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Identification and quantification of bioactive compounds in different extracts of *morinda lucida* benth (rubiaceae) root using GC–MS analysis

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Abstract

This study aims to analyze and describe the chemical constituents of various crude extracts derived from the root of *Morinda lucida* Benth (Rubiaceae) an ethnomedicinal plant commonly found in Nigeria. The root of *Morinda lucida* was dried at room temperature and pulverized into powder. The soxhlet extraction technique was used with solvents of different polarities, namely hexane, chloroform, and methanol, to obtain three distinct extracts. Subsequently, gas chromatography-mass spectrometry analysis was carried out on the extracts. A total of 69 compounds were identified from the different extracts of *M. lucida* root. The hexane extract had 3 major compounds and 8 minor ones, with diethyl phthalate being the most prominent with 87.10% peak area. The chloroform extract had 24 compounds, with phthalic acid, 2-Ethylhexyl isohexyl ester being the highest with 16.61% peak area. Six of these compounds had more than 5%, while the remaining 18 ranged from 2.50% to 1.00%. The ethanol extract contained 36 compounds, with 6 compounds being greater than 5% and the remaining 30 less than 5%. The highest percentage in the ethanol extract was 2-Pyrrolidinone, 1-methyl-, at 16.05%. In terms of the biological and pharmacology benefits, these chemicals are regarded as crucial. Also, each of the three extracts has a few similarities in their physicochemical properties that can be related to the natural substances that are abundantly found in *M. lucida* root. The GC-MS analysis of different extracts of *M. lucida* revealed the presence of several bioactive compounds that have potential therapeutic properties.

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Keywords: GC-MS, *M. lucida* roots, bioactive compounds, anthraquinone

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1. Introduction

Plants are rich sources of chemical compounds with great biological and pharmacological importance [1]. Several parts of *M. lucida* like the leaves, seeds, twigs and stem/ stem bark has been used in Africa to treat several ailments ranging from Inflammation, Typhoid fever, Diabetes, Abdominal pains, dysmenorrhoea, splenomegaly, Helminthiasis, trypanosomiasis, Sickle cell disease [2]. *M. lucida,* which thrives across the tropical parts of Central and West Africa, is still one of the medicinal plants frequently collected and used in African traditional medicine. [3–5]. Several studies have been conducted on the bioactive compounds of *M. lucida* Benth using GC-MS

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analysis. Fortunately, hardly anyone placed much interest in the root for instance Okoha et al. (2011)[6] investigated the composition of the volatile oils present in the leaf and root of *M. lucida* using GC-MS analysis, 50 and 18 compounds were identified in the leaf and root volatile oil respectively which serves as an indicator of how rich *M. lucida* is with phytocompounds.

Furthermore, the bioactive secondary metabolites in certain parts of the plant, such as the seed and flower, have not been thoroughly screened, and the ones that have been screened were usually tested with aqueous or alcoholic extracts [2]. There is a scarcity of literature that utilizes GC-MS to identify the phyto compounds in various extracts of the roots of *M. lucida*.

Several researches have been reported on the biologically active compounds of the leaves and steam bark of *M. lucida* but very little has been reported on the roots of *M. lucida*. In fact there is scarce information on the GC-MS analysis of the root extracts from solvents such as hexane and chloroform.

This research aims at helping to expand the resource base of natural sources of biologically active substances, thereby improving the quality of life and enriching the diet that is relevant in the modern world. Thus, the output could be useful in the development of new drugs or natural products from *Morinda lucida* Benth for the management of various diseases.

2. Materials and methods

2.1. Plant sample

In August 2022, fresh *M. lucida* roots were collected, in Iwo. A botanist and herbarium curator identified the plant's leaves and flowers assigned them the herbarium accession number BUH036 and added them to the Herbarium collection.

2.2. Extraction of crude extracts

M. lucida root was dried for seven days at room temperature. The *M. lucida* roots were reduced to a powdery consistency. Soxhlet extraction was performed on this powdered material using n-hexane, chloroform, and ethanol as solvents. The resulting extracts were evaporated to dryness using a digital Stuart rotary evaporator (RE300DB) which was equipped with a Heidolph Rotavac valve control.

