Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Research Article

Estimation of Bio-active Compounds in Stem of *Anogeissus rotundifolia* (Indrdok) by Gas Chromatography-Mass Spectrometry.

Premlata Singariya[*1], Krishan Kumar Mourya[2] , Arun Meena[3], Padma Kumar[1]

[1]UGC Post-doctoral fellow, Laboratory of Tissue Culture and Secondary Metabolites, Department of Botany, University of Rajasthan, Jaipur- 302004.

[2]Assistant Director, Rural Veterinary Polyclinic, Hingonia, Jaipur (Rajasthan).

[3]Jr. Application Scientist (GCMS), University Science Instrumentation Centre, University of Rajasthan, Jaipur,302004

Received 26 May 2018; received in revised form 23 June 2018; accepted 23 June 2018

***Corresponding author E-mail address:** premlatasingariya@gmail.com*

ABSTRACT

The investigation was carried out to determine the possible bioactive components of methanolic extracts of *Anogeissus rotundifolia* (stem) using Gas chromatography-Mass spectrometry (GC-MS). All the samples were dried firstly at 60°C for 2 days in an oven after that leave it on room temperature. They were then macerated to powder form with a mixer grinder. The powder was stored in air sealed polythene bags at room temperature before extraction. The chemical compositions of the methanolic extracts of *Anogeissus rotundifolia* (stem) were investigated using Thermo G C 1300 and "TSQ 8000 "Triple quadruple GC-MSMS SYSTEM with auto sampler Al 1310 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) library. GC-MS analysis of the extract reveals the identification of forty nine compounds. This is the first report of identification of components from the stem of *Anogeissus rotundifolia* by GC-MS. Most of the compounds in the list are bioactive and possess medicinal properties.

KEYWORDS

Anogeissus rotundifolia, stem, Gas chromatography-Mass spectrometry, bioactive components.

1. INTRODUCTION

Evaluation of the medicinal importance of this selected plant (*Anogeissus rotundifolia*); the methanolic extract of stem was analyzed for the first time using Gas chromatography-Mass spectrometry (GC-MS). This specific work will help to identify the compounds of therapeutic value. GC-MS is one of the best scientific tool to identify the bioactive constituents of alcohols, acids, ester, steroids, long chain, branched chain hydrocarbons, phenolic compounds etc. (Amakrishnan, 2011; Singariya et al., 2015b; Singariya et al., 2016c; Singariya et al., 2017b).This plant is used in traditional treatments to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal/ folk medicine. Natural products or secondary metabolites have been a source of new generation of antibiotic drugs for centuries.

A. rotundifolia a medium sized tree, about 6 m long. Young parts (branches, leaves and inflorescence) cinereo-tormentose. Leaves alternate, many younger ones elliptic or suborbiculate, the mature ones orbiculate or sub orbiculate, slightly broader than long, upto 2cm in diameter, apex obtuse or emarginated, generally mucronate, silvery pubescent, petioles upto 3mm long.

2. MATERIALS AND METHODS

2.1. Plant material:

Anogeissus rotundifolia (stem) were collected in the month of August 2015 from the Central Arid Zone Research Institute (CAZRI), Jodhpur (Rajasthan). Plants samples were identified and deposited in the herbarium (herbarium no. RUBL211363), Department of Botany, University of Rajasthan, Jaipur. The collected plant materials were transferred to the laboratory, cleaned with water and selected plant parts were separately shade dried (Singariya et al., 2012l) until weight has been constant.

2.2. Preparation of plant extracts:

The collected plant materials were shade dried, powered with the help of grinder (Singariya et al., 2012m) and passed through 40mm meshes and stored in clean container for further use (Singariya et al., 2012o). The dried powder material was extracted with acetone by using the Soxhlet apparatus (Subramanian and Nagarajan, 1969) for 18 hours at a temperature not exceeding the boiling point of the respective solvent (Singariya et al., 2013b; Singariya et al., 2012k). The obtained extracts were filtered by using Whatman No. 1 filter paper and then concentrated at 40^0C by using an evaporator (Singariya et al., 2012p) and stored the residual extracts in refrigerator at 4^0C in small and sterile amber colour glass bottles (Singariya et al., 2012n) for subsequent use in the further antimicrobial, anti-fungal and phyto-chemical analysis. The extract contains both polar and non-polar phyto-components.

