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ESTIMATION OF BIO-ACTIVE COMPOUNDS IN INDIAN PERENNIAL GRASS *PANICUM ANTIDOTALE* BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

The investigation was carried out to determine the possible bioactive components of methonolic extracts of *Panicum antidotale* (whole plant) 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 methonolic extracts of *P. antidotale* were investigated using Thermo G C 1300 and "TSQ 8000 "Triple quadrupole GC-MS MS 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 whole plant of *P. antidotale* by GC-MS. Most of the compounds in the list are bioactive and possess medicinal properties..

Keywords: *Panicum antidotale*, Gas chromatography-Mass spectrometry, Bioactive components.

INTRODUCTION

Taking into consideration of the medicinal importance of this plant, the methonolic extracts of *Panicum antidotale* (whole plant) were analyzed for the first time using Gas chromatography-Mass spectrometry (GC-MS). This work will help to identify the compounds of therapeutic value. GC-MS is one of the best techniques to identify the bioactive constituents of alcohols, acids, ester, long chain, branched chain hydrocarbons, steroids, phenolic compounds etc [1-4].

The genus *Panicum* comprises over 500 species distributed mostly in tropical and subtropical regions of the world. *P. antidotale* (blue panic or Giant panic) is a native of Southeast Asia. *P. antidotale* is a perennial grass, growing to 3 m (9ft 10in) in height. It is a robust and shortly rhizomatous with very deep root system. changes and alterations in genetic and biochemical attributes. Alteration in various mechanisms by plants to produce resistance against drought, tolerance at the cellular level is essential, because the cellular processes are most sensitive due to change in cell turgor under drought. Reduced cell turgor may lead to impaired growth in most plant species. The smoke of the burning plant is used to fumigate wounds and as a disinfectant in the treatment of smallpox.

In the last 40-50 years there has been an exponential growth in the field of herbal medicine. Medicinal plants are used in traditional treatments to cure variety of diseases. Natural products (secondary metabolites) have been a source of drugs for centuries. In the present study methonolic extracts of *P. antidotale* were analyzed by GC-MS technique to study the major and minor phyto-constituents of the vegetative parts of the whole plant.

Panicum antidotale 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. RUBL211364), Department of Botany, University of Rajasthan, Jaipur. The collected plant materials were transferred immediately to the laboratory cleaned with water and selected plant parts were separately shade dried [5] until weight has been constant.

Preparation of plant extracts

The collected plant materials were shade dried, powered with the help of grinder [6] and passed through 40mm meshes and stored in clean container for further use

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[7]. The dried powder material was extracted with acetone by using the Soxhlet apparatus [8] for 18 hours at a temperature not exceeding the boiling point of the respective solvent [9-10]. The obtained extracts were filtered by using Whatman No. 1 filter paper and then concentrated at 40^0C by using an evaporator [11] and stored the residual extracts in refrigerator at 4^0C in small and sterile amber colour glass bottles for subsequent use in the further antimicrobial, anti-fungal and phyto-chemical analysis [12]. The extract contains both polar and non-polar phyto-components.

Gas chromatography-Mass spectrometry analysis

Gas chromatography-Mass spectrometry (GC-MS) analysis of these extracts was carried out by following the method [13]. 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.

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. [14-15] and WILEY-8 LIB. [16-17] library sources were used for matching the identified components from the plant material having more than 62,000 patterns [18-19]. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library [20-21]. The name, molecular weight and structure of the components of the test materials were ascertained [22].

RESULTS

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 ***P. antidotale*** [23] are presented in tables. 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. Totally 50 bio-active constituents were identified in the present study from the acetone extracts of the whole plant of ***P. antidotale*** which including both major and minor constituents.

