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Original Article

Column Chromatography Purified Phytochemicals Identified in *Phragmytes vallatoria* Leaf Ethanolic Extract

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ABSTRACT

Phragmytes vallatoria belongs to the family poaceae. The complexity of leaf ethanolic extract can be simplified through column chromatography. Different solvent mixtures were used in elution systems *i.e.*, hexane, ethyl acetate and methanol. The concentrated ethanolic leaf extract of 100 g was fractionated by column chromatography on silica gel (60-120 mesh). Major fractions were (hexane + ethyl acetate 9:1 and ethyl acetate + methanol 8.5: 1.5 fraction) and identified the major phytochemicals through NIST Electron Ionization Mass database. The identified compounds were 9, 12, 15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-, Androstane-11, 17-dione, 3-hydroxy-, (3.alpha. 5. alpha)-, 9,12-Octadecadienoic acid ethyl ester and 9-12-15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-. Only one peak was observed in ethyl acetate and methanol (8.5: 1.5) fraction and it was subjected to GC-MS and HPLC. The structures were (Benzenamine, 3-(methylthio)-, Morpholine, Phenanthrene and 3-Eicosene, (E).

Keywords: *Phragmytes vallatoria*, Phytochemicals, Leaf ethanolic extract, Column chromatography.

INTRODUCTION

Phragmytes vallatoria belongs to the family poaceae and it is wide spread throughout India. It has different types of applications in the field of medicine and agriculture. Medicinally it has the properties of diuretic, animistic, diaphoretic; wound healing, diabetes, arthritis, rheumatism, antiemetic and febrifuges[1]. Further it is used as building material, in fibre, pulp and paper sisling. The earlier study proved that the antidiabetic activity of *Phragmytes vallatoria* leaf ethanolic extract in STZ

induced Diabetic rats[2]. *Phragmytes vallatoria* crude extract as peripheral application has wound healing activity [(3)]. The GC-MS analysis of ethanolic extract of *Phragmytes vallatoria* revealed the presence of fatty acids and plasticizer compounds. Hence, the study was under taken to investigate the presence of phytochemicals in leaf ethanolic extract of *Phragmytes vallatoria* and the complexity of leaf ethanolic extract can be simplified through column chromatography.

MATERIALS AND METHODS

Collection of plant material

Phragmytes vallatoria is obtained from Chirala (Prakasam district, Andhra Pradesh, India). The leaves were collected and shade dried and powdered. The powdered leaves were extracted with ethanol using soxhlet apparatus. The extract was concentrated by rotary evaporator under vacuum and was further subjected to column chromatography.

Isolation of fractions from Ethanolic extract of *Phragmytes vallatoria* leaf

Different solvent mixtures were used in elution systems *i.e.*, hexane, ethyl acetate and methanol. The concentrated ethanolic leaf extract of 100g was fractionated by column chromatography on silica gel (60-120 mesh). The fractions were collected and subjected for further analysis.

HPLC analysis of the fractions

The HPLC was run in an Alliance 1200 series apparatus using a Xterra RP-18column (250x4.6mm, 5 micron). The samples were eluted in a linear gradient of acetonitrile and water containing 0.1% TFA from 0 to 45% in 45 minutes, followed by a linear gradient of acetonitrile and water containing 0.1% TFA from c45 to 100% in 15 minutes. The flow rate was kept constant at 0.5 ml/min. The chromatogram was measured at 300nm and 360nm. All reagents employed in this investigation were of analytical grade. High-purity water was obtained by passing water though a Milli-Q treatment system (Millipore, USA) and the HPLC mobile phase was prepared using Milli-Q water.

GC-MS analysis

GC-MS analysis was performed with GC Clarus 500 Perkin Elmer equipment. Compounds were separated in Elite-1 capillary column (100% Di methyl poly siloxane), 30×0.25×1µmdf. The samples were injected at a temperature of about 250 °C with a split ratio of 10: 1 with a flow rate of helium 1 ml/min. Mass detector turbo mass gold-perkin elmer was used as detector.

MS-programme

The constituents were identified after comparison with those available in the computer library (NIST ver. year 2005) attached to the instrument.

Liquid chromatography-mass spectrometry (LC-MS) analysis

Ethanolic extract of leaf was analyzed using LC-MS. The LC system consisted of an Agilent Technologies Series 1200 system (Agilent, USA) equipped with an automatic degasser, a quaternary pump, and an auto sampler. Chromatographic separations were performed using YMC-pack ODS-Aq (50X4.6nm, 3µm), and the column temperature was maintained at 25°C and the sample injection volume was maintained as 12 µl. The mobile phase consisted of acetonitrile and 0.1% aqueous formic acid using gradient elution (0–2 min, 45–80% acetonitrile; 2–5 min, 80% acetonitrile; 5 – 5.1 min 80–45% acetonitrile) and was delivered at a flow rate of 0.8 mL/min. The mass analyser operated with an ESI source, ion mode: positive, mass range: m/z 10–1500, scan speed: 2500 u/s, ESI at 400µL/min, selected ion monitoring of m/z 609.3, scan

range: 100–650 m/z. The ion source temperature was held at 650^{o}C and target ions were monitored at m/z 609.3.

RESULTS

HPLC analysis

The samples were analyzed by HPLC coupled with a photo diode array detector (DAD). Peaks were identified by HPLC retention times and % area, which are summarized in Table-1.

DISCUSSION

From the study it can be concluded that ethanolic extract of *Phragmytes vallatoria* is having the phytochemicals, antioxidants, fatty acids and plasticizer compounds. Further, the polyunsaturated fatty acid (*Z, Z*)-9, 12-octadecadienoic acid (LA), a conjugated linoleic acid identified are known to have antioxidant property that can protect membranes from harmful compounds[4]. 9, 12, octadecanoic acid (z,z)-has the property of anti-inflammatory and anti arthritic as reported by earlier worker[5].

REFERENCES

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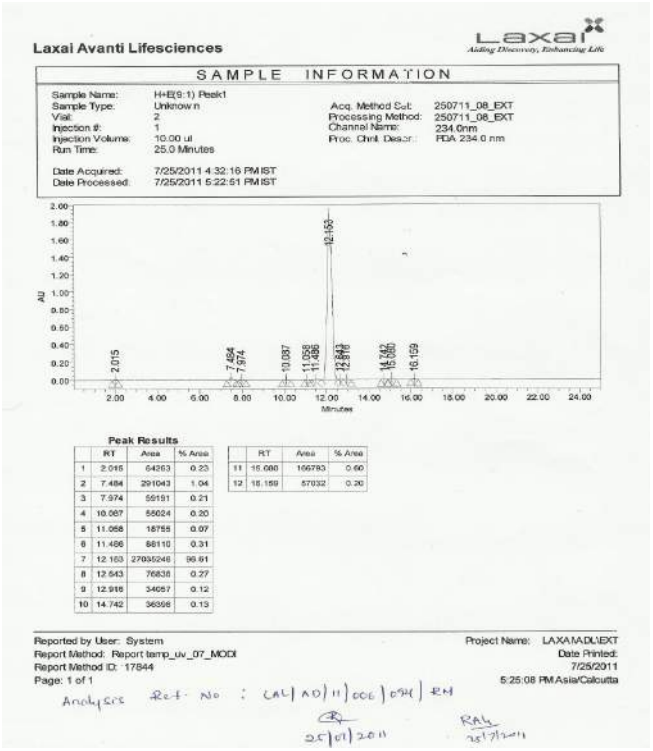

