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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL STUDIES ON THE STEM OF *CENCHRUS CILIARIS* AND *CENCHRUS SETIGERUS*

DR. PREMLATA SINGARIYA*[1], DR. PADMA KUMAR[1], DR. KRISHAN K MOURYA[2]

[1]Laboratory of Tissue Culture and Secondary Metabolites, Department of Botany, University of Rajasthan, Jaipur- 302004,[2]Nodal Officer, Animal Husbandry Department, Pahari (Bharatpur) Rajasthan,Email: premlatasingariya@gmail.com

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ABSTRACT

The stems of *Cenchrus ciliaris* (CAZRI-358) and *Cenchrus setigerus* (CAZRI-76) were evaluated against medically important bacteria viz. *Proteus merabilis, Klebsiella pnemoniae, Agerobacterium tumefaciens* and one fungi *Aspergillus niger*. The dried and powdered stems were successively extracted with a series of non polar to polar solvents using soxhlet assembly. The antimicrobial assay was done by both disc diffusion and serial dilution methods. Glacial acetic acid extract of *C. ciliaris* showed highest activity against *P. merabilis* to varying degrees, but it had no inhibitory effect on fungal species (*Aspergillus niger*) by any extract of both the grasses. Gentamycin, the standard antibacterial drug used was effective in inhibiting these bacteria. The effect on *P. merabilis* and *A. tumefaciens* were comparable to that of gentamycin. Ketoconazole the standard anti fungi used was effective against the fungi. The extract of *Cenchrus* grasses also significantly (P>0.005) inhibited the bacterial growth. The inhibitory effect is very identical in magnitude and comparable with that of standard antibiotics used.

Keywords: *Cenchrus ciliaris, Cenchrus setigerus*, *Proteus merabilis, Klebsiella pnemoniae, Agerobacterium tumefaciens* and *Aspergillus niger.*

INTRODUCTION

Antimicrobial resistance to anti microbial agents has lead to treatment failure and the shift of medical care from orthodox to herbal medicine. This development has led to increased search to unfold new, broad spectrum, potent antimicrobial agents. Most of the herbal medicines in use await validation of their claimed effects and possibly the development of novel antimicrobial drugs from them[1]. Natural plants derived compounds contribute a lot in fight against pathogens. Various plant extracts have also been examined for their antibacterial activity with the objective of exploring environmentally safe alternatives of plant disease control[2].

Cenchrus grasses (C_4 grasses) are gaining attention in various field of research, as they are best suited to the present environmental conditions. C_4 grasses are more competitive under the conditions of high temperature, solar radiation and low moisture[3]. These grasses are more efficient at gathering Carbon dioxide and utilizing nitrogen from the atmosphere and recycled N in the soil[4-5]. This grass has excellent soil binding capacity which helps to conserve soil in desert areas[6]. However, *Cenchrus* is most suitable and highly nutritive grasses for desert environmental conditions, still no antimicrobial work yet have been done on this grass.

Klebsiella is the genus name for one of these bacteria found in the respiratory, intestinal, and urino-genital tracts of animals and man. When *Klebsiella* bacteria get outside of the gut, however, serious infection can occur. Klebsiella pneumonia more frequently causes lung destruction and pockets of pus in the lung (known as abscesses). The mortality rate for untreated cases is around 90%. There may also be pus surrounding the lung (known as empyema), respiratory infections, such as bronchitis, which is usually a hospital-acquired infection[7-8]. *Proteus merabilis* is a rod shaped bacterium causes obstruction and renal failure. It can also cause wound infections, septicemia and pneumonias, mostly in hospitalized patients. *A. tumefaciens* (Plant pathogen) uses horizontal gene transf er to cause tumors "crown gall disease" in plants. It can be responsible for opportunistic infections in humans with weakened immune systems[9-10].

The objective of present study is to evaluate the *in vitro* anti microbial properties of crude extracts of *Cenchrus* grass in different polar solvents with gentamycin and ketoconazole against different species of bacteria and fungi.

