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EVALUATION OF ANTIMICROBIAL ACTIVITY OF CRUDE FLAVONOIDS IN MEDICINALLY IMPORTANT ARID ZONE PLANT- *CLERODENDRUM PHLOMIDIS* LINN.

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ABSTRACT

Medicinal plants are source of many valuable drugs and the isolation, identification, purification and characterization of bioactive compounds of these plants are beneficial for healthy living of human beings. Several wild plants and herbs species used traditionally in medicinal field as rich source of medicine. This article is based on the evaluation of the crude flavonoid (free and bound) of Clerodendrum phlomidis against some pathogen. The benzene extracts of plants parts and callus culture (leaves, root and calli) were found to be effective 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.). However, aqueous extracts of C. phlomidis did not show any inhibitory effect against tested pathogenic fungal and bacterial strains. This investigation concluded that the flavonoids extracts of C. phlomidis are effective for both antifungal and antibacterial activities.

KEY WORDS Clerodendrum phlomidis, flavonoids, Antimicrobial activity, Inhibition zone, Minimum inhibitory concentration.

INTRODUCTION

Clerodendrum phlomidis plant species is placed in family Verbenaceae, commonly known as Glory Bower, Arni and Aginmentha. It is found in arid zones and cultivated for its medicinal

value. It is one of the highly traded medicinal plants from tropical forests. The whole plant of C. phlomidis used for ailments involving swellings, joint pains, inflammatory and the whole plant is used to treat diabetes [1, 2]. The leaves and roots are used for malaria and as febrifuge, digestive disorders, stomach pain, asthma, rheumatisms and inflammatory disease, skin diseases [3, 4] cough, cold, anemia and nervous disorders. [5]. Over the past few decades, herbal and Ayurveda drugs has become a subject of world importance for both medicinal and economic implications [6]. The World Health Organization has estimated that approximately 80% of world's population relies on plant based traditional medicines for primary health care of which 85% are plant extracts. In recent years, an increase in popularity and acceptance of herbal medicines has swept the Western world. Many individuals are now taking greater responsibility for their own health and quality of life, taking confidence in herbal and alternative therapies as "natural, safe and effective" [7]. 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 [8]. Plant cells produce a vast amount of natural products. Some flavonoids are formed as antimicrobial barrier in plants response to microbial infection. Although thousands of wild plants species have been use to test the biological activity. Antimicrobial drug resistance is a global problem today as the resistant microorganisms have emerged and spread throughout the world because of their genetic plasticity [9]. Some of these raw drugs are collected from the local communities and used as a folk healer in the local areas; many other raw drugs are collected in large quantities and traded in the market as the raw material for many herbal industries [10]. The present study was undertaken to screen for antimicrobial activity of various fractions of the plant extract of *C.* phlomidis against different types of pathogens.

MATERIAL AND METHODS

Plant Material

Plants of C. phlomidis were collected from Jhalana Hills, Jaipur. This plant was identified and voucher specimen of it was deposited to the Herbarium, Botany Department, University of Rajasthan, Jaipur (C. phlomidis → RUBL NO 20646). Various plant parts (roots, stem, and leave) and calli of *C. phlomidis* were collected, dried, powdered and crude extracts of flavonoids of above parts used for antimicrobial activity. The calli induced in C. phlomidis was also studied for the estimation of some metabolites. The calli of C. phlomidis is raised from leaf explants on Murashige and Skoog's (1962) medium supplemented with varied concentration of auxin and cytokinin. The induction of callus in C. phlomidis takes places

when MS medium was supplemented with NAA+2,4D (10.7µM+4.53µM), the callus mass was yellow in color and globular in nature. However, later on, calli turned dark brown as the time increased due to synthesis of phenolic compounds.

Test Microorganisms

In vivo and in vitro antimicrobial activity was evaluated against common pathogenic gram positive and gram negative bacterial and some fungal strains. Gram positive bacteria - Bacillus subtilis (ATCC 6633) (B. s.), Staphylococcus aureus (ATCC 25923) (S.a.) and 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 provided by 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 [11]. 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 [12]. 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

