

Qualitative Investigation and Screening of Antimicrobial Activity of Stem Extract of Clerodendrum Infortunatum Plant

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ABSTRACT

Clerodendrum infortunatum is also known as hill glory bower. *Clerodendrum infortunatum* plants are widely distributed throughout the whole world. Up to now, many species of *C. infortunatum* have been described in various indigenous systems of medicine that are used in preparation of folklore medicines for the treatment of various life-threatening disease, and more of the *Clerodendrum infortunatum* have been very well studied for their chemical constituents and biological activities. It also used in Unani, Ayurveda, and Siddha system of medicine for many years. In the *Clerodendrum Infortunatum* many compounds, including monoterpenes and its derivatives, sesquiterpene, di-terpenoids, tri-terpentine, flavonoid, quercetin, acacetin, gallic acid, sterols and flavonoid glycoside, phenylethanoid glycoside, steroids and steroid glycosides, cyclohexylethanoids, anthraquinone, cyanogenic glycosides, and others have been isolated and identified. In the present study, Chloroform, pet.ether, and water stem extract *Clerodendrum infortunatum* obtained by Soxhlet extraction was screened to detect the presence or absence of several bioactive compounds which are reported to cure different diseases. Anti-microbial analysis of stem extract was carried out against *Lacto-bacillus*, *E.coli* and *Staphylococcus aureus* organisms by agar well diffusion method. It was observed that the zone was recorded against this organism. The results indicate that the chloroform, pet.ether and water extract of *C. infortunatum* is having anti-microbial efficiency in controlling the microorganisms. So, *clerodendrum infortunatum* is the plant which are beneficial on human health.

Keywords- *Clerodendrum Infortunatum*, qualitative investigation and anti-microbial activity.

I. INTRODUCTION

Clerodendrum Infortunatum is a plant (Fig. 1) from the Lamiaceae family that has been used as medicine in India for centuries. In Hindi and Malayalam, the plant is referred to as Peruvellam and Bhand, respectively. It is frequently observed in India's arid plains and lands. The therapeutic qualities of leaves, bark, roots, flowers, stems, and seeds are present. The plant's many parts have been used to cure digestive issues, anaemia, malaria, inflammatory illnesses, tumors, snake bites, and more. In all parts of life, plants have been a beneficial starting point for the creation of drugs. Lead optimization programs are used to create safe and

efficacious medicines by using phytochemicals as templates. Many active substances, including Alkaloids, terpenoids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, and flavonoids that are deposited in their respective sections, such as leaves, flowers, bark, seeds, fruits etc(1).





Figure 1: *Clerodendrum Infortunatum* plant

As stated by the World Health Organisation "(2) Approximately 75% of people on the planet use plants as medicine for a variety of ailments. The widespread use of medicinal plants in healthcare is mostly due to a number of causes, including the adverse effects of synthetic pharmaceuticals, the high expense of industrialised medicines, and the lack of public access to pharmaceutical and medical treatment. (3). Plants are always surrounded by an enormous number of potential enemies such as bacteria, viruses, fungi, insect(4). Natural products have been a consistently successful source in drug discovery and silly offer more opportunities to find antimicrobial drugs(5).

II. PARTS OF CLERODENDRUM INFORTUNATUM

The following are some of the parts of the *clerodendrum infortunatum* that have varied chemical contents that produce various biological actions or responses in the body:

- ❖ Leaves
- ❖ Flowers
- ❖ Seeds and fruits
- ❖ Roots and stem

Leaves:

Simple, opposite, villous-pubescent leaves with denticles that are elliptic, broadly elliptic, ovate or elongate ovate, 3.5–20 cm (1.4–7.9 in) wide, and 6–25 cm (2.4–9.8 in) long. Few-flowered, terminal, pedunculated cyme with pubescent white blooms that have a dull-pink or pinkish-purple throat. Scutellarin and hispidulin-7-glucuronide are two chemical substances that have been isolated from leaves. (6) analgesic, antimicrobial, anticonvulsants, antioxidant which are given by *C. infortunatum* leaves.

