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In Vivo and *In Vitro* Antimicrobial Efficacy of Crude Extracts From *Clerodendrum Inerme* Linn

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ABSTRACT

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as source for new drugs it is essential to study medicinal plants .The present investigation was undertaken to evaluate the antimicrobial activity of crude extract isolated from callus and different plant parts (leaf, stem and root) of a medicinal plant - *Clerodendrum inerme*. *In vivo* and *in vitro* antimicrobial efficacy of various extracts of *Clerodendrum inerme* was assessed by disc diffusion method against Gram positive bacteria - *Bacillus subtilis* (ATCC 6633) (*B.s.*), *Staphylococcus aureus* (ATCC 25923) (*S.a.*) Gram negative bacteria - *Escherichia coli* (ATCC 25922) (*E.c.*), *Psedomaonas aeruginosa* (ATCC 27853) (*P.a.*) and fungal strains *Aspergillus niger* (ATCC 16404) (*A.n.*), *Aspergillus flavus* (ATCC 9807) (*A.f.*), *Candida albicans* (ATCC 5027) (*C.a.*) and *Candida glabrata* (ATCC 66032) (*C.g.*)on the basis of inhibition zone and minimum inhibitory concentration. The crude extract of selected plant parts and callus with methanol showed significant and more pronounced antimicrobial activity on the used microorganisms.

Keywords: *Clerodendrum inerme*, Antimicrobial activity, Inhibition zone, Minimum inhibitory concentration

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INTRODUCTION

Traditional medicines are used to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against microbial organisms. Medicinal plants are a source of great raw material with economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country[1]. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment[2]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds[3]. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like phenolic compounds which are part of the essential oils as well as in tanning.[4]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[5].

Flavonoids gained recent interest because of their broad pharmacological and antimicrobial activity [6]. Plants produce a huge variety of secondary compounds as natural protection against microbial and insect attack. Many of these compounds have been used in the form of whole plants extract for food or medicinal application in man.[7]. Flavonoid are a broad group of secondary metabolites with varies and important roles in plant physiology as well as they have gained recent interest because of their board pharmacological activity. Putative therapeutic effects of many traditional medicines may be ascribed to the presence of flavonoids[8, 9].

Flavonids occur as aglycones, glycosides and methylated derivative and are widely distributed in the plant kingdom. They have been reported to possess a variety of biological activities including antiallergic, antidiabetic, anti-inflammatory, antiviral, antiproloferative and anticancinogenic, hepatoprotective and antioxidant activities. Since these secondary metabolites are synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in-vitro* to be effective antimicrobial substances against a wide array of microorganisms [10-12].

MATERIAL AND METHODS

Plant Materials

A regular collection of various plants of *C. inerme* was done from the campus of University of Rajasthan, Jaipur. The plant was identified and voucher specimen of was deposited to the Herbarium, Botany Department, University of Rajasthan, Jaipur (*C. inerme* → RUBL NO 20620). Collected plants parts as well as the callus culture was separately washed with running water to remove dust, shade dried, powdered and all parts were crushed with motel and pestle.

TISSUE CULTURE

Selection of explants

Various explants (nodal segments and leaves) collected from *C. inerme* were used for the establishment of tissue culture and shoot proliferation on Murashige and Skoog's medium.

Callus induction

The callus induction was initiated by swelling of the explants. Callus induction from different explants *viz.* young leaves, nodes, internodes and shoot tips of *C. inerme* was observed within 10-14 days of inoculation in MS medium supplemented with various concentrations of auxin and cytokinin. The calli production was observed in all the concentrations of growth hormones tried. But, the leaf explants showed maximum production of callus (80.6%) when MS medium was supplemented with NAA+Kn+2, 4-D (5.37µM+4.53µM+4.65µM). The unorganized callus mass of *C. inerme* was creamish yellow in color and fragile in nature. The color of calli turned creamish brown as the time of transfer increased. It may be due to synthesis of phenolic compounds.

