

## Phytochemical screening and evaluation of antibacterial, anti-diabetic and antioxidant activities of leaf and flower extracts of *Clerodendrum infortunatum* L.

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| <p><b>Original Research Article</b><br/>Received on January 06, 2026<br/>Revised on January 11, 2026<br/>Accepted on February 05, 2026<br/>Published on February 09, 2026</p> <p><b>Article Authors</b><br/>Anusha P. G., Jisha S.,<br/>Deepthi G. R., Santhi W. S.</p> <p><b>Corresponding Author Email</b><br/><a href="mailto:santhiws2020@gmail.com">santhiws2020@gmail.com</a></p> | <p>Medicinal plants have long been used to prevent and treat diseases, containing complex bioactive compounds with diverse pharmacological potentials. They form the basis of many traditional medicine systems and continue to inspire modern pharmaceuticals. <i>Clerodendrum infortunatum</i> L., commonly found in tropical South and Southeast Asia, particularly India, has a rich history in traditional medicine, though detailed scientific studies on its bioactive compounds and therapeutic effects remain limited. This study aimed to investigate the phytochemical constituents and assess the in vitro antimicrobial, antioxidant, and anti-diabetic activities of distilled water, isopropyl alcohol, and chloroform extracts from the leaves and flowers of <i>Clerodendrum infortunatum</i> L. Fresh leaves and flowers of <i>Clerodendron infortunatum</i> L. were collected from natural habitat, Alappuzha district, Kerala. Cold extraction method was used for the preparation of. Qualitative phytochemical screening was carried out by using standard procedures. The antimicrobial, antioxidant, and anti-diabetic activities were evaluated using the agar well diffusion method, DPPH free radical scavenging assay, and alpha-amylase inhibitory assay, respectively. Phytochemical screening of <i>Clerodendrum infortunatum</i> revealed alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, and glycosides, in various flower extracts showing a broader and more intense profile, especially in chloroform and isopropyl alcohol. Flower extracts exhibited stronger antibacterial activity, while leaf extracts, particularly chloroform extracts, showed higher antioxidant and anti-diabetic potential. These findings highlight the plant's medicinal value as a source of natural antibacterial, antidiabetic, and antioxidant agents.</p> |
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### HOW TO CITE THIS ARTICLE

Anusha P. G., Jisha S., Deepthi G. R., Santhi W. S. (2026) Phytochemical screening and evaluation of antibacterial, anti-diabetic and antioxidant activities of leaf and flower extracts of *Clerodendrum infortunatum* L., *International Journal of Agricultural Invention*, 11(1): 16-23. DOI: 10.46492/IJAI/2026.11.1.3

Plants have been used as sources of medicine since ancient times, playing a vital role in maintaining human health and combating diseases. Medicinal plants are utilized successfully across all continents, forming an integral part of traditional healthcare systems. In Asia, in particular, the practice of herbal medicine is highly developed and well documented, reflecting a long history of ethnobotanical knowledge and cultural tradition. Phytochemicals are biologically active, naturally occurring chemical compounds derived from plants.

Phytochemicals occur in different plant parts including roots, stem, leaves, flowers, fruits, seeds etc. They contribute towards aroma, colour and flavour of plants. These compounds also have many biological and pharmacological properties. *Clerodendrum infortunatum* L., (fig 1) commonly known as “Bhant” or “Hill Glory Bower,” is a medicinal plant widely found in the tropical regions of South and Southeast Asia, particularly in India. Under APG IV system of flowering plant classification, this plant is placed in the Lamiaceae family (Chase *et al.*, 2016).

For generations, local communities have used various parts of this plant leaves, roots, and flowers treat common ailments such as fever, skin infections, wounds, and respiratory issues. Despite its rich history in traditional medicine, there is still a need for detailed scientific evaluation of its bioactive properties (Das *et al.*, 2014). This study aims to investigate the phytochemical constituents and evaluate the *in vitro* antibacterial, antioxidant, and antidiabetic activities of the leaf and flower extracts of *Clerodendrum infortunatum* L, using three different solvents, chloroform, isopropyl alcohol, and distilled water.



