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PHYTOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA* BARK EXTRACT USING GC-MS ANALYTICAL TECHNIQUE AND ITS PHARMACEUTICAL POTENCY

[1]Jyoti Rathore * , [2]Bharti Patel , [3]Sudeep Kumar Mandal, [4]Jayshree Swarnkar

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Abstract

Moringa Oleifera plant exhibit many medicinal activities due to which it plays a significant role in herbal pharmaceuticals. The chemical phytoconstituents in the final extract of herbal medicines have a significant impact on their effectiveness. The conjugated approach Gas Chromatography-Mass Spectrometry (GC-MS) is used to pinpoint the chemical components in plant samples. This research work focused on extraction of compounds from bark of *Moringa Oleifera* plant belonging to family Moringaceae via Soxhlet extractor using solvents Benzene, Chloroform, Ethyl acetate and Hexane.The extract was subjects for GC-MS for detection of phytochemical constituents which play role in pharmaceutical sector. Phytochemical named dimethyl sulfoxide, tridecane, eicosane, dotriacontane, tetrapentacontane, n-hexadecanoic acid, 2,4-dimethyldodecane, 2,6,10-trimethyldodecane, heptadecane, heneicosane, dotriacontane and many more were detected from bark of *M.Oleifera* . More than 50 phytoconstituents were identified from *M.Oleifera* bark using four non polar solvents. Identified phytochemicals are associated with pharmacological activities.

Keywords - *Moringa Oleifera* , Mass Spectrometry, Phytoconstituents, Pharmacological.

[2]Royal College of Pharmacy, [3,4]Kalinga University
joychemistryjrc@gmail.com

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Introduction

The importance of medicinal plants in both modern medicine and the preservation of biodiversity is well acknowledged. People lack access to information about the healing potential of the plants in many rural areas of India. Recording traditional knowledge has contributed to the development of many of the most important medications currently in use, as over 80% of the world's population lives in low-income countries and relies on plant resources for treatment. For many who reside distant from urban centres, plants may be their only source of healthcare. The traditional use of plants as medicines is not well documented. Outside of its home climate, the Indian medicinal plant moringa (Moringa oleifera Lam) is well known. The family Moringaceae and the genus Brassica both contain the species Moringa oleifera. The family Moringaceae, which includes the genus Moringa, contains 13 different species. Because of its natural origin and lack of side effects, moringaoleifera is a preferred option in underdeveloped nations. The other significant and economically significant species of Moringa are *M. oleifera, M. arborea, M. longitude, M. drouhardii, M. ovalifolia, M. borziana, M. rival, M. hildebrandtii, M. corcanensis, M. stenopetala, M. ruspoliana, M. pygmaea*, and *M. Peregrine.*

Fig no. 01 – *M.Oleifera* Plant and *M.Oleifera* bark powder

Moringa has anti-inflammatory, anti-viral, anti-depression, and anti-viral properties. It has been discovered that giving animals moringa can enhance their overall health, reduce seizures, ease digestive problems, prevent cancer, and help them lose weight. Additionally, research has shown that moringa has potent antibacterial capabilities against a wide range of bacteria and yeasts, including Helicobacter pylori (H. pylori), Candida, E. coli, and Salmonella. An aqueous extract of the leaves of the Moringa oleifera plant has been found to lower high blood pressure (BP) in studies on animals and in clinical trials on humans. The eNOS-NO-sGC pathway is activated by *Moringa oleifera* extract, which lowers arterial blood pressure and eases the flow of tiny resistance arteries.

Pharmacological activities of *M.Oleifera*

1. Antioxidant activity

Strong in-vitro anti-oxidant and radical scavenging properties can be seen in extracts of the leaves and roots of *Moringa oleifera* that are both alcohol- and water-based (methanolic and ethanolic). Its leaves contain antioxidants that may shield animals from illnesses brought on by oxidative stress. It suggests that *Moringa*

Oleifera leaf extract guards against the oxidative harm caused by a high-fat diet.

2. **Antiepileptic activity**

Swiss albino mice were used to examine the anti-convulsant effects of an ethanolic extract of Moringaconcanensis leaves (200 mg/kg, i.p.) using MES- and PTZ-induced seizures. When PTZ was administered to animals, their seizure activity stopped. As the ethanolic extract of Moringaconcanensis leaves inhibited both MES- and PTZ-induced lengthening of the hind limbs and seizures, there may be numerous processes at play.