The callus was harvested at different time intervals of 2, 4, 6 and 8 weeks of sub-culturing and growth index was calculated. Three replicates of each of the tissue samples were examined and the mean values recorded. The maximum growth index was observed in 6 weeks old tissues grown as static cultures in both the plants, which decreased subsequently in 8 weeks old tissues. Callus growth exhibited a gradual increase from 2 weeks onwards following the sigmoid pattern of growth. The maximum growth index (5.21mg/g. d.w) in six weeks old and minimum (1.57 mg/g. d.w) in 2-weeks old cultures were observed in *C. inerme*.

Preparation of plant extracts

All the extracts obtained by successive maceration of the powdered (Leaves, Stem, Root and Calli) of *Clerodendrum inerme*. Linn (with Petroleum ether, Chloroform, Ethyl acetate, Ethanol, Chloroform and water) using soxhlet's apparatus for 12-14 hours on a water bath separately. The 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 5c in the refrigerator unit used for various qualitative tests for the identification of different plant constituents.

ANTIMICROBIAL SCREENING

Test microorganisms

In vivo and *in vitro* antimicrobial activity was evaluated against common pathogenic microorganisms such as Gram positive bacteria - *Bacillus subtilis* (ATCC 6633) (*B. s.*), *Staphylococcus aureus* (ATCC 25923) *(S. a.)* Gram negative bacteria - *Escherichia coli* (ATCC 25922) (*E. c.*), *Psedomaonas aeruginosa* (ATCC 27853) and fungal strains *Aspergillus niger* (ATCC 16404) (*A. n.*), *Aspergillus flavus* (ATCC 9807) (*A. f.*), *Candida albicans* (ATCC 5027) (*C. a.*) and *Candida glabrata* (ATCC 66032) (*C. g.*). 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.

ANTIMICROBIAL ACTIVITY

Disc Diffusion Method

Antimicrobial assay of the crude extracts was performed against eight tested pathogenic strains by disc diffusion method[13]. 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. Nutrient agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculums. 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 allowed to stand at room temperature for1hr for extract to diffuse into the agar and then they were incubated at 35 ± 2°C for 24 h, except for *C. albicans* which was incubated at 29 ± 2°C.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[14]. 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 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.

RESULTS AND DISCUSSION

Antimicrobial and Anti-Fungal Activity

In the current research, the antimicrobial activities of various extract of *in vitro* (callus) and *in vivo* (different plant parts) of *Clerodendrum inerme* Linn (Verbenaceae) were evaluated.

Among crude plant extracts in various solvents the methanol extract of *C. inerme* showed more pronounced antimicrobial activity than other extracts. The methanolic leaves extract exhibited highest zone of inhibition against S. aureus (16.67 ±0.47mm) C. albicans (15.0 ±0.0mm) with low MIC values (0.078 mg/ml).

Table 1and 2 are demonstrates the anti-microbial activity of various extracts of *C. inerme* against some pathogenic micro organisms on the basis of inhibition zone (IZ) and minimum inhibitory concentration (MIC) values.

Result of phytochemical screening showed the presence of sterols, tannins, sugars, glycosides etc. in the solvents used. The presence of these secondary metabolites confirms plant use for pharmaceutical manufacturing and drug discovery. Along with biological studies, isolation and identification studies of chemical constituents and its correlation with the biological activities of the genus has also been studied. The major chemical components reported from the genus are phenolic, steroids, di and triterpenes, flavonoids, volatile oils, etc diseases. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity.

It is well know that biological activities such as antimicrobial activities and antioxidant activities are important for healthy living of human beings. Now a day, multiple drug resistance of both human and plant pathogenic microorganisms is quite prevalent due to indiscriminate use of commercial antimicrobial drugs for the treatment of infectious diseases [15]. This situation forced the scientists to search an alternative of it by discovering more and more antimicrobial substances. Medicinal plants play an important role in this respect [16].

