Original Research Article

GC-MS Based Metabolite Profiling and Phytochemical Screening of Different Solvent Extracts of *Moringa Oleifera* Seeds

Abstract

The present study aimed at screening the different crude extracts of *M. oleifera* seeds for their active constituents and to explore the metabolites present using Gas chromatography-Mass spectrometry. The phytochemical screening of the different crude extracts revealed the presence of tannis, flavonoids, cardiac glycosides, saponins, steroids, terpenoids and alkaloids in various quantities. In the metabolite profiling of the seeds, the presence of fatty acid was found to predominate in the n-hexane, ethyl acetate and methanol extracts. GC-MS analysis confirmed the occurrence of forty-one (41) compounds in n-hexane extract, where major compound includes cis-vaccenic acid (9.2 %), cis-13-octadecenoic acid (7.1 %) and n-hexadecanoic acid (4.1 %). Thirty-nine (39) compounds were detected in the GC-MS profile of ethyl acetate extract. The major compounds identified were cis-vaccenic acid (7.5 %), 9-octadecenoic acid (7.2 %) and palmitoleic acid (6.0 %), while methanol extract profile revealed the presence of 39 detectable compounds, including cis-13-octadecenoic acid (14.7 %), ethyl oleate (7.3 %), and cis-vaccenic acid (6.3 %) as the major components present in *M. oleifera* seeds. In conclusion, extraction solvents influenced the recovery of phytoconstituents; and the therapeutically valued metabolites obtained will serve as a guide on further drug discovery process.

Keywords: *Moringa oleifera* seeds; phytochemical screening; GC-MS analysis; metabolite profiling

1. Introduction

Moringa oleifera (MO) is a resilient tree cultivated mainly within the tropical and sub-tropical regions of the world whose origin has been linked to two continents of Asia and Africa [1]. MO is among the major plants in the Moringaceae family representing one of the most valuable and widely used ethnomedicinal plant species occupying the food-medicine interface [2,3]. The plant (*Moringa oleifera)* parts such as the leaves, seeds, stem, roots, flowers and bark are considered medicinal. The seeds which have been tagged the most important part of the plant are brownish with semi-permeable seed hull has been reported to improve overall health in patients due to the presence of chemical substances often referred to as secondary metabolites [4].

Previous studies have reported the use of *Moringa oleifera* bioactive compounds in functional foods and several commercial food uses. The plant may be used for various food technology applications, such as antimicrobial agents, antioxidant, and food fortification, including nutritional and technological applications, because of high amount and the quality of bioactive components [5,6,7].

Gas chromatography-mass spectrometry is one of the base analytical platforms used in plant metabolite profiling which have been increasingly applied and proved to be a valuable method for the identification and quantification purpose by matching the mass spectra of the unknown compound with reference spectra [8]. In phytochemical screening, the medicinal value of plant lies in the phytochemical (bioactive) constituents of the plant which shows various physiological effect on human body.

Phytochemicals are biochemical metabolites that occur naturally in plants with nutritional value to human life. These secondary metabolites include alkaloids, flavonoids, steroids, glycosides, gums, phenols, tannins and terpenoids [9,10]. However, the extraction and purification of phytochemicals from the plant material are generally affected by various factors including time, temperature, solvent concentration and solvent polarity. Depending on chemical nature, various phytochemicals are extracted in solvents of different polarity as no single solvent may be reliable to extract all the phytochemicals present in the plant material [11].

Therefore, the use of different solvent polarity in this study, for the Phytochemical screening of *M. oleifera* seed samples from Bauchi, North Eastern Nigeria will not only help to reveal the constituents of the seed extracts and the one that predominates over the others but also to extract the maximum amount of required phytochemicals that can be used in the synthesis of more potent drugs for curing various diseases. Further, the Gas Chromatogram Mass Spectrometric method (GCMS) was used to carry out metabolite profiling of the different crude extracts of *M. oleifera* seeds.

