6,7,4′-trimethoxyflavone (5).[30]

PHARMACOLOGICAL ACTIVITIES:

The genus *Clerodendrum* contain many plant species that are being used in various health care systems for the treatment of various disorders including life threatening diseases. The following pharmacological actions are reported for *Clerodendrum* species.

Anti-Diabetic activity: The counter diabetic action of *Clerodendrum inerme (L) Gaertn* was assessed utilizing in vivo streptozotocin-actuated diabetes in mice, and in vitro thinks about. The leaves of *C. inerme*

were separated in oil ether, methanol pursued by fluid dissolvable. Methanolic concentrate of leaves of *Clerodendrum inerme* at 200 mg/kg demonstrated an extremely progressive and potent decrease in glucose level[31]

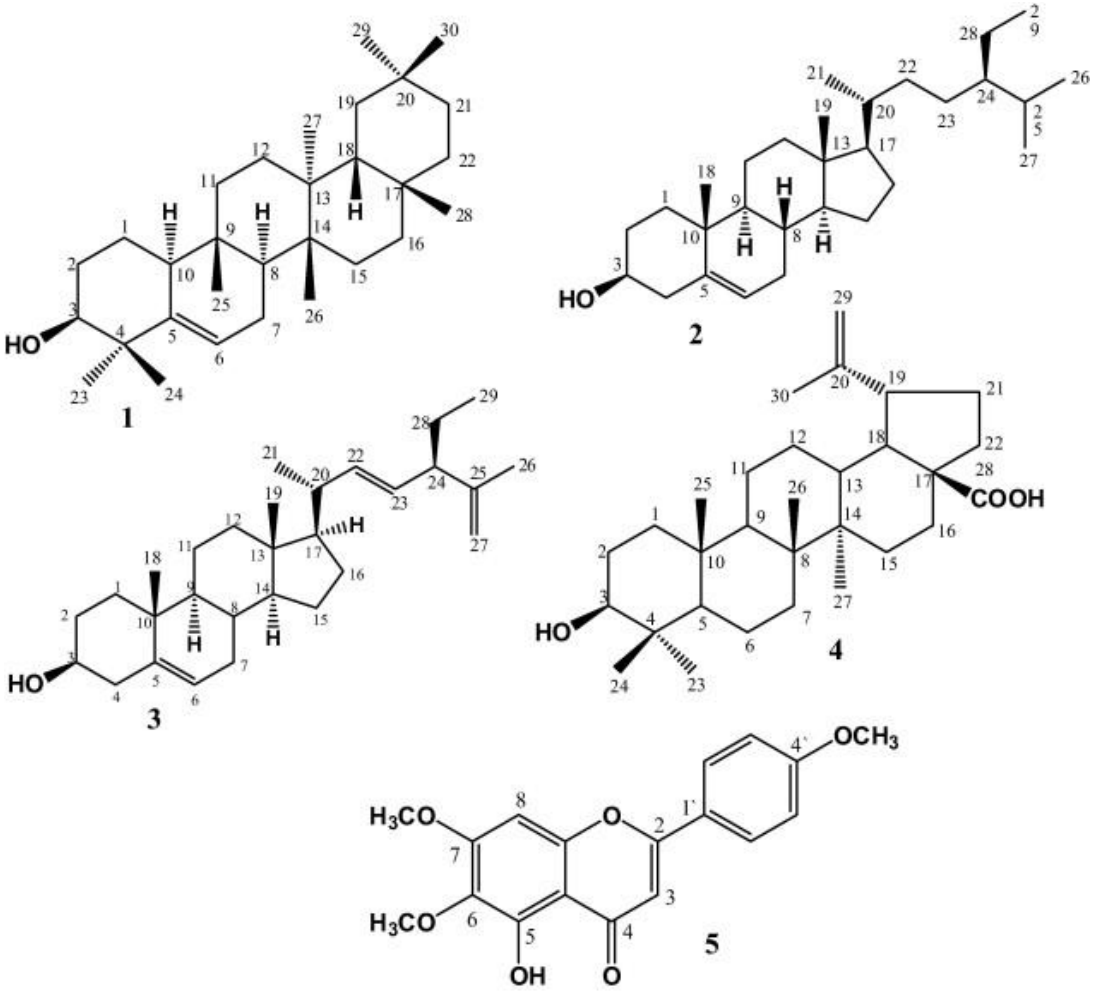


Fig. 6 Chemical structure of important constituent of *Clerodendruminerme (L) Gaertn*

Anti-oxidant activity: The reducing power assay was dictated by following strategy, 0.5ml of concentrates (200 to 1000µg/ml) was blended with 0.5ml of 0.2 M phosphate support (pH 6.6) and 0.5ml potassium ferrocyanide (1%). after incubating the blend at 50ºC for 20 min., 0.5ml of 10% trichloroacetic corrosive was included, centrifugation was completed at 3000 rpm for 10 min. 1ml of supernatant was blended with 1ml of refined water and 0.2ml FeCl3 (0.1%) and the absorbance was estimated at 700nm[32]