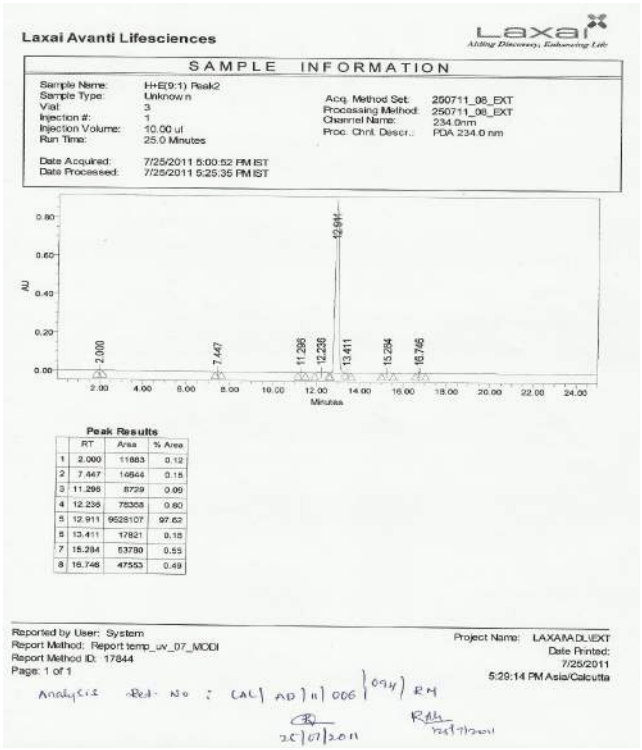

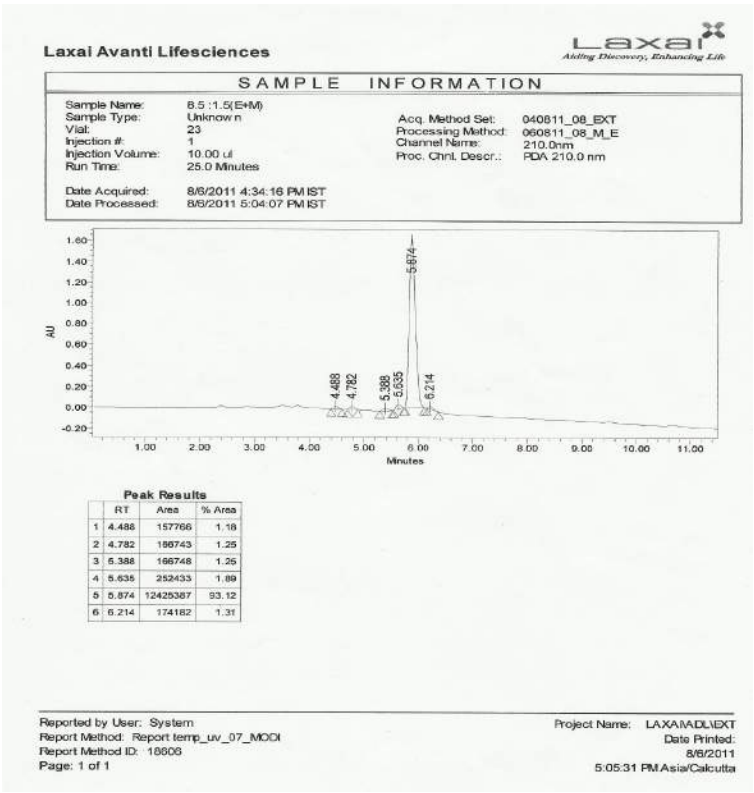

Table-1 HPLC Analysis of the fractions

GC-MS Analysis of the compounds

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ALS Vial : 3 Sample Multiplier: 1

Integration Parameters: 281210-002.E
Integrator: ChemStation

Method : D:\msdchem\1\DATA\2011\JUIY-11\050711\010.D\G60-17-300-6HM.M
Title :

Signal : TIC: 003.D\data.ms

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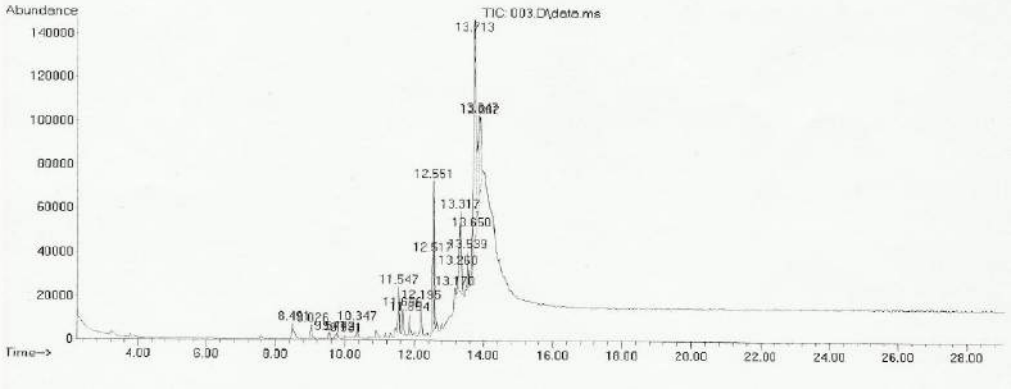
1	8.491	559	564	588	M2	7177	386123	7.91%	2.591%
2	9.026	606	612	625	M2	6540	217317	4.45%	1.458%
3	9.541	654	658	666	M2	2826	80131	1.64%	0.538%
4	9.773	670	679	682	M5	2406	104753	2.14%	0.703%
5	9.981	694	697	701	M	1518	33889	0.69%	0.227%
6	10.347	727	730	735	M2	6285	144453	2.96%	0.969%
7	11.547	832	838	843	M3	22338	587834	12.04%	3.944%
8	11.656	843	847	856	M	11973	331441	6.79%	2.224%
9	11.854	861	865	872	M	9439	273428	5.60%	1.835%
10	12.195	887	896	901	M	15889	422611	8.65%	2.836%
11	12.517	920	924	925	M	36451	829184	16.98%	5.564%
12	12.551	925	927	932	M	70691	1613130	33.01%	10.818%
13	13.170	978	983	986	M3	5884	144524	2.96%	0.970%
14	13.260	986	991	992	M4	10262	221722	4.54%	1.488%
15	13.317	992	996	1005	M	36709	1468710	30.07%	9.855%
16	13.539	1013	1016	1021	M	16880	392100	8.03%	2.631%
17	13.650	1022	1026	1028	M	20556	405972	8.31%	2.724%
18	13.713	1028	1032	1039	M4	107724	4884152	100.00%	32.774%
19	13.847	1039	1044	1046	M2	41294	1497169	30.65%	10.046%
20	13.882	1046	1047	1053	M4	34950	865103	17.71%	5.805%

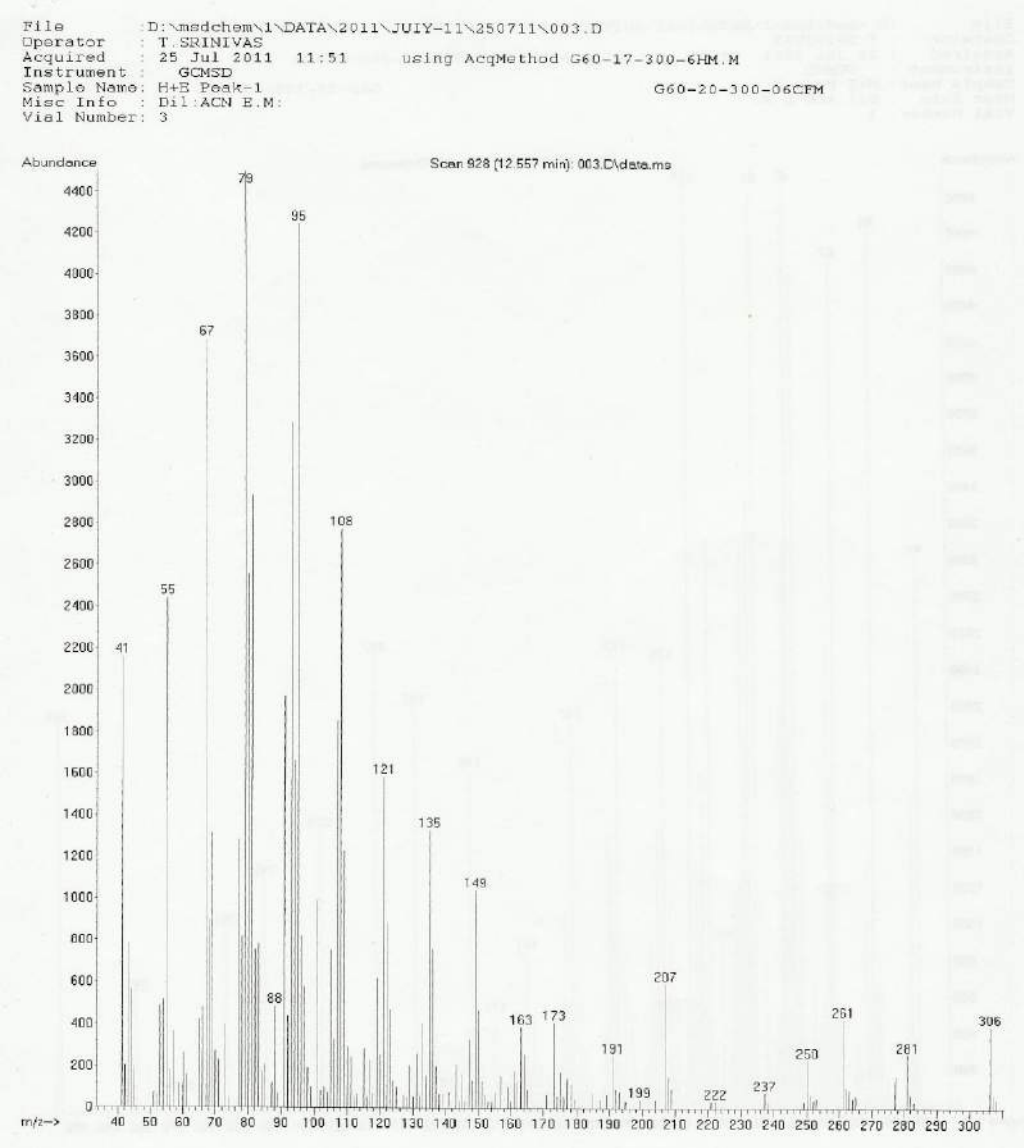
Sum of corrected areas: 14902745

G60-17-300-6HM.M Mon Jul 25 12:32:24 2011



(Hexane + Ethyl acetate 9:1)

