



## Study of antibacterial activity and phytochemical screening of some medicinal plants occurring in Barpeta District, Assam, India

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### ABSTRACT

The present study was aimed to determine phytochemical constituents of the leaf of four medicinally important plants. *Ficus hirta* Vahl, *Clerodendrum colebrookianum* Walp., *Clerodendrum infortunatum* L., and *Dillenia indica* L. Further, their antibacterial activity was studied against two pathogenic bacteria viz. *Escherichia coli* and *Staphylococcus aureus*. The *in vitro* antibacterial activity of aqueous, ethanol and ethyl acetate extracts of the four medicinal plants were evaluated against the bacteria was determined by agar disc diffusion method. The ethanol and ethyl acetate extracts showed better antibacterial activity compared to the aqueous extract against the tested bacterial strains. Amongst the plant species screened extract of *Clerodendrum viscosum* showed best antimicrobial activity against both *Escherichia coli* and *Staphylococcus aureus*. The presence of active compounds such as sterols, terpenoids, alkaloids, carbohydrates, tannins, glycosides, saponins, proteins, carotenoids, flavonoids, and amino acids was observed in various solvents of *Clerodendrum colebrookianum* and *Clerodendrum infortunatum*. These medicinal plants with huge reservoir of important phytochemical compounds can be applied as an important source for drug development and other pharmaceutical applications.

**Keywords-** Medicinal plant, antibacterial activity, phytochemical screening, *Clerodendrum colebrookianum* Walp., Assam.

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### INTRODUCTION

Since the time of human civilization, medicinal plants have been playing an indispensable role on the health and healing of man. Plants are a large source of natural antioxidants thus the use of traditional medicine is widespread and might lead for the development of novel drugs (1). Plants have been a source of medicinal agents as many naturally occurring compounds present, show to possess antimicrobial activities and act as a source of antimicrobial agents against many pathogens (2). Moreover, many active components of herbal medications have the advantage of being incorporated with numerous other substances that seem to be inactive. Still, these interrelated components give the plant altogether a safety and productivity much superior to that of its isolated and pure active components (3).

Conventional healing methods throughout the world that employ herbal remedies are a principal source for the finding of new antibiotics. It has already produced abundant compounds and are potent against numerous antibiotic-resistant strains of bacteria. Hence, it specifies the demand for additional research into traditional health approaches. Need of the moment is to bring out a number of medicinal plants for promising biological activity.

*Ficus hirta* Vahl, *Clerodendrum colebrookianum* Walp., *Clerodendrum infortunatum* L. and *Dillenia indica* L. are commonly known as Hairy fig, glory bower, Hill glory bower and Elephant apple respectively. They are used to treat diarrhoea, diabetes, tumour, cancer, jaundice, blood pressure, menstruation pain, pneumonia, pox, constipation (4,5,6,7,8). Several investigations were carried out on antidiabetic, antioxidant, phytochemical, antimicrobial, Anti-neuroinflammatory and other aspects of the plants (*Ficus hirta* Vahl, *Clerodendrum colebrookianum* Walp., *Clerodendrum infortunatum* L. and *Dillenia indica* L.) by different workers (4,6,9,10). Different parts of *C. infortunatum* is known to have hepato-protective and antimicrobial activities and are used traditionally to cure skin diseases as well as diseases like rheumatism, asthma, alopecia, etc. (11).

The aim of this study is to evaluate the phytochemical contents and antimicrobial activity of some medicinal plants of Assam, used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms.

## MATERIAL AND METHODS

### Collection of Plant material

Leaves of *Ficus hirta* Vahl, *Clerodendrum colebrookianum* Walp., *Clerodendrum infortunatum* L. and *Dillenia indica* L. were collected from Barpeta town, Barpeta District, Assam (26.3216° N, 90.9821° E) during 2020-21. The details of the medicinal plant including the parts screened, their families, vernacular names, and ethnobotanical uses are given in Table 1. For antimicrobial and phytochemical analysis, fresh, disease free and tender leaves of selected plants were collected from the study area. Collected plant materials were cleaned thoroughly and washed under running water to remove dust and extra debris. The material were air-dried under shade at room temperature. Completely dried materials were ground to get fine powder and were dipped in different solvents -aqueous, ethanol and ethyl acetate extract by maceration technique at room temperature (12).