2.3. Gas chromatography-Mass spectrometry analysis:

Gas chromatography-Mass spectrometry (GC-MS) analysis of these extracts was carried out by following the method of Hema et al., 2010. The GC-MS analysis of the extracts was performed using a GC-MS Thermo G C 1300 and "TSQ 8000 "Triple quadrupole GCMSMS SYSTEM with auto sampler Al 1310. Gas Chromatography 1300 with a fused GC column TG-5MS AMINE. The column length was 30 m with internal diameter; coated film 0.25µm with flow rate 10 ml/m

in and the condition were as follows: PTV Temp. Program: 70 °C, hold 1.00 min, 10 °C/min to 280 °C, hold 15 min. Carrier gas helium flow rate 1ml/min, split ratio 1:50. GC is equipped with auto-sampler AI 1300 and sample volume was 1µ litre. The elutes were automatically passed into a mass spectrometer. GC mass Spectrum analysis was conducted using TSQ8000 with transfer line temperature 270°C and ion source temperature 230°C in EI mode. Mass scan time was 4 min with full Scan MS. The mass spectrum was also equipped with a computer fed NIST mass Spectra data library.

2.4. Identification of Components:

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of standard and Technology NIST-08 LIB. (Singariya et al., 2016a; Mc-Lafferly, 1989) and WILEY-8 LIB. (Singariya et al., 2016d; Stein, 1990) library sources were used for matching the identified components from the plant material having more than 62,000 patterns (Singariya et al., 2015c; Singariya et al., 2012s). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library (Singariya et al., 2015a; Singariya et al., 2014). The name, molecular weight and structure of the components of the test materials were ascertained (Singariya et al., 2012t).

3. RESULTS AND DISCUSSION

The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) in the methanolic extracts of the whole plant of *A. rotundifolia* are presented in tables 1 followed by (Singariya et al., 2012q; Singariya et al., 2012r). The GC-MS analysis of the extracts showed the presence of phyto-components, the phyto-components of the above said plant extract are presented in Table-1 and the GC-MS chromatogram with peak area of each extract is also given figure-1 (Singariya et al., 2013a). Totally 50 bio-active constituents were identified in the present study from the acetone extracts of the whole plant of *A. rotundifolia* which including both major and minor constituents.

Table-1: Total Bio-active compounds of *A. rotundifolia* (stem) by Gas Chromatography- Mass Spectrometry.

S. No.	RT	Compound Name	Area	Area %	RSI
1.	4.22	Bis(1-hydroxycyclohexyl) peroxide	74219	0.16	831
2.	4.75	2 Thiophenecarboxylic acid, 5 (1,1dimethylethoxy)	884863	1.89	752
3.	5.04	Trimethyl(3,3-difluoro-2-propenyl)silane	585735	1.25	908
4.	5.19	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	137550	0.29	839
5.	6.01	2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate	384871	0.82	922
6.	6.24	Tricyclohexanone triperoxide	141962	0.30	893
7.	6.72	1-[[3's-Hydroxy-2'R-butoxy]methyl]thymine, 1',3'-cyclic	760150	1.62	775

		phenyl phosphonate			
8.	7.68	2Propoxysuccinic acid, dimethyl ester	267139	0.57	758
9.	7.85	[1(Diethylamino) ethylidenimino] sulfurpentafluoride	842559	1.80	802
10.	8.05	2 Butanol, 2 nitroso, acetate (ester)	1320585	2.82	816
11.	8.57	3,4-Dihydro-5-methyl-4-[(7Z)-pentadecenyl]-2H-pyrrole	96327	0.21	758
12.	9.10	3-Octyne-2,5-dione, 6,6,7-trimethyl-	157275	0.34	761
13.	9.33	3-Dodecen-1-ol, (Z)-	47137	0.10	883
14.	9.53	Cyclopentadecanone, 4-methyl-	66197	0.14	856
15.	9.63	Phosphonic acid, methyl-, dicyclopentyl ester	347989	0.74	783
16.	9.88	Disulfide, propyl 1(propylthio) ethyl	1345276	2.87	937
17.	10.39	DL-4,5-Octanediol	2843620	6.07	896
18.	10.57	Sydnone, 3,3' tetramethylene di-	1604474	3.43	864
19.	11.06	Cyclopropane, 1-(1-methylethyl)-2-nonyl-	195563	0.42	664
20.	11.44	Ephedrine acetate	514835	1.10	798
21.	11.70	2-(1-Methylcyclohexyloxy)-tetrahydropyran	209253	0.45	751
22.	12.20	Oxalic acid, dicyclobutyl ester	58193	0.12	887
23.	12.43	N-Vinylpyridinium bromide	4240575	9.06	814
24.	12.92	Cyclohexanebutanoic acid, á-oxo-, methyl ester	552062	1.18	844
25.	13.05	Butanimidamide, N-(1-chloro-2-methyl-1-butenyl)-2-methyl-	674185	1.44	914
26.	13.14	Carbonic acid, allyl nonyl ester	342996	0.73	852
27.	13.49	2 Formyl 9 [á dribofuranosyl] hypoxanthine	3323520	7.10	875
28.	14.07	1,5cis,8cisUndecatriene3,7diolbis(trimethylsilyl)ether	120635	0.26	728
29.	14.39	Butyl pentyl carbonate	861789	1.84	865
30.	15.05	Methyl 4-methyl-4-nitroso-2-trimethylsiloxy-pentanoate	139414	0.30	845
31.	15.10	2-Propanone, 1,1,1,3,3-pentafluoro-	102404	0.22	773
32.	15.18	4-Methyl-5-nonanone	74899	0.16	722