Fig 1. Habit of *Clerodendrum infortunatum* L

## Materials and Methods

### Collection of Plant Materials and Preparation of Extracts

Fresh leaves and flowers of *Clerodendron infortunatum* L. were collected from natural habitat, Alappuzha district of Kerala, India. Leaves and flowers were separated, washed carefully with tap water, rinsed with distilled water, air dried, and shade dried. The dried materials were then coarsely powdered and stored at room temperature until further use. Extracts were prepared using the cold extraction method with distilled water, isopropyl alcohol, and chloroform as solvents. The extraction was carried out in a 1:25 ratio (1 part plant material to 25 parts solvent) for 24 hours at room temperature.

All extracts were filtered using Whatman No. 1 filter paper. The solvent was evaporated and the residues were stored at 4 °C till use. 0.1 mg of the extract was dissolved in 1 ml of dimethyl sulfoxide for further analysis.

### Qualitative Phytochemical Analysis

The extract was tested for the presence of bioactive compounds by using following standard methods (Trease and Evans, 1989; Sofowara, 1993).

#### Test for Alkaloids

Crude extract was mixed with 2 ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

#### Test for Carbohydrate

The solvent extracts were dissolved separately in 5 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

**Barfoed's Test:** Crude extract when mixed with 2 ml of Benedict's reagent and boiled, a reddish-brown precipitate formed which indicated the presence of the carbohydrates.

#### Test for Reducing Sugar

**Fehling's Test:** The extract was diluted in 5 ml of distilled water and filtered. 1 ml of the filtrate was treated with 1 ml of Fehling's solution A and B and boiled in a water bath. A reddish precipitate obtained would show the presence of sugar.

#### Test for Flavonoids

**Ferric Chloride Test:** To the 1ml of solvent extract few drops of 10% ferric chloride solution is added and a green precipitate indicate the presence of flavonoids.

#### Test for Phenolic Compounds

**Iodine Test:** To 1 ml of the extract few drops of dil. Iodine solution was added. A transient red colour indicates the presence of phenolic compounds.

### Test for Tannins

**Braymer's Test:** To the 1 ml of the extract 3ml distilled water is added and adds 3 drops 10% Ferric chloride solution. A Blue-green colour indicates the presence of tannins.

### Test for Phlobatannins

To the 2 ml aqueous extract add 2 ml of 1% HCl and boiled. A red precipitate indicates the presence of phlobatannins.

### Test for Terpenoids

2 ml chloroform is added to the 5 ml plant extract, (evaporated on water bath) and add 3mL conc. H<sub>2</sub>SO<sub>4</sub> (boiled on water bath). A grey coloured solution indicates the presence of Terpenoids.

### Test for Quinones

**Conc. HCl Test:** To the 2 ml extract add 2 ml of conc. HCl, a green colour is obtained by the presence of quinones.

### Detection of Phytosterols

**Salkowski's Test:** Add a few drops of conc. H<sub>2</sub>SO<sub>4</sub> in the 2 ml of the extract and shaken well and allowed to stand, a red layer is obtained in the lower part indicate phytosterols.

### Test for Carboxylic Acid

**Effervescence Test:** To 1 ml plant extract add 1 ml sodium bicarbonate solution. Appearance of effervescence indicates the presence of carboxylic acid.

### Test for Saponins

**Froth Test:** Approximately 2 ml of the plant extract was mixed with 5ml of distilled water in a test tube and shaken vigorously for 30 seconds. The mixture was allowed to stand undisturbed for 15 minutes. The formation of a stable, persistent froth ( $\geq 1$  cm in height) was indicative of the presence of saponins.

### Test for Glycosides

To 1 ml of the extract, 2 ml of glacial acetic acid containing a trace of ferric chloride was added.

This was followed by the careful addition of 1 ml concentrated sulfuric acid along the side of the test tube. The appearance of a brown ring at the interface confirmed the presence of cardiac glycosides.

### Test for Steroids

About 2 ml of the extract was dissolved in 2 ml of chloroform, and 2 ml of concentrated sulfuric acid was added slowly along the sides of the test tube. The formation of a reddish-brown ring at the interface was taken as a positive indication of steroids.

