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Research Article

ANTI- MICROBAL EFFICACY OF MEDICINALLY IMPORTANT PLANTS (*C. PHLOMIDIS*) USED IN FOLKARIC MEDICINES IN ARID ZONE

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ABSTRACT
The present investigation was undertaken to evaluate *in vivo* antimicrobial activity of different extracts (Methanol, Benzene and Aqueous) of *Clerodendrum phlomidis* plants parts. In vivo antimicrobial efficacy of various extracts of *Clerodendrum phlomidis* 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* (ATCC 5027) and *Candida glabrata* (ATCC 66032). The methanol leaf extract exhibited highest zone of inhibition against the bacterial stain in *S. aureus* (15.6±0.6mm) with low MIC values (0.078 mg/ml) and in the case of fungal strains *C. albicans* (14.0±0.0mm) with low MIC values (0.156 mg/ml).However, none of activity is shown by aqueous extract against pathogenic bacteria. Result of the present investigation indicates that *Clerodendrum phlomidis* possess compounds with antimicrobial properties and hence can be exploited for future natural plant based antimicrobial agents.
KEY WORDS: Antimicrobial activity, *Clerodendrum phlomidis,* Minimum inhibitory concentration

INTRODUCTION

Since ancient time, plants have been traditional source of medicines. They are reservoir of chemical agents with therapeutic properties flavors, dyes, oils and resins[1]. The potential of higher plants used as new drugs is still unexplored. Thus, plants continued to be one of the most important sources of modern medicines used for the treatment of human diseases. Nature gives us a rich botanical wealth and large number of diverse type of plants grows in different area of the country. Thousand of the species known as medicines and still they are used. Plants are important therapeutic aids for alleviating various ailments of human beings. Crude plant extract often consist of complex mixtures of active compounds[2, 3].

These reason support the use of crude, chemically unrefined plant extracts containing mixtures of bioactive plant compounds rather than of the use of pure individual compounds[4, 5]. Medicinal plants have an enormous therapeutic potential of antimicrobial activity. Drug resistance developing in pathogenic microorganisms against commonly used antibiotics has necessitated a search for new antimicrobial compound from biological sources (plants) [6]. These valuable plants utilized in the treatment of infectious diseases. The beneficial medicinal effects of plants materials typically result from, the combination of secondary products present in plants. These compounds are flavonoids, sterols, alkaloids, prytherins and fatty acids, which are able to produce definite physiological action on body[7, 8].

C. phlomidis L. (Verbenaceae) commonly known as Arni. It is a small, deciduous to semi-evergreen aromatic tree found throughout India, Sri Lanka, Burma and Pakistan. It is one of the highly traded medicinal plants from tropical forests. The whole plant of *C. phlomidis* is used for ailments involving swellings, joint pains, inflammatory and the whole plant is used to treat diabetes[9], anti-disrrhoeal[10] and roots are used in cough, cold, anemia and nervous disorders[11]. This situation forced the scientists to search some other new antimicrobial substances. Medicinal plants play a great role in drug discovery[12]. Keeping the antimicrobial activity in view, the plant parts were investigated for their antimicrobial principles.

MATERIAL AND METHODS

Plant Materials

A regular collection of various plant parts of *C. phlomidis* was collected from University Campus, Jaipur. The plant was identified and voucher specimen was deposited to the Herbarium, Department of Botany, University of Rajasthan, Jaipur RUBL NO 20646. The various plant parts (leaves, stem and stem) of *C. phlomidis* were separately washed with running water to remove dust, shade dried, powdered and all parts were crushed with motal and pestle.

Preparation of Extracts

The powdered Leaf, stem and root (250 g.) of *C. phlomidis* was extracted with methanol, benzene 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°C in the refrigerator until used for further studies.

ANTIMICROBIAL SCREENING

Test Microorganisms

In vivo antimicrobial activity was evaluated against common pathogenic microorganisms, Gram positive - *Bacillus subtilis* (ATCC 6633) (*B. s.*), *Staphylococcus aureus* (ATCC 25923) *(S. a.)* Gram negative- *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. 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°C for 24 h for bacterial strains. 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.

