Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 1445-1450
Received: 04-11-2017
Accepted: 05-12-2017

Sunita Arora
Department of Botany, Jai Narain vyas University, Jodhpur, Rajasthan, India

Ganesh Kumar
Department of Botany, Jai Narain vyas University, Jodhpur, Rajasthan, India

Correspondence
Sunita Arora
Department of Botany, Jai Narain vyas University, Jodhpur, Rajasthan, India

Phytochemical screening of root, stem and leaves of *Cenchrus biflorus* Roxb

Sunita Arora and Ganesh Kumar

Abstract

The present investigation was carried out to characterize the possible bioactive phytochemical constituents from the leaf of *Cenchrus biflorus* Roxb. (Poaceae) using various solvents i.e. methanol, ethyl acetate and hexane by Gas Chromatography-Mass Spectrometry technique. The phytochemical compounds were investigated using GC-MS technique, while the mass spectra of the compounds found in the extracts was matched with the National Institute of Standards and Technology (NIST) and Willey 8 library. Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester, Octadecanoic acid, 2,3-dihydroxypropyl ester, A’-Neogammacer-22(29)-en-3-one, Humulane-1, 6-dien-3-ol, n-Hexadecanoic acid, Hexadecanoic acid methyl ester, Stigmasterol, Stigmast-5-en-3-ol, (3.beta.)-, Ergost-5-en-3-ol, (3.beta.,24r)-, Vitamin E, Undecane, Guanosine, Squalene, gamma.-sitosterol, p-Tert butylcalix[4] arene, Behenyl behenate, 1H-Indene, 1-hexadecyl-2,3-dihydro-, D-ribose, 2-deoxy-bis (thioheptyl)-, Pentadecanoic acid, Octadecanoic acid, and Octadecanoic acid, 3-oxo-, ethyl ester were present in various crude extract of *C. biflorus*. Further investigation on other aspects may create a new platform for pharmaceutical companies to produce new herbal drugs.

Keywords: Anticancer, Antioxidant, Behenic alcohol, *Cenchrus biflorus* Roxb., Gas Chromatography – Mass Spectrometry, famine food

Introduction

Medicinal plants are best known source of drugs and herbal formulations used for treatment of various ailments. According to the World Health Organization (1999), an estimated 80% of people living in developing countries rely on harvested wild plants for their primary health care [1]. Medicinal plants have been a major source of therapeutic agents since long back ago. Indian flora consists of more than 2200 species of medicinal and aromatic plants. The interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products. 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 [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 [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 [4]. The extraction of bioactive compounds from plants for therapeutic targets also needs active principle to be identified [5]. 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 separately [6].

Cenchrus biflorus Roxb. commonly known as “Bhurat” belongs to Poaceae family. It is most common and abundant grass distributed throughout tropical Africa, Arabia, Pakistan, India and Australia, grows mostly on sandy soil. Bhurat is very much used at the time of food scarcity as “famine food”. The grain is pounded and mixed with “Bajra Atta” to make a roti known as “Sogra” that is consumed by villagers and local persons. “Bhurat-Bread” is said to be a very nutritious food also considered as staple food just after rains in some rural and interior areas. It is extremely drought and heat tolerant species. *Cenchrus biflorus* Roxb. a tufted, annual, erect, rooting at lower nodes, slender branches more often arises from lower nodes. Leaves are linear, acuminate with smooth surface. Inflorescence is a dense raceme with purplish flower that bears 1-3 deciduous involucres. The rachis is not straight it shows angular deviations throughout the length. The burrs (spiny projections) can strongly adhere to any object for dissemination. Earlier reports suggested that *Cenchrus biflorus* Roxb. is a potential grass of

'Thar Desert'. However, to date, there are no reports regarding exploitation of root, stem and leaves of *Cenchrus biflorus*.

Materials and Methods

Collection of Plant Material

Fresh, disease free plant samples were collected from Barmer district of Rajasthan (India), during the rainy season. 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, dried under shade for 20 days and grinded into fine powder. After sieving they were transferred to airtight polyethylene zipper bags, labeled and stored till further use.

