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Metabolite Profiling And Antimicrobial Activity Using GCMS And LC-MS Of Senna Occidentalis L. Root Extract

Ram Chandra Bharti1*, Anil Kumar[2], Garima Gupta[3], Akansha Chauhan [4]

[1*] School of Pharmacy, Monad University, Hapur, U.P., India
[2*] Monad College of Pharmacy, Monad University, Hapur, U. P., India

Abstract

Senna occidentalis L. is a medicinal plant known for its traditional use in various therapeutic applications. In this study, we aimed to elucidate the metabolite profile of *Senna occidentalis* L. root extract using Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). The phytochemical compositionof the extract was analyzed to identify and characterize the bioactive compounds present. TheGC-MS analysis revealed a diverse array of volatile and non-volatile compounds in the *Sennaoccidentalis* L. root extract, including alkaloids, terpenoids, flavonoids, and other secondary metabolites. LC-MS/MS further provided a comprehensive identification of the polar and non-polar constituents, allowing for a more in-depth understanding of the chemical composition. To explore the potential therapeutic applications of the identified compounds, the antimicrobial activities of the *Senna occidentalis* L. root extract were evaluated against a panel of pathogenic microorganisms. The extract exhibited notable antimicrobial effects, demonstrating its efficacyagainst both Gram-positive and Gram-negative bacteria, as well as fungi. The findings from this study contribute valuable insights into the chemical composition of *Senna occidentalis* L.Roots and highlight its potential as a source of bioactive compounds with antimicrobial properties. Further research is warranted to isolate and characterize specific compounds responsible for the observed activities, paving the way for the development of novel pharmaceuticals or natural products for therapeutic applications.

Keywords: Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography- Mass Spectrometry (LC-MS/MS), Phytochemical, Antimicrobial properties.

Introduction

A microscopic organism is known as a microorganism or microbe. The term "microbiology" refers to the study of microorganisms. Bacteria, fungi, archaea, and protists are all examples of microorganisms. Prions and viruses are not considered microbes because they are considered non-living in general(Tamang et al., 2016).

Currently, there is a lot of debate over how life is organized and categorized, especially when it comes to the study of microorganisms. The essential division between prokaryotes (cells lacking internal membrane-bound organelles, the Monera, including the majority of microbes) & eukaryotes (cells containing membrane-bound organelles - protists, fungi, plants, & animals) separates living beings into two groups. The two kingdoms of plants and animals were a convenient division of living things before the invention of the microscope. But this division was inadequate what about fungi Today's kingdom taxonomists have established five or six kingdoms (Monera, Protoctista, Fungi, Plantae, & Animalia, with Archaea as the sixth), neither of which include viruses (or prions). Viruses are thought to straddle the living and non-living spectrum. The question of whether or not viruses are live things. Because they don't fulfil all the requirements of the widely recognized definition of life, the majority of virologists consider them to be non-living. For instance, many viruses do not react to environmental changes, which is a defining characteristic of living things. Additionally, viruses can only multiply by invading a host cell. As a result, they are unable to reproduce on their own. A recently identified infectious agent is called prion. They are proteins that can change properly folded proteins into abnormally folded ones because of their abnormal folding (folding is a feature of proteins that allows them to take a shape that is crucial for their function). The development of genetic analysis has both made the issue simpler and more complicated. Dr Carl Woeser discovered using DNA sequence analysis in the 1970s to suggest the split of life into three domains: bacteria, archaea, & eukaryota. The tree of life he derived from the sequence data reveals the reasons behind this divide. This tree of life demonstrates unequivocally that microorganisms make up the vast bulk of life on Earth. 99% of the microbes on Earth, according to scientists, have not yet been named (Mishra et al., 2021).

MATERIAL AND METHODS

METHODS

Collection and Authentication of *Senna occidentalis*.

The plant and leaves of *Senna occidentalis*. Were collected from nearby areas of Hapur (UP) inDecember 2022. A herbarium was prepared and authenticated by Dr Sunita Garg, Former Chiefscientist Head, RHMD, CSIR – NIScPR, and Mr R S Jayasomu, Chief Scientist, Head, RHMD,CSIR – NIScPR, Reference number NIScPR/RHMD/Consult/2022/4262-63. A voucher specimen of the plant was preserved at the Raw Material Herbarium and Museum, Delhi (RHMD) for future reference.

