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Gas Chromatography-Mass Spectrometry (GC-MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of *Cenchrus setigerus* Vahl (Poaceae)

Sunita Arora and Ganesh Kumar

Abstract
Cenchrus setigerus Vahl belongs to Poaceae family, an important fodder plant which is traditionally used as famine food during drought. The objective of this study was to characterize the chemical constituents in different crude extracts of the root, stem and leaves of *Cenchrus setigerus* by using modern sensitive gas chromatography–mass spectrometry technique. GC–MS analyses showed that majority of these identified compounds in various crude extracts contain normal hydrocarbons, fatty acid, fatty acid esters, terpeniods, phytosterols, alkaloids and glycosides. The high percentage of compounds that were identified in the crude extracts shows chemical and biological importance are Hexadecanoic acid, 1 -(hydroxymethyl)-1,2-ethanediyl ester, p-Tert butylcalix arene, Stigmasta-5, 22-dien-3-ol, 2-Amino-9-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-3,9-dihydro-purin-6-one, Hexadecanoic acid, Pentadecanoic acid, Octadecanoic acid, i-Propyl 9,12,15-octadecatrienoate, Eicosane Tetracontane and Vitamin E. Plant secondary metabolites are commercially important and are used by pharmaceutical industry as well as the traditional practitioners. Therefore the identified good numbers of chemical compounds from various extracts of *Cenchrus setigerus* show antioxidant, antibacterial, anti-inflammatory, anticancer and anti-HIV activity. Hence the present study focused on bioactive phytoconstitutes of *Cenchrus setigerus* Vahl justifying the use of this plant to treat various ailments.

Keywords: Anti-HIV, betulin, *Cenchrus setigerus* Vahl, Methyl commate B and C, p-Tert butylcalix [4] arene, Vitamin E

1. Introduction

Medicinal plants are of great interest to mankind worldwide since long back ago. The medicinal value of plants is due to presence of various bioactive compounds with interesting pharmacological activities such as anticancer, anti-inflammatory, antibacterial, antifungal and antioxidant (Ammal and Bai, 2013) [1]. Nowadays, there is manifold increase in medicinal plant based industries due to the increase in the interest of use of medicinal plants throughout the world (Ullah *et al*., 2013) [2]. Secondary metabolites are produced within plants, which are of great pharmacological importance having differences in molecular structure and their property. Natural crude drug extracts, isolated from plant species can be prolific sources for such new drugs (Mulula *et al*., 2017) [3]. 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 formulations (Karimi and Jaafar, 2011) [4]. The extraction of bioactive compounds from plants for therapeutic targets also needs active principle to be identified (Vuorela *et al*., 2004) [5]. Gas chromatography coupled to mass spectrometry (GC-MS) has commonly been used for analysis of bioactive compounds. 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 separately (Bai *et al*., 2014) [6].

Cenchrus setigerus Vahl (C_4 plant), belongs to Poaceae family, commonly known as "Kala Dhaman" is highly nutritious grass. It is best suited for desert environmental conditions, considered as an excellent pasture for cattle's. This 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 (Singariya *et al*., 2012) [7]. In extreme conditions when food is in scarce, seeds of this grass are consumed by tribals or originals as famine food.

C_4 plants are more efficient at gathering CO_2 and utilizing nitrogen from the atmosphere and recycling of N in the soil. It is more competitive under the conditions of high temperature, solar radiation and low moisture (Agrawal, 2007) [8]. This grass has a potential of excellent soil binding capacity due to adventitious root system which goes deeper below the soil, and helps to conserve soil in dry areas. The objective of this study was to determine the possible bioactive components of *C. setigerus* using GC-MS analysis.

2. Material and methods

2.1 Collection of plant material

C. setigerus plant materials were collected from Jodhpur of Rajasthan, India, during the period 2015-2016. Further identification and authentication of the specimens was done from Botanical survey of India, Jodhpur (Raj.) The plant parts were thoroughly washed with tap water followed by distilled water, were dried under shade for 20 days and ground into fine powder. After sieving they were transferred to airtight polyethylene zipper bags, labeled and stored till further use.

2.2 Preparation of Plant Extracts

The powdered plant samples (5 g) were successively extracted with methanol and ethyl acetate. The extraction was done by hot continuous soxhlet extraction method (Harborne, 1984) [9]. The extracts were stored at -4 °C till further use.

