Research Article

BIO ACTIVITY OF CRUDE EXTRACTS OF LEAVES OF *CENCHRUS* GRASS IN DIFFERENT POLAR SOLVENTS AGAINST SOME PATHOGENIC MICROBES

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ABSTRACT

The aim of present study is to investigate the antimicrobial activity of *Cenchrus ciliaris* (CAZRI-358) and *Cenchrus setigerus* (CAZRI-76) in order to use it as a possible source for new antimicrobial substances against important human pathogens. Crude extracts of leaf of *C. ciliaris* and *C. setigerus* were evaluated against six medically important bacteria viz. *Escherichia coli* (Gram-ve), *Raoultella planticola* (Gram-ve), *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeruginosa* (Gram-ve), *Bacillus Subtilis* (Gram +ve), *Enterobactor aerogens* (Gram-ve), one yeast *Candida albicans* and one fungi *Aspergillus flavus*. The dried and powdered leaves were successively extracted with hexane, toluene, isopropyl alcohol, acetone and ethanol using soxhlet assembly. The antimicrobial activity assay was done by both disc diffusion and serial dilution methods. Iso propyl alcohol extract of *C. ciliaris* (CAZRI-358) showed highest activity against *Staphylococcus aureus*. Disc diffusion and serial dilution methods were used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) at concentrations of 0.469mg/ml to 15mg/ml. Iso propyl alcohol extract of *C. ciliaris* was the most active one with MIC of 0.469mg/ml against *R. planticola*. The present investigation provides a scientific basis for the use of these plant extracts in home-made remedies and their possible application against micro-organisms.

Keywords: *Bacillus subtilis*; *Candida albicans*; *Cenchrus setigerus*; *Enterobactor aerogens*; *Raoultella planticola*.

INTRODUCTION

Presently, A huge number of research have been carried out to extract various natural products for antimicrobial activity in whole world[1] because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics[2] in current clinical use and non-availability and high cost of new generation of antibiotics with limited effective span have resulted in increase in morbidity and mortality, Therefore, there is a need to look for substances from other sources with proven antimicrobial activity[3]. A number of plants have been documented for their biological[4-5] and antimicrobial properties[6-9].

Cenchrus grasses are known as C_4 grasses and gaining attention in various field of research, as they are best suited to the present environmental conditions. These grasses are more competitive under the conditions of high temperature, solar radiation and low moisture[10]. C_4 grasses are more efficient at gathering Carbon dioxide and utilizing nitrogen from the atmosphere and recycled N in the soil[11-12]. This grass has excellent soil binding capacity which helps to conserve soil in desert areas[13]. However, *Cenchrus* is most suitable and highly nutritive grasses for desert environmental conditions, still no antimicrobial work yet have been done on this grass.

E. coli is the culprits for human urinary tract infections[14]. *P. aeruginosa* is involved in respiratory tract, urinary tract[15], bloodstream, and central nervous system infections of nosocomial origin[16] and this pathogen is becoming resistant against gentamycin, ciprofloxacin[17]. Major causative agent of nosocomial infections is *S. aureus* along with *E. coli*. *Raoultella planticola* has been determined to cause severe pancreatitis in one case[18]. *C. albicans* is notorious for causing candidiasis. *B. subtilis* can contaminate food. *E. aerogens* is nosocomial and pathogenic bacterium that cause opportunistic infections including most type of infections.

MATERIALS AND METHODS

Experimental design: Crude extracts of leaves of *C. ciliaris* (CAZRI-358) and *C. setigerus* (CAZRI-76) were prepared with a series of non polar to polar solvents by hot extraction method[19] in soxhlet assembly. Different extracts were then screened for antimicrobial activity by disc diffusion Assay[20] against a few medically important bacteria, fungi and yeast. The fraction showing best activity was then used for determining of minimum inhibitory concentration (MIC) by tube dilution method[21] and minimum bactericidal/fungicidal concentration (MBC/MFC).

