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EFFICACY OF *CLERODENDRUM INERME* L. (GARDEN QUININE) AGAINST SOME HUMAN PATHOGENIC STRAINS

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ABSTRACT

The present investigation was undertaken to evaluate *in vitro* antimicrobial activities of different extracts (ethanol, benzene and aqueous) of *Clerodendrum inerme* plant parts. *In vitro* antimicrobial efficacy of various extracts of *C. inerme* was assessed by disc diffusion method against Gram positive - *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923) Gram negative- *Escherichia coli* (ATCC 25922), *Psedomaonas aeruginosa* (ATCC 27853) and fungal strains *Aspergillus niger* (ATCC 16404), *Aspergillus flavus* (ATCC 9807), *Candida albicans* (ATCC5027) and *Candida glabrata* (ATCC 66032). The methanol leaves extract exhibited highest zone of inhibition against *S. aureus* and *A. niger* (16.67 ±0.47 and 15.0±0.0 mm, respectively) with low MIC values (0.78 mg/ml for each). However, none of activity is shown by aqueous extract against tested pathogenic strains. Results of the present investigation indicate that *Clerodendrum inerme* possess compounds with antimicrobial properties and hence can be exploited for future natural plant based antimicrobial agents.

KEY WORDS

Clerodendrum inerme, antimicrobial activity, minimum inhibitory concentration.

INTRODUCTION

Medicinal and aromatic plants are potential source of raw materials used for manufacture of drugs and perfumery products. More than a quarter of all the medicines used in the world today contain natural compounds derived from plants that often serve as lead molecules whose activities can be enhanced by manipulation through combinations with chemicals and by synthetic chemistry that can be exploited in the field of new drugs research and development. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Bandow *et al.,* 2003).

Clerodendrum inerme L., (Verbenaceae) commonly known as garden quinine is a perennial shrub found throughout India. Traditionally, whole plant parts of *C.*

inerme are used as to treat coughs, scrofulous infection, venereal infection, skin diseases and Bariberi diseases (Kirtikar and Basu 1991). It is also used as febrifuge, vermifuge and antioxidant (Anonymous 1992, Kanchanapoom *et al.* 2001). Biologically active compounds like sterols, diterpenes, flavones and iridoids have been isolated from *C. inerme* (Kanchanapoom *et al.,* 2001; Pandey *et al.,* 2006). Aerial parts of *C. inerme* showed potent anti-viral activity against Hepatitis B virus (Mehdi *et al.* 1997).

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Scazzocchio *et al.,* 2001). For a long period of time, plants have been used because of their antimicrobial traits, which are due to compounds known by their active substances which may represent new source of anti-microbial with stable, biologically effective components that can establish a scientific base for the use of plants in modern medicine (Kelmanson, *et al*., 2000; Ahmad and Beg, 2001). In the present investigation, the antimicrobial potential of *C. inerme* leaf and root extracts has been evaluated against common pathogens.

MATERIAL AND METHODS

Plant material

Plants of *C. inerme* were collected from the campus of University of Rajasthan, Jaipur. The plant was identified and voucher specimen of each of them was deposited to the Herbarium, Botany Department, University of Rajasthan, Jaipur (RUBL NO.-20620). The various plant parts (leaves and roots) of *C. inerme* were separated, washed with running water to remove dust and shade dried.

Preparation of extracts

The powdered leaves and roots (500g) of *C. inerme* were extracted with benzene, methanol and aqueous using soxhlet's apparatus for 12-14 hours on a water bath separately. The organic extracts were separately filtered with Whatmann No. 1 filter paper and evaporated to dryness on water bath to obtain semi-solid mass. However, aqueous extraction is performed by using hot water maceration. The dried extracts were stored at 5^0C in the refrigerator until used for further studies.

Antimicrobial Screening

Test microorganisms

In vitro antimicrobial activity was evaluated against common pathogenic microorganisms, Gram positive - *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923) Gram negative- *Escherichia coli* (ATCC 25922), *Psedomaonas aeruginosa* (ATCC 27853) and fungal strains *Aspergillus niger* (ATCC 16404), *Aspergillus flavus* (ATCC 9807), *Candida albicans* (ATCC5027) and *Candida glabrata* (ATCC 66032). All the tested microorganisms were obtained from Batra Hospital and Medical Research Centre (BHMRC), New Delhi. The bacterial cultures were grown and maintained on Nutrient Broth medium at 37^0C for 24h while the fungal cultures were maintained on Potato Dextrose Agar slants and incubated at 27^0C for 48h.

Antimicrobial activity

Antimicrobial assay of the crude extracts was performed against ten tested pathogenic strains by disc diffusion method (Gould and Bowie, 1952). The nutrient agar plates and potato dextrose agar plates were seeded with suspension (10^6 cfu/ ml) of the bacterial and fungal strains vice- versa. The empty sterilized Whatmann No.1 filter paper disc (6 mm) were impregnated with 1mg/ml of extracts dried and placed aseptically on seeded plates with the help of a sterile forceps. Finally, the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes

(Pre diffusion time). The standard discs (6 mm) impregnated with antibiotics chloroamphenicol and nystatin (2µg/ml) was used as positive control. The plates were incubated at 37^0C for 24 h and 25^0C for 48 h for bacteria and fungi, respectively. The diameter of the inhibition zone (mm) was measured. The experiment was done in triplicate and the mean values (±SD) calculated for conclusion.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method (Basri and Fan, 2005). For broth dilution, 1ml of standardized suspension of a strain (10^6cfu/ ml) was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at 37ºC for 24h (for bacterial strains) and 25ºC for 48h (for fungal strains) and observed for visible growth after vortexing the tubes gently. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation.