The major constituents were N,N-Dimethyl-2-isopropoxyethylamine (52.37%); Sucrose (17.52%); [1-(Diethylamino) ethylidenimino] sulfur pentafluoride (6.93%); 2,6,10,14- Tetramethyl pentadecan-2-ol (3.43%); 1,3-Oxazolidine, 4-methyl-cis-5-phenyl-2- (4-cyanophenyl)- (2.97%); à-D-Glucopyranoside, methyl 3,6-anhydro- (2.13%); 2-Methyl-2- chloro-3-nitroso-4-cyclohexyloxy-butane (0.93%); Disulfide, methyl 2-methyl-1-(methylthio) propyl (0.91%); 4-[(Bicyclo[2.2.1] hepta-2,5-dien-7-yl) oxy] benzonitrile (0.84%) and Propylamine, N,N,2,2-tetramethyl-, N-oxide (0.82%) (table-2). The minor constituents were 3-(Dimethylamino-carbonyl)-1,2-diphenyl-cyclopropene(0.15%); 2Thiophene carboxylicacid,5 (1,1dimethylethoxy) (0.14%); 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methyl but-2- enyl) –cyclohexane (0.14%); 1H-Imidazole -1-ethanol, 2-methyl-à-phenyl- (0.14%); 3Methyl heptyl acetate (0.13%); Carbonochloridimidothioic acid, [(trifluoromethyl)thio]-, anhydrosulfide with thiohypochlorous acid (0.11%); 2-(Phenoxymethyl) benzoic acid (0.11%) and Ethanethioic acid, S-(2-methylbutyl) ester (0.10%) (Table-3).

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 *P. antidotale*. 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. The best hit for the prevailing compounds in the chromatogram (Fig. 2).

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Table 1. Total Bio-active compounds of *P. antidotale* by Gas Chromatography-Mass Spectrometry

S.No.	RT	Compound Name	Area	Area %	RSI
1.	4.49	(Z)-9-Hydroxy-2,4-dimethyl-non-7-enoic acid lactone	77009	0.26	802
2.	4.67	Butanimidamide, N-(1-chloro-2-methyl-1-butenyl)-2-methyl-	93487	0.32	915
3.	4.74	Propylamine, N,N,2,2-tetramethyl-, N-oxide	242117	0.82	822
4.	5.05	Heptacosanoic acid, 25-methyl-, methyl ester	55402	0.19	768
5.	5.20	Heptane, 1(2propenyloxy)	98515	0.34	734
6.	7.54	DL-4,5-Octanediol	79716	0.27	838
7.	7.74	[1-(Diethylamino)ethylidenimino]sulfur pentafluoride	2034087	6.93	828
8.	8.05	2Thiophenecarboxylicacid,5(1,1dimethylethoxy)	42322	0.14	781
9.	9.12	Tricyclohexanone triperoxide	98840	0.34	843

