



Research Article

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

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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 Wiley 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 phytochemicals 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 phytochemicals 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%), Stigmasta-5-en-3-ol, (3.β)- (8.50%), Ergost-5-en-3-ol, (3.β)-,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 9,12,15-octadecatrienoic acid (z,z,z)- (10.67%), Stigmasta-5,22-dien-3-ol (8.75%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.96%) and Stigmasta-5-en-3-ol, (3.β)- (3.49%), Ergost-5-en-3-ol, (3.β)-,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 possess 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.

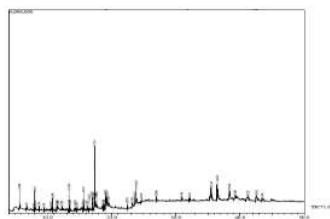


Figure 1: GC-MS Chromatogram of *Cenchrus ciliaris* L. methanolic root extract

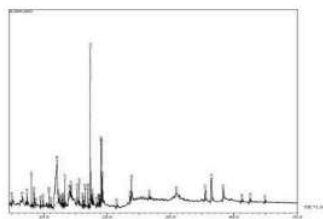


Figure 2: GC-MS Chromatogram of *Cenchrus ciliaris* L. methanolic stem extract

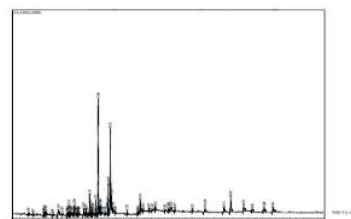


Figure 3: GC-MS Chromatogram of *Cenchrus ciliaris* L. methanolic leaf extract

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	C ₁₁ H ₂₄ O	Bactericidal
3	8.056	Naphthalene	128	C ₁₀ H ₈	Antiseptic, Carcinogenic
4	9.479	2-Undecanone	170	C ₁₁ H ₂₂ O	Natural non-toxic insect repellent
5	10.806	1-Tetradecene	196	C ₁₄ H ₂₈	Anti tuberculosis
6	12.250	Naphthalene, 2-bromo-	207	C ₁₀ H ₇ Br	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	Anti-arthritis, 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	C ₁₅ H ₃₀ O ₂	Lubricants, Adhesive agents
17	19.567	n-Nonadecanol-1	284	C ₁₉ H ₄₀ O	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	C ₁₈ H ₃₆ O	Sex Pheromone
21	24.639	2-Hexadecyloxirane	268	C ₁₈ H ₃₆ O	Antibacterial, Antimicrobial, Antioxidant, Antipyretic, AntiInflammatory, Analgesic
22	30.936	gamma.-Tocopherol	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, Artherosclerosis
24	36.461	Stigmasta-5,22-dien-3-ol	412	C ₂₉ H ₄₈ O	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

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

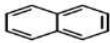
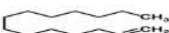
S. No.	RT (min.)	Compound	Mol. Weight	Mol. formula	Bioactivities
1	7.339	2,3-Dihydro-3,5- dihydro -6-methyl-4h-pyran-4-one	144	C ₆ H ₈ O ₄	Antimicrobial, AntiInflammatory
2	8.050	Napthalene	204	C ₁₀ H ₈	Antiseptic, Carcinogenic
3	8.429	2,3-Dihydro-benzofuran	120	C ₈ H ₈ O	In treatment of Diabetic Retinopathy and Arthritis
4	9.879	2-Methoxy-4-vinylphenol	150	C ₉ H ₁₀ O ₂	Antibacterial
5	13.347	9-Eicosene	280	C ₂₀ H ₄₀ O	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	C ₁₆ H ₃₀ O	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	C ₁₈ H ₃₆ O	Sex pheromone
17	26.656	1-Hentetracontanol	593	C ₄₁ H ₈₄ O	Antimicrobial

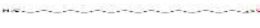
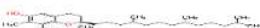
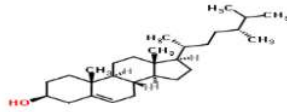
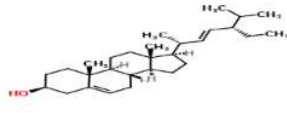
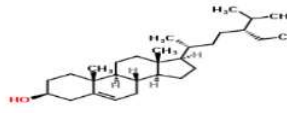
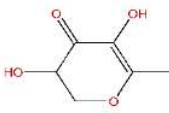
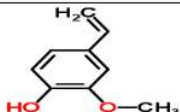
Table 3: Bioactivity of phytochemical compounds identified in the methanol extract of leaf

