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GC-MS Analysis of Phytochemical Compounds of Normal and Leaf Galls of *Madhuca longifolia* (Koenig) j.f.Macb.

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Abstract*: Plant galls are manifestations of growth and of differentiation induced on a plant by animal or plant parasite. Madhuca longifolia (Koenig) J.F. Macb.is widely distributed in tropical mixed deciduous forests in India in the states of Uttar Pradesh, Bihar, Maharashtra, Madhya Pradesh, Kerala, and Rajasthan. Leaf galls of Madhuca longifolia induced by insects of order diptera and hymenoptera present unique pattern of chemical perturbations which normally do not occur in normal morphogenesis of the involved organ. The plant – pathogen interaction leads to production of increased secondary metabolites owing to the stress conditions. The secondary metabolites are supposed to provide resistance against pathogen. GC-MS analysis of the normal and galled leaf tissue of Madhuca longifolia lead to the finding that under stressed conditions larger number of fatty acids and secondary metabolites were produced. Further studies on the efficacy of these fatty acids and secondary metabolites can result into various findings and discovery of novel and useful secondary metabolites resulting in increased resistance against pathogen to host plant and biomedical importance. The fatty acids (oleic acid and palmitic acid) were found in higher amount in dipteran and hymenopteran galls, So these galls can be widely used in traditional medicines and food industry.*

Keywords: *Diptera and hymenoptera, GC-MS, Leaf galls, Madhuca longifolia, Plant-pathogen interaction*

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I. Introduction

Madhuca longifolia, commonly known as ***Mahwa*** or ***Mahua*** is an Indian tropical tree found largely in the central and north Indian plains and forests. It is a fast-growing tree that grows to approximately 20 meters in height, possesses evergreen or semi-evergreen foliage, and belongs to the family Sapotaceae. It is adapted to arid environments, being a prominent tree in tropical mixed deciduous forests in India in the states of Chhattisgarh, Jharkhand, Uttar Pradesh, Bihar, Maharashtra, Madhya Pradesh, Kerala, Gujarat and Orissa and Rajasthan. It is found in deciduous forest throughout the India. The fruit contains 51% valuable oil known as mohua oil or butter of commerce that is used for cooking, illumination, soap and candle making. This tree's bark, leaves, fruits, flowers and seeds everything is useful in making of drugs[1].Bark of *Madhuca longifolia* is used in Rheumatism, ulcers, bleedings and tonsillitis[2].Three different types of galls i.e.(1) Adaxial gall, caused by dipteran **Mohwadiplosis orientalis Rao**, (Dipteran adaxial gall, DAG), (2)Marginal gall caused by insect **dipteran cecidomyiid** (Dipteran marginal gall, DMG) and (3) Abaxial gall caused by insect of **hymenopteran chalcidoiid** (Hymenopteran abaxial gall, HAbG) have been reported on the leaf of *Madhuca longifolia*. These galls vary in their size, shape, colour and distribution. These pathogens cause biotic stress to the plant. A stress can leads to various results. Stress can have a devastating impact on plant growth and yield [3] or can result into enhancement of production of secondary metabolites [4].These secondary metabolites are capable of triggering changes into plants cell which helps to overcome the stress [5].Present study reveals the phytochemical compounds comparative analysis of stress (Galled leaf) and non-stressed (Normal leaf) condition of *Madhuca longifolia* Plant.

II. Material And Methods

2.1 Dry Powder Preparation-

Leaves of *Madhuca* tree were collected from field area of Bharatpur district of state Rajasthan. The normal leaf and galled leaves were separated from plant and washed with tap water to remove soil particle followed by distilled water. Dried normal leaf and galled leaf tissue were pulverized to powder using mechanical grinder.

2.2Preparation of Extract-

About 5 gm powder of normal and infected galled leaves of *Madhuca* was weighed and was extracted with methanol (70-80ºC) by hot continuous percolation method in soxhlet apparatus for 24 hours. The extract

was taken and filtered through whatmann filter paper. Then extract was concentrated by rotary evaporator to obtain extract.