### Antibacterial Studies

Agar well diffusion method is widely used to evaluate antimicrobial activity. These bacterial strains are procured from Microbial Type Culture Collection (MTCC, Chandigarh, India) were employed in the present study to investigate the antibacterial properties. Nutrient agar and nutrient broth were used for storage and sub-culturing of the bacterial pathogens. The Gram-negative organisms such as *Salmonella typhi* and *Bacillus subtilis*, and Gram-positive organism, *Staphylococcus aureus* were employed as test pathogens. The antimicrobial activity of *Clerodendrum infortunatum* leaf and flower extracts (chloroform, isopropyl alcohol, and distilled water) was assessed using the Agar well diffusion assay (Perez *et al.*, 1990) against *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella typhi*. After sterile nutrient agar was poured into Petri dishes (25.0 mL per plate for ~4 mm thickness) and allowed to solidify, wells of 5.0 mm diameter were punched using a sterile cork borer (Tippayatum and Chonhenchob, 2007). Each agar plate was inoculated with 100  $\mu$ L of an actively growing bacterial suspension (*S. aureus*, *B. subtilis*, or *S. typhi*) using a sterile swab to ensure uniform distribution. Plates were allowed to dry at room temperature. Then, 100  $\mu$ L of each extract (chloroform, isopropyl alcohol, and distilled water) from the leaf and flower samples were pipetted into the respective wells. A sterile ampicillin disc (10  $\mu$ g) was placed at the center of each plate to serve as a positive control. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition around each well and the antibiotic disc was measured in millimeters using a transparent ruler.

Each experiment was conducted in duplicate, and the results were recorded and considered for analysis (Oke *et al.*, 2001). Antibiotic ampicillin was used as Positive control. Solvents used for preparation of extracts were (distilled water, chloroform, isopropyl alcohol) were used as negative control for that particular extract.

### Antioxidant Free Radical Scavenging Assay

The antioxidant activity of different extracts (chloroform, distilled water, and isopropyl alcohol) of leaf and flower parts of *Clerodendrum infortunatum* was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, as per the modified method of (Sharma and Thakur, 2022). One milliliter of each plant extract (at a specific concentration, e.g., 1 mg/mL) was taken in separate test tubes. To this, 2 ml of 0.1 mM DPPH solution prepared in methanol was added. The reaction mixture was mixed well and incubated in dark at room temperature for 30 minutes to ensure complete reaction. A control solution was prepared by mixing 1 ml of the respective solvent (chloroform, distilled water, or isopropyl alcohol) with 2 ml of DPPH solution. Methanol was used as the blank. After incubation, the absorbance of all samples, controls, and blank was measured at 517 nm using a UV-visible spectrophotometer. The degree of discoloration indicates the scavenging potential of the extract due to hydrogen-donating ability. The assay was carried out in triplicate.

**Calculation: Percentage inhibition = (Absorbance of control – Absorbance of sample) ×100 / Absorbance of control**

### Anti Diabetic Assay

Alpha-amylase inhibitory assay was carried out. A starch solution (1% W/V) was prepared by stirring 0.52 g starch in 50 ml of 0.1M of phosphate buffer (pH 6.9). To the reaction mixture containing extract 100 ml (1%) starch solution was added and incubated at 37 degree C for 10 minutes. The reaction was stopped by adding 1.5 ml DNSA (1g of 3,5 di nitro salicylic acid, 30 g of sodium potassium tartarate and 20 ml of 2 N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 minutes.

The reaction mixture diluted with 2.2 ml of water and absorbance was read at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 ml in distilled water. Control, representing 100% enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol (Ali *et al.*, 2006).

**Calculation: Percentage inhibition = (Absorbance of control – Absorbance of sample) ×100 / Absorbance of control**

### Results and Discussion

#### Qualitative Phytochemical Analysis of Leaf and Flower Extracts

The results of preliminary qualitative phytochemical screening are summarized in tables 1 and 2.

**Table 1. Phytochemical analysis of leaf extracts**

| Phytochemicals analyzed | Extracts        |            |                   |
|-------------------------|-----------------|------------|-------------------|
|                         | Distilled Water | Chloroform | Isopropyl Alcohol |
| Alkaloids               | ++              | +          | +                 |
| Flavonoids              | ++              | ±          | ++                |
| Phenolic compounds      | ++              | ±          | ++                |
| Tannins                 | +               | -          | ±                 |
| Phlobatannins           | +               | -          | ±                 |
| Terpenoids              | ±               | +          | +                 |
| Quinones                | ±               | +          | +                 |
| Carbohydrates           | ++              | -          | ±                 |
| Proteins                | +               | -          | ±                 |
| Amino acids             | +               | -          | ±                 |
| Carboxylic acids        | +               | ±          | ±                 |
| Saponins                | ±               | -          | ±                 |
| Glycosides              | ±               | -          | ±                 |
| Steroids                | ±               | ±          | ±                 |

**Note:** ++ –Strongly present, + –Present, ± –Moderately present / slightly present / Trace amount- – Absent

The results revealed the presence of various phytochemicals. In the aqueous extract, both leaf and flower samples tested positive for alkaloids, carbohydrates, and proteins and amino acids. While flavonoids, phenolic, tannins, phlobatannins, and carboxylic acids were also detected in both flower and leaf extracts, supporting their traditional uses in inflammation and wound healing.