Anti-Carcinogenic activity: *Clerodendrum inerme (L) Gaertn* is employed by Indian ancient practitioners for the treatment of various ailments, as well as cancer. The *Clerodendrum inerme* exerts its chemo preventive action by modulating macromolecule peroxidation and inhibitor defence mechanisms. Oral administration of liquid leaf extract of *Clerodendrum inerme* at a dose of five hundred mg/kg body weight considerably prevented the tumour formation and histopathological abnormalities. Oral administration of *Clerodendrum inerme* protected the amount of blood and tissue lipids, cell surface glycoconjugates, and red

corpuscle diffusion fragility and membrane sure accelerate or activity throughout DMBA induced oral carcinogenesis[33]

Anti-microbial activity: Hamid et.al reported that the specific media plates were vaccinated with inoculums of 106 sizes, a sterile swab is dipped into weakened culture inoculums, the agar surface of the plates is spread utilizing spreader. Cups are created by utilizing borer. The glasses were loaded up with 500µg/ml, plant separates, which were put in mugs with the assistance of a sterile pipette. The plates were permitted to remain at room temperature for 30 minutes. (Pre-dissemination time) and afterward brooded at 370C for 24 hrs if there should be an occurrence of microscopic organisms and 48 hrs for parasites. The zones of restraint were recorded after indicated time. The trials were rehashed thrice. [34]

Anti-malarial activity: *C. inerme* inhibit the growth of larvae of *Aedes aegypti*, *Culexquinque fasciatus* and *Culex pipiens*at 80 and 100 ppm concentration of petroleum ether and ether extracts and was found to have antimalarial activities.[36]

Anti-haemolytic activity

Some researchers reported the antibacterial activity of ethyl acetate extract of C. inerme on human pathogens. It is reported for its other biological activity such as antihaemolytic effect[44]

Insecticidal properties

The leaf extract of the plant has been shown to contain insecticidal properties against mosquitoes. Various solvent extracts of plant materials have been tested against mosquitoes. Therefore, it was thought rewarding to investigate the dry powder of leaf material as source of insecticidal properties against the mosquito larvae. The effect of sundried leaf powder of *Clerodendrum inerme (L) Gaertn* against fourth in star larvae of A. aegypti[45] .

Anti-carcinogenic activity

Clerodendrum inerme is used by Indian traditional practitioners for the treatment of various ailments, including cancer. The *Clerodendrum inerme (L) Gaertn* exerts its chemopreventive action by modulating lipid peroxidation and antioxidant defence mechanisms[46]. Oral administration of aqueous leaf extract of *Clerodendrum inerme (L) Gaertn* at a dose of 500 mg/kg body weight significantly prevented the tumor formation and histopathological abnormalities. Oral administration of *Clerodendrum inerme (L) Gaertn* protected the levels of blood and tissue lipids, cell surface glycoconjugates, red blood cell osmotic fragility and membrane bound enzyme activity during DMBA induced oral carcinogenesis[47] .

Anti-feedants activity

3-Epicaryoptin isolated from the leaves is responsible for growth inhibition and antifeedant activities in housefly and mosquito. Three new neo-clerodanediterpenoids, namely inermes A, inermes B and 14,15-dihydro-15b-methoxy-3-epicaryoptin were found in the hexane extract of aerial parts of C. inerme. 14, 15-Dihydro-15-hydroxy-3-epicaryoptin has also been isolated as an epimeric mixture[48]

Other biological activities

C. inerme extracts showed hypotensive effects in dogs. The methanolic extract of leaf extracts of C. inerme showed antispasmodic activity in mouse[49]. Its leaves have been shown to possess antimicrobial activity andare reported to be cardiovascular system active. They also stimulate uterine motility in rats and inhibit intestinal motility. The plant contains mainly iridoids, flavonoids, diterpenes, sterols, triterpenes and neolignans[50]. Organic extracts of C. inerme showed strong uterine stimulant activity, when tested in female rats and rabbits[51] and also showed strong antihemolytic activity in human adults at 0.02-2.0 mg/mL, with inhibition of phospholipase at 0.05-1.5 mg/mL[52]

Table 3. Pharmacological Activities reported in *Clerodendrum inerme* by various authors.