RESULT AND DISCUSSION

Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicines. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. They are more readily degraded in the environment than synthetic compounds. The bioactive components of plants have different solubility in different extracting solvents (Oloke and Kolawole, 1998). Several reports have shown that bioactive compounds from plants have control on the growth of pathogenic strains (Taylor *et al.,* 1996; Singh *et al.,* 2002; Kagale *et al.,* 2004; Abed, 2007).

In the present investigation, *in vitro* antimicrobial efficacy of the crude extracts of *C. inerme* (leaves and roots) was quantitatively assessed on the basis of inhibition zone and minimum inhibitory concentration. The methanol and benzene extracts of *C. inerme* exhibited varying degree of inhibitory effect against all tested pathogenic strains (Table 1 and 2). None of the activity is shown by aqueous extracts. The most susceptible bacterium and fungi are *S. aureus* and *A. niger,* respectively. The inhibition zones (IZ) were in the range of 7.0±0.0 to 16±0.47 mm for most of the tested strains. The MIC of crude extracts of leaves and roots was determined at the concentrations ranging from 0.078 to 0.625 mg/ml.

Crude methanol extract of *C. inerme* (leaves and roots) showed more pronounced antimicrobial activity than other extracts. The methanol leaves extract exhibited highest zone of inhibition against *S. aureus* and *A. niger* (16.67 ±0.47 and 15.0±0.0 mm, respectively) with low MIC values (0.78 mg/ml for each). The methanol roots extract was also found to effective against *S. aureus* and *A. niger* (15.0 ±0.0 and 11.0±0.0mm, respectively) with low MIC values (0.78 mg/ml and 0.156 mg/ml, respectively). However, aqueous extracts of both plant parts were inactive against tested pathogenic fungal and bacterial strains. Among bacterial pathogens, gram positive bacterial strains were found to be more susceptible than gram negative bacterial strains. This may attributed to the fact that cell wall in gram positive bacteria consist of a single layer, whereas, gram negative cell wall is multilayered structure bounded by an outer cell membrane (Yao and Moellering, 1995).The findings of the present investigation suggest that *C. inerme* is source of biologically active compounds which may potentially prove to be efficient natural antimicrobial agents.

Table. 1
Antimicrobial activity of Clerodendrum inerme L (inhibition zone).

Tested microorganisms	Plant part assayed						Control	
	Leaf			Roots				
	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous	C	N
Bacillus subtilis	9.34± 0.94	9.0±0.0	-	13.0± 0.0	8.67± 0.47	-	20.2 ± 0.3	-
Staphylococcus aureus	16.67± 0.47	10.0± 1.41	-	15.0± 0.0	11.0±0.8 2	-	12.0 ± 0.0	-
Escherichia coli	7.0± 0.0	8.67± 0.47	-	10.0± 1.41	7.0± 0.0	-	24.8 ± 0.3	-
Pseudomans aeruginosa	8.4± 0.94	7.0± 0.0	-	7.34± 0.47	8.0± 0.81	-	15.0 ± 0.0	-
Aspergillus niger	15.0± 0.0	13.34± 0.47	-	11.0± 0.0	10.0± 0.0	-		17.7± 0.3
Aspergillus flavus	11.0± 0.81	9.0± 0.0	-	12.0± 0.0	9.34± 0.94	-		13.3± 0.6
Candida albicans	14.0±0.0	10.0± 1.41	-	11.0±0.81	10.0± 0.0	-		15.2± 0.3
Candida glabrata	10.0±0.0	9.34± 0.47	-	9.0±0.0	7.0± 0.0	-		12.0± 0.0

Control: C = chloroamphenicol and N = nystatin at 2µg/disc; Diameter of inhibition zone (mm) including the diameter of disc (6mm) values are mean (±SD)

Table. 2
Antimicrobial activity of Clerodendrum inerme L (MIC).

Tested microorganisms	Plant part assayed					
	Leaf			Roots		
	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous
Bacillus subtilis	0.312	0.625	-	0.312	0.625	-
Staphylococcus aureus	0.078	0.312	-	0.078	0.312	-
Eschericia coli	0.625	0.625	-	0.312	0.625	-
Pseudomans aeruginosa	0.625	0.625	-	0.312	0.625	-
Aspergillus niger	0.078	0.312	-	0.156	0.312	-
Aspergillus flavus	0.312	0.625	-	0.625	0.625	-
Candida albicans	0.156	0.625	-	0.312	0.625	-
Candida glabrata	0.625	0.625	-	0.625	0.625	-

CONCLUSION

The present investigation revealed that the various extracts from leaves and roots of *C. inerme* exhibited antimicrobial properties which explain the basis for its use in traditional medicines. However, methanol extracts exhibited significant inhibitory activity against tested pathogenic microorganisms.

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