2.3 GC-MS analysis-

The GC-Ms analysis of methanolic extract of normal and galled leaves of *Madhuca longifolia* was carried out on Shimadzu QP-2010 plus with thermal desorption system TD 20. It includes auto sampler and a gas chromatograph which interfaced to a mass spectrophotometer. The column size of this system is 30m × 0.25mm i.d × 0.26µm with a film thickness of 0.26mm, composed of 5MS (5% diphenyl/ 95% dimethyl poly siloxane). Helium gas (99.999%) was used as carrier gas at constant flow rate of 1ml/min. The 2µl injection volume of sample was utilized with split ratio of 10:1.The injector temperature was programmed initially at 280 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (for 4 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were analyzed using electron impact ionization at 70 eV. The total running time for each sample was 45 min.

2.4 Identification of phytochemicals-

Interpretation of phytochemical present in the sample was conducted using NIST, having more than 62,000 patterns and Wiley8 Library. The comparison of unknown spectrum with known spectrum of various components was done by stored spectrum of NIST library and Wiley8 Library. The name, molecular weight and structure of the components were ascertained.

III. Result

GC-MS is a combined technique of Gas Chromatography with Mass Spectrometry. MS is wide ranging analytical technique, which identifies the charged species according to their mass to charge ratio (M/Z). GC-MS is one of the best techniques to identify the constituents of volatile compounds. The GC-MS analysis of normal and galled leaf (DAG Dipteran adaxial gall, DMG Dipteram marginal gall and HAbG Hymenopteran abaxial gall) of *Madhuca longifolia(*Koenig) j.f.Macb. showed the presence of forty one (figure1) fifty two(Figure2), thirty four(figure3) and thirty three (figure4) phytochemical compounds respectively. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), area %, compound name, of normal and leaf galls are presented in Table 1,2,3 and 4 respectively.