Extraction

The dried leaves were subjected to size reduction with the help of a stainless-steel grinder andthe fine powder of the leaves Extraction was with a hot soxhlet extraction process using Petroleum ether as a solvent the extract was concentrated to dryness with the help of a water bath and finally air-dried. The obtained dried extracts of Senna occidentalis. Leaves were weighed and extractive value was calculated. It was kept in an airtight container stored in a desiccator and used for investigation of their potential.

Fig. 15 Extraction Process

PRELIMINARY PHYTOCHEMICAL TESTS

The extract obtained from ethanol solvent was subjected to various chemical tests fordetermination of phytoconstituents present.

Test for Alkaloids

The extract was dissolved individually in dilute Hydrochloric acid and filtered.

- **Mayer's Test:** Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a cream-coloured precipitate indicates the presence of alkaloids.
- **Wagner's Test:** Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide).The formation of a brown/ reddish precipitate indicates the presence of alkaloids.
- **Dragendorff's Test:** Filtrate was treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). The formation of a red precipitate indicates the presence of alkaloids.
- **Hager's Test:** Filtrate was treated with Hager's reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of a yellow-coloured precipitate.

Test for Carbohydrates

- **Molisch's Test:** 3-5 drops of extract were treated with a few drops of ethanolic α-naphtholsolution in a test tube. Add 3-5 drops of conc. H_2SO_4 from the sides of the test tube. Formation of the violet ring at the junction of two liquids indicates the presence of Carbohydrates.
- **Benedict's test:** Equal volume of Benedict's reagent and test solution in a test tube and heat in a boiling water bath for 5 minutes. The solution appears green, yellow, or red colourindicating the presence of reducing sugars.
- **Fehling's Test:** Mix 1ml Fehling's A & Fehling's B 1ml solution, boil for one minute. Addan equal volume of the test solution and heat in a boiling water bath for 5-10 minutes. FirstYellow, the brick red precipitate indicates the presence of reducing sugars.
- **Barfoed's Test:** The extract was treated with Barfoed's reagent heated for 1-2 minutes ina boiling water bath and cool. The formation of a red precipitate indicates the presence ofMonosaccharides.

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RESULTS AND DISCUSSION

EXTRACTIVE VALUES (%) AND APPEARANCE OF EXTRACTS OF ROOTS SENNA OCCIDENTALIS L.

Results of (%) extractive values and the appearance of extracts are presented in Table 9.

Table 8 Extraction yield (%) of ethanolic extract

Petroleum ether Extractive value (%)	Appearance	Ethanolic Extractive value (%)	Appearance
0.5	Oily	4.3	Greasy

PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT

Preliminary phytochemical screening of ethanolic extract revealed the presence of alkaloids,saponins, phenols and tannins, Flavonoids, Terpenoids, and Steroids.

S. No.	Phytochemical	Ethanolic extract of *Senna Occidentalis* L.
1	Carbohydrates	+
2	Saponins	+
3	Alkaloids	–
4	Glycosides	–
5	Steroids	–
6	Flavonoids	–
7	Phenolics and Tannins	+
8	Coumarin Glycosides	–
9	Fat and Oils	-

Present (+), Absent (-)

Qualitative phytochemical screening *Senna Occidentalis* L leaves extract. The presence of alkaloids was confirmed by Hager's, Mayer's, Wagner's, and Dragendorff's tests. The presence of phenol was confirmed by litmus, ferrous chloride, and phthalein dye tests. The presence of saponin was confirmed by the foam test. For the confirmation of flavonoids sulfuric acid test and lead acetate test were performed. While the presence ofsteroids was confirmed through the Libermann-Burchard test.

PRELIMINARY PHYTOCHEMICAL SCREENING OF PETROLEUM EXTRACT

Preliminary phytochemical screening of petroleum extract revealed the presence of alkaloids,saponins, phenols and tannins, Flavonoids, Terpenoids, and Steroids.

S. No.	Phytochemical	Petroleum extract of *Senna Occidentalis* L.
1.	Saponins	-
2.	Alkaloids	-
3.	Glycosides	-
4.	Steroids	-
5.	Flavonoids	-
6.	Phenolics and Tannins	+
7.	Coumarin Glycosides	-

Present (+), Absent (-)

Qualitative phytochemical screening *Senna Occidentalis* L leaves extract. The presence of alkaloids was confirmed by Hager's, Mayer's, Wagner's, and Dragendorff's tests. The presence of phenol was confirmed by litmus, ferrous chloride, and phthalein dye tests. The presence of saponin was confirmed by the foam test. For the confirmation of flavonoids sulfuric acid test and lead acetate test were performed. While the presence of steroids was confirmed through the Libermann-Burchard test.