Flowers:

Clerodendrum infortunatum is a little shrub with gorgeous centre panicles of long-lasting white and purple flowers. The awful, ugly leaves that give rise to its Latin name, *infortunatum*, seem unfair given how lovely the blossoms are. The chemical components that are separated from the blossoms or flowers include fumaric acid, caffeic acid esters, B-sitosterol, apigenin, acacetin, etc (7). It given Anthelmintics pharmacological action in the body.

Seeds And fruits:

The fruit is a beautiful dark metallic blue

drupe with a diameter of 1 cm (0.4 in). Fruit typically contains four dry nutlets, and the seeds may or may not have endosperm. The seeds contain 24-methyl-sterols (24-methylcholestanol, 24-methylcholesterol, 24-methyl-22-dehydrocholesterol, and 24-methyl-22-dehydroclerothero), 24-methyl-sterols (clerosterol and 22-dehydroclerosterol), and 24-beta-ethyl-22-dehydrocholestanol. (8).

Roots and aerial parts:

Major part of the *Clerodendrum infortunatum* plant include the roots. The root is then used to cure many diseases, like anti-tumor, cancer, and cytotoxic effects on the body. so that the in vitro and in vivo root extracts of *C. infortunatum*. 24 beta-ethylsterols, clerosterol and 22-dehydroclerosterol, 24-methyl-sterols (24-methylcholestanol, 24-methylcholesterol, 24-methyl-22-dehydrocholesterol, and 24-methyl-22-dehydrocholestanol) and 24 beta-ethyl-22-dehydrocholestanol are found in the seeds. (8) The stem of *c.infortunatum* (Hill Glory Bower) are hollow. 24 ethylcholesta-5,22E diene-3B-ol these are the compound found in aerial parts(9). which give different pharmacological action in the body.

Scientific classification

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Lamiales
Family	Lamiaceae
Genus	Clerodendrum
Species	Clerodendrum infortunatum
Synonyms	Clerodendrum visosum vent Clerodendrum Calycinum turcz

Ethnobotanical uses of C. Infortunatum

According to the "sohel *et al*" the plant is found in Bhola district, Bangladesh which are isolated from the different chemical constituents from the leaf, root, fruit which are used to cure scabies, fever and anthelmintic activities in the living beings. (10) In Cachar district, Assam, (India) "Das *et al*" extract different constituents from the leaf which are used to treatment of Diabetes, Dyworming, and Dysentery (11). Other way that Pregnant ladies apply leaf juice around their vaginal opening to facilitate uncomplicated birth which is given by "Prasad *et al*" isolated from the Wayanad district, Kerala, (India) (12). "Rao *et al*" are the personnel which are given the various compounds from the root in Visakhapatnam district, Andhra Pradesh, (India)(13). According to the "Lal and Singh" from Hazaribag district, Jharkhand, (India) which are

extracted compound from the lead and root can be prevented and used to control swelling, stomachic and malaria (14).“ Rahmatullah *et al*” which are derived phytochemical constituent from the leaf extract from Mymensingh district (Bangladesh) To treat diabetes, crushed leaves of *C. infortunatum* and *Catharanthus roseus* are dissolved in water, and a teaspoon of this mixture is administered before meals(15).



Figure 2: *Clerodendrum infortunatum*

Geographical Regions Of *C.infortunatum*

In *Celerodendrum Infortunatum* Plant which have various species That are found in tropical regions of different country such India, Pakistan, Bangladesh, Myanmar, Thailand, Malaysia, The Andaman Islands, and Sri Lanka (16) . In india it is found in Malappuram district (Kerala), Visakhapatnam district (Andhra Pradesh), Jalpaiguri district (West Bengal), Nadia district (West Bengal), Sivasagar district (Assam), Wayanad district (Kerala), Barpeta district (Assam), Sonebhadra district (Uttar Pradesh), Wardha district (Maharashtra), Madhya Pradesh, Uttar Pradesh, Uttarakhand, India (17).