2. Materials and Methods

2.1. Plant Material Collection

Mature seeds of *Moringa oleifera* were collected from the open market, Bauchi, North Eastern Nigeria and identified by a taxonomist in the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, North Eastern Nigeria. The matured seeds were cracked (exerting manually pressure on them) and the shells were carefully removed. The matured seed kernels obtained were dried under shade for three weeks to prevent the loss of bioactive ingredients contained in the seed sample. The dried seed kernels were pulverized into fine powder using a laboratory mortar and pestle, packed into air tight glass containers and kept in a dark, cool place for further use at room temperature.

2.2 Chemicals and Reagents

All chemicals and reagents used in this research were of the analytical grade and procured from Sigma- Aldrich (USA).

2.3. Preparation of Extracts

Three (3) different solvent viz., methanol, ethyl acetate and n-hexane were used in the sequential cold extraction method with increasing polarity for solvent-extraction of the grounded *M. oleifera* seed kernels. 100 g of the pulverized seed kernels were separately soaked in 300 cm^3 of solvent for 72 hr in an enclosed glass jar and filtered by Whatman filter paper. The procedure with each solvent was repeated, evaporated to dryness and stored at 4 ^{O}C in a refrigerator for further studies.

2.4. Qualitative Phytochemcial Screening of Different Solvent Extracts of *Moringa Oleifera* Seeds

Phytochemical profiling of different crude extract of *Moringa oleifera* seeds were carried out using the procedures as described by [12,13,14].

Test for Tannins

2 g of each sample were weighed into the beaker and 100 cm^3 of water added and allowed to soak for two hours thoroughly. The extracts were treated with drops of ferric chloride.
Observation: The development of a deep blue black color indicates the presence of tannins.

Test for Flavonoids

2 g of each sample were soaked with 100cm^3 of distilled water and allowed to stay for 48 hr, and filtered thereafter. The filtrate was kept in a conical flask with free drops of magnesium powder; and concentrated sulphuric acid (H_2SO_4) were added.
Observation: A formation of a reddish precipitate indicates the presence of flavonoids.

Test for cardiac glycosides:

0.5 % (w/v) extract was weighed and 2 ml of glacial acetic acid with few drops of 5 % ferric chloride were mixed together. This was under layered with 1 ml of concentrated sulphuric acid.

Observation: The formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Test for Saponins

2 g of each sample were weighed and added to 2 cm^3 of distilled water in a test tube. The extract obtained was shaken vigorously accordingly.

Observation: The formation of foam (persistent frothing) indicates the presence of saponins

Test for Steroids

2 g of water extract were obtained and drops of formaldehyde and concentrated sulphuric acid (H_2SO_4) were added.

Observation: The formation of a reddish brown colour indicates the presence of steroids.

Test for Terpenoids

(Salkowski test): Dried extract (50 mg) was taken and soaked in 5 mL of ethanol. Extract was mixed in 2 mL of chloroform. It was slightly warmed, and then cooled. 3 ml of concentrated H_2SO_4 was added slowly along the sides of test tubes.

Observation: A redish brown colored precipitation was formed at the interface indicating the presence of terpenoids .

Test for Alkaloids

2 g of each sample were weighed into a 200 ml flask and 95 % ethanol was added and left for four hrs. The sample was filtered and few drops of Wagner's reagent (iodine crystals and potassium iodide) were added to filtrate.

Observation: A yellowish coloration indicates the presence of alkaloids

2.5. Quantitative Phytochemcial Analysis of Different Solvent Extracts of *Moringa Oleifera* Seeds

The presence of the Phytochemicals in different crude extracts of *Moringa oleifera* seeds was quantified using the procedures as described by [15,16].

Estimation of Tannins

1 g of each sample was extracted with 25 ml 80:20 acetone: 10 % glacial acetic acid for 4 hrs. It was then filtered and measured at 500 nm absorbance. The absorbance of the reagent blank was also measured. A standard graph with10, 20, 30, 40, 50 mg/100 g of tannic acid was

made. The concentration of tannins was read taking into consideration the dilution factor.

Estimation of Flavonoids

1 g of each sample was extracted with 10 ml of 80 % methanol and left to stand for 2 hrs. It was filtered through Whatman filter paper into a petri-dish, evaporated to dryness in an oven at 40 ^{o}C and weighed.