MATERIAL AND METHODS

Experimental design

Crude extracts of Stems *of Cenchrus ciliaris* (CAZRI-358) and *Cenchrus setigerus* (CAZRI-76) were prepared with a series of non polar to polar solvents by hot extraction method[11] in soxhlet assembly. Different extracts were then screened for antimicrobial activity by disc diffusion Assay[12] against a few medically important bacteria and fungi. The fraction showing best activity was then used for determining of minimum inhibitory concentration (MIC) by tube dilution method[13] and minimum bactericidal/fungicidal concentration (MBC/MFC).

Collection of plant material

Stems of *C. ciliaris* and *C. setigerus* 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 shade dried for one week. Then 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[14] in different polar solvents selected.

Extraction procedure

Each plant part (10 gm) was sequentially extracted with different solvents (250 ml) according to their increasing polarity (hexane < petroleum ether < toluene < benzene < iso propyl alcohol < chloroform < ethyl acetate < acetone < ethanol < glacial acetic acid < water) 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 40^0C by using an evaporator[15]. The residual extracts were stored in refrigerator at 4^0C in small and sterile glass bottles. Percent extractive values were calculated by the following formula (table-4).

$$\text{Percent Extracts} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Drugs and chemicals used

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

Chemicals

Hexane, toluene, iso propyl alcohol, acetone and ethanol, Nutrient Agar (for bacteria), Sabouraud Dextrose Agar (for fungi).

Micro-organisms

The organisms used in this study were three Gram-negative bacteria and one fungus.

Proteus merabilis (MTCC-3310), *Klebsiella pnemoniae* (MTCC-4030), *Agerobacterium tumefaciens* (MTCC-431) and one fungi *Aspergillus niger* (MTCC-282).

Test pathogenic microorganisms were procured from Microbial Type Culture Collection, IMTECH, Chandigarh, India. The reference strains of bacteria were maintained on nutrient agar slants, sub cultured regularly (after every 30 days) and stored at 4°C as well as at –80°C by preparing suspensions in 10% glycerol.

Screening for antimicrobial activity

Bacterial strains were grown and maintained on Nutrient Agar medium, while fungi and fungi were maintained on Sabouraud Dextrose Agar medium. Disc diffusion assay was performed for screening. NA and SDA base plates were seeded with the bacterial and fungal inoculum, respectively (inoculum size 1×10^8 CFU/ml for bacteria and 1×10^7 cell/ml for fungi). Sterile filters paper discs (Whatman no. 1, 5mm in diameter) were impregnated with 100 µl of each of the extracts (100 mg/ml) to give a final concentration of 1 mg/disc and left to dry in vaccuo so as to remove residual solvent, which might interfere with the determination. Petri plates were pre-seeded with 15 ml of growth agar medium and 1.0 ml of inoculum[16]. Extract discs 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)[17]. The inhibition zones were measured and compared with the standard reference antibiotics[18-22]. AI for each extract was calculated (Table 2).

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

Broth dilution method

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 (for bacteria 1×10^8 CFU/ml and 1×10^7 cell/ml for fungi) and the tubes were incubated at 37°C for 24 h for bacteria and 28°C for 48 h for fungi in a BOD incubator and observed for change in turbidity after 24 h compared with the growth and in controls[16]. 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. 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[23-24]. The turbidity of the test tube was interpreted as visible growth of microorganisms.

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[25]. The tubes were incubated aerobically at 37°C for 24 h for bacteria and 28°C for 48 h for fungi. 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 Mueller Hinton 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.

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 or compound isolated and 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 and fungi. Data were analyzed by one-way ANOVA and p values were considered significant at $p > 0.005$.[2]

RESULTS

Quantitative estimation

The preliminary phyto–profiling for the stems of *C. ciliaris* and *C. setigerus* were carried out according to Farnsworth[28] wherein the consistency was found to be sticky in the high polar solvent extracts whereas the low polar solvent extracts were found to be non-sticky which supported by Singariya[12]. The yield (%) of the extracts was also analyzed where in the highest yields were recorded for *C. ciliaris* (6.48) in acetone extracts and for *C. setigerus* (4.33) in glacial acetic acid extracts.