33.	15.43	lGalalidooctose	133943	0.29	861
34.	16.03	2H-Pyran, 2-(tert-butylthio)tetrahydro-	411428	0.88	807
35.	16.48	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	11771430	25.15	875
36.	16.60	2,6,10,14-Tetramethylpentadecan-2-ol	5063131	10.82	925
37.	16.84	Sucrose	47559	0.10	915
38.	16.96	5Thiodglucopyranose	93334	0.20	859
39.	17.08	meso-2,5-Dimethyl-3,4-hexanediol	121512	0.26	874
40.	18.18	Phenylacetaldehyde N-methyl-N-formylhydrazone	150497	0.32	685
41.	18.67	Diazene, [(hydroxyimino)(4-nitrophenyl)methyl]phenyl-	197777	0.42	747
42.	19.80	áDGlucosyloxyazoxymethane	122365	0.26	836
43.	20.10	DglyceroDmannoHeptitol	69455	0.15	861
44.	20.18	à-D-Glucose	79559	0.17	898
45.	20.33	1Nitro1deoxydglycerolmannoheptitol	166498	0.36	873
46.	20.59	Isosorbide Dinitrate	4223709	9.02	909
47.	21.60	2-Pentanone, 1,3-dimethoxy-3-methyl-	300686	0.64	764
48.	25.74	9-Azabicyclo[6.1.0]nonane, 9,9'-azobis-[1à,8à,9[E(1'R*,8'S*)]]-	289750	0.62	842
49.	25.88	Cyclohexanemethyl propanoate	163019	0.35	868
50.	28.67	2Pentoxytetrahydropyran	90134	0.19	797

The major constituents were Acetic acid, trifluoro-, 3,7-dimethyloctyl ester (25.15%); 2,6,10,14-Tetramethyl pentadecan-2-ol (10.82%); N-Vinyl pyridinium bromide (9.06%); Isosorbide Dinitrate (9.02%); 2 Formyl 9 [á dribofuranosyl] hypoxanthine (7.10%); DL-4,5- Octanediol (6.07%); Sydnone, 3,3' tetramethylene di- (3.43%); Disulfide, propyl 1(propylthio) ethyl (2.87%); 2 Butanol, 2 nitroso, acetate (ester) (2.82%) and 2 Thiophenecarboxylic acid, 5 (1,1dimethylethoxy) (1.89%) (Table-2).

Table-2: Major Bio-active compounds of *A. rotundifolia* (stem) by Gas Chromatography- Mass Spectrometry.

S. No.	**RT**	**Compound Name**	**Area %**	**RSI**
1.	16.48	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	25.15	875

2.	16.60	2,6,10,14-Tetramethylpentadecan-2-ol	10.82	925
3.	12.43	N-Vinylpyridinium bromide	9.06	814
4.	20.59	Isosorbide Dinitrate	9.02	909
5.	13.49	2 Formyl 9 [á dribofuranosyl] hypoxanthine	7.10	875
6.	10.39	DL-4,5-Octanediol	6.07	896
7.	10.57	Sydnone, 3,3' tetramethylene di-	3.43	864
8.	9.88	Disulfide, propyl 1(propylthio) ethyl	2.87	937
9.	8.05	2 Butanol, 2 nitroso, acetate (ester)	2.82	816
10.	4.75	2 Thiophenecarboxylic acid, 5 (1,1dimethylethoxy)	1.89	752

Table-3: Minor Bio-active compounds of *A. rotundifolia* (stem) by Gas Chromatography- Mass Spectrometry.

S. No.	**RT**	**Compound Name**	**Area %**	**RSI**
1.	8.57	3,4-Dihydro-5-methyl-4-[(7Z)-pentadecenyl]-2H-pyrrole	0.21	758
2.	16.96	5Thiodglucopyranose	0.20	859
3.	28.67	2Pentoxytetrahydropyran	0.19	797
4.	20.18	à-D-Glucose	0.17	898
5.	4.22	Bis(1-hydroxycyclohexyl) peroxide	0.16	831
6.	15.18	4-Methyl-5-nonanone	0.16	722
7.	20.10	D glycero Dmanno Heptitol	0.15	861
8.	9.53	Cyclopentadecanone, 4-methyl-	0.14	856
9.	12.20	Oxalic acid, dicyclobutyl ester	0.12	887
10.	16.84	Sucrose	0.10	915

