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An Activity guided Isolation and Evaluation of various Solvent Extracts of the Leaves of Anjan Grass

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Abstract

Plan: *The present study was undertaken to identify the possible bioactive components and antimicrobial activity in various polar solvents from the leaves of Anjan grass (Cenchrus ciliaris) (Poaceae).*

Materials and methods: *New compounds were identified by GC-MS analysis. Antimicrobial activity was evaluated against Proteus mirabilis, Klebsiella pneumoniae, Agrobacterium tumefaciens and Aspergillus niger using disk diffusion method followed by MIC by broth dilution method.*

Outcome: *Various aromatic, steroids, fatty acids and esters from C. ciliaris were identified. The prevailing compounds in the ethyl acetate extract of C. ciliaris were 4,22-stigmastadiene-3-one (2.41%), cyclopentacycloheptene (azulene) (1.81%), ergost-5-en-3-ol (3β, 24R)- (campesterol) (1.63%) and myristic acid, isopropyl ester (0.17%). The highest antimicrobial activity was exhibited by the glacial acetic acid extract against P. mirabilis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. However, isolation of individual photochemical constituents and subjecting it to biological activity will definitely give fruitful results.*

Key words: *Antibacterial activity, Azulene, Campesterol, Cenchrus grass and Myristic acid, isopropyl ester.*

1. Introduction

Anjan grass (*Cenchrus ciliaris*) is gaining attention in various field of research[1-4], as this is best suited to the present environmental conditions. This grass is more efficient at gathering CO_2 and utilizing nitrogen from the atmosphere and recycled N in the soil[1-2]. It has excellent soil binding capacity which helps to conserve soil in desert areas[3] and are more competitive under the conditions of high temperature, solar radiation and low moisture[4]. However, *C. ciliaris* is most suitable and highly nutritive grasses for desert environmental conditions.

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The chemical analysis of ethyl acetate extract of *C. ciliaris* (Anjan grass) showed a mixture of long-chain hydrocarbons, carboxyl esters, alcohols, acids, alkaloids, steroids, amino and nitro compound etc.

Phytochemical screening using the pharmacognostic methods revealed the presence of flavonoids, steroids and alkaloids. Taking into consideration of the medicinal importance of this plant, the ethyl acetate extract of *C. ciliaris* was analyzed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compound etc.
The objectives of this investigation was carried out to determine the possible bioactive components of *C. ciliaris* using GC-MS analysis and *in vitro* estimation of metabolites, photosynthetic pigments of seedlings and antimicrobial activity of extracts in various polar solvents from the leaves of *C. ciliaris* were done.

2. Materials and Methods

2.1. Identification of Components by GC-MS[5]

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained[5].

2.2. Antimicrobial Activity

2.2.1. Plant material[6]

Leaves of *C. ciliaris* (RUBL-14118) were collected in the month of August 2009 from the CAZRI, Jodhpur (Rajasthan). Plants samples were identified and deposited in the herbarium, department of botany, university of Rajasthan, Jaipur. The collected plant materials were transferred immediately to the laboratory cleaned with water and selected plant parts were separately shade dried until weight has been constant. Shade dried parts were powdered with the help of grinder[6].

2.2.2. Preparation of extracts[7-11]

Crude extracts of leaves of *C. ciliaris* were prepared with a series of non polar to polar solvents by hot extraction method[7] in soxhlet assembly for 18 hours at a temperature not exceeding the boiling point of selected polar solvents following the method of Subramanian and Nagarjan[8]. The obtained extracts were filtered by using Whatman No. 1 filter paper and then concentrated at 40^0C by using an evaporator and stored in amber colour bottle for subsequent use in the further antimicrobial, anti-fungal and phyto-chemical analysis[9]. Different extracts were then screened for antimicrobial activity by disc diffusion Assay[10] against a few medically important bacteria and fungi. The fraction showing best activity was then used for determining of MIC by tube dilution method[11] and minimum bactericidal/fungicidal concentration (MBC/MFC).