GC Chromatogram of *senna occidentalis* L root ethanol acid extract.

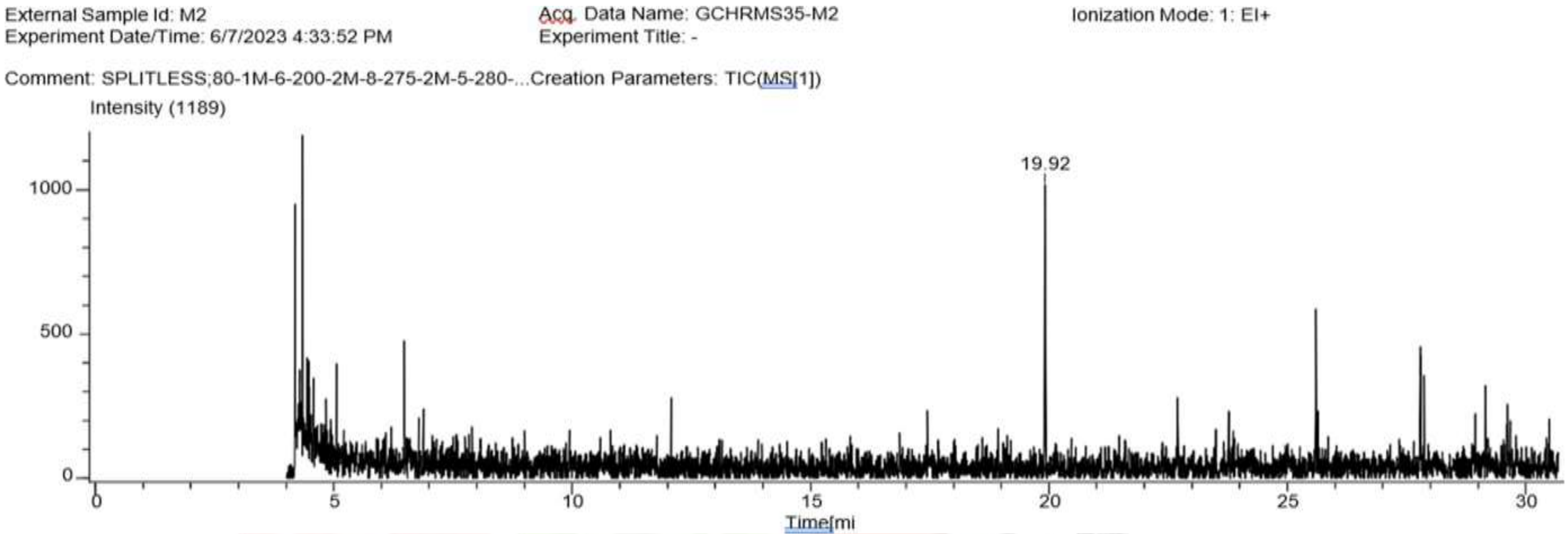

Fig. 16 GC Chromatogram of senna occidentalis L root ethanol acid extract.

Table 9 GC Chromatogram of senna occidentalis L root ethanol acid extract.

Peak no.	Time (min.)	Type	Peak width FWH (min.)	Area (intens. *sec)	Height	Description	Start Point		End Point	
							Time (min.)	Height	Time (min.)	Height
1	19.92	BB	0.0177	1183.37	994.00		19.89	47	19.95	1

NIST MS SPECTRA FOR COMPOUND OPTEND FROM senna occidentalis L root extract.HIT FOR COMPOUNDS 1RT = 19.92