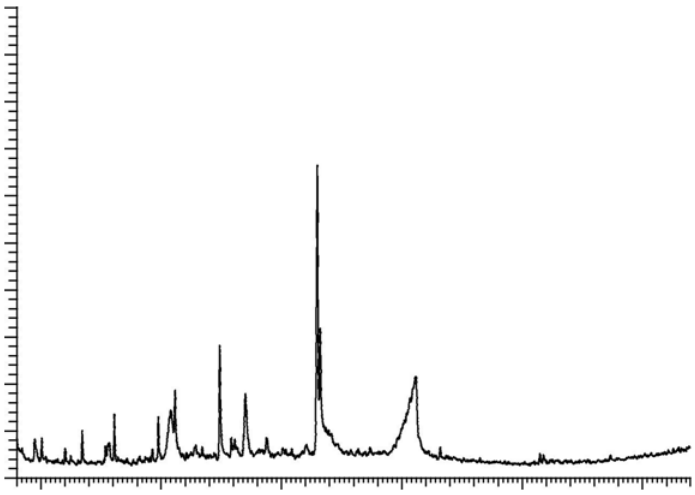
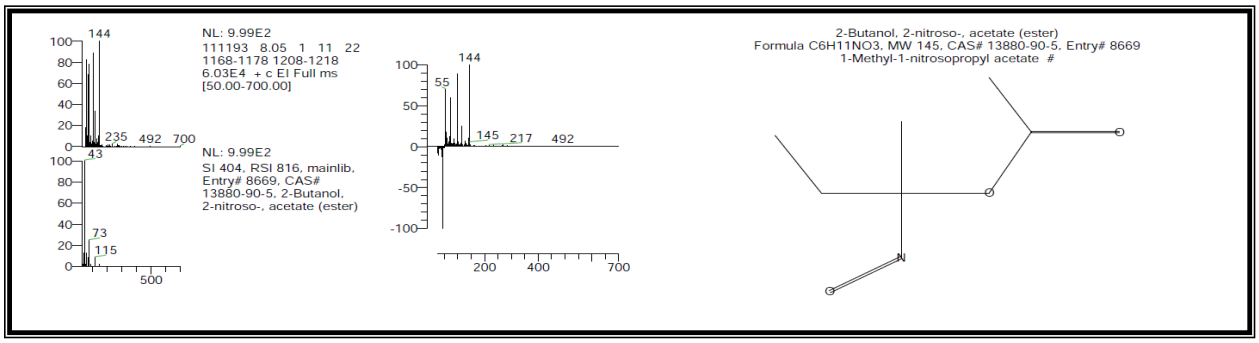
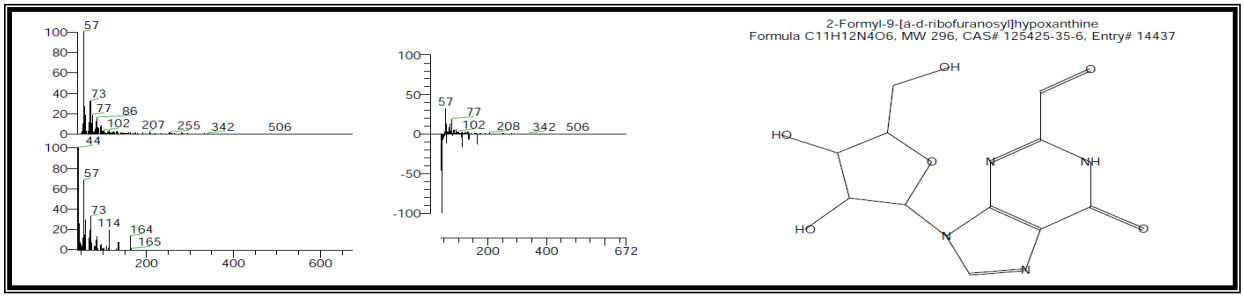


Fig. 1. Chromatogram of Methanolic extract of whole plant of *A. rotundifolia* by GC-MS

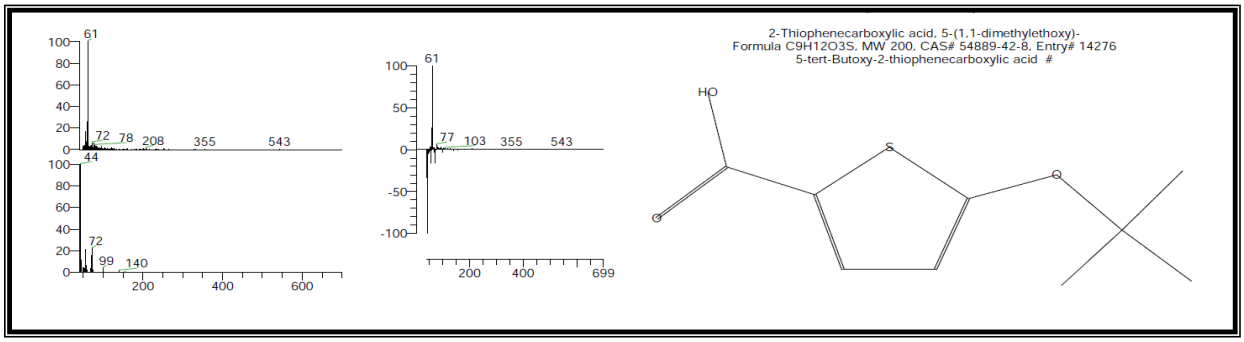
The GC-MS chromatogram with peak area has shown in fig-1. The aim of the present study is to provide more information about the essential phyto-constituents of *A. rotundifolia*. The results from the present investigation were very encouraging and indicates that this plant should be studied more extensively to explore its potential to use as plant medicinal nutritive.