2.3. The GC–MS analysis

The bioactive compounds present in the different extracts of *M. lucida* root were analyzed by GC-MS. The analysis was performed using an 8860A gas chromatograph coupled to a 5977C inert mass spectrometer with an electron impact source. Separation of the compounds was carried out on an HP-5 capillary column coated with 5% of Phenyl Methyl Siloxane. The carrier gas was helium at a constant flow rate of 1.573 ml/min. Samples were injected with 50:1 split mode and a purge flow of 21.5 ml/min at 0.50 min. The oven temperature was programmed from 40°C to 270°C with a run time of 30.25 min. The mass spectrometer was operated in electron-impact ionization mode, and possible compounds were scanned from m/z 50 to 550 amu at a scan rate of 2.62s/scan. The relative quantity of the compounds in each extract was expressed as a percentage based on the peak area produced in the chromatogram. The mass spectral data were compared with those in NIST 14 Mass Spectral Library to identify the compounds.

2.4. Determination of chemical components

The identification of bioactive compounds present in various extracts of *M. lucida* was carried out by matching the spectra obtained from GC analysis on the HP-5 capillary column coated with 5% of Phenyl Methyl Siloxane with those from the NIST 14 library. The identification was based on the retention time of the compounds and their spectra.

3. Results

3.1. Percentage yield

From three batches of approximately 100g of the dried powdered root, the mean of the percentage yield and the standard deviation are presented in Table 1.

Table 1. Percentage yield of extracts.

Solvent	Mean % yield	Standard deviation
Hexane	0.92	0.04
Chloroform	2.64	0.07
Ethanol	3.34	0.06

3.2. Physical properties

All the extracts from *M. lucida had* varying shades ranging from yellow to orange. The n-hexane extract was yellow and had some traces of oil, it also had a distinct odor compared to the other extracts. The chloroform extract was dark orange with a reddish look and less gummy in nature; The ethanol extract was orange in color with an agreeable odor and had a gummier nature when compared to the other extracts.

3.3. Bioactive compounds present in the extracts

Tables 2-4 show the compounds present in the ethanol, hexane, and chloroform extracts obtained from *M. lucida* root. In the chloroform extract, the three major compounds by abundance were 2-Ethylhexyl isohexyl ester Phthalic acid (24.92%) alizarin (10.85%), and 3-Hydroxy-1-methoxy anthraquinone (14.05%). The n-hexane crude extract contained Diethyl Phthalate (87.10%) followed by 1-methyl-2-Pyrrolidinone (8.01%) and Mesitylene (2.62%). The ethanol crude extract had 1,4-benzene dicarboxylic acid, mono (1-methyl ethyl) ester (5.62%) Diethyl Phthalate (5.50%) and 2-methyl-Benzofuran (5.65%) among other compounds. Figures 1-3, show the retention time, molecular formula and percentage peaks corresponding to the bioactive compounds present in the extract.

Table 2. GC-MS analysis of hexane extract of *M. lucida* root.

S/No	Retention Time (Min)	Compound	Formula	Peak area (%)
1	3.259	1,2,4-trimethyl-Benzene	C_9H_{12}	0.66
2	3.259	1,2,3-trimethyl-Benzene	C_9H_{12}	0.66
3	3.310	Mesitylene	C_9H_{12}	2.62
4	3.917	1-methyl-2-Pyrrolidinone	C_5H_9NO	8.01
5	9.496	10-methyl-, methyl ester Undecanoic acid	$C_{13}H_{26}O_2$	0.53
6	10.051	gamma.-Dodecalactone	$C_{12}H_{22}O_2$	0.55
7	10.051	2(3H)-Furanone, 5-heptyldihydro-	$C_{11}H_{20}O_2$	0.55
8	10.280	Diethyl Phthalate	$C_{12}H_{14}O_4$	87.10
9	15.565	6-Octadecenoic acid (Z)	$C_{18}H_{34}O_2$	0.53
10	15.565	cis-10-Heptadecenoic acid, methyl ester	$C_{18}H_{34}O_2$	0.53
11	15.565	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	0.53

Table 3. Chemical compounds from chloroform extract of *M. lucida* root.