Preparation of Plant Extracts

The powdered plant parts (5g) were successively extracted with methanol, ethyl acetate and hexane. The extraction was done by hot continuous soxhlet extraction [7] method. The extracts were stored at -4 °C till further use.

Principle of GC-MS

GC-MS is a combination of two different analytical techniques. Gas Chromatography (GC) and Mass Spectrometry (MS) is used to analyze complex organic and biochemical mixtures [8]. The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility [9] by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column [8]. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed [9]. GC-MS is widely used for research purposes in many fields and is emerging as an important technique in pharmaceutical science.

GC-MS Analysis

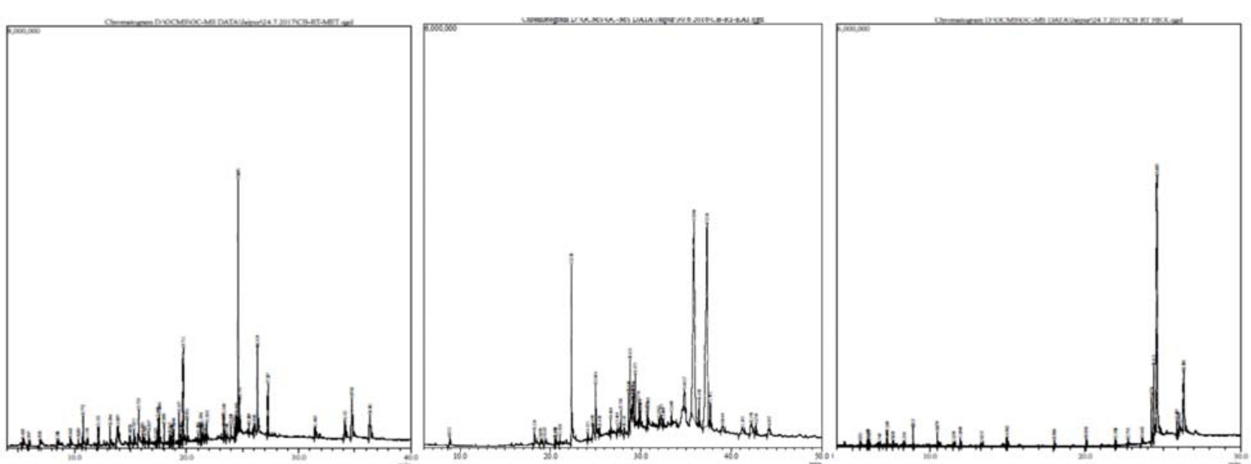
Gas chromatography-Mass spectrometry analysis of the extracts was performed using a GC-MS (Model; QP 2010 ultra series, Shimadzu, Tokyo, Japan) equipped with thermal desorption system TD 20. Injection Mode: Split, Flow Control Mode: Linear Velocity, Pressure: 81.9 kPa, Linear Velocity: 40.5 cm/sec, Purge 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 each extract constituents was expressed as percentage with peak area.

Identification of Components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST), USA and WILEY- 8 library. Library possesses 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 structure and weight of the compounds of the test samples were ascertained.