 ISSN 2321-547X



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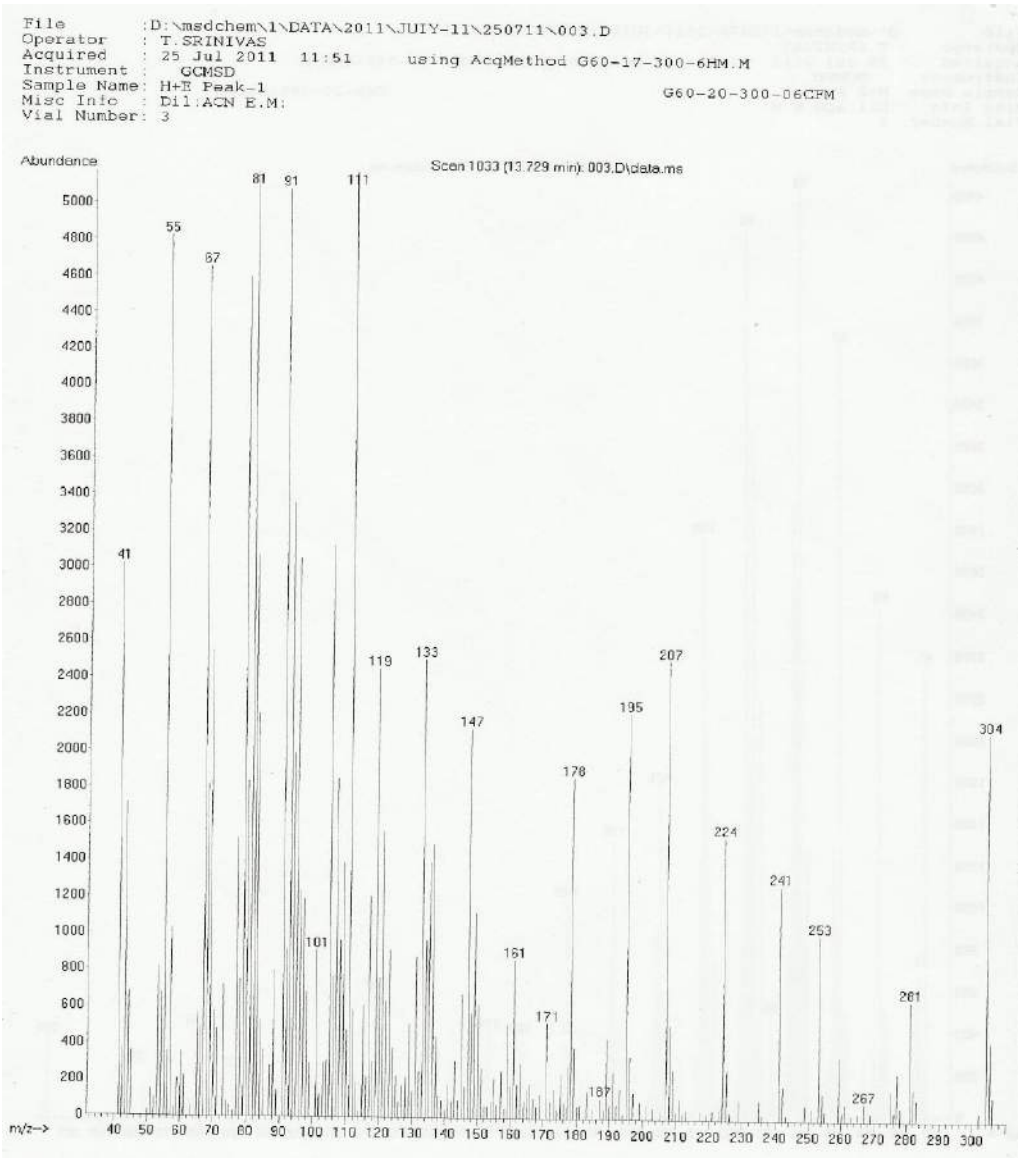

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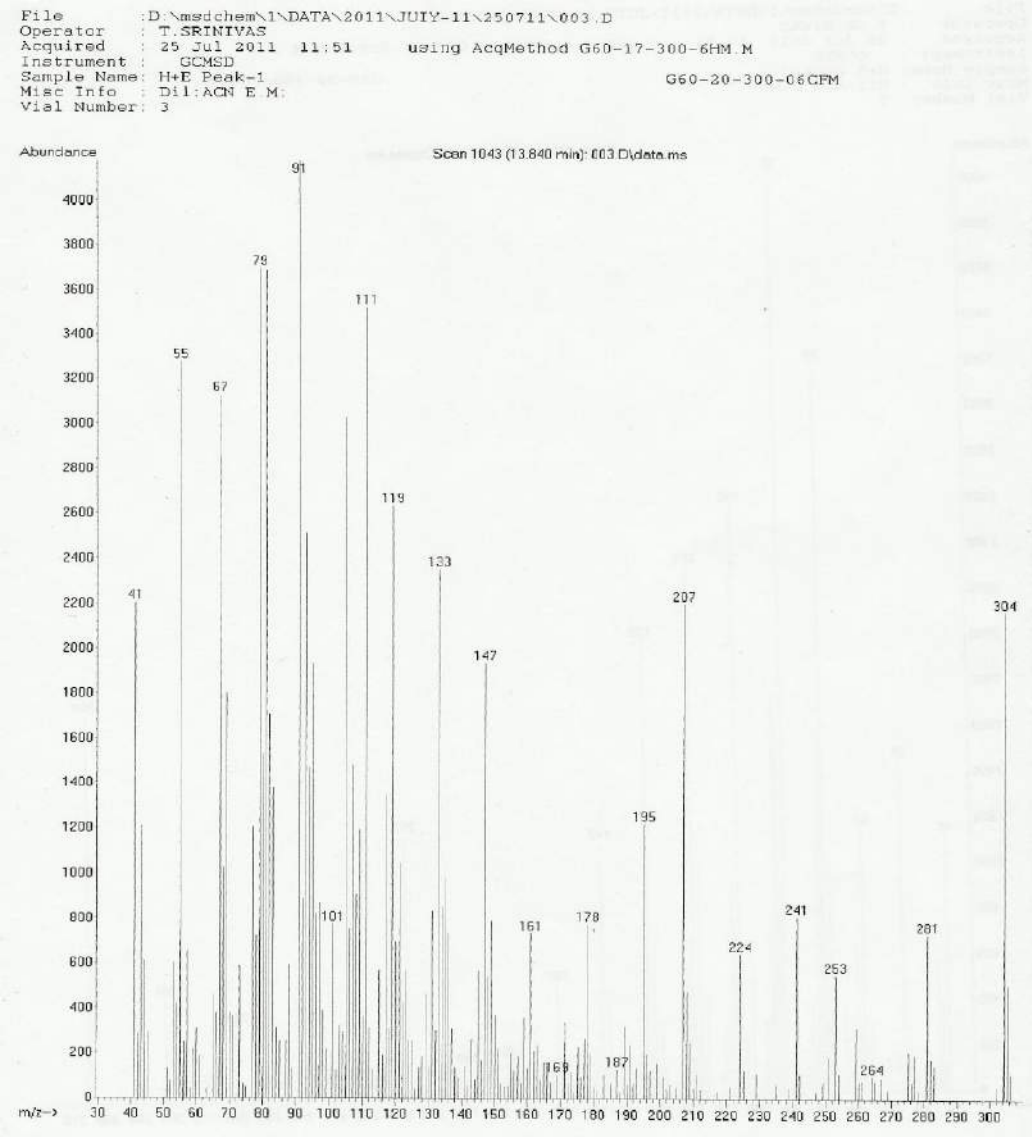