S/No	Retention Time (Min)	Compound	Formula	Peak area (%)
	5.857	Sulfurous acid, butyl hexadecyl ester	$C_{21}H_{44}O_3S$	1.04
1	7.047	Tridecane	$C_{13}H_{28}$	1.37
2	8.168	Sulfurous acid, butyl nonyl ester	$C_{13}H_{28}O_3S$	1.00
3	9.433	Butylated Hydroxytoluene	$C_{15}H_{24}O$	1.01
4	13.473	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	5.04
5	13.473	Pentadecanoic acid	$C_{15}H_{30}O_2$	5.04
6	13.713	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	2.21
7	14.760	9,10-Anthracenedione, 2-methyl-	$C_{15}H_{10}O_2$	1.95
8	14.760	9,10-Anthracenedione, 1-methyl-	$C_{15}H_{10}O_2$	1.95
9	15.481	1-Hydroxy-4-methyl anthraquinone	$C_{15}H_{10}O_3$	1.75
10	15.481	1-Hydroxy-2-methyl anthraquinone	$C_{15}H_{10}O_3$	1.75
11	16.563	9,10-Anthracenedione, 2-hydroxy-1- methoxy-	$C_{15}H_{10}O_4$	1.96
12	16.912	6-Methoxy-3-phenyl-4H-chromen-4-on	$C_{16}H_{12}O_3$	2.11
13	16.958	2,3-Dihydro-5-phenyl-1H-1,4-benzodiazepine-2-thione	$C_{15}H_{13}O_2N2S$	1.37
14	17.318	Ergolin-7-one, 6-methyl-8-methylene	$C_{16}H_{16}N_2O$	1.03
15	17.713	Alizarin	$C_{14}H_8O_4$	10.85
16	17.942	4H-1-Benzopyran-4-one, 5-hydroxy-7 -methoxy-2-phenyl-	$C_{17}H_{14}O_5$	1.59
17	17.942	9,10-Anthracenedione, 1,5-dimethoxy-	$C_{16}H_{12}O_4$	1.59
18	18.462	benzaldehyde, 2,4-dimethoxy-5-2-phenylethenyl-	$C_{17}H_{16}O_3$	5.45
19	18.634	3-Hydroxy-1-methoxy anthraquinone	$C_{15}H_{10}O_4$	14.05
20	18.897	Phthalic acid, 2-Ethylhexyl isohexyl ester	$C_{22}H_{34}O_4$	16.61
21	20.631	2-Amino-3-cyano-4-phenyl-5-carboethoxy-6-methyl-4H-pyran	$C_{16}H_{16}N_2O_3$	2.50
22	20.980	4H-1-Benzopyran-4-one, 5-hydroxy-7 -methoxy-2-phenyl-	$C_{16}H_{14}O_4$	2.21
23	20.980	9,10-Anthracenedione, 1,8-dimethoxy-	$C_{16}H_{12}O_4$	2.21

4. Discussion

Medicinal plants contain phytochemicals that have therapeutic activities and can be used to treat various diseases. Some of these phytochemicals are responsible for the distinct features of the plants, such as their smell, color, and taste, while others have both culinary and medicinal uses [7]. The different compounds identified from each of the mass spectra fragmentation patterns are listed in Tables 2-4. A total of eleven (11) compounds were identified consisting of three (3) major compounds and eight (8) minor compounds in the hexane extract. Diethyl Phthalate has the highest percentage (87.10). A total of twenty-four (24) compounds were identified in the chloroform extract consisting of six (6) constituents having percentages higher than 5.00% and eighteen constituents ranges between 2.50% and 1.00%. Phthalic acid, 2-Ethylhexyl isohexyl ester has the highest percentage (16.61%). The ethanol extract had a total of thirty-six (36) compounds. It consists of six (6) compounds that have percentages above 5% and thirty (30) compounds with less than 5%. 2-Pyrrolidinone, 1-methyl- (16.05%) had the highest percentage. The three crude extracts had no compound in common, although 1-methyl-2-Pyrrolidinone and Diethyl Phthalate were identified in both n-hexane (8.10%, 87.01%) and ethanol (16.05%, 5.05%) extracts respectively. The compounds identified in the chloroform extract are completely different from the other

Table 4. Chemical compounds from ethanol extract of *M. lucida* root.