| Sr.No. | EXTRACT | PLANT PART | ACTIVITY | AUTHOR AND YEAR |
|---|---|---|---|---|
| 1 | Hexane and ethyl acetate extracts | Leaves and stems | Anti-fungal activity | Rajasekaran ANITHA, Ponnusamy KANNAN et.al -2006 [37] |
| 2 | Ethanolic extract | Leaves | Hepatoprotective activity. | M.George&joseph et.al-2008[38] |
| 3 | Alcohol & chloroform extract | Leaves | Anti-Microbial activity | Hamid *et al*, -2008[34,40] |
| 4 | Chloroform &Ethanolic extract | Leaves | Anti-diuretic activity by flame spectrophotometry | GarimaUpmanyu et al -2011[41] |
| 5 | Methanol extract | Aerial parts | Anti-oxidant activityby reducing power assay. | Prasad M.P., Sushant S. and Chikkaswamy B.K., et.al -2012 [32] |
| 5 | Pet ether and ether extract | Leaves | Anti-malarial activity | P. Verma*et al*. 2013[36] |
| 6 | Methanol extract | Leaves | Anti-spasmodic activity. | S P Gupta, et.al -2013[32] |
| 7 | Aqueous extract | Leaves | Anti-proliferative action | S P Gupta, et.al. 2013 [32] |
| 9 | Aqueous extract | Leaves | Analgesic and anti-pyretic activity | M. Thirumal et al-2013[42] |
| 11 | Methanol extract | Aerial parts | Anti-inflammatory activity. | S.R.M. Ibrahim et.al. -2014[35] |
| 12 | Petroleum ether | Leaves | Anti-Diabetic action | Ali Esmail Al-Snafi, et.al, 2016[31] |
| 13 | Methanol & Aqueous | Leaves | Anti-anxiety activity. | Laila Anwar et.al -2016 [43] |

TRADITIONAL USES:

*Clerodendrum inerme* was used as a febrifugal and uterine stimulant, a pest control agent and antiseptic, to arrest bleeding, treatment of asthma, hepatitis, ringworm and stomach pains, the roots are boiled in oil and used in rheumatic affections[31].it is an important medicinal plant used in various skin diseases. In siddha

medicine it is used under the names of chankankuppi and pechagnan. In various literature related to healthcare.

*C. inerme* have been accounted for its antimalarial exercises in view of the nearness of unpleasant taste. Natural products are like fruits used in food poisoning[33]

## MATERIAL AND METHODS

### Collection and Authentication of *Clerodendrum inerme (L) Gaertn*

The plant of Clerodendrum inerme were collected January 2022, from pioneer pharmacy degree college ,Vadodara, Gujarat. The plant was identified by comparing its morphological and microscopical with description given in different standard texts and floras25. Besides these, the plant was then identified and authenticated by Dr.P.K.PATEL and a voucher specimen was deposited. For further confirmation, the microscopic characters of this plant was studied and compared with available literature as mentioned above.

### Pharmacognostic Investigation[53-54]

### Morphology of *Clerodendrum inerme*

The morphology or macroscopical description of a crude drug include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc. were studied and compared with available literature as mentioned above.

### Microscopic Evaluation[55,56]

a) Transverse section of the leave of *Clerodendrum inerme (L) Gaertn*

The material to be sectioned is held between the thumb and four finger of the left hand. Using sharp razor blade held in the right hand, thin section was made the razor blade across the object in quick successions. Transferred the sections in to watch glass containing water, added chloral hydrate to these sections, boiled, filtered and the sections were stained with phloroglucinol and hydrochloric acid (1:1) and the same mounted in glycerin and observed under low power and High power of microscope.

b) Powder microscopy of leaves of *Clerodendrum inerme (L) Gaertn*

Leaves of *Clerodendrum inerme* are dried in a shade at 40°C for 2-3 days to make it moisture free and grounded using electric grinder and 60# powder was prepared. The powdered slide was treated with phloroglucinol and a drop of concentrated hydrochloric acid to stain the lignified elements. The powdered slide was also treated with iodine to stain the starch grains.

PHYTOCHEMICAL STUDY

Determination of Extractive values[57,58]

The determination of Extractive values helps to determine the number of soluble constituents in a given amount of medicinal plant material, when extracted with solvents.

The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the

nature of drug and solvent used. The use of single solvent can also be used by means of providing preliminary information of quality of a particular drug sample.

Determination of water-soluble extractive:

Place about 4 gm of coarsely powdered air-dried material, accurately weighed, in a glass- stoppered conical flask. Macerated with 100 ml of the chloroform-water (0.25 % chloroform in water) for 6 hours, shaking frequently, and then allow standing for 18 hours. Filter rapidly taking care not to lose any solvent, transfer 25 ml of the filtrate to a tared flat-bottomed dish and evaporate to dryness on a water-bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay. Calculate the content of extractable matter in mg per g of air-dried material.

Determination of alcohol soluble extractive:

Alcohol-soluble extractive value was obtained by following the same procedure as described for water soluble extractive using alcohol instead of Water.

