

INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

SCREENING AND EVALUATION OF BIOACTIVE COMPONENTS OF CENCHRUS CILIARIS L. BY GC-MS ANALYSIS

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Article Received on: 19/05/17 Approved for publication: 19/06/17

DOI: 10.7897/2230-8407.08699

ABSTRACT

Plants are rich source of secondary metabolites with interesting biological activities. *Cenchrus ciliaris* L. is an important forage plant belonging to grass family (Poaceae). The objective of the present investigation was to characterize possible bioactive phytochemical constituents from the whole plant of C. ciliaris L. using methanol by gas chromatography-mass spectrometry (GC-MS) analysis. The shade-dried plant powder was extracted with methanol using Soxhlet extractor and crude extract was subjected to GC-MS. The phytochemical constituents were investigated using Perkin-Elmer gas chromatography-mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) and Willey 8 library. Maximum % area is found for Stigmasta-5, 22-Dien-3-ol, is present in maximum amount (12.68%) with RT = 36.461 min. in the methanolic root extract. 6,6-Dideutero-nonen-1-ol-3, is present in maximum amount (31.92 %) with RT=12.088 min. in the methanolic stem extract and Pentadecanoic acid is present in maximum amount (20.04%) with RT= 16.328 min. in the leaf extract of *Cenchrus ciliaris* L. Green plants synthesize and preserve a variety of biochemical compounds, these products include flavonoids, phenols, saponins, terpenes, steroids and glycosides. C4 plants, specifically in warm conditions may show more potentialities to defend themselves. These compounds are commercially important and are used by pharmaceutical industries as well as the traditional practitioners for making herbal/synthetic drug formulations.

Keywords: Cenchrus ciliaris L., Gas Chromatography – Mass Spectroscopy, Secondary metabolites, Pharmaceutical, Antibiotics, Therapeutic drugs.

INTRODUCTION

Plants defend themselves from pathogens and other herbivore enemies by elaborating a variety of bioactive secondary metabolites that may have multiple molecular sites of action. Accordingly, exploitation of these useful plants has spread rapidly to safeguard increasing population from various pathogens and ailments. Many plants are good sources of antioxidants and other bioactive compounds containing phenolics, alkaloids, amino acids, ascorbic acid etc. Due to increasing demand, seeking therapeutic drugs from plants has grown tremendously. Such preparations contain various bioactive compounds of high therapeutic value and becoming popular in the area of medicine for their less expense and less side effects etc., compared to modern allopathic drugs. The traditional medicines in the last few decades emerged to have immense acknowledgements and it is estimated that 80% of community depend on traditional medicine for their primary healthcare¹. More than 70% of India's 1.1 billion population still use non-allopathic systems of medicine². Natural bioactive phytocompounds have been suggested as alternative sources for antibiotics. The chemical features of these constituents differ considerably among different species. This approach is alluring, in part, because they constitute a potential source of bioactive compounds that have been professed by the general public as comparatively safe and often act at multiple and novel target sites, thereby increasing the potential for resistance³. Extraction is the main step for the recovery and isolation of bioactive components from plant parts. The analysis and extraction of plant matrices play an important role in the development, modernization and quality control of herbal formulations⁴. The

extraction of bioactive compounds from plants for therapeutic targets also needs active principle to be identified⁵.

GC-MS method can serve as an interesting tool for identification of active principles of herbs. It combines two analytical techniques to a single method of analyzing mixtures of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy analyzes each of the components separately⁶.

Cenchrus ciliaris, commonly known as Dhaman grass, is extremely variable, tufted and perennial species. This grass is gaining attention in various field of research as it is more competitive in high temperature, solar radiation and low moisture. It is most suitable and highly nutritive grass for environmental conditions of desert⁷.

MATERIALS AND METHODS Collection of plant material

Fresh, disease free plants of *C. ciliaris* were collected from natural habitats of Jodhpur (Rajasthan) during July to October. Further identification and authentication of the specimens was done from Botanical survey of India, Jodhpur (Raj.) The samples were washed thoroughly in running tap water to remove soil particles and other adhered debris and finally washed with sterile distilled water. The vegetative plant was dried under shade at room temperature and powdered using an electric grinder. After sieving the powder was transferred to airtight polyethylene zipper bags.