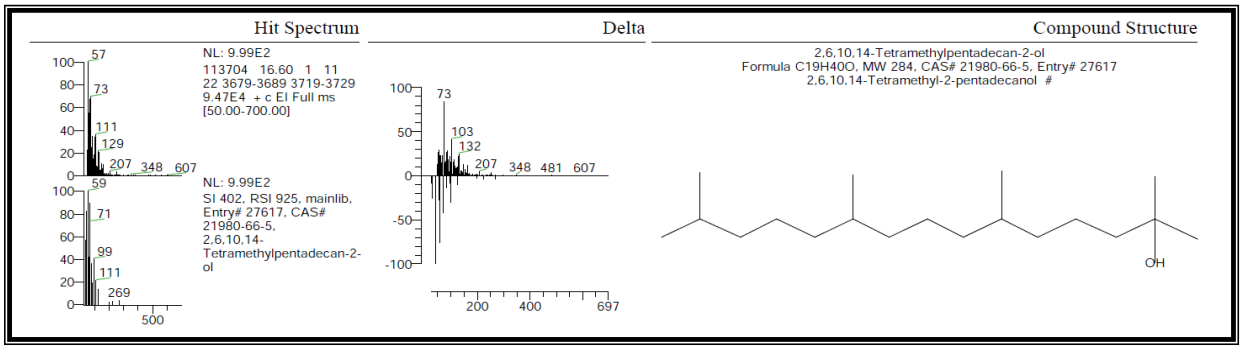
2 Butanol, 2 nitroso, acetate (ester)



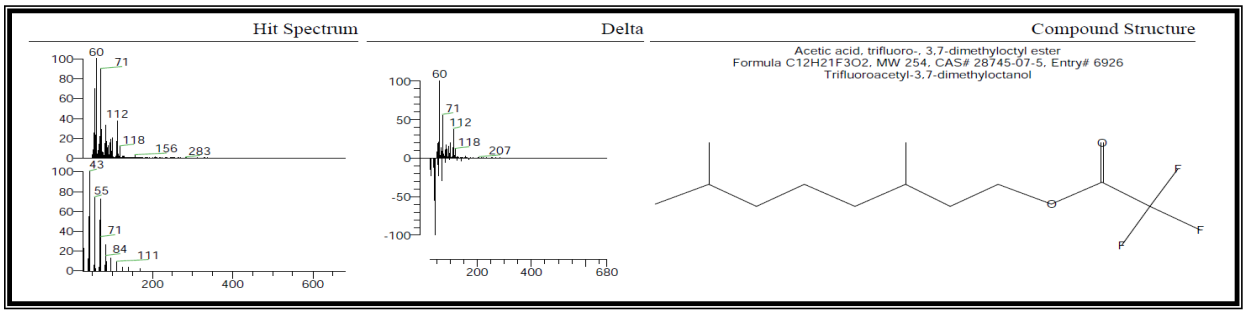
2 Formyl 9 [á dribofuranosyl] hypoxanthine



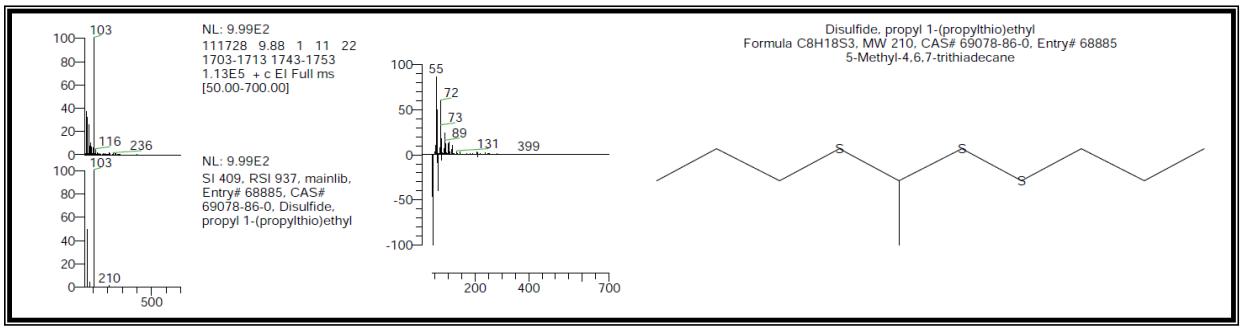
2 Thiophenecarboxylic acid, 5 (1,1dimethylethoxy)