3. **Anticancer activity**

Strong anti-tumor properties are present in ethanol-based extracts of *Moringa oleifera's* leaves and seeds. Thiocarbamates and isothiocyanates were connected to an inhibitor of tumour promoter activity. The presence of three substances related to thiocarbamates and isothiocyanates, which are known to inhibit Epstein-Barr virus replication in response to the tumour promoter leukocidin B-4, was the cause of the in vivo anticancer effect.

Materials & Method

Moringa oleifera Bark was collected from regions of Korba district Chhattisgarh state india.

Preparation of extract

Moringa oleifera Bark was cleaned, dried and converted into fine powder. Extraction carried out in Soxhlet extractor. 50gms of *Moringa oleifera* Bark powder was subjected for extraction in nonpolar solvents separately for Ethyl acetate, hexane, Benzene & Chloroform for 24hrs and the extract was filtered. Than the extract was collected and evaporated to dryness by using vaccum distillation unit .The final residue was obtained and subjected to GC-MS analysis.

Fig no. 02 – Soxhlet Extraction of *M.Oleifera* Bark

GC-MS Analysis

A Perkin Elmer Clarus 500 gas chromatography was used to complete the GC-MS study which was fitted with a 100% Dimethyl polysiloxane-based Elite-I fused silica capillary column (30m x 0.25mm ID x 1df). An electron ionisation device with an ionising energy of 70 eV was employed for GC-MS detection. The carrier gas was helium gas (99.99%), which was used at a continuous flow rate of 1 ml/min and an injection volume of 2 l. The split ratio was 10:1, with the injector temperature being 2500 °C and the ion source heat being 2800 °C. The oven temperature was set to rise from 110°C (isothermal for 2 min) to 2000°C, then to 2800°C, and finally to 2800°C for a final 9 min isothermal period. At a scan interval of 0.5 seconds, 70eV, and fragment sizes ranging from 45 to 450 Da, mass spectrum data were collected. The duration of the GC was 36 minutes. The software used to handle mass spectra and chromatograms was a Turbo mass. The relative percent amount of each component was estimated by comparing its average peak area to the total areas.

Result & Discussion

The extract collected after soxhletion subjected under investigation for

percentage yield.The percentahe yield was reported in sequence higher to lower i.e n-hexane > chloroform> benzene> ethyl acetate(Table no. 01). More than 50 phytochemicals including dimethyl sulfoxide, tridecane, eicosane, dotriacontane, and tetrapentacontane, were present in the *M.oleifera* bark hexane extract . The existence of 40 phytochemicals was revealed in the *M.Oliefera* bark benzene extract including substantial amounts of 2,4,10-Trimethyltridecane, 2,4-Di-tert-butylphenol, heneicosane, and heptadecane. And 80 phytochemicals were detected in the chloroform fraction of the bark extract including high concentrations of n-hexadecanoic acid, 2,4-dimethyldodecane, 2,6,10-trimethyldodecane, heptadecane, heneicosane, and dotriacontane. Dimethylsulfoxide, undecane, tetradecane, heptadecane, dotriacontane, heneicosane, squalene, and n-hexadecanoic acid were among the 50 phytochemicals identified in the ethyl acetate fraction of the *M.Oleifera* bark extract that were found to be present.(Figure no.2- 6 & Table no.2-5) The identified chemical components are in the forefront of diverse therapeutic effects. Dimethylsulfoxide identified is FDA approved for treatment of painful bladder syndrome symptoms, Hexadecane reported for exhibiting antibacterial , antioxidant activities. Hexadecane is commonly known as cetane which is a alkane hydrocarbon. The compound Eicosane also known as icosane exerts strong anti-inflammatory ,analgesic and antipyretic activity. Dotriacontane exhibits antispasmodic, antibacterial, antioxidant, anti microbial and antiviral potency.