Collection of plant material: Leaves of *C. ciliaris* (CAZRI-358) *and C. setigerus* (CAZRI-76) were collected in the month of August from the Central Arid Zone Research Institute, Jodhpur, Rajasthan. The collected plant

materials were transferred immediately to the laboratory cleaned with water and selected plant parts were separately shade dried for one week. Shade dried leaves were powdered with the help of grinder. Fine powder of each sample was stored in clean container to be used for Soxhlet extraction following the method of Subramanian and Nagarjan, (1969)[22] in different polar solvents selected.

Extraction procedure: Leaves (10 gm) were sequentially extracted with different solvents (250 ml) according to their increasing polarity (hexane < toluene < isopropyl alcohol < acetone < ethanol) by using Soxhlet apparatus for 18 hours at a temperature not exceeding the boiling point of the respective solvent. The obtained extracts were filtered by using Whatman No. 1 filter paper and then concentrated at 40°C by using an evaporator. The residual extracts were stored in refrigerator at 4°C in small and sterile glass bottles. Total yield were calculated.

Drugs and chemicals used

Drugs: Gentamycin (for bacteria) and Ketoconazole (for yeast and fungi)

Chemicals: hexane, toluene, isopropyl alcohol, acetone and ethanol, Nutrient Agar (for bacteria), Sabouraud Dextrose Agar (for yeast and fungi).

Micro-organisms: The organisms used in this study were four Gram-negative bacteria, two Gram positive bacteria, one yeast and one fungi namely.

Bacteria: *Raoultella planticola* (Gram-ve) (MTCC-530), *Escherichia coli* (Gram-ve) (MTCC-46), *Pseudomonas aeruginosa* (Gram-ve) (MTCC-1934), *Bacillus subtilis* (Gram+ve) (MTCC-121), *Enterobactor aerogens* (Gram-ve) (MTCC-111), *Staphylococcus aureus* (Gram+ve) (MTCC-3160).

Yeast: *Candida albicans* (MTCC-183)

Fungi: *Aspergillus flavus* (MTCC-277)

Screening for antimicrobial activity: Bacterial strains were grown and maintained on Nutrient Agar medium, while yeast and fungi were maintained on Sabouraud Dextrose Agar medium. Petri plates were pre-seeded with 15 ml of growth agar medium and 1.0 ml of inoculum (inoculum size 1×10^8 CFU/ml for bacteria and 1×10^7 cell/ml for yeast and fungi). Extract discs (10mcg/disc) were then placed on the seeded agar plates. Each extract was tested in triplicate with gentamycin (10mcg/disc) and ketoconazole (10mcg/disc) as standard for bacteria and fungi, respectively. The plates were kept at 4°C for 1 h for diffusion of extract, thereafter were incubated at 37°C for bacteria (24 h) and 27°C for fungi (48 h)[23]. The inhibition zones were measured and compared with the standard reference antibiotics. Activity index for each extract was calculated (Table 1).

$$\text{Activity index (AI)} = \frac{\text{Inhibition Zone of the sample}}{\text{Inhibition Zone of the standard}}$$

Table 1: Inhibition zone (mm)* and Activity index of Leaves of *Cenchrus* grass in different polar solvents against tested pathogens.