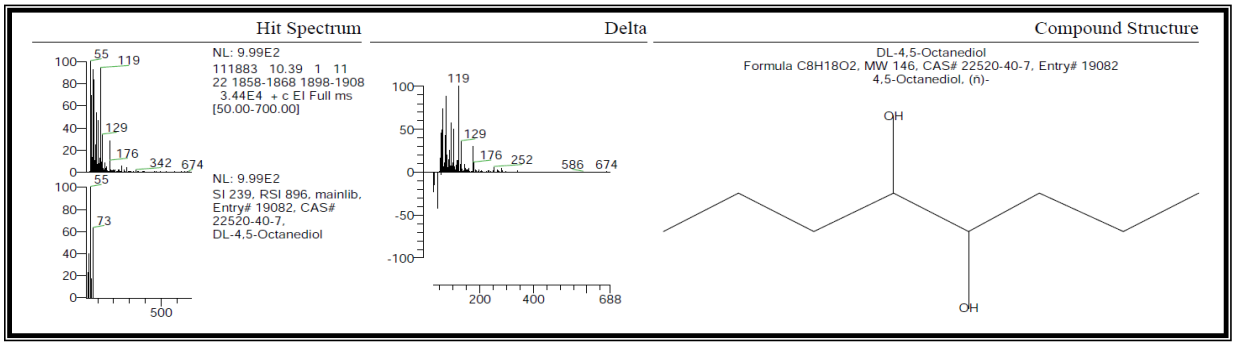
2,6,10,14-Tetramethylpentadecan-2-ol



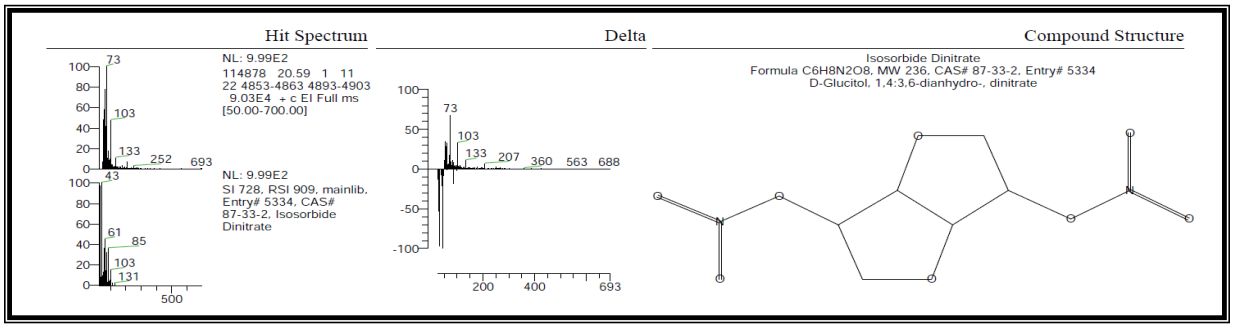
Acetic acid, trifluoro-, 3,7-dimethyloctyl ester



