Original Article

Absence of antibiotic activities of *Cenchrus setigerus* and *cenchrus ciliaris* seed extracts in different polar solvents

Premlata Singariya, Padma Kumar, Krishan K. Mourya[1]

Department of Botany, Laboratory of Tissue Culture and Secondary Metabolites, University of Rajasthan, Jaipur, [1]Department of Animal Husbandry, Government of Rajasthan Veterinary Hospital, Pahari (Bharatpur), Rajasthan, India

Abstract

Aim: The aim of present study is to investigate the antibiotic activity of seeds of *Cenchrus setigerus* and *Cenchrus ciliaris* extracts, in order to use them as a possible source of new anti-microbial substances against important human pathogens. **Materials and Methods:** Crude extracts of seeds of both species of *Cenchrus* grass were evaluated against some important G-ve bacteria. *Escherichia coli*, *Raoultella planticola*, *Enterobacter aerogenes* and one fungus *Aspergillus flavus*. The dried and powdered seeds were successively extracted with hexane, toluene, isopropyl alcohol, acetone and ethanol using the soxhlet assembly. The antimicrobial activity assay was done by both disc diffusion and serial dilution methods. **Results:** The results indicate that all the extracts, in different polar solvents did not show any antibacterial activity against *R. planticola* or any antifungal activity against *A. flavus*. **Conclusion:** All extracts in the different polar solvents did not have or had very less antibacterial and antifungal activities.

Key words: Antibiotic, *Aspergillus flavus*, *Cenchrus*, hexane, toluene

INTRODUCTION

Thar desert occupies diverse habitat and landscape that support distinctive plant species. About 682 plant species have been identified including 107 grasses.[1] *Cenchrus setigerus* and *Cenchrus ciliaris* (C_4 grasses) are 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[2] and are more efficient at gathering CO_2 and utilizing nitrogen from the atmosphere and recycled N in the soil.[3,4] *Cenchrus* L. (Poaceae) is highly nutritious grass and considered excellent for pasture in hot, dry areas and is valued for its production of palatable forage and intermittent grazing during droughty periods in the tropics. The grass, fed green, turned into silage, or made into hay is said to increase flow of milk in cattle and impart a sleek and glossy appearance. This grass has excellent soil binding capacity which helps to conserve soil in desert areas.[5] 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.[6] *Raoultella planticola* has been determined to cause severe pancreatitis in one case.[7] *E. aerogenes* is a nosocomial and pathogenic bacterium that causes opportunistic infections including most types of infections. *Aspergillus* species are the most common mold causing severe infections.[8,9]

Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.4103/0976-9234.116756

MATERIALS AND METHODS

Experimental design

Crude extracts of seeds of *Cenchrus ciliaris* (CAZRI-358)

Address for correspondence:
Dr. Premlata Singariya, Dr. D. S. Kothari Post Doctoral Fellow, Shri Kalyan Nagar, Kartarpura, Jaipur, Rajasthan, India.
E-mail: premlatasingariya@gmail.com

and *Cenchrus setigerus* (CAZRI-76) were prepared with a series of non polar to polar solvents by hot extraction method[10] in soxhlet assembly. Different extracts were then screened for antimicrobial activity by DDA[11] 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[12] and minimum bactericidal/fungicidal concentration (MBC/MFC).

Collection of plant material

Seeds of *C. ciliaris* and *C. setigerus* were collected in the month of August from the CAZRI, Jodhpur, Rajasthan. Selected plant seeds were separately shade dried and powdered with the help of grinder.[13] 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

Seeds were sequentially extracted with different solvents according to their increasing polarity (hexane <toluene <isopropyl alcohol <acetone <ethanol) by using the 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 and stored in amber color bottle for subsequent use in the further antimicrobial and phytochemical analysis.[15]

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

Drugs and chemicals used

Drugs

Gentamycin (for bacteria) and ketoconazole (for fungi)

Chemicals

Hexane, toluene, isopropyl alcohol, acetone and ethanol, Nutrient Agar (NA medium for bacteria), Sabouraud Dextrose Agar (SDA medium for fungi).

Table 1: Name of the tested pathogens (bacteria and fungi)

Pathogens	Name of pathogens	G+ve/G−ve	Specimen no.
Bacteria	*Escherichia coli*	G−ve	MTCC-46
	Raoultella planticola	G−ve	MTCC-530
	Enterobacter aerogenes	G−ve	MTCC-111
Fungi	*Aspergillus flavus*	-	MTCC-277

Micro-organisms

The organisms used in this study were namely [Table 1]. 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 (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 colonies were maintained on NA medium and fungi were maintained on SDA medium. DDA[16] 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). DDA was performed for screening by standard method.[12] Bacterial growths were seen after a minimum of 18 h and occasionally until 24 h.[17] Activity index for each extract was calculated.