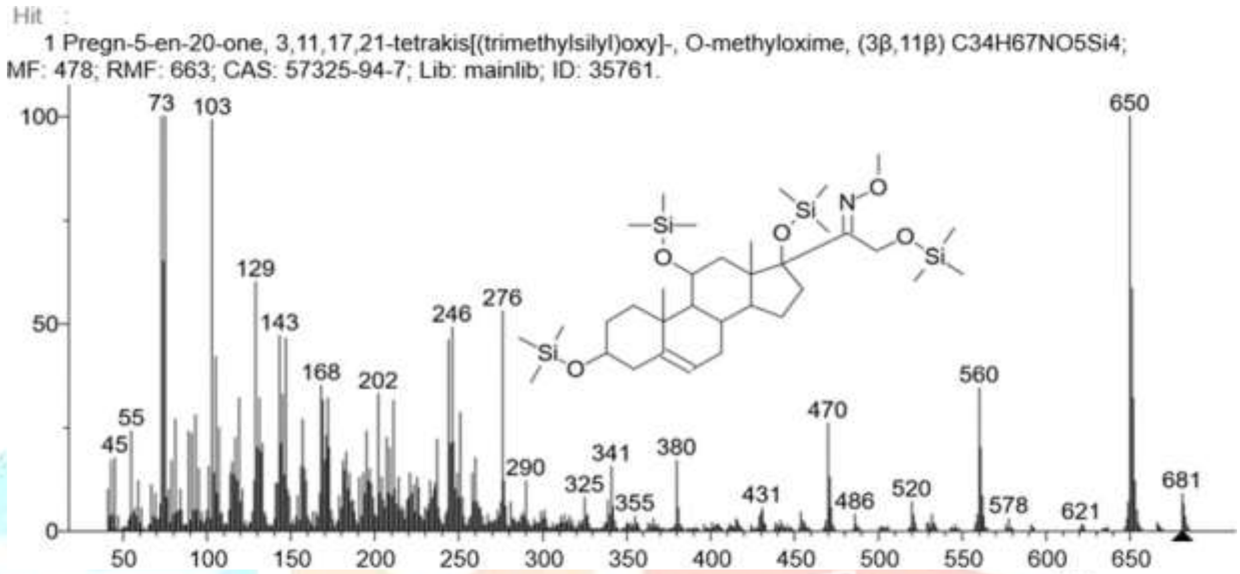

Fig. 17 HIT FOR COMPOUNDS 1RT = 19.92

Table 10 NIST MS SPECTRA FOR COMPOUND OPTEND FROM senna occidentalis L root extract.

Peaks found incom. 1RT 19.92	53	73.08	128.31	196.69	198.81	233.19	246.02	308.10	365.53
Peaks found inNIST MS SPECTRA	45	55	73	103	129	143	168	202	246