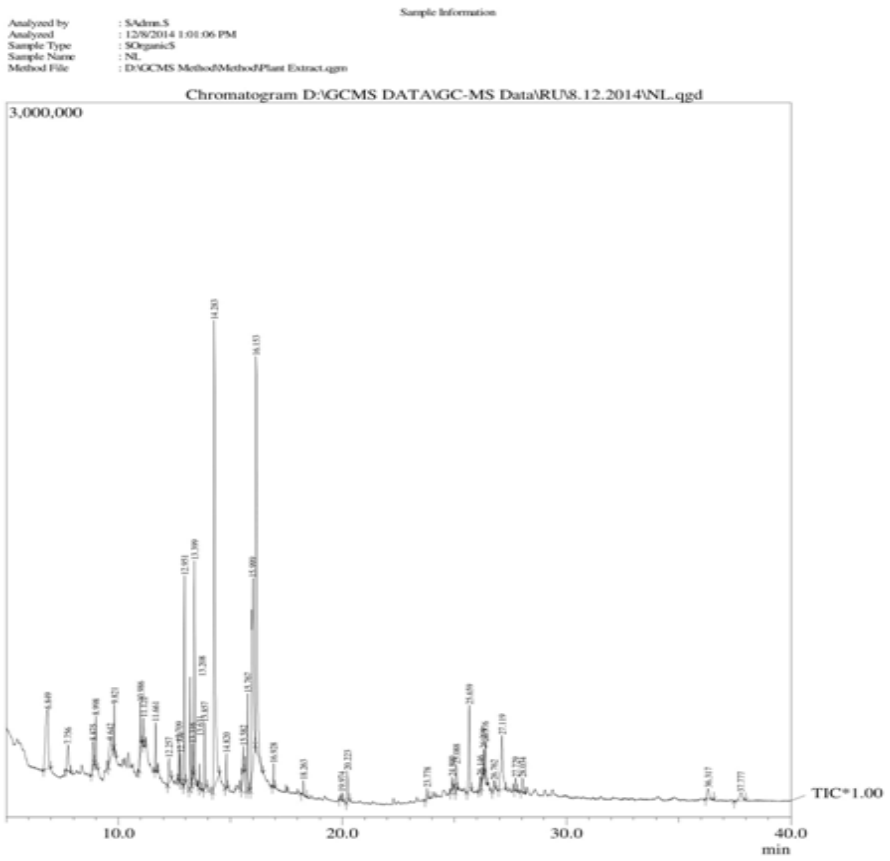

Figure 1:- Shows GC-MS Chromatogram of Normal Leaf Part of *Madhuca longifolia*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	6.849	2150118	5.72	Cyclohexasiloxane, dodecamethyl-
2	7.756	611936	1.63	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester
3	8.875	487687	1.30	3,8-DIMETHYLENE-1-CYCLOOCTENE
4	8.998	586458	1.56	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsilox
5	9.642	696439	1.85	1-Methyl-1-(3-methylbutyl)oxy-1-silacyclobutane
6	9.821	460883	1.23	Cyclohexasiloxane, dodecamethyl-
7	10.986	542648	1.44	Cyclooctasiloxane, hexadecamethyl-
8	11.122	276236	0.74	Ethyl citrate
9	11.661	374940	1.00	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadeca
10	12.257	448362	1.19	TETRADECANOIC ACID
11	12.709	271774	0.72	Cyclohexasiloxane, dodecamethyl-
12	12.779	351723	0.94	2-Ethylhexyl salicylate
13	12.951	1679877	4.47	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
14	13.208	949946	2.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
15	13.316	230733	0.61	BENZOIC ACID, 2,4-BIS(TRIMETHYLSILOXY)-, TRIME
16	13.399	1905136	5.07	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
17	13.631	264699	0.70	6,6-DIMETHYL-4-CYCLOOCTEN-1-ONE
18	13.857	716521	1.91	Hexadecanoic acid, methyl ester
19	14.283	6671346	**17.75**	**n-Hexadecanoic acid**
20	14.820	286752	0.76	1,1,3,3,5,5,7,7,9,9,11,11,13,13-TETRADECAMETHYL-HE
21	15.582	588016	1.56	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
22	15.767	889117	2.37	Octadecanoic acid, methyl ester
23	15.999	4843130	12.89	
24	16.153	5301355	**14.11**	**Octadecanoic acid**
25	16.928	189740	0.50	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxan
26	18.263	123972	0.33	SILICATE ANION TETRAMER
27	19.974	131382	0.35	SILICATE ANION TETRAMER
28	20.223	446374	1.19	1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER
29	23.778	148611	0.40	DECANEDIOIC ACID, DIDECYL ESTER
30	24.890	96744	0.26	Dodecane, 2-methyl-

31	25.088	299157	0.80	LUP-20(29)-ENE-3,28-DIOL, (3.BETA.)-
32	25.659	1271701	3.38	Farnesyl bromide
33	26.146	73137	0.19	Nonane, 5-(2-methylpropyl)-
34	26.299	359945	0.96	ERGOSTA-7,22-DIEN-3-OL, (3.BETA.,22E)-
35	26.376	332538	0.88	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.b
36	26.762	151683	0.40	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-me
37	27.119	1399571	3.72	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
38	27.729	183399	0.49	
39	28.034	260669	0.69	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-
40	36.317	240844	0.64	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B
41	37.777	284639	0.76	Thunbergol
		37579938	100.00	

Table 1:- Compounds identified from methanolic extract of Normal Leaf Part of *Madhuca longifolia* using GC-MS analysis.

