ORIGINAL ARTICLE

Comparative bioactivity of dhaman grass root extracts in different polar solvents against plant and human pathogens

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Background: *Cenchrus ciliaris* L. (Poaceae) is a very suitable and highly nutritive grass for environmental conditions of the desert; yet, no antimicrobial work has been done on this grass. **Aim:** To estimate *in vitro* the antibiotic activity of root extracts of *C. ciliaris* in various polar solvents to use the grass as a possible source for new antimicrobial compounds against important plant and human pathogens. **Settings and Design:** The antibiotic activity of *C. ciliaris* root extracts were evaluated against a few medically important pathogens including Gram-negative bacteria *Proteus mirabilis, Klebsiella pneumoniae, Agrobacterium tumefaciens,* and the fungi, *Aspergillus niger.* **Materials and Methods:** Dried, powdered, and weighted root material was successively extracted with different polar solvents [hexane, petroleum ether, toluene, benzene, isopropyl alcohol, chloroform, ethyl acetate, acetone, ethanol, glacial acetic acid (GAA), and water] using a Soxhlet assembly. Antibiotic activity was performed by using disc diffusion assay followed by determination of minimum inhibitory concentrations by broth dilution method, against sensitive bacteria (with good inhibition zone). Most of the extracts, at higher concentrations, showed varying degrees of inhibitory activity against selected bacteria. **Statistical Analysis:** Mean value and standard deviation were calculated for the test bacteria and fungi. Data were analysed by one-way analysis of variance (ANOVA) and *P* values were considered significant at $P<0.05$. **Results and Conclusions:** Results revealed that the highest antibiotic activity was exhibited by the water and GAA extracts against *P. mirabilis,* followed by isopropyl alcohol extract against *K. pneumoniae* and *A. tumefaciens.* Water extract was observed to be the most active extract with maximum zone of inhibition against *A. tumefaciens* (plant pathogen) as compared to all other extracts.

Key words: Antibiotic activity, *Cenchrus* grass, minimum inhibitory concentration, one of inhibition

INTRODUCTION

The use of higher plants and their preparations to treat infectious and noninfectious diseases is an age-old practice and was the only method available in the past. Though the use of natural sources for curing diverse forms of ailments is an age-old practice, scientific analysis of different natural sources for their possible medicinal potency is comparatively recent in origin.[1] Microbial resistance to antimicrobial agents has led to failure of treatment and the shift of medical care from orthodox to herbal medicine. Most of the herbal medicines in use await validation of their claimed effects and possibly the development of novel antimicrobial drugs from them.[2] Plant-derived compounds contribute a lot in the fight against pathogens.[3] Various plant extracts can serve both as potential antimicrobial crude drugs as well as a source for new anti-infective agents.[4]

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Cenchrus ciliaris (C_4 grass) is gaining attention in various field of research, as it is best suited to the present environmental conditions. This grass flourishes more under conditions of high temperature, solar radiation, and low moisture,[5] and is very efficient in collecting carbon dioxide and utilising nitrogen from the atmosphere and recycling it into the soil.[6,7] *C. ciliaris* (dhaman grass) has excellent soil-binding capacity which helps to conserve soil in desert areas.[8] The grass is most suitable and highly nutritive for environmental conditions of the desert; yet, no antimicrobial work has been done on this grass.

Klebsiella pneumoniae frequently causes lung destruction and pockets of pus in the lungs (known as abscesses). The mortality rate for untreated cases is around 90%. It may also cause empyema (pus surrounding the lung) and respiratory infections such as bronchitis, which is usually a hospital-acquired infection.[9] *Proteus mirabilis,* a rod-shaped bacterium, causes obstruction and renal failure. It can also cause wound infections, septicemia, and pneumonia, mostly in hospitalised patients. *Agrobacterium tumefaciens* (plant

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pathogen) uses horizontal gene transfer to cause tumours or 'crown gall disease' in plants. It can be responsible for opportunistic infections in humans with weakened immune systems.[10,11]

MATERIALS AND METHODS

Experimental Design: Antimicrobial Activity

Plant material

Roots of *C. ciliaris* (CAZRI-358) were collected in the month of August from the Central Arid Zone Research Institute (CAZRI), Jodhpur (Rajasthan, India). The collected plant materials were transferred immediately to the laboratory, cleaned with water, and separately dried in the shade till constant weight was achieved. The shade-dried roots were powdered with a grinder.[12]

Drugs and Chemicals Used

Drugs

Gentamycin (for bacteria) and ketoconazole (for fungi).

Chemicals

Hexane, petroleum ether, toluene, benzene, isopropyl alcohol, chloroform, ethyl acetate, acetone, ethanol, GAA, water, Muller-Hinton agar (MHA), nutrient agar (for bacteria), and Sabouraud dextrose agar (for fungi).

