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GC-MS Analysis of Phytochemical Compounds in the Ethanolic Extract of Root of *Lawsonia inermis Linn*.

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Abstract : The aim of the present study is to investigate the ethanolic extract of root of *Lawsonia inermis* for its phytochemical compounds using GC-MS. The shade dried root of plant powder *Lawsonia inermis* was extracted with ethanol and crude ethanolic extract was obtained. The GC-MS analysis was performed by using Perkin Elmer Clarus 500 model with the software Turbomass ver 5.2. The GC-MS analysis shows different peaks with low and high molecular weight determining the presence of 41 phytochemical compounds. The Phytochemical compounds in the ethanolic extract of *Lawsonia inermis* have been screened by using GC-MS analysis. The presence of these compounds may proceed to find out various therapeutic activities.
Keywords: *Lawsonia inermis* Ethanol extract; GC-MS analysis; Phytochemical compounds.

Introduction

For millennia, people around the world have healed the sick with herbal derived remedies, and handed down through generations. Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness [1]. In developing countries, the practice of medicine still relies heavily on plant and herbal extracts for the treatment of human ailments. Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants, and many of them have been used as traditional medicines [2, 3, 4]. An estimate of the World Health Organization (WHO) states that around 85-90% of the world's population consumes traditional herbal medicines.[5]. Screening of active components from plants has direct to the development of new medicinal drugs which have efficient protection and treatment role against various diseases [6]. Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [7].

Lawsonia inermis is an important medicinal plant in the Indian system of Medicine. It is commonly called henna, which grows in warm and arid regions. The dye derived from green leaves of henna is used to decorate the body with intricate designs and the principle coloring matter is lawsone, 2-hydroxy-1, 4-naphthoqunone [8]. Root is considered as a potent medicine for gonorrhoea and herpes infection. Root is astringent may be pulped and used for sore eyes. Pulped root may also be applied to the heads of children for boils. Cambodians drink a decoction as a diuretic. Decoction of the root generally in combination with prepared indigo as a powerfulabortifacient. The root is supposed to be useful in treatment of hysteria and nervous disorders. [9-11]. Therfore this is an attempt to determine the Phytochemical compounds present in the Ethanol extract of root of *Lawsonia inermis* by Gas chromatography and Mass spectroscopy (GC-MS) technique.

Materials and Methods

Collection and Identification of Plant materials

Dry roots of *Lawsonia inermis* were collected from Thirunelveli district, Tamil Nadu, India. This root was identified and authenticated by Dr. V. Chelladurai, Research officer - Botany (scientist C), Central council for research in Ayurveda and Siddha, Govt. of India; Thirunelveli. The roots were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags till use.

Preparation of extracts

The dried roots were extracted with ethanol using soxhlet extractor. The extract which is obtained is concentrated with rotary evaporator till dry powder was obtained. The final concentrated extract is analyzed by using GC-MS.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS was performed by using PerkinElmer Clarus 500 Model and the software used is Turbomass ver 5.2. The fused silica column was packed with Elite -5MS(5% Phenyl 95% dimethylpolysiloxane,30m x 250µm)The oven temperature was set up from 50ºC with an increase of 8 ^{o}C/min to 220 ^{o}C for 5 min and 7ºC /min to 280 ºC for 15 mins. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. An aliquot of 2µl of sample was injected into the column with the injector temperature at 280ºC and the Split ratio of 10:1. The ionizing energy of 70 eV was used and the electron ionization is involved. The mass range is 40-600amu.The Inlet line temperature was 200 ºC and source temperature was 150 ºC Total GC running time was 50 minutes. The detection employed in NIST 2005 library.

Identification of compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technique (NIST Version-Year 2005) having more patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST data library (version 2005). The name, molecular weight, molecular formula and structure of the components of the test material were determined.