Estimation of Cardiac Glycosides

1 g of each sample with 40 ml of water was extracted and placed in an oven at 100 ^{0}C for 15 min. Then, to 1 ml of the extract dissolved in 5 ml of water was added 2 ml of glacial acetic acid followed by one drop of iron chloride ($FeCl_3$) and 1 ml of H_2SO_4. The absorbance was then measured at 410 nm.

Estimation of Saponnins

1 g of each sample was dispersed in 15 ml of 20 % ethanol. The suspension was put inside the water bath at 55 ^{0}C for 4 hrs. The mixture was filtered and the residue re-extracted with another 15 ml of 20 % ethanol twice. The extract was reduced to about 5 ml in the oven. The concentrate was transferred into a 250 ml separating funnel and 5 ml of petroleum ether was added and mixed vigorously. The petroleum ether layer was discarded and 3 ml of butanol was added to the aqueous layer. The extract was washed twice with 5 ml of 5 % sodium chloride. The remaining solution was poured into a weighed petri-dish, evaporated to dryness in the oven and the residue was weighed.

Estimation of Steroids

1 ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4 N, 2 ml) and iron (III) chloride (0.5 % w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5 % w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2 ^{0}C for 30 mins with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Estimation of Terpenoids

1 g of each sample was weighed into 250 ml beaker and 10 ml petroleum ether was added. It was allowed to extract for 15 min and was filtered. The absorbance was then read at 420 nm.

Estimation of Alkaloids

1 g of each sample (W) was extracted with 20 ml of 10 % acetic acid in ethanol, mixed and allowed to stand for 4 hrs. The extract was filtered through whatman filter paper. The filtrate was evaporated to about a quarter of its original volume and one drop of concentrated

ammonia was added. The extract was filtered through weighed (W1) whatman filter paper. The filter paper was dried in the oven at 60 ^{o}C. The dried filter paper was weighed to a constant Weight (W_2)

$$\% \, Alkaloids = \frac{(W_2 - W_1)}{W} \text{ X } 100$$

2.6. GC-MS Analysis

GC-MS analysis on the hexane, ethyl acetate and methanol extract of *M. oleifera* seeds was carried out using agilent technologies 7890A GC and 5977B MSD with experimental conditions of GC-MS system were as follows: Hp 5-MS capillary standard non-polar column, dimension: 30 M, ID: 0.25 mm, film thickness: 0.25µm. flow rate of mobile phase (carrier gas: Helium) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40 °C raised to 250 °C at 5 °C/min and injection volume was 1 µl. Samples dissolved in methanol were run fully scan at a range of 40-650 m/z and the results were compared by using NIST mass Spectral library search programme.

2.7. Data analysis

The procedures for extraction and phytochemical analysis were replicated four times. Data were analyzed using one-way analysis of variance (One-way ANOVA). Values were considered statistically significant difference at $p \leq 0.05$ with results expressed as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Extracts Yield

The percentage yield of the n-hexane, ethyl acetate and methanol obtained from the *Moringa oleifera* seed extracts is given as 17.25 ± 0.84, 18.44 ± 0.03 and 8.15 ± 0.52 %. The extract percentage varied greatly between different solvents used for the extraction.

3.2. Phytochemical Screening

The phytochemical screening of *Moringa oleifera* seeds shows the presence of some phyto-constituents which are analyzed and quantified as presented in Table **1** and Table **2** respectively.

Table 1: Results of Qualitative Phytochemcial screening from three (3) different solvent extracts of *Moringa Oleifera* seeds.

Phytochemicals	MeOH	EtOAc	n-HX
Tannins	+ + +	+ + +	+ + +
Flavonoids	+ + +	+ +	+ +
Cardiac glycosides	+ + +	+ + +	+
Saponins	+ + +	+ +	+ + +
Steroids	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+

Key: - MeOH = Methanol Extract
EtOAc = Ethyl acetate Extract
n-HX = n-Hexane Extract
+ + + = Highly Present
+ + = Moderately Present
+ = Slightly Present

Table 2: Results of Quantitative Phytochemcial Analysis from three (3) different solvent extracts of *Moringa Oleifera* seeds.