Preparation of extracts

Crude extracts of the roots of *C. ciliaris* were prepared using a series of nonpolar to polar solvents by hot extraction method[13] in Soxhlet assembly. Different extracts were then screened for antimicrobial (antibiotic) activity by 'disc diffusion assay' against selected medically important bacteria and fungi. The fraction showing best activity was then used for determining minimum inhibitory concentrations MIC by broth dilution method[14] and minimum bactericidal/fungicidal concentrations (MBC/MFC).

Micro-organisms

The organisms selected for the study were three Gram-negative bacteria *P. mirabilis* MTCC-3310, *K. pneumoniae* MTCC-4030, *A. tumefaciens* MTCC-431, and one fungus (*Aspergillus niger* MTCC-282). Test pathogenic micro-organisms were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference strains of bacteria were maintained on nutrient agar slants, subcultured regularly (after every 30 days) and stored at 4°C as well as at –80°C by preparing suspensions in 10% glycerol.

Preparation of test pathogens and disc diffusion assay

Bacterial strains were grown and maintained on nutrient agar medium, whereas fungus was maintained on Sabouraud dextrose agar medium. Disc diffusion assay was performed for screening using standard method.[15] Activity index (AI) for each extract was calculated [Table 1].

$$AI = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Serial dilution method

MIC was determined as the least extract concentration which inhibited the growth of the test organisms.[16,17] Bacterial and fungal suspensions were used as negative control, whereas the broth-containing standard drug was used as positive control.

Determination of MBC/MFC

Equal volumes of the various concentrations of each extract and nutrient broth were mixed in microtubes to make up 0.5 mL of solution, and 0.5 mL of McFarland standard of the organism suspension was added to each tube.[18] The tubes were incubated aerobically and MBC was determined by subculturing and incubation at 37° C for bacteria (24 h) and 27°C for fungi (48 h). The highest dilution that yielded no single bacterial/fungal colony was taken as the MBC/MFC.[19]

Table 1: Zone of inhibition (mm) and activity index of root extracts of *Cenchrus ciliaris*

Polar solvents	Bioactivity of root extracts of *C. ciliaris* against pathogens							
	P. mirabilis		*K. pneumoniae*		*A. tumefaciens*		*A. niger*	
	ZOI	AI	ZOI	AI	ZOI	AI	ZOI	AI
Water	28.83±0.24	2.403	7.33±0.23	0.367	10.33±0.26	0.646	-	-
Acetic acid	28.50±0.21	2.403	18.33±0.25	0.917	16.5±0.64	1.031	-	-
Ethanol	-	-	-	-	10.67±0.24	0.762	-	-
Acetone	8.33±0.24	0.694	12.17±0.24	0.609	-	-	-	-
Ethyl acetate	19.67±0.26	2.459	7.5±0.64	0.375	8.17±0.26	0.681	-	-
Chloroform	-	-	11.33±0.24	0.567	7.17±0.23	0.398	-	-
Isopropyl alcohol	12.67±0.27	1.056	14.67±0.23	0.734	13.5±0.64	0.964	-	-
Benzene	8.50±0.64	1.063	7.5±0.64	0.375	8.67±0.24	0.619	-	-
Toluene	8.17±0.24	0.681	-	-	8.67±0.23	0.361	-	-
Petroleum ether	-	-	-	-	8.83±0.24	0.736	-	-
Hexane	-	-	-	-	8.67±0.24	0.723	-	-

All values are mean±SD; n –3; ZOI – Zone of Inhibition (mm±S.D.); AI – Activity index; *P. mirablils – Proteus mirabilis*; *K. pneumonia – Klebsiella pneumoniae*; *A. tumefaciens – Agrobacterium tumefaciens; A. Niger – Aspergillus niger*

Total activity determination

Total activity is the volume at which the test extract can be diluted with the ability to kill micro-organisms. 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.[20]

Extract per g dried plant part
Total activity = MIC of extract

Stastical Analysis

Mean value and standard deviation were calculated for each test bacteria and fungus. Data were analysed by one-way ANOVA and *P* values were considered significant at $P < 0.05$.

RESULTS

Zone of Inhibition and Activity Index

Antimicrobial activity [assessed in terms of Zone of Inhibition (ZOI) and Activity Index (AI)] of the root extracts in different polar solvents, tested against selected micro-organisms, were recorded [Figure 1]. In the present study, a total of 11 extracts of the roots of selected grass were tested for their bioactivity; all the extracts showed significant antimicrobial potential against test microbes. *A. tumefaciens* is most susceptible organism in the investigations.[21,22] However, according to the ZOI, *P. mirabilis* was the most susceptible organism. The assay was repeated thrice and mean of the three experiments was recorded. The diameter of the ZOI was measured and commercial disc of antibiotics was used as positive control (standard).[23]

P. mirabilis

Water extract showed the highest activity (ZOI- 28.83±0.24 mm, AI- 2.403), followed by GAA extract (ZOI- 28.50±0.21 mm, AI- 2.403).