S. No.	RT (min.)	Compound	Mol. Weight	Mol. formula	Bioactivities
1	7.339	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran	144	C ₆ H ₈ O ₄	Antimicrobial, AntiInflammatory
2	9.878	2-Methoxy-4-vinylphenol	150	C ₉ H ₁₀ O ₂	Antibacterial
3	10.807	1-Tetradecene	196	C ₁₄ H ₂₈	Antituberculosis
4	11.654	Guanosine	283	C ₁₀ H ₁₃ N ₅ O ₅	Cytotoxic against T cells lines, Anti viral against Vero cells infected with HSV-1
5	12.207	Heptadecane	240	C ₁₇ H ₃₆	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 acid, diethyl ester	222	C ₁₂ H ₁₄ O ₄	Cosmetics, Insecticides, Plasticizer
9	14.364	n- Tridecan-1-ol	200	C ₁₃ H ₂₈ O	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, Hypocholesterolemic
13	16.133	2,6,10-Trimethyl, 14-ethylene-14-pentadecene	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
16	16.390	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r ⁺ , (e)]]	296	C ₂₀ H ₄₀ O	Antimicrobial, Sedatives and anesthetics
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, Hepatoprotective, 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
30	27.001	Squalene	410	C ₃₀ H ₅₀	Antioxidant, Anticancer Pesticide, Sunscreen, Perfumery, Chemo preventive
31	30.927	gamma.-Tocopherol	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	C ₂₉ H ₄₈ O	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	
3	9-Eicosene, (E)-	Alkene	
4	1-Tetradecene	Olefins	

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

19	Tetradecanoic acid	Myristic acid	
20	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	
21	9,12-Octadecadienoic acid (z,z)-, methyl ester	Fatty acid methyl ester	
22	9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)-	Fatty acid methyl ester	

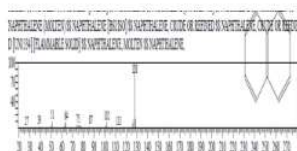


Figure 4: Mass spectrum of Naphthalene

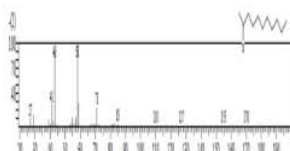


Figure 5: Mass spectrum of 2-Undecanone

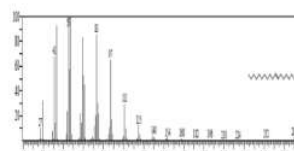


Figure 6: Mass spectrum of 9-Eicosene, (E)-

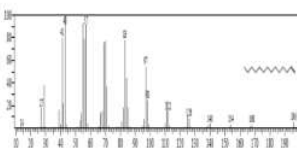


Figure 7: Mass spectrum of 1-Tetradecene

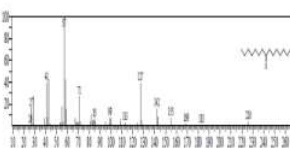


Figure 8: Mass spectrum of 8-Pentadecanone

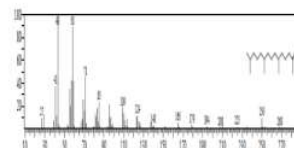


Figure 9: Mass spectrum of 2-Pentadecanone, 6, 10, 14-trimethyl-

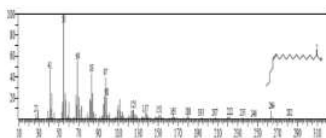


Figure 10: Mass spectrum of cis-13-Octadecenoic acid

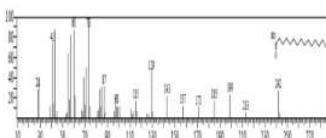


Figure 11: Mass spectrum of Pentadecanoic acid

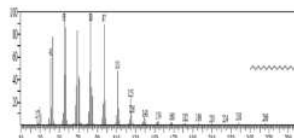


Figure 12: Mass spectrum of n-Nonadecanol-1

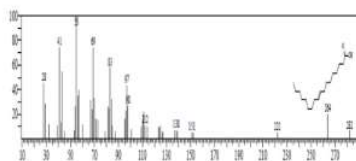


Figure 13: Mass spectrum of 9-octadecenoic acid

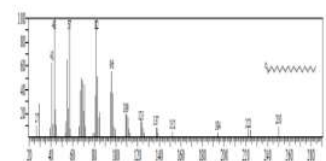


Figure 14: Mass spectrum of Octadecanal

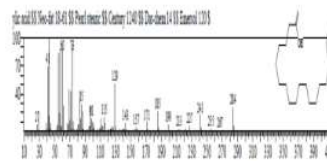


Figure 15: Mass spectrum of Octadecanoic acid

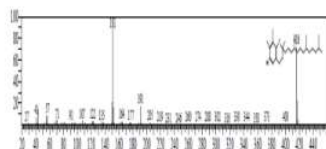


Figure 16: Mass spectrum of γ-Tocopherol

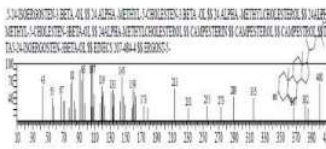


Figure 17: Mass spectrum of ERGOST-5-EN-3-OL, (3. BETA., 24R)-

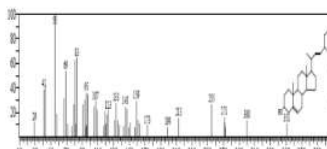
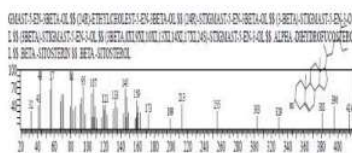


Figure 18: Mass spectrum of Stigmasta-5, 22-dien-3-ol



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