Preparation of plant extracts

The powdered components (5 g) were successively extracted with methanol. Crude extracts of diff. plant parts were prepared with methanol by using hot extraction method⁸ in soxhlet assembly. The extracts were stored at -4 °C for further use. Repeated extraction was done if needed with same solvent until a colorless extract was obtained. All the plant extracts were evaporated to dryness. The GC-MS analysis was performed at AIRF (Advanced Instrumentation Research Facility), Jawaharlal Nehru University, New Delhi, India. Identification of phytoconstituents was based on Willey 8 and NIST libraries attached to GC-MS instrument. Retention indices of extracted compounds were compared with stored components & results were tabulated.

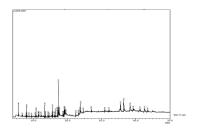
RESULTS AND DISCUSSION

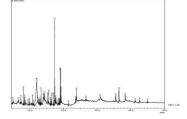
Gas chromatography coupled with mass spectrometry is a commonly used technique for separating and identifying the components of complex volatile mixtures, it can be a valuable tool assisting in the separation and identification of isolated components. GC is known for its high resolution separation of structurally similar compounds. The use of electron ionization in mass spectrometry produces distinctive mass spectral fragmentation patterns enabling mass spectra for unknown to be searched against libraries⁹.

GC-MS chromatogram of the methanol extracts of root, stem, and leaf of *C. ciliaris* L. revealed 46, 46 and 66 peaks (Figure 1-3) indicating the presence of 42, 44 and 61 bioactive compounds respectively. The GC-MS analysis of methanol extract of whole

plant of *C. ciliaris* L. revealed the presence of 147 phytoconstituents that could contribute the medicinal quality of the plant. 26, 17 & 35 phytochemicals (table 1-3) were identified in root, stem & leaf extract respectively with various biological activities. Structure and nature of common phytocompounds were identified in the methanolic extract of whole plant (Table 4). Mass spectrum of different compounds are shown (Figure 4-25).

Stigmasta-5, 22-Dien-3-OL¹⁰ is present in maximum amount (12.68%), followed by Pentadecanoic acid (11.35%), Stigmast-5-en-3-ol, (3.beta.)- (8.50%), Ergost-5-en-3-ol, (3.beta.,24r)-(7.08%) and Octadec-9-Enoic acid (4.55%) in methanolic root extract. 6,6-Dideutero-nonen-1-ol-3 is present in maximum amount (31.92%), followed by Pentadecanoic acid (11.50%), Stigmasta-5,22-dien-3-ol (6.22%), 9,12,15-octadecatrienoic acid,(z,z,z)- (5.43%), and 10,12-Hexadecadien-1-ol (4.49%) in methanolic stem extract and Pentadecanoic acid is present in maximum amount (20.04%), followed by 9,12,15octadecatrienoic acid (z,z,z)- (10.67%), Stigmasta-5,22-dien-3-(8.75%). Hexadecanoic acid. 2-hvdroxv-1ol (hydroxymethyl)ethyl ester (3.96%) and Stigmast-5-en-3-ol, (3.beta.)- (3.49%), Ergost-5-en-3-ol, (3.beta., 24r)- (2.99%) and Vitamin E (2.82%) in methanolic leaf extract of C. ciliaris L. Phytosterols have been clinically proved to reduce blood cholesterol and scientific reports suggest that they posses antioxidant activity¹¹. Beta-sitosterol is mainly known and used for its cholesterol lowering property¹². Activities revealed by other valuable compounds have been tabulated. They are being used in a variety of ways in cure of various ailments & in supplementing and supplanting human health and welfare¹³⁻¹⁸.