K. pneumoniae

Isopropyl alcohol and acetone extract showed the highest activity after GAA extracts (ZOI: 14.67 ± 0.23 mm, AI: 0.734 and ZOI: 12.17 ± 0.24 mm, AI: 0.609).

A. tumefaciens

Isopropyl alcohol and ethanol extract showed the highest activity after GAA extracts (ZOI: 13.50 ± 0.64 mm, AI: 0.964 and ZOI: 10.67 ± 0.24 mm, AI: 0.762, respectively against *A. tumefaciens)*.

A. niger

There was an absence of antifungal activity.

MIC and MBC/MFC

MIC and MBC/MFC values [Table 2] were evaluated for those plant extracts which showed activity in disc diffusion assay. The range of MIC and MBC/MFC of extracts recorded was 0.234-15 mg/mL. In the present investigation, the lowest MIC value of 0.234 mg/mL was recorded for GAA extracts against *K. pneumoniae, P. mirabilis,* and

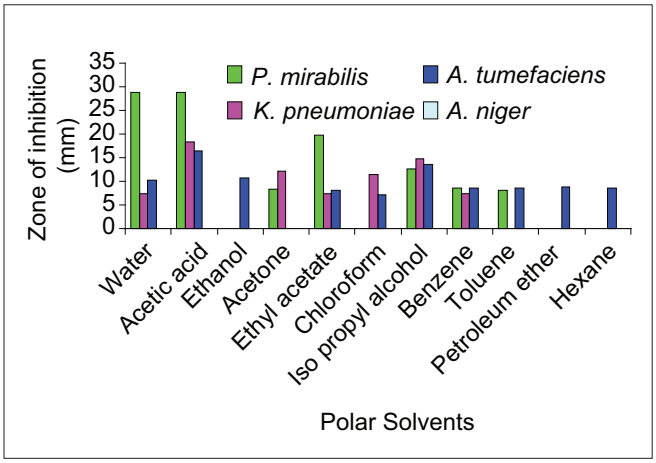

Figure 1: Zone of inhibition (mm) of root extracts of *Cenchrus ciliaris* against pathogens in different polar solvents

Table 2: MIC and MBC/MFC of root extracts of *Cenchrus ciliaris*

Polar solvents	Bioactivity of root extracts of C. ciliaris against pathogens							
	P. mirabilis		*K. pneumoniae*		*A. tumefaciens*		*A. niger*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Water	0.938	0.938	7.5	15	3.75	7.5	-	-
Acetic acid	0.234	0.469	0.234	0.468	0.234	0.468	-	-
Ethanol	-	-	-	-	1.875	3.75	-	-
Acetone	7.5	15	1.875	1.875	-	-	-	-
Ethyl acetate	1.875	1.875	15	15	7.5	15	-	-
Chloroform	-	-	1.875	1.875	15	15	-	-
Isopropyl alcohol	3.75	7.5	1.875	1.875	1.875	1.875	-	-
Benzene	7.5	15	7.5	15	3.75	3.75	-	-
Toluene	7.5	15	-	-	3.75	7.5	-	-
Petroleum ether	-	-	-	-	3.75	7.5	-	-
Hexane	-	-	-	-	7.5	15	-	-

MIC: Minimum inhibitory concentration (mg/mL); MBC– Minimum bactericidal concentration (mg/mL); MFC– Minimum fungicidal concentration (mg/mL); *P. mirablils – Proteus mirabilis; K. pneumonia – Klebsiella pneumoniae; A. tumefaciens – Agrobacterium tumefaciens; A. Niger – Aspergillus niger*

A. tumefaciens indicating significant antimicrobial potential of the test extract. MIC and MBC values were found equal for GAA extracts showing bactericidal properties of the extract.

Total Activity

Most of the GAA extracts showed high values of total activity against *P. mirabilis, K. pneumoniae,* and *A. tumefaciens* (180.34 mL), which prove the high potential of the extract to inhibit the growth of the test micro-organisms, even at low concentrations [Table 3].

Phytochemical Estimation

The phytochemical estimation for the roots of *C. ciliaris* were carried out according to Farnsworth.[24] The consistency was found to be sticky in the high polar solvent extracts whereas the low polarity solvent (hexane, petroleum ether, toluene, benzene, ethyl acetate) extracts were found to be nonsticky[25] [Table 4], except water extracts (with high polarity) which were found to be nonsticky. The yield (mg/10 g ± S.D.) of the extracts was also analyzed, wherein the highest yield was recorded for acetone and ethyl acetate extracts, 615 ± 14.31 and 527 ± 10.23 (mg/10 g ± S.D.), respectively [Figure 2].