2.3 GC-MS analysis

Gas chromatography-Mass spectrometry (GC-MS) analysis of the extracts was performed using a GC-MS (Model; QP 2010 ultra-series, Shimadzu, Tokyo, Japan) equipped with thermal desorption system TD 20. Injection Mode: Split, Flow Control Mode: Linear Velocity, Pressure: 81.9 kPa, Linear Velocity: 40.5 cm/sec, Purge Flow: 3.0 mL/min, Split Ratio: 50.0. For GC-MS detection [GC-2010], Helieum gas (99.99%) was used as a carrier gas at a constant flow rate- total flow: 64.7 mL/min. and column flow: 1.21 mL/min. injector and mass transfer line temperature were set at 200 and 240°C respectively. The oven temperature was programmed (Column Oven Temp.: 80.0°C and Injection Temp.: 260.00°C). Total running time of GC-MS is 46.28 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total area, software adopted to handle mass spectra and chromatograms was a Turbomass. The relative percentage of the each extract constituents was expressed as percentage with peak area.

3. Results and discussion

The analysis and extraction of plant material play an important role in the development, modernization and quality control of herbal formulations. Hence the present study was aimed to find out the bioactive compounds of high therapeutic value present in the methanol and ethyl acetate extract of *C. setigerus* by using Gas chromatography and Mass spectroscopy. The common active principles with their % peak area, retention time and biological activity in the methanol and ethyl acetate extracts of root, stem and leaf of *C. setigerus* are presented in table 1. Total 65 major, bio-active constituents were identified in the present study in different extracts of *C. setigerus*. GC-MS chromatogram of the methanol and ethyl acetate extracts of root, stem and leaves of *C. setigerus* are shown in figure 1. Molecular structure of important compounds are shown in figure 2. Hexadecanoic acid, 1 -(hydroxymethyl)-1,2-ethanediyl ester is present in maximum amount (21.37%) followed by p-Tert butylcalix [4] arene (11.96%), Pentadecanoic acid (9.49%), Stigmasta-5,22-dien-3-ol (6.67%), Octadec-9-enoic acid (6.65%), Phenol (4.01%) and Ergost-5-en-3-ol, (3.beta.,24r)- (3.74%) in methanol root extract.

2-Amino-9-(3,4-dihydroxy-5-hydroxy methyl-tetrahydro-furan-2-yl)-3,9-dihydro-purin-6-one is present in maximum amount (33.93%) followed by Pentadecanoic acid (14.97%), Stigmasta-5, 22-dien-3-ol (7.54%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (4.34%), Ergost-5-en-3-ol, (3.beta.,24r)- (4.10%) and Stigmast-5-en-3-ol, (3.beta.)- (3.66%) in methanolic stem extract.

Pentadecanoic acid is present in maximum amount (13.52%) followed by i-Propyl 9,12,15-octadecatrienoate (7.88%) and Stigmasta-5, 22-dien-3-ol (7.75%), Ergost-5-en-3-ol, (3.beta.)- (6.99%), Tetracontane (5.96%), p-Tert butylcalix[4] arene (5.94%),Vitamin E (4.04%), Stigmast-5-en-3-ol, (3.beta.)- (4.23%) and Methyl commate B (2.68%) in methanolic leaf extract of *C. setigerus*.

Pentadecanoic acid is present in maximum amount (13.47%), followed by Octadecanoic acid (12.30%), Stigmasta-5, 22-dien-3-ol (2.99%), Stigmast-5-en-3-ol, (3.beta.)- (2.47%), Ergost-5-en-3-ol, (3. beta., 24r)- (2.24%) and Squalene (1.10%) in ethyl acetate root extract.

p-Tert butylcalix [4] arene is present in maximum amount (11.95%) followed by Pentadecanoic acid (8.43%), Octadecanoic acid (6.59%), Hexadecanoic acid, methyl ester (5.36%), Stigmasta-5, 22-dien-3-ol (5.33%) and Ergost-5-en-3-ol, (3. beta., 24r)- (3.51%) in ethyl acetate stem extract.

Tetracontane is present in maximum amount (27.24%), followed by Stigmasta-5, 22-dien-3-ol (9.15%), Hexadecanoic acid (8.11%), Stigmast-5-en-3-ol, (3.beta.)- (4.35%), Vitamin E (4.15%), Ergost-5-en-3-ol, (3.beta.)- (3.23%) and and Methyl commate B (2.31%) in ethyl acetate leaf extract of *C. setigerus*.