LCMS

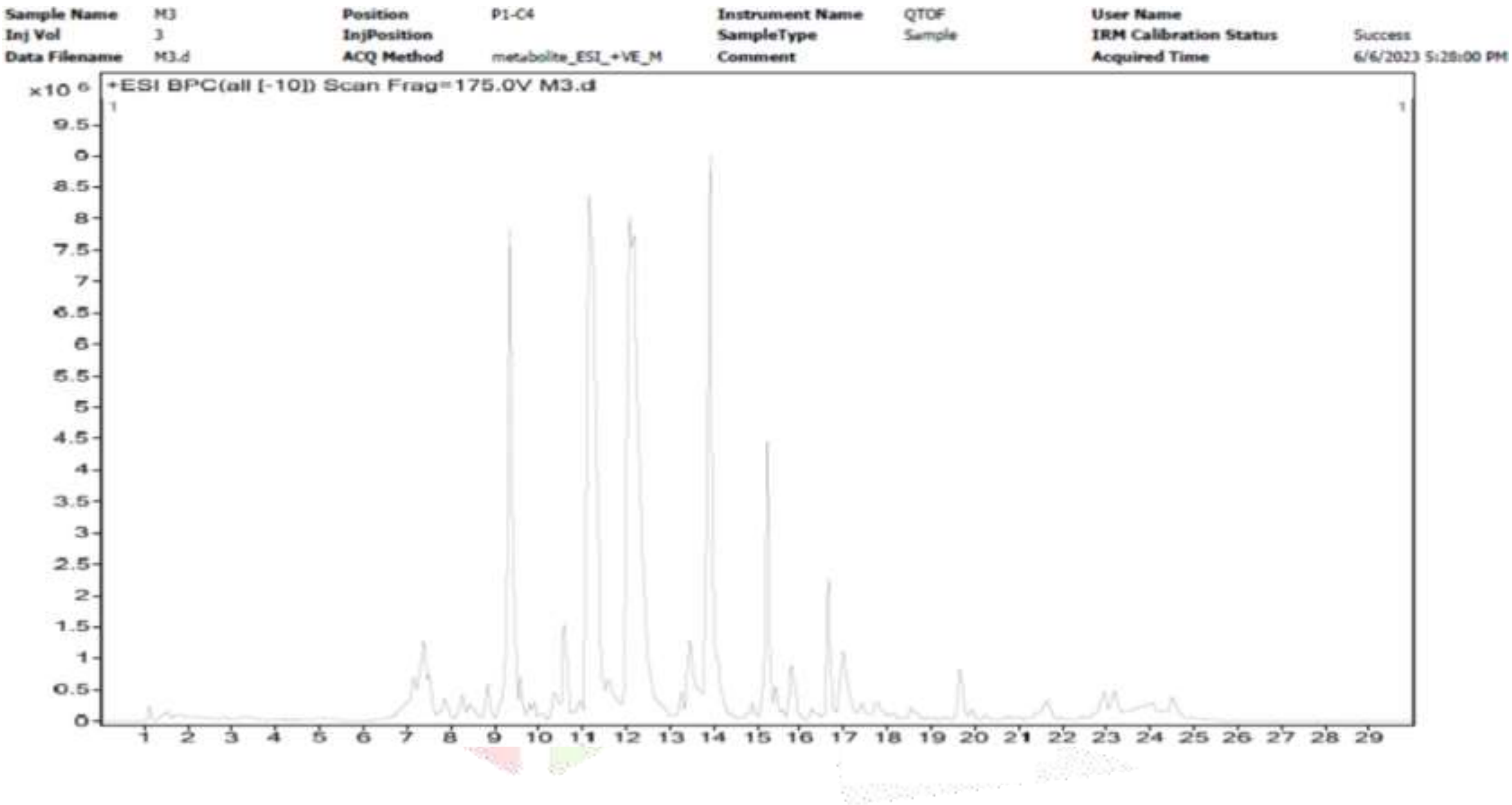

Fig. 18 LCMS chromatogram

Table 11 LCMS (during analysis P polarity: positive and algorithm: Auto MS/MS)

Cpd	Name	Formula	Score	Mass	Mass (DB)	Base Peak	m/z	RT	Height	Score (DB)	Hits (DB)
1	Brassilexin	C9 H6 N2 S	45.27	174.0275	174.0252	116.9938	175.035	1.198	102735	45.27	10
2	Cyclohexylamine	C6 H13 N	9.69	99.0978	99.1048	122.0949	122.0951	6.972	75446	9.69	10
3	N-heptanoyl-homoserine lactone	C11 H19 N O3	84.25	213.1345	213.1365	214.1406	214.1418	6.985	218115	84.25	1
4	Viloxazine	C13 H19 N O3	99.61	237.1366	237.1365	242.1156	260.1259	7.126	197169	99.61	10
5	N-heptanoyl-homoserine lactone	C11 H19 N O3	85.52	213.1346	213.1365	214.1414	214.1419	7.262	378336	85.52	1
6	1-(2,3-Dihydro-6-methyl-1H-pyrrolizin-5-yl)-1,4-pentanedione	C13 H17 N O2	98.86	219.1261	219.1259	242.1152	242.1153	7.337	106262	98.86	10
7	Viloxazine	C13 H19 N O3	99.03	237.1368	237.1365	242.115	260.126	7.407	125746	99.03	10
8	N-heptanoyl-homoserine lactone	C11 H19 N O3	86.47	213.1346	213.1365	214.1417	214.1419	7.549	124045	86.47	1
9	N6,N6-Dimethyladenosine	C12 H17 N5 O4	51.98	295.1251	295.1281	164.0913	296.1325	7.713	106484	51.98	8
10	Diethyl (2R,3R)-2-methyl-3-hydroxy succinate	C9 H16 O5	96.61	204.0999	204.0998	153.0535	227.0892	7.821	116540	96.61	10
11	Unknown					136.1103	228.1574	7.869	156081		
12	(Z)-3-(1-Formyl-1-propenyl)pentanoic acid	C9 H12 O5	85.34	200.0688	200.0685	223.0582	223.058	7.977	80702	85.34	10