Table 1: Preliminary Phyto-Profile for Stems of *Cenchrus* Grass In Different Polar Solvent.

S.No.	Solvents	Preliminary phyto-profile for Stems of *Cenchrus* grass			
		Color		Consistency	
		C. ciliaris	*C. setigerus*	*C. ciliaris*	*C. setigerus*
1.	Water	Brown	Brick red	Sticky	Nonsticky
2.	Glacial acetic acid	Very dark green	Dark green	Sticky	Sticky
3.	Ethanol	Yellow	Yellow	Nonsticky	Nonsticky
4.	Acetone	Dark brown	Coffee color	Sticky	Nonsticky
5.	Ethyl acetate	Light green	Yellowish green	Nonsticky	Nonsticky
6.	Chloroform	Yellow	Greenish brown	Sticky	Sticky
7.	Iso propyl alcohol	Brown	Colorless	Nonsticky	Nonsticky
8.	Benzene	Yellow	Yellow	Nonsticky	Nonsticky
9.	Toluene	Colorless	Brown	Nonsticky	Nonsticky
10.	Petrolium ether	Very light Yellow	Light Yellow	Nonsticky	Nonsticky
11.	Hexane	Very dark green	Dark Brown	Sticky	Sticky

Antimicrobial activity

Antimicrobial activity (assessed in terms of inhibition zone and activity index) of the stem extracts in different polar solvents, tested against selected microorganisms were recorded (Table 2). In the present study total 22 extracts of stem of selected grasses were tested for their bioactivity, among which 19 extracts showed significant antimicrobial potential against test microbes. However, only three extracts (petroleum ether extract of *C. setigerus* and hexane extract of both the species of *Cenchrus* grass) showed no activity against any selected microorganism at tested concentration. Most susceptible organism in the investigation was *A. tumefaciens* against which, most of the plant extracts showed inhibition zone. But, according the zone of inhibition *P. merabilis* was the most susceptible organism. The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The assay was repeated twice and mean of the three experiments was recorded. Maximum antibacterial activities were observed by water and glacial acetic acid extracts in *Cenchrus* grass.

Table 2: Inhibition Zone (Mm)* And Activity Index of Stems Of *Cenchrus* Grass In Different Polar Solvents Against Tested Pathogens.

S. No.	Solvents with Polarity Index	Plants	Bio-activity of stem extracts against pathogens							
			Proteus merabilis		*Klebsiella pnemoniae*		*Agerobacterium tumefaciens*		*Aspergillus niger*	
			IZ	AI	IZ	AI	IZ	AI	IZ	AI
1.	Water	*C.s.*-76	-	-	-	-	9.5±0.64	0.475	-	-
	(9)	*C.c.*-358	10.67±0.26	0.889	9.5±0.64	0.475	8.17±0.27	0.511	-	-
2.	Glacial acetic acid	*C.s.*-76	38.83±0.21	3.236	26.5±0.64	1.325	25.67±0.22	1.167	-	-
	(6.2)	*C.c.*-358	30.67±0.24	2.556	10.5±0.64	0.525	30.67±0.26	1.917	-	-
3.	Ethanol	*C.s.*-76	7.67±0.24	0.548	-	-	9.17±0.27	0.459	-	-
	(5.2)	*C.c.*-358	-	-	8.67±0.21	0.434	9.33±0.23	0.666	-	-
4.	Acetone	*C.s.*-76	-	-	-	-	15.5±0.64	0.705	-	-
	(5.1)	*C.c.*-358	-	-	8.33±0.26	0.417	9.5±0.64	0.679	-	-
5.	Ethyl acetate	*C.s.*-76	8.83±0.24	0.631	10.5±0.64	0.525	12.33±0.23	0.771	-	-
	(4.4)	*C.c.*-358	30.83±0.23	3.854	-	-	8.33±0.22	0.694	-	-
6.	Chloroform	*C.s.*-76	13.17±0.27	1.463	8.17±0.23	0.409	10.17±0.24	0.509	-	-
	(4.1)	*C.c.*-358	-	-	10.83±0.27	0.542	8.33±0.22	0.463	-	-
7.	Iso propyl alcohol	*C.s.*-76	14.67±0.28	1.630	9.67±0.24	0.484	11.33±0.22	0.755	-	-
	(3.9)	*C.c.*-358	8.83±0.24	0.736	8.33±0.24	0.417	9.67±0.24	0.691	-	-
8.	Benzene	*C.s.*-76	-	-	-	-	8.33±0.21	0.694	-	-
	(2.7)	*C.c.*-358	8.33±0.24	1.041	-	-	-	-	-	-
9.	Toluene	*C.s.*-76	-	-	-	-	8.33±0.25	0.694	-	-
	(2.4)	*C.c.*-358	-	-	7.17±0.23	0.359	11.5±0.64	0.479	-	-
10	Petrolium	*C.s.*-76	-	-	-	-	-	-	-	-
	Ether (0.1)	*C.c.*-358	-	-	-	-	8.17±0.22	0.681	-	-
11.	Hexane	*C.s.*-76	-	-	-	-	-	-	-	-
	(0.1)	*C.c.*-358	-	-	-	-	-	-	-	-