Table 1: Bioactivity of common phytocompounds identified in root, stem and leaves of *C. setigerus*

S. No	Compound	Plant Part	Solvent	RT (Min.)	Area (%)	Bioactivity
1	Naphthalene	Root	M, E	8.057, 8.068	2.74, 0.61	Antiseptic, Carcinogenic
		Stem	M, E	8.048, 8.062	2.11, 0.31	
2	Pentadecanoic acid	Root	M, E	17.354, 17.375	9.49, 13.47	Lubricants, Adhesive agents
		Stem	M, E	15.259, 17.366	14.97, 8.43	
3	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Root Stem	M, E E	17.111, 17.111 17.108	0.30, 3.05 2.34	Antimicrobial

4	Ergost-5-en-3-ol, (3.beta., 24 R)-	Root Stem	M, E M, E	35.542, 35.559 35.493, 35.538	3.74, 2.24 4.10, 3.51	Liver disease, Jaundice, Arthrosclerosis
5	Stigmasta-5,22-Dien-3-ol	Root Stem Leaf	M, E M, E M, E	36.487, 36.483 36.451, 36.478 36.454, 36.474	6.67, 2.99 7.54, 5.33 7.75, 9.15	Synthetic Progesterone Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive, Hypo-cholesterolemic
6	Stigmast-5-en-3-ol, (3.beta.)-	Root Stem Leaf	E M, E M, E	38.378 38.326, 38.372 38.336, 38.355	2.47 3.66, 2.40 4.23, 4.35	Anti-inflammatory, Antipyretic, Anti-ulcer, Antiarthritic
7	p-Tert butylcalix [4] arene	Root Stem	M, E E	23.860, 23.944 23.956	11.96, 1.51 11.95	Membrane carriers for the transport of chiral amino acids, used as extractants, transporters, and optical sensors
8	Octadecanoic acid	Root Stem Leaf	M, E M, E M, E	19.279, 19.288 19.267, 19.279 19.274, 19.284	2.33, 12.30 1.50, 6.59 2.03, 3.58	Antifungal, Antitumor, Antibacterial
9	Hexadecanoic acid, methyl ester	Root Stem	E M, E	17.014 17.007, 17.010	3.80 1.30, 6.59	Antioxidant, Nematicide, Insecticide, Lubricant, Antiandrogenic, Hemolytic, Hypo –cholesterolemic
10	Squalene	Root Leaf	E E	27.003 27.000	1.10 0.29	Antioxidant, Anticancer Pesticide, Sunscreen, Perfumery, Chemo preventive
11	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl-, [R-[R*R*,$^-$(E)]]	Stem Leaf	M, E M, E	18.866, 18.867 18.868, 18.880	0.47, 0.90 2.12, 2.45	Antimicrobial, Sedatives and Anesthetics
12	Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl)ethyl ester	Stem Leaf	M E	23.442 23.464	4.34 1.89	Antioxidant
13	Tetracontane	Stem Leaf	E M, E	31.588 30.526, 31.605	4.87 5.96, 27.24	Anti Inflammatory