### Phytochemical screening

Phytochemical screening of all crude extracts was performed following standard protocols described by different authors (13,14). Aqueous, ethanol and ethyl acetate extracts of *Ficus hirta*, *Clerodendrum colebrookianum*, *Clerodendrum infortunatum* and *Dillenia indica* give a positive indication about the presence of flavonoid, steroid, saponin, starch, anthraquinone, terpenoid and tannin.

### Preparation of extracts

For aqueous extraction, 10 g of air-dried powder was added to distilled water and boiled on slow heat for 2 h. The mixture was then filtered through 8 layers of muslin cloth and then centrifuged was done at 5000g for 10 min. The supernatant was collected. After 6 h, the supernatant collected at an interval of every 2 h, was pooled together and concentrated to make the final volume one-fourth of the original volume (15). It was then autoclaved at 121°C temperature and stored at 4°C. For solvent extraction, 10 g of air-dried powder was taken in 100 ml of organic solvent (ethanol or ethyl acetate) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. The supernatant was collected after 24 hours and the solvent was evaporated to make the final volume to one-fourth of the original volume and stored at 4°C in airtight bottles (15).

### Antimicrobial assay

In vitro antimicrobial activity was examined for aqueous and ethanol extracts from four medicinal plants used by traditional healers. Microorganisms were obtained from Guwahati Neurological Research Centre, Guwahati, Assam, India. Microorganisms were stored at 4°C on nutrient agar slants. The pathogens include *Escherichia coli* and *Staphylococcus aureus*.

The antimicrobial assay was carried out by agar disc diffusion method (16). The molten Mueller Hinton Agar (Hi-Media) was inoculated with the 100 µl of the inoculum ( $1 \times 10^8$  CFU) and poured into the sterile Petri plates (Hi-media). For agar disc diffusion method, 100 µl of the test compound was poured on the disc (0.7cm) (Hi-Media), allowed to dry and was plated Mueller Hinton agar plate. The plates were incubated overnight at 37°C. After 24 hrs, the diameter of the zone of inhibition was measured. Pure solvents were used as the negative control. The result was obtained by measuring the zone diameter. The experiment was performed in three replicates and the mean values are presented. The results were compared with the standard antibiotic piperacillin (100 µg /disc).

### Statistical Analysis

All the results are the mean of three observations. Variation of data was expressed in Standard deviation (SD) of three observations.

## RESULTS AND DISCUSSION

Plants extracted medicines have acquired a significant contribution towards human health, thus, they have provided a source of inspiration for the development of medicine. Previous studies reported that the presence of antibacterial substances in the higher plants is well accepted (17). The phytochemical screening of *Ficus hirta*, *Clerodendrum colebrookianum*, *Clerodendrum infortunatum* and *Dillenia indica* showed the presence of flavonoid, steroid, saponin, starch, anthraquinone, terpenoid, tannin, glycoside and carbohydrate (Table 2). Phytochemical investigation of *Ficus hirta* Vahl. (Moraceae) fruits led to isolation of two carboline alkaloids, five sesquiterpenoids/norsesquiterpenoids, three flavonoids, and one phenylpropane-1,2-diol (18). Phytochemical analysis of the medicinal plants in the present study indicated the presence of sterols, terpenoids, alkaloids, carbohydrates, tannins, glycosides, saponins, proteins, carotenoids, flavonoids, and amino acids in various solvents of *Clerodendrum colebrookianum* and *Clerodendrum infortunatum*. The study indicated that the ethanolic extract of leaves of *Clerodendrum*