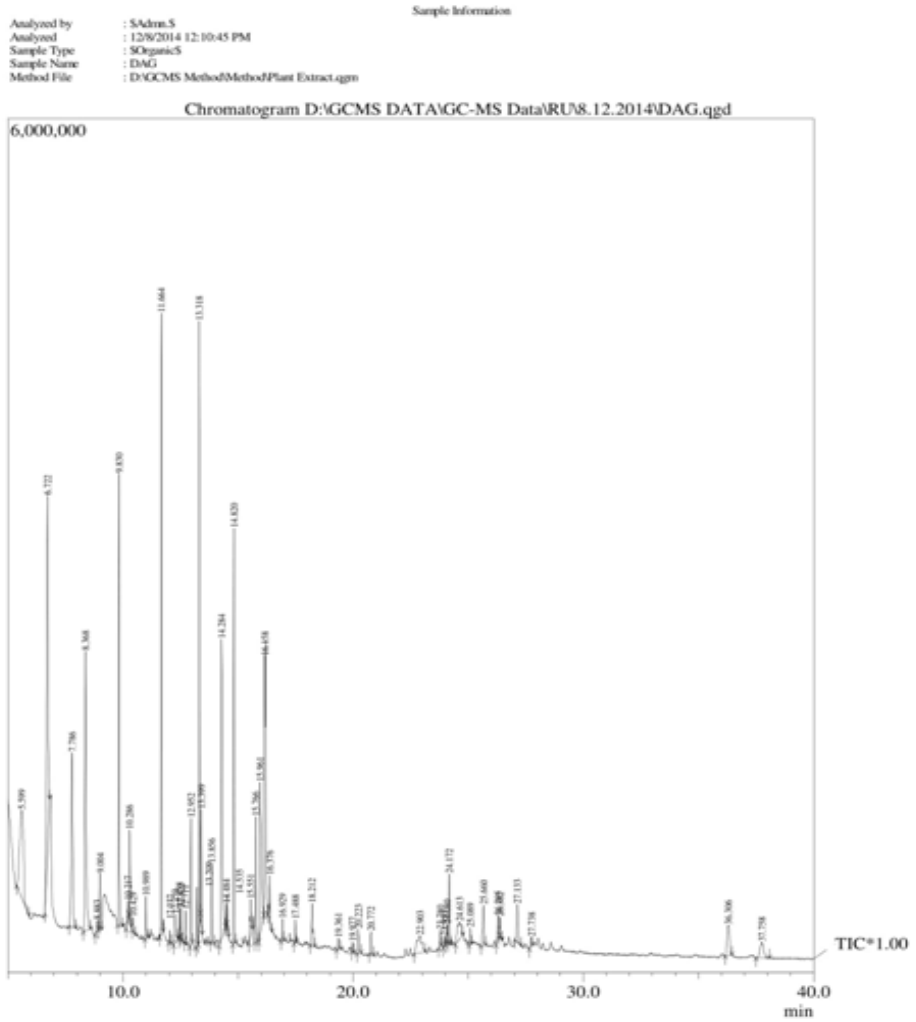

Figure 2:- Shows GC-MS Chromatogram of Dipteran Adaxial Gall (DAG) of *Madhuca longifolia*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	5.599	7097019	6.42	1,1,1,3,5,7,9,9,9-NONAMETHYLPENTASILOXANE
2	6.722	11275642	**10.20**	**1H-PYRROLE-3-PROPANOIC ACID, 4-METHYL-2-[(PHE**
3	7.786	5976101	5.40	BENZENEACETIC ACID, 3-METHOXY-.ALPHA.,4-BIS[(
4	8.368	7357476	**6.65**	**1,2-DIBENZOYLBENZO[G]INDOLIZINE**
5	8.883	326328	0.30	CYCLOHEPTANE, 1,3,5-TRIS(METHYLENE)-
6	9.004	1029922	0.93	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsilox
7	9.830	7002733	6.33	1,1,3,3,5,5,7,7,9,9,11,11,13,13-TETRADECAMETHYL-HE
8	10.217	303184	0.27	E-15-Heptadecenal
9	10.286	1410445	1.28	PHOSPHONIC ACID, METHYLENEBIS-, TETRAKIS(TRI
10	10.429	334310	0.30	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER
11	10.989	655369	0.59	Cyclooctasiloxane, hexadecamethyl-
12	11.664	7689541	6.95	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadeca
13	12.037	213353	0.19	2-(4-Oxo-5-(4-pyridinylmethylene)-2-thioxo-1,3-thiazolidin-3
14	12.259	957450	0.87	TETRADECANOIC ACID
15	12.458	377650	0.34	1-Nonadecene
16	12.522	372864	0.34	TETRADECANE
17	12.711	444374	0.40	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecameth
18	12.952	1725824	1.56	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
19	13.209	841570	0.76	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R
20	13.318	7086835	6.41	Cyclooctasiloxane, hexadecamethyl-
21	13.399	1382986	1.25	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
22	13.856	1351881	1.22	Hexadecanoic acid, methyl ester
23	14.284	6952390	6.29	n-Hexadecanoic acid
24	14.484	233913	0.21	9-Tricosene, (Z)-
25	14.535	393305	0.36	TETRADECANE
26	14.820	5217962	4.72	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadeca
27	15.551	1042473	0.94	9-Octadecenoic acid (Z)-, methyl ester
28	15.766	1930180	1.75	Octadecanoic acid, methyl ester
29	15.961	5401473	**4.89**	**Oleic Acid**