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

Serial dilution method

MICs are considered as the "gold standard" for determining the susceptibility of the organisms to antimicrobials.[18] MIC of antibiotics was evaluated (thrice) using standard micro broth dilution method against inoculum *Escherichia coli* organisms.[19] Bacterial and fungal suspensions were used as negative control, while broth containing standard drug was used as positive control.

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 0.5 ml of solution. 0.5ml of McFarland standard of the organism suspension was added to each tube.[20,21] 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 MHA and further incubated for 24 h. The highest dilution that yielded no single bacterial colony was taken as the MBC.[22] 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.[23]

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

RESULTS

Antimicrobial activity

Antimicrobial activity [assessed in terms of zone of inhibition (ZOI) in mm* and activity index] of the seeds of *C. ciliaris* and *C. setigerus* extracts in different polar solvents, tested against selected microorganisms were recorded [Table 2]. In the present study total ten extracts of selected plant were tested for their bioactivity, among which all these extracts showed insignificant antimicrobial potential against test microbes. However, all these extracts showed no activity or very less activity against *E. coli*, *R. planticola*, *E. aerogens* and antifungal activity against *A. flavus* at tested concentration. Highest antimicrobial activities were recorded for *C. ciliaris* in hexane (ZOI-9.17 ± 0.24 and AI-0.459) and for *C. setigerus* in acetone (ZOI-8.50 ± 0.64 and AI-0.425) against *E. aerogens* and *E. coli*, respectively.

MIC and MBC/MFC

MIC and MBC/MFC values [Table 3] were evaluated for those plant extracts, which were showing activity in disc diffusion assay. The range of MIC and MBC/MFC of extracts recorded was 3.75-15 mg/ml. In the present investigation lowest MIC value 3.75 mg/ml was recorded for *C. ciliaris* (against *E. aerogens*) in hexane extract.

Total activity

Total activity indicates the volume at which extract can be diluted with still having ability to kill microorganism. Seed extracts of *C. ciliaris* in hexane showed high values of TA 7.73 ml against *E. aerogenes* [Table 4].

Preliminary phyto–profiling

The preliminary phyto–profiling for the seeds of *Cenchrus* extracts were carried out according to Farnsworth[24] wherein the consistency was found to be sticky in the hexane and toluene 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* (685 ± 11.78 mg/10 g ± S.D.) followed by ethanolic and iso propyl alcohol extract of *C. setigerus* (346 ± 13.46 mg/10 g ± S.D.) and (346 ± 10.13 mg/10 g ± S.D.) respectively [Table 5].

Table 2: Inhibition zone (mm)* and activity index of seeds of *Cenchrus* grass in different polar solvents against tested pathogens

Solvents (polarity)	Species	IZ / AI	Bio-activity of seed extracts of *Cenchrus* grass			
			E. coli	*R. planticola*	*E. aerogens*	*A. flavus*
Hexane (0.1)	CAZRI-76	IZ	-	-	-	-
		AI	-	-	-	-
	CAZRI-358	IZ	-	-	9.17±0.24	-
		AI	-	-	0.459	-
Toluene (2.4)	CAZRI-76	IZ	-	-	-	-
		AI	-	-	-	-
	CAZRI-358	IZ	-	-	-	-
		AI	-	-	-	-
Isopropyl Alcohol (3.4)	CAZRI-76	IZ	-	-	-	-
		AI	-	-	-	-
	CAZRI-358	IZ	-	-	-	-
		AI	-	-	-	-
Acetone (5.1)	CAZRI-76	IZ	8.50±0.64	-	-	-
		AI	0.425	-	-	-
	CAZRI-358	IZ	-	-	-	-
		AI	-	-	-	-
Ethanol (5.2)	CAZRI-76	IZ	-	-	-	-
		AI	-	-	-	-
	CAZRI-358	IZ	-	-	-	-
		AI	-	-	-	-

All values are mean±SD, *n*-3, CAZRI-76: *Cenchrus setigerus*; CAZRI-358: *Cenchrus ciliaris*; IZ: Inhibition zone in mm±S.D., AI: Activity index

Table 3: Minimum inhibitory concentration and (MBC/MFC) of seeds of *Cenchrus* in different solvents against tested pathogens