10.	9.44	4-[(Bicyclo[2.2.1]hepta-2,5-dien-7-yl)oxy]benzonitrile	246440	0.84	833
11.	9.53	Carbonochloridimidothioic acid, [(trifluoromethyl)thio]-, anhydrosulfide with thiohypochlorous acid	32256	0.11	767
12.	9.88	Disulfide, methyl 2-methyl-1-(methylthio)propyl	267330	0.91	862
13.	10.32	[1,4]Dioxino[2,3-b]-1,4-dioxin, hexahydro-2,3,6,7-tetramethyl-	115317	0.39	855
14.	10.38	Arsine, trihexyl	50156	0.17	755
15.	10.42	meso-2,5-Dimethyl-3,4-hexanediol	47567	0.16	809
16.	10.55	2-Methyl-2-chloro-3-nitroso-4-cyclohexyloxy-butane	272987	0.93	868
17.	10.74	4-Propionyloxypiperidine	117260	0.40	807
18.	11.12	4-Butyl-5-(1-methylethenyl)-6-(3-methylbutyl)-2H-pyran-2-one	25875	0.09	710
19.	11.38	3-Selenetanol, 3-(4-methoxyphenyl)-	129756	0.44	767
20.	11.69	Isosorbide Dinitrate	73994	0.25	747
21.	11.89	3Methylheptylacetate	39371	0.13	836
22.	12.99	Cyclobutanecarboxylic acid, hexyl ester	208886	0.71	717
23.	13.48	Sucrose	5142906	17.52	880
24.	14.19	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methyl but-2-enyl)-cyclohexane	42228	0.14	726
25.	14.36	1,6-Methanonaphthalene-1,9(2H)-diol, octahydro-,(1à,4aà,6á,8aà,9S*)-	110856	0.38	705
26.	15.01	Pyrazinamine, 3,4,5,6-tetrahydro-6-imino-N-phenyl-4-(phenylmethyl)-	50529	0.17	790
27.	15.23	2-Dodecen-1-ol, 12-chloro-	83201	0.28	860
28.	15.97	à-D-Glucopyranoside, methyl 3,6-anhydro-	624922	2.13	812
29.	16.45	Cyclopropane, 1-(1-methylethyl)-2-nonyl-	76587	0.26	874
30.	16.56	2,6,10,14-Tetramethylpentadecan-2-ol	1007926	3.43	910
31.	16.73	5-Thio-d-glucopyranose	175602	0.60	855
32.	16.88	Ethanethioic acid, S-(2-methylbutyl) ester	28648	0.10	858
33.	18.69	4,8-Etheno-1H-cyclohepta[c]furan-1,3(4H)-dione, 3a,5,6,7,8,8a-hexahydro-, (3aR,4-trans,8-trans,8a-cis)-	226465	0.77	801
34.	19.31	Benzene, p-di-tert-butoxy-	121990	0.42	595
35.	19.53	Sydnone, 3-(phenylmethyl)-	97846	0.33	808
36.	19.62	2-(Phenoxymethyl)benzoic acid	31751	0.11	605
37.	21.05	Phenylacetaldehyde N-methyl-N-formylhydrazone	28025	0.10	629
38.	22.52	Bicyclo[3.2.1]oct-3-en-2-one,3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol- 5- yl)-6-methyl-5-(2-propenyl)-, [1R-(6-endo,7-exo,8-syn)]-	63936	0.22	732
39.	23.33	(9S,10R)-9,10-Epoxy-3Z,6Z-heneicosadiene	83169	0.28	698
40.	24.31	N,N-Dimethyl-2-isopropoxyethylamine	15375893	52.37	919
41.	26.00	N,N-Dimethyl-3-methoxy-4-methylphenethylamine	194165	0.66	948
42.	28.01	5-Benzoyl-4-amino-3-(2-dimethylaminoethylthio)thieno[2,3-c]isothiazole	53618	0.18	730
43.	28.19	1,3-Oxazolidine, 4-methyl-cis-5-phenyl-2-(4-cyanophenyl)-	871433	2.97	772
44.	30.21	Chloromethyl 2chloroundecanoate	55626	0.19	874
45.	30.27	Manganese, tricarbonyl[(1,2,3,4,5-ü)-1-[(dimethylamino)sulfonyl]-2-(1- hydroxy-1-methylethyl)-2,4-cyclopentadien-1-yl]-	48559	0.17	807
46.	30.77	Hydrazine, phenylsulfinyl	79981	0.27	816
47.	30.88	1H-Imidazole-1-ethanol, 2-methyl-à-phenyl-	40810	0.14	806
48.	31.43	3-(Dimethylamino-carbonyl)-1,2-diphenyl-cyclopropene	44892	0.15	804
49.	31.48	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane	71849	0.24	883
50.	31.72	3Tosylsedoheptulose	76451	0.26	822

Table 2. Major Bio-active compounds of *P. antidotale* by Gas Chromatography- Mass Spectrometry.

S. No.	RT	Compound Name	Area %
Major constituents			
1.	24.31	N,N-Dimethyl-2-isopropoxyethylamine	52.37
2.	13.48	Sucrose	17.52
3.	7.74	[1-(Diethylamino)ethylidenimino]sulfur pentafluoride	6.93
4.	16.56	2,6,10,14-Tetramethylpentadecan-2-ol	3.43
5.	28.19	1,3-Oxazolidine, 4-methyl-cis-5-phenyl-2-(4-cyanophenyl)-	2.97
6.	15.97	à-D-Glucopyranoside, methyl 3,6-anhydro-	2.13
7.	10.55	2-Methyl-2-chloro-3-nitroso-4-cyclohexyloxy-butane	0.93
8.	9.88	Disulfide, methyl 2-methyl-1-(methylthio)propyl	0.91
9.	9.44	4-[(Bicyclo[2.2.1]hepta-2,5-dien-7-yl)oxy]benzonitrile	0.84
10.	4.74	Propylamine, N,N,2,2-tetramethyl-, N-oxide	0.82

Table 3. Minor Bio-active compounds of *P. antidotale* by Gas Chromatography- Mass Spectrometry.