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.

Inhibition Zone and Activity Index

Glacial acetic acid extract of *C. setigerus* and ethyl acetate extracts of *C. ciliaris* were showed highest activity (IZ- 38.83±0.21 mm, AI- 3.236 and IZ- 30.83±0.23 mm, AI- 3.854) respectively against *P. merabilis* followed by GAA extract of *C. ciliaris* (IZ- 30.67±0.26 mm, AI- 1.917) against *A. tumefaciens* and *C. setigerus* (IZ- 26.50±0.64 mm, AI- 1.325) against *K. pnemoniae*. (table 2)

Antibacterial assay

From the results of the antimicrobial screening (Tables 2), the GAA and ethyl acetate extracts have significant antimicrobial activities compared to the other solvent extracts with respect to the tested bacteria *P. merabilis*. The ANOVA analysis revealed that GAA extracts of both the grasses showed highly significant inhibitory effect ($p > 0.005$) when compared with Gentamycin which are used as positive controls. The toluene extracts of *C. setigerus* did not show any inhibitory effects.

MIC and MBC/MFC

MIC and MBC/MFC values (Table 3) were evaluated for those plant extracts, which were showing activity in diffusion assay. The range of MIC and MBC/MFC of extracts recorded was 0.117 - 15 mg/ml. In the present investigation lowest MIC value 0.117 mg/ml was recorded for GAA extracts of *C. setigerus* against *P. merabilis* followed by 0.234 mg/ml for *C. ciliaris* extracts against *P. merabilis* and *A. tumefaciens* by ethyl acetate and GAA extracts respectively indicating significant antimicrobial potential of test extracts. MIC and MBC/MFC values were found equal shows bactericidal properties of *Cenchrus* grass. (Table-3).

Total activity

Total activity indicates the volume at which extract can be diluted with still having ability to kill microorganism. GAA extracts of *C. setigerus* and *C. ciliaris* grasses showed high values of TA (370.09 ml and 178.63 ml respectively) against *P. merabilis* which prove the potential to inhibit the growth of the test microorganisms, even at low concentration. Amount of extract isolated from *Cenchrus* grass and total activity (TA) of different extracts were calculated and recorded (Table 4).

Overall, the test pathogens were more sensitive to GAA, iso propyl alcohol, acetone and ethyl acetate extracts than to the other extracts. This suggests that some of the active compounds in the crude extracts are polar and thus dissolved readily in these polar extracts while the less polar or non polar extracts may have dissolved out non-polar compounds that possess less antimicrobial activity. Previous studies have noted alcohols to be reliable and consistent solvents for the extraction of antimicrobial substances from medicinal plants[29].