Solvents with polarity	Species	IZ and AI	Bio-activity of leaf extracts of *Cenchrus* grass against test pathogens							
			Escherichia coli	*Staphylococcus aureus*	*Raoultella planticola*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Enterobactor aerogens*	*Aspergillus flavus*	*Candida albicans*
Hexane (0.1)	C.s.-76	IZ	-	9.33±0.29	-	-	7.33±0.27	-	-	-
		AI	-	0.583	-	-	0.458	-	-	-
	C.c.-358	IZ	-	-	-	-	10.83±0.22	7.33±0.23	-	-
		AI	-	-	-	-	0.417	0.367	-	-
Toluene (2.4)	C.s.-76	IZ	-	-	-	-	14.5±0.64	-	-	-
		AI	-	-	-	-	0.518	-	-	-
	C.c.-358	IZ	-	8.50±0.64	-	-	-	-	-	-
		AI	-	0.531	-	-	-	-	-	-
Isopropyl Alcohol (3.4)	C.s.-76	IZ	9.50±0.64	17.67±0.28	10.33±0.25	8.50±0.64	18.67±0.29	9.33±0.21	-	8.5±0.64
		AI	0.475	1.104	0.517	0.405	0.667	0.467	-	0.531
	C.c.-358	IZ	-	35.83±0.24	30.67±0.25	14.67±0.27	-	-	-	-
		AI	-	2.756	1.534	0.815	-	-	-	-
Acetone (5.1)	C.s.-76	IZ	-	10.50±0.64	7.17±0.24	7.17±0.27	11.83±0.24	9.33±0.21	-	11.67±0.22
		AI	-	0.656	0.359	0.478	0.592	0.467	-	0.729
	C.c.-358	IZ	-	11.17±0.24	-	8.17±0.21	8.17±0.28	-	-	-
		AI	-	0.859	-	0.454	0.292	-	-	-
Ethanol (5.2)	C.s.-76	IZ	8.17±0.24	8.50±0.64	7.33±0.25	-	14.83±0.24	-	-	-
		AI	0.409	0.531	0.367	-	0.618	-	-	-
	C.c.-358	IZ	-	-	8.33±0.24	-	8.33±0.23	8.17±0.24	-	9.83±0.25
		AI	-	-	0.417	-	0.521	0.409	-	0.819

Abbreviations: All values are mean ± SD, n=3 (p>0.005), C.s.-76= *Cenchrus setigerus* (CAZRI-76), C.c.-358= *Cenchrus ciliaris* (CAZRI-358), IZ= Inhibition zone in mm±S.D., AI= Activity index.

Determination of minimum inhibitory concentration (MIC): Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against test pathogens. To measure the MIC values, various concentrations of the stock, 15, 7.5, 3.75, 1.875, 0.938, 0.469, 0.234, 0.117, 0.059, 0.029mg/ml were assayed against the test pathogens. Plant extracts were re-suspended in acetone (which has no activity against test microorganisms) to make 15mg/ml final concentration and then two fold serially diluted. 1 ml of each extract was added to test tubes containing 1 ml of sterile NA media (for bacteria) and SDA (for fungi). The tubes were then inoculated with standard size of microbial suspension and the tubes were incubated at 37°C for 24 h for bacteria and 28°C for 48 h for yeast in a BOD incubator and observed for change in turbidity after 24 h compared with the growth and in controls[24]. A tube containing nutrient broth and inoculum but no extract was taken as control. The least extract concentration which inhibited the growth of the test organisms was taken as MIC[24]. Bacterial and fungal suspensions were used as negative control, while broth containing standard drug was used as positive control. Each extract was assayed in duplicate and each time two sets of tubes were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the test tubes. The MIC values were taken as the lowest concentration of the extracts in the test tubes that showed no turbidity after incubation. The turbidity of the test tube was interpreted as visible growth of microorganisms. (Table 2).

Determination of Minimum bactericidal/fungicidal concentration (MBC/MFC): Equal volume of the various concentration of each extract and nutrient broth mixed in micro-tubes to make up 0.5ml of solution. 0.5ml of McFarland standard of the organism suspension was added to each tube. The tubes were incubated aerobically at 37°C for 24 h for bacteria and 28°C for 48 h for yeast. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculum. The MBC was determined by sub culturing the test dilution on nutrient Agar and further incubated for 24 h. The highest dilution that yielded no single bacterial colony was taken as the Minimum bactericidal Concentration[26]. MBC was calculated for some of the extracts showed high antimicrobial activity against highly sensitive organisms. (Table 2).