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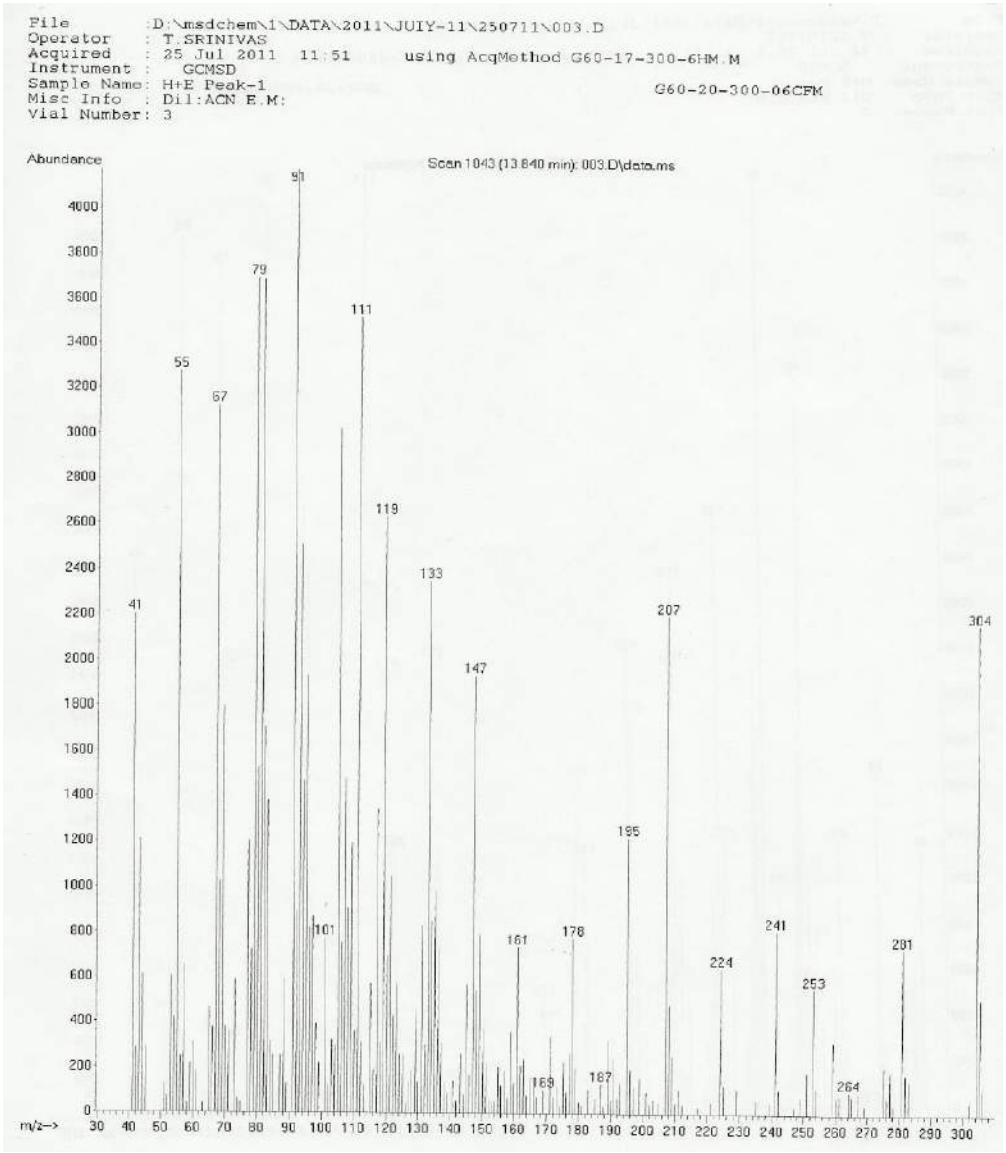

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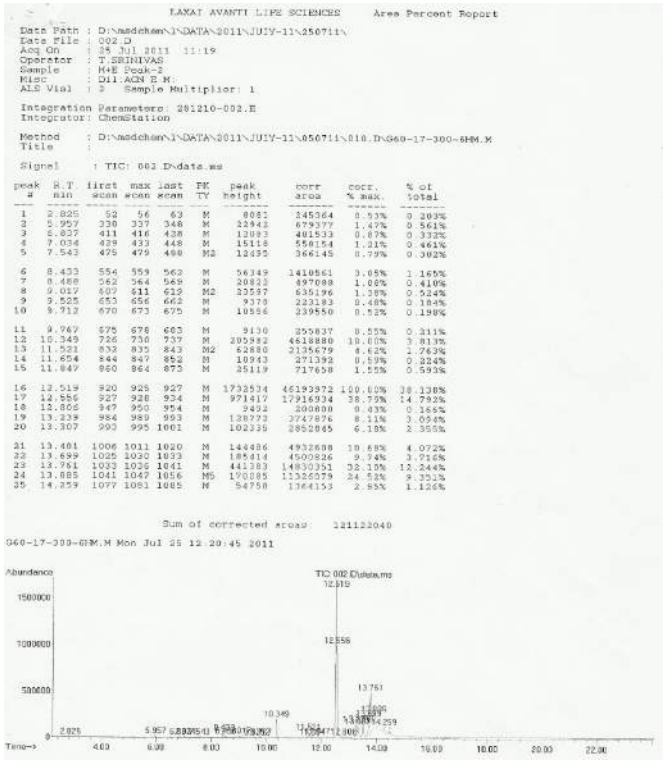

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ALS Vial : 2 Sample Multiplier: 1

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Integrator: ChemStation

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Title :

Signal : TIC: 002.D\data.ms

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2	5.957	330	337	348	M	32943	679377	1.47%	0.561%
3	6.837	411	416	428	M	12083	401533	0.87%	0.332%
4	7.034	429	433	448	M	15118	558154	1.21%	0.461%
5	7.543	475	479	490	M2	13455	366145	0.79%	0.302%
6	8.433	554	559	562	M	56349	1410561	3.05%	1.165%
7	8.488	562	564	569	M	20823	497088	1.08%	0.410%
8	9.017	607	611	619	M2	23587	635196	1.38%	0.524%
9	9.525	653	656	662	M	9378	223183	0.48%	0.184%
10	9.712	670	673	675	M	10556	239550	0.52%	0.198%
11	9.767	675	678	683	M	9130	255837	0.55%	0.211%
12	10.349	726	730	737	M	205982	4618880	10.00%	3.813%
13	11.521	832	835	843	M2	62880	2135679	4.62%	1.763%
14	11.654	844	847	852	M	10943	271392	0.59%	0.224%
15	11.847	860	864	872	M	25119	717658	1.55%	0.593%
16	12.519	920	925	927	M	1732534	46193972	100.00%	38.138%
17	12.556	927	928	934	M	971417	17916934	38.79%	14.792%
18	13.806	947	950	954	M	9492	200800	0.43%	0.166%
19	13.239	984	989	993	M	138773	3747876	8.11%	3.094%
20	13.307	993	995	1001	M	102335	2852845	6.18%	2.355%
21	13.481	1006	1011	1020	M	144486	4902808	10.68%	4.072%
22	13.699	1025	1030	1033	M	185414	4500826	9.74%	3.716%
23	13.761	1033	1036	1041	M	441383	14830351	32.10%	12.244%
24	13.885	1041	1047	1056	M5	170385	11326579	24.52%	9.351%
35	14.259	1077	1081	1085	M	54758	1364153	2.95%	1.126%

Sum of corrected areas: 121122040

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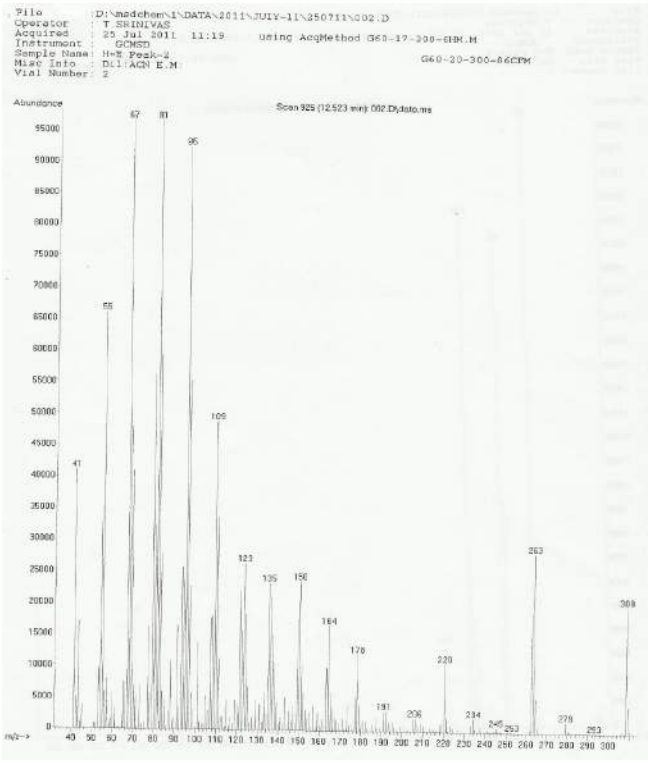