13	N-[2-(4-Hydroxyphenyl)ethyl]benzamide	C15 H15 N O2	70.97	241.109	241.1103	242.1153	242.1156	8.473	138668	70.97	10
14	Fluenetil	C16 H15 F O2	78.64	258.1055	258.1056	144.0651	259.1126	8.574	80366	78.64	2
15	Unknown					228.1574	228.1578	8.93	204264		
16	2-Phenyl-2-butenal	C10 H10 O	46.5	146.0727	146.0732	169.0747	169.062	8.961	154699	46.5	10
17	Glycerol 1-propanoate diacetate	C10 H16 O6	46.81	232.0951	232.0947	233.1267	233.1024	9.193	103923	46.81	2
18	Dioscoretine	C13 H23 N O3	71.09	241.1657	241.1678	242.1727	242.173	9.213	195844	71.09	2
19	Gentisyl alcohol	C7 H8 O3	92.73	140.0484	140.0473	163.0376	163.0376	9.35	817508	92.73	10
20	6-beta-D-Glucopyranosyl-4',5-dihydroxy-3',7-dimethoxyflavone	C23 H24 O11	73.99	476.1278	476.1319	315.0839	477.1352	9.465	127142	73.99	9
21	Picrotoxinin	C15 H16 O6	94.19	292.0947	292.0947	300.0603	315.0837	9.466	165103	94.19	10
22	Dioscoretine	C13 H23 N O3	84.37	241.1657	241.1678	242.1732	242.173	9.487	753494	84.37	2
23	11-amino-undecanoic acid	C11 H23 N O2	97.02	201.1732	201.1729	122.0951	224.1624	9.498	110711	97.02	4
24	N-Glycosyl-L-asparagine	C10 H18 N2 O8	86.06	294.1079	294.1063	191.0319	295.115	9.555	126205	86.06	6
25	Quercetin	C15 H10 O7	77.19	302.0399	302.0426	303.0473	303.0471	9.613	107246	77.19	10
26	Gentisyl alcohol	C7 H8 O3	90.51	140.0484	140.0473	163.0372	163.0375	9.617	150601	90.51	10

27	Terbutaline	C12 H19 N O3	48.37	225.1335	225.1365	180.1002	226.1416	9.637	81818	48.37	3
28	Vanillin acetate	C10 H10 O4	78.64	194.0582	194.0579	189.0524	217.0475	10.371	84054	78.64	10
29	2-Methoxy-4-(4-methyl-1,3-dioxolan-2-yl)phenol	C11 H14 O4	86.28	210.0894	210.0892	233.0781	233.0786	10.375	207524	86.28	10
30	Ethyl vanillin isobutyrate	C13 H16 O4	85.78	236.1053	236.1049	189.0531	259.0945	10.426	218106	85.78	10
31	MeIQ	C12 H12 N4	94.9	212.1052	212.1062	191.0686	235.0944	10.59	219952	94.9	10
32	Unknown					301.0659	300.2138	10.704	85282		
33	Unknown					218.2092	218.2096	10.712	217477		
34	Phaseolic acid	C13 H12 O8	96.81	296.0529	296.0532	273.0373	319.0421	10.74	99514	96.81	10
35	2-Methoxy-4-(4-methyl-1,3-dioxolan-2-yl)phenol	C11 H14 O4	82.96	210.0898	210.0892	215.0684	233.0789	10.754	76051	82.96	10
36	(+)-Sophorol	C16 H12 O6	79.26	300.0607	300.0634	286.0438	301.0679	10.837	219547	79.26	10
37	Unknown					186.1838	234.2044	10.863	91870		
38	Pinacidil	C13 H19 N5	55.59	245.16	245.164	172.1309	246.1675	10.879	81859	55.59	1
39	Metyrosine	C10 H13 N O3	64.19	195.0891	195.0895	144.0436	218.0795	10.883	89708	64.19	9
40	Glu-P-1	C11 H10 N4	78.48	198.0894	198.0905	179.0687	221.0786	10.985	126474	78.48	10