Table no. 01
Percentage yield of *M. Oliefera* bark

Extract	**Percentage yield**
	M. oleifera bark
Ethyl acetate	17%
Chloroform	20%
Benzene	25%
n-hexane	33%

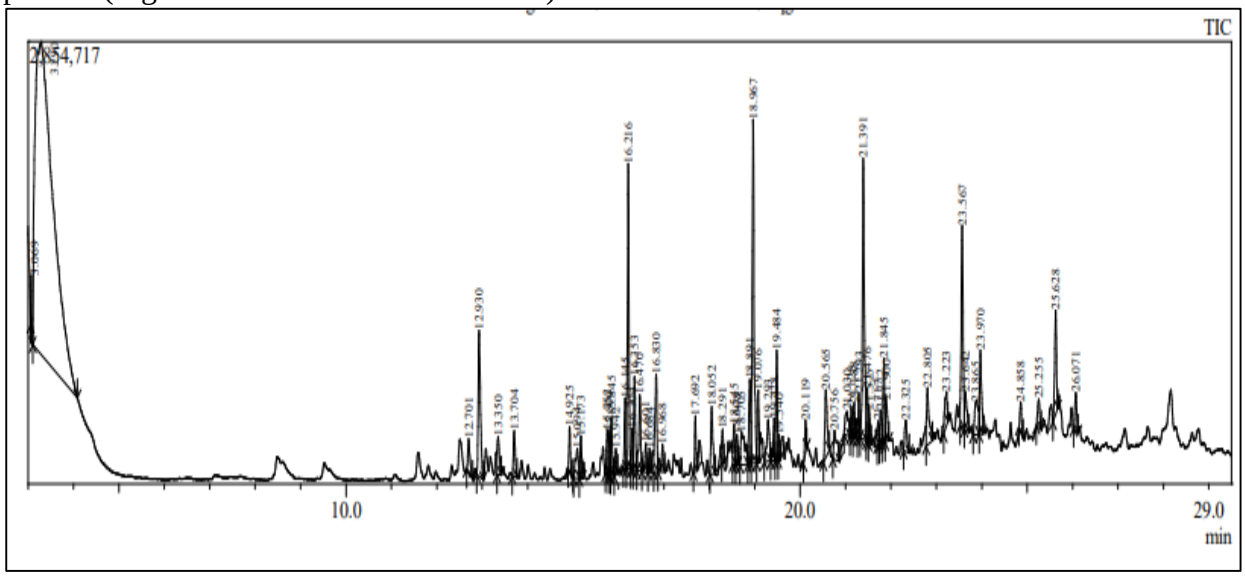

Fig no.03 – GC-MS Spectra of *M.Oleifera* Bark Hexane extract

Table no. 02- Phyto Compounds identified by GC-MS for hexane bark extract of *M. oleifera*

Peak	R.Time	F.Time	Area	Area%	Height	Basem/z	Name
1	3.069	3.100	417594	0.33	359845	63.05	DimethylSulfoxide
2	3.290	4.085	55524517	44.44	2019461	63.05	DimethylSulfoxide
3	12.701	12.790	678782	0.54	214700	57.15	2,4-Dimethyldodecane
4	12.930	13.025	3153649	2.52	940492	57.15	Hexadecane
5	13.350	13.420	730554	0.58	245621	57.15	Tridecane
6	13.704	13.770	821689	0.66	292612	57.15	Hexadecane
7	14.925	14.985	621973	0.50	289737	57.15	Tetradecane
8	15.094	15.135	842984	0.67	190963	57.15	Tridecane,2,5-dimethyl-
9	15.173	15.210	665168	0.53	272648	57.15	Heptadecane
10	15.752	15.780	689462	0.55	281560	57.15	Heptadecane
11	15.800	15.825	645888	0.52	273119	57.15	2,6,10-Trimethyltridecane
12	15.845	15.895	747219	0.60	377598	57.15	Tetradecane, 5-methyl-
13	16.145	16.175	1303513	1.04	452873	57.15	Undecane,2,4-dimethyl-
14	16.216	16.270	4596748	3.68	1971374	57.15	Eicosane
15	16.304	16.320	561207	0.45	267599	57.15	Heptadecane
16	16.353	16.400	1485617	1.19	604836	57.15	Heptadecane
17	16.470	16.515	1493571	1.20	490962	191.25	2,4-Di-tert-butylphenol
18	16.830	16.870	1884935	1.51	632038	57.15	Heptadecane
19	16.968	17.025	638016	0.51	177716	57.15	Eicosane
20	17.692	17.735	706352	0.57	359420	57.15	Hexadecane
21	18.052	18.130	1335486	1.07	434959	57.15	Heneicosane
22	18.291	18.325	438727	0.35	215986	57.15	Pentadecane,2,6,10-trimethyl-
23	18.545	18.580	620453	0.50	256444	57.15	Heptadecane
24	18.608	18.635	481735	0.39	195623	57.15	Heneicosane
25	18.705	18.850	1388224	1.11	214516	57.15	Heptadecane
26	18.891	18.920	1510714	1.21	545780	57.15	Heneicosane
27	18.967	19.040	6330644	5.07	2219462	57.15	Eicosane
28	19.076	19.110	1110349	0.89	469183	57.15	Eicosane
29	19.428	19.450	823361	0.66	274007	57.15	Heneicosane
30	19.484	19.515	1629938	1.30	710307	57.15	Eicosane
31	20.119	20.145	396541	0.32	213443	57.15	Octadecane
32	20.565	20.645	1485984	1.19	436496	57.15	Heneicosane
33	20.756	20.870	932813	0.75	188832	57.15	Heneicosane