Laboratory Method

Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new syn-thetized molecules as potential antimicrobial agents (18). However, when we reviewed the published articles on the anti-microbial effect of these natural products, the comparison between results is often difficult, because of the use of different non-standardized approaches inoculum preparation techniques, inoculum size, growth medium, incubation conditions and endpoints determination(19). The fact that a plant extract exhibits antimicrobial activity is of interest, but this preliminary part of data should be trustworthy and allow researchers to compare results, avoiding work in which researchers use the antimicrobial activity investigation only as a complement to a phytochemical study(20).



Figure 3: Mixture Grinder

A variety of laboratory methods can be used to evaluate or screen the *in vitro* antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-dif-fusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as poisoned food technique. To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometric methods are re-commended, which provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent) and the cell damage inflicted to the test microorganism(21).

Owing to the new attraction to the properties of new anti-microbial products like combating multidrug-resistant bacteria, it is important to develop a better understanding of the current methods available for screening and/or quantifying the anti-microbial effect of an extract or a pure compound for its applications in human health, agriculture and environment. Therefore, in this review, the techniques for evaluating the *in vitro* antimicrobial activity were discussed in detail(22).



Figure 4: Soxhlet extractor

It was originally designed for the extraction of a lipid from a solid material. Typically, Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in

that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. In this extraction, a small amount of dry sample is placed in a thimble, which is placed in a distillation flask containing the solvent of particular interest. After reaching an overflow level, the solution of the thimble-holder is aspirated by a siphon, which unloads the solution back into the distillation flask(23).

by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, lacto-bacillus, Haemophilus influenzae, Haemophilus parainfluenzae, Neisseria gonorrhoeae and Neisseria meningitidis, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones (24). Antibigram provides qualitative results by categorizing bacteria as susceptible, intermediate or resistant (25). Therefore, it is a typing tool based on the resistance phenotype of the microbial strain tested, its outcomes also guide clinicians in the appropriate selection of initial empiric treatments, and antibiotics used for individual patients in particular situations (26). However, since the bacterial growth inhibition does not mean the bacterial death, this method cannot distinguish bactericidal and bacteriostatic effects.

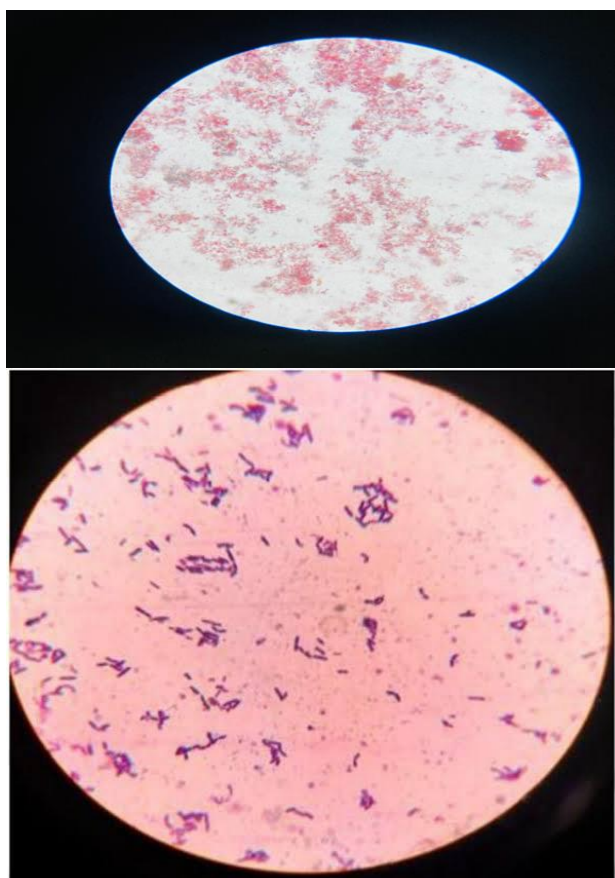


Figure 5: Lacto-Bacillus Bacteria

Lactobacillus is a genus of gram-positive, aerotolerant anaerobes or microaerophilic, rod-shaped,