2.2.3. Micro-organisms

The organisms used in this study were three G-ve bacteria and one fungus, viz., *Proteus mirabilis* (MTCC-3310), *Klebsiella pneumoniae* (MTCC-4030), *Agrobacterium tumefaciens* (MTCC-431) and *Aspergillus niger* (MTCC-282). Selected microorganisms were procured from IMTECH, Chandigarh, India.

2.2.4. Preparation of test pathogens and Disc diffusion assay[12-13]

Initial screening of different extracts for their antibacterial activity carried out using MHA and NA media did not reveal any significant difference, thus further studies were carried out using NA medium only[12], while fungi were maintained on SDA medium. DDA was performed for screening by standard method[13]. Activity index for each extract was calculated (Table 2).

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

2.2.5. Serial dilution method (Determination of Minimum inhibitory concentration)

MICs are considered as the "gold standard" for determining the susceptibility of the organisms to antimicrobials[14]. MIC was determined as the least extract concentration which inhibited the growth of the test organisms. Bacterial and fungal suspensions were used as negative control, while broth containing standard drug was used as positive control.

2.2.6. Determination of Minimum bactericidal/fungicidal concentration (MBC/MFC)[15-17]

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[15-16]. The tubes were incubated aerobically and MBC was determined by sub culturing and further incubated for 24 h. The highest dilution that yielded no single bacterial colony was taken as the MBC[17].

2.2.7. Total activity (TA) determination[18-19]

Total activity is the volume at which the test extract can be diluted with the ability to kill microbes. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract/compound isolated and is expressed in ml/g[18-19].

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

3. Results and Discussion

3.1. GC-MS analysis

Gas Chromatography - Mass Spectroscopy (GC-MS) was done using the database of National Institute of standard and Technology (NIST). The prevailing compounds in the ethyl acetate extract of *C. ciliaris* were 4,22-stigmastadiene-3-one (2.41%, retention time- 37.288 min. and area- 1293164) (Figure-1), Azulene (Cyclopentacycloheptene) (1.81%, retention time- 8.026 min. and area- 971301) (Figure-2). It is use in salves and ointments; azulene is thought to assist in calming a wide variety of skin irritations, wrinkles, skin blemishes and conditions because of its soothing properties, anti-inflammatory effects and antibacterial properties. Ergost-5-en-3-ol, (3β, 24R)- (Campesterol) (1.63%, retention time- 35.520 min. and area- 872955) (Figure-3) and Myristic acid, isopropyl ester (0.17%, retention time- 15.415 min. and area- 90383) (Figure-4). However, till date there was no report on the presence of sterols, aromatic compounds and fatty acid/ esters from *C. ciliaris.*

3.2. Antimicrobial Activity

3.2.1. Phyto-chemical estimation[20]

The phyto–chemical estimation for the leaves of *C. ciliaris* were carried out according to Farnsworth[20] 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 et. al[21]. The yield (mg/10 gm ± S.D.) of the extracts was also analyzed where in the highest yields were recorded for *C. ciliaris* (641 ± 16.25) in acetone extracts (Table 1).

3.2.2. Total activity

Total activity indicates the volume at which extract can be diluted with still having ability to kill microorganisms. Most of the extracts showed high values of TA against *P. mirabilis, K. pneumoniae, A. tumefaciens* and *A. niger* which proves the potential to inhibit the growth of the test microorganisms, even at low concentration. Maximum TA values calculated were 204.27 ml against *P. mirabilis, K. pneumoniae* and *A. tumefaciens* followed by 117.84 ml against *A. niger* respectively (table 1).