DISCUSSION

Results of the present study showed that 19/22 extracts tested inhibited the growth of selected pathogens, indicating broad spectrum bioactive nature of selected two plants (9/11 in *C. setigerus* and 10/11 in *C. ciliaris*). It indicates that *C. ciliaris* has broad spectrum bioactive nature than *C. setigerus.* GAA and ethyl acetate extracts *Cenchrus* express maximum antimicrobial activities respectively by suppressing the growth of all microbes under investigation. In the present study, most of the extracts of *Cenchrus* grass were found to be potent inhibitor of tested organisms except *A. niger*. Excellent antimicrobial activities were observed by GAA

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. Some extracts were found to be bactericidal in nature against *P. merabilis, K. pnemoniae* and *A. tumefaciens.*

Table 3: Minimum Inhibitory Concentration (Mic) And Minimum Bactericidal / Fungicidal Concentration (Mbc/Mfc) Of Stems Of *Cenchrus* Grass In Different Polar Solvents Against Tested Pathogens.

S.No.	Solvents with Solubility in water %	Plants	(MIC) and (MBC/MFC) of stem extracts against pathogens							
			P. m.		*K. p.*		*A. t.*		*A. n.*	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
1.	Water	*C.s.*-76	-	-	-	-	7.5	15	-	-
	(100)	*C.c.*-358	3.75	7.5	3.75	7.5	7.5	15	-	-
2.	Glacial acetic acid (100)	*C.s.*-76	0.117	0.117	0.234	0.468	0.234	0.468	-	-
		C.c.-358	0.234	0.469	1.875	3.75	0.234	0.234	-	-
3.	Ethanol	*C.s.*-76	7.5	15	-	-	3.75	7.5	-	-
	(100)	*C.c.*-358	-	-	7.5	15	1.875	3.75	-	-
4.	Acetone	*C.s.*-76	-	-	-	-	1.875	1.875	-	-
	(100)	*C.c.*-358	-	-	7.5	15	7.5	15	-	-
5.	Ethyl acetate	*C.s.*-76	7.5	7.5	1.875	3.75	1.875	3.75	-	-
	(8.7)	*C.c.*-358	0.234	0.234	-	-	7.5	15	-	-
6.	Chloroform	*C.s.*-76	3.75	3.75	7.5	7.5	3.75	3.75	-	-
	(0.815)	*C.c.*-358	-	-	3.75	3.75	7.5	15	-	-
7.	Iso propyl alcohol (100)	*C.s.*-76	1.875	1.875	3.75	3.75	1.875	3.75	-	-
		C.c.-358	7.5	15	7.5	15	3.75	7.5	-	-
8.	Benzene	*C.s.*-76	-	-	-	-	3.75	3.75	-	-
	(0.18)	*C.c.*-358	7.5	15	-	-	-	-	-	-
9.	Toluene	*C.s.*-76	-	-	-	-	3.75	7.5	-	-
	(0.051)	*C.c.*-358	-	-	15	15	0.938	1.875	-	-
10	Petrolium ether (0.1)	*C.s.*-76	-	-	-	-	-	-	-	-
		C.c.-358	-	-	-	-	7.5	7.5	-	-
11.	Hexane	*C.s.*-76	-	-	-	-	-	-	-	-
	(0.001)	*C.c.*-358	-	-	-	-	-	-	-	-

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); *P. m. - Proteus merabilis*; *K. p. - Klebsiella pnemoniae*; *A. t. - Agerobacterium* tumefaciens; *A. n. - Aspergillus niger.*

Table 4: Total Activity of Stems of *Cenchrus* Grass In Different Polar Solvents Against Tested Pathogens.