Total activity (TA) determination: Total activity is the volume at which the test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract is expressed in ml/g[27].

$$\text{Total Activity} = \frac{\text{Extract per gram dried plant part}}{\text{MIC of extract}}$$

Statistical Analysis: Mean value and standard deviation were calculated for each test bacteria, yeast and fungi. Data were analyzed by one-way ANOVA and p values were considered significant at $p > 0.005$[28].

RESULTS

Preliminary phyto–profiling: The preliminary phyto–profiling for the leaves of *Cenchrus* extracts were carried out wherein the consistency was found to be sticky in the hexane extracts whereas all other extracts were found to be non-sticky. The yield mg/g (w/w) of the extracts was also analyzed wherein the highest yield was found in acetone extract of *C. ciliaris* (641±16.25 mg/10g ± S.D.) and in ethanol extract of *C. setigerus* (427±12.38 mg/10g ± S.D.) (Table 3)**.**

Antimicrobial activity: Antimicrobial activity (assessed in terms of inhibition zone and activity index) of the leaf extracts against selected microorganisms were recorded. In the present study total 10 extracts of leaves of selected grasses were tested for their bioactivity, among which all the extracts showed significant antimicrobial potential against test microbes. However, all the extracts of *C. ciliaris* did not produce any type of activity against *E. coli* and *A. flavus* was the most resistant pathogen in this study. Most susceptible organism in the investigation was *B. subtilis* against which, most of the plant extracts showed inhibition zone.

Antibacterial activity: Maximum antibacterial activities were observed for iso propyl alcohol extracts by the both species of *Cenchrus* grass; *C. ciliaris* against *S. aureus* followed by *C. setigerus* against *B. subtilis*. Inhibition Zone and Activity Index was found to be maximum in *C. ciliaris* IZ- 35.83±0.24 mm, AI- 2.756 against *S. aureus* followed by *C. setigerus* IZ- 18.67±0.29 mm, AI- 0.667 against *B. subtilis*.

Antifungal activity: Maximum antifungal activity was observed by acetone extract of *C. setigerus*. Inhibition Zone and Activity Index was found to be maximum in *C. setigerus* IZ- 11.67±0.22 mm, AI- 0.729 followed by *C. ciliaris* IZ- 9.83±0.25 mm, AI- 0.819 against *C. albicans*.

Minimum inhibitory concentration (MIC) and Minimum bactericidal/fungicidal concentration (MBC/MFC): MIC and MBC/MFC values (Table 1) were evaluated for those plant extracts, which were showing activity in diffusion assay. The range of MIC and MBC/MFC of extracts recorded was 0.469 - 15 mg/ml. In the present investigation lowest MIC value was recorded by iso propyl alcohol extracts, 0.469 mg/ml for *C. ciliaris* against *R. planticola* and 0.938 mg/ml against *S. aureus* as well as for *C. setigerus* against *B. subtilis*. MIC and MBC/MFC values were found equal for 7 times of *C. setigerus* and 4 times of *C. ciliaris*. Iso propyl alcohol extract of *C. ciliaris* showed bactericidal effect against *S. aureus*, *R. planticola* and *P. aeruginosa*.

Total activity: Total activity indicates the volume at which extract can be diluted with still having ability to kill microorganism. Most of the extracts showed high values of TA against *S. aureus, R. planticola*, *B. subtilis* and yeast *C. albicans* which proves the potential to inhibit the

growth of the test microorganisms, even at low concentration. Maximum TA values were recorded 42.48 ml, 38.08 ml, 34.19 ml and 17.81 ml against *R. planticola*, *B. subtilis*, *S. aureus* and yeast *C. albicans* respectively. Amount of extract isolated from plant parts and total activity was calculated and recorded (Table 4).

Table 2: Minimum inhibitory concentration and (MBC/MFC) of Leaves of *Cenchrus* in different polar solvents against tested pathogens.