**Table 2. Phytochemical analysis of flower extracts**

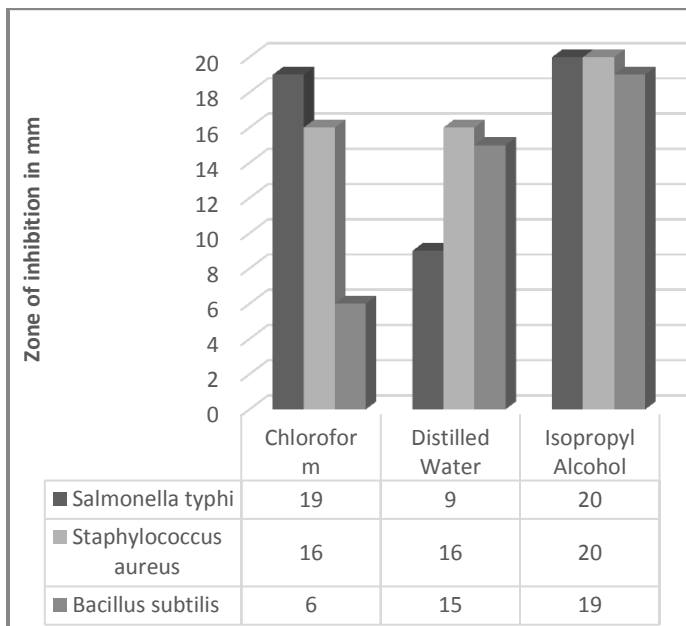
| Phytochemicals Analyzed | Extracts        |            |                   |
|-------------------------|-----------------|------------|-------------------|
|                         | Distilled Water | Chloroform | Isopropyl Alcohol |
| Alkaloids               | ++              | +          | +                 |
| Flavonoids              | ++              | +          | ++                |
| Phenolic compounds      | ++              | +          | ++                |
| Tannins                 | +               | -          | +                 |
| Phlobatannins           | +               | -          | +                 |
| Terpenoids              | ±               | +          | +                 |
| Quinones                | ±               | +          | +                 |
| Carbohydrates           | ++              | -          | ±                 |
| Proteins                | +               | -          | ±                 |
| Amino acids             | +               | -          | ±                 |
| Carboxylic acids        | +               | ±          | ±                 |
| Saponins                | ±               | -          | ±                 |
| Glycosides              | ±               | -          | ±                 |
| Steroids                | ±               | ±          | ±                 |

**Note:** ++ –Strongly present, + –Present, ± –Moderately present / slightly present / Trace amount, -- Absent

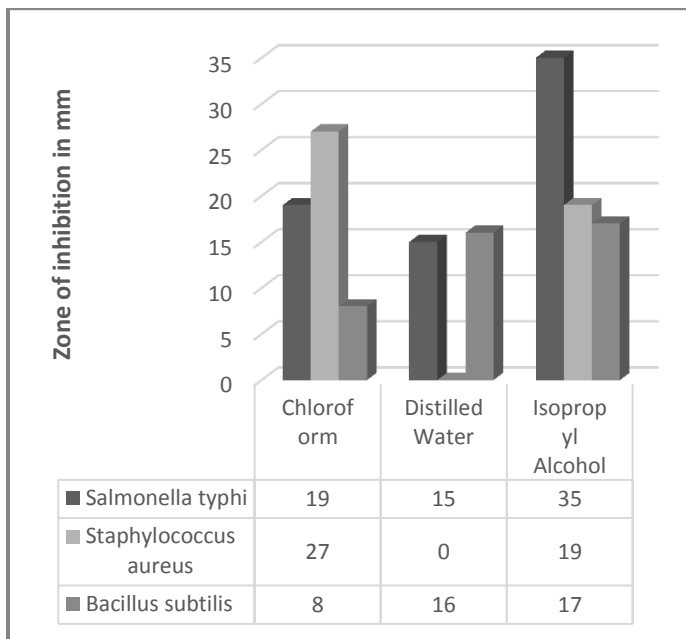
The chloroform extract showed a relatively lower presence of polar compounds including carbohydrates and proteins, but tested positive for alkaloids, terpenoids, and quinones, suggesting the extraction of more lipophilic bioactive compounds (Kumari and Singh, 2025; Pawar, 2023). The isopropyl alcohol extract exhibited a strong presence of phenolic compounds, flavonoids, and terpenoids in both leaf and flower extracts. This solvent effectively extracted both moderately polar and nonpolar constituents. Water proved to be the most effective solvent for extracting a wide range of constituents from *Clerodendrum infortunatum*, especially hydrophilic compounds such as alkaloids, phenolics, and carbohydrates. Moreover, the presence of these phytoconstituents validates the ethnomedicinal applications of *Clerodendrum infortunatum* and encourages further exploration into its pharmacological properties.