Determination of ash values[58,59]

Determination of total ash:

Accurately weighed 2 g of the powdered drug was taken in a crucible and it was incinerated at a temperature not exceeding 4500C in muffle furnace until free from carbon. The sample was cooled and weighed. The residue was collected on an ashless filter paper and. The percentage of ash was calculated with reference to the air-dried drug.

Determination of acid-insoluble ash:.

The ash obtained as described above was boiled for 5 min. with 25 ml of dilute hydrochloric acid. The insoluble matter was collected in a Gooch crucible or on an ashless filter paper and washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

Determination of water-soluble ash:

The ash obtained as described in the determination of total ash was boiled for 5 min with 25 ml of water and insoluble matter was collected in a Gooch crucible, or on an ashless filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 4500C.

Weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS[60]

The powder of dried leaves of Clerodendrum inerme was subjected to systemic preliminary phytochemical screening after extraction using appropriate solvents. The extracts were subjected for phytochemical investigation by qualitative chemical tests.

Extraction of plant *Clerodendrum inerme (L) Gaertn*:

Dried and coarsely 500 g powdered leaves of Clerodendrum inerme was extracted with petroleum ether by maceration for two days. Filter and mark are extracted with 60% (v/v) ethanol and distilled water for ethanol and aqueous extract respectively in soxhlet apparatus for 36 hr. Filter the filtrate. The filtrate was concentrated on water bath using petridish. The temperature was maintained at 55 0c.

Qualitative chemical identification[56,57]

The extracts were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils and fats, proteins an amino acid, flavonoids, saponins, etc. using reported methods.

Alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were tested carefully & treated with alkaloid reagents.

i. Mayer's Test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow cream precipitate indicated the presence of alkaloids.

ii. Wagner's Test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. Formation of brown or reddish-brown precipitate indicated the presence of alkaloids.

iii. Dragendorff's Test: Filtrates were treated with Dragendorff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicated the presence of alkaloids.

iv. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow coloured precipitate indicated the presence of alkaloids.

Proteins and Amino acids

i. Millon's Test: The extracts were treated with 2 ml of Mallon's reagent. The formation of white precipitate, which turned to red upon heating, indicated the presence of proteins and amino acids.

ii. Biurets Test: The extracts were treated with 1ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purplish violet colour indicated the presence of proteins.

iii. Ninhydrin Test: To the extracts, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicated presence of amino acid.

Carbohydrates: Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

i. Benedict's test: Filtrates were treated with Benedict's reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

ii. Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.

iii. Fehling's Test: Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. A red precipitate was formed which indicated the presence of carbohydrates.

iv. Barfoed's Test: Filtrates were treated with Barfoed's reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

Flavonoids: To a 2-3 ml of ethanolic extract, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid was added. Pink red or red coloration of the solution indicated the presence of flavonoids in the drug.

Phenols: A drop of ethanolic extract was spotted on a filter paper and a drop of phosphomolybdic acid reagent was added on it. The spot was then exposed to ammonia vapor. Blue coloration of the spot indicated the presence of phenols.

Glycosides: Extracts were hydrolyzed with dilute hydrochloric acid and the hydrolysate was subjected to glycosides tests.

i. Modified Borntrager's Test: The extracts were treated with ferric chloride solution and heated on boiling water bath for about 5 mins. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. The formation of rose pink or cherry red colour in the ammonical layer indicated the presence of anthranol glycoside.

ii. Legal's Test: The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to red colour indicated the presence of cardiac glycosides.

iii. Baljit Test: The extract of drug was treated with sodium picrate and the formation of a yellowish orange colour confirmed the presence of cardiac glycosides.

iv. Killer killani Test: Take 0.5g of dried extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solutions. This was then under laid with 1 ml of concentrated H2SO4. A brown ring obtained at the presence of a cardenolides

Saponins

Froth's Test: The extracts (alcoholic and aqueous) were diluted with 20 ml of distilled water separately and further shaken for 15 mins in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicated the presence of saponins.

Tannins (Phenolic compounds)

i. Ferric chloride Test: The extract was treated with few drops of neutral ferric chloride solution (5%). The formation of bluish black color indicated the presence of phenolic nucleus.

ii. Lead acetate Test: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

iii. Alkaline reagent Test: The extracts were treated with few drops of sodium hydroxide separately. Formation of intense yellow color, which turned colorless on addition of few drops of dilute acid, indicated the presence of flavonoids.

iv. Shinoda Test: The extracts were treated with few fragments of magnesium metal separately, followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta colour indicated the presence of flavonoid.

v. Vanillin hydrochloric Test: The extracts were treated with few drops of vanillin hydrochloride reagent. The formation of pinkish red colour indicated the presence of tannins.