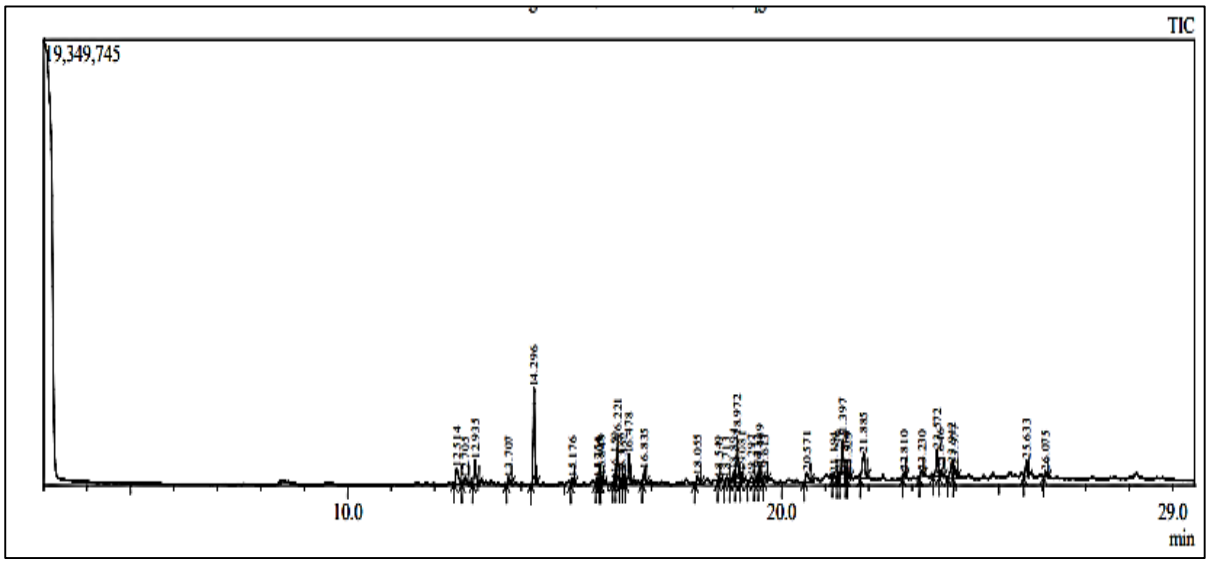

Fig no. 04- GC-MS Spectra of M.*oleifera* Bark Benzene extract

Table no. 03- Phyto Compounds identified by GC-MS for benzene bark extract of *M. oleifera*

Peak	R.Time	F.Time	Area	Area%	Height	Name
1	12.514	12.630	2907612	3.83	683008	Benzene,1,3-bis(1,1-dimethylethyl)-
2	12.705	12.795	946745	1.25	253166	Tetradecane, 5-methyl-
3	12.935	13.030	3392464	4.47	1050045	Hexadecane
4	13.707	13.775	865184	1.14	308690	Hexadecane
5	14.296	14.350	10487287	13.83	4167692	Ethanol,2-(2-butoxyethoxy)-,acetate
6	15.176	15.215	638601	0.84	266748	Heptadecane
7	15.756	15.785	701133	0.92	277844	Heptadecane
8	15.805	15.830	628347	0.83	264865	2,6,10-Trimethyltridecane
9	15.849	15.900	694874	0.92	339478	Tetradecane, 5-methyl-
10	16.150	16.175	1135130	1.50	436194	Heptadecane
11	16.221	16.270	4661564	6.15	1924535	Eicosane
12	16.308	16.325	550497	0.73	249803	Heptadecane
13	16.352	16.390	829970	1.09	380557	Hexadecane
14	16.478	16.530	3369062	4.44	1257306	2,4-Di-tert-butylphenol
15	16.835	16.875	1871794	2.47	642285	Eicosane
16	18.055	18.135	1239033	1.63	386223	Heneicosane
17	18.549	18.580	542505	0.72	233739	Heptadecane
18	18.713	18.795	991201	1.31	214097	Heneicosane
19	18.894	18.925	1713357	2.26	515521	Heneicosane