Solvents	Species	MIC MBC/MFC	Bio-activity of leaf extracts against pathogens							
			E. c.	*S. a.*	*R. p.*	*P. a.*	*B. s.*	*E. a.*	*A. f.*	*C. a.*
Hexane	C.s.-76	MIC	-	3.75	-	-	7.5	-	-	-
		MBC/MFC	-	7.5	-	-	15	-	-	-
	C.c.-358	MIC	-	-	-	-	3.75	7.5	-	-
		MBC/MFC	-	-	-	-	7.5	15	-	-
Toluene	C.s.-76	MIC	-	-	-	-	1.875	-	-	-
		MBC/MFC	-	-	-	-	1.875	-	-	-
	C.c.-358	MIC	-	3.75	-	-	-	-	-	-
		MBC/MFC	-	7.5	-	-	-	-	-	-
Isopropyl alcohol	C.s.-76	MIC	7.5	1.875	3.75	3.75	0.938	3.75	-	7.5
		MBC/MFC	7.5	3.75	7.5	7.5	0.938	7.5	-	15
	C.c.-358	MIC	-	0.938	0.469	1.875	-	-	-	-
		MBC/MFC	-	0.938	0.469	1.875	-	-	-	-
Acetone	C.s.-76	MIC	-	3.75	7.5	7.5	3.75	3.75	-	1.875
		MBC/MFC	-	3.75	15	15	3.75	7.5	-	1.875
	C.c.-358	MIC	-	1.875	-	7.5	7.5	-	-	-
		MBC/MFC	-	3.75	-	15	15	-	-	-
Ethanol	C.s.-76	MIC	7.5	3.75	7.5	-	1.875	-	-	-
		MBC/MFC	7.5	7.5	15	-	3.75	-	-	-
	C.c.-358	MIC	-	-	3.75	-	7.5	7.5	-	3.75
		MBC/MFC	-	-	7.5	-	15	15	-	3.75

C.s.-76= *Cenchrus setigerus* (CAZRI-76) ; C.c.-358= *Cenchrus setigerus* (CAZRI-358) ; MIC - Minimum inhibitory concentration (mg/ml); MBC - Minimum bactericidal concentration (mg/ml); MFC - Minimum fungicidal concentration (mg/ml); *E. c. - Escherichia coli; S. a. - Staphylococcus aureus; R. p. - Raoultella planticola; P. a. - Pseudomonas aeruginosa*; *B. s. - Bacillus subtilis*; *E. a. - Enterobactor aerogens; A. f. - Aspergillus flavus*; *C. a. - Candida albicans.*

Table 3: Preliminary phyto-profile for leaves of *Cenchrus* grass in different polar solvent

Solvents	Boiling point of solvents (°C)	Solubility in Water (%)	Plants	Total yield mg/10gm±S.D.	Color	Consistency
Hexane	65-70	0.001	C.s.-76	153±16.68	Dark brown	Sticky
			C.c.-358	179±16.84	Very dark green	Sticky
Toluene	109-111	0.051	C.s.-76	200±12.74	Brown	Nonsticky
			C.c.-358	144±8.42	Light yellow	Nonsticky
Isopropyl alcohol	81-83	100	C.s.-76	357±9.81	Colorless	Nonsticky
			C.c.-358	199±13.68	Yellow	Nonsticky
Acetone	55-56	100	C.s.-76	334±12.87	Pale green	Nonsticky
			C.c.-358	641±16.25	Yellow	Nonsticky
Ethanol	78.4	100	C.s.-76	427±12.38	Yellow	Nonsticky
			C.c.-358	431±11.37	Yellow	Nonsticky

C.s.-76= *Cenchrus setigerus* (CAZRI-76) ; C.c.-358= *Cenchrus setigerus* (CAZRI-358)

Table 4: Total activity of leaves of *Cenchrus* in different polar solvents against tested pathogens