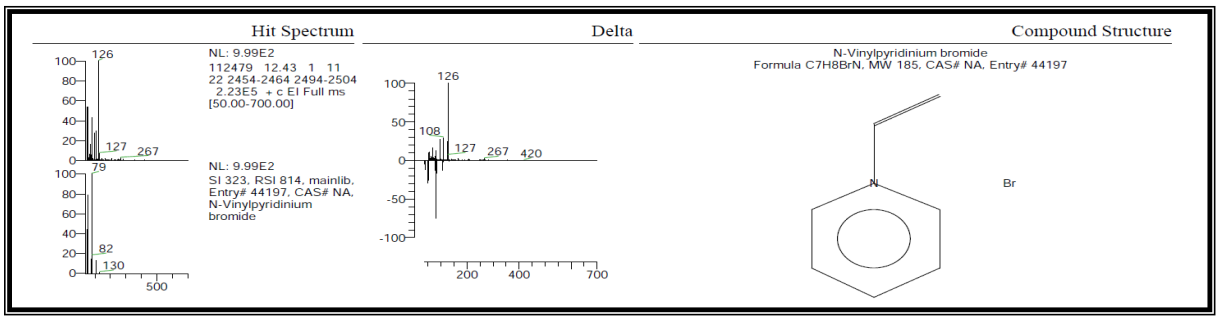
Disulfide, propyl 1(propylthio) ethyl



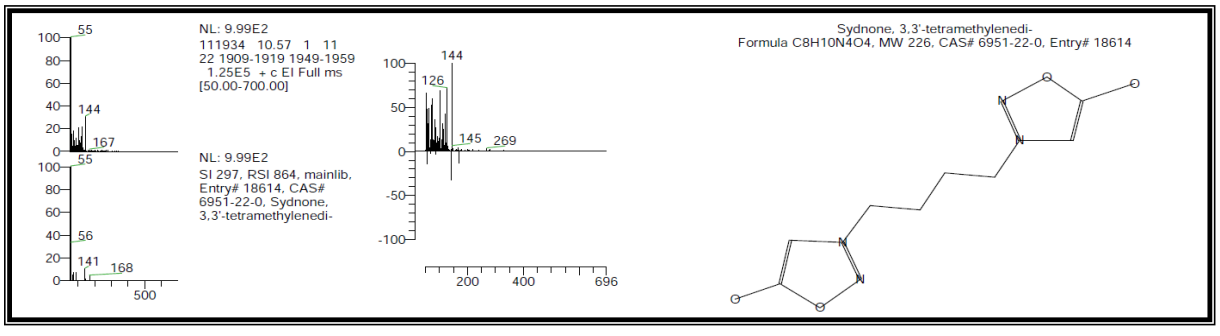
DL-4,5-Octanediol



Iso-sorbide Di-nitrate



N-Vinylpyridinium bromide



Sydnone, 3,3' tetramethylene di-

Fig. 2. The best hit for the prevailing compounds in the chromatogram.

Phytochemical constituents such as tannins, flavonoids, steroids and several other aromatic compounds of C_4 grasses that serve as defense mechanisms against predation by many microorganisms, insects and herbivores. (Lutterodt *et al*. 1999; Marjorie 1999). This may therefore explain the demonstration of antimicrobial activity by the plant extracts. The demonstration of the antimicrobial activity against both bacteria and fungi may be indicative of the presence of broad spectrum antibiotic compounds (Srinivasan *et al*. 2001). This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times. There are so many types of genes and proteins present in desertic plants so it can grow easily in stress condition (Goswami et al., 2016b; Singariya et al., 2009). At the cellular level, plant cell responds to these stresses by the activation of cascades of molecular mechanisms involved in stress perception, signal transduction and the expression of specific stress related genes and metabolites. A number of genes are induced by exposure to such condition, those that protect against environmental stresses directly (Shinozaki and Yamaguchi-Shinozaki, 1997).

4. CONCLUSION

Therapeutic mechanism of a plant can be better understood with a proper investigation of its active ingredients. In the present study, 50 components from the methanolic extracts of stem of *A. rotundifolia* were identified by GC-MS analysis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. These active principles provide inspiration for further investigation to achieve lead molecules in the discovery of novel herbal drugs. However, isolation of individual photochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *A. rotundifolia* contains various bioactive compounds. So it is recommended as a plant of phyto-pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

5. ACKNOWLEDGEMENT

Authors are expressing their thanks to UGC for providing the funds for the project under Post-doctoral fellowship scheme and also thankful to Department of USIC, University of Rajasthan, Jaipur for providing Gas chromatography-Mass spectrometry (GC-MS) facility.

7. REFERANCES

1. Amakrishnan, N. (2011). GC-MS Analysis of *Rumex vesicarius* L.International Journal of Drug Development & Research, 3(2), 272-279.
2. Goswami, B., Singariya, P., Mourya, K. K., & Gadi, B. R. (2016b). Abiotic Stress Induced Proteins in Plants. Plant Stress Physiology, 173-193. Editor: P. C. Trivedi. Pointer Publishers, Jaipur. ISBN 13: 9788171328321.
3. Hema, R., Kumaravel, S., Gomathi, S., & Sivasubramaniam, C. (2010). Gas Chromatography-Mass Spectroscopic analysis of *Lawsonia inermis* leaves. New York Science Journal, 3, 141-143.
4. Mc-Lafferly, F. W. (1989). Registry of mass spectral data, ed. 5, Wiley New York.

5. Shinozaki, K., & Yamaguchi-Shinozaki, K. (1997). Gene expression and signal transduction in water Stress response. Plant Physiology, 115(2), 327-334.

6. Singariya, P. (2009). Effect of Sub-Optimal Environment and PGR's on Metabolic Pattern of Certain Species of *Cenchrus*. Ph. D. Thesis, Jai Narian Vyas University, Jodhpur (Rajasthan).

7. Singariya, P., Kumar, P., & Mourya, K. K. (2012k). Evaluation of Indian Ginseng Against Different Bacteria and Fungi. Asian Journal of Pharmaceutical and clinical Research, 5(2), 145-148.

8. Singariya, P., Kumar, P., & Mourya, K. K. (2012L). Estimation of Bio-activity of Arial parts of *Withania somnifera* Against the Bacterial and Fungal Microbes. International Journal of Pharmacy and Pharmaceutical science, 4(3), 553-557.

9. Singariya, P., Kumar, P., & Mourya, K. K. (2012m). Qualitative and Pharmacological examination of Root extracts of *Withania somnifera* against Human and Plant Pathogens. Asian Journal of Research in Chemistry, 5(6), 733-737.

10. Singariya, P., Kumar, P., & Mourya, K. K. (2012n). Screening for Antimicrobial Potency of Methanolic Extract of Indian Ginseng. International Journal of Pharmacy and Technology, 4(2), 4537-4548.