S. No.	RT	Compound Name	Area %
Minor constituents			
1	31.43	3-(Dimethylamino-carbonyl)-1,2-diphenyl-cyclopropene	0.15
2	8.05	2Thiophenecarboxylicacid,5(1,1dimethylethoxy)	0.14
3	14.19	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methyl but-2-enyl)-cyclohexane	0.14
4	30.88	1H-Imidazole-1-ethanol, 2-methyl-à-phenyl-	0.14
5	11.89	3Methylheptylacetate	0.13
6	9.53	Carbonochloridimidothioic acid, [(trifluoromethyl)thio]-, anhydrosulfide with thiohypochlorous acid	0.11
7	19.62	2-(Phenoxymethyl)benzoic acid	0.11
8	16.88	Ethanethioic acid, S-(2-methylbutyl) ester	0.10
9	21.05	Phenylacetaldehyde N-methyl-N-formylhydrazone	0.10
10	11.12	4-Butyl-5-(1-methylethenyl)-6-(3-methylbutyl)-2H-pyran-2-one	0.09

Fig 1. Chromatogram of Methanolic extract of whole plant of *P. antidotale* by GC-MS

Data File:		4		Injection Volume(µl):	1.00	
Sample ID:		4		Acquisition Date:	09/11/15 04:14:54 PM	
Run Time(min):		32.67		Comments:		
Scans:		9605		Low Mass(m/z):	50	
High Mass(m/z):		700		Instrument Name:	TSQ 8000	
RT: 4.00 - 32.00		SM: 15G				NL:
	100					
						4.00E6
	90			24.31		TIC MS 4
	80					
Relati	70					
	60					
	50					
	40					
			13.48			
	30					
	20	7.74				
			16.56			28.19
	10	10.55				
					26.00	