S/No	Retention time (Min)	Compound	Formula	Peak area (%)
1	3.659	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	2.24
2	3.722	(+)-Diethyl L-tartrate	$C_8H_{14}O_6$	0.82
3	4.020	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	16.05
4	4.329	4H-Furo3,2-bpyrrole-5-carboxylic acid, 4-(2-oxopropyl)-	$C_{12}H_{13}NO_4$	1.06
5	4.483	Benzenemethanol, 3-fluoro-	C_7H_7FO	1.53
6	4.529	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy)-	$C_9H_9N_3O_3$	0.97
7	4.581	Cyclohexa-2,5-diene-1,4-dione, 2-methyl-5-(4-morpholinyl)-	$C_{11}H_{13}NO_3$	0.82
8	4.626	Succinic acid, 2,4,6-trichlorophenyl 2-methoxyphenyl ester	$C_{17}H_{13}Cl_3O_5$	0.99
9	4.661	2-Bromo-4-chloroaniline	C_6H_5BrClN	0.85
10	4.712	Benzaldehyde, 3-methyl-	C_8H_8O	6.78
11	5.284	Diglycolic acid, 2-chloro-6-fluoro phenyl ethyl ester	$C_{12}H_{12}ClFO_5$	1.90
12	5.347	N,N'-(2-Hydroxytrimethylene)diphth alimide		5.42
13	5.588	Benzofuran, 2-methyl-	C_9H_8O	5.65
14	6.412	4-Methylphthalaldehyde	$C_9H_8O_2$	3.20
15	8.792	trans-Isoeugenol	$C_{10}H_{12}O_2$	1.30
16	8.792	Phenol, 2-methoxy-5-(1-propenyl)-,(E)-	$C_{10}H_{12}O_2$	1.30
17	8.792	Eugenol	$C_{10}H_{12}O_2$	1.30
18	9.107	1H-Inden-1-one, 2,3-dihydro-7-hydroxy-3-methyl-	$C_{10}H_{10}O_2$	2.04
19	9.559	Benzoic acid, 4-ethoxy-, ethyl ester	$C_{11}H_{14}O_3$	1.30
20	10.257	Diethyl Phthalate	$C_{12}H_{14}O_4$	5.50
21	10.366	1-Adamantanecarboxamide, N,N-dimethyl-,	$C_{13}H_{21}NO$	2.29
22		Benzamide, 3-methoxy-N-isobutyl-	$C_{12}H_{17}NO_2$	
23	11.287	2-(n-Propyl)oxybenzylidene acetophenone	$C_{18}H_{18}O_2$	0.65
24	12.769	5-Amino-1-(4-amino-furazan-3-yl)-1H-1,2,3triazole-4-carbonitrile	$C_5H_4N_8O$	2.01
25	13.146	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-3-oxo-1-buteny l perhydro-, methyl ester	$C_{15}H_{23}NO_3$	0.73
26	13.146	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	$C_{11}H_{13}NO_3$	0.73
27	13.444	4-Phenyl-3,4-dihydro isoquinoline	$C_{15}H_{13}N$	0.80
28	13.713	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	1.08
29	13.713	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	1.08
30	13.713	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	1.08
31	14.211	4-Hydroxyphenyl pyrrolidinyl thion	$C_7H_6N_4OS$	1.51
32	14.211	7-Methyl -2-phenyl-1H-indole	$C_{15}H_{13}N$	1.51
33	15.252	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	0.82
34	15.481	1H-Benzo4,5furo3,2-findole	$C_{14}H_9NO$	0.79
35	18.886	Methyl 2-oxo-5,6,7,8-tetrahydro-1H-quinoline-3-carboxylate	$C_{11}H_{13}NO_3$	2.54
36	18.949	1,4-benzenedicarboxylic acid, mono(1-methylethyl) ester	$C_{11}H_{12}O_4$	5.62

extracts.

Several studies have been reported on biological activities of some of the identified compounds in the extracts. For example, the repellency of naturally occurring or related compounds such as gamma.-Dodecalactone ($C_{12}H_{22}O_2$) against bed bugs has been reported [8]. Anthraquinones are known secondary metabolites from numerous plants. The chloroform extract contain anthraquinones such as 2-methyl-9,10-Anthracenedione, 1-methyl-9,10-Anthracenedione, 1-Hydroxy-4-methylanthraquinone, 1-Hydroxy-2-methylanthraquinone, 2-hydroxy-1- methoxy-9,10-Anthracenedione, 1,5-dimethoxy-9,10-Anthracenedione, 1,8-dimethoxy-9,10-Anthracenedione, Alizarin, 3-Hydroxy-1-methoxyanthraquinone.