11. Singariya, P., Kumar, P., & Mourya, K. K. (2012o). Insignificant Antimicrobial Activities and Phyto-chemical screening in different extracts of Indian Ginseng. Journal of Pharmaceutical Negative Results, 3(1), 41-45.

12. Singariya, P., Kumar, P., & Mourya, K. K. (2012q). Identification of Steroid Compound using Preparative Thin Layer Chromatography, GC-MS & Anti-microbial and Antioxidant Properties of *Cenchrus setigerus* (Poaceae). International Journal of Pharmacy and Life Sciences, 3(8), 1909-1916.

13. Singariya, P., Kumar, P., & Mourya, K. K. (2012r). Identification of New Bioactive Compounds by GC-MS and Estimation of Physiological and Biological Activity of Kala Dhaman (*Cenchrus setigerus*). International Journal of Pharmaceutical and Biological Archive, 3(3), 610-616.

14. Singariya, P., Kumar, P., & Mourya, K. K. (2012s). Comparative Assessment of Bio-efficacy of Leaf Extract of Anjan grass in Polar Solvents and Compound Identification in Ethyl Acetate Extract. Hygeia journal for drugs and medicines, 4(2), 49-56.

15. Singariya, P., Kumar, P., & Mourya, K. K. (2012t). Isolation of Some New Steroids and Evaluation of Bio-activity of*Cenchrus ciliaris*. International Journal of Research in Pharmaceutical Science, 3(4), 678-684.

16. Singariya, P., Kumar, P., & Mourya, K. K. (2013a). Antimicrobial activity and identification of 4,22-stigmastadiene -3-one and some other compounds in Motha Dhaman grass from Tribal area of Western Rajasthan.Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 83(3), 415–421. DOI 10.1007/s40011-012-0135-9.

17. Singariya, P., Kumar, P., & Mourya, K. K. (2013b). Absence of Antibiotic Activities of *Cenchrus setigerus* and *Cenchrus ciliaris* Seed extracts in Different Polar Solvents. Journal of Pharmaceutical Negative Results, 4(1), 71-75.

18. Singariya, P., Kumar, P., & Mourya, K. K. (2014). Isolation of New Steroids and Evaluation of Bio-activity of Kala Dhaman Grass (*Cenchrus setigerus*). Brazilian Archives

of Biology and Technology, 57(1), 62-69.

19. Singariya, P., Kumar, P., Mourya, K. K. (2012p). Evaluation of Antimicrobial Activity of Leaf extracts of Winter Cheery (*Withania somnifera*).International Journal of Pharm Tech Research, 4(3), 1247-1253.

20. Singariya, P., Mourya, K. K., & Gadi, B. R. (2016d). Evaluation of Microcidal and Nitrogen assimilatory enzymes activity and identification of β – Sitosterol in C4 Grasses of Thar Desert. Environmental Impact on Biodiversity, 113-131. Editor: B. R. Bamniya and B. R. Gadi. Today & Tomorrow's Printers and Publishers, New Delhi - 110 002. ISBN-p- 81-7019-547-7

21. Singariya, P., Mourya, K. K., & Kumar, P. (2015a). Gas Chromatography-Mass Spectrometric Analyses of Acetone extract of Marwar Dhamangrass for bio-active compounds. Plant Archives, 15(2), 1065-1074.

22. Singariya, P., Mourya, K. K., & Kumar, P. (2015b). Gas Chromatography-Mass Spectrometric analysis of acetone extract of *Cenchrusciliaris* (Dhaman grass). International journal of Science and Nature, 6(4), 652-661.

23. Singariya, P., Mourya, K. K., & Kumar, P. (2015c). Identification of some bio-active compounds of ethyl acetate extract of *Cenchrusciliaris* by gas chromatography-mass spectrometric analysis. Life Science Bulletin, 12(2), 141-148.

24. Singariya, P., Mourya, K. K., & Kumar, P. (2016a). Identification of some bio-active compounds of iso-propyl alcohol extract of motha dhaman grass by gas chromatography-mass spectrometric analysis. Life Sciences Leaflets, 72(2), 122-135.

25. Singariya, P., Mourya, K. K., & Kumar, P. (2016c). Identification of Bioactive components of Isopropyl alcohol extract of Bird wood grass using gas chromatography- mass spectrometry. Biotechnology in crop improvement, Chapter-10, 118-130, Editor: P. C. Trivedi. Pointer Publishers, Jaipur. ISBN 978-81-7132-8550.

26. Singariya, P., Mourya, K. K., & Kumar, P. (2017b). Evaluations of Organic Compounds in Ethyl Acetate extracts of Marwar Dhaman By gas chromatography- mass spectrometry. Journal of Plant Science, 33(1), 17-27.

27. Stein, S. E. (1990). National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02, USA, 1990.