	0					
	5	10	15	20	25	30

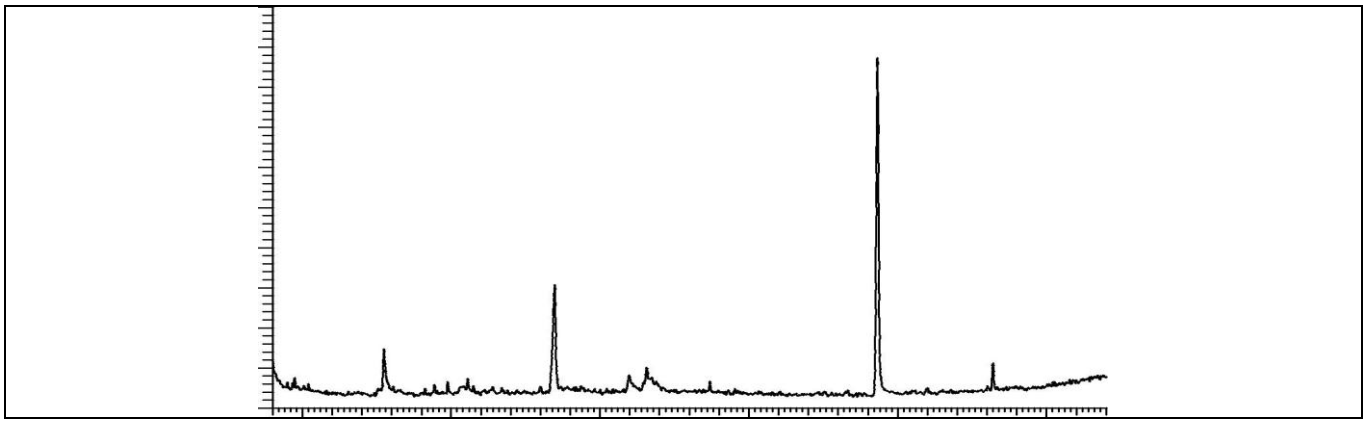
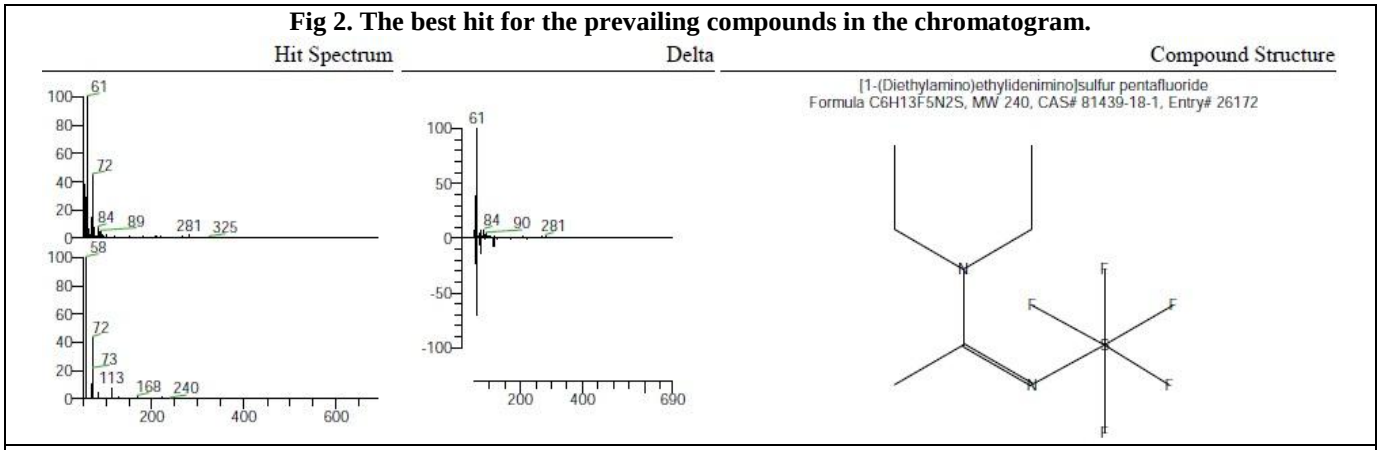
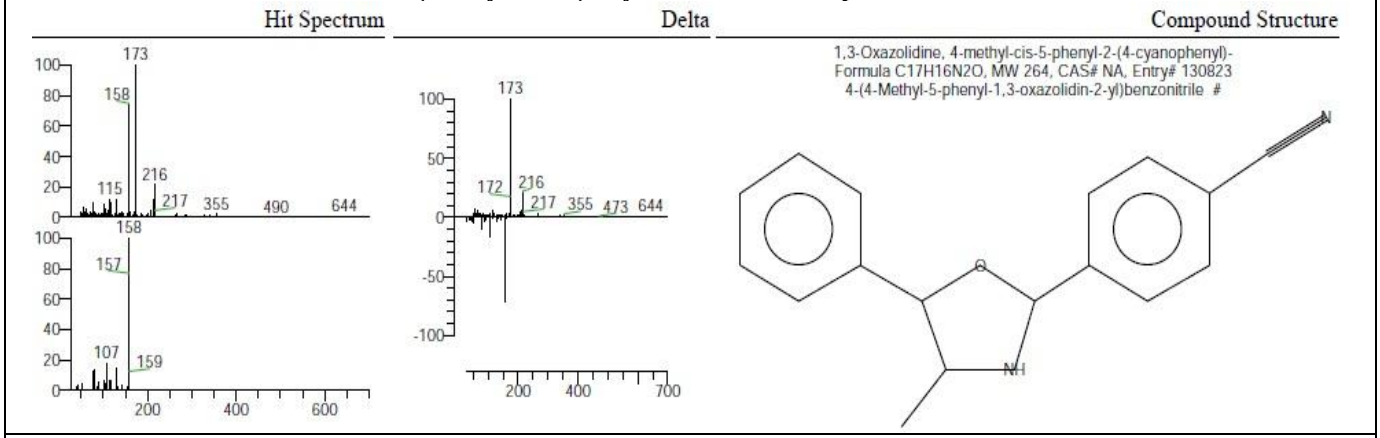


