IN VITRO SCREENING OF ANTIMICROBIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL SCREENING OF – SURAN

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Summary

Ethno medical uses of suran , botanically equated as tubers of Amophophallus paeoniifolius (Dennst.) Nicolson (Araceae), in the skin diseases hinted about its possible antimicrobial activity during the literature survey. To lay down scientific basis for the ethno medical usage an attempt was made to asses antifungal and antimicrobial properties of the various crude extracts of the drug by using cup-plate diffusion method against common pathogens viz., E.coli, S.aureus, E.faecalis, K.pneumoniae, C.albicans and A.fumigatus. Among the different extracts, the methonolic extract of A. paeoniifolius found relatively effective.

Key words: Antimicrobial activity, *Amorphophallus paeoniifolius*, MIC Values, DIZ, Teat organisms , TLC.

Introduction

Presently the herbal medicines are back into prominence, as the conventional synthetic medicines are on the wane because of development of microbial drug resistance against potent antimicrobial agents. The potentiality of higher plants in producing varieties of secondary metabolites possessing antimicrobial property, targeted to defy invasions by microbes is core-matrix of developing phytomedicines against drug resistance microbes. The exhaustive literature survey hinted about the possible antimicrobial property of the tubers of Amorphophallus peoniifolius (Dennst.) Nicolson (Family:Araceae). So, the sensitivity testing using some fungal pathogens and MIC determination against some bacteria was undertaken .

Literature survey revealed that the powdered tuber as an ingredient of medicines for cholera and constipation and plant juice in sores [1] in treatment of arsas (Piles), haemophilic conditions, skin diseases, intestinal worms and obesity [2] restorative in dyspepsia, debility [3,4]. The tubers are reported in management of hemorrhoids [5] to have antiprotease activity [6] and analgesic activity of methanolic extract [7] .

The tuber is reported with sterols, fat, proteins, carbohydrates, various vitamins, amino acids and minerals and abundant calcium oxalate crystals causing irritation and itching[8].

Materials and Methods

Plant material

The tubers of Amorphophallus paeoniifolius were collected from cultivated lands from Hassan district of Karnataka and authenticated by Dr.Kotresh, Botany department, Karnataka University, Dharwad. The voucher specimens of these plants and tubers were preserved in the herbarium of the pharmacognosy department of this institution.

Evalution of Antimicrobial activity :

Preparation of extracts

The tubers were dried in shade by making into chips, pulverized in a mechanical grinder to 40# ; it was extracted with petroleum ether, hydroalcohol (70%), methanol and water by soxhlation, exhaustively ; all extracts were dried in Rotary Vacuum Evaporator and over a desiccator and stored in air tight containers at 4^0C.

Test organisms

Bacterial and fungal strains were obtained from microbial type culture collection (MTCC) viz., E.coli ATCC 10536 ; S.aures ATCC 11632 ; E.faecalis ; K.pneumoniae ATCC 10031 ; C.albicans ATCC 1013 and A.fumigatus procured from Department of Microbiology, SDM-DCR Dharwad , Karnataka.

Preparation of test solution

Formulation of petroleum ether extract was made by dissolving extract in 5% dimethyl sulphoxide (DMSO). Whereas 70% hydroalcoholic, methanolic and aqueous extracts were added with distilled water, such that the final stock solutions were of 500 mg/ml concentration. Further, two fold serial dilutions of various extracts were reconstituted from them.

Atimicrobial activity :

Determination of MIC and DIZ

MIC values of each of four extracts mentioned above against selected bacterial strains were determined by macro broth dilution assay method[9]. Two fold serial dilution of the extracts of A. paeoniifolius (1–500mg /ml) were prepared in tubes with Mueller Hinton Broth as diluent. Duplicate tubes of each dilution were seeded with 0.1ml of the known stains of test organisms to the standard concentration (5 x 10^5 cfu/ml). Ciprofloxacin (1mg/ml) was taken as experimental positive control. The tubes were incubated at 37^0C for 24 hours. The lowest concentration of the extract showing absence of growth and clarity was taken as the MIC.

Antifungal activity was determined by measuring Diameter of the Inhibition Zone(DIZ) in mm , using cup-plate method against C.albicans and A.fumigatus. Fluconazole (22mg/ml) was taken as experimental positive control. The culture plates were incubated at 37^0C for 72 hours, prior to calculation of the averages of triplicate readings[10].