Solvents	Species	MIC	Bio-activity of seed extracts against pathogens			
		MBC/MFC	*E. coli*	*R. planticola*	*E. aerogens*	*A. flavus*
	CAZRI-76	MIC	-	-	-	-
Hexane		MBC/MFC	-	-	-	-
	CAZRI-358	MIC	-	-	3.75	-
		MBC/MFC	-	-	7.5	-
	CAZRI-76	MIC	-	-	-	-
Toluene		MBC/MFC	-	-	-	-
	CAZRI-358	MIC	-	-	-	-
		MBC/MFC	-	-	-	-
	CAZRI-76	MIC	-	-	-	-
Isopropyl		MBC/MFC	-	-	-	-
Alcohol	CAZRI-358	MIC	-	-	-	-
		MBC/MFC	-	-	-	-
	CAZRI-76	MIC	7.5	-	-	-
Acetone		MBC/MFC	15	-	-	-
	CAZRI-358	MIC	-	-	-	-
		MBC/MFC	-	-	-	-
	CAZRI-76	MIC	-	-	-	-
Ethanol		MBC/MFC	-	-	-	-
	CAZRI-358	MIC	-	-	-	-
		MBC/MFC	-	-	-	-

CAZRI-76: *Cenchrus setigerus*; CAZRI-358: *Cenchrus ciliaris*; MIC: Minimum inhibitory concentration (mg/ml); MBC: Minimum bactericidal concentration (mg/ml); MFC: Minimum fungicidal concentration (mg/ml)

Table 4: Total activity of seeds of *Cenchrus* in different solvents

Solvents	Species	Total activity of seed extracts against pathogens			
		E. coli	*R. planticola*	*E. aerogens*	*A. flavus*
Hexane	CAZRI-76	-	-	-	-
	CAZRI-358	-	-	7.73	-
Toluene	CAZRI-76	-	-	-	-
	CAZRI-358	-	-	-	-
Isopropyl	CAZRI-76	-	-	-	-
Alcohol	CAZRI-358	-	-	-	-
Acetone	CAZRI-76	3.45	-	-	-
	CAZRI-358	-	-	-	-
Ethanol	CAZRI-76	-	-	-	-
	CAZRI-358	-	-	-	-

CAZRI-76: *Cenchrus setigerus*; CAZRI-358: *Cenchrus ciliaris*

Table 5: Preliminary phyto-profile for seeds of *Cenchrus* grass in different polar solvent

Solvents	Plants	Total yield (%)	Color	Consistency
Hexane	CAZRI-76	1.28	Brick red	Sticky
	CAZRI-358	2.90	Brick red	Sticky
Toluene	CAZRI-76	1.74	Light brown	Sticky
	CAZRI-358	2.43	Brown	Sticky
Isopropyl	CAZRI-76	3.46	Cream color	Nonsticky
Alcohol	CAZRI-358	1.02	Colorless	Nonsticky
Acetone	CAZRI-76	2.59	Dark yellow	Nonsticky
	CAZRI-358	6.85	Light yellow	Nonsticky
Ethanol	CAZRI-76	3.46	Yellow	Sticky
	CAZRI-358	1.82	Yellow	Nonsticky

CAZRI-76: Cenchrus setigerus; CAZRI-358: *Cenchrus ciliaris*

CONCLUSION

Cenchrus ciliaris and *C. setigerus* extracts in different polar solvents at a tested concentration were not showing ZOI against the selected pathogens [Table 1] although, water, chloroform and acetic acid extract of seeds of this grass show good activity against these pathogens supported by different researchers.[25-27] But, in this study these extracts lack of antimicrobial activity well be useful to avoid any study repeated in this direction in the future.

Disclaimer

The opinions expressed in this publication are those

of the authors and do not necessarily represent those of SCIBIOLMED.ORG. Authors are responsible for their citing of sources and the accuracy of their references and bibliographies. The editors cannot be held responsible for any lacks or possible violations of third parties' rights.