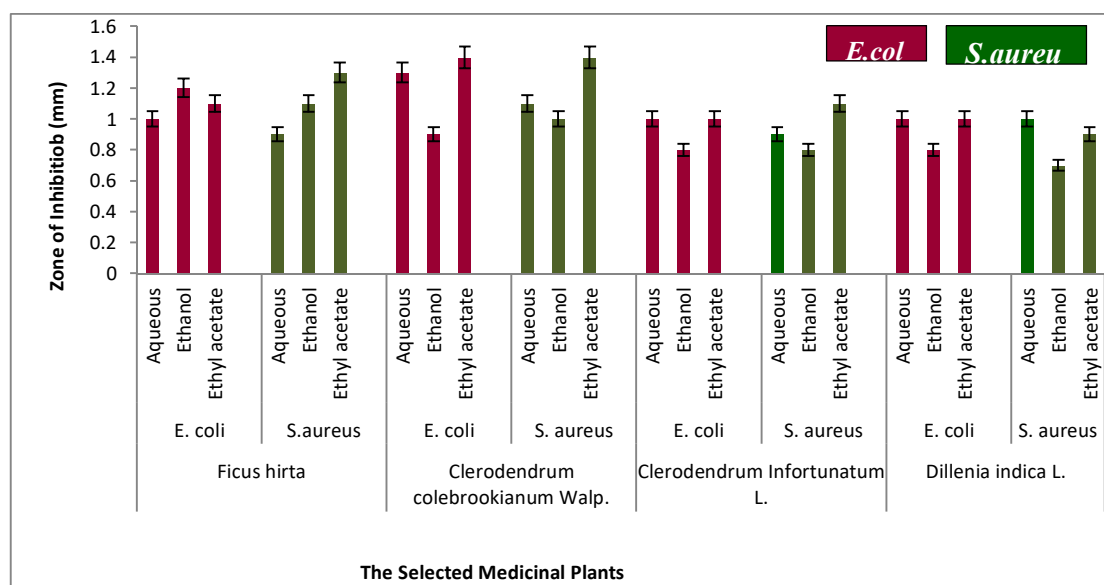
*infortunatum* contained the high presence of phytochemical components which has also been reported by some previous studies (19, 20). The phytochemical screening of the leaves and stem bark extracts showed the presence of saponins, glycosides, phenols, alkaloids, flavonoids, tannins, carbohydrates and resins in both leaf and stem bark extract of *Dillenia indica* (21).

The type of solvent used in the extraction procedure specifies the isolation of botanical compounds from plant material. Primarily, the traditional healers use water as the solvent but we found in this study the plant extracts by Ethyl acetate provided more consistent antimicrobial activity compared to those extracted by water and Ethanol.

In sensitivity test of disc agar diffusion method all aqueous, ethanol and ethyl acetate extract showed a very good zone of inhibition. It was observed that in case of *Ficus hirta*, ethanol extract and ethyl acetate extract showed the highest range of anti-microbial activity against *E. coli* and *S. aureus*. However, in case of *Dillenia indica*, ethyl acetate and aqueous extract show highest range of anti-microbial activity against *E. coli* and *S. aureus*. Nkop and Zudnou (2020) reported that ethanol extracts of the leave and stem bark of *Dillenia indica* exhibit moderately proven potential to contain antimicrobial agents of pharmacological interest. Similarly, both *Clerodendrum colebrookianum* and *Clerodendrum infortunatum* showed highest range of anti-microbial activity against *E. coli* and *S.aureus* in aqueous and ethyl acetate extract. The aqueous and ethyl acetate extracts showed better antibacterial activity than aqueous extract against the tested bacterial strains. Amongst the plant species screened ethanol extract of *Clerodendrum colebrookianum* showed best antimicrobial activity. The ethnolic extract of *Clerodundron infortunatum* showed average zone of inhibition against both gram positive (*S. mutans*) and gram negative (*P. aeruginosa*) bacteria (22). The results of antibacterial activity of all the four plants against the investigated bacterial strains are shown in Fig 1.