3.2.3. Antibacterial/Antifungal activity

Maximum antibacterial/ antifungal activities were observed by GAA extracts of *C. ciliaris*. Antimicrobial activity (assessed in terms of ZOI and AI) of the leaves extracts in different polar solvents, tested against selected microorganisms were recorded ZOI- 36.50 ± 0.64 mm, AI- 3.042, against *P. mirabilis* followed by the same extract (ZOI-27.67 ± 0.24 mm, AI- 1.384 and ZOI- 25.83 ± 0.23 mm, AI- 1.614) against *K. pneumoniae* and *A. tumefaciens* respectively and ZOI- 7.67 ± 0.25 mm, AI- 0.592, against *A. niger* (table 2).

3.2.4. MIC and MBC/MFC

MIC and MBC/MFC values (table 3) were evaluated for those plant extracts, which were showing activity in DDA. The range of MIC and MBC/MFC of extracts recorded was 0.234 - 15 mg/ml. In the present investigation lowest MIC value 0.234 mg/ml was recorded for GAA extracts of *C. ciliaris* against *P. mirabilis*, *K. pneumonia* and *A. tumefaciens* indicating significant antimicrobial potential of test extracts. MIC and MBC/MFC values were found equal for GAA extracts of *C. ciliaris* showing bactericidal properties of test extracts.

4. Conclusion

In the present study most susceptible organism[22-23] in the investigation was *A. tumefaciens* against which, most of the plant extracts showed inhibition zone supported by some previous studies[22-23]. But, according the zone of inhibition *P. mirabilis* 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. Maximum antibacterial activities were observed by glacial acetic acid (GAA) and isopropyl alcohol extracts in *C. ciliaris*. In the light of the fact that microorganism are becoming resistant against the drugs in use, present investigation is of great significance to identified new bio-active compound in this species, as far as the future drugs are concerned and uses of selected plants by the pharmaceutical industries for preparing plant based antimicrobials drugs. *C. ciliaris* 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.

Table 1: Primary Phyto-chemical estimation and Total activity of leaf extract of *C. ciliaris* in different polar solvents

Polar	*Primary Phyto-chemical estimation*			*Total activity*			
Solvents	*Total Yield (mg/10 gm±S.D.)*	*Color*	*Consistency*	*P. m.*	*K. p.*	*A. t.*	*A. n.*
Water	556±13.89	Dark brown	Sticky	14.83	-	-	-
Acetic acid	478±17.32	Very dark green	Sticky	204.27	204.27	204.27	117.84
Ethanol	431±11.37	Yellow	Nonsticky	5.75	5.75	22.99	-
Acetone	641±16.25	Yellow	Nonsticky	-	8.55	34.19	-
Ethyl acetate	434±11.13	Green	Nonsticky	6.84	11.57	-	-
Chloroform	456±18.69	Dark green	Sticky	-	24.32	-	-
Iso propyl alcohol	199±13.68	Yellow	Nonsticky	2.65	5.31	10.61	-
Benzene	214±9.36	Yellow	Nonsticky	-	-	-	-
Toluene	144±8.42	Light yellow	Nonsticky	-	-	3.84	-
Petrolium ether	210±13.45	Yellowish green	Nonsticky	-	-	1.4	-
Hexane	179±16.84	Very dark green	Sticky	-	-	-	-

P. m.- Proteus mirabilis, K. p.- Klebsiella pneumoniae, A. t.- Agrobacterium tumefaciens, A. t.- Aspergillus niger

Table 2: Zone of Inhibition (mm)* and Activity index of leaf extract of *Cenchrus ciliaris*