Phytochemicals		MeOH	EtOAc	n-HX
Tannins	(mg/g)	302.90	300.80	299.30
Flavonoids	(mg/g)	218.80	134.70	123.60
Cardiac glycosides	(mg/g)	233.30	218.20	91.36
Saponins	(mg/g)	213.30	171.10	205.90
Steroids	(mg/g)	18.84	27.33	25.74
Terpenoids	(mg/g)	75.30	74.33	78.25
Alkaloids	(mg/g)	20.18	14.07	30.88

Results of the phytochemical screening of the different crude extracts show the presence of tannis, flavonoids, cardiac glycosides, saponins, steroids, terpenoids and alkaloids with different quantities. This study established that *M. oleifera* seeds contained: tannins, steroids, terpenoids, flavonoids, cardiac glycosides, saponins and alkaloids which have been identified by other researchers [17,18].

Tannins are medicinally significant due to their astringent properties. They promote rapid healing and the formation of new tissues on wounds and inflamed mucosa. Tannins are used in the treatment of varicose ulcers, haemorrhoids, minor burns, frostbite, as well as inflammation of gums. Internally tannins are administered in cases of diarrhoea, intestinal catarrh, and in cases of

heavy metal poisoning as an antidote. In recent years, these compounds have demonstrated their antiviral activities for treatment of viral diseases including AIDS (https:// www. Pharmacy 180. com).

Flavonoids are water-soluble polyphenolic molecules used for anti-inflammatory activity, enzyme inhibition, antimicrobial activity, estrogenic activity, antiallergic activity, antioxidant activity, antiulcerogenic activity, vascular activity and cytotoxic antitumor activity [19]. Flavonoids in the duodenal tract lower the risk of heart diseases. In addition, flavonoids protect ulcer development by initiating a gastric mucosa cover, increasing capillary resistance, and improve microcirculation, which renders the cells less injurious to precipitating factors [20].

Cardiac glycosides are known to work by inhibiting the Na^+ / k^+ pump. This causes an increase in the level of sodium ions in the myocytes, which then lead to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for contraction of the heart muscle, which improves cardiac output and reduces distention of the heart; thus, they are used in the treatment of congestive heart failure and cardiac arrhythmia. They are also used to strengthen a weakened heart and allow it to function more efficiently, though the dosage must be controlled carefully, since the therapeutic dose is close to the toxic dose [21].

According to [22], Saponins are used for antimicrobial activity and inhibit mould as they have haemolytic activities, cholesterols binding usage, also in treatment of yeast and fungal infections. Saponins also prevent cancer cell multiplication, thus inhibiting unwanted cancerous cell generation in the body [23]. Saponin content of the sample may be the reason for its usage as natural antibiotic and aids in the fight of infection and microbial invasion [14].

Plant steroids are known to be important for their cardiotonic activities; they possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics. They are routinely used in medicine because of their profound biological activities [21].

The biological properties of terpenoids include cancer chemo-preventive effects, antimicrobial, antifungal, antiviral, anti-hyperglycemic, anti-inflammatory, anti-parasitic activities and memory enhancers [24]. The presence of terpenoid found in the seed of *M. oleifera* is suggestive of its antifungal and antibacterial activities and this can be attributed to their membrane disruption and inhibitory action on bacterial cell or fungus.

Alkaloids are significant for protection against microbial and pesticide activities as it is used by ethnomedicinal practitioners for analgesic, antispasmodic and antimicrobial treatment. Alkaloids have many pharmacological functions such as antimalarial, antihypertensive, anticancer, antifungal and antibacterial abilities in treatment of diseases or illnesses [25].

3.3. Metabolite Profiling of Different Crude Extracts

The Gas Chromatogram Mass Spectrometric (GCMS) analysis shows the distinct chromatogram of *M. oleifera* seeds extracted in different solvents in Figure **1** and the identified compounds with their peak number, retention time (RT), and peak area (%) are presented in Table **3**.