**Antibacterial Studies**

The present study reveals that the leaf and flower extracts of *Clerodendrum infortunatum* exhibit considerable antibacterial activity, with variations observed depending on the extract type, plant part, and target microorganisms.



**Fig 2. Antibacterial analysis of leaf extracts**



**Fig 3. Antibacterial analysis of flower extracts**

In this study antibiotic *Penicillin* was used as the positive control and it was observed that the microorganisms tested were resistant to *Penicillin* as no zone of inhibition was obtained for the antibiotic. The results are summarized in fig 2 and 3. The antimicrobial effects of *Clerodendrum infortunatum* leaf extracts varied with the type of solvent used for preparing extracts. The extracts demonstrated notable antimicrobial efficacy, showing inhibitory effects against both Gram-positive and Gram-negative bacteria, indicating a broad spectrum of antibacterial potential.

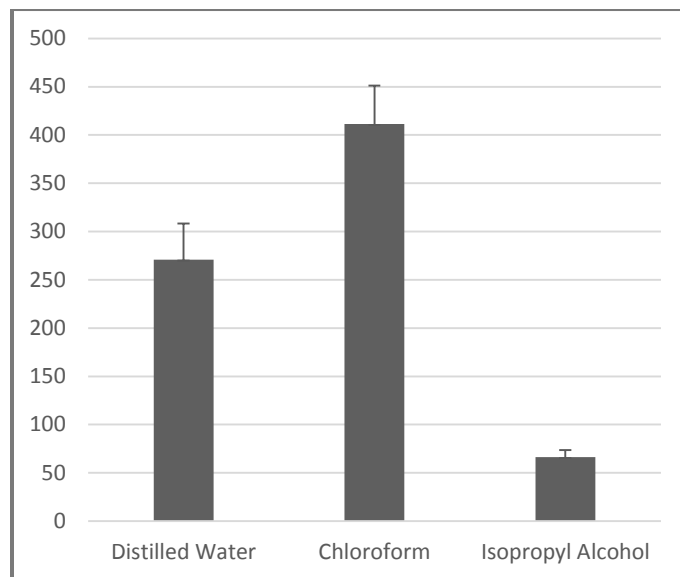
The flower extracts demonstrated stronger and broader antibacterial activity compared to leaf extracts, especially when extracted using chloroform and ethanol. The results suggest that the major antibacterial constituents present in the flowers are relatively less soluble in water. In general, flower extracts had superior efficacy against both Gram-positive and Gram-negative strains, showcasing the potential of *Clerodendrum infortunatum* flowers as a source of natural antimicrobials. Taken together, the tested microorganisms, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis* demonstrated differential sensitivity to the leaf and flower extracts prepared with chloroform, distilled water, and isopropyl alcohol.

The chloroform extracts derived from both the leaf and flower parts of the plant demonstrated significant antibacterial activity, suggesting the presence of bioactive compounds with potent antimicrobial potential. Gram-positive strains, such as *Staphylococcus aureus* and *Bacillus subtilis*, were generally more susceptible to both leaf and flower extracts, while Gram-negative *Salmonella typhi* exhibited lower sensitivity. This difference is likely due to the structural variations in bacterial cell walls, with Gram-positive bacteria being more permeable to phytochemicals. Overall, the results revealed that *Clerodendrum infortunatum* extracts were potentially effective in suppressing microbial growth and can be used as a source of potential natural antimicrobial agent (Aparna *et al.*, 2021). This finding is noteworthy because the microorganisms evaluated showed resistance to penicillin, yet demonstrated susceptibility to the plant extracts tested, indicating the potential of these extracts as alternative antimicrobial agents.