Steroids and Triterpenoids

i. Libermann-Burchard test: To one ml of ethanolic extract of drug, one ml of chloroform and 2 to 3 ml of acetic anhydride was added. To the above mixture, 1 to 2 drops of concentrated Sulphuric acid was added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of triterpenoids.

ii. Salkowski's test: Treat extract in chloroform with few drops of concentrated Sulfuric acid, shake well and allow standing for some time, red colour appears in the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicating the presence of triterpenoids.

Fixed Oils and Fats

i. Stain test: Small quantity of extracts was pressed between two filter papers separately. An oily stain on filter paper indicated the presence of fixed oil.

ii. Saponification test: The extracts were heated on water bath with 0.5 N alcoholic potassium hydroxide solutions. Formation of soap indicated the presence of fixed oils and fats.

**RESULTS ANDDISCUSSION**

Identification and Authentication of *Clerodendrum inerme*

The plant of *Clerodendrum inerme* were collected January 2022, from pioneer pharmacy degree college ,Vadodara, Gujarat. The plant was identified by comparing its morphological and microscopical with description given in different standard texts and floras25. Besides these, the plant was then identified and authenticated by Dr.P.K.PATEL and a voucher specimen was deposited.

Fig. 9 Herb of *Clerodendruminerme (L) Gaertn*

Morphology of leaf and Leaflet of *clerodendruminerme*

Leaves: *Clerodendrum inerme* is an evergreen sprawling shrub 1-1.8 m tall. The stem is woody and smooth. The leaves are ovate to elliptical, 5-10 cm long and 2-5 cm wide. The leaves have acute to acuminate tip, smooth and slightly shiny upper surface, pinnate venation, margins entire, leaves opposite and simple.

Stem: Stems were quadrangular, 7 to 12 cm in length. Generally branching starts from the base and internodes were short and 0.8 to 1.5 cm long.

Root: Roots of plant were thin, rough, 5 to15 cm in length, and 0.3 to 2.5 cm in diameter, externally light yellow while internally Creamish white.

4.3 Microscopic evaluation of *Clerodendrum inerme*

Transverse section of *Clerodendrum inerme* leaves showed epidermal layer of thin walled, columnar palisade, covered externally with thick cuticle; cells flat at base, mostly pointed but a few flattened at apex, followed by 4-5 layers of tangentially elongated, thin walled, parenchymatous cells. Generally parenchymatous cells are spongy in nature. Epidermis contains some bicellular uniseriate covering trichomes. Both covering and glandular trichomes are present. The covering trichomes are uni-seriate, multicellular, warty and with blunt apex. The glandular trichomes have one stalk consisting of one cell and multicellular head. The mesophyll has spongy parenchyma and palisade parenchyma in it. Palisade cells are radially elongated, single layer and compactly arranged. Spongy parenchyma are several layers, loosely arranged consisting of micro-sphenoidal crystals and vascular strands. In the midrib, strips of collenchymas appear below the upper and above the lower epidermis followed by the cortical parenchymatous cells containing calcium oxalate. In the centre part of midrib it contains Phloem which covers xylem. Phloem and xylem both are lignified. Transverse section shows a bifacial structure. The lower epidermis is similar to that of the upper one but has more number of trichomes and stomata when compared with upper epidermis.

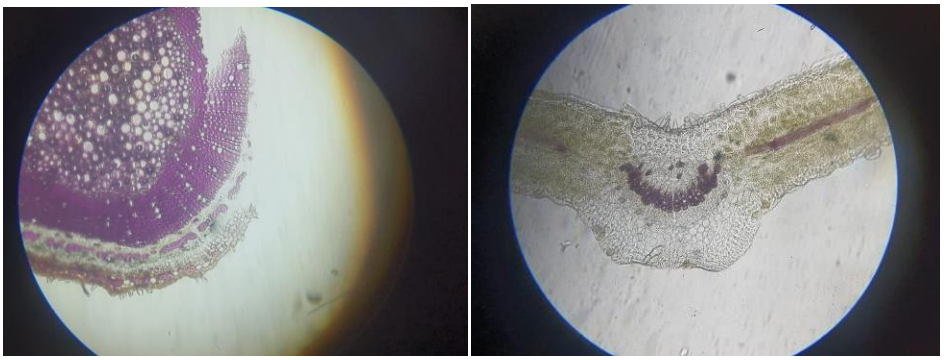

Fig. 10 Transverse section of *Clerodendrum inerme (L) Gaertn* leaves through midrib

Powder study of *Clerodendrum inerme (L) Gaertn* leaves.

The fragments of the epidermis of the leaf were composed of cells containing yellowish- brown pigment. Cells were polygonal and regular with thick walls and a small lumen from which radiate distinct pits. The fragments of the lamina were composed of a single layer of colorless cells with a very characteristic appearance. They were polygonal in appearance with the rods of thickening joining the upper and lower walls. Parenchyma cells were wavy surface containing spongy look. The epidermis contained two different types of trichomes. Among them one was bicellular uniseriate covering trichomes and other was sessile trichomes. Leaf contains stomata which were generally anisocytic but in rare cases paracytic stomata were also seen. Xylem and phloem were lignified and xylem was reticulate type, sometimes bordered pittedtype.