Polar Solvents	*Bio-activity of leaf extracts of Cenchrus ciliaris against pathogens*							
	Proteus mirabilis		*Klebsiella pneumoniae*		*Agrobacterium tumefaciens*		*Aspergillus niger*	
	ZOI	AI	ZOI	AI	ZOI	AI	ZOI	AI
Water	9.17±0.25	0.764	-	-	-	-	-	-
Acetic acid	36.50±0.64	3.042	27.67±0.24	1.384	25.83±0.23	1.614	7.67±0.25	0.592
Ethanol	7.17±0.24	0.359	8.33±0.24	0.417	9.83±0.22	0.702	-	-
Acetone	-	-	9.83±0.21	0.492	13.83±0.23	0.988	-	-
Ethyl acetate	7.17±0.24	0.896	8.67±0.24	0.434	-	-	-	-
Chloroform	-	-	12.67±0.26	0.634	-	-	-	-
Iso propyl alcohol	8.33±0.23	0.694	9.5±0.64	0.475	14.33±0.25	1.024	-	-
Benzene	-	-	-	-	-	-	-	-
Toluene	-	-	-	-	9.67±0.21	0.403	-	-
Petrolium ether	-	-	-	-	7.17±0.21	0.598	-	-
Hexane	-	-	-	-	-	-	-	-

All values are mean ± SD, n=3, ZOI= Zone of Inhibition (mm±S.D.), AI=Activity index

Table 3: MIC and MBC/MFC of leaf extract of *Cenchrus ciliaris* in different polar solvents

Polar Solvents	Bio-activity of leaf extracts of *Cenchrus ciliaris* against pathogens							
	P. mirabilis		*K. pneumoniae*		*A. tumefaciens*		*A. niger*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Water	3.75	7.5	-	-	-	-	-	-
Acetic acid	0.234	0.234	0.234	0.468	0.234	0.468	7.5	7.5
Ethanol	7.5	15	7.5	15	1.875	3.75	-	-
Acetone	-	-	7.5	7.5	1.875	3.75	-	-
Ethyl acetate	7.5	15	3.75	3.75	-	-	-	-
Chloroform	-	-	1.875	1.875	-	-	-	-
Isopropyl alcohol	7.5	15	3.75	7.5	1.875	3.75	-	-
Benzene	-	-	-	-	-	-	-	-
Toluene	-	-	-	-	3.75	7.5	-	-
Petrolium ether	-	-	-	-	15	15	-	-
Hexane	-	-	-	-	-	-	-	-

MIC-Minimum inhibitory concentration (mg/ml), MBC-Minimum bactericidal concentration (mg/ml), MFC-Minimum fungicidal concentration (mg/ml),

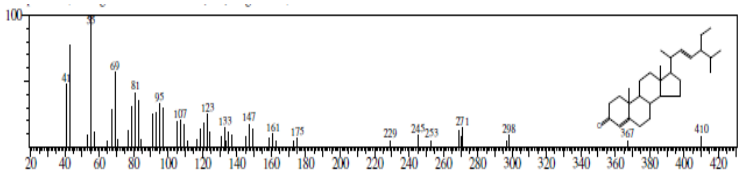

Figure 1: Mass Spectrum of 4,22-Stigmastadiene-3-one (RT- 37.288 min.)

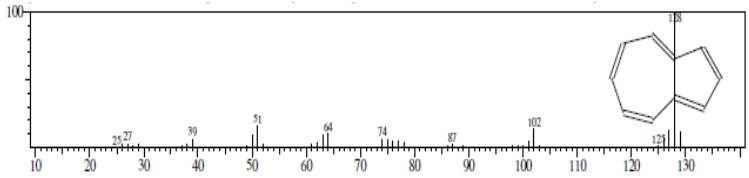

Figure 2: Mass Spectrum of Cyclopentacycloheptene (Azulene) (RT- 8.026 min.)

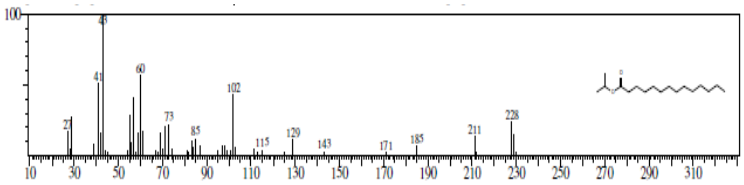

Figure 3: Mass Spectrum of Ergost-5-en-3-ol, (3β, 24R)- (Campesterol) (RT- 35.550 min.)