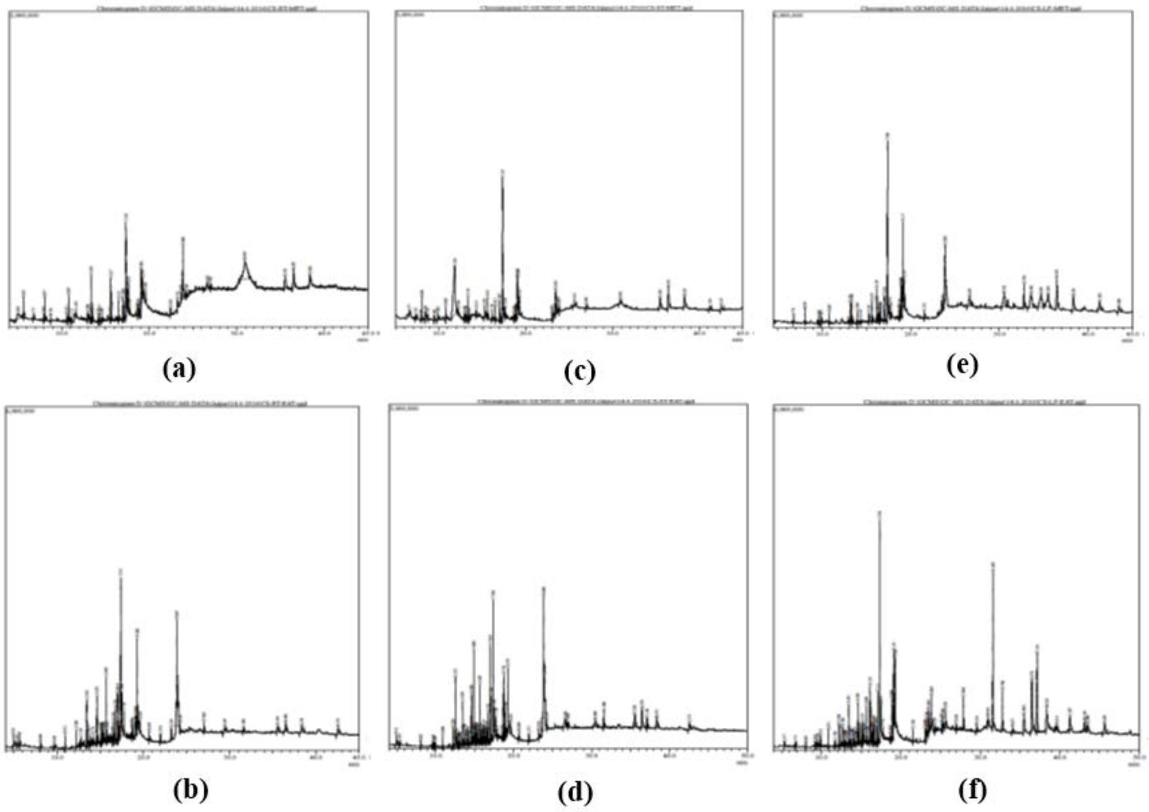
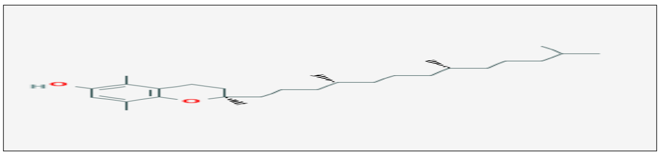
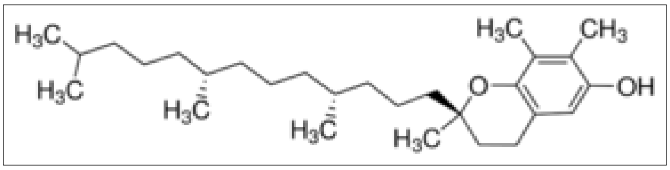


Fig 1: GC-MS Chromatogram of *Cenchrus setigerus* Vahl methanolic and ethyl acetate extract of root (a, b), stem (c, d) and leaf (e, f).



(a)



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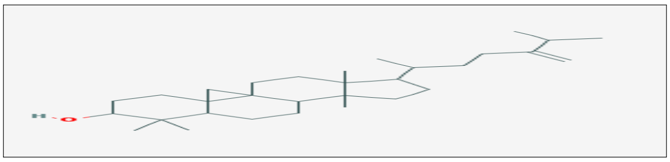

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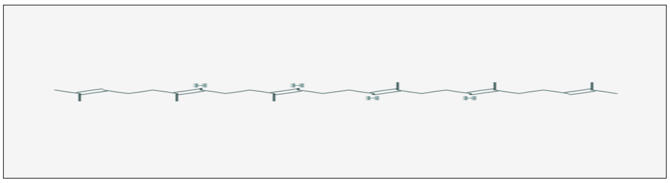