A number of species of the genus *Clerodendrum* also showed their antimicrobial action. Antimicrobial drug resistance is a world wild problem[17]. Drug resistant infectious microorganisms are those, which are not killed or inhibited by antimicrobial compounds. Antimicrobial resistance has been reported in most predominant pathogenic microorganisms [18]. Screening of Indian plants for biological activity has been reviewed by a number of workers [19, 20, 21]. Antimicrobials from plant tissue cultures has been also reported by [22; 23, 24, 25, 26, 27].

Table1. Antimicrobial activity of crude extracts in organic solvents of *C. inerme* on the basis of inhibition zone (IZ).

Tested strains	Plant part assayed												Control	
	Leaf			Stem			Roots			Calli				
	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous	C	N
B s.	9.34± 0.94	9.0± 0.0	-	7.9± 0.39	12.2± 0.21	-	13.0 ±0.0	8.67 ±0.47	-	8.0±1.0 0	7.44 ±0.44	-	20.2±0 .3	-
S. a.	**16.67 ±0.47**	10.0 ±1.41	-	15.6± 0.62	10.3± 0.41	-	15 ±0.00	11 ±0.8	-	12 ±0.00	10.0 ±0.85	-	12.0± 0.0	-
E. c.	7.0± 0.0	8.67± 0.47	-	9.03± 0.45	11.6± 0.29	-	10 ±0.41	7.0 ±0.0	-	9.43±1. 2	8.16±0. 18	-	24.8± 0.3	-
P.a.	8.40 ±0.94	7.3±0. 47	-	10.2± 0.39	8.1± 0.0	-	7.44 ±0.47	8.0 ±0.8	-	14 ±2.60	8 ±0.95	-	15.0± 0.0	-
A. n.	14.2 ±0.44	13.34 ±0.47	-	12.2± 0.0	11± 0.0	-	11 ±0.0	10 ±0.0	-	9.7 ±0.9	7.0± 0.57	-		17.7±0 .3
A. f.	11.0 ±0.81	9.0 ±0.0	-	10.3± 0.41	9.63± 0.28	-	12 ±0.0	9.34 ±0.94	-	11.7 ±0.9	10 ±0.79	-		13.3± 0.6
C. a.	**15.0 ±0.0**	10.0 ±1.41	-	13.8± 0.55	12.2± 0.21	-	11± 0.81	10± 0.0	-	8.0± 0.57	7.0 ±0.89	-		15.2± 0.3
C. g.	10.0 ±0.0	9.34± 0.47	-	8.12± 0.39	7.9± 0.31	-	9.0 ±0.0	7.0± 0.0	-	11 ±0.57	9.34 ±0.47	-		12.0± 0.0

Abbreviations: *B. s. = Bacillus subtilis, S. a. = Staphylococcus aureu, E.c.= Escheria coli, P. a..= Pseudomonas aeruginosa*; *A. n. = Aspergillus niger, A. f. = Aspergillus flavus, C. a. = Candida albicans, C. g. = Candida glabrata;* Control: C = *chloroamphenicol and N = nystatin at 2µg/disc;* Diameter of inhibition zone (mm) including the diameter of disc (6mm) values are mean (±SD); IZ= Inhibition zone (mm).

Table 2: Antimicrobial activity of crude extracts in organic solvents of *C. inerme* on the basis of Minimum inhibitory concentration (MIC) values.