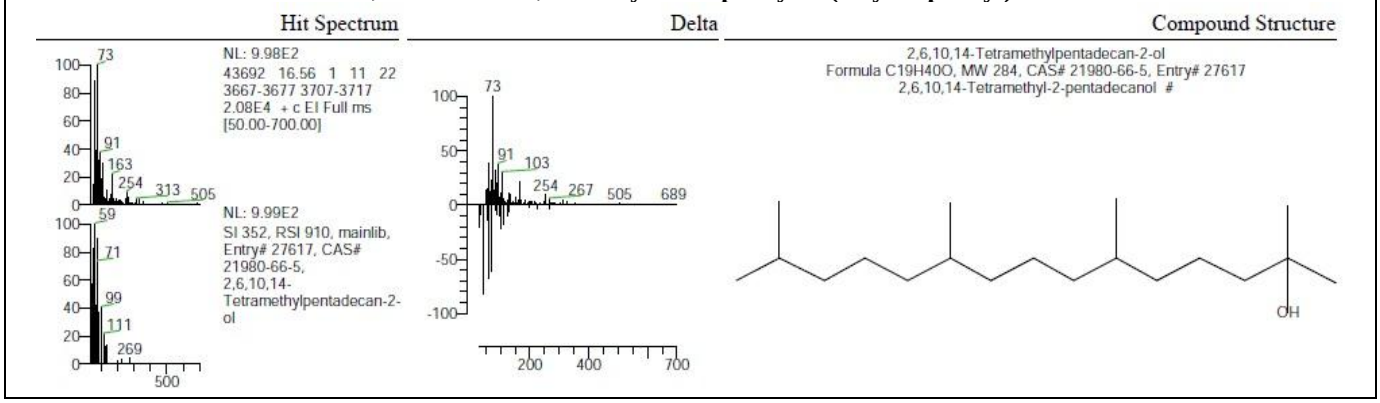
Fig 2. The best hit for the prevailing compounds in the chromatogram.