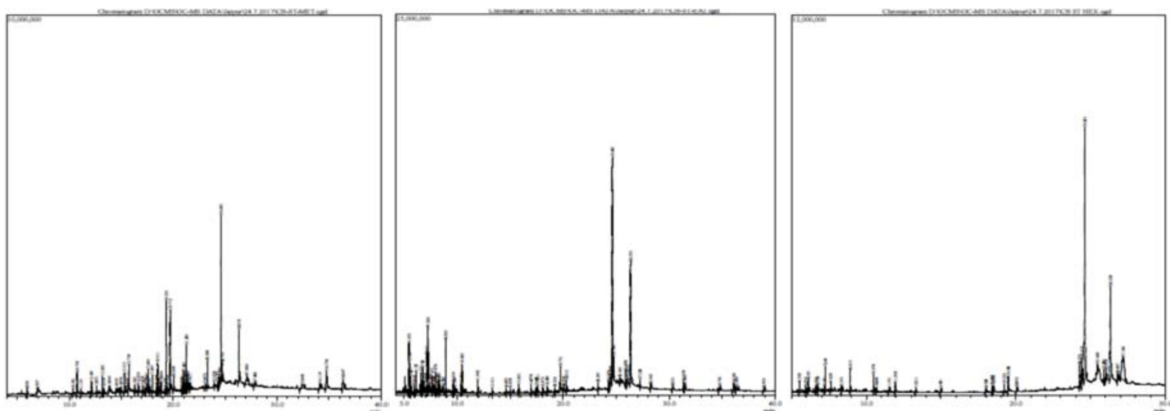
Results

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 present in methanol, ethyl acetate and hexane extract of *C. biflorus* by using Gas chromatography and Mass spectroscopy. The active principles with their molecular formula (MF), molecular weight (MW) and biological activity in different plant parts of *C. biflorus* are presented (Table 1). Total 57 bio-active constituents were identified in the present study including both major and minor constituents. GC-MS chromatogram of the methanol, ethyl acetate and hexane extract of root, stem and leaves of *C. biflorus* (Figure 1-3)



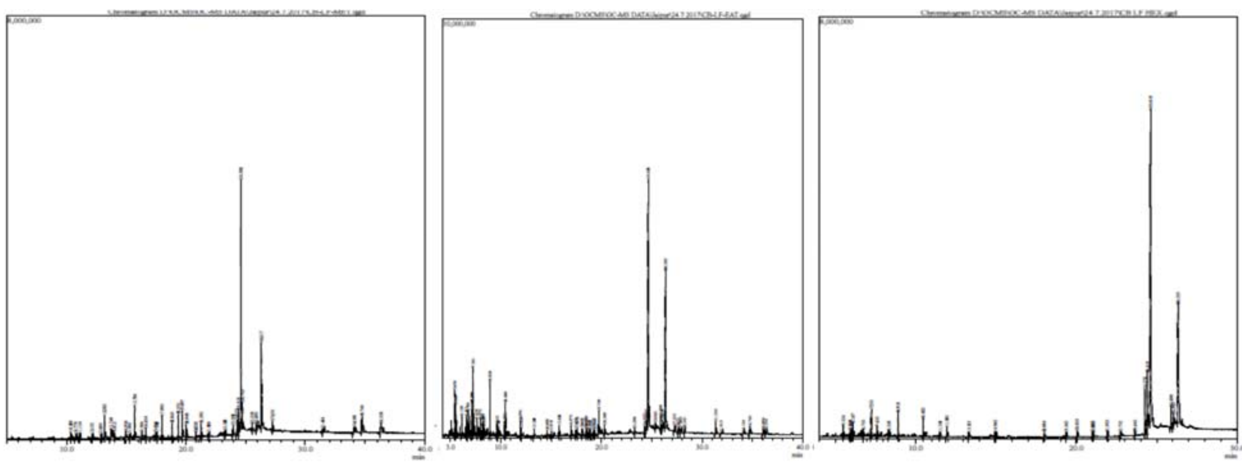
(a) (b) (c)

Fig 1: GC-MS Chromatogram of *Cenchrus biflorus* root extract in (a) methanol, (b) ethyl acetate and (c) hexane.



(a) (b) (c)

Fig 2: GC-MS Chromatogram of *Cenchrus biflorus* stem extract in (a) methanol, (b) ethyl acetate and (c) hexane.



(a) (b) (c)

Fig 3: GC-MS Chromatogram of *Cenchrus biflorus* leaf extract in (a) methanol, (b) ethyl acetate and (c) hexane.

Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester is present in maximum amount (20.33%) followed by Stigmasterol (8.70%), Octadecanoic acid, 2,3-dihydroxypropyl ester (8.40%), n-Hexadecanoic acid (6.96%), Stigmast-5-en-3-ol, (3.beta.)- (5.83%), Guanosine (4.11%) and Squalene (4.01%) in methanolic root extract. A'-Neogammacer-22(29)-en-3-one is present in maximum amount (35.32%) followed by Humulane-1, 6-dien-3-ol (32.62%), Pentadecanoic acid (7.31%), Stigmast-5-en-3-ol, (3.beta.)- (2.46%), Pentacosane (1.95%) and Octadecanoic acid (1.89%) in ethyl acetate root extract. Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester is present in maximum amount (49.29%) followed by Octadecanoic acid, 2,3-dihydroxypropyl ester (16.20%), D-ribose, 2-deoxy-bis (thioheptyl)- (10.22%) and Undecane (3.45%) in hexane root extract of *C. biflorus.*

Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester is present in maximum amount (18.94%) followed by n-Hexadecanoic acid (9.21%), Octadecanoic acid, 2,3-dihydroxypropyl ester (7.23%), Hexadecanoic acid methyl ester (6.60%), Stigmasterol (5.60%), p-Tert butylcalix[4] arene (4.58%) and. gamma.-sitosterol (3.23%) in methanolic stem extract. Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester is present in maximum amount (28.29%) followed by Octadecanoic acid, 2,3-dihydroxypropyl ester (15.87%), Undecane (7.05%), n-Hexadecanoic acid (2.81%) and Stigmast-5-en-3-ol, (3.beta.)- (1.03%) in ethyl acetate stem extract. Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester is present in maximum amount (37.67%) followed by Behenyl behenate (16.67%), Octadecanoic acid, 2,3-dihydroxypropyl ester (16.28%), 1H-Indene, 1-hexadecyl-2,3-dihydro- (4.73%) and Undecane (3.88%) in hexane stem extract of *C. biflorus.*

Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester is present in maximum amount (35.79%) followed by Octadecanoic acid, 2,3-dihydroxypropyl ester (15.42%), Stigmasterol (4.80%), Stigmast-5-en-3-ol, (3.beta.)- (3.77%), 1H-Indene, 1-hexadecyl-2,3-dihydro- (3.52%), n-Hexadecanoic acid (2.70%) and Ergost-5-en-3-ol, (3.beta.,24r)- (2.25%) in methanolic leaf extract. Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester is present in maximum amount (31.68%) followed by Octadecanoic acid, 2,3-dihydroxypropyl ester (20.14%), Undecane (6.27%), 1H-Indene, 1-hexadecyl-2,3-dihydro- (2.64%), n-Hexadecanoic acid (2.52%), Tetracontane (1.67%) and Stigmasterol (1.44%) in ethyl acetate leaf extract. Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester is present in maximum amount (43.78%) followed by Octadecanoic acid, 2,3-dihydroxypropyl ester (23.72%), 1H-Indene, 1-hexadecyl-2,3-dihydro- (8.81%), Undecane (4.19%) and Octadecanoic acid, 3-oxo-, ethyl ester (4.03%) in hexane leaf extract of *C. biflorus.*

Table 1: Bioactivity of phytocompounds identified in different plant parts of *Cenchrus biflorus*