Results and Discussion

GC–MS chromatogram of the ethanol extract of root of *Lawsonia inermis* (Fig. 1) clearly showed fourty one peaks indicating the presence of fourty one phytochemical compounds

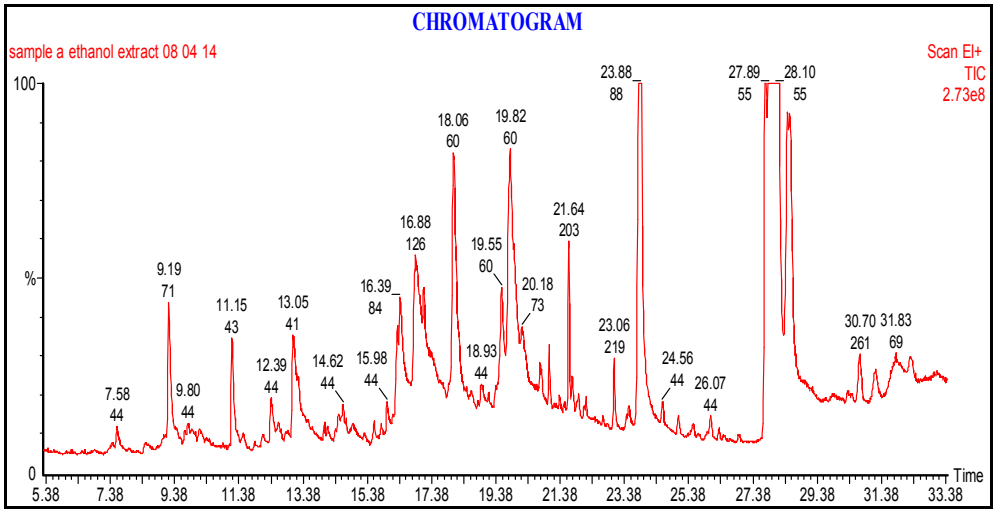


Fig. 1: The GC - MS Chromatogram of ethanol extracts of roots of *Lawsonia inermis*

GC–MS chromatogram of the ethanol extract of root of *Lawsonia inermis* (Fig. 1) clearly shows 41 peaks indicating the presence of 41 phytochemical compounds. The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula. The table 1 shows the compound name with its molecular formula, Retention time, Peak area and % Peak area. The results reveal the presence of Propanoic acid, 2-hydroxy-, ethyl ester, (S)- (0.0768 %), Furfural (0.1258 %),Phenol (0.0985%), Pantolactone (2.8043%), 2,5-Dimethyl-4-hydroxy-3(2H)-furanone(0.1844%), Phenol, 2-methoxy-(0.4438%), 1-Penten-4-one, 2-acetyl-1-dimethylamino-((Z)- or (E)-) (0.2981%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(3.0692%), Octanoic Acid (0.5415%), Benzenecarboxylic acid (0.3254%), Dianhydromannitol (1.3691%), Isosorbide(0.6070%), 2-Furancarboxaldehyde,5-(hydroxymethyl)-(3.6541%),2-Methoxy-4-vinyl phenol (0.2117%), Flucytosine(0.2586%),Phenol, 2,6-dimethoxy-(0.3026%), Hydroquinone(0.1595%), 1,4-Naphtha lenedione (0.4559%),1,4-Benzenediol, 2-methoxy-(0.3501%), 2-Pyrrolidinecarboxylic acid-5-oxo-ethyl ester(0.4166%), 1,2,3-Benzenetriol (6.7698%), 1,4-Naphthalenediol(0.5808%), D-Allose(9.1236%), 3-Hydroxy-4-methoxybenzoic acid(0.4677%), Ethyl á-d-riboside(1.8216%), á-D-Glucopyranoside, methyl (6.6434%), Benzyl Benzoate(0.6915%), Quinolin-8-amine, 5,6-dimethoxy-4-methyl-(2.0762%), 5-Amino-7,8-dimethoxyisoquinoline(0.2026%), Pentadecanoic acid, ethyl ester(0.1154%), 3,5-di-tert-Butyl-4-hydroxy benzaldehyde (1.0592%), (E)-9-Octadecenoic acid ethyl ester(0.1128%), Hexadecanoic acid, ethyl ester(28.1707%), 1,4-Dihydroxy-3-methylnaphthalene-2-carboxylic acid, methyl ester(0.4004%), 9-Octa decenoic acid (Z)-, methyl ester(0.1766%), Tridecanoic acid, methyl ester(0.1604%), 9,12-Octa decadienoic acid, ethyl ester(3.5158%), 9-Octadecenoic acid, ethyl ester(13.4028%), 6-Octadecenoic acid, (Z)-(6.1856%), 3-t-Butyl-4,5-diphenyl-1H-pyrazole(1.3687%), Benzoic acid, 2-hydroxy-5-iodo-, methyl ester(1.2015%). The spectrum sketch out of GC-MS confirmed the presence of 41 components with the retention time 4.34, 4.91, 8.48, 9.19, 9.69, 9.79, 10.16, 11.15, 11.15, 12.14,12.39, 12.60, 13.05, 14.04, 14.13, 14.62, 14.93, 15.98, 16.31, 16.39, 16.87,17.13, 18.06, 18.93, 19.55, 19.82, 21.04, 21.64, 21.74, 22.19, 23.06, 23.15, 23.88, 24.56, 26.33, 26.94, 27.77, 27.89, 28.10, 30.70, 31.20 min respectively which is shown in Figure. 1. The individual fragmentation patterns of necessary components were illustrated in Figures A-S. The phytochemical compounds recognized through GC-MS analysis showed many biological activities are listed in Table 2.