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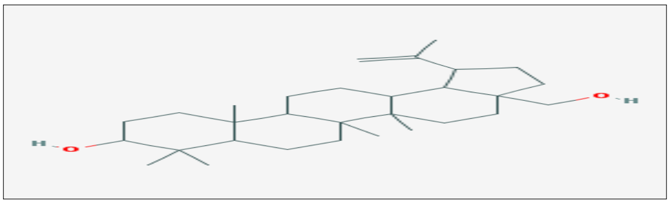
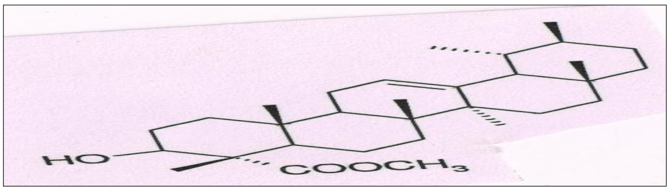
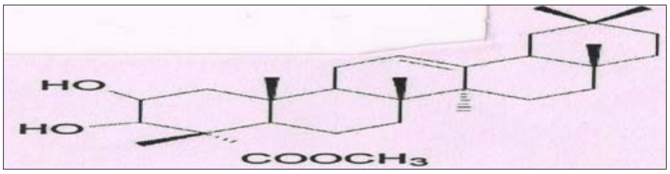


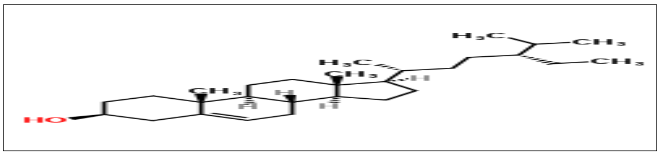
(e)



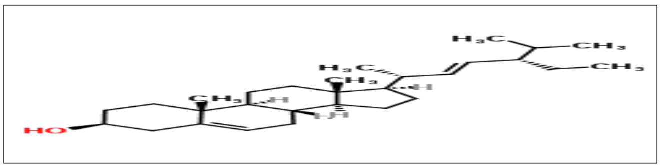
(f)



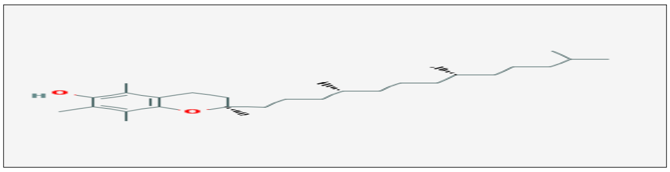
(g)



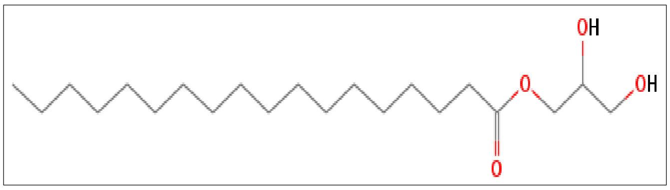
(h)



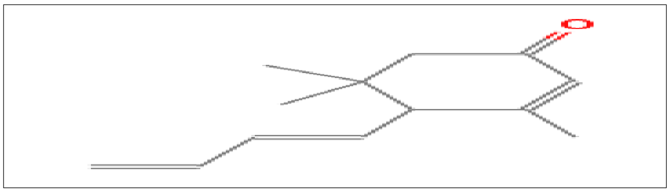
(i)



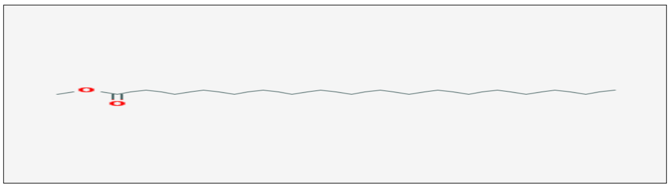
(j)



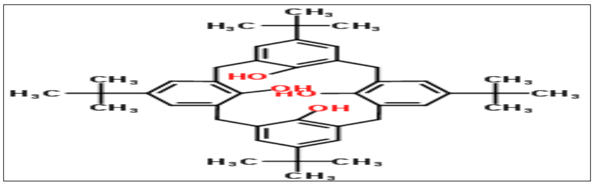
(k)



(l)



(m)



(n)

Fig 7: Molecular structure of (a) β-tocopherol, (b) γ-tocopherol, (c) 9.19- cyclolanostan-3-ol.24-methylene -3. beta., (d) Squalene, (e) lup-20 (29)- ene-3, 28-diol, (f) Methyl commate B, (g) Methyl commate C, (h) Stigmast-5-en-3-ol, (3.beta.)-, (i) Stigmasta-5, 22-dien-3-ol, (j) Ergost-5-en-3-ol, (3.beta.)-, (z,z,z)-, (k) Vitamin E, (l) Octadecanoic acid, 2,3-dihydroxypropyl ester (m) Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (n) Tert butylcalix [4] arene