Tested strains	Leaf			Stem			Roots			Cali		
	Methanol	**Benzene**	**Aqueous**	**Methanol**	**Benzene**	**Aqueous**	**Methanol**	**Benzene**	**Aqueous**	**Methanol**	**Benzene**	**Aqueous**
B s.	0.312	0.625	-	0.156	0.625	-	0.312	0.625	-	0.625	0.156	-
S. a.	**0.078**	0.312	-	0.312	0.312	-	0.312	0.312	-	0.312	0.625	-
E. c.	0.312	0.625	-	0.625	0.625	-	0.625	0.312	-	0.625	0.625	-
P.a.	0.625	0.625	-	0.312	0.312	-	0.312	0.625	-	0.625	0.625	-
A. n.	0.156	0.312	-	0.312	0.625	-	0.156	0.312	-	0.625	0.156	-
A. f.	0.312	0.625	-	0.312	0.625	-	0.625	0.312	-	0.312	0.312	-
C. a.	**0.078**	0.625	-	0.156	0.312	-	0.312	0.625	-	0.625	0.156	-
C. g.	0.625	0.625	-	0.156	0.312	-	0.625	0.625	-	0.312	0.625	-

Abbreviations: *B. s. = Bacillus subtilis, S. a. = Staphylococcus aureu, E.c.= Escheria coli, P. a..= Pseudomonas aeruginosa*; *A. n. = Aspergillus niger, A. f. Aspergillus flavus, C. a. = Candida albicans, C. g. = Candida glabrata;* Control: C = *chloroamphenicol and N = nystatin at 2µg/disc;* Diameter of inhibition zone (mm) including the diameter of disc (6mm) values are mean (±SD); MIC= minimum inhibitory concentration.

Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of health care since ancient times [28, 29, 30].

In the present study, crude extracts of various parts of *C. inerme* is tested for their antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* as test bacteria and *A. niger, A. flavus, C. albicans* and *C. glabrata* as test fungi. Among all crude extracts, methanol extracts of *C. inerme* (various parts) showed significant antimicrobial activity against all tested bacterial and fungal strains. *In vitro* antimicrobial efficacy of the crude extracts of *C. inerme* (leaves, roots, stem and calli) was quantitatively assessed on the basis of inhibition zone and minimum inhibitory concentration. The crude extracts of *C. inerme* exhibited varying degree of inhibitory effect against all tested pathogenic strains.

CONCLUSION

The result of this study justifies the use of *Clerodendrum inerme* Linn as traditional medicine. Our results indicate that, the plant extract is non toxic. There is a need for further studies on this plant to ascertain the active compounds so as to maximize the widely used medicinal plant in the development of antimicrobial drugs.

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REFERENCES

1. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4:685-688.
2. Santos, PRV, Oliveira, ACX, Tomassini, TCB. Controle microbiógico de produtos fitoterápicos. Rev. Farm. Bioquím. 1995; 31: 35-38.
3. Ellof, JN. Which extractant should be used for the screening and isolationof antimicrobial components from plants? *J. Ethnopharmacol*. 1998; 60: 1-6,
4. Selvamohan T, Ramadas V, S. Shibila Selve Kishore.. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Advances in Applied Sciences Research. 2012; 3 (5):3374-3381.
5. Srivastava J, Lambert J, Vietmeyer N. Medicinal palnts: An expanding role in development. World Bank Technical Paper. No 320 1996.