Out of 41 compounds identified in the chromatogram of n-hexane extract, the major compounds included cis-vaccenic acid (9.2 %), cis-13-octadecenoic acid (7.1 %) and n-hexadecanoic acid (4.1 %). Among the 39 compounds detected in the GC-MS profile of ethyl acetate extract, The major compounds identified were cis-vaccenic acid (7.5 %), 9-octadecenoic acid (7.2 %) and palmitoleic acid (6.0 %), while methanol extract profile revealed the presence of 39 detectable compounds, including cis-13-octadecenoic acid (14.7 %), ethyl oleate (7.3 %), and cis-vaccenic acid (6.3 %) as the major components.

The metabolites present in the crude extracts were separated according to their groups as presented in Figure **2-4**. Fatty acids (68 %), esters (12 %), aldehyde (7 %), alkane (5 %), alkene (3 %), benzene derivatives (3 %) and pentose sugars ((2 %) were found to be present in n-hexane seed extract metabolite profile.

Whereas metabolite profiling of ethyl acetate seed extract revealed the presence of fatty acids (79 %), esters (8 %), aldehyde (2 %), alkane (5 %), carboxylic acid (3 %) and steroids (3 %), the metabolite profiling of methanol seed extract revealed the presence of fatty acids (67 %), esters (26 %), aldehyde (2 %), alkane (2 %) and steroids (3 %).