Solvents	Species	Total activity of leaf extracts against pathogens							
		E. c.	*S. a.*	*R. p.*	*P. a.*	*B. s.*	*E. a.*	*A. f.*	*C. a.*
Hexane	C.s.-76	-	4.08	-	-	2.04	-	-	-
	C.c.-358	-	-	-	-	4.77	2.39	-	-
Toluene	C.s.-76	-	-	-	-	10.67	-	-	-
	C.c.-358	-	3.84	-	-	-	-	-	-
Isopropyl alcohol	C.s.-76	4.76	19.04	9.52	9.52	38.08	9.52	-	4.76
	C.c.-358	-	21.23	42.48	10.61	-	-	-	-
Acetone	C.s.-76	-	8.91	4.45	4.45	8.91	8.91	-	17.81
	C.c.-358	-	34.19	-	8.55	8.55	-	-	-
Ethanol	C.s.-76	5.69	11.39	5.69	-	22.77	-	-	-
	C.c.-358	-	-	11.49	-	5.75	5.75	-	11.49

C.s.-76= *Cenchrus setigerus* (CAZRI-76) ; C.c.-358= *Cenchrus setigerus* (CAZRI-358); *E. c. - Escherichia coli*; *S. a. - Staphylococcus aureus*; *R. p. - Raoultella planticola*; *P. a. - Pseudomonas aeruginosa*; *B. s. - Bacillus subtilis*; *E. a. - Enterobactor aerogens.*

DISCUSSION

Results of the present study showed that 10/10 extracts tested inhibited the growth of selected bacteria and fungi, indicating broad spectrum bioactive nature of selected two plants (5/5 in *C. setigerus* and 5/5 in *C. ciliaris*). Iso propyl alcohol and acetone extracts in both species of *Cenchrus* express maximum antibacterial and antifungal activities respectively by suppressing the growth of all microbes under investigation. In the present study, most of the extracts of *C. setigerus* were found to be potent inhibitor of tested organisms except *A. flavus*. Excellent antibacterial activities were observed by iso propyl alcohol and antifungal activities by acetone extracts in both species of *Cenchrus* were shown by low MIC and MBC/MFC values. MBC/MFC values were found higher than the MIC values of the extracts against microorganisms tested; indicate the bacteriostatic/fungistatic effects of the extracts. 7 times of *C. setigerus* and 4 times of *C. ciliaris* were found to be bactericidal/fungicidal in nature. Iso propyl alcohol extracts of both species of *Cenchrus* were act as bactericidal against *E. coli, R. planticola, B. subtilis, S. aureus and P. aeruginosa.* Iso propyl alcohol extract of *C. setigerus* and ethanolic extract of *C. ciliaris* were recorded as fungicidal against *C. albicans*.

Gram positive bacteria *B. subtilis, S. aureus* were the most susceptible organisms after Gram negative bacteria *E. coli, R. planticola, P. aeruginosa.* and *E. aerogens*, which supported the finding that plant extracts are usually more active against Gram positive bacteria than Gram negative[9]. Susceptibility differences between Gram-positive and Gram-negative bacteria may be due to cell wall structural differences between these classes of bacteria. The Gram-negative bacterial cell wall outer membrane appears to act as a barrier to many substances including synthetic and natural antibiotics[29].

CONCLUSION

Extracts under this study not only inhibit the bacterial/fungal growth but the IZ developed, was more or less permanent when compared with the IZ developed by the standard drug used, as after sometime bacterial/fungal colonies could be easily seen in IZ developed by standard drugs. In the light of the fact that microorganism are becoming resistant against the drugs in use, present investigation is of great significance, as far as the future drugs are concerned and uses of selected plants by the pharmaceutical industries for preparing plant based antimicrobials drugs. *Cenchrus* grass easily grows in harsh climatic conditions or xeric conditions and requires less care; hence its use as raw material for preparing drugs would definitely be economical.

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