The Rubiaceae genus, to which *M. lucida* belong, has a lot of anthraquinone compounds, particularly in the roots. Natural anthraquinones exhibit a wide range of bioactivities, including antioxidant, anticancer, anti-inflammatory, immunosuppressive, diuretic, cathartic, laxative, antimicrobial, vasorelaxant, and phytoestrogen activities, according to research [9–15]. This has aroused the interest of researchers to work on them as potential drugs or drug lead for the prevention and treatment wide range of diseases.

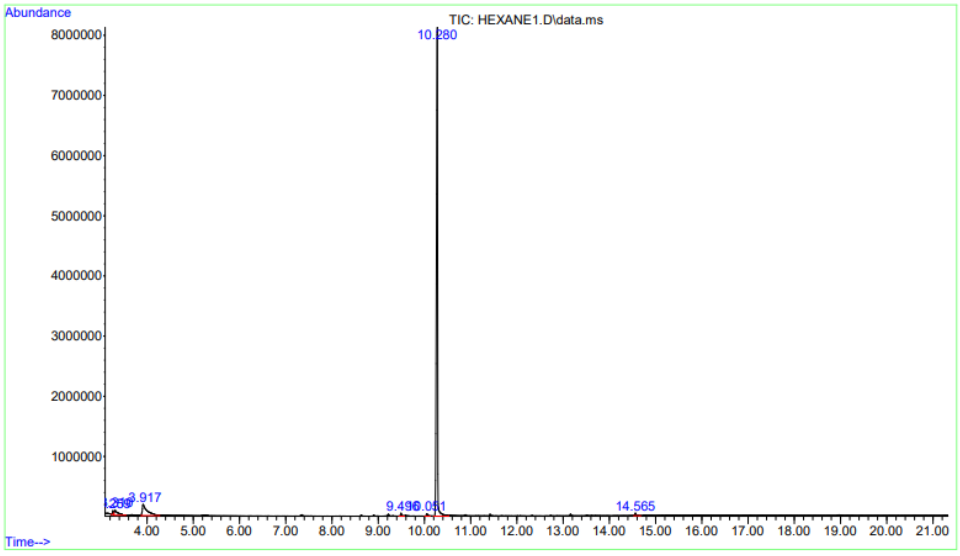

Figure 1. Chromatogram of bioactive constituents found in the hexane extract.

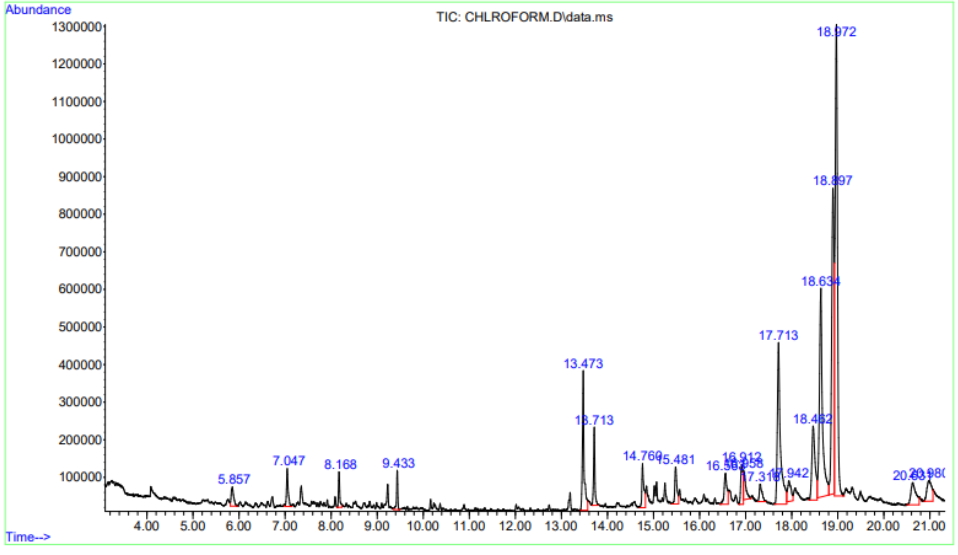

Figure 2. Chromatogram of bioactive constituents found in the chloroform extract.

