

Qualitative investigation and screening of antimicrobial activity of stem extract in *Clerodendrum infortunatum* plant

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Abstract- *Clerodendrum infortunatum* is known as hill glory bower and a plant which is 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 diseases, and much of this plant have been very well studied for their chemical constituents and biological activities. It also is used in Unani, Ayurveda, and siddha system of medicine for many years. From *Clerodendrum infortunatum* many compounds, including mono-turpentine and its derivatives, sesquiterpene, diterpenoids, tri-terpentine, flavonoid, quercetin, acacetin, gallic acid, sterol 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 of *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 *lactobacillus*, *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 extracts of *C. infortunatum* are having anti- microbial efficiency in controlling the microorganisms. So, *Clerodendrum infortunatum* is the plant which are beneficial on human health.

Keyword: *Clerodendrum infortunatum*, qualitative investigation and anti-microbial activity.

Introduction

Clerodendrum infortunatum is a plant (Figure 1) from the Verbenaceae 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 found in India's arid plains and lands. The therapeutic qualities in leaves, bark, roots, flowers, stems, and seeds are present. The plant's many parts have been used to cure digestive issues, anemia, malaria, inflammatory illnesses, tumors, snakebites, and more. These plants have been 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 different sections, such as leaves, flowers, bark, seeds, fruits etc. (Figure 1).



Figure 1 *Clerodendrum infortunatum* plant

According to the World Health Organization^[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 offer more opportunities to find antimicrobial drugs^[5].

Benefits of *clerodendrum infortunatum*

Clerodendrum infortunatum provide Health benefits, financial benefits, and society-wide benefits.

Health benefits

Medicinal plants can enhance the immune response to disease agents and a potential source of bio-molecules to treat diseases like cancer, diabetes, and hypertension. These herbs are also known for their reputation for fewer side effects.

Financial benefit

People who harvest, process, and distribute medicinal plants for sale can earn financial benefits. Local traders can also sell their products at higher prices and open new markets.

Society-wide benefit

Medicinal plants provide society wide income and livelihood for people in the area. They also play a role in strengthening health care opportunity for people with noncommunicable disease.

Aim and Objective

- To study about qualitative investigation of its plant.
- To study about preparation of stem extract by using different extract solvent.

- To create antimicrobial agent by using stem extract from *Clerodendrum infortunatum* plant.

- MIC (Minimum Inhibitory Concentration)

Material and Methods

- Proper and timely collection of the *Clerodendrum infortunatum* plant
- Authentication by the expert
- Adequate drying
- Grinding
- Extraction

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 0C.for 72 hour. The stem dried stem were grind into a powder from this material kept in air tight bottle for other extraction^[6].



Figure-2 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^[7].



Figure 3 Chloroform extract.



Figure 4 Water extract.



Figure 5 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 added to 2ml of petroleum ether extract, it gave deep yellow color so this solution was indicating that flavonoid was present. 2-3 drop of sodium hydroxide were added to 2ml of chloroform extract, it became colorless by adding few drop of dilute HCL it give yellow color which indicated flavonoid were present. 2-3 drop of NaOH were added 2 ml of water a yellow color appeared which indicated flavonoid was present⁽⁸⁾.

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 desired 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⁽⁹⁾.

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 added few drop of ferric chloride solution gave colorless precipitate which indicated there is no phenol compound. Similarly, in chloroform extract it gave

greenish color which indicates that was presence of phenol.

Test for anthraquinone

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

Test for terpenoids

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

Test for tannins

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

Test for steroids

This test is given by Lieberman 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 showed there was presence of steroids in the pet.ether and the aqueous solution didn'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 gave deep blue color which indicated presence of glycosides.

Phytochemical analysis of steam extract

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⁽¹⁰⁾.



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

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⁽¹¹⁾.

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. Anti-microbial analysis of stem extract was carried out against lacto-bacillus organisms by agar well diffusion method. It was observed that the zone of was recorded against lacto-bacillus organism. The results indicates that the chloroform, pet.ether and water extract of *C. amboinicus* is having anti- microbial efficiency in controlling the microorganisms.



Figure-6 Zone of inhibition of Lactobacillus from Pet.ether extract (C.infortunatum plant)

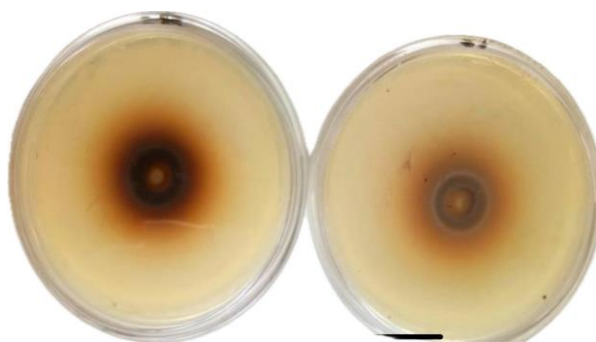


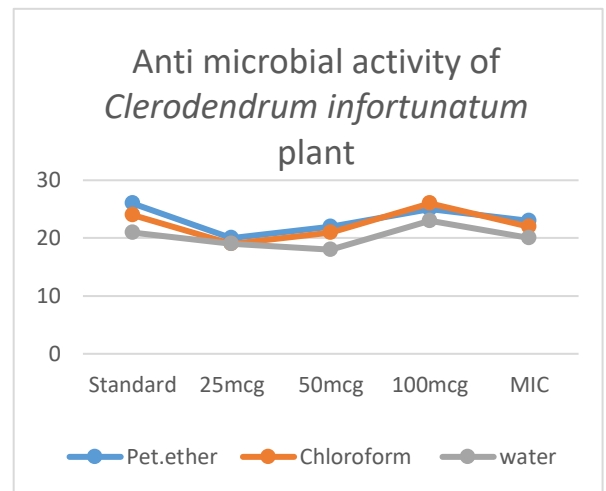
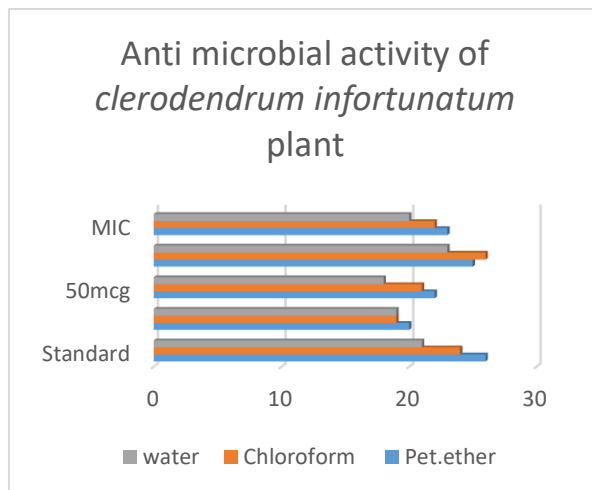
Figure-7 Zone of inhibition of Lactobacillus from chloroform extract (C.infortunatum Plant)



Figure-8 Zone of inhibition of lactobacillus from Water extract (C.infortunatum Plant)

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



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.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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