20	18.972	19.045	5482141	7.23	2011520	Eicosane
21	19.081	19.115	1000858	1.32	427766	Eicosane
22	19.432	19.455	724100	0.96	256925	Heneicosane
23	19.489	19.525	1514898	2.00	655322	Eicosane
24	19.613	19.665	743541	0.98	269817	Eicosane
25	20.571	20.660	1712860	2.26	431504	Heneicosane
26	21.299	21.330	658363	0.87	322990	Dotriacontane
27	21.397	21.460	5366931	7.08	1669439	Eicosane
28	21.885	21.965	4444699	5.86	1084948	l-(+)-Ascorbicacid2,6-dihexadecanoate
29	22.810	22.850	465694	0.61	219475	Dotriacontane
30	23.230	23.260	826199	1.09	208481	Tetracosane
31	23.572	23.625	3744680	4.94	1160060	Dotriacontane
32	23.646	23.735	882685	1.16	256608	Dotriacontane
33	23.912	23.945	2383930	3.14	640128	Octadecanoicacid
34	23.977	24.040	1737396	2.29	521607	Tetrapentacontane
35	25.633	25.685	2471752	3.26	716081	Dotriacontane
36	26.075	26.130	637830	0.84	230219	Dotriacontane

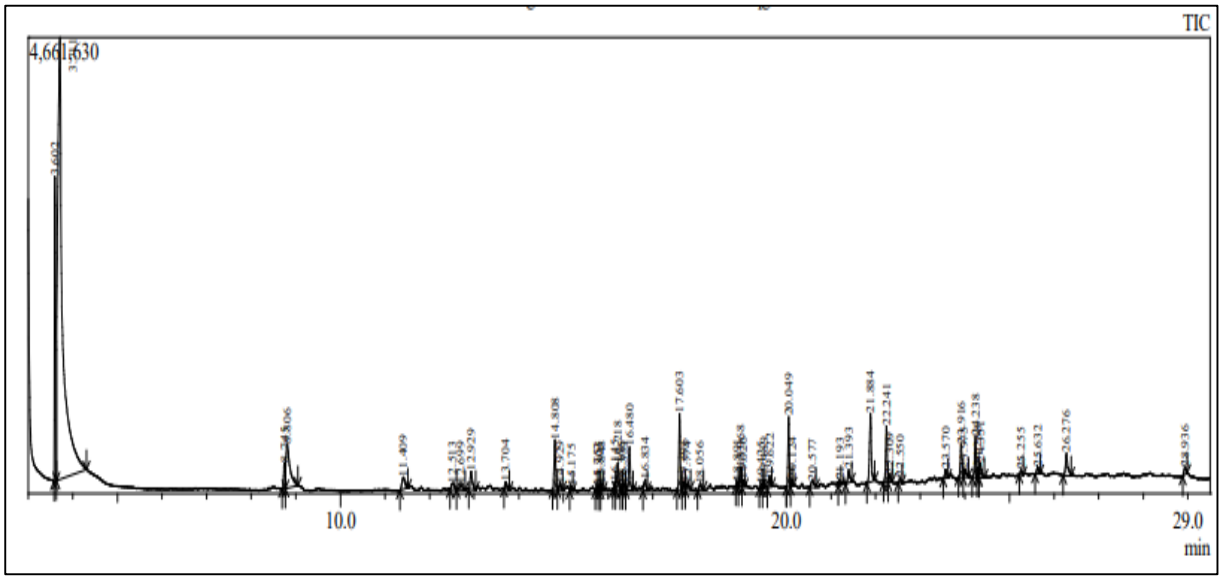

Fig no. 05 – GC-MS Spectra of *M.Oleifera* Bark ethyl acetate extract

Table no. 04 – Phyto Compounds identified by GC-MS for ethyl acetate bark extract of *M. oleifera*