Two derivatives of quinoline namely Methyl 2-oxo-5,6,7,8-tetrahydro-1H-quinoline-3-carboxylate and 4-Phenyl-3,4-dihydro isoquinoline were identified in the ethanol extract. Numerous natural products, particularly alkaloids [16] with intriguing biological activities, contain the quinoline ring structure. Novel quinolone derivatives are biologically active substances with a variety of pharmacological activities. The quinoline nucleus offers a variety of therapeutic activities with many pharmaceuticals having quinolone nuclei [17]. Quinoline has been described to have additional bioactivities such as anti-inflammatory, anticonvulsant, analgesic, anthelmintics, antimicrobial, hypoglycemic, and anticancer. Quinoline has several anti-malarial derivatives, including quinine, chloroquine, amodiaquine, and primaquine [17].

Eugenol is a naturally occurring substance that has a wide range of potential applications and is widely distributed in nature. It serves as a feedstock for several applications, including biotransformations, bio-based epoxy resins, natural chemicals, and pharmaceuticals with enhanced physicochemical qualities. Due to its numerous active sites, eugenol has medicinal properties, and by altering it, it can produce several derivatives with therapeutic potential for a wide range of ailments [18]. Several researchers have analysed different plant parts using Gas Chromatorgraphy for example the seeds [19] and leaves [20] which is imples that the use of GC-MS to analyse plant parts is of great benefit. GC and GC-MS analysis of the derivative are useful for identification and screening of brassinolide (being a potent plant growth stimulator) in plants [21] aslo GC-MS-based methods has been successfully utilized for phytochemical pro-

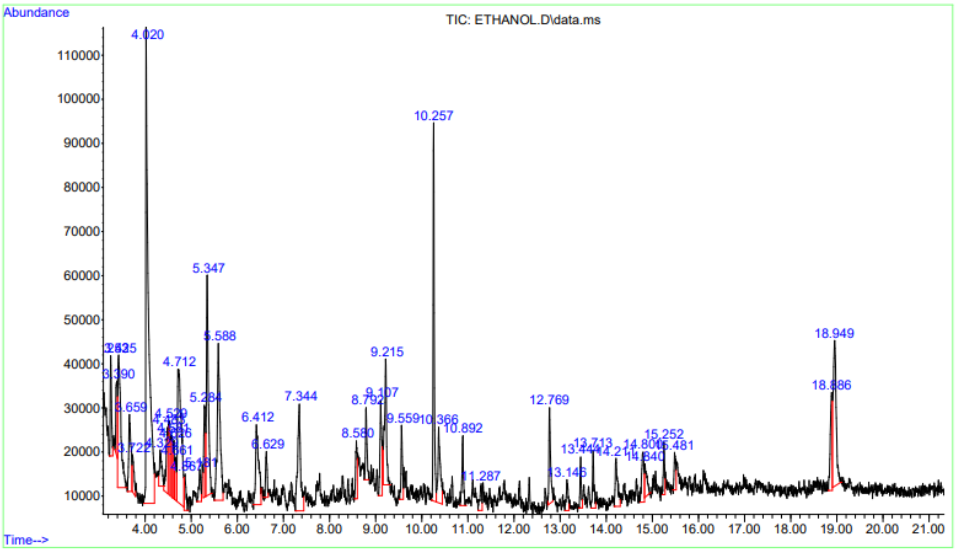

Figure 3. Chromatogram of bioactive constituents found in the ethanol extract.

filing and standardization of plant material [22].The GC-MS technique is specific and sensitive, and can be used for simultaneous identification and determination of a wide range of phenolic and terpenic compounds in different plants even at trace levels [23].

5. Conclusion

In conclusion, the analysis of different extracts of *M. lucida* revealed several bioactive compounds that have potential therapeutic properties. The identified compounds include terpenoids, flavonoids, alkaloids, and phenolic compounds. Some of these compounds have been reported to exhibit various biological activities. Further studies are needed to isolate and characterize the individual compounds identified in the GC-MS analysis and to evaluate their safety and efficacy in vivo. Thus, a recommendation is that more research should be carried out as this GC-MS analysis using more solvents since just three solvents has proven that a wide range of compounds with possibly promising biological activities is richly embedded in *M. lucida* roots. The results of this study may contribute to the development of new drugs and therapies for the treatment of various diseases.

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