S.No.	Polar solvents (boiling point °C)	plants	Total Yield (%)	Total activity of stem extracts			
				P. m.	*K. p.*	*A. t.*	*A. n.*
1.	Water	C.s.-76	1.19	-	-	1.59	-
	(100)	C.c.-358	2.78	7.41	7.41	3.71	-
2.	Glacial acetic acid (118.1)	C.s.-76	4.33	370.09	185.04	185.04	-
		C.c.-358	5.43	146.58	18.29	146.58	-
3.	Ethanol	C.s.-76	3.48	4.64	-	9.28	-
	(78.4)	C.c.-358	4.26	-	5.68	22.72	-
4.	Acetone	C.s.-76	3.20	-	-	17.07	-
	(55-56)	C.c.-358	6.48	-	8.64	8.64	-
5.	Ethyl acetate	C.s.-76	1.44	1.92	7.68	7.68	-
	(76-77.5)	C.c.-358	4.18	178.63	-	5.57	-
6.	Chloroform	C.s.-76	3.57	9.52	4.76	9.52	-
	(60-62)	C.c.-358	4.43	-	11.81	5.91	-
7.	Iso propyl	C.s.-76	2.58	13.76	6.88	13.76	-
	alcohol (81-83)	C.c.-358	1.09	1.45	1.45	2.91	-
8.	Benzene	C.s.-76	1.93	-	-	5.15	-
	(79-81)	C.c.-358	1.97	2.63	-	-	-
9.	Toluene	C.s.-76	1.38	-	-	3.68	-
	(109-111)	C.c.-358	0.71	-	0.47	7.57	-
10	Petrolium	C.s.-76	2.45	-	-	-	-
	ether (60-80)	C.c.-358	2.82	-	-	3.76	-
11.	Hexane	C.s.-76	1.37	-	-	-	-
	(65-70)	C.c.-358	2.34	-	-	-	-

C.s.-76= *Cenchrus setigerus* (CAZRI-76); C.c.-358= *Cenchrus setigerus* (CAZRI-358),
P. m. - Proteus merabilis*; *K. p. - Klebsiella pnemoniae*; *A. t. - Agerobacterium* tumefaciens; *A. n. - Aspergillus niger.

Extracts under 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.