41	Picrotoxinin	C15 H16 O6	96.8	292.0951	292.0947	300.0604	315.0842	11.206	409150	96.8	10
42	Picrotoxinin	C15 H16 O6	99.35	292.0946	292.0947	300.0598	315.0837	11.487	496539	99.35	10
43	Picrotoxinin	C15 H16 O6	98.74	292.0945	292.0947	300.0598	315.0837	11.751	376861	98.74	10
44	Ethyl vanillin isobutyrate	C13 H16 O4	85.91	236.105	236.1049	189.0521	259.0943	11.811	109944	85.91	10
45	Picrotoxinin	C15 H16 O6	96.64	292.0953	292.0947	300.0603	315.0846	12.023	1427029	96.64	10
46	Primidone	C12 H14 N2 O2	86.18	218.1053	218.1055	167.059	241.0946	12.037	122071	86.18	6
47	Unknown					299.0519	300.0602	12.094	243235		
48	Quercetin	C15 H10 O7	87.57	302.0407	302.0426	303.0473	303.048	12.158	3240505	87.57	10
49	Unknown					184.1312	300.214	12.276	201772		
50	Picrotoxinin	C15 H16 O6	99.43	292.0944	292.0947	300.0599	315.0836	12.29	1560968	99.43	10
51	Primidone	C12 H14 N2 O2	75.1	218.1054	218.1055	167.0585	241.0946	12.307	82773	75.1	6
52	Unknown					246.2404	246.2409	12.381	356553		
53	Unknown					228.2306	290.2666	12.413	160253		
54	Quercetin	C15 H10 O7	81.42	302.0401	302.0426	303.0464	303.0473	12.419	289350	81.42	10

55	Cyclic de-hypoxanthine futalosine	C14 H14 O7	99.47	294.0737	294.074	302.0388	317.0629	12.49	185091	99.47	10
56	3,9-Dimethoxypterocarpan	C17 H16 O4	64	284.1022	284.1049	125.0583	307.0913	12.493	154922	64	8
57	Unknown					125.0578	306.2609	12.539	105885		
58	Pseudopurpurin	C15 H8 O7	53.49	300.0249	300.027	303.0471	301.0319	12.562	300013	53.49	1
59	N-heptanoyl-homoserine lactone	C11 H19 N O3	81.29	213.1346	213.1365	112.0739	214.1417	12.576	100549	81.29	1
60	Picrotoxinin	C15 H16 O6	84.52	292.0941	292.0947	300.0597	315.0834	12.579	215080	84.52	10
61	Quercetin	C15 H10 O7	80.83	302.04	302.0426	303.047	303.0473	12.73	153859	80.83	10
62	Terbutaline	C12 H19 N O3	82.21	225.1344	225.1365	124.0738	226.1417	12.941	134239	82.21	3
63	Quercetin	C15 H10 O7	77.83	302.0398	302.0426	303.0466	303.047	13.051	162970	77.83	10
64	Unknown					242.2826	242.2819	13.075	82750		
65	(+)-Sophorol	C16 H12 O6	65.48	300.0607	300.0634	286.0442	301.068	13.297	383556	65.48	10
66	Cyclic de-hypoxanthine futalosine	C14 H14 O7	99.04	294.0735	294.074	317.0624	317.0627	13.499	383598	99.04	10
67	(+)-Sophorol	C16 H12 O6	65.7	300.0607	300.0634	286.0442	301.0681	13.642	151752	65.7	10
68	N-heptanoyl-homoserine lactone	C11 H19 N O3	72.12	213.1373	213.1365	134.0582	236.1261	13.689	138667	72.12	3

69	Isomyristicin	C11 H12 O3	86.1	192.0789	192.0786	215.0691	215.0682	13.695	91673	86.1	10
70	Phytosphingosine	C18 H39 N O3	79.55	317.2903	317.293	256.2608	318.2976	13.967	215670	79.55	1
71	Unknown					242.2457	334.2921	14.039	203966		
72	Unknown					242.2453	290.2663	14.04	129651		
73	Aminopentol	C22 H47 N O5	75.02	405.3418	405.3454	300.2867	406.3492	14.092	81493	75.02	1
74	Leucyl-Valine	C11 H22 N2 O3	87.33	230.1613	230.163	101.0335	231.1685	14.171	325087	87.33	6
75	Lauroyl diethanolamine	C16 H33 N O3	63.57	287.2432	287.246	116.069	288.2505	14.195	126319	63.57	1
76	Phytosphingosine	C18 H39 N O3	79.71	317.2903	317.293	167.0318	318.2976	14.328	113989	79.71	1
77	Tutin	C15 H18 O6	96.51	294.1097	294.1103	167.0322	317.0988	14.406	118025	96.51	10
78	Quercetin	C15 H10 O7	77.81	302.0398	302.0426	303.0463	303.047	14.605	110323	77.81	10
79	8-Deoxy-11,13-dihydroxygrosheimin	C15 H20 O5	82.5	280.1306	280.1311	303.0465	303.1197	14.624	123398	82.5	10
80	3-Hydroxy-6,8-dimethoxy-7(11)-eremophilen-12,8-olide	C17 H26 O5	74.68	310.1771	310.178	231.0991	333.1665	14.816	127175	74.68	10
81	Picrotoxinin	C15 H16 O6	82.15	292.0939	292.0947	297.0736	315.0832	14.876	135955	82.15	10

