



EVALUATION OF ANTIBACTERIAL ACTIVITY IN *CLERODENDRUM PHLOMIDIS* LINN. AND *CLERODENDRUM INFORTUNATUM* LINN.

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Abstract:

The present study was conducted to investigate the antibacterial potential of crude extracts of *Clerodendrum phlomidis* Linn. and *Clerodendrum infortunatum* Linn. by Disc Diffusion method. Extracts of polar and non polar solvents of the selected plant parts were subjected to antibacterial activity against two gram positive such as *Staphylococcus aureus*, *Bacillus subtilis* and two gram negative pathogens such as *E. coli* and *Klebsiella pneumoniae*. Amongst all plants studied Ethanolic root extract of *Clerodendrum infortunatum* showed maximum zone of inhibition against *Staphylococcus aureus*.

Keywords: *Clerodendrum phlomidis*, *Clerodendrum infortunatum*, disc diffusion, zone of inhibition, pathogens.

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicine (Cragg and Newman 2001).

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency (Ge et al. 2002; Nair and Chanda 2005; Neogi et al.2008).

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans et al. 2002). Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today, (Hussain and Gorski 2004).The primary benefits of using plant based medicines are that they are safer than synthetic drugs.

In the present study extracts of some polar and non polar solvents of *Clerodendrum phlomidis* Linn. and *Clerodendrum infortunatum* Linn. of family Verbanaceae

were studied for antibacterial activity against two Gram positive and two Gram negative bacteria. *Clerodendrum phlomidis* is commonly known as Agnimantha. Its root is a part of important Ayurvedic formulation known as Dashmoolarishta. *Clerodendrum infortunatum* Linn commonly known as Khanduchakka, is used in stomach pain, all types of worm infection, malarial fever and for many types of skin disease, (Ray 2012).

Materials And Methods Plant materials

Fresh roots and leaves of *Clerodendrum phlomidis* Linn. and *Clerodendrum infortunatum* Linn. were collected from Nagpur and Amravati region, Maharashtra. The Collected plant materials were washed under tap water, dried in shade and then homogenized to fine powder and stored in airtight bottles. Air dried powdered material was extracted with petroleum ether, ethyl acetate, acetone, ethanol and water in a soxhlet extractor.

Microorganisms

The microorganisms were obtained from Department of Veterinary Microbiology and Animal Biotechnology, Nagpur Veterinary College, Nagpur. The bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4 °C. Antibacterial activity against two gram positive as *Staphylococcus aureus*, *Bacillus subtilis* and two gram negative pathogens such as *E. coli* and *Klebsiella pneumoniae*.

Antibiotics

Chlorenpanicol (30mcg) and Gentamycin(10 mcg) were used as antibiotics for antibiotic susceptibility test against the bacteria . All antibiotic discs were purchased from Hi-Media, Bombay, India.

Antibiotic susceptibility test:

In vitro antibiotic susceptibility of individual isolates was determined by single disc diffusion method (Bauer, *et al.*, 1966). Commercially available antibiotic discs (Himedia Laboratories Ltd., Mumbai) were used.

The nutrient broth was inoculated with single isolated colony and tubes were incubated at 37° C for 16 – 18 hrs. 0.1 ml broth culture was uniformly spread over Mueller-Hinton agar plate using sterile cotton swab and was allowed to stand for 15 minutes. 0.15 mg of crude extracts were impregnated on sterile 6 mm diameter autoclaved filter paper disc and allowed to dry. The inoculum was allowed to dry and antibiotic discs were placed on it using sterile forceps. The plate was then incubated for 24 hrs. at 37°C and observed after overnight incubation. The diameter of zones of inhibition was measured to nearest millimeter. The effects were compared with that of the standard antibiotics chloramphenicol for Gram positive bacteria and gentamycin for Gram negative bacteria. The experiment was performed in triplicates.