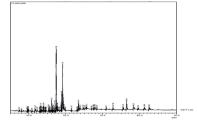


Figure 3: GC-MS Chromatogram of

Cenchrus ciliaris L. methanolic leaf extract

Figure 1: GC-MS Chromatogram of *Cenchrus ciliaris* L. methanolic root extract

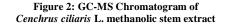


 Table 1: Bioactivity of compounds identified in the methanolic extract of root

S. No.	RT (min.)	Compound	Mol. weight	Mol. formula	Bioactivities
1	6.767	Hexanoic acid,2-Ethyl-	144	C ₈ H ₁₆ O ₂	Plasticizer, Lubricants
2	7.944	1-Undecanol	172	C11H24O	Bactericidal
3	8.056	Naphthalene	128	C10H28	Antiseptic, Carcinogenic
4	9.479	2-Undecanone	170	C ₁₁ H ₂₂ O	Natural non-toxic insect repellant
5	10.806	1-Tetradecene	196	C14H28	Anti tuberculosis
6	12.250	Naphthalene, 2-bromo-	207	C10H7Br	Dye preparation
7	13.344	9-Eicosene, (E)-	280	$C_{20}H_{40}$	Antimicrobial, Cytotoxic
8	13.495	1,2-Benzenedicarboxylic acid, Diethyl ester	222	$C_{12}H_{14}O_4$	Cosmetics, Insecticides, Plasticizer
9	14.316	8-Pentadecanone	226	C ₁₅ H ₃₀ O	Hepatotoxic, Demyelination, Conjunctivitis
10	14.585	Heptadecane, 2,6,10,15- tetramethyl-	296	C ₂₁ H ₄₄	Sex hormone in Algae
11	15.617	n-Heptadecanol-1	256	C ₁₇ H ₃₆ O	Antiarthritis, In treatment of skin diseases
12	15.686	Tetradecane	198	C ₁₄ H ₃₀	Antifungal, Antibacterial, Nematicidal
13	16.198	2-Pentadecanone, 6,10,14- Trimethyl-	268	C ₁₈ H ₃₆ O	Allelopathic, Antibacterial

14	17.007	Octadecanoic acid, Methyl ester	298	C ₁₉ H ₃₈ O ₂	Antifungal, Antibacterial, Antimicrobial, Emulsifier, Perfumery Industry
15	17.267	cis-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$	Therapeutic uses in medicine, surgery
16	17.354	Pentadecanoic acid	242	C15H30O2	Lubricants, Adhesive agents
17	19.567	n-Nonadecanol-1	284	C19H40O	Antimicrobial, Cytotoxic
18	19.074	Octadec-9-enoic acid	282	C ₁₈ H ₃₄ O ₂	Antihypertensive, Increases HDL & decrease LDL
19	19.271	Octadecanoic acid	284	$C_{18}H_{36}O_2$	Antifungal, Antitumor, Antibacterial
20	22.432	Octadecanal	268	C18H38O	Sex Pheromone
21	24.639	2-Hexadecyloxirane	268	C ₁₈ H ₃₆ O	Antibacterial, Antimicrobial, Antioxidant, Antipyretic, AntiInflammatory, Analgesic
22	30.936	.gammaTocopherol	416	C ₂₈ H ₄₈ O ₂	Antioxidant, Cardio protective, Anticancer, AntiInflammatory
23	35.547	Ergost-5-en-3-ol, (3.beta.,24r)-	400	C ₂₈ H ₄₈ O	Liver disease, Jaundice, Arthrosclerosis
24	36.461	Stigmasta-5,22-dien-3-ol	412	C29H48O	Synthetic Progesterone
25	38.346	Stigmast-5-en-3-ol, (3.beta.)-	414	C ₂₉ H ₅₀ O	AntiInflammatory, Antipyretic, Anti ulcer, Antiarthritic
26	43.463	Androst-4-en-3-one, 17-hydroxy-, (17.beta.)-	288	$C_{19}H_{28}O_2$	Regulation of spermatogenesis

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Table 2: Bioactivity of compounds identified in the methanolic extract of stem

S. No.	RT (min.)	Compound	Mol. Weight	Mol. formula	Bioactivities
		2,3-Dihydro-3,5- dihydro -6-methyll-			Antimicrobial,
1	7.339	4h-pyran-4-one	144	$C_6H_8O_4$	AntiInflammatory
2	8.050	Napthalene	204	$C_{10}H_{8}$	Antiseptic, Carcinogenic
3	8.429	2,3-Dihydro-benzofuran	120	C ₈ H ₈ O	In treatment of Diabetic Retinopathy and Arthritis
4	9.879	2-Methoxy-4-vinylphenol	150	$C_9H_{10}O_2$	Antibacterial
5	13.347	9-Eicosene	280	C20H40O	Antimicrobial, Cytotoxic
6	14.317	8-Pentadecanone	226	C ₁₅ H ₃₀ O	Hepatotoxic, Demyelination, Conjunctivitis
7	9.470	2-Undecanone	170	C ₁₁ H ₂₂ O	Natural non-toxic insect repellent
8	15.258	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	Antioxidant, Anticancer, Hypocholesterolemic
9	16.509	8-Octadecanone	268	C ₁₈ H ₃₆ O	Antimicrobial
10	17.009	Hexadecanoic acid, methyl ester	270	C ₁₇ H ₃₄ O ₂	Antioxidant, Antifungal, Hypocholesterolemic, Antimicrobial, Pesticide Nematicide
11	17.675	n-Nonadecanol-1	284	C ₁₉ H ₄₀ O	Antimicrobial, Cytotoxic
12	18.688	9,12-Octadecadienoic acid (z,z)-,methyl ester	294	C ₁₉ H ₃₄ O ₂	Hepatoprotective, Anti-histaminic, Antieczemic, Hypocholesterolemic
13	18.758	9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)	292	C ₁₉ H ₃₂ O ₂	AntiInflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective
14	19.045	10,12-Hexadecadien-1-ol	238	C16H30O	Sex pheromone
15	21.509	9-Octadecanoic acid (z)-	282	C ₁₈ H ₃₄ O ₂	Antihypertensive, Increases HDL & decrease LDL
16	23.624	Octadecanal	268	C18H36O	Sex pheromone
17	26.656	1-Hentetracontanol	593	C41H84O	Antimicrobial