[1-(Diethylamino)ethylidenimino]sulfur pentafluoride

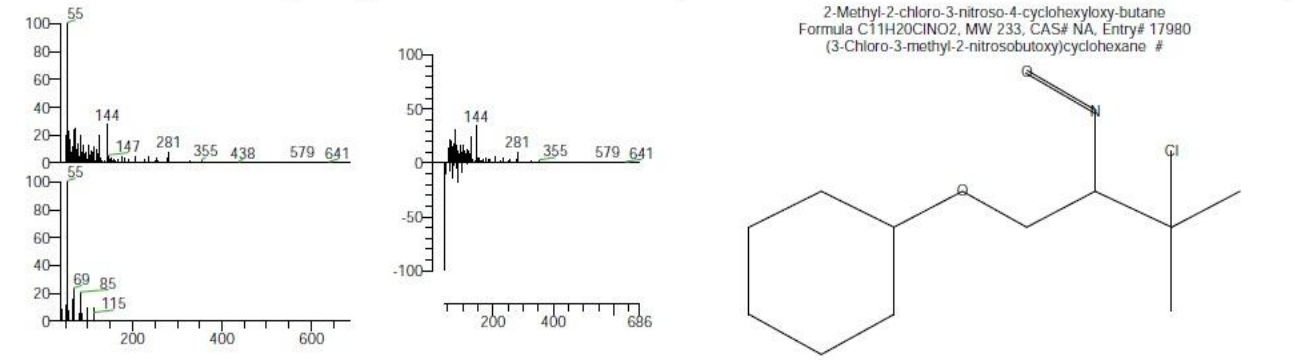


1,3-Oxazolidine, 4-methyl-cis-5-phenyl-2-(4-cyanophenyl)-



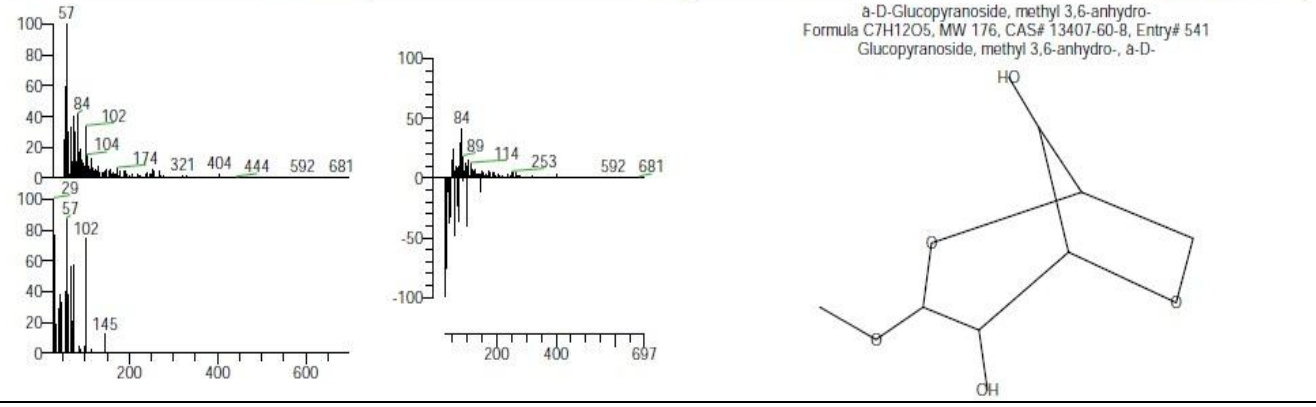
2,6,10,14-Tetramethylpentadecan-2-ol

Hit Spectrum | Delta | Compound Structure



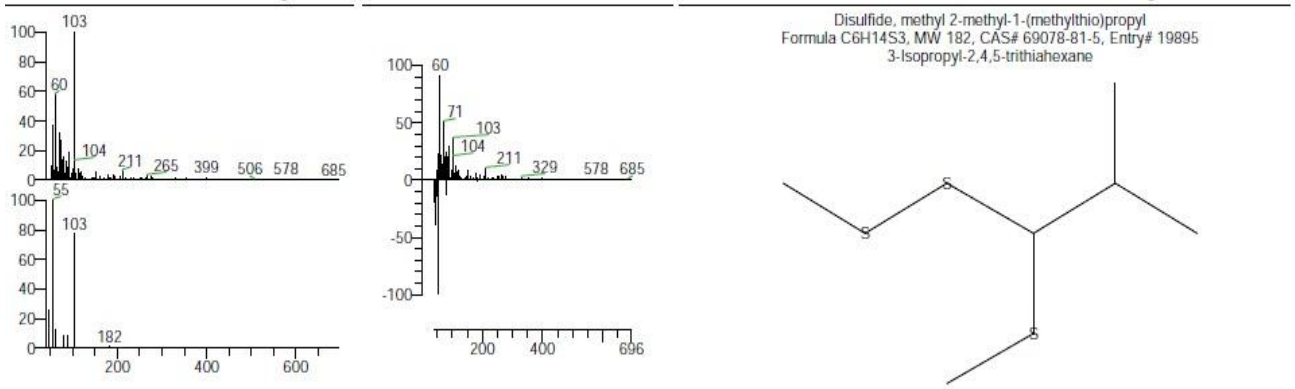
2-Methyl-2-chloro-3-nitroso-4-cyclohexyloxy-butane