REFERENCES

1. Khan TI, Frost S. Floral biodiversity: A question of survival in the Indian Thar Desert, The Environmentlist 2001;21:231-6.
2. Agrawal P. Ecophysiological and Biochemical studies Related to drought adaptation in grasses of Indian Desert. Ph.D. Thesis, J. N. Vyas University. 2007.
3. Bessman SP. Ammonia Metabolism in Animals: Symposium on Inorganic Nitrogen Metabolism. In: W.D. Mcelory and B. Glass (eds.) The Johns Hopkins Press. 1956
4. 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.
5. Sinha RK, Bhatia S, Vishnoi R. Desertification control and rangeland management in the Thar desert of India. In: Rala Report no. 1996;200:115-23.
6. Venier AG, Talon D, Party I, Mercier-Girard D, Bertrandx. Patient and bacterial determinants involved in symptomatic urinary tract infections caused by *E. coli* with and without bacteraemia. Clin Microbial Infect 2007;13:205-8.
7. Alves MS, Riley LW, Moreira BM. A case of severe pancreatitis complicated by *Raoultella planticola* infection. J Med Microbiol 2007;56:696-8.
8. Anaissie EJ, Bodey GP, Rinaldi MG. Emerging fungal pathogens. Eur J Clin Microbiol Infect Dis 1989;8:323-30.
9. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 2002;34:909-17.
10. Harborne JB. Phytochemical Methods, London, New York: Chapman and Hall; 1984. p. 5-6.
11. Singariya P, Kumar P, Mourya KK. Antimicrobial Activity of Fruit coat (Calyx) of *Withania somnifera* against some Multi Drug Resistant Microbes. Int J Biolog Pharm Res 2012;3:252-8.
12. Singariya P, Kumar P, Mourya KK. Evaluation of Antibacterial Activity and Preliminary Phytochemical Studies on the Stem of *Cenchrus ciliaris* and *Cenchrus setigerus.* Asian J Pharm Clin Res 2012;5:163-7.
13. Hussain A, Zaman MK, Ramteke AM. Antibacterial Activity of Trunk Bark of Alstonia scholaris, Asian J of Pharm and Clin Res 2010;3:46-7.
14. Subramanian SS, Nagarjan S. Flavonoids of the seeds of *Crotolaria retusa* and *Crotolaria striata*. Curr Sci 1969;38:65.
15. Dey AC. Indian Medicinal Plants Used in Ayurvedic Preparations. In: Singh B, Singh MP, Editors. Dehradhun: 1980. p. 202.
16. Andrews JM. BSAC standardized disc susceptibility testing method. J Antimicrob Chemother 2001;4:43-57.
17. Brindha D, Arthi D. Antimicrobial activity of white and pink *Nelumbo nucifera gaertn* flowers. JPHRC 2010;2:147-55.
18. Moideen MMJ, Raffick MM. Antibacterial, Antifungal Activity and Phytochemical Analysis of *Sanseviveria roxburghiana* Root. Int J Phytopharmacol 2012;3:21-6.
19. Liu CP, Tsai WJ, Lin YL, Chen CF, Kuo YC. The extracts from *Nelumbo nucifera* suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells. Life Sci 2004;75:699-716.
20. Singariya P, Mourya KK, Kumar P. Comparative Microcidal Activity of *Withania somnifera* and *Cenchrus setigerus* against the Pathogenic Micro-organisms. Int J Pharmacy Pharm Sci 2011;3:511-5.
21. Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, *et al.* Phytochemical extraction and antimicrobial properties of different medicinal plants*: Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). J Microbiol Antimicrobials 2011;3:1-7.
22. Cheesbrough M. Medicinal laboratory manual for tropical countries, ELBS reprinted Edition. 1993.
23. Singariya P, Mourya KK, Kumar P. Bio Activity of crude extracts of Leaves of *Cenchrus* Grass in different polar solvents against some pathogenic microbes. Int J Pharm Sci Rev Res 2011;11:124-9.
24. Farnsworth NR. Biology and Phytochemical screening of plants. Pharm Sci 1966;55:225-76.
25. Singariya P, Mourya KK, Kumar P. Preliminary Phyto-profile and Pharmacological Evaluation of some Extracts of *Cenchrus* grass against Selected Pathogens. J Pharm Sci Res 2011;3:1387-93.
26. Singariya P, Mourya KK, Kumar P. Antimicrobial Activity of the Crude Extracts of *Withania somnifera* and *Cenchrus setigerus In-vitro*. Pharmacog J 2011;3:60-5.
27. Singariya P, Kumar P, Mourya KK. Phyto-chemical Screening and Antimicrobial Activities of Dhaman grass and Indian Ginseng. J Pharmacy Res 2012;5:135-9.

How to cite this article: Singariya P, Kumar P, Mourya KK. Absence of antibiotic activities of *Cenchrus setigerus* and *cenchrus ciliaris* seed extracts in different polar solvents. J Pharm Negative Results 2013;4:71-5.

Source of Support: Nil. **Conflict of Interest:** None declared.