30	16.158	8191749	**7.41**	**Octadecanoic acid**
31	16.376	693620	0.63	Hexadecane
32	16.929	307965	0.28	Heptasiloxane, hexadecamethyl-
33	17.488	318072	0.29	1,1,3,3,5,5,7,7,9,9,11,11,13,13-TETRADECAMETHYL-HE
34	18.212	1028568	0.93	DOCOSANE
35	19.361	221409	0.20	Hexadecane
36	19.977	188678	0.17	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-ICOSA
37	20.223	582366	0.53	Di-n-octyl phthalate
38	20.772	544055	0.49	DOCOSANE
39	22.903	1840007	1.66	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B
40	23.780	313056	0.28	DOCOSANE
41	23.950	205468	0.19	Olean-12-en-28-al
42	24.080	417377	0.38	NERYL LINALOOL ISOMER
43	24.172	1251614	1.13	Squalene

Peak#	R.Time	Area	Area%	Name
44	24.613	1460612	1.32	
45	25.089	308989	0.28	METHYL COMMATE B
46	25.660	1150717	1.04	1-BROMO-3,7,11-TRIMETHYL-DODECA-2,6,10-TRIENE
47	26.295	486535	0.44	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
48	26.382	381368	0.34	Stigmasterol
49	27.133	1153005	1.04	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
50	27.738	248072	0.22	NAPHTHALENE, DECAHYDRO-4A-METHYL-1-METHY
51	36.306	1698693	1.54	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B
52	37.758	1191256	1.08	<NO NAME>
		110567804	100.00	

Table 2:- Compounds identified from methanolic extract of Dipteran Adaxial Gall (DAG) Part of *Madhuca longifolia* using GC-MS analysis.