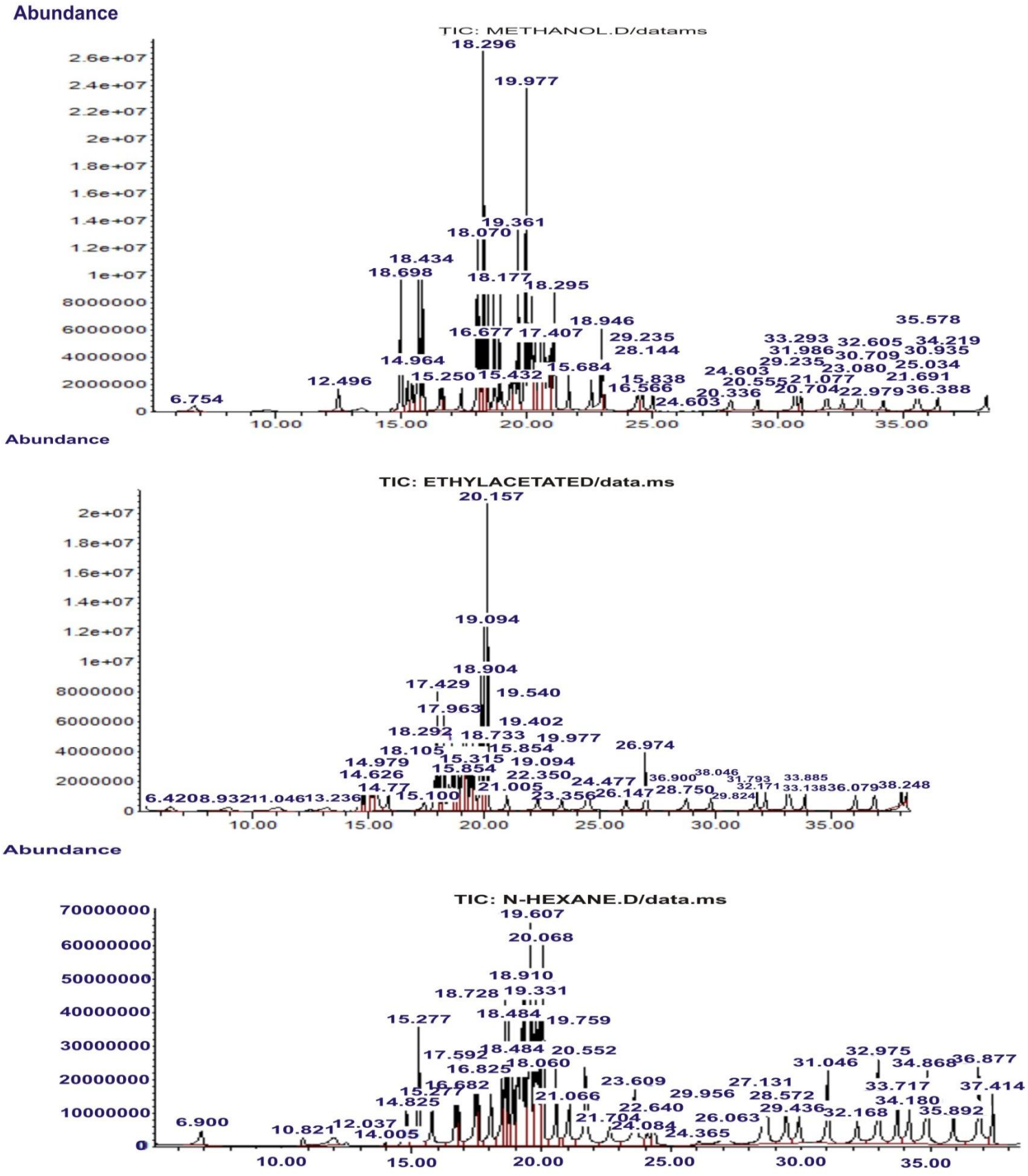

Fig 1: GC-MS total ion chromatogram (TIC) of analysis of different solvent extract of *M. oleifera seeds* (a) methanol (b) ethyl acetate (c) n-hexane extracts.

TABLE 3: GC-MS profile of different solvent extracts of *M.Oleifera* seeds.

S/No.	Name of the compound	Peak number	R. time	Peak area (%)
	Meethanol extract			
1	i-Propyl 11-octadecenoate	1	6.7541	0.6114
2	Tetradecanoic acid	2	12.4961	1.1609
3	Hexadecanoic acid, ethyl ester	3	14.9649	3.8727
4	Palmitoleic acid	4	15.2507	1.1767
5	n-Hexadecanoic acid	5	15.4329	1.3757
6	n-Hexadecanoic acid	6	15.6841	3.7183
7	n-Hexadecanoic acid	7	15.8386	4.1149
8	n-Hexadecanoic acid	8	16.5666	1.1567
9	9-Octadecenoic acid (Z)-, methyl ester	9	16.6779	0.6626
10	n-Hexadecanoic acid	10	17.4072	0.8883
11	(E)-9-Octadecenoic acid ethyl ester	11	18.0703	5.7297
12	Ethyl Oleate	12	18.1776	1.788
13	Ethyl Oleate	13	18.2957	7.3084
14	Ethyl Oleate	14	18.4341	2.7745
15	trans-13-Octadecenoic acid	15	18.6982	3.2282
16	9-Octadecenoic acid	16	18.9461	3.3272
17	9-Octadecenoic acid	17	19.3613	1.9484
18	9-Octadecenoic acid	18	19.5166	5.9079
19	cis-13-Octadecenoic acid	19	19.9773	14.7341
20	cis-Vaccenic acid	20	20.3369	2.0479
21	cis-13-Octadecenoic acid	21	20.5553	3.6374
22	cis-Vaccenic acid	22	20.7044	6.3623
23	cis-Vaccenic acid	23	21.0774	4.8199
24	trans-13-Octadecenoic acid	24	21.6913	0.9768
25	2-Dodecenal, (E)-	25	22.979	3.1444
26	Erucic acid	26	23.0806	0.6988
27	cis-Vaccenic acid	27	24.4398	1.2767
28	cis-Vaccenic acid	28	24.6033	0.9618
29	Docosanoic acid, ethyl ester	29	25.0343	0.8863
30	1-Nonadecene	30	28.1447	0.9056
31	Octadecanoic acid	31	29.2357	0.7399
32	cis-11-Eicosenoic acid	32	30.7099	1.1787
33	cis-Vaccenic acid	33	30.9358	0.7171
34	Oleic Acid	34	31.9862	0.7868
35	Methyl 6-O-[1-methylpropyl]-.beta.-d-galactopyranoside	35	32.6052	0.4741
36	cis-13-Octadecenoic acid	36	33.2935	1.6854
37	Heneicosanoic acid, isopropyl ester	37	34.2197	0.6211
38	Oleic Acid	38	35.5786	1.8018
39	Heneicosanoic acid, isopropyl ester	39	36.3882	0.7926