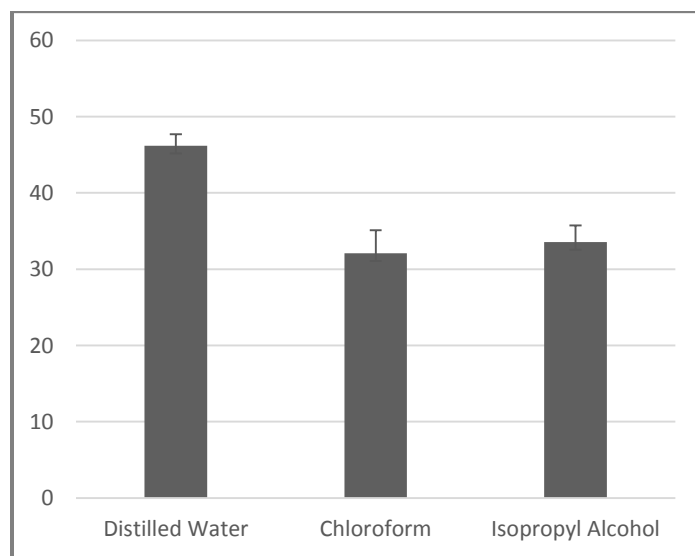
### Antioxidant Studies

DPPH (1,1-diphenyl-2-picryl hydrazyl) is a stable free radical that exhibits a maximum absorbance at 517 nm in methanol. Its color changes from deep purple to yellow when it accepts an electron or hydrogen atom from antioxidant molecules (or antioxidant extracts), resulting in the formation of a stable, non-radical (diamagnetic) molecule. The results of antioxidant DPPH assay of leaf and flower extracts of *Clerodendrum infortunatum* are summarised in fig 4 and 5.

The leaf extracts of *Clerodendrum infortunatum* demonstrated strong antioxidant activity across all solvents, particularly in chloroform and distilled water extracts (fig 4).