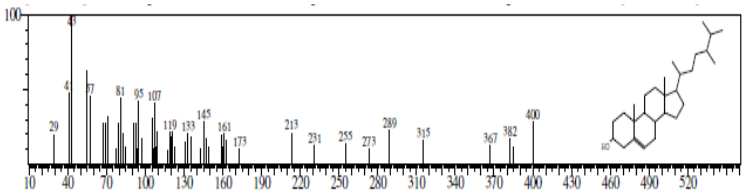

Figure 4: Mass Spectrum of Myristic acid, isopropyl ester (RT- 15.415 min.)

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References

1. Bessman SP. *Ammonia Metabolism in Animals: Symposium on Inorganic Nitrogen Metabolism.* Mc-Elry, Glass (editors). The Johns Hopkins Press, **1956**.
2. 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**.
3. Sinha RK, Bhatia S, Vishnoi R. Desertification control and rangeland management in the Thar desert of India. *In: Rala Report no. 200*: 1996, 115-23.
4. Agrawal P. *Ecophysiological and Biochemical studies Related to drought adaptation in grasses of Indian Desert.* Ph.D. Thesis, J. N. Vyas University, **2007**.
5. Muthulakshmi A, Jothibai-Margret R, Mohan VR. GC-MS Analysis of Bioactive Components of *Feronia elephantum* Correa (Rutaceae). *J of Applied Pharm Sci*. **2012**, 2(2): 69-74.
6. Hussain A, Zaman MK, Ramteke AM. Antibacterial Activity of Trunk Bark of *Alstonia scholaris*. *Asian J of Pharm and Clin Res*. **2002**, 3(4): 46-7.
7. Harborne JB. *Phytochemical Methods*, Chapman and Hall, London, New York. **1984**, 5-6.
8. Subramanian SS, Nagarjan S. Flavonoids of the seeds of *Crotolaria retusa* and *Crotolaria striata*. *Current Sci.* **1969**, 38: 65.
9. Dey AC. *Indian Medicinal Plants Used in Ayurvedic Preparations*. Singh B, Singh MP. (Eds), Dehra Dun. **1980** p.p. 202.
10. Andrews JM. BSAC standardized disc susceptibility testing method. *J Antimicrob Chemother.* **2001**, 4: 43-57.

11. 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.
12. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol.* **2002**, 81: 81-100.
13. Singariya P, Kumar P, Mourya KK. Phyto-chemical Screening and Antimicrobial Activities of Dhaman grass and Indian Ginseng. *J of Pharm Res.* **2012a**, 5(1): 135 9.
14. Moideen MMJ, Raffick MM. Antibacterial, Antifungal Activity and Phytochemical Analysis of *Sanseviveria roxburghiana* Root. *I J of Phytopharmacol.* **2012**, 3(1): 21-6.
15. Singariya P, Mourya KK, Kumar P. Antimicrobial Activity of the Crude Extracts of *Withania somnifera* and *Cenchrus setigerus In-vitro*. *Pharmacognosy journal.* **2012d**, 4(27): 60-5.
16. Bhattacharya S, Zaman MK, Haldar PK. Antibacterial Activity of stem Bark and Root of Indian *Zanthoxylum nitidum*. *Asian J of Pharm and Clin Res.* **2009**, 2(1): 30-4.
17. 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.
18. 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): 124-9.
19. Eloff JN. Quantifying the bioactivity of the plant extracts during screening and bioassay-guided fractionation. *Phytomedicine*. **2004**, 11(4): 370-1.
20. Farnsworth NR. Biology and Phytochemical screening of plants. *Pharm Sci.* **1966**, 55: 225-76.
21. 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): 1387-93.
22. 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.* **2012b**, 5(1): 163-7.
23. Singariya P, Kumar P, Mourya KK. Antimicrobial Activity of Fruit coat (Calyx) of *Withania somnifera* against some Multi Drug Resistant Microbes. *I J of Biol and Pharm Res.* **2012c**, 3(2): 252-8.