non-spore-forming bacteria. (27) Until 2020, the genus Lactobacillus comprised over 260 phylogenetically, ecologically, and metabolically diverse species; a taxonomic revision of the genus assigned lactobacilli to 25 genera. (28)

Lactobacillus species constitute a significant component of the human and animal microbiota at a number of body sites, such as the digestive system, and the female genital system(29). Lactobacillus exhibits a mutualistic relationship with the human body, as it protects the host against potential invasions by pathogens, and in turn, the host provides a source of nutrients(30). Lactobacilli are among the most common probiotic found in food such as yogurt, and it is diverse in its application to maintain human well-being, as it can help treat diarrhea, vaginal infections, and skin disorders such as eczema(31).

Lactobacilli are homofermentative, i.e. hexoses are metabolised by glycolysis to lactate as major end product, or heterofermentative, i.e. hexoses are metabolised by the Phosphoketolase pathway to lactate, CO₂ and acetate or ethanol as major end products(32). Most lactobacilli are aerotolerant and some species respire if heme and menaquinone are present in the growth medium. Aerotolerance of lactobacilli is manganese-dependent and has been explored (and explained) in Lactiplantibacillus plantarum (previously Lactobacillus plantarum)(33).

Lactobacillus species are dominant in yogurt, cheese, and sourdough fermentations. The antibacterial and antifungal activity of lactobacilli relies on production of bacteriocins and low molecular weight compounds that inhibits these microorganism(34).

III. METHOD AND MATERIALS

Plant collection

These are the plant which are collected from majhau village, dehradun district (Uttarakhand). First the fresh stem of the collected plant were separated, wash cleanly several time using tapwater, thereafter the stem were rinsed by using distilled water, and then shade dried at 28 °C for 72 hour. The stem dried stem were grind into a powder from this material kept in air tight bottle for other extraction(35).



Figure 6: Dried stem powder of clerodendrum infortunatum

Preparation of extracts

Crude plant extracts were prepared by Soxhlet extraction method. About 35gm of powdered *Clerodendrum Infortunatum* plant material was uniformly packed into a thimble and extracted with 400ml of different solvents separately. Solvents used were, Petroleum ether, Water and chloroform. The process of extraction continued for 24 hours or till the solvent in siphon tube of an extractor became colorless. After that the extracts were taken in the beakers and kept on hot plate and heated at 30-40°C till all the solvents got evaporated. Dried extracts were kept in refrigerator until use(36).



Figure 7: Chloroform extract.



Figure 8: Water extract.



Figure 9: pet.ether extract

Qualitative analysis of *Clerodendrum Infortunatum* plant

Stem extracts prepared in three different solvents (water, petroleum ether, chloroform) were used for experimental purpose.

Test for flavonoids:

2-3 drop of sodium hydroxide were add to 2ml of petroleum ether extract it give deep yellow color so this solution indicating that flavonoid is present. 2-3 drop of sodium hydroxide were add to 2ml of chloroform extract it become colorless by adding few drop of dilute HCL it give yellow color which indicate flavonoid is present. 2-3 drop of NaOH were add 2 ml of water a yellow color appear which indicate flavonoid is present(37).

Test for alkaloids:

Few drops of Mayer's reagent were added to 1 mL of chloroform extract. A yellowish precipitate was formed, indicating the presence of alkaloids. 1ml of water extract which added few drop of Mayer's reagent desire precipitate was formed which indicating alkaloids are present in the solution. few drop of Meyer's reagent were added to 1ml of pet. ether it give yellow precipitate which indicate there is alkaloids present(38).