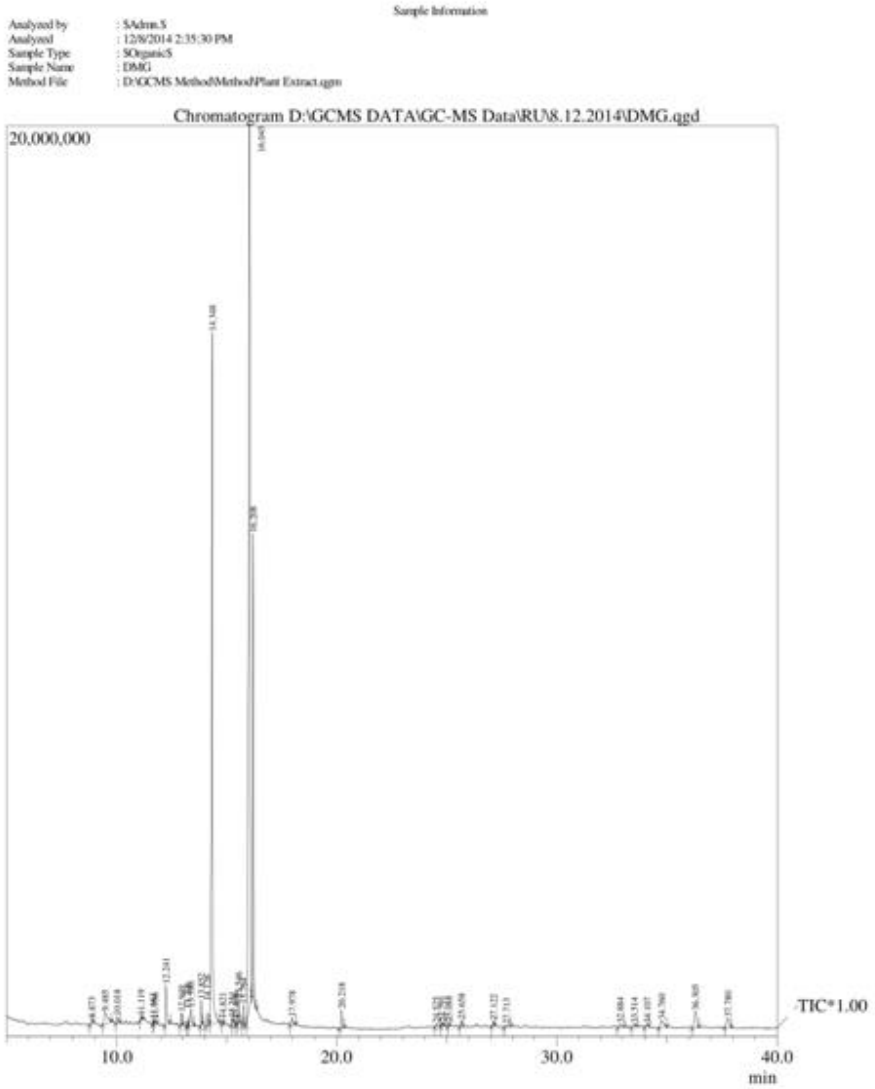

Figure 3:- Shows GC-MS Chromatogram of Dipteran Marginal Gall (DMG) of *Madhuca longifolia*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.873	503661	0.25	Cyclodecane
2	9.485	2812845	1.39	.beta.-D-Glucopyranose, 1,6-anhydro-
3	10.018	594617	0.29	N,N-BIS(2-HYDROXYETHYL)DODECANAMIDE
4	11.119	180140	0.09	1,2,3-PROPANETRICARBOXYLIC ACID, 2-HYDROXY-,
5	11.663	149731	0.07	Homogentisic acid, bis(tert-butyldimethylsilyl)-, tert-butyldime
6	11.756	266986	0.13	OCTADECANOIC ACID, METHYL ESTER
7	12.241	2933262	1.44	TETRADECANOIC ACID

8	12.949	555294	0.27	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
9	13.206	195864	0.10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
10	13.390	2010095	0.99	1,2-BENZENEDICARBOXYLIC ACID, DINONYL ESTER
11	13.852	1247487	0.61	Hexadecanoic acid, methyl ester
12	14.126	1134366	0.56	9-Hexadecenoic acid
13	14.348	55932276	**27.55**	**n-Hexadecanoic acid**
14	14.821	235638	0.12	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester
15	15.251	268466	0.13	Heptadecanoic acid
16	15.406	263737	0.13	n-Heptadecanol-1
17	15.546	1813313	0.89	9-Octadecenoic acid (Z)-, methyl ester
18	15.764	959494	0.47	Octadecanoic acid, methyl ester
19	16.045	94985459	**46.78**	**Oleic Acid**
20	16.208	24191853	**11.91**	**Octadecanoic acid**
21	17.978	817674	0.40	Eicosanoic acid
22	20.218	1356312	0.67	Di-n-octyl phthalate
23	24.525	375335	0.18	2-Piperidinone, N-[4-bromo-n-butyl]-
24	24.791	369947	0.18	2-Piperidinone, N-[4-bromo-n-butyl]-
25	25.088	230550	0.11	Betulin
26	25.658	512591	0.25	GERMACRENE B
27	27.122	384904	0.19	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
28	27.713	205445	0.10	LUP-20(29)-ENE-3,28-DIOL, (3.BETA.)-
29	32.884	432107	0.21	METHYL COMMATE C
30	33.514	377369	0.19	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,
31	34.107	444040	0.22	d-Norandrostane (5.alpha.,14.alpha.)
32	34.760	1610725	0.79	Lupeol
33	36.305	2821674	1.39	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B
34	37.780	1865903	0.92	Thunbergol
		203039160	100.00	

Table 3:- Compounds identified from methanolic extract of Dipteran Marginal Gall (DMG) Part of *Madhuca longifolia* using GC-MS analysis.