All over the world population depends on the herbs for the treatment of various ailments before the event of modern medicine. They use the plant organs such as flower, leaves, roots and seeds also [13]. In the present investigation all the crude flavonoid extracts of Clerodendrum phlolomidis were subjected to screening against bacterial and fungal species. It is clear from the table 1 that most of the extracts of plants parts and calli of C.phlomidis are effective against both organisms; bacterial and fungal. Flavonoids are known to be synthesized by plants in response to microbial infection. Among crude solvent extracts of free and bound flavonoids of C. phlomidis, The free and bound flavonoids rich fraction from various plant parts and callus culture of C. phlomidis (leaf, stem, roots and calli) also showed significant inhibitory potential against tested pathogenic strains. All pathogens growth was inhibited by flavonoids rich fraction of C. phlomidis. The bound flavonoids rich fraction exhibited maximum zone of inhibition against Staphylococcus aureus (15.2±0.68mm) with low MIC values (0.078 mg/mland A. niger (18.3±0.37mm) with low MIC values (0.156 mg/ml). The benzene extracts of other plants parts and callus culture (leaves, root and calli) were also found to be effective against pathogenic strains. However, aqueous extracts of C. phlomidis did not show any inhibitory effect against tested pathogenic fungal and bacterial strains. The MIC value of leaf extract is good in compression other plants parts and calli. C. viscosum is also showed the antibacterial activity [14]. Similarly number of species of Clerodendrum were listed in ancient texts for their antimicrobial activities like Clerodendrum inerme [15], C. phlomidis [16, 17], C. wildii [18], C. serratum [19, 20, 21] which have been reported for their antibacterial activity against gram positive and gram negative pathogens. According to the literature whole biodiversity is full of valuable plants and all plants showed the presence of bioactive compounds like glycosides, alkaloids, terpenoides, flavonoids, phenols which account their usefulness as medicinally important plants [22]. Many workers have reported antimicrobial activity from different plants [23-32] which can enhance the development of phytomedicines to act against microbes (33-36). In this research article it is clear that leaves of Clerodendrum phlomidis are more effective in controlling bacterial and

fungal pathogens in compression the other plants parts and calli. In this investigation it is certain that most effective are bound flavonoids which showed maximum zone of inhibition..

Table 2: Antimicrobial Activity of Extracts of Flavonoids (Free And Bound) of *C. Phlomidis* on The Basis of Minimum Inhibitory Concentration (MIC).

Tested Strains	PLANT PART ASSAYED							
	LEAF		ROOTS		STEM		CALLI (6 WEEKS)	
	FREE	BOUND	FREE	BOUND	FREE	BOUND	FREE	BOUND
B s.	0.312	0.625	-	0.156	0.625	-	0.312	0.625
S. a.	0.156	**0.078**	-	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.	0.312	**0.156**	-	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
C. g.	0.625	0.625	-	0.156	0.312	-	0.625	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.

Table 1: Antimicrobial Activity Of Crude Extracts Of Flavonoids (Free And Bound) Of C. Phlomidis On The Basis Of Inhibition Zone (IZ).

Tested Strains	PLANT PART ASSAYED								Control	
	LEAF		ROOTS		STEM		CALLI (6 WEEKS)			
	FREE	BOUND	FREE	BOUND	FREE	BOUND	FREE	BOUND	C	N
B s.	10.8±0.43	12.7±0.28	7..93±0.31	9.3±0.45	8.14±0.32	9.63±0.28	8.64±0.27	11.9±0.46	20.2± 0.3	-
S. a.	13.1±0.52	**15.2±0.68**	11.1±0.66	12.2±0.28	9.72±0.21	12.0±0.36	7.91±0.39	9.72±0.33	12.0± 0.0	-
E. c.	11.1±0.66	14.1±0.56	12.8±0.26	13.8±0.55	8.30±0.33	10.8±0.43	8.14±0.33	10.8±0.43	24.8± 0.3	-
P.a.	12.2±0.28	13.2±0.52	8.25±0.28	10.3±0.41	11.1±0.66	14.1±0.56	11.9±0.46	9.67±0.38	15.0± 0.0	-
A. n.	16.6±0.33	**18.3±0.37**	9.24±0.26	12.0±0.2	7.34±0.47	9.3±0.33	8.91±0.39	13.8±0.55	-	17.7± 0.3
A. f.	8.94±0.27	9.61±0.39	10.2±0.39	11.2±0.33	10.8±0.38	12.2±0.28	10.8±0.89	7.23±0.23	-	13.3± 0.6
C. a.	7.0±0.28	11.63±0.29	12.2±0.37	14.6±0.29	9.3±0.33	13.0±0.01	9.3±0.45	13.8±0.55	-	15.2± 0.3
C. g.	9.72±0.21	10.8±0.38	9.24±0.26	11.2±0.39	7.23±0.23	9.72±0.33	8.91±0.39	7.91±0.39	-	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).

CONCLUSION

The above finding clearly indicates that the selected plant species of Clerodendrum contains many bioactive compounds. From the current results it can be concluded that Clerodedrum pholomidis may be beneficial as a source of ingredient to the pharmaceutical industries for the development of new bioactive compound flavonoids as an antimicrobial agent.

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