S. No.	Compound	Mol. wt.	Mol. formula	Plant part			Bioactivity
				Root	Stem	Leaf	
1	2,3-DIhydro-3,5-Di hydroxyl-6-Methyl-4H-Pyran	144	$C_6H_8O_4$	+			Antimicrobial, AntiInflammatory
2	2-Methoxy-4-Vinylphenol	150	$C_9H_{10}O_2$	+	+	+	Antibacterial
3	Naphthalene	128	$C_{10}H_{28}$	+	++	+	Antiseptic, Carcinogenic
4	1-Tetradecene	196	$C_{14}H_{28}$	+			Antituberculosis
5	Heptadecane	240	$C_{17}H_{36}$	+	+	+	Antioxidant
6	Dodecanoic acid	200	$C_{12}H_{24}O_2$		+		Antimicrobial
7	n- Tridecan-1-ol	200	$C_{13}H_{28}O$		+		Natural mosquito control agent
8	2-Methyltetracosane	352	$C_{25}H_{52}$	+			Free radical scavenging
9	2-Methylhexacosane	380	$C_{27}H_{56}$			+	Antimirobial, decreases Blood Cholesterol
10	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276	$C_{17}H_{24}O_3$		++	+	Antimicrobial
11	Neophytadiene	296	$C_{20}H_{38}$	+	+	+	Antiproliferative
12	2-Pentadecanone, 6,10,14-Trimethyl-	268	$C_{18}H_{36}O$	++	+	+	Allelopathic, Antibacterial
13	2-Hexadecen-1-OL, 3,7,11,15-Tetramethyl-, [R-[R*R*,-(E)]]	296	$C_{20}H_{40}O$		+		Antimicrobial, Sedatives and anesthetics
14	Hexadecanoic Acid	256	$C_{16}H_{32}O_2$	+	++	++	Antitumor
15	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O$	+	+	+++	Antioxidant, Insecticide, hemolytic, Hypo-cholesterolemic
16	Pentadecanoic Acid	242	$C_{15}H_{30}O_2$	++			Lubricants, Adhesive agents
17	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	+	++	+	Antioxidant, Anticancer, Hypocholesterolemic
18	Tetracontane	562	$C_{40}H_{82}$	+	+	+	Anti Inflammatory
19	9,12-Octadecadienoic acid (z,z,)-	280	$C_{18}H_{32}O_2$		+		AntiInflammatory, Antibacterial, Antiarthritic, Hepatoprotectiv, Anti-histaminic, Anticoronary
20	9,12-Octadeca dienoic acid (z,z,)-, methyl ester	294	$C_{19}H_{34}O_2$		+		Hepatoprotective, Anti-histaminic, Antieczemic, Hypocholesterolemic
21	Octadecanoic acid	284	$C_{18}H_{36}0_2$	++		+	Antifungal, Antitumor, Antibacterial
22	Squalene	410	$C_{30}H_{50}$	++	+	+	Antioxidant, Anticancer Pesticide, Sunscreen, Perfumery, Chemo preventive
23	Vitamin E	430	$C_{29}H_{50}O_2$			+	Antiaging, Analgesic, antidiabetic**,** Antidermatitic, Antileukemia, Anticancer, Vasodilator, Hepatoprotective, Hypocholesterolemic, Antibronchitic, Anticoronary
24	Ergost-5-en-3-ol, (3.beta.,24r)-	400	$C_{28}H_{48}O$	++	+	++	Liver disease, Jaundice, Arthrosclerosis
25	Stigmasta-5,22-dien-3-ol	412	$C_{29}H_{48}O$	++	++	++	Synthetic Progesterone
26	Stigmast-5-en-3-ol, (3.beta.)-	414	$C_{29}H_{50}O$	++	+	++	Anti Inflammatory, Antipyretic, Anti ulcer, Antiarthritic
27	1-Undecanol	172	$C_{11}H_{24}O$			+	Bactericidal activity
28	Tetradecane	198	$C_{14}H_{30}$		++	++	Antifungal, Antibacterial, Nematicidal
29	8-Pentadecanone	226	$C_{15}H_{30}O$	+	+	+	Hepatotoxic, Demyelination, Conjunctivitis
30	n-Nonadecanol-1	284	$C_{19}H_{40}O$		+	+	Antimicrobial, Cytotoxic
31	Octadec-9-enoic acid	282	$C_{18}H_{34}O_2$			+	Antihypertensive, Increases HDL & decrease LDL
32	Pentadecane	212	$C_{15}H_{32}$	+		++	Suger-phosphatase inhibitor, Chymosin inhibitor, Antibacterial
33	Hexadecane	226	$C_{16}H_{34}$		+		Antifungal, Antibacterial, Antioxidant
34	Eicosane	282	$C_{20}H_{42}$	+	+	+	Antifungal, Antitumor, Antibacterial, Larvicidal, Cytotoxic, Antimicrobial
35	8-Octadecanone	268	$C_{18}H_{36}O$	+	+	+	Antimicrobial
36	Octadecane	254	$C_{18}H_{38}$			+	Lubricants, Anticorrosion agents
37	2,3-Dihydrobenzo-furane	120	C_8H_8O	+		+	In treatment of Diabetic Retinopathy and Arthritis
38	Phenol	94	C_6H_6O	+			Antiseptic, Disinfectant and Cosmetics
39	Heneicosane	296	$C_{21}H_{44}$		+		Ovi-position Attractant Pheromone
40	Nonane	128	C_9H_{20}	+	++	++	Antibacterial, antifungal
41	Undecane	156	$C_{11}H_{24}$	+	++	++	Mild sex attractant for Moths and Cockroaches
42	Dodecane	170	$C_{12}H_{26}$		++	++	Food additives, Antifungal
43	1-Nonadecene	266	$C_{19}H_{38}$	++	++	++	Antifungal, anticancer
44	Behenic alcohol	326	$C_{22}H_{46}O$			+	Antiviral activity against HSV
45	1H-Indene, 1-hexadecyl-2,3-dihydro-	342	$C_{25}H_{42}$	+	++	+++	Anticancer
46	Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl)ethyl ester	330	$C_{19}H_{38}O_4$	++	+++	+++	Antioxidant
47	Octadecanoic acid, 2,3-dihydroxypropyl ester	358	$C_{21}H_{42}O_4$	++	+++	+++	Anticancer, antimicrobial
48	14-.Beta.-H-Pregna	288	$C_{21}H_{36}$	+			Prevention and treatment of Diabetic retinopathy
49	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	+	+		Antioxidant, Nematicide, Insecticide, Lubricant, Antiandrogenic, Hemolytic, Hypo –cholesterolemic
50	p-Tert butylcalix[4] arene	648	$C_{44}H_{56}O_4$		+		Membrane carriers for the transport