Table 1: Phytochemical compounds identified in ethanol extract of roots of *Lawsonia inermis*

S.No.	Peak Name	Molecular formula	Molecular weight	Retention Time	% Peak area
1	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-	$C_5H_{10}O_3$	118	4.34	0.0768
2	Furfural	$C_5H_4O_2$	96	4.91	0.1258
3	Phenol	C_6H_6O	94	8.48	0.0985
4	Pantolactone	$C_6H_{10}O_3$	130	9.19	2.8043
5	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128	9.69	0.1844
6	Phenol, 2-methoxy-	$C_7H_8O_2$	124	9.79	0.4438
7	1-Penten-4-one, 2-acetyl-1-dimethylamino- ((Z)- or (E)-)	$C_9H_{15}NO_2$	169	10.16	0.2981
8	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	11.15	3.0692

9	Octanoic Acid	$C_8H_{16}O_2$	144	11.51	0.5415
10	Benzenecarboxylic acid	$C_7H_6O_2$	122	12.14	0.3254
11	Dianhydromannitol	$C_6H_{10}O_4$	146	12.39	1.3691
12	Isosorbide	$C_6H_{10}O_4$	146	12.60	0.6070
13	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	13.05	3.6541
14	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	14.04	0.2117
15	Flucytosine	$C_4H_4FN_3O$	129	14.13	0.2586
16	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154	14.62	0.3026
17	Hydroquinone	$C_6H_6O_2$	110	14.93	0.1595
18	1,4-Naphthalenedione	$C_{10}H_6O_2$	158	15.98	0.4559
19	1,4-Benzenediol, 2-methoxy-	$C_7H_8O_3$	140	16.31	0.3501
20	2-Pyrrolidinecarboxylic acid-5-oxo-, ethyl ester	$C_7H_{11}NO_3$	157	16.39	0.4166
21	Name: 1,2,3-Benzenetriol	$C_6H_6O_3$	126	16.87	6.7698
22	1,4-Naphthalenediol	$C_{10}H_8O_2$	160	17.13	0.5808
23	D-Allose	$C_6H_{12}O_6$	180	18.06	9.1236
24	3-Hydroxy-4-methoxybenzoic acid	$C_8H_8O_4$	168	18.93	0.4677
25	Ethyl á-d-riboside	$C_7H_{14}O_5$	178	19.55	1.8216
26	á-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194	19.82	6.6434

27	Benzyl Benzoate	$C_{14}H_{12}O_2$	212	21.04	0.6915
28	Quinolin-8-amine, 5,6-dimethoxy-4-methyl-	$C_{12}H_{14}N_2O_2$	218	21.64	2.0762
29	5-Amino-7,8-dimethoxyisoquinoline	$C_{11}H_{12}N_2O_2$	204	21.74	0.2026
30	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	270	22.19	0.1154
31	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	234	23.06	1.0592
32	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	23.15	0.1128
33	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	23.88	28.1707
34	1,4-Dihydroxy-3-methylnaphthalene-2-carboxylic acid, methyl ester	$C_{13}H_{12}O_4$	232	24.56	0.4004
35	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	26.33	0.1766
36	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	26.94	0.1604
37	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	27.77	3.5158
38	9-Octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310	27.89	13.4028
39	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	28.10	6.1856
40	3-t-Butyl-4,5-diphenyl-1H-pyrazole	$C_{19}H_{20}N_2$	276	30.70	1.3687