S/No.	Name of the compound	Peak number	R. time	Peak area (%)
	Ethyl acetate extract			
1	Thiomorpholine	1	6.4205	0.865
2	9-Oxabicyclo[6.1.0]nonane, cis-	2	8.9237	1.0745
3	Oleic Acid	3	11.0468	1.3271
4	Oleic Acid	4	13.2362	1.1119
5	Palmitoleic acid	5	14.6262	1.7349
6	Palmitoleic acid	6	14.77	1.3448
7	n-Hexadecanoic acid	7	14.9797	2.3994
8	n-Hexadecanoic acid	8	15.1005	2.6182
9	n-Hexadecanoic acid	9	15.3157	4.3008
10	n-Hexadecanoic acid	10	15.8542	1.4272
11	n-Hexadecanoic acid	11	17.4298	1.0725
12	Palmitoleic acid	12	17.9633	6.0682
13	9-Octadecenoic acid	13	18.1052	1.9866
14	9-Octadecenoic acid	14	18.2928	7.2481
15	9-Octadecenoic acid	15	18.7333	2.7108
16	9-Octadecenoic acid	16	18.9044	1.9373
17	9-Octadecenoic acid	17	19.094	4.1765
18	9-Octadecenoic acid	18	19.2405	3.7652
19	cis-Vaccenic acid	19	19.4022	2.8164
20	cis-Vaccenic acid	20	19.5406	3.8954
21	9-Octadecenoic acid, (E)-	21	19.8304	5.8265
22	9-Octadecenoic acid	22	19.9778	6.4626
23	cis-Vaccenic acid	23	20.1576	7.5035
24	9-Octadecenoic acid	24	21.0054	1.075
25	cis-Vaccenic acid	25	22.3505	2.0112
26	Oleic Acid	26	23.3567	1.034
27	cis-11-Eicosenoic acid	27	24.4777	2.4671
28	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	28	26.147	1.3625
29	9-Octadecenal, (Z)-	29	26.9741	3.636
30	Oleic Acid	30	28.7503	1.7494
31	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	31	29.8243	1.4891
32	Oleic Acid	32	31.793	2.0251
33	Butoxyacetic acid	33	32.1711	1.4582
34	Oleic Acid	34	33.1388	3.4251
35	Heneicosanoic acid, isopropyl ester	35	33.885	1.2771
36	cis-11-Eicosenoic acid	36	36.0798	1.6578
37	Oleic Acid	37	36.9009	1.1962
38	1,4,10,13-tetraoxa-7,16-dithiacyclooctadecane	38	38.0466	0.2059
39	Oleic Acid	39	38.2485	0.2571

Table 4: GC-MS profile of different solvent extracts of *M.Oleifera* seeds in n-Hexane extract.