Peak	R.Time	F.Time	Area	Area%	Height	Name
1	3.602	3.630	6471900	9.67	3115031	DimethylSulfoxide
2	3.711	4.310	36635199	54.72	4514984	DimethylSulfoxide
3	8.745	8.765	645802	0.96	266542	Acetophenone
4	8.806	9.050	2801988	4.19	451924	Acetophenone
5	11.409	11.515	572442	0.86	125055	1-Dodecene
6	12.513	12.605	290397	0.43	62194	Benzene,1,3-bis(1,1-dimethylethyl)-
7	12.699	12.795	259362	0.39	56765	Undecane,2,4-dimethyl-
8	12.929	13.030	611616	0.91	182154	Hexadecane
9	13.704	13.785	189679	0.28	67413	Hexadecane
10	14.808	14.865	1219349	1.82	508725	1-Pentadecene
11	14.929	14.985	155963	0.23	67628	Tetradecane
12	15.175	15.210	125465	0.19	49355	Heptadecane
13	15.757	15.780	124672	0.19	52983	Heptadecane
14	15.805	15.830	144823	0.22	56534	2,6,10-Trimethyltridecane
15	15.848	15.900	146022	0.22	73537	Tetradecane, 5-methyl-
16	16.145	16.170	187045	0.28	76021	Undecane,2,4-dimethyl-
17	16.218	16.275	728381	1.09	275814	Heptadecane
18	16.306	16.330	118027	0.18	51428	Heptadecane
19	16.353	16.395	159731	0.24	71504	Heptadecane
20	16.480	16.565	1093496	1.63	441710	2,4-Di-tert-butylphenol
21	16.834	16.880	354459	0.53	112171	Eicosane
22	17.603	17.655	1612923	2.41	772772	1-Heptadecene
23	17.696	17.735	164724	0.25	82105	Hexadecane
24	17.774	17.850	173534	0.26	57684	Carbonicacid,decylundecylester
25	18.056	18.140	181314	0.27	54137	Heptadecane
26	18.891	18.925	98748	0.15	51595	Heneicosane
27	18.968	19.000	441826	0.66	193900	Eicosane
28	19.425	19.460	121389	0.18	42973	Eicosane
29	19.489	19.520	196635	0.29	85673	Eicosane
30	19.622	19.670	289792	0.43	118732	Tetradecanoicacid
31	20.049	20.095	1510902	2.26	718035	1-Nonadecene
32	20.124	20.155	121754	0.18	70935	Octadecane

33	20.577	20.660	308688	0.46	63761	Heneicosane
34	21.193	21.220	107370	0.16	46320	Dotriacontane
35	21.393	21.460	414328	0.62	133179	Eicosane
36	21.884	21.975	1968291	2.94	702433	n-Hexadecanoic acid
37	22.241	22.280	1339318	2.00	574282	1-Nonadecene
38	22.309	22.370	192805	0.29	75882	Heneicosane
39	22.550	22.590	106556	0.16	54930	Pentadecanal-