Test for phenol:

Few drop of ferric chloride solution were added to 1ml of water extract. A dark green precipitate was formed, indicating presence of phenol. 1 ml of pet. ether extract add few drop of ferric chloride solution give colorless precipitate which indicate there is no phenol compound. Similarly in chloroform extract it give greenish color was formed which indicate there is presence of phenol.

Test for anthraquinone

In anthraquinone test, Bromine test is used. 2ml of bromine to add equal volume of pet ether extract to give pink precipitate which indicate there is presence of anthraquinone. In water and chloroform extract which do not give pink precipitate which mean there is no presence of anthraquinone

Test for terpenoids:

5ml of pet. ether, and aqueous solution was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid were carefully added a reddish brown coloration of the interface was formed to show the presence of terpenoids.

Test for tannins:

In the tannins test ferric reagent is use in this test 3 drop of ferric reagent were added to a 2ml of sample (chloroform, pet. ether, water) extract the chloroform extract and pet. extract give gray color which indicate the presence of tannin water doesn't give color which indicate there is no presence of tannin.

Test for steroids

This test is given by Liebermann Burchard reaction to the chloroform solution in a test tube few drop of acetic anhydride was added 1ml of concentrated sulphuric acid allowed to stand a reddish ring was

formed which mean there is presence of steroids in the pet.ether and aqueous solution doesn't give positive reaction

Test for glycosides:

In glucosides test at first 0.5ml of glacial acetic acid and 2-3 drop of ferric chloride was mixed with Chloroform, pet.ether, water then 1 ml of concentrated H2SO4 then water and pet.ether gives deep blue color which indicate presence of glycosides.

Table 1 Phytochemical analysis of steam extract

S.No	Test	Pet.ether	Chloroform	Water
1	Flavonoids	+	+	+
2	Alkaloids	+	+	+
3	Phenol	-	+	+
4	Anthraquinone	+	-	-
5	Terpenoids	+	-	+
6	Tannins	+	+	-
7	Steroids	-	+	-
8	Glycosides	+	-	+

Preparation of agar media

In a beaker, 28 grams of the dehydrated powder or lab-prepared media is added to 1000 milliliters of distilled water. The suspension is then heated to boiling to dissolve the medium completely. The dissolved medium is then autoclaved at 15 lbs pressure (121°C) for 15 minutes. Once the autoclaving process is complete, the beaker is taken out and cooled to a temperature of about 40-45°C. The media is then poured into sterile Petri plates under sterile conditions. Once the media solidifies, the plates can be placed in the hot air oven at a lower heat setting for a few minutes to remove any moisture present on the plates before use(39).

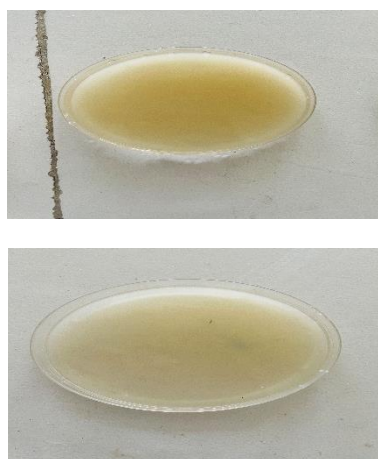


Figure 10: Agar media

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the

entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested(40).