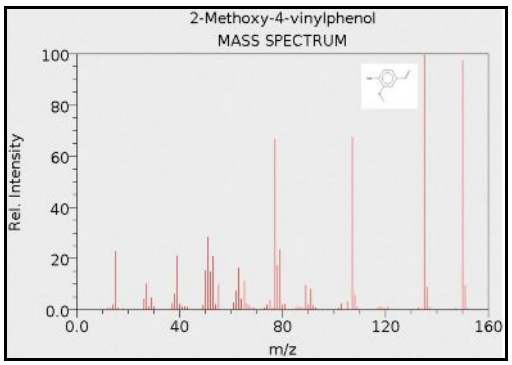
41	Benzoic acid, 2-hydroxy-5-iodo-, methyl ester	$C_8H_7IO_3$	278	31.20	1.2015

Table 2: Biological activities of phytochemical compounds identified in Ethanol extract of roots of Lawsonia *inermis*

No.	Name of the compound	**Biological Activity
1.	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-	No Activity reported
2.	Furfural	Antiseptic, Flavor, Fungicide, Insecticide, Irritant, Pesticide [12]
3.	Phenol	Antiseptic, Topical Anesthetic [13]
4.	Pantolactone	No Activity reported
5.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	Antioxidative [14]
6.	Phenol, 2-methoxy-	Expectorant, antiseptic and local anesthetic [15]
7.	1-Penten-4-one, 2-acetyl-1-dimethylamino- ((Z)- or (E)-)	No activity reported
8.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	No Activity reported
9.	Octanoic Acid	Candidicide, Flavor, Fungicide, Perfumery, Pesticide [12]
10.	Benzenecarboxylic acid	Perfumes, flavorings and anti-fungal agent. [16]
11.	Dianhydromannitol	Antimicrobial [12]
12.	Isosorbide	Diuretic [17]
13.	2-Furancarboxaldehyde,-5-(hydroxymethyl)-	Antimicrobial [12]
14.	2-Methoxy-4-vinylphenol	Flavor and Perfumery [12]
15.	Flucytosine	Antifungal agent [18]
16.	Phenol, 2,6-dimethoxy-	No Activity reported
17.	Hydroquinone	Catastrophic Activity [19]
18.	1,4-Naphthalenedione	Antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory and antipyretic [20]
19.	1,4-Benzenediol, 2-methoxy-	No Activity reported
20.	2-Pyrrolidinecarboxylic acid-5-oxo-, ethyl ester	No Activity reported

21.	1,2,3-Benzenetriol	Antioxidant, Antiseptic, Antibacterial, Antidermatitic, Fungicide, Pesticide, Antimutaginic Dye [12]
22.	1,4-Naphthalenediol	Larvicidal activity [21]
23.	D-Allose	Anti-oxidative activity[22]
24.	3-Hydroxy-4-methoxybenzoic acid	No Activity reported
25.	Ethyl á-d-riboside	No Activity reported
26.	á-D-Glucopyranoside, methyl	No Activity reported
27.	Benzyl Benzoate	Scabicide and pediculicide [23]