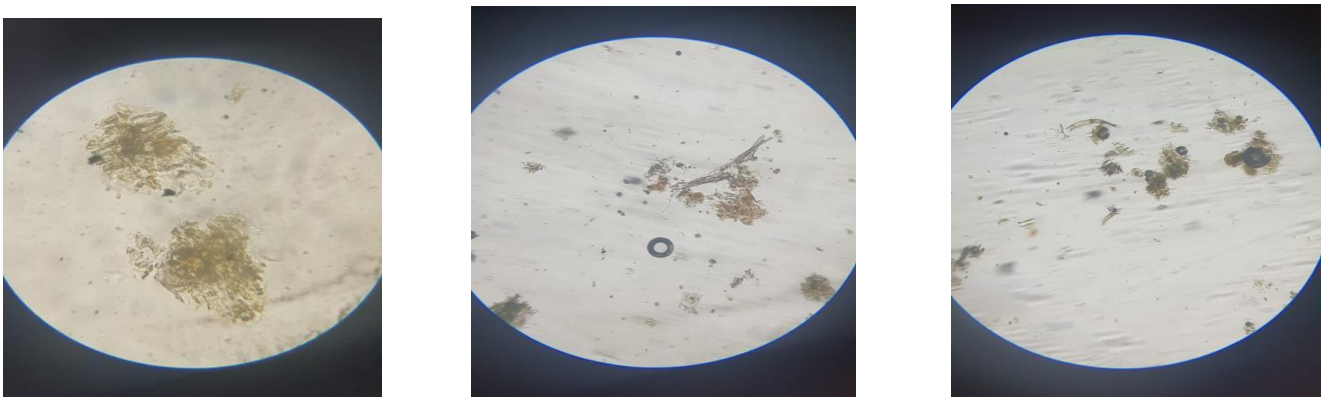

Fig. 11 Powder study of *Clerodendrum inerme (L) Gaertn*

Phytochemical Parameters of *Clerodendrum inerme* Leaves:

Phytochemical Parameters of *Clerodendrum inerme* Leaves:

Table 4. Physical parameter of *Clerodendrum inerme.* leaves

| Sr. No. | Physical Parameters | % w/w |
|---|---|---|
| **1** | Total ash | **12.05** |
| **2** | Acid-insoluble ash | **4.63** |
| **3** | Water soluble ash | **1.24** |

| | | |
|---|---|---|
| **4** | Sulfated ash | **1.11** |
| **5** | Alcohol soluble extractive | **3.69** |
| **6** | Water soluble extractive | **5.15** |
| **7** | Petroleum Ether soluble ctive | **2.50** |
| **8** | Chloroform soluble extractive | **2.31** |
| **9** | Moisture content | **56.86** |
| **10** | **Foreign matter** | **1.89** |

In preliminary study *Clerodendrum inerme* leaves showed total ash (12.05%), acid-insoluble ash (4.63%), water soluble ash (1.24%) and sulfated ash (1.11%). Petroleum Ether soluble extractive were higher (2.50%) than water soluble extractive (5,15%), alcohol soluble extractive (3.69%) and chloroform soluble extractive (2.31%).

Quantitative microscopy of Aerial part of *Clerodendrum inerme (L) Gaertn*

Quantitative microscopy of Aerial part of *Clerodendrum inerme.* was done and stomatal index, vein-islet number, vein termination number and palisade ratio were determined which are given in Table 5

Table 5 Quantitative microscopy of leaf of *Clerodendrum inerme.*

| Sr. | Determination | Value ( per sq. mm ) |
|---|---|---|
| **i)** | Stomatal index Upper epidermis Lower epidermis | **13.25-16.35** **12.15-15.45** |
| **ii)** | Vein-islet number | **23-33** |
| **iii)** | **Vein-termination number** | **35- 46** |

Preliminary Phytoprofile

The percentage of different chemical constituents in the crude drug can be detected by subjecting them to successive extraction using solvents in the order of increasing polarity. The extract obtained were then dried completely and kept in vacuum desiccators. They were then subjected to qualitative chemical tests in order to detect the various chemical constituents present in them. The *Clerodendrum inerme* extracts were screened for various chemical investigations and the results are mentioned in Table no.7

Table 7. Preliminary Phytoprofile of aerial parts of *Clerodendrum inerme.*

| Sr. No. | Solvent | Color and consistency after drying | Average value (%w/w) |
|---|---|---|---|
| **1.** | Petroleum ether (60-80°c) | Yellowish, solid mass | **2.50** |
| **2.** | Ethyl acetate | Greenish, sticky mass | **3.21** |
| **3.** | Chloroform | Greenish, sticky mass | **2.13** |
| **4.** | Methanol | Greenish yellow, sticky mass | **4.21** |
| 5. | Water | Dark Brown solid mass | 6.61 |

Tests for preliminary Phytochemical screening of powder of aerial part of *Clerodendrum inerme*:-

Qualitative chemical examination of various successive extracts of powder indicated the presence of carbohydrates, steroids, Triterpenoid glycosides, alkaloid, phytosterols, steroids, mucilage.