Results and Discussion:

The genus *Clerodendrum* has been cited in many indigenous systems of health care for the treatment of variety of disorders. Root and leaf crude extracts of *C.phlomidis* and *C. infortunatum* in five solvents were subjected to screening against two Gram positive and two Gram negative bacteria. **Fig.1** and **Fig.2** indicated the zone of inhibition produced by plant extract against pathogenic bacteria. In *C.phlomidis* roots, among the five extracts studied, Ethyl acetate and ethanol extracts have shown the antibacterial activity. Whereas in *C. infortunatum*, activity was found in acetone, ethanol and aqueous roots extract. *S.aureus* is sensitive to Petroleum ether, ethanol root extract and leaf extract of ethyl acetate, acetone, ethanol and aqueous extract of *C.phlomidis*.

Among the five extracts petroleum ether and aqueous root extracts of *C.phlomidis* does not exhibit antibacterial activity against all the tested bacteria. However only aqueous leaf extract of *C.phlomidis* shows antibacterial activity against *B.subtilis*. Petroleum ether, acetone and ethyl acetate leaf extract of *C.phlomidis* exhibit significant activity against *S.aureus*. *Klebsiella pneumoniae* is sensitive to the aqueous leaf extract of *C.phlomidis* and not sensitive to any leaf extract of *C. infortunatum*. *B.subtilis* is sensitive to acetone, ethyl acetate

and aqueous root extracts of both *C.phlomidis* and *C. infortunatum*.

Ethyl acetate root and leaf extract of *C. phlomidis* showed 11 mm and 12 mm zone of inhibition against *S.aureus* whereas no antibacterial activity was observed in ethyl acetate root and leaf extract of *C. infortunatum* against *S. aureus*.

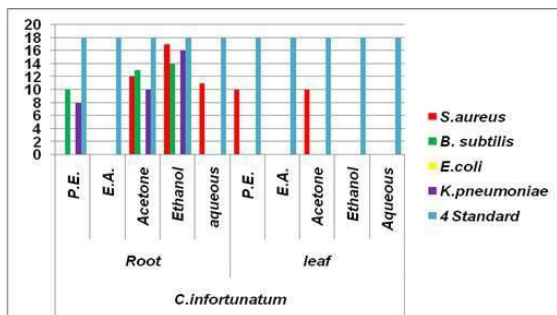
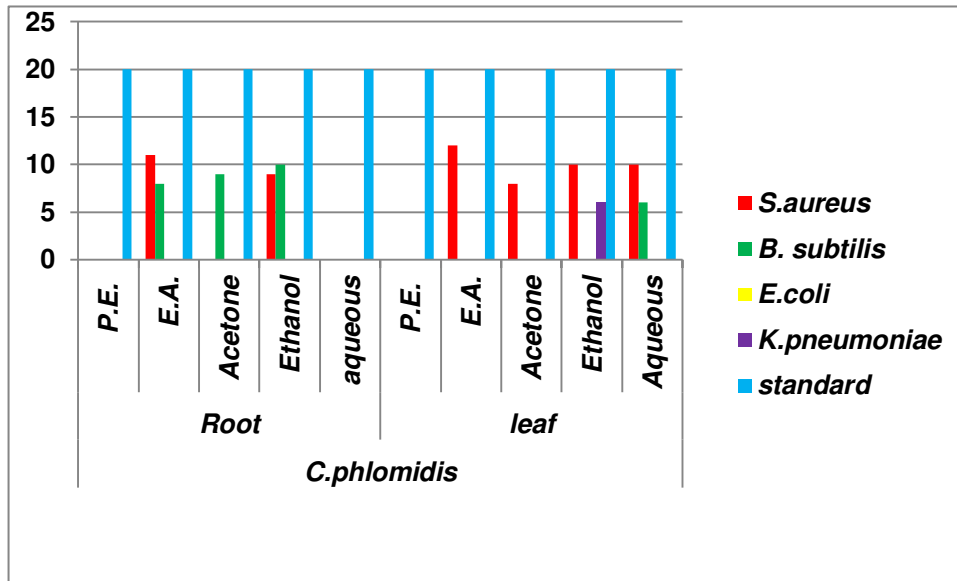
The Ethanolic extract was most active against all the tested bacteria. Against *Staphylococcus aureus* and *Bacillus subtilis*, ethanol root extract of *Clerodendrum infortunatum* showed highest zone of inhibition 17 mm and 14mm respectively. No zone of inhibition was observed in *E. coli* whereas against *Klebsiella pneumoniae*, ethanol root extract of *Clerodendrum infortunatum* showed 16 mm zone of inhibition. Some other species of the genus *Clerodendrum* have also been reported for their antibacterial activity.