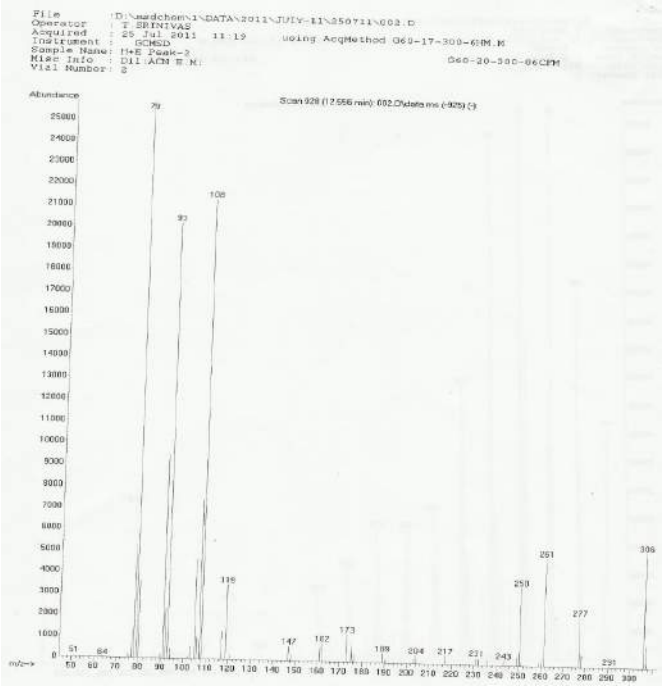

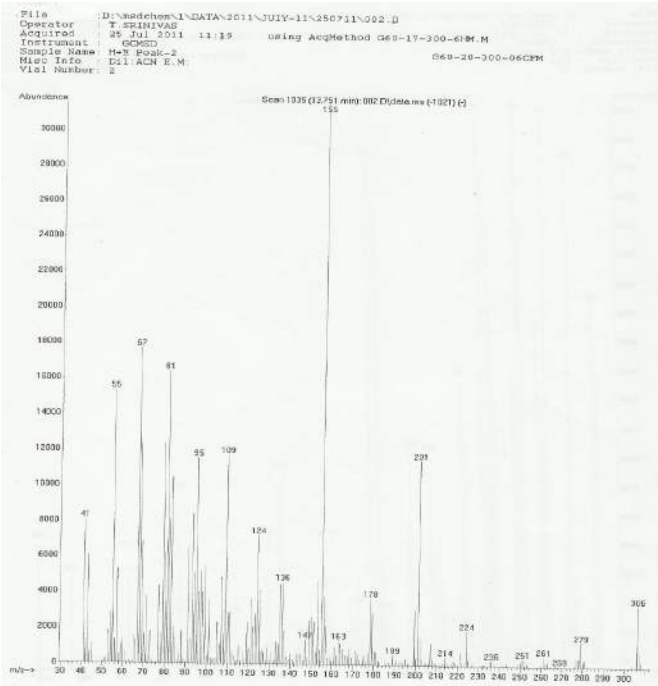

(Hexane + Ethyl acetate 9:1)

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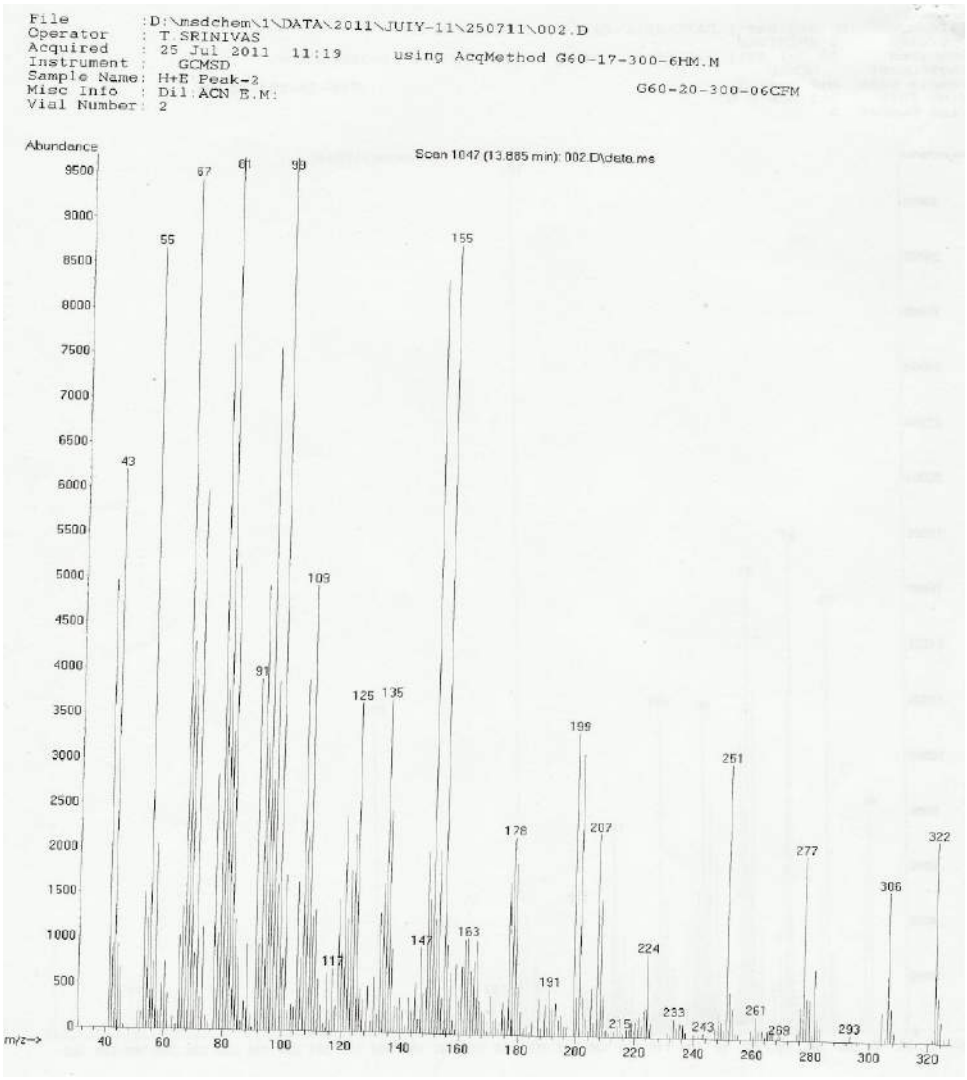

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LAXAI AVANTI LIFE SCIENCES Area Percent Report

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ALS Vial : 17 Sample Multiplier: 1

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Title :

Signal : TIC: 020.D\data.ms

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2	3.102	67	72	86	M3	4505	247119	4.33%	1.428%
3	4.602	201	206	232	M	13567	774902	13.59%	4.477%
4	7.678	477	482	503	M	44599	1783224	31.27%	10.304%
5	8.156	521	525	530	M	5621	129288	2.27%	0.747%
6	8.549	565	569	575	M3	3715	94543	1.66%	0.546%
7	8.827	580	585	603	M	18907	622852	10.92%	3.599%
8	9.792	667	671	678	M	228253	5702681	100.00%	32.950%
9	9.902	678	681	693	M2	32180	926031	16.24%	5.351%
10	10.201	704	708	716	M	31566	822831	14.43%	4.754%
11	10.736	747	756	764	M4	17107	559256	9.81%	3.231%
12	11.040	772	783	793	M2	39524	1050620	18.43%	6.071%
13	12.080	872	876	882	M	32788	745945	13.08%	4.310%
14	13.034	958	962	968	M	23251	537568	9.43%	3.106%
15	13.953	1040	1044	1052	M	13769	357225	6.26%	2.064%