Table 3: Bioactivity of phytochemical compounds identified in the methanol extract of leaf

S. No.	RT (min.)	Compound	Mol. Weight	Mol. formula	Bioactivities
1	7.339	2,3-Dihydro-3,5-dihydroxy-6-			Antimicrobial,
		methyl-4h-pyran	144	$C_6H_8O_4$	AntiInflammatory
2	9.878	2-Methoxy-4-vinylphenol	150	C ₉ H ₁₀ O ₂	Antibacterial
3	10.807	1-Tetradecene	196	C14H28	Antituberculosis
					Cytotoxic against T cells lines, Anti viral
4	11.654	Guanosine	283	$C_{10}H_{13}N_5O_5$	against Vero cells infected with HSV-1
5	12.207	Heptadecane	240	C17H36	Antioxidant
6	12.964	Dodecanoic acid	200	$C_{12}H_{24}O_2$	Antimicrobial

7	13.390	Megastigmatrienone	190	C ₁₃ H ₁₈ O	Aroma
8	13.493	1,2-Benzene dicarboxylic	222	C ₁₂ H ₁₄ O ₄	Cosmetics, Insecticides, Plasticizer
Ũ	101170	acid, diethyl ester		012111404	
9	14.364	n- Tridecan-1-ol	200	C13H28O	Natural mosquito control agent
10	14.466	5-Octadecene, (E)-	252	C ₁₈ H ₃₆	Stronger sexual characters
11	14.589	2-Methyltetracosane	352	C ₂₅ H ₅₂	Free radical scavenging
12	15.260	Tetradecanoic acid	228	C ₁₄ H ₂₈ O ₂	Antioxidant, Anticancer,
				- 14 28 - 2	Hypocholesterolemic
13	16.133	2,6,10-Trimethyl, 14-ethylene- 14-pentadecne	296	$C_{20}H_{38}$	Antiproliferative
14	16.204	2-Pentadecanone, 6,10,14- Trimethyl-	268	C ₁₈ H ₃₆ O	Allelopathic, Antibacterial
15	16.328	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	Lubricants, Adhesive agents
		2-Hexadecen-1-ol, 3,7,11,15-			Antimicrobial, Sedatives and anesthetics
16	16.390	tetramethyl-, [r-[r*r*, (e)]]	296	$C_{20}H_{40}O$	
17	16.520	1,2-Benzenedicarboxylic Acid, bis(2-Methylpropyl) Ester	278	$C_{16}H_{22}O_4$	Antimicrobial, Antifouling
18	17.011	Hexadecanoic acid, methyl ester	270	C ₁₇ H ₃₄ O	Antioxidant, Insecticide, hemolytic, Hypo – cholesterolemic
19	17.183	13-Docosanoic acid	338	C ₂₂ H ₄₂ O ₂	Surfactant, Precursor to Biodiesel fuel
20	17.268	cis-13-Octadecenoic acid	282	C ₁₈ H ₃₄ O ₂	Therapeutic uses in medicine, surgery
21	17.681	Hexadecanoic acid , ethyl ester	284	C ₁₈ H ₃₆ O ₂	Antioxidant, hypocholesterolemic Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor
22	18.330	Heptadecanoic acid	270	C ₁₇ H ₃₄ O ₂	Antimicrobial
23	18.688	9,12-Octadecadienoic acid (z,z,)-, methyl ester	294	$C_{19}H_{34}O_2$	Hepatoprotective, Anti-histaminic, Antieczemic, Hypocholesterolemic
24	18.758	9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)-	292	$C_{19}H_{32}O_2$	AntiInflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective
25	19.051	9,12-Octadecadienoic acid (z,z,)-	280	C ₁₈ H ₃₂ O ₂	AntiInflammatory, Antibacterial, Antiarthritic, Hepatoprotectiv, Anti-histaminic, Anticoronary
26	19.133	9,12,15-Octadecatrienoic acid, (z,z,z)-	278	$C_{18}H_{30}O_2$	Preventive against cardiovascular diseases
27	19.281	Octadecanoic acid	284	C ₁₈ H ₃₆ O ₂	Antifungal, Antitumor, Antibacterial
28	19.506	(E)-9-Octadecenoic acid ethyl ester	310	C ₂₀ H ₃₈ O ₂	Steroids and primer pheromone
29	19.846	Phytol, acetate	338	$C_{22}H_{42}O_2$	flavor and fragrance
				22 72 2	Antioxidant, Anticancer Pesticide, Sunscreen,
30	27.001	Squalene	410	C ₃₀ H ₅₀	Perfumery, Chemo preventive
31	30.927	.gammaTocopherol	416	$C_{28}H_{48}O_2$	Antioxidant, Cardio protective, Anticancer, Anti-inflammatory
32	32.784	Vitamin E	430	C ₂₉ H ₅₀ O ₂	Antiaging, Analgesic, antidiabetic, Antidermatitic, Antileukemia, Anticancer, Vasodilator, Hepatoprotective, Hypocholesterolemic, Antibronchitic, Anticoronary
33	35.513	Ergost-5-en-3-ol, (3.beta.,24r)-	400	C ₂₈ H ₄₈ O	Liver disease, Jaundice, Arthrosclerosis
34	36.475	Stigmasta-5,22-dien-3-ol	412	C29H48O	Synthetic Progesterone
35	38.337	Stigmast-5-en-3-ol, (3.beta.)-	414	C ₂₉ H ₅₀ O	Anti Inflammatory, Antipyretic, Anti ulcer, Antiarthritic