							of chiral amino acids, used as extractants, transporters, and optical sensors
51	Methyl stearate	298	$C_{19}H_{38}O_2$	+	+	+	Antidiarrheal, cytotoxic and Antiproliferative
52	Guanosine	283	$C_{10}H_{13}N_5O_5$	+	+	+	Cytotoxic against T cells lines, Anti viral against Vero cells infected with HSV-1
53	Tetracosane	338	$C_{24}H_{50}$		+		Cytotoxicity against colon, breast and gastric cancer cells
54	A'-Neogammacer-22(29)-en-3-one	424	$C_{30}H_{48}O$	+			Antioxidant, Hypocholesterolemic
55	Humulane-1, 6-dien-3-ol	222	$C_{15}H_{26}O$	+			Hypocholesterolemic
56	Bis(tridecyl)phthalate	530	$C_{34}H_{58}O_4$	+	+	+	Plasticizer, used to soften PVC
57	Behenyl behenate	649	$C_{44}H_{88}O_2$		+		Emollient and skin conditioning

Presence in anyone solvent= +; presence in any two solvent= ++; presence in all solvent= +++

4. Discussion
Phytosterols have been clinically proved to reduce blood cholesterol and scientific reports suggest that they possess antioxidant activity [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 [11]. Ergost-5-en-3-ol, (3. beta., 24r)- (Campesterol) and Stigmasta-5, 22-dien-3-ol (Stigmasterol) shows anticancerous activity [12]. Stigmast-5-en-3β-ol (β-Sitosterol), a phytosterol shows antiinflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and oestrogenic effects. Beta-sitosterol is mainly known and used for its cholesterol lowering property [13]. A'-Neogammacer-22(29)-en-3-one, a phytosterol, commonly known as moretenone possess antioxidant and hypocholesterolemic activity. Squalene is a triterpene that has antibacterial, antioxidant, pesticide, antitumor, cancer preventive, immunostimulant, chemopreventive and lipoxygenase inhibitor activity [14]. Vitamin E, the most potent lipid-soluble antioxidant is a group of eight compounds, but only two of them forms, α-tocopherol and γ- tocopherol, are found in the human body, is thought to play an important role in skin protection [15]. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) 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-Dodecaene-25,26,27,28-Tetrol, commonly known as p-tert butylcalix[4] arene with a supramolecular chemistry used as extractants, transporters and optical sensors in medical science [16]. Octadecanoic acid, 2, 3-dihydroxypropyl ester belongs to the family of monoacylglycerols, is used as a food additive shows antimicrobial and anticancer activities [17]. Humulane-1, 6-dien-3-ol, a plant stanol is a bioactive component with similar functions as that of cholesterol in mammals. Plant stanols are 5α-saturated derivatives of plant sterols [18]. Plant sterols/ stanols occurring naturally in the diet, reduces cardiovascular diseases and have a modest hypocholesterolaemic effect. 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.