Antibacterial activity of *C. infortunatum* has also been reported earlier (Cheng *et al.*, 2001; Mahato & Chaudhary, 2005). Some other species of the genus *Clerodendrum* have also been reported for their antibacterial activity. Misra *et al.* (1995) reported hexane extracts of *C. colebrookianum* at concentrations of 1000 and 2000 ppm showed strong antibacterial activities against various Gram positive and Gram negative pathogens such as *S. aureus*, *S. haemolyticus*, *E. coli*, *P. aeruginosa*. (Oly 2011). Sathish (2013) reported *Clerodendrum phlomidis* as important medicinal plant as its ethanol, petroleum ether, ethyl acetate and chloroform root extracts showed good antimicrobial activity against all organisms except *Pseudomonas aeruginosa*. Kutty *et al* (2011) studied Chloroform and Ethanol extracts of roots of *C. infortunatum*. They found the marked activities against *E.coli*, *K.pneumoniae* and *Staphylococcus aureus*.

Ethanolic extract of *C.infortunatum* exhibited significant antimicrobial activity comparable to the standard drug tetracycline against *E.coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, (Rajurkar2011).

Conclusion:

From the results it can be concluded that ethanol extracts of the plants showed good antibacterial activity than petroleum ether, acetone, ethyl acetate and aqueous extracts. It is evident that the Gram- positive bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis* are more sensitive to the plant extracts than the Gram-negative bacteria i.e. *E.coli* and *Klebsiella pneumoniae*.





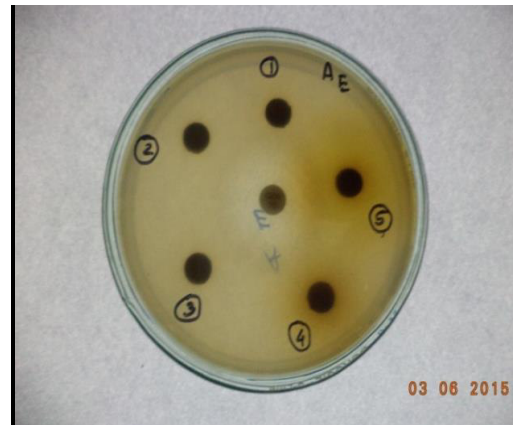
Clerodendrum phlomidis Linn.



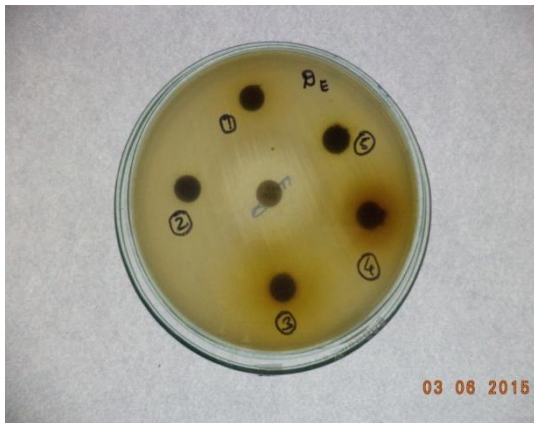
Clerodendrum infortunatum Linn.



Staphylococcus aureus



Bacillus subtilis



E.coli



Klebsiella pneumoniae

Plates showing zone of inhibition

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