Table 4: Structure and nature of compounds identified in the methanolic extract of whole plant

S. No.	Compound	Nature	Structure
1	Naphthalene	Polycyclic aromatic hydrocarbon	
2	2-Undecanone	Ketone	H ₂ C
3	9-Eicosene, (E)-	Alkene	1. 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
4	1-Tetradecene	Olefins	CH ₃ CH ₃

5	8-Pentadecanone	Ketone	н,есн,
6	2-Pentadecanone, 6,10,14-Trimethyl-	Sesquiterpenoids	
7	cis-13-Octadecenoic acid	Elaidic acid	110- J
8	Pentadecanoic acid	Fatty acid	
9	n-Nonadecanol-1	Fatty alcohol	п,вон
10	9-octadecenoic acid	Oleic acid	
11	Octadecanal	Fatty Aldehyde	H 1 1 2
12	Ocadecanoic acid	Stearic acid	Her Lander Call
13	γ-Tocopherol	Vitamin E compound	
14	Ergost-5-en-3-ol, (3.beta.,24r)-	Campesterol	
15	Stigmasta-5,22-dien-3-ol	Stigmasterol	H ₃ C, CH ₃ CH, CH ₃
16	Stigmast-5-en-3-ol, (3.beta.)-	β-Sitosterol	H ₃ C CH ₃ CH ₃ CH ₃ CH ₃ CH ₃ CH ₃ CH ₃
17	2,3-Dihydro-3,5-dihydroxy-6-methyl- 4h-pyran	Pyranone	но
18	2-Methoxy-4-vinylphenol	Phenolic compound	

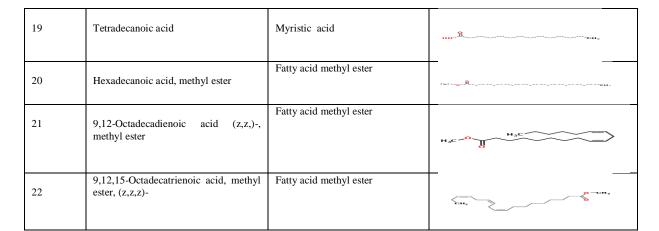




Figure 4: Mass spectrum of Naphthalene

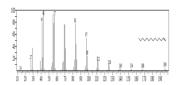


Figure 7: Mass spectrum of 1-Tetradecene

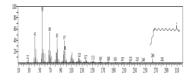


Figure 10: Mass spectrum of cis-13-Octadecenoic acid

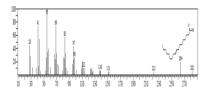


Figure 13: Mass spectrum of 9-octadecenoic acid

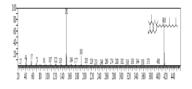
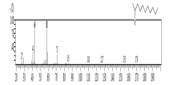
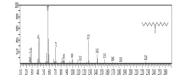