4. Discussion

Phytosterols have been clinically proved to reduce blood cholesterol and scientific reports suggest that they possess antioxidant activity (Zawistowski, 2010) [10]. Phytosterols indirectly (in-vivo as a dietary supplement) and directly (in tissue culture media) inhibit the growth and metastasis of prostate cancer PC-3 cells (Awad *et al.*, 2001) [11]. Ergost-5-en-3-ol, (3. beta., 24r)- (Campesterol) and Stigmasta-5, 22-dien-3-ol (Stigmasterol) shows anticancerous phytosterols (Bradford and Awad, 2007) [12]. Stigmast-5-en-3β-ol (β-Sitosterol), a phytosterol shows anti-inflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and oestrogenic effects. Beta-sitosterol is mainly known and used for its cholesterol lowering property (Patra *et al.*, 2010) [13]. Octadecanoic acid (Stearic acid) shows hypocholesterolemic property and is used in cosmetics, flavor, lubricant, perfumery, propecic and suppository (Markkas and Govindharajalu, 2015) [14]. lup-20 (29)- ene-3, 28-diol a triterpene, commonly known as betulin reported to exhibit anti-human immunodeficiency virus (anti-HIV), anticarcinogenic, anti-flu, anti-inflammatory, immuno-modulator, hepatoprotector, antihypoxic, anti-allergen, anti-tuberculosis, antitumor, anti-viral, aphidifuge, cytotoxic, hypolipemic, detoxicant (detoxicating agent), adaptogenic and anti-oxidant activities. It also prevents hyperlipidosis and acts as prostaglandin-synthesis and topoisomerase- II-inhibitor (Collins *et al*., 1991) [15]. Squalene is a triterpene that has antibacterial, antioxidant, pesticide, antitumor, cancer preventive, immunostimulant, chemopreventive and lipoxygenase inhibitor activity (Sermakkani and Thangapandian, 2012) [16]. Methyl commate B and C is a triterpene glycoside in nature. Triterpene glycosides are natural, highly polar compounds with low volatility, first discovered in higher plants. Triterpene glycosides are well-known for their cytotoxic, antibacterial, antimicrobial, antiviral, insecticide, nematicide anticoagulant, hemolytic, antiparasitic wound healing and antitumor activities (Bahrami and franco, 2016) [17]. Vitamin E is a group of eight different compounds, but only two of the forms, α-tocopherol and γ-tocopherol, are commonly found in the human body. Vitamin E is the most potent lipid-soluble antioxidant in vivo. It is thought to play an important role in skin protection (Mitchel and McCann, 2003) [18]. 9.19-cyclolanostan-3-ol.24-methylene-3.beta. acts as an anti-HIV compound, used to prevent the HIV virus (Florence and Jeeva, 2015) [19]. Hexadecanoic acid, 1 - (hydroxymethyl)-1,2-ethanediyl ester, a fatty acid ethyl ester exhibits antioxidant, hypocholesterolemic, antiandrogenic, hemolytic and alpha reductase inhibitor activity. 5,11,17,23-TetratertButylpentacyclo[19.3.1.1~3,7~.1~9,13~.1~15,19~]Octacosa1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-Dodecaene25,26,27,28-Tetrol, commonly known as p-tert butylcalix [4] Arene with a supramolecular chemistry used as extractants, transporters and optical sensors in medical science (Li *et al*., 2001) [20].

In future, the isolation and purification of above mentioned compounds analyzed from various parts of this plant may be fruitful for the pharmaceutical companies to formulate novel drugs and herbal medications for treating various ailments. So this plant can be recommended as a plant of pharmaceutical importance. However further studies are needed to undertake its bioactivity, toxicity and ethical profile.

5. Conclusion

It can be concluded that *Cenchrus setigerus* is a rich source of novel and biologically active metabolites. Secondary or primary metabolites produced by this plant of poaceae may be of great interest for the pharmaceutical industry and medicinal research. Present investigation presents adequate data on the phytochemical constituents of the "Kala Dhaman" in two polar solvent for the synthesis of novel antibiotics and other herbal formulations. Bioactive compounds found in this grass await a major breakthrough for their potential application as natural antioxidants and pharmaceutical products.

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