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Research Article

In vitro anti-microbial activity of combined isolates from ethyl acetate extract of *Cynodon dactylon* and *Piper betle*

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ABSTRACT

The antibacterial effect of isolated compound from *Cynodon dactylon* and *Piper betle* was evaluated on both Gram-positive and Gram-negativie bacteria. The *in-vitro* antibacterial activity was performed by Cylinder or cup plate method. The dried materials were coarsely powdered using an electric blender. Powdered materials (500g) were then packed in soxhlet apparatus and successively extracted with petroleum ether, Benzene, chloroform, ethyl acetate, methanol and water. Each time before extraction with the next solvent, the powdered materials were dried in hot air oven at below 50ºC. About 2 gms of the concentrated ethyl acetate extract was mixed with suitable quantity of silica gel (100-200 mesh). The elute was concentrated by evaporating the solvent and the residues named as *CD* isolate (90% of Chloroform: 10 % of Ethyl acetate) and *PB* isolate (50% of Toluene: 50 % of Ethyl acetate) were identified by High performance Thin Layer Chromatography. The selected isolates from *Cynodon dactylon* and *Piper betle* and the combination of both were found to have anti-microbial activity in the present study. The MIC value of CD Isolate against gram positive bacteria was 4.4 ± 0.5 mg/ml and against gram negative bacteria was 4.5 mg/ml**.** The MIC value of *PB* Isolate against gram positive bacteria was 4.2 mg/ml and against gram negative bacteria was 4.1 mg/ml. The MIC values of Test Antibiotics were found to be 0.001- 0.412 mg/ml and 0.008 -0.412 mg/ml against gram positive bacteria and gram negative bacteria respectively. The result data revealed that the isolates of individual and combined samples were showed better results for both Gram positive and Gram negative organisms. In that it was very effective against S.Typhi when compare to other organisms which are compared with standard solution. The results obtained in the present study suggest that the isolates can be used in treating diseases caused by the test organisms.

Keywords: *Cynodon dactylon; Piper betle;* Cylinder or cup plate method

INTRODUCTION

Plants and plant-derived products are part of health care system since ancient human civilization. The need of new chemical entities (NCEs) for health care is explored and served through the plant sources. In India the health care goes back to 5000 years BC. Polyherbal behavior is said to be a current pharmacological principle having the benefit of producing utmost therapeutic worth with minimum side effects(Patrick Ekong Ebong et al.,2008) Clinical microbiologists are paying attention in the area of antimicrobial plant extracts intended for two reasons. First, it is very probable that these phytochemicals will locate their way into the weapon store of antimicrobial drugs prescribed by physicians. The antimicrobial property of numerous naturally occurring compounds has been known for decades. In recent times, many plants have established special notice as sources of novel antimicrobial agents(Patel A.Vet al.,2005 Dhar et al., 1995). *Cynodon dactylon* (L.) Pers (Gramineae family), generally known as “Njem” in Morocco, possesses various medicinal property (Chopra et al., 1982). *Piper betle* belongs to the family piparaceae. . In the South East Asia region, *Piper betle L*. are among the plants that have been associated with the control of caries and periodontal diseases (Parmar et al., 1997, Ponglux et al., 1987). The present work was planned to evaluate the effect of isolated fractions on microbes by antimicrobial activity in In-vitro anti-microbiological model.

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MATERIALS AND METHODS

The plant materials such as *Cynodon dactylon* and *Piper betle* were collected from Local market in that *Cynodon dactylon* were collected from Departmental medicinal garden of Annamacharya college of Pharmacy, Rajampet, Andhrapradesh. The collected plant materials were identified and authentified by Dr. Madha-

Table 1: MIC value of gram positive bacteria and gram negative bacteria

Test organism	Minimum Inhibitory Concentrations (mg/ml)							
	TET	NEO	ERY	CHL	REF	*CD*	*PB*	*CD+PB*
Staphylococcus aureus	0.004	-	-	-	-	4.0	4.5	Combined
Staphylococcus epidermis	-	0.412	-	-	-	-	-	-
Coryne bacterium species	0.016	-	-	-	-	-	-	-
Bacilus subtilis	-	-	-	-	0.001	5.0	4.2	Combined
Streptococcus griseus	0.016	-	-	-	-	-	-	-
E.coli	-	-	-	0.008	-	4.5	4.1	Combined
Proteus vulgaris	-	-	0.412	-	-	-	-	-
Salmonella typhi	-	-	0.128	-	-	4.1	4.0	Combined

Standard MIC value of test Drugs ranges between: 0.05 - 20mg/ml; -- : Symbol denotes, In case of antibiotics the test was not performed; -- : Symbol denotes, In case of test Drugs, the result was negative.