Table 3: Total activity of root extracts of *Cenchrus ciliaris*

Polar solvents	Total activity of root extracts of *C. ciliaris* against pathogens			
	P. *mirabilis*	K. pneumoniae	A. tumefaciens	A. niger
Water	45.44	5.68	11.36	-
Acetic acid	180.34	180.34	180.34	-
Ethanol	-	-	26.56	-
Acetone	8.20	32.80	-	-
Ethyl acetate	28.11	3.51	7.03	-
Chloroform	-	21.28	2.66	-
Isopropyl alcohol	4.51	9.01	9.01	-
Benzene	2.81	2.81	5.63	-
Toluene	1.29	-	2.59	-
Petroleum ether	-	-	6.67	-
Hexane	-	-	3.29	-

P. mirablils – Proteus mirabilis; K. pneumonia – Klebsiella pneumoniae; *A. tumefaciens – Agrobacterium tumefaciens; A. Niger – Aspergillus niger*

DISCUSSION

The present study showed that all the tested extracts inhibited the growth of selected bacteria and fungi, indicating the broad-spectrum bioactive nature of *C. ciliaris*. After GAA extracts, the test pathogens were more sensitive to ethyl acetate, water, isopropyl alcohol, and ethanol extracts as compared to the other extracts. The observation suggests that some of the active compounds in the crude extracts were polar and thus dissolved readily in these extracts, It means low polarity solvent extracts shows less antimicrobial activity. Previous studies indicate that alcohols are reliable and consistent solvents for the extraction of antimicrobial substances from medicinal plants.[26] Excellent antibacterial and antifungal activities were observed by GAA extracts with low MIC and MBC/MFC values. MBC/MFC values were found to be higher than MIC values of the extracts against the micro-organisms tested, indicating the bacteriostatic/ fungistatic nature of the extracts. *A. tumefaciens* was noted to be the most susceptible organism.

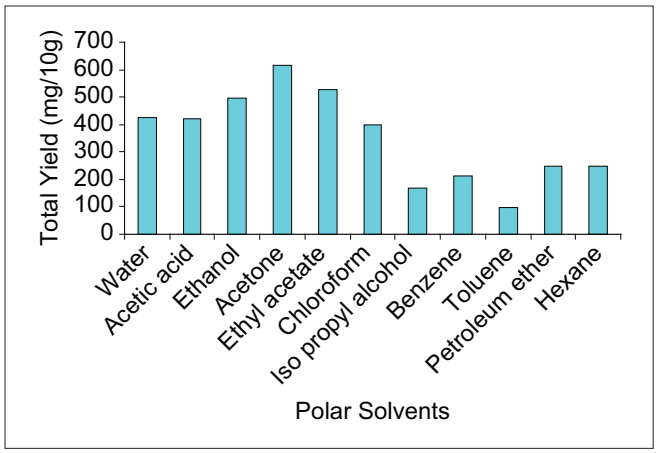

Figure 2: Total yield (mg/10 g) of root extracts of *Cenchrus ciliaris* in different polar solvents

Table 4: Phytochemical estimation of root extracts of *Cenchrus ciliaris* in different polar solvents

Polar solvents	Primary phytochemical estimation of root extracts of *C. ciliaris*		
	Total yield (mg/10 g±S.D.)	Color	Consistency
Water	426±12.52	Light yellow	Nonsticky
Acetic acid	422±12.36	Very dark green	Sticky
Ethanol	498±15.69	Coffee brown	Sticky
Acetone	615±14.31	Brown	Sticky
Ethyl acetate	527±10.23	Light yellow	Nonsticky
Chloroform	399±15.48	Brown	Sticky
Isopropyl alcohol	169±10.83	Brown	Sticky
Benzene	211±13.76	Yellow	Nonsticky
Toluene	097±08.61	Colorless	Nonsticky
Petroleum ether	250±12.48	Light yellow	Nonsticky
Hexane	247±17.46	Brown	Nonsticky

P. mirablils – Proteus mirabilis; K. pneumonia – Klebsiella pneumoniae; A. tumefaciens – Agrobacterium tumefaciens; A. Niger – Aspergillus niger

The extracts under study not only inhibit the bacterial/fungal growth but the ZOI developed was more or less permanent when compared with the ZOI developed by the standard drug used, as after sometime, bacterial/fungal colonies could be easily seen in the ZOI developed by standard drugs. In the light of the fact that micro-organisms are becoming resistant against the drugs in use, the present investigation is of great significance, as far as future drugs are concerned. The selected plant (*C. ciliaris*) could be a good option for pharmaceutical industries for the preparation of plant-based antimicrobial drugs.

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