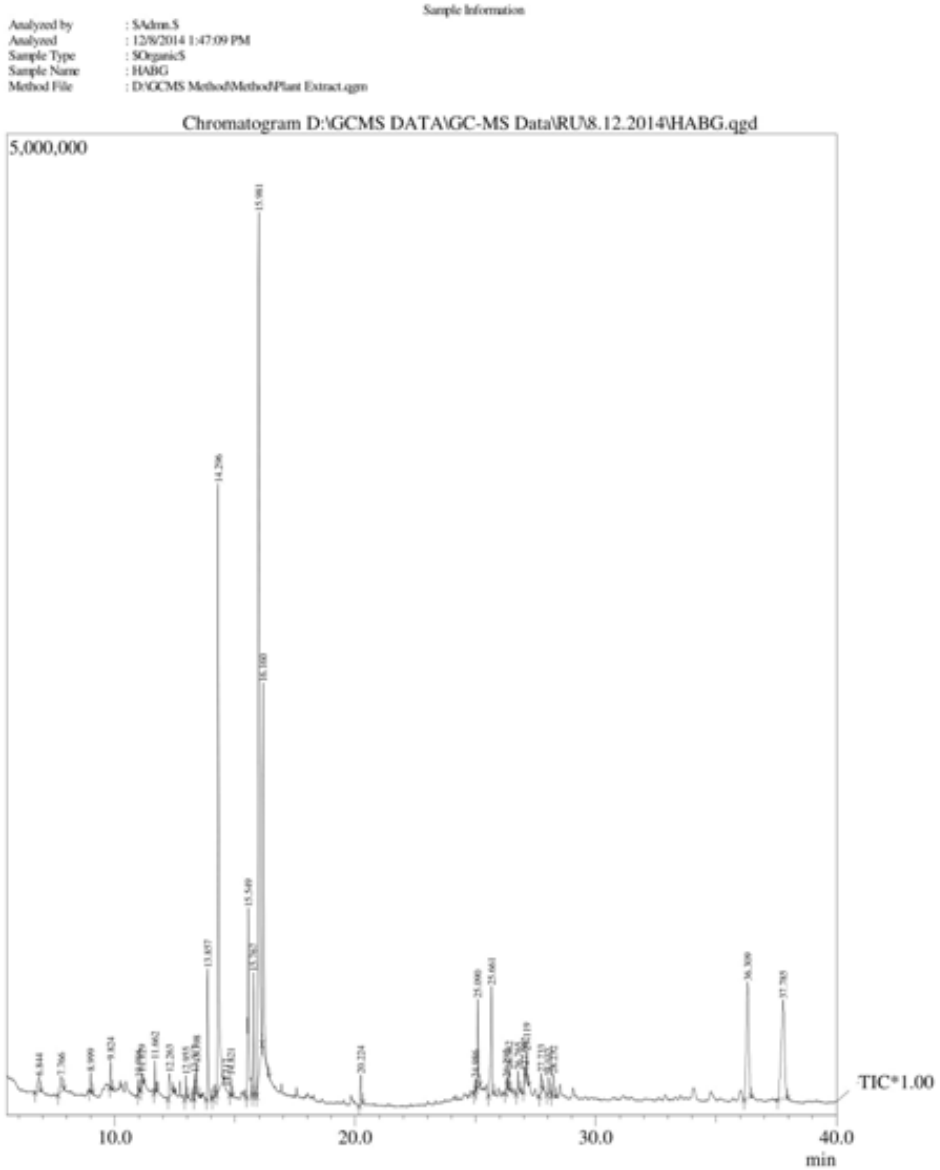

Figure 4:- Shows GC-MS Chromatogram of Hymenopteran Abaxial Gall (HAbG) of *Madhuca longifolia*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	6.844	486032	0.85	Cyclohexasiloxane, dodecamethyl-
2	7.766	509043	0.89	Octadecanoic acid, 4-hydroxybutyl ester
3	8.999	246260	0.43	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tet
4	9.824	314928	0.55	Cyclohexasiloxane, dodecamethyl-
5	10.986	132969	0.23	3,4-DIHYDROXYMANDELIC ACID-TETRATMS
6	11.129	192892	0.34	
7	11.662	316570	0.56	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadeca

8	12.263	651448	1.14	TETRADECANOIC ACID
9	12.955	244328	0.43	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
10	13.317	163858	0.29	BENZOIC ACID, 2,4-BIS(TRIMETHYLSILOXY)-, TRIME
11	13.398	419271	0.74	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl]-,
12	13.857	1775474	3.11	Hexadecanoic acid, methyl ester
13	14.117	235617	0.41	9-OCTADECENOIC ACID (Z)-
14	14.296	9644491	**16.91**	**n-Hexadecanoic acid**
15	14.821	229339	0.40	Methyl .beta.-d-ribofuranoside
16	15.549	3563063	6.25	9-Octadecenoic acid (Z)-, methyl ester
17	15.767	1393803	2.44	Octadecanoic acid, methyl ester
18	15.981	15445783	**27.08**	**Oleic Acid**
19	16.160	5182611	**9.09**	**Octadecanoic acid**
20	20.224	534804	0.94	1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER
21	24.986	173552	0.30	Betulin
22	25.090	1231910	2.16	GAMMA-NEOCLOVEN
23	25.661	2141675	3.75	Farnesyl bromide
24	26.292	149533	0.26	10-12-Pentacosadiynoic acid
25	26.382	293790	0.52	(-)-Isolongifolol, methyl ether
26	26.765	426056	0.75	.beta.-Humulene
27	27.042	169142	0.30	METHYL COMMATE C
28	27.119	347397	0.61	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
29	27.733	377146	0.66	NAPHTHALENE, DECAHYDRO-4A-METHYL-1-METHY
30	28.032	347285	0.61	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-
31	28.232	493661	0.87	Betulin
32	36.309	4307324	7.55	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B
33	37.785	4898259	8.59	(-)-Globulol
		57039314	100.00	

Table 4:- Compounds identified from methanolic extract of Hymenopteran Abaxial Gall(HAbG) of *Madhuca longifolia* using GC-MS analysis.

IV. Discussion

The GC-Ms analysis showed that the methanolic extract of normal leaf of *Madhuca longifolia* had fewer compounds than the leaf galls (DAG, DMG and HAbG)extract of *Madhuca* plant. Normal leaf extract showed the major compound present as n-Hexadeconoic acid (Palmitic acid) with 17.75% peak area which

retention time is 14.283. The next highest found compound was octadecanoic acid (Stearic acid), which retention time is16.153 with 14.11%peak area. In the galled leaf extract the highest found compound was Oleic acid (with 46.78% peak area in Dipteran marginal gall, DMG).However oleic acid was absent in the normal leaf and was present in the highest quantity (with 46.78% peak area) in dipteran marginal gall. The Hymenopteran abaxial gall (HAbG) shows much concentration (27 folds) of oleic acid rather than normal leaf with 27.08% peak area which retention time is 15.981. The another maximum found compound was hexadecanoic acid, (Palmitic acid) which is 10 times more(with peak area 27.55%) in DMG in comparition of normal leaf and less in DAG and HAbG. The octadecanoic acid (stearic acid) and stigmasterol compounds was found less (with peak area in DAG-1.82%, DMG-0.19% HAbG-1.22%) as compared to the normal leaf (peak area 4.41%).In dipteran marginal gall (DMG) a another important compound hematinic acid was found with 10.20% peak area which retention time is 6.722%.Oleic acid is monounsaturated omega -9- fatty acid which decreases LDL cholesterol and blood pressure[6].This fatty acid has much economic and commercial significance and is used widely in food industry and pharmaceuticals. Hexadecanoic acid is a saturated fatty acid. The hematinic acid is a multi-vitamins iron, B12 and folate. Its deficiency can lead to anaemia. The effect of hexadecanoic acid have been the focus of several dietary guidelines targeting the reduction of cardiovascular disease (CVD)[7,8], obesity related diseases and recently, cancer prevention[9]. Other various compounds such as various fatty acids, secondary metabolites sterols such as Hematinic acid (multivitamin iron, Sterols (Stigmasterol, ergasterol and squaline), triterpenes and phenols were also found.

V. Conclusion

This study showed that the Galled Leaf (under Stressed condition) led to production of more phytochemical compounds than the normal leaf (under non-stressed condition) of *Madhuca longifolia* plant. Oleic acid is only compound which is not found in normal leaf but in galled leaf is found in highest amount under stressed conditions and can be exploited commercially.

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