Table 2: Effect of Cd Isolate against selected micro organism

Isolates	Organism	Drug Concentrations in µg and zone of inhibition in mm				Antibiotic 10µg
		100µg	150µg	200µg	250µg	
CD	*Staphylococcus aureus*	Negative	Negative	Negative	15	20
	Bacillus subtilis	Negative	Negative	Negative	10	20
	E-coli	Negative	Negative	Negative	10	22
	Salmonella Typhi	Negative	Negative	Negative	10	16.0

Table 3: Effect of PB Isolate against selected micro organism

Isolates	Organism	Drug Concentrations in µg and zone of inhibition in mm					Antibiotic 10µg
		100µg	200µg	300µg	400µg	500µg	
PB	*Staphylococcus aureus*	Negative	Negative	Negative	Negative	18	20
	Bacillus subtilis	Negative	Negative	Negative	Negative	10	20
	E-coli	Negative	Negative	Negative	Negative	12	22
	Salmonella Typhi	Negative	Negative	Negative	Negative	15	16.0

Table 4: Effect of Cd + Pb Isolate against selected micro organism

Isolates	Organism	Drug Concentrations in µg and zone of inhibition in mm	Antibiotic 10µg
		125µg + 250µg	
CD+PB	*Staphylococcus aureus*	Synergism (19)	21
	Bacillus subtilis	Synergism (19)	27
	E-coli	Synergism (20)	35
	Salmonella Typhi	Synergism (19)	16.6

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Preparation of extract

The freshly collected plant materials were washed; shadow dried and then dried in hot air oven at a temperature not more than 50°C. The dried materials were coarsely powdered using an electric blender. Powdered materials (500g) were then packed in soxhlet apparatus and successively extracted with petroleum ether, Benzene, chloroform, ethyl acetate, methanol and water. Each time before extraction with the next solvent, the powdered materials were dried in hot air oven at below 50°C. Finally extracts were concentrated in rotary evaporator at a temperature not more than 50°C and then, dried under vacuum desiccators. The dried extracts thus obtained were used for Isolation (C. K. Kokate et al., 22nd edition).

Isolation

About 2 gms of the concentrated ethyl acetate extract was mixed with suitable quantity of silica gel (100-200 mesh) to ensure the free flow of the extract along with adsorbent it was packed in the column through the funnel, then petroleum ether was added through the column and kept aside over night. Then the column was eluted with different organic solvents. The fraction 100ml each of the elute from the column was collected into series of 500ml glass beakers. The elute was concentrated by evaporating the solvent and the residues named as CD isolate (90% of Chloroform: 10 % of Ethyl acetate) and PB isolate (50% of Toluene: 50 % of Ethyl acetate) were identified by High performance Thin Layer Chromatography.

Test Organisms

Microbes are the important parameters which are essential for the biological screenings. For the current

research, microbes are selected and procured from the National research laboratories such as National Collection of Industrial Micro-organisms (NCIM) department of National Chemical Laboratory (NCL) pune (NCIM Catalogue).

Experimental Procedure

The antimicrobial study was carried out by Cylinder cup plate method. According to these methods, the antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

Determination of Minimum Inhibitory Concentration

Dilutions of the antibiotic ranging from 0.001-1.0 mg/ml in DMSO, dilutions of the isolates ranging from 0.05-25 mg/ml in DMSO were prepared and incorporated (added) into the wells of pre-sterilized and pre-inoculated Nutrient agar medium plates with test organisms. Plates were incubated at 370 C for 24 hrs under aerobic conditions.

Cylinder- cup plate method

After determining the MIC values, for the further sensitivity tests, doses of test drugs were fixed as 250µg/ml, 500µg/ml. And the doses of standards were fixed as 10µg/ml. Then microbiological assay was carried out by Diffusion method under aseptic conditions. Then the inoculated medium was poured in to the pre-labeled petridishes and allowed for solidification. After solidification, cups (wells) were made in the agar medium by using sterile glass borers. Different concentrations of Isolates of individual and combined form (tests) and standard drugs were introduced in to the corresponding wells of the petriplates and then plates were kept for incubation at 37°C for 24 hrs After incubation, cultures were examined for the growth of bacteria and zones of inhibition formed by them. The zones of inhibition were measured by using Antibiotic zone reader (Bacteriological analytical manual 2002, Janssen et al., 1976).

RESULTS AND DISCUSSION

As per the standards, The MIC values of Antibiotics must be range between 0.001- 1.0 mg/ml, and the MIC of Test Drugs must be range between 0.05 -20 mg/ml. The results obtained from our MIC studies have shown that, the MIC value of CD Isolate against gram positive bacteria was 4.4 ± 0.5 mg/ml and against gram negative bacteria was 4.5 mg/ml. The MIC value of PB Isolate against gram positive bacteria was 4.2 mg/ml and against gram negative bacteria was 4.1 mg/ml. The MIC values of Test Antibiotics were found to be 0.001-0.412 mg/ml and 0.008 -0.412 mg/ml against gram positive bacteria and gram negative bacteria showed in Table 1. MIC values are the important parameters for microbial sensitivity tests. With known MIC values, the doses of isolates were fixed for microbial sensitivity tests, and tests were performed against both gram positive and gram negative bacteria. The result data revealed that the isolates of individual and combined samples were showed better results for both Gram positive and Gram negative organisms Table 2, 3 and Table 4. In that it was very effective against S.Typhi when compare to other organisms which are compared with standard solution. The constituent molecules obtained from these isolates from ethyl acetate extracts can be good candidates for further activities.

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