RESULT AND DISCUSSION

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. 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[15]. The antimicrobial efficacy of the extracts of *C. phlomidis* leaf, stem and root were quantitatively assessed on the basis of inhibition zone (Table 1) and minimum inhibitory concentration (MIC) (Table 2). In the present investigation, all the extracts (Methanol, benzene) were found to be effective against tested pathogenic stains expect aqueous extract.

Methanol extract showed more pronounced antimicrobial activity than other extracts. Among the tested bacterial strain, the most susceptible bacterium to the extract (Methanol, benzene) was *S. aureus*, which is known to play significant role in skin diseases[16]. It indicates that root of *C. phlomidis* may possess compounds with antimicrobial properties which are effective against cough, cold, anemia, nervous disorders[17] and demulcent in gonorrhea and the juice of the roots [18]and leaves[19] are used as bitter tonic. Earlier, an ethanolic leaf extracts of *C. phlomidis* showed inhibition against *Plasmodium falciparum*[20] and anti-diarrheal activity[10], psycho-pharmacological effects[3] and anti-feedent activity[21] the study concluded that the activity might be due to the presence of bioactive compounds. In antibacterial screening, the methanol extract showed maximum inhibitory effect against *S. aureus* (15.6±0.29mm) with MIC value of (0.78mg/ml) while benzene extract shown minimum inhibitory effect against *Psedomaonas aeruginos* (7.34±0.47mm) with MIC value of (0.625mg/ml). However, aqueous extracts of *C. phlomidis* did not show any inhibitory effect against tested pathogenic fungal and bacterial strains.

In antifungal screening, the methanol extract shown maximum inhibitory effect against *Candida albicans* (14.0±0.00mm) with MIC value of 0.156 mg/ml while benzene extract showed minimum inhibitory effect against *Aspergillus flavus* (9.0±0.0mm) with low MIC value of 0.625mg/ml. Aqueous extracts of *C. phlomidis* did not show any inhibitory effect against tested pathogenic fungal and bacterial strains. The antifungal activity of *C. phlomidis* is well documented[22].

Present study is an effort towards this direction. In this study, IZ and MIC values have been evaluated for each extract. For most of the extracts MIC values recorded were very low, indicating strong bio-efficacy of the plant. In the current investigation *C. phlomidis* showed its antimicrobial potential against test pathogens. These activities are due to the presence of flavonoids[7] and sterols [8.] *C. phlomidis* has previously been studied for a number of biological activities. Still the literature available is meager. This study suggested that the chemical constituent investigated can be utilized in biological pesticide formulations.

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

Tested strains	Plant part assayed									Control	
	Leaf			Stem			Roots				
	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous	Methanol	Benzene	Aqueous	C	N
B s.	8.28± 0.23	15.3± 0.37	-	11.1± 0.66	12.2± 0.21	-	10.8 ±0.0	8.67 ±0.47	-	10.0±1.00	9.0 ±0.40
S. a.	**15.6 ±0.6**	10.8 ±0.28	-	9.24± 0.24	10.3± 0.41	-	13.6±0.29	11 ±0.8	-	12 ±0.00	10.0 ±0.85
E. c.	14.6± 0.29	8.67± 0.47	-	12.8± 0.45	11.6± 0.29	-	12.2 ±0.41	7.0 ±0.0	-	9.43±1.2	8.16±0.18
P.a.	10.8±0.36	7.34±0.47	-	10.2± 0.39	8.1± 0.26	-	7.34 ±0.47	8.0 ±0.8	-	14 ±2..60	9.72 ±0.95
A. n.	11.2± 0.33	13.3 ±0.47	-	12.2± 0.0	11.0± 0.0	-	12.3±0.02	10 ±0.0	-	9.7 ±0.9	8.48± 0.2
A.f	10.8±0.27	9.0 ±0.0	-	10.3± 0.41	9.63± 0.28	-	12 ±0.0	9.34 ±0.94	-	11.7 ±0.9	10.8 ±0.79
C. a.	**14.0 ±0.0**	10.0 ±1.41	-	9.8± 0.55	8.2± 0.21	-	11± 0.81	10± 0.0	-	8.0± 0.57	7.0 ±0.89
C. g.	10.1 ±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

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. phlomidis* on the basis of Minimum inhibitory concentration (MIC)

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

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