Table 1.
MIC Values of different extracts of *Amorphophallus paeoniifolius* against test organisms. (mg /ml)

Extracts	Test organisms			
	E.Coli ATCC 10536	**S. aureus ATCC 11632**	**E. faecalis**	**K. pneumoniae ATCC 10031**
Pet. Ether	62.5	250	R	31.25
Hydro alcohol 70%	250	32.25	31.25	4
Methanol	62.5	16	31.25	4
Aqueous	62.5	31.5	31.25	4

R – Resistant.

Table 2.
Antifungal activity of different extracts of *Amorphophallus paeoniifolius* against test organisms. (DIZ in mm)

Extracts	C.aibicans ATCC 1013			A.fumigatus		
	500	**250**	**100**	**500**	**250**	**100**
Pet. Ether	25mm	21mm	16mm	20mm	16mm	13mm
Hydro alcoholic (70%)	R	R	R	R	R	R
Methanol	24mm	20mm	14mm	22mm	18mm	16mm
Aqueous	20mm	R	R	12mm	R	R

Values are mean of triplicates R – Resistant.

Preliminary phytochemical screening :

All the extracts were subjected to preliminary phytochemical screening [11] and recorded in Table 3. As the methonolic extract showed broad spectrum activity, the same when subjected to thin layer chromatography revealed the presence of coumarins and flavonoids significantly {12} as consolidated in Table 4. As coumarins proved to posses antimicrobial [13], anti-inflammatory[14] and cytotoxic activities [15] , the further studies to isolate them is in progress.

Table 3 : Phytochemical screening of the tuber of Amorphophallus paeoniifolius.

Phytoconstiluents	extracts of tuber			
	P	A	M	W
Sterols	+	+	+	-
Saponins	-	-	-	-
Tannins	-	+	+	+
Flavonoids	-	+	+	-
Carbohydrates	-	+	+	+
Starch	-	-	-	+
Protein and amino acids	-	+	+	+
Alkaloids	-	-	-	-
Volatile oil	-	-	-	-
Fixed oil / Fat	+	-	-	-
Coumarins	-	+	+	-
Triterpenoids	+	+	+	-

P: Petroleum ether; A: HydroAlcohol; M: Methanol; W: Aqueous; '+' – Present; '-' – Absent.

Table-4: TLC screening of Methonlolic extract of tuber of Amorphophallus paeoniifolius.

Adosorbent	Solvent system	Detecting Reagent	Observation	Inference	Rf Values	
					Under UV light 365nm	After acid spray and heated at 110^0C
Silica gel 60GF 254 precoated sheet	**n-Butanol: Acetic Acid : Water (4:1:5)**	**NP/PEG & UV**	**Yellow/ Orange**	**Flavonoids present**	**0.31, 0.27,0.41, 0.65,82, 0.92**	**0.27,0.31, 0.41,0.51, 0.65,0.82, 0.92**
		NH_3/KOH	**Light Blue**	**Coumarins present**		
Silica gel 60GF 254 precoated sheet	**10% Acetic Acid**	**NP/PEG & UV**	**----**	**Flavonoids absent**	**0.27**	**0.13, 0.27, 0.92**
		NH_3/KOH	**Deep Blue**	**Coumarins present**		

Results and Discussions

The results of elaborate macro tube dilution assay of four extracts are indicated the presence of antimicrobial properties of the four crude extracts of the tuber of Amorphophallus paeoniifolius as consolidated in Table 1 & 2. The assay revealed that the K. pneumoniae remained highly sensitive to all extracts whereas the methonolic extract possess broad spectrum antimicrobial activity, notably it appeared highly effective on a notorious hospital pathogen, S.aureus, may be due to presence of coumarins significantly. In addition petroleum extract showed good antimicrobial activity which may be due to phytosterols present in them.

Conclusion

Although the tubers Amorphophallus paeioniifolius are reported to be used in skin diseases, the DIZ values against C. albicans & A. fumigatus found in the present study were not very encouraging, because extracts did not produce significant DIZs at concentrations less than 100 mg/ml. However, it can be concluded that the methonolic extract exerted more of antibacterial action than antifungal action, whereas petroleum ether extract appeared vice versa.

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