Hit Spectrum | Delta | Compound Structure



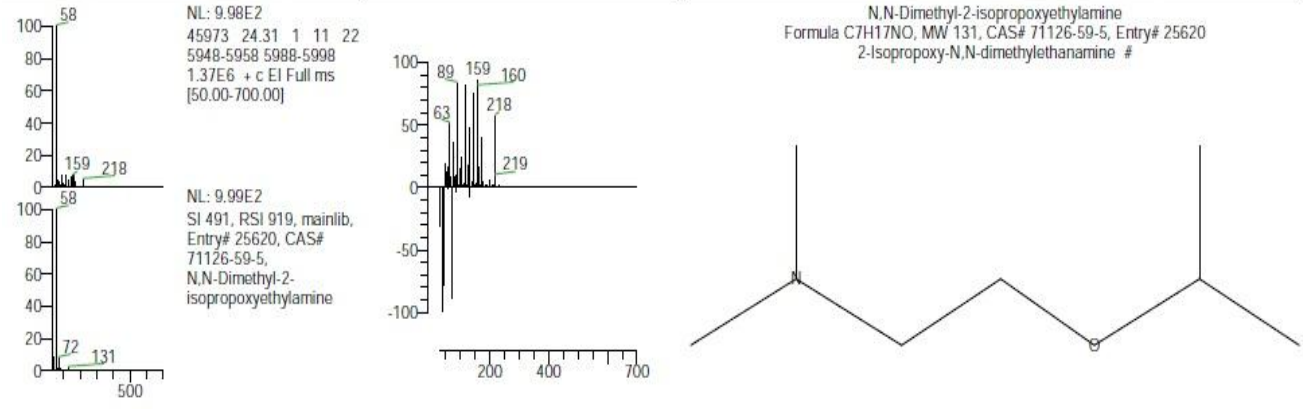
à-D-Glucopyranoside, methyl 3,6-anhydro

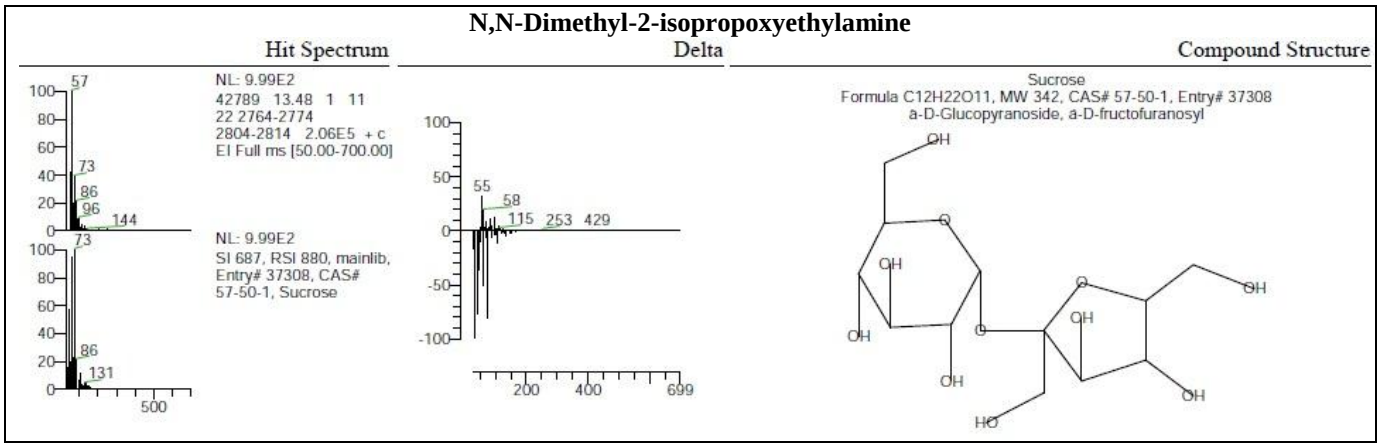
Hit Spectrum | Delta | Compound Structure



Disulfide, methyl 2-methyl-1-(methylthio)propyl

Hit Spectrum | Delta | Compound Structure





DISCUSSION

Phytochemical constituents such as tannins, flavonoids, steroids and several other aromatic compounds of C_4 grasses that serve as defence mechanisms against predation by many microorganisms, insects and herbivores. 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. This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times.

Antibiotic activity of C_4 grasses were recorded higher against G+ve organism (*Bacillus subtilis, Staphylococcus aureus*) as compare to the G-ve bacteria (*Escherichia coli, Raoultella planticola, Enterobactor aerogens, Proteus mirabilis, Klebsiella pneumoniae, Agrobacterium tumefaciens* and *Pseudomonas aeruginosa*) which supported the finding that plant extracts are usually more active against G+ve bacteria than G-ve bacteria [16, 24-43]

CONCLUSION

Therapeutic mechanism of a plant can be better understood with a proper investigation of its active ingredients. In the present study, 49 components from the methanolic extracts of the whole plant of *P. antidotale* 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, *P. antidotale* 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.

There are so many type of genes and proteins present in perennial grasses so it can grow easily in stress condition [44-45]. 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 [46].

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