28	Quinolin-8-amine, 5,6-dimethoxy-4-methyl-	Fungicides, virucides [12]
29.	5-Amino-7,8-dimethoxyisoquinoline	No Activity reported
30.	Pentadecanoic acid, ethyl ester	No Activity reported
31.	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	No Activity reported
32.	(E)-9-Octadecenoic acid ethyl ester	Antioxidant, anti-inflammatory[12]
33.	Hexadecanoic acid, ethyl ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide,Lubricant, Antiandrogenic, Flavor [12]
34.	1,4-Dihydroxy-3-methylnaphthalene-2-carboxylic acid, methyl ester	No Activity reported
35.	9-Octadecenoic acid (Z)-, methyl ester	Anti inflammatory,Antiandrogenic , Cancer preventive , Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge [12]
36.	Tridecanoic acid, methyl ester	No Activity reported
37.	9,12-Octadecadienoic acid, ethyl ester	Nematicide, hepatoprotective, antihistaminic, anticoronary [12]
38.	9-Octadecenoic acid, ethyl ester	No Activity reported
39.	6-Octadecenoic acid, (Z)-	No Activity reported
40.	3-t-Butyl-4,5-diphenyl-1H-pyrazole	No Activity reported
41.	Benzoic acid, 2-hydroxy-5-iodo-, methyl ester	No Activity reported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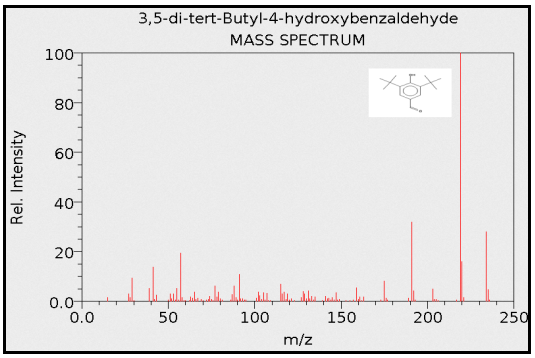
m/z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Rel. Intensity
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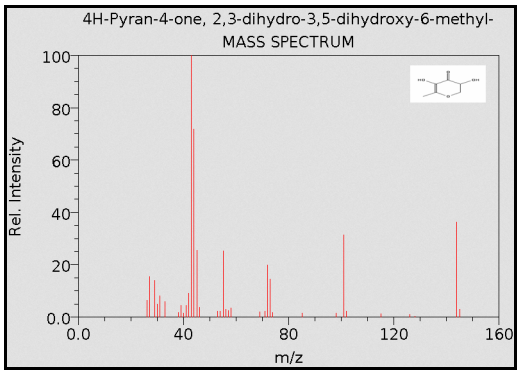
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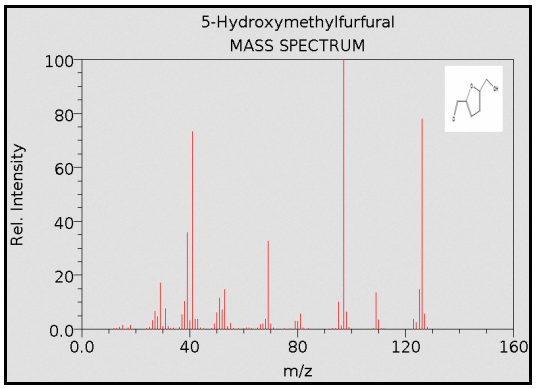
Rel. Intensity
m/z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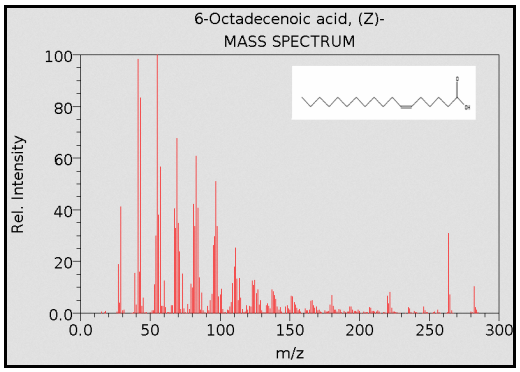
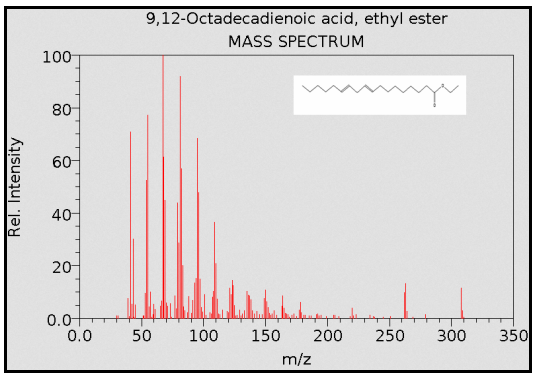