Phytosterols were detected by Libermann Burchard test and salkowaski reaction, carbohydrates by molisch's, Fehling's and Benedict's test, saponin by foam test, flavonoids by shinoda test, tannins and phenolics by Lead acetatetest.

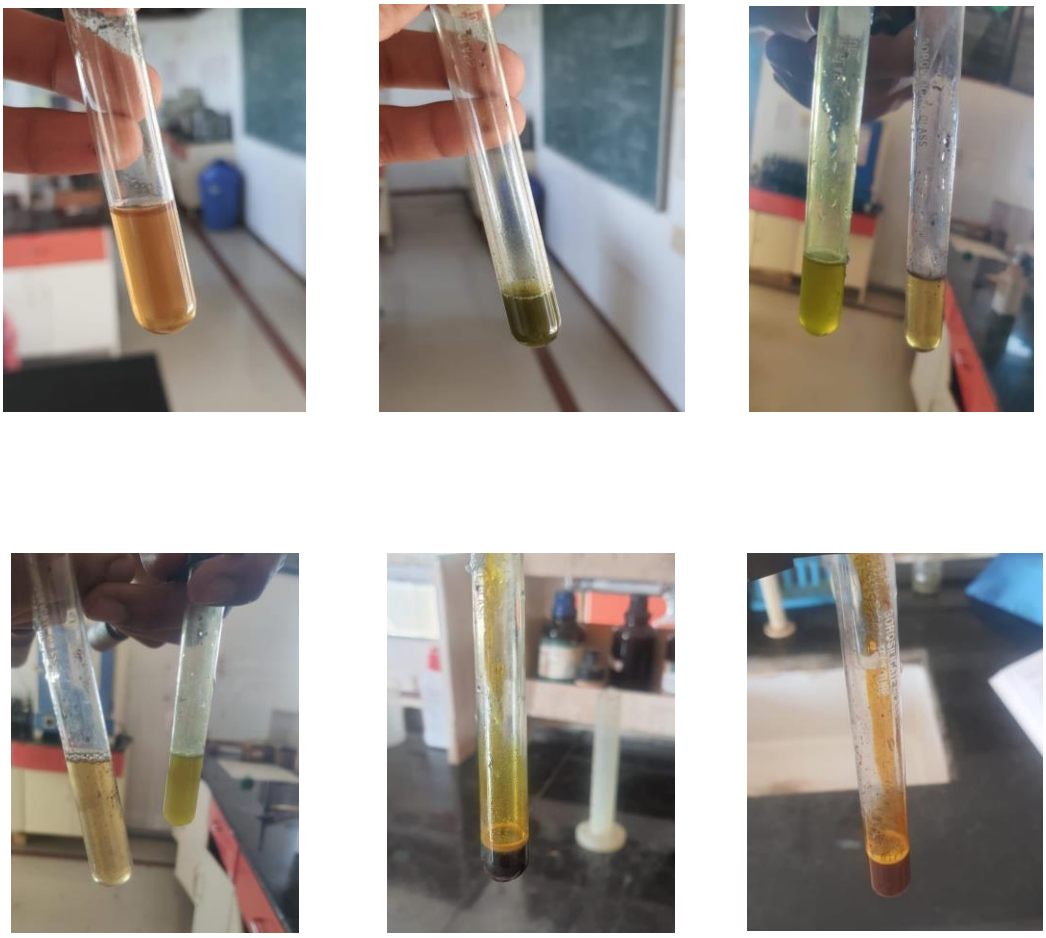

Fig. 12 Positive result of chemical test for preliminary testing

Table 8. Test for Preliminary Phytochemical screening of aerial parts of *Clerodendrum inerme.*