Sum of corrected areas: 17306861

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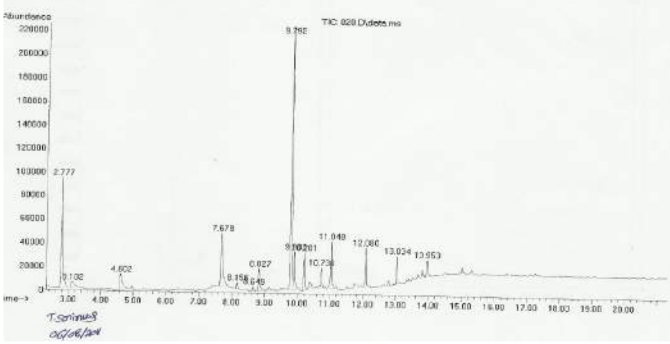
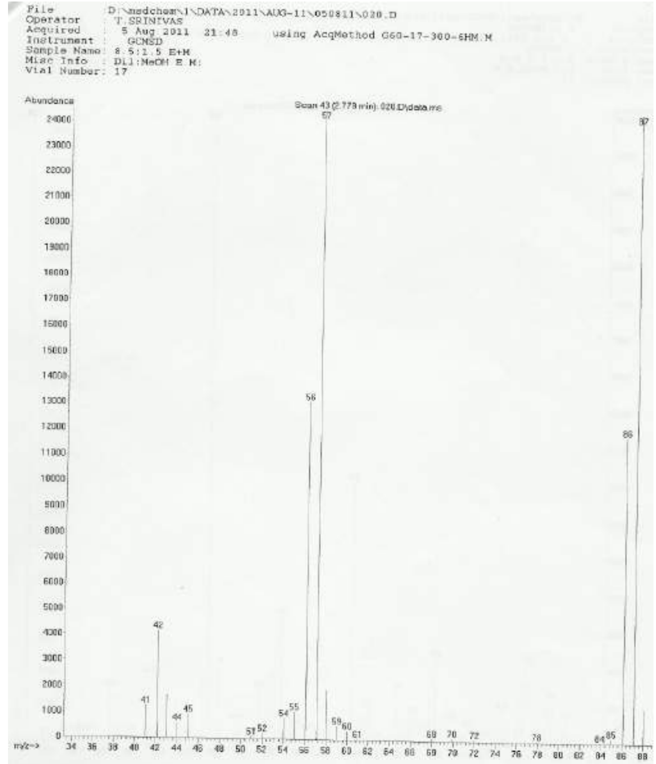
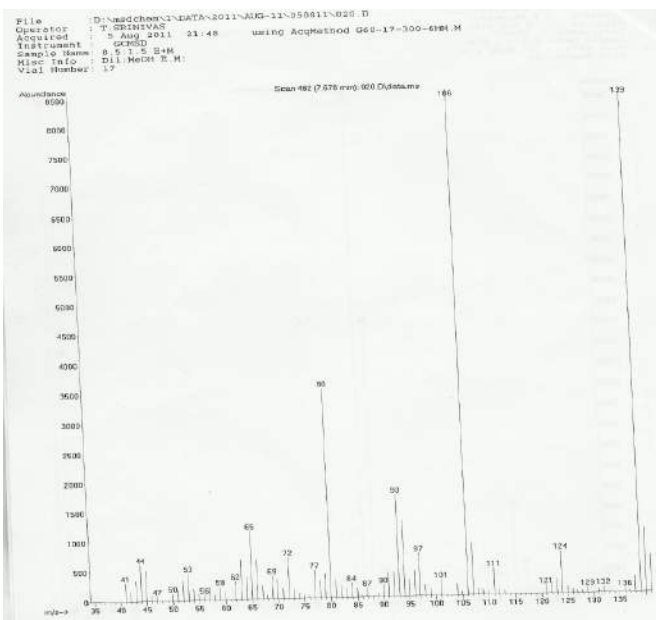
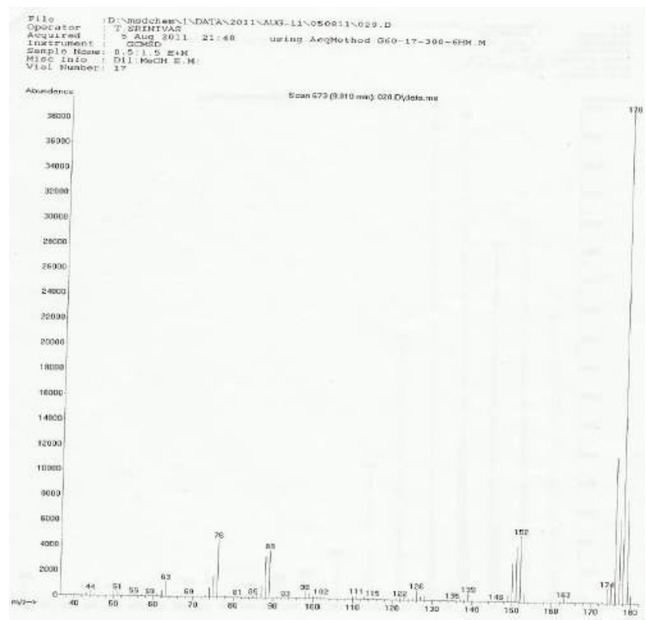
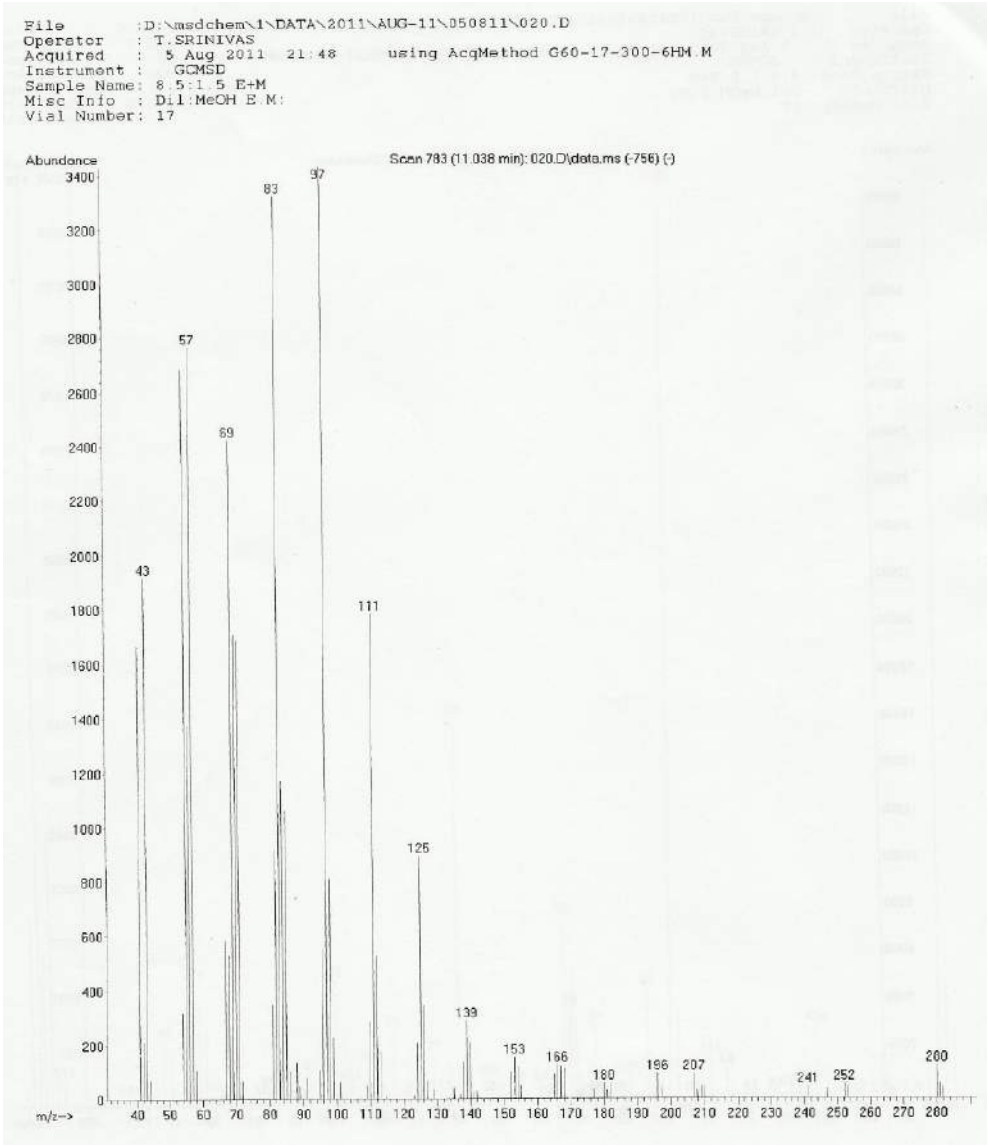