6-Octadecenoic acid, (Z)-
MASS SPECTRUM
Rel. Intensity
m/z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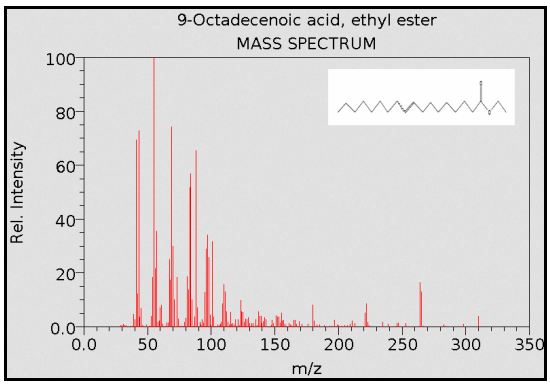
MASS SPECTRUM
Rel. Intensity
m/z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MASS SPECTRUM
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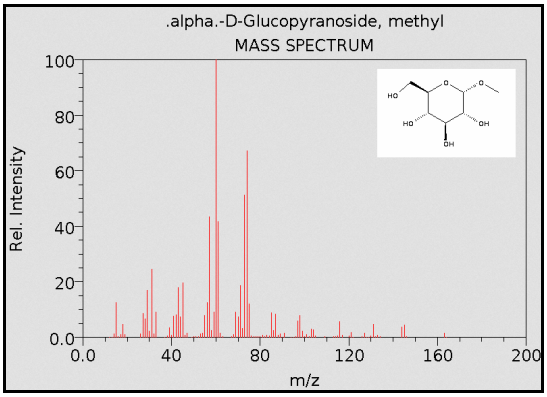
m/z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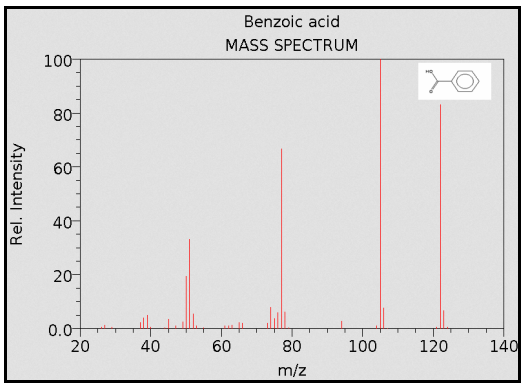
MASS SPECTRUM
Rel. Intensity
m/z


Benzoic acid
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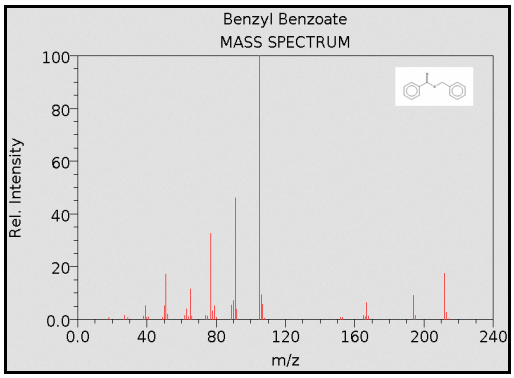
Benzyl Benzoate
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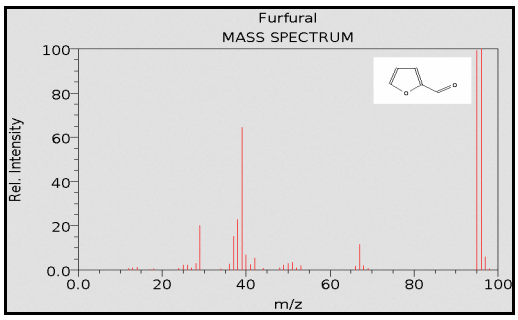
Rel. Intensity
m/z