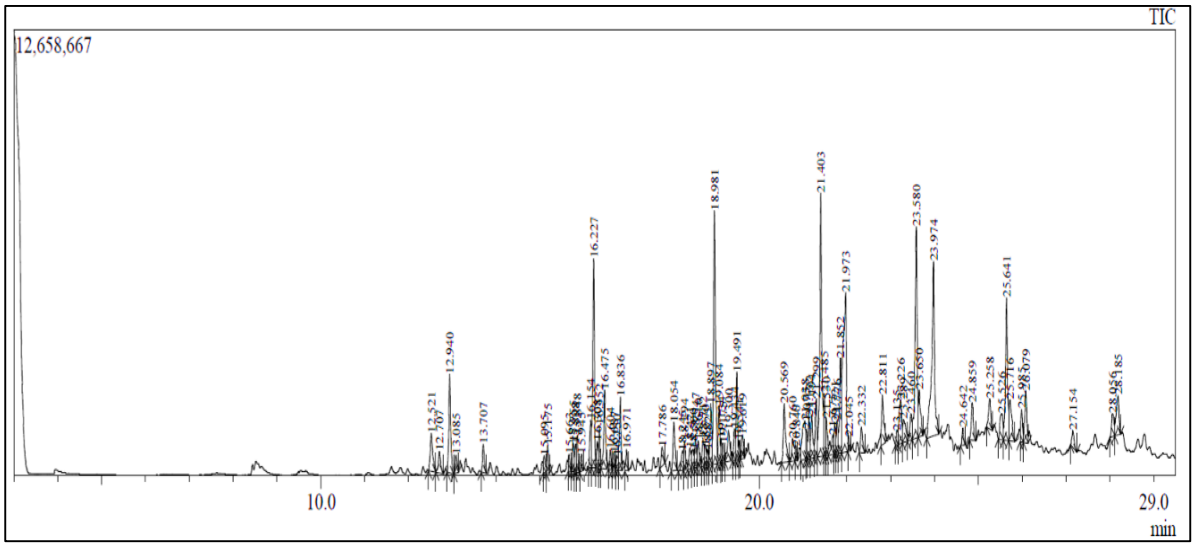

Fig no. 06 – GC-MS Spectra of M.Oleifera bark Chloroform extract

Table no. 05 – Phyto Compounds identified by GC-MS for chloroform bark extract of *M. oleifera*

Peak	R.Time	F.Time	Area	Area%	Height	Basem/z	Name
1	12.521	12.625	4411654	1.26	1061254	175.25	Benzene,1,3-bis(1,1-dimethylethyl)-
2	12.707	12.795	2245744	0.64	607319	85.20	2,4-Dimethyldodecane
3	12.940	13.035	8686836	2.48	2785352	57.15	Hexadecane
4	13.085	13.145	1698824	0.48	513939	57.15	Dodecane,2,6,11-trimethyl-
5	13.707	13.780	2217540	0.63	805808	57.15	Dodecane,4,6-dimethyl-
6	15.095	15.135	1224356	0.35	481616	57.15	Hexadecane
7	15.175	15.215	1939944	0.55	784030	57.15	Heptadecane
8	15.663	15.710	974842	0.28	471309	57.15	Heptadecane
9	15.755	15.780	1920529	0.55	820074	57.15	Heptadecane
10	15.808	15.825	1751184	0.50	783216	71.20	2,6,10-Trimethyltridecane
11	15.848	15.900	2209136	0.63	1042643	85.20	Tetradecane, 5-methyl-
12	16.154	16.175	3411230	0.97	1338651	57.15	Heptadecane

13	16.227	16.275	14961783	4.26	5927034	57.15	Heptadecane
14	16.308	16.325	1597946	0.46	755550	57.15	Heptadecane
15	16.352	16.390	2559408	0.73	1209462	57.15	Eicosane
16	16.475	16.520	6203893	1.77	2239529	191.25	2,4-Di-tert-butylphenol
17	16.687	16.720	997391	0.28	482575	71.15	Eicosane
18	16.836	16.875	6008614	1.71	2080194	57.15	Eicosane
19	16.971	17.025	1521088	0.43	601249	71.15	Eicosane
20	17.786	17.850	1736082	0.49	537301	57.15	Heptadecane
21	18.054	18.135	4331995	1.23	1378495	57.15	Heneicosane
22	18.245	18.270	1742385	0.50	533804	85.20	2,4-Dimethyldodecane
23	18.294	18.330	2077669	0.59	827792	57.15	Octadecane
24	18.547	18.580	2433234	0.69	948049	57.15	Heptadecane
25	18.610	18.635	1841331	0.52	748683	57.15	Heneicosane
26	18.712	18.740	2118606	0.60	804952	57.15	Undecane,2,4-dimethyl-
27	18.897	18.930	5090621	1.45	1724932	57.15	Heneicosane
28	18.981	19.050	21043173	6.00	7174328	57.15	Eicosane
29	19.084	19.115	3758880	1.07	1617230	57.15	Eicosane
30	19.434	19.455	2192542	0.62	899237	57.15	Eicosane

Conclusion -

This research work focused on analyzing the phytochemical constituents from M.Oleifera bark extract.The sample were investigated for percentage yield & GC-MS study. The presence of bioactive compounds played a significant role, via this compounds pharmaceutical product can be formulated exhibiting the specific activity.From this activity approximately 30-50 compounds were reported. Further isolation and characterization of this compounds can be done for its potency via invivo studiy. This work reported the significant role as title of the study indicates .

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