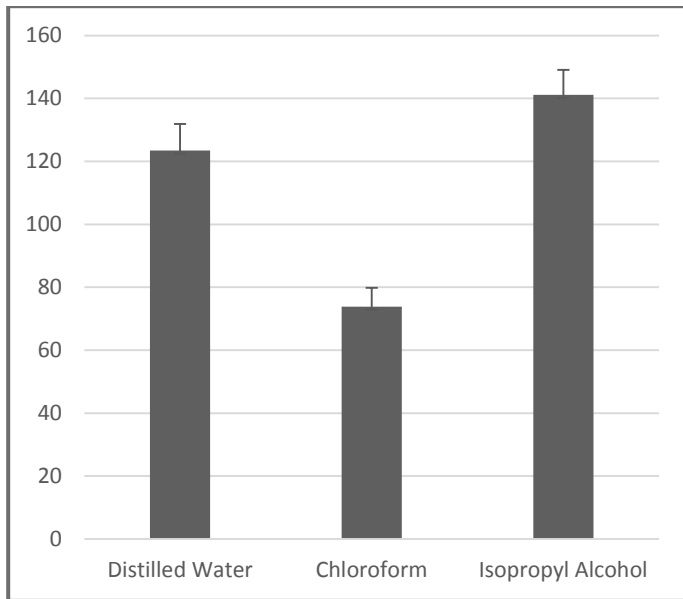


**Fig 4. Antioxidant analysis of leaf extracts**

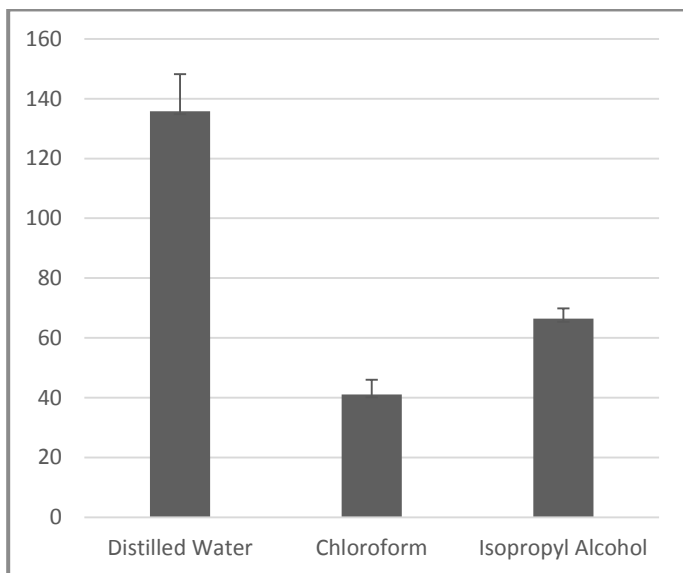


**Fig 5. Antioxidant analysis of flower extracts**

These findings suggest that the leaf is rich in antioxidant phytoconstituents, particularly those soluble in chloroform (non-polar) and water (polar), which may include phenolic compounds, flavonoids, and tannins. In contrast, both the flower and leaf extracts showed variable and comparatively lower antioxidant activity, with notable variations depending on the solvent used for extraction (fig 5).



**Fig 6. Percentage of Inhibition in leaf extracts**



**Fig 7. Percentage of Inhibition in flower extracts**

The results of this part of the study highlight a clear distinction in antioxidant potential between leaf and flower extracts of *Clerodendrum infortunatum*. Leaf extracts, particularly in chloroform and water, demonstrated significantly higher antioxidant activity, likely due to a higher concentration of bioactive compounds such as phenols, flavonoids, and tannins, which are known for their free radical scavenging properties (Khalaf *et al.*, 2008). This may indicate that the flowers possess fewer antioxidant constituents, or they may contain interfering or unstable compounds that reduce the effectiveness of the assay.

Additionally, the strong performance of chloroform leaf extract suggests that non-polar antioxidant compounds in the leaf are particularly potent antioxidant potential. This is supported by previous phytochemical screenings that showed the presence of alkaloids, flavonoids, and tannins in these fractions.

### Antidiabetic Studies

Alpha amylase digests carbohydrates and increases the glucose level in diabetic patients. Inhibiting the activity of enzyme can reduce the post prandial hyperglycemia, and reduce the risk of developing diabetes. This assay was performed using the 3,5-dinitrosalicylic acid method (DNSA method). Here the three different extracts (Chloroform, Distilled water and Isopropyl alcohol) *Clerodendrum infortunatum* leaf and flower were evaluated for antidiabetic activity and the results are summarized in fig 6 and 7. The isopropyl alcohol extract of *Clerodendrum infortunatum* leaves exhibited the highest antidiabetic activity among all leaf extracts, followed by the distilled water extract. In contrast, among the flower extracts, the distilled water extract demonstrated the strongest antidiabetic activity. Both leaf and flower extracts demonstrated considerable antidiabetic potential. Among the different solvent extracts, the distilled water extracts exhibited the strongest activity, followed by the isopropyl alcohol extracts, whereas the chloroform extracts showed comparatively lower efficacy. This suggests that polar solvents, such as distilled water and isopropyl alcohol, are more effective in extracting antidiabetic compounds compared to the non-polar solvent, chloroform.

The observed antidiabetic activity, as indicated by percentage inhibition, is therefore strongly dependent on solvent polarity, with polar solvents facilitating the extraction of more bioactive constituents responsible for the activity (Kumar *et al.*, 2025). According to the above results leaves of *Clerodendrum infortunatum* generally had higher and more consistent antidiabetic potential than flowers, except in distilled water, where both are comparably highly expressed. Based on the phytochemical, antibacterial, antioxidant and antidiabetic studies, *Clerodendrum infortunatum* exhibits significant medicinal potential, with both leaf and flower extracts showing bioactivity.

Both the leaf and flower extracts demonstrated strong antibacterial activity against both Gram-negative and Gram-positive microorganisms. In addition, they exhibited excellent antioxidant and antidiabetic properties, indicating broad-spectrum bioactivity and significant therapeutic potential. However, when all results are considered collectively, the leaf extracts appear to possess greater overall efficacy, suggesting a higher potential for pharmaceutical and therapeutic applications.

## Conclusion

To conclude with, the findings of this study confirm the medicinal value of *Clerodendrum infortunatum*, showcasing its strong potential as a source of natural antibacterial, antidiabetic, and antioxidant agents. While both the leaf and flower parts are rich in bioactive compounds, the leaf extract, particularly in chloroform and isopropyl alcohol solvents, demonstrated greater antibacterial activity and a broader spectrum of phytochemicals. Overall, *Clerodendrum infortunatum*, holds promising potential in pharmaceutical applications, warranting further exploration for drug development and clinical applications. Nevertheless, further studies are required to confirm these findings and to isolate and characterize the active compounds responsible for these activities.

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