Anti-Microbial Activity

The chloroform, pet.ether, and water extract was prepared by using the Soxhlet apparatus of Clerodendrum infortunatum to study its antimicrobial potential. Antimicrobial analysis of extract was carried out against lacto-bacillus organisms. The zone of inhibition in mm for the tested organism with the Chloroform, pet.ether and water extract of Clerodendrum infortunatum and by agar well diffusion method. In the present study, Chloroform, pet.ether, and water, stem extract Clerodendrum infortunatum obtained by Soxhlet extraction was screened to detect the presence or absence of several bioactive compounds which are reported to cure different diseases. Antimicrobial analysis of stem extract was carried out against lacto-bacillus organisms by agar well diffusion method. It was observed that the zone of inhibition was recorded against lacto-bacillus organism. The results indicate that the chloroform, pet.ether and water extract of C. amboinicus is having anti-microbial efficiency in controlling the microorganisms.



Figure 11: Zone of inhibition of Lactobacillus from Pet.ether extract(C.infortunatum plant)



Figure 12: Zone of inhibition of Lactobacillus from chloroform extract(C.infortunatum Plant)



Figure 13: Zone of inhibition of lactobacillus from Water extract (C.infortunatum Plant)

Table 2 Minimum inhibitory concentration of *Clerodendrum Infortunatum* plant

Extract	Standard(amoxicillin) in mm	25mcg	50mcg	100mcg	MIC
Pet.ether	26	20	22	25	23
Chloroform	24	19	21	26	22
Water	21	19	18	23	20

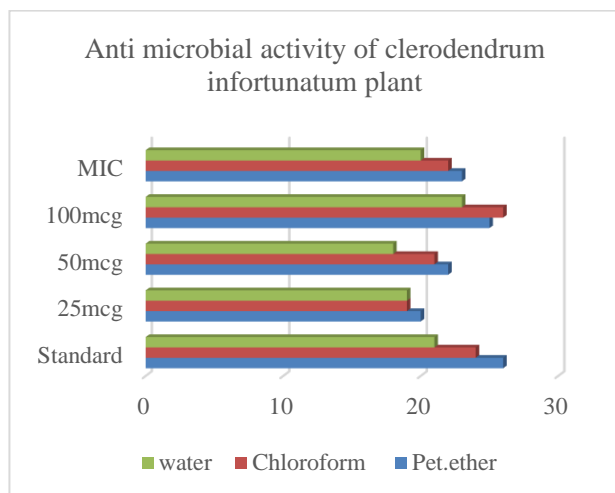
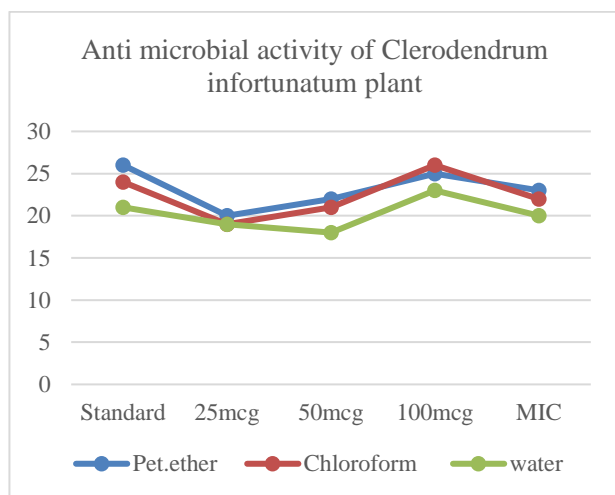


Fig: 14 Antimicrobial activity of Clerodendrum

IV. CONCLUSION

Numerous phytochemicals, including alkaloids, flavonoids, steroids, saponins, and tannins, were identified by the phytochemical study conducted in this paper. The phytochemical component verified the use of stem as a medical treatment. Indians today use medicinal plants and phytochemicals extensively for health treatment. *Clerodendrum infortunatum* can be identified with the help of this investigation. Investigations on the antimicrobial efficacy of *Clerodendrum infortunatum*'s water, pet.ether and chloroform extract against organisms resistant to many drugs have been conducted.

The findings of the investigation demonstrate that the dried stem powder extract of *Clerodendrum infortunatum* in various solvents exhibits a strong antimicrobial action against microbes that are resistant to drugs, including lacto bacillus.

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