82	Atenolol	C14 H22 N2 O3	73.89	266.1635	266.163	187.0846	289.1525	14.889	84515	73.89	7
83	Tocopheronic acid	C16 H22 O5	98.56	294.1462	294.1467	260.1016	317.1354	14.94	138718	98.56	9
84	Unknown					225.147	225.1703	15.197	83211		
85	Sphinganine	C18 H39 N O2	80.76	301.2954	301.2981	302.3014	302.3027	15.234	267264	80.76	1
86	Phytosphingosine	C18 H39 N O3	79.88	317.2902	317.293	270.2755	318.2975	15.317	150886	79.88	1
87	Unknown					198.1464	300.2134	15.529	87598		
88	Sphinganine	C18 H39 N O2	79.8	301.2953	301.2981	302.302	302.3026	15.532	80223	79.8	1
89	(+)-Sophorol	C16 H12 O6	77.98	300.0607	300.0634	269.0422	301.0681	15.825	1014831	77.98	10
90	Cajanin	C16 H12 O6	62.71	300.0604	300.0634	269.0418	301.0677	16.034	162032	62.71	10
91	Unknown					212.2358	304.2971	16.233	80566		
92	6-Hydroxypentadecanedioic acid	C15 H28 O5	91.31	288.1928	288.1937	209.1149	311.1822	16.295	126759	91.31	4
93	Tocopheronic acid	C16 H22 O5	91.49	294.1464	294.1467	167.0667	317.1354	16.305	116034	91.49	9
94	Unknown					330.3328	330.3338	16.632	122464		

95	L-phenylalanyl-L-hydroxyproline	C14 H18 N2 O4	88.97	278.1255	278.1267	106.0646	301.1148	17.141	90583	88.97	10
96	Quercetin	C15 H10 O7	82.01	302.0402	302.0426	303.0469	303.0474	17.353	100758	82.01	10
97	Oxacyclotetradecan-2-one	C13 H24 O2	97.8	212.1779	212.1776	235.1671	235.1671	19.637	88083	97.8	10
98	6-Oxabicyclo[3.1.0]hexane-2-undecanoic acid methylester	C17 H30 O3	88.64	282.2185	282.2195	263.1977	305.2079	19.934	83170	88.64	10
99	Farnesyl pyrophosphate	C15 H28 O7 P2	87.47	382.1304	382.131	105.0686	383.1376	22.788	83596	87.47	10
100	Unknown					283.261	512.4993	23.2	96894		

Results- Unknown Compounds.

Compound 11-found to be unknown having m/z 228.1574, RT 7.869, Compound 15-found to unknown having m/z 228.1578, RT 8.93, Compound 32-found to be unknown having m/z 300.2138, RT 10.704, Compound 33-found to unknown having m/z 218.2096, RT 10.712,Compound 37-found to unknown having m/z 234.2044, RT 10.863, Compound 47-found to unknown having m/z 300.0602, RT 12.094, Compound 49-found to unknown having m/z 300.214, RT 12.276, Compound 52-found to unknown having m/z 246.2404, RT 12.381, Compound 53-found to unknown having m/z 290.2666, RT 12.413, Compound 57-found to unknown having m/z 306.2609, RT 12.539,Compound 64-found to unknown having m/z 242.2819, RT 13.075, Compound 71-found to unknown having m/z 334.2921, RT 14.039, Compound 72-found to unknown having m/z 290.2663, RT 14.04, Compound 84-found to unknown having m/z 225.1703, RT 15.197, Compound 87-found to unknown having m/z 300.2134, RT 15.529, Compound 91-found to unknown having m/z 304.2971, RT 16.233

Compound 94-found to be unknown having m/z 330.3338, RT 16.632, Compound 100-found to be unknown having m/z 512.4993, RT 23.2 detectedby Auto MS/MS

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