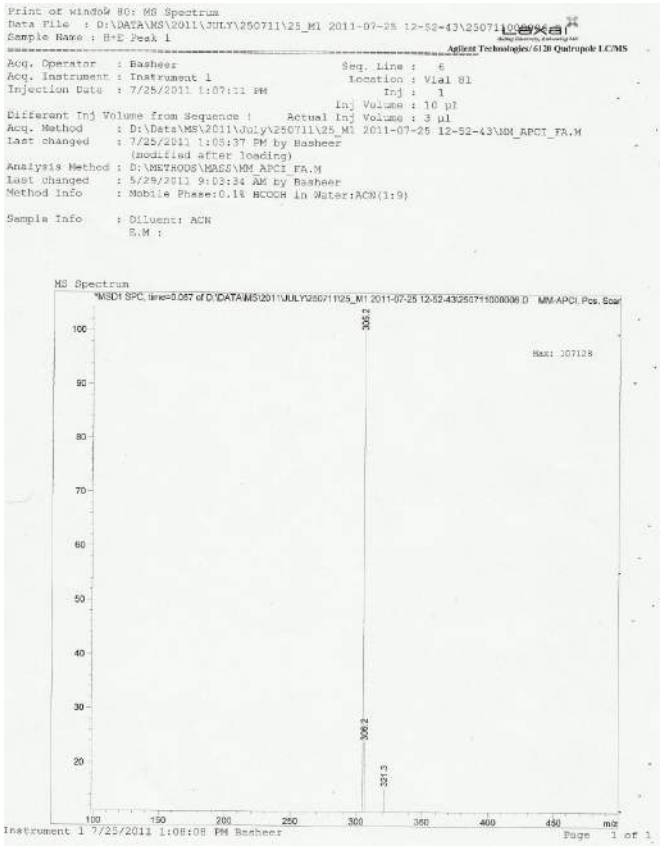
(Ethyl acetate + Methanol 8.5:1.5)

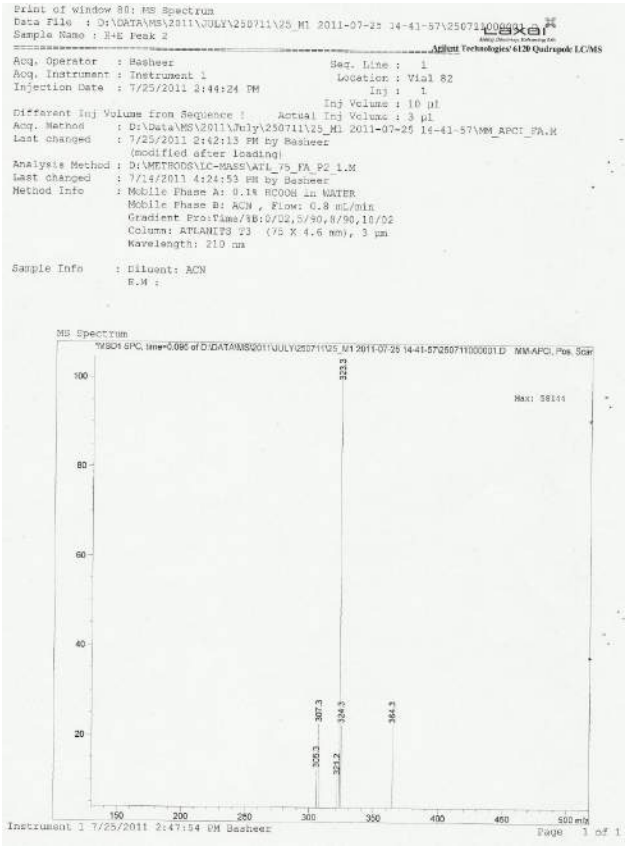


(Ethyl acetate + Methanol 8.5:1.5)

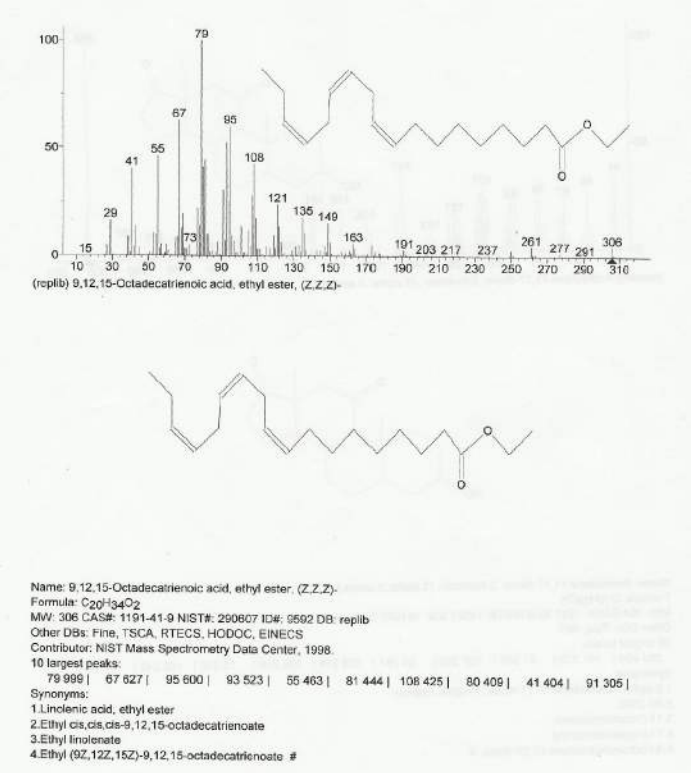
LC-MS Analysis of the ethanolic leaf extract

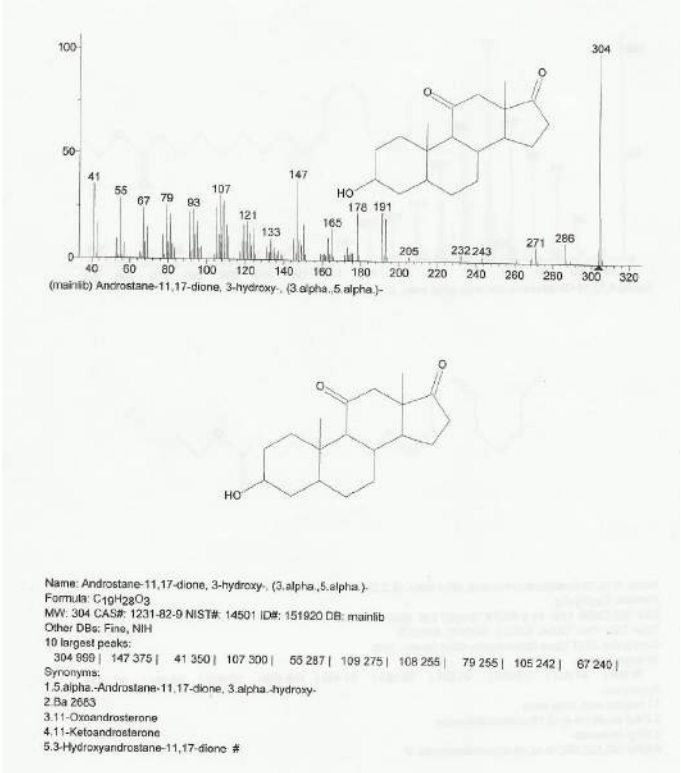
Leaf ethanolic extract analyzed by LC-MS and found the compounds with different masses.