Conclusion
It can be concluded that *Cenchrus biflorus* 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 "Bhurat" in three polar solvents 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.

Acknowledgement
We are 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.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare that we have no conflict of interest.

References
1. World Health Organization. Drug information. WHO, Geneva. 1999; 13(4):230-233.
2. Bijauliya RK, Alok S, Singh M, Mishra SB. Morphology, Phytochemistry and Pharmacology of *Syzygium cumini* (Linn.) - an overview. Int J Pharm Sci Res. 2017; 8(6):2360-2371.
3. Mulula A, Ntumba K, Mifundu MM, Taba KM. Phytochemical screening, antibacterial and antioxidant activities of aqueous and organics stem extracts of *Strophanthus hispidus* DC. Int J Pharm Sci Res. 2017; 8(1):95-100.
4. Karimi E, Jaafar HZE. HPLC and GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of *Labisia pumila* Benth. Molecules. 2011; 16(8):6791-6805.
5. Vuorelaa P, Leionen M, Saikku P, Tammela P, Rauha JP, Wennberg T *et al*. Natural products in the process of finding new drug candidates. Curr Med Chem. 2004; 11(11):1375-1389.
6. Bai S, Seasotiya L, Malik A, Bharti P, Dalal S. GC-MS analysis of chloroform extract of *Acacia nilotica* L. leaves. J Pharmacog Phytochem. 2014; 2(6):79-82.
7. Harborne JB. Methods of plant analysis. In: Harborne JB, editor. Phytochemical Methods. London: Chapman and Hall; 1984, 5-6.
8. Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 6th ed. Boston, USA: Cengage Learning, 2007.
9. Oregon State University. GC-MS: How does it Work? Environmental Health Sciences Center Corvallis, 2012 http://www.unsolvedmysteries.oregonstate.edu/MS_05.
10. Zawistowski J. Tangible health benefits of phytosterol functional foods. In: J. Smith, E. Charter editors.

Functional Food Product Development. Oxford, UK: Wiley Blackwell, 2010, 362-387

11. Awad AB, Fink CS, Williams H, Kim U. *In vitro* and *in vivo* (SCID mice) effects of phytosterols on the growth and dissemination of human prostate cancer PC-3 cells. Euro J Cancer Prev, 2001; 10(6):507-13.
12. Bradford PG, Awad AB. Phytosterols as anticancer compounds. Mole Nutr Food Res. 2007; 51(2):161-170.
13. Patra A, Jha S, Murthy PN, Manik, Sharone A. Isolation and characterization of stigmast- 5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders. Int J Pharm Sci Res. 2010; 1(2):95-100.
14. Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia italica* leaf methanol extract. Asian J Pharm Clin Res. 2012; 5(2):90-94.
15. Mitchel RE, McCann RA. Skin tumor promotion by vitamin E in mice: amplification by ionizing radiation and vitamin C. Cancer Detect Prev. 2003; 27:102-108.
16. Li D, Saldeen T, Romeo F, Mehta JL. Different isoforms of tocopherols enhance nitric oxide synthase phosphorylation and inhibit human platelet aggregation and lipid peroxidation: implications in therapy with vitamin E. J Cardiovasc Pharmacol Ther. 2001; 6(2):155-161.
17. Chinnu K, Muthukumaran M, Srinivasan M, Subasubramanian V. GC-MS analysis of some bioactive constituents from isolated βeta-glucan from *Chroococcus turgidus*. Int J Inst Pharm Life Sci. 2014; 4(6):69-74.
18. Gylling H, Plat J, Turley S, Ginsberg HN, Ellegard L, Jessup W *et al*. Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease. Atherosclerosis. 2014; 232(2):346-360.