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REFERENCES

1. Otimenyin O-Sunday, Uguru MO, Ogbonna A. Antimicrobial and hypoglycemic effects of *Momordica balsamina.* Linn. Journal of Natural Products 2008; 1:03-09.
2. Gracelin D, Herin Sheeba, Britto A, John De. *Datura metel* linn.- A plant with potential as Antimicrobial agent. I. J. of applied biology and pharmaceutical technology 2011; 2(2):429-433.
3. Agrawal P. Ecophysiological and Biochemical studies Related to drought adaptation in grasses of Indian Desert. Ph.D. Thesis, J. N. Vyas University 2007.
4. Bessman SP. Ammonia Metabolism in Animals: Symposium on Inorganic Nitrogen Metabolism. Mc Elry and Glass (eds.) The Johns Hopkins Press, 1956.
5. Singariya P. Effect of Sub-Optimal Environment and PGR's on Metabolic Pattern of Certain Species of *Cenchrus*. Ph.D Thesis, J. N. Vyas University, 2009.
6. Sinha RK, Bhatia S, Vishnoi R. Desertification control and rangeland management in the Thar desert of India. In: Rala Report no. 200, 115-123; 1996.
7. Eickhoff TC. Klebsiella pneumoniae infection: a review with reference to the water-borne epidemiologic significance of K. pneumoniae presence in the natural environment. National Council of the Paper Industry for Air and Stream Improvement, Inc. Technical Bulletin no. 254, New York, N.Y, 1972.
8. Martin WJ, Yu KW, Washington JA. Epidemiological significance of *Klebsiella pneumoniae* - a 3 month study. Mayo C. in. Proc. 1971; 46:785-793.
9. Hulse M, Johnson S, Ferrieri P. *Agerobacterium* infections in humans: experience at one hospital and review. Clin. Infect. Dis. 1993; 16(1): 112–117. PMID 8448285.
10. Dunne WM, Tillman J, Murray JC. Recovery of a strain of *Agerobacterium radiobacter* with a mucoid phenotype from animmuno compro mised- child with bacteremia. J. Clin. Microbiol. 1993; 31(9): 2541-3. PMC 265809PMID 8408587.
11. Sunday OO, Uguru OM, Ode A, Siniya KA. Anti-convulsant, analgelsic and mechanism of anti-inflammatory effects of *M. balsamina* linn. Presented at the Neuroscience Conference (2007) in San diego, CA, USA. A-31047-SfN.
12. Singariya P, Mourya KK, Kumar P. Preliminary Phyto-profile and Pharmacological Evaluation of some Extracts of *Cenchrus* grass against Selected Pathogens. J. Pharm. Sci. and Res 2011a; 3(8): 1387-1393.
13. Deborah KB, Mecky IN, Olipa D, Cosam GJ, Zakaria HM. Screening of Tanzania Medicinal Plants for Anti-Canddia activity. BMC Complementary and Alternative Medicine 2006; 6: 1-29.
14. Subramanian SS, Nagarjan S. Flavonoids of the seeds of *Crotolaria retusa* and *Crotolaria striata*. Current Sci 1969; 38: 65.
15. Bhattacharya S, Zaman MK, Haldar PK. Antibacterial Activity of stem Bark and Root of Indian Zanthoxylum nitidum, Asian Journal of Pharmaceutical and Clinical Research 2009; 2(1): 30-34.
16. Singariya P, Mourya KK, Kumar P. Bio Activity of crude extracts of Leaves of *Cenchrus* Grass in different polar solvents against some pathogenic microbes. I. J. of Pharm. Sci. Review and Res 2011b; 11(1): 124-129.
17. Upadhyay B, Singh KP, Kumar A. Pharmacognostical and antibacterial studies of different extracts of *Euphorbia hirta* L. J. Phytol 2010; 2(6): 55-60.
18. Cynthia H, O'Callaghan. Assessment of a new antibiotic. In. Hugo WB, Russel AD, editors. Pharmaceutical Microbiology. 3.Oxford: Blackwell Scientific Publications; 1983, Pp. 122-134.
19. Yamac M, Bilgili F. Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushroom isolates. Pharm Biol 2006; 44: 660-667.
20. Reeves W, Andrew, White. Clinical antimicrobial assay, Oxford University Press, New York, USA, 1999; Pp 25.
21. Aneja KR. Experiments in Microbiology Plant Pathology Tissue Culture and Mushroom Production 2003.
22. Hussain A, Zaman MK, Ramteke AM. Antibacterial Activity of Trunk Bark of Alstonia scholaris, Asian Journal of Pharmaceutical and Clinical Research 2010; 3(4): 46-47.
23. Pepeljnjak S, Kalodera Z, Zovko M. Investigation of antimicrobial activity of *Pelarogarium radula* (Cav.) L'Herit. Acta Pharm 2005; 55: 409-415.
24. Vimala Y, Elizabeth KM. Antimicrobial activity of *Decalpis hamiltonii* on some microbial isolates of spoiled vegetables and pathogenic microorganisms. Indian J Microbiol 2006; 46: 397-399.
25. Shahidi-Bonjar, GH. Evaluation of Antibacterial properties of Iranian Medicinal plants against *Micrococcus aureus, Serratia marcescens*, *Klebsiella pneunomiae* and *Bordella bronchoseptica*. Asian Journal of Sciences 2004; 3(1):82-86.
26. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in southwest Nigerian unorthodox medicine for antimethicillin resistant *Staphylococcus aureus* activity. BMC Complementary and Alternative Medicine 2005; 5:6.
27. Eloff JN. Quantifying the bioactivity of the plant extracts during screening and bioassay-guided fractionation. Phytomedicine 2004; 11(4): 370-371.
28. Farnsworth NR. Biology and Phytochemical screening of plants. Pharm Sci 1966; 55: 225-76.
29. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmocol 1998; 62:183-193.