Furfural
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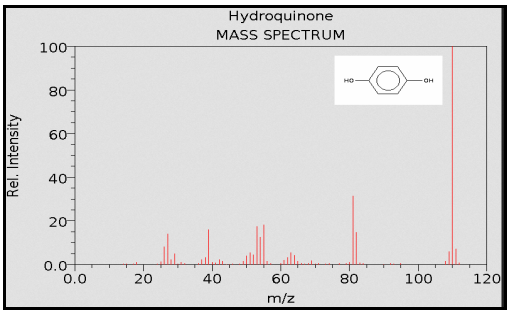
Hydroquinone
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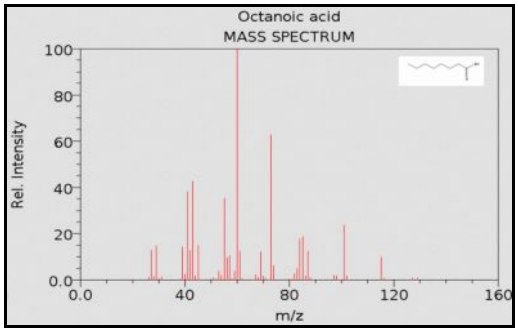
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MASS SPECTRUM
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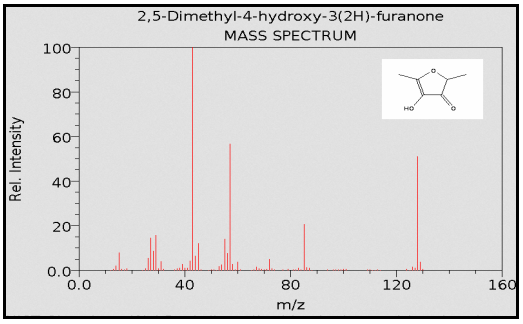
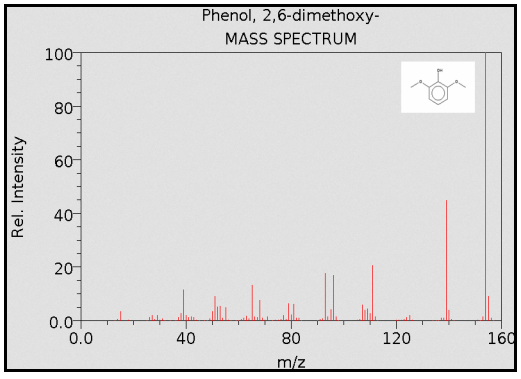
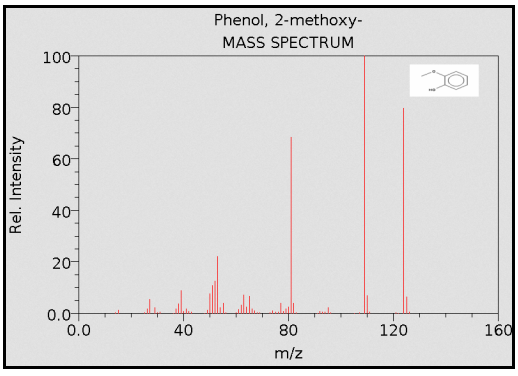
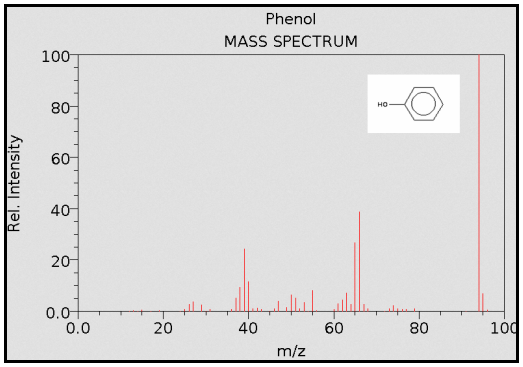
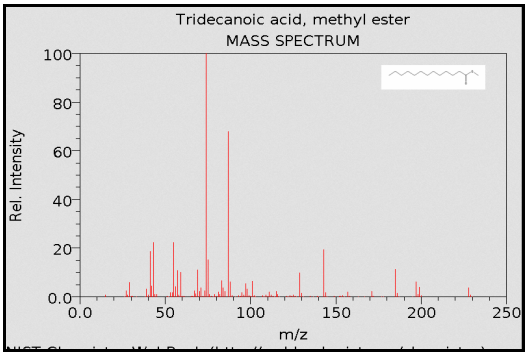
m/z


2,5-Dimethyl-4-hydroxy-3(2H)-furanone
MASS SPECTRUM
Rel. Intensity
m/z


Phenol, 2,6-dimethoxy-
MASS SPECTRUM
Rel. Intensity
m/z


Phenol, 2-methoxy-
MASS SPECTRUM
Rel. Intensity
m/z


Phenol
MASS SPECTRUM
Rel. Intensity
m/z


Tridecanoic acid, methyl ester
MASS SPECTRUM
Rel. Intensity
m/z

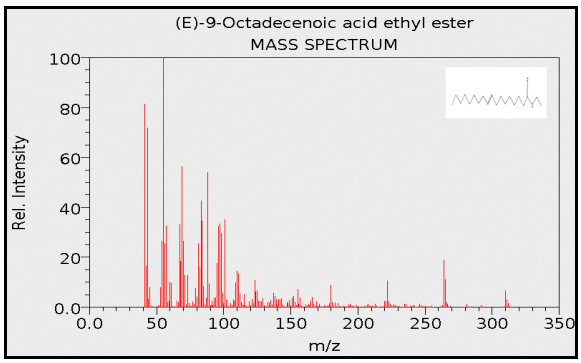


Fig. A-S: The individual fragmentation pattern of the important compounds

Conclusion

In the present study, fourty one phytochemical constituents have been identified from the ethanol extract of roots of *Leptadenia reticulata* by Gas Chromatogram - Mass Spectrometry (GC - MS) analysis. The presence of these phytochemical constituents justifies the use of this plant for various ailments by traditional practitioners. Isolation of individual photochemical constituents and subjecting it to biological activities are being undertaken.

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