6. Manimozhi DM, Sankaranarayanani S, Sampathkumar G. Evaluating the antibacterial activity of falvonoids extracted from Ficus benghalensis. International journal of pharmaceutical and biological research. 2012; 3(1): 7-15
7. Wallace RJ. Antimicrobial properties of plant secondary metabolites. Proceedings of the Nutrition Society. 2004; 63 (4):621-629.
8. Saskia A, Acker BEV, Dirk-Jan Den Berg, Michel NJL. Tromp, Desiree H. Griffioen, Wout P. Van Bennekom, Wim JF Van Der Vijgh, Aalt Bast. Structural Aspects of antioxidant activity of flavonoids: free rad boil med. 1996; 20 (3):331-342.
9. Schultz SC, Bahraminejad S, Asenstorfer RE, Riley IT. Analysis of the antimicrobial activity of flavonoids and sapnins isolated rom the shoots of oats (Aveena sativa L.). Journal of phyto path 2008;156:1-7
10. Harborne JB. The flavonoids:Advances in research science 1986.
11. Cowan MM. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999; 12:564-582.
12. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In the flavonoids: Advances in Research Science 1986. Chapman & Hall: Londan, 1993; 619-652.
13. Gould JC, Bowie JH. The determination of bacterial sensitivity to antibiotics. Edinb. Med. J. 1992; 59: 178.
14. Basri DF, Fan SH. The potential of aqueous and acetone extracts galls of *Quercus infectoria* as antibacterial agents. Indian J Phrmacol. 2005; 37:26-29.
15. Service RF. Antibiotics that resist resistance. *Science* 1995; 270: 724– 727
16. Cordell G A. Biodiversity and drug discovery a symbiotic relationship. *Phytochem*. 20005; 5: 463–480
17. Abdullah WB, Anowa H, Doli G, Amina TS, Kamrun N, Korshed A, Neor A, Aliya, N, Balakrish N, Stephen L, Robert B. Bacteremic Typhoid fever in children in an urban slum, Bangladesh. Emerg. Infect. Dis. 2005; 11(2): 326-329.
18. Fox KK, Knapp JS, Holmes KK, Hook EW, Judson FN, Thomson SE, Washington JA, Whittington WL. Antimicrobial resistance in *Neisseria gonorrhea* in the United States, 1988-1994: the emergence of decreased susceptibility to the fluoroquinolones. J. Infectious Diseases. 1997; 175: 1396- 1403.
19. Narayanan N, Thirugnanasambantham P, Viswanathan S, Rajarajan S, Sukumar E. Comparative antibacterial activities of *Clerodendrum serratum* and *Premna herbacea*. Indian J. Pharm Sci. 2004; 66:543.

20. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol. 2005; 29: 41-47.
21. Parkesh J, Chanda SV. *In vitro* antimicrobial activity and photochemical analysis of some Indian medicinal plants. Turk. J. Boil. 2007; 31: 53-58.
22. Sarin R, Khandelwal S. Study of bioactivity of flavonoids from *Ociumum sanctum* Linn. against *Pseudomonas, Staphylococcus* (gram positive), *Escherchia coli* (gram negative) bacteria and *Candida albicans* (fungus). Flora and Fauna 2006; 11: 163-167.
23. Rishi A, Sarin R. Flavonoid from *Gloriosa superba* L. tissue culture. J. Indian bot. Sco. 2009; 89 (1&2): 71-73.
24. Malwal M, Sarin R. Antibacterial activity of *Murraya koengii* (L.) Spreng. Oil on few clinically important bacteria. The Bioscan. 2009; 4(4): 653-654.
25. Malwal M, Sarin R. Chemical characterization and antimicrobial screening of volatile components of *Murraya koengii* (L.) Spreng – An Indian aromatic tree Journal of Pharmacy Research. 2010; 3 (8):1782 -1784.
26. Chahal JK, Sarin R, Malwal M Efficacy of *Clerodendrum inerme* L. (Garden quinine) against some human pathogenic strains Intl. J. Pharma and Biosciences. 2010; 1(4): 219-223.
27. Bansal S, Malwal M, Sarin R. Antibacterial Efficacy of some plants used in folkaric medicines in arid zone Journal of Pharmacy Research. 2010; 3:2640-2642.
28. Vyas A, Sarin R Analysis of the phytochemical content and antimicrobial activity of Nyctanthes arbor-tristis. Int. J. Pharma. Bio. Sci. 2013; 4:201-206.
29. Vyas A, Sarin R. Analysis of the phytochemical content and antimicrobial activity of Nyctanthes arbor-tristis. Int. J. Pharm. Bio. Sci. .2013; 4 :201-206)
30. Sarin R, Samariya S. Screening for Antimicrobial efficacy of phytochemicals extracted from two medicinally important plants of Cucurbitaceae. American J. Pharm. Health Res.2014; 2(3):99-106

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