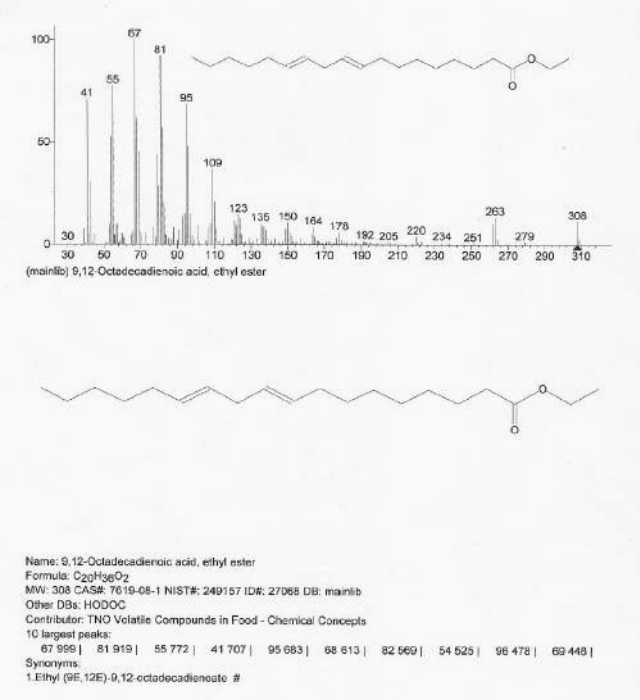
Possible structures through NIST

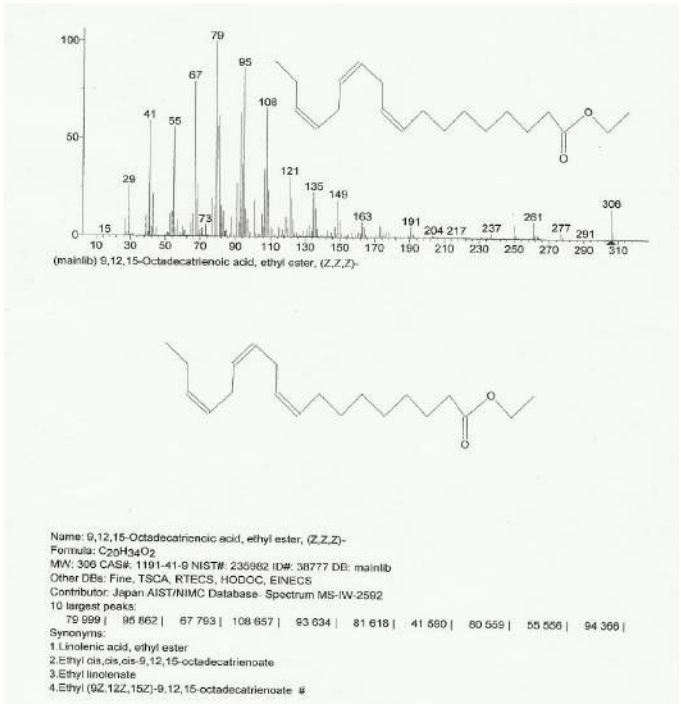


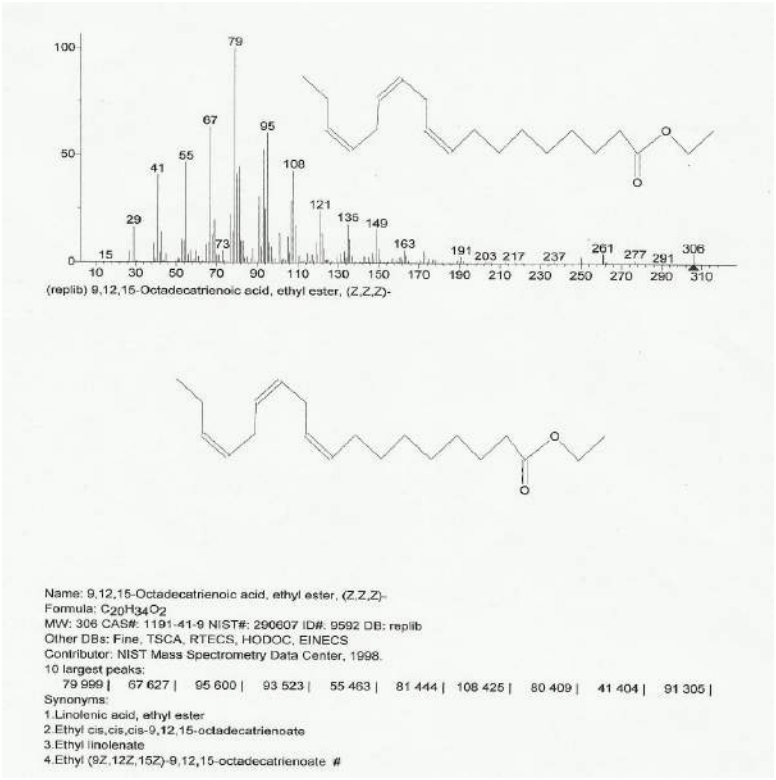


(Hexane + Ethyl acetate 9:1, Peak-1)

 ISSN 2321-547X

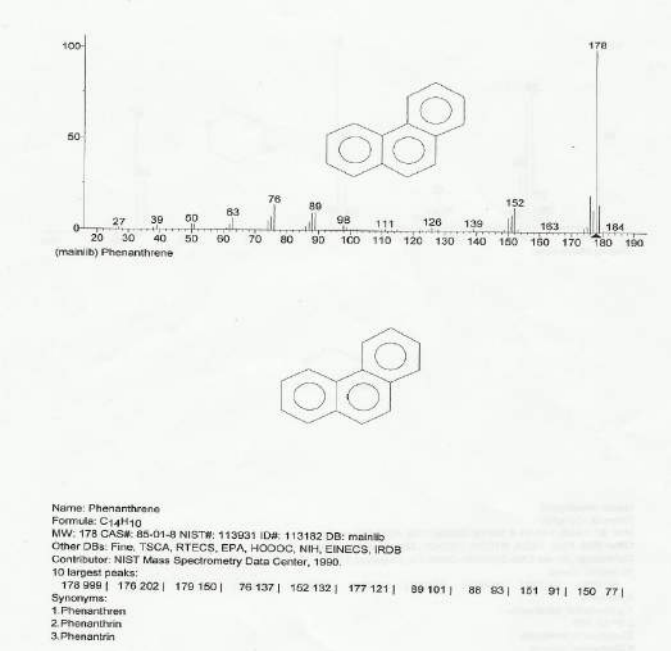


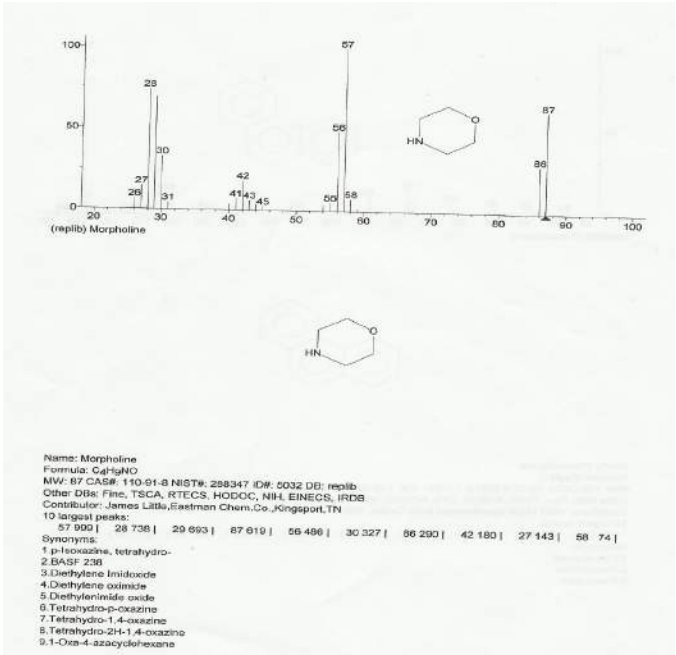




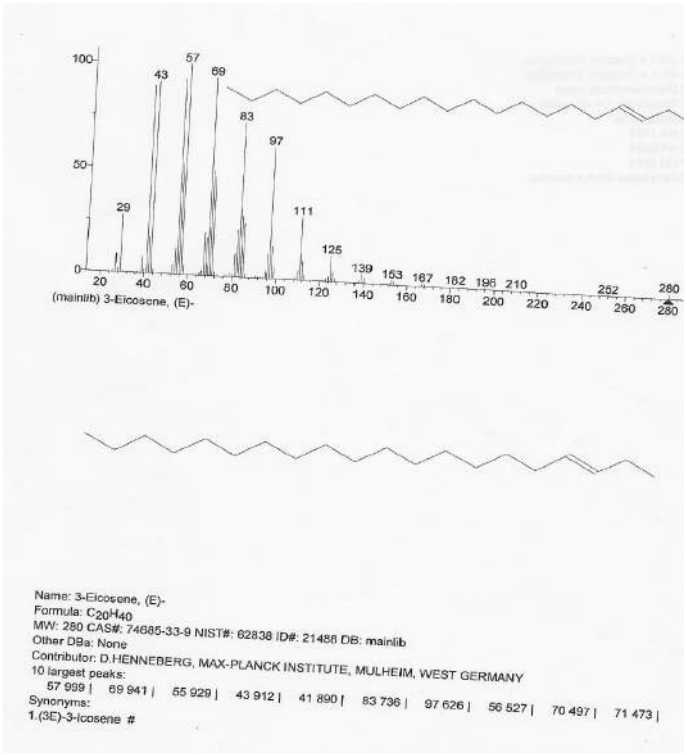
(Hexane + Ethyl acetate 9:1, Peak-2)

 ISSN 2321-547X





10.2H-1,4-Oxazine, tetrahydro-
11.4H-1,4-Oxazine, tetrahydro-
12.Diethyleneimide oxide
13.Tetrahydro-1,4-isoxazine
14.Drewamine
15.NA 1760
16.NA 2054
17.UN 2054
18.Tetryhydro-2H-1,4-oxazine



(Ethyl acetate + Methanol 8.5:1.5)