Figure 16: Mass spectrum of γ -Tocopherol



Δ

Figure 5: Mass spectrum of 2-Undecanone



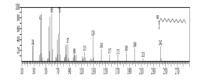


Figure 11: Mass spectrum of Pentadecanoic acid

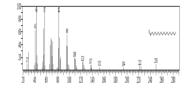


Figure 14: Mass spectrum of Octadecanal

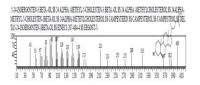


Figure 17: Mass spectrum of ERGOST-5-EN- Figure 18: Mass spectrum of Stigmasta-5, 22-3-OL, (3. BETA ., 24R)-

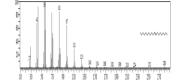


Figure 6: Mass spectrum of 9-Eicosene, (E)-

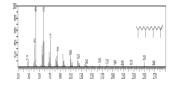


Figure 8: Mass spectrum of 8-Pentadecanone Figure 9: Mass spectrum of 2-Pentadecanone, 6, 10, 14-trimethyl-

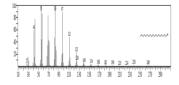


Figure 12: Mass spectrum of n-Nonadecanol-1

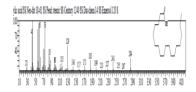
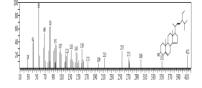


Figure 15: Mass spectrum of Ocadecanoic acid



dien-3-ol

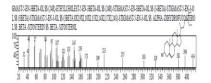


Figure 19: Mass spectrum of Stigmast-5-en-3ol, (3.beta.)-

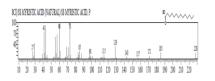


Figure 22: Mass spectrum of Tetradecanoic acid

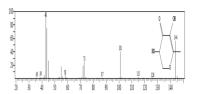


Figure 20: Mass spectrum of 2, 3-Dihydro-3, 5-dihydroxy-6-methyl-4h-pyran

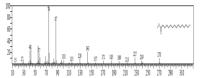
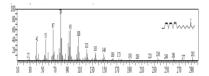


Figure 23: Mass spectrum of Hexadecanoic acid, methyl ester



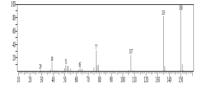


Figure 21: Mass spectrum of 2-Methoxy-4vinylphenol

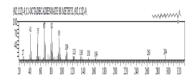


Figure 24: Mass spectrum of 9, 12-Octadecadienoic acid (z, z,)-, methyl ester



CONCLUSION

22 bioactive compounds were identified in methanol extract of whole plant of \overline{C} . ciliaris by GC-MS analysis. The results revealed presence of higher no. of compounds in methanol extract of leaf followed by root and stem. GC-MS study has exploited the potential of C. ciliaris. This plant can be a good source of phytoconstituents like alkane, carboxylic acid, phytosterol, aldehyde, ketone, fatty acid ester, fatty alcohol, terpenes and Vitamin E. Ergost-5-en-3-ol, (3.beta., 24r)-(Campesterol) and Stigmasta-5, 22-dien-3-ol (Stigmasterol) both are anti-cancerous phytosterols. Stigmast-5-en-3β-ol (β-Sitosterol), a phytosterol shows anti-inflammatory, anti-pyretic, anti-arthritic, anti-ulcer, insulin releasing and estrogenic effects. In future, the isolation and purification of above mentioned compounds analyzed from various parts of this plant would be fruitful for the pharmaceutical companies to formulate novel drugs and herbal medications for treating various ailments. It could be concluded that C. ciliaris L. contains numerous bioactive compounds. So it's recommended as a plant of pharmaceutical importance. However further studies are needed to undertake its bioactivity and toxicity profile.

ACKNOWLEDGEMENTS

I am grateful to the Department of Botany, Jai Narain Vyas, University, Jodhpur & AIRF (Advanced Instrumentation Research Facility), Jawaharlal Nehru University, New Delhi, for assisting laboratory & Instrumentation facilities.

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Cite this article as:

Sunita Arora *et al.* Screening and evaluation of bioactive components of *Cenchrus ciliaris* L. by GC-MS analysis. Int. Res. J. Pharm. 2017;8(6):69-76 http://dx.doi.org/10.7897/2230-8407.08699

Source of support: Nil, Conflict of interest: None Declared

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