## CONCLUSION

Among the medicinal plants studied in the present work, *Clerodendrum colebrookianum* showed the most promising antimicrobial properties in aqueous and ethyl acetate extract against both *E. coli* and *S. aureus* indicating the potential for discovery of antibacterial principles. Further phytochemical studies are required to study the types of compounds responsible for the antibacterial effects of these medicinal plants. Leaf extract of *Ficus hirta* Vahl, *Clerodendrum colebrookianum* Walp., *Clerodendrum infortunatum* L. and *Dillenia indica* L possess different phytochemical components which have been reported for many important applications against different human pathogens, including those that cause several infections like malaria, eczema, typhoid, stomach disorder, etc. The present study indicated ethyl acetate as the most effective solvent for extraction of phytochemical components from *Clerodendrum colebrookianum* Walp., which also showed the highest zone of inhibition against both the studied bacterial pathogens. Thus, the use of these medicinal plants with rich source of phytochemical compounds and antibacterial activities could be an important source for drug development and pharmaceuticals.



**Figure.1** Zone of inhibition obtained against the studied microbial pathogens by different extract of *Ficus hirta*, *Clerodendrum colebrookianum* Walp., *Clerodendrum Infortunatum* L. and *Dillenia indica*. The bars represent the standard deviation of three observations.

**Table 1. Ethnobotanical uses of selected plants**

Scientific name	Family	Common name	Part used	Ethnobotanical uses
<i>Ficus hirta</i> Vahl.	Moraceae	Dimaru (Ass)	Root, Leaf	Roots are used by Hakka people in southern China to improve fatigue resistance as they have anti-inflammatory activity (23). It is used for the treatment of constipation, inflammation, postpartum hypogalactia, cancer and tumors (6). Leaves are used for skin diseases, cough, typhoid, dysentery.
<i>Clerodendrum colebrookianum</i> Walp.	Verbenaceae	Nefafu (Ass)	Leaf	It is used in treatment of high blood pressure, stomach disorder, hypertension, diabetes, etc. (24, 25). It has been used for the treatment of cough, dysentery, headache, stomach disorder, colic pain, hypertension, helminthic infections (26).
<i>Clerodendrum Infortunatum</i> L.	Verbenaceae	Bhat-ful (Ass)	Leaf	It is used in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy (27). Used to treat disorders such as headache, toothache, rheumatism, swelling, skin diseases, fever, diabetes, malaria, burns, tumor and epilepsy (11). Leaves are used for fever, skin diseases, intestinal infection, snake bite, etc.
<i>Dillenia indica</i> L.	Dilleniaceae	Ou-tega (Ass)	Leaf, stem, Bark, fruit	Diseases like leucoderma, skin rash, skin itches and eczema can be treated by different parts of the plant (28). Fruit is used for diabetes, diarrhea and dysentery, reduce dandruff (29). It is used to treat cancer and diarrhea (30). It is used to treat fever and cough and constipation (31).

**Table 2. Phytochemical screening of *Ficus hirta*, *Clerodendrum colebrookianum* Walp., *Clerodendrum Infortunatum* L. and *Dillenia indica***

PLANT	SOLVENT	FLAVONOIDS	STEROIDS	SAPONINS	STARCH	ANTHRAQUINONE	TERPENOIDS	TANNINS
<i>Ficus hirta</i> Vahl.	Aqueous	+	+	-	+	-	-	+
	Ethanol	-	+	-	+	-	+	-
	Ethyl acetate	+	+	-	+	-	-	+
<i>Clerodendrum colebrookianum</i> Walp.	Aqueous	+	-	-	+	+	-	+
	Ethanol	+	+	+	+	+	-	+
	Ethyl acetate	+	+	+	+	+	+	+
<i>Clerodendrum infortunatum</i> L.	Aqueous	+	+	+	+	+	+	-
	Ethanol	+	+	+	-	-	+	+
	Ethyl acetate	+	+	-	-	-	+	+
<i>Dillenia indica</i> L.	Aqueous	+	+	+	+	-	-	-
	Ethanol	+	+	+	+	+	+	-
	Ethyl acetate	+	+	+	-	-	-	+

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**CONFLICT OF INTEREST**

The authors declared no conflict of interest

**AUTHOR'S CONTRIBUTION**

All the authors designed the experiment and approved the manuscript. Nibedita Das and Kishore Deka performed the ethnobotanical studies and the phytochemical analysis. Antibacterial activities were done by Sashi P. Devi and Dhritiman Chanda. The manuscript was written by Nibedita Das and reviewed by the coauthors.

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## DATA AVAILABILITY

All the required data are present in the manuscript

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