| SR NO | Tests of phytoconstituents | P. ether extract | Ethyl acetate extract | Chloroform extract | Methanol Extract | Water Extract |
|---|---|---|---|---|---|---|
| **1** | Tests for alkaloids | | | | | |
| | a) Mayer'sreagent | * | * | -ve | -ve | **-ve** |
| | b) Dragendorff'sreagent | * | * | +ve | +ve | **-ve** |
| | c) Hager'sreagent | * | * | * | * | * |
| | d) Wagner's reagent | * | * | * | * | * |
| **2** | Tests for flavonoids | | | | | |
| | a) Shinodatest | * | * | * | * | * |
| | b) Fluorescence test | * | * | * | * | * |
| | c) FeCl3test | * | * | * | +ve | **-ve** |
| | d) Lead acetate test | * | * | * | +ve | **-ve** |
| **3** | Tests for saponins | | | | | |
| | a) Froth test | * | * | * | * | * |
| | b) Hemolyticzone | * | * | * | * | * |
| **4** | Tests for carbohydrates | | | | | |
| | a) Molisch'stes | * | * | * | -ve | **+ve** |
| | b) Fehling's solutiontest | * | * | * | -ve | **-ve** |
| | c) Benedict'stest: | * | * | * | -ve | **+ve** |
| | Tests for cardiac glycoside | | | | | |
| | a) Legal'stest | * | | * | * | * |
| | b) Keller Killiani'stest | * | | * | * | * |
| | c) Baljettest | * | | * | * | * |
| **6** | Tests for fixed oil andfat | | | | | |
| | a) Spot test | | | | | |
| | b) Saponification test | * | * | * | * | * |
| | | * | * | * | * | * |
| **7** | Tests for sterols and triterpenoids | | | | | |
| | a) Libermann-burchard`stest | * | * | * | * | * |
| | b) Salkowski reaction | * | * | * | * | * |
| **8** | Tests for anthraquinone glycosides | | | | | |
| | a) Borntrager'stest | * | * | * | * | * |
| | b) Modifying borntrager'stest | * | * | * | * | * |

| | | | | | | |
|---|---|---|---|---|---|---|
| **9** | Tests for phenolic compounds<br>a) Test withFecl3<br>b) Test with folin-ciocalteureagent | <br>*<br>* | <br>*<br>* | <br>*<br>* | <br>+ve<br>* | <br>**-ve**<br>* |
| **10** | Tests forcoumarins<br>a) Withammonia<br>b) With hydroxylamine hydrochloride | <br>*<br>* | <br>*<br>* | <br>*<br>* | <br>*<br>* | <br>*<br>* |
| **11** | **Tests for tannins**<br>**a) Test withgelatin**<br>**b) Reaction with leadacetate** | <br>*<br>* | <br>*<br>* | <br>*<br>* | <br>*<br>**+ve** | <br>*<br>**-ve** |

SUMMARY AND CONCLUSION

Transverse section of *Clerodendrum inerme* leaves shown epidermal layer of thin walled, columnar palisade, covered externally with thick cuticle; cells flat at base, mostly pointed but a few flattened at apex, followed by 4-5 layers of tangentially elongated, thin walled, parenchymatous cells. Generally parenchymatous cells were spongy in nature. Epidermis contains some bicellular uniseriate covering Trichomes. Some times also sessile glandular Trichomes are present but they were only at lower epidermis. In the centre part of midrib it contains Phloem which covers xylem. Phloem and xylem both were lignified. Microscopy of leaf powder of *Clerodendrum inerme* Linn showed the presence of epidermis and hypodermis of leaves, parenchyma, lamina, stomata (Paracytic and Anisocytic), bicellular uniceriate covering trichomes and sessile glandular trichomes, anastomus and pitted xylem vessel.

*Clerodendrum inerme.* leaves contain total ash (12.05%), acid-insoluble ash (4.63%), water soluble ash (1.24%) and sulfated ash (1.11%). Petroleum Ether soluble extractive were higher (2.50%) than water soluble extractive (5,15%), alcohol soluble extractive (3.69%) and chloroform soluble extractive (2.31%). Ethanol extract of Clerodendrum inerme.leaves was dark yellowish, semisolid and the yield 14.52% w/w while Aqueous extract was greenish, semisolid and the yield 16.37% w/w. Qualitative chemical examinations of ethanol and aqueous extracts revealed the presence of Alkaloid, Carbohydrates, Flavonoids, Saponins, Steroids, Triterpenoids, Glycosides and Tannins.

Ethno-medical importance of various species of Clerodendrum genus has been reported in various indigenous systems of medicines and as folk medicines. The genus is being used as medicines specifically in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of various life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension The researches on Clerodendrum inerme provide the proof that it contains chemical constituents like Triterpenoids, Tannins, diterpenoids, Alkaloids, glycosides, phenols, flavonoids, and, volatile oils and steroids. However, the researches also

prove that it is used as anti-diabetic, anti–microbial anti-inflammatory, anti-hepatotoxic activity, anti-malarial, anti-oxidant. These plant mainly used for the traditional purpose like febrifugal and uterine stimulant. Ethno-medical importance of various species of Clerodendrum genus has been reported in various indigenous systems of medicines and as folk medicines. Along with biological studies, isolation and identification studies of chemical constituents and its correlation with the biological activities of the genus has also been studied. Also phytochemical investigation of plant done.

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