S/No.	Name of compound	Peak number	R. time	Peak area (%)
	n-Hexane extract			
1	9-Octadecenal, (Z)-	1	6.9	0.9048
2	Dodecanoic acid	2	10.8215	0.2558
3	9,12-Octadecadienal	3	12.0373	0.9665
4	Hexadecanoic acid, methyl ester	4	14.0052	0.0793
5	Palmitoleic acid	5	14.8252	1.3877
6	n-Hexadecanoic acid	6	15.2774	4.16
7	cis-Vaccenic acid	7	15.7925	1.8416
8	9-Octadecenoic acid (Z)-, methyl ester	8	16.683	1.9308
9	Octadecane, 1-(ethenyloxy)-	9	16.8257	1.2534
10	cis-Vaccenic acid	10	17.4762	2.7971
11	n-Hexadecanoic acid	11	17.5924	1.3165
12	9-Octadecenoic acid	12	18.0605	2.2242
13	9-Octadecenoic acid	13	18.4824	2.0439
14	9-Octadecenoic acid	14	18.6162	2.8174
15	9-Octadecenoic acid	15	18.7286	2.064
16	9-Octadecenoic acid	16	18.9101	2.0405
17	cis-Vaccenic acid	17	19.331	9.2691
18	9-Octadecenoic acid	18	19.6072	7.1072
19	cis-13-Octadecenoic acid	19	19.759	7.142
20	9-Octadecenoic acid	20	20.068	5.5633
21	trans-13-Octadecenoic acid	21	20.5528	1.7679
22	cis-Vaccenic acid	22	21.0663	1.7891
23	9-Octadecenoic acid	23	21.7044	3.7429
24	cis-Vaccenic acid	24	22.6401	1.5163
25	9-Octadecenoic acid, (E)-	25	23.6093	3.5895
26	Bis(2-ethylhexyl) phthalate	26	24.0841	0.5855
27	1-Nonadecene	27	24.3659	1.1787
28	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	28	26.0638	0.183
29	Oleic Acid	29	27.1318	1.5644
30	trans-13-Octadecenoic acid	30	28.5725	3.5768
31	cis-13-Octadecenoic acid	31	29.4362	1.6566
32	trans-13-Octadecenoic acid	32	29.9566	1.2894
33	9-Octadecenoic acid	33	31.0465	2.7763
34	trans-13-Octadecenoic acid	34	32.1689	1.2494
35	Oleic Acid	35	32.9756	3.5438
36	1,4,10,13-tetraoxa-7,16-dithiacyclooctadecane	36	33.7171	1.8288
37	2-O-Mesyl arabinose	37	34.1801	1.5401
38	Z-10-Tetradecen-1-ol acetate	38	34.868	3.1484
39	Oleic Acid	39	35.8922	1.5354
40	Z-10-Tetradecen-1-ol acetate	40	36.8778	3.4061
41	13-Octadecenal, (Z)-	41	37.4142	1.3666

Class of Metabolite	Metabolite number
Fatty acid	26
Ester	10
Aldehyde	1
Alkene	1
Steroid	1

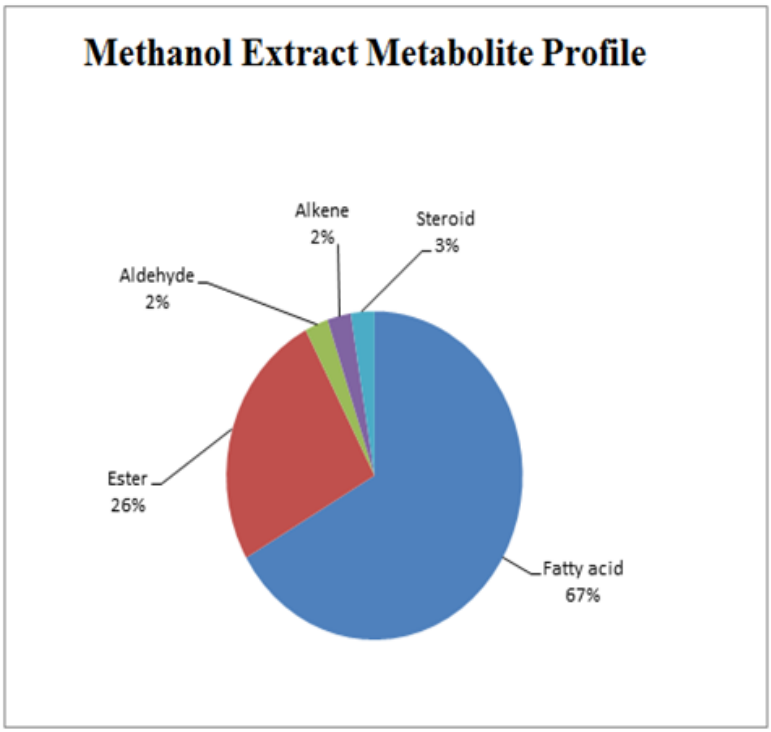

FIG.2: THE ABUNDANCE OF EACH PRIMARY AND SECONDARY METABOLITES OF METHANOL EXTRACTS OF *MORINGA OLEIFERA* SEEDS

Class of Metabolite	Metabolite number
Fatty acid	31
Aldehyde	1
Ester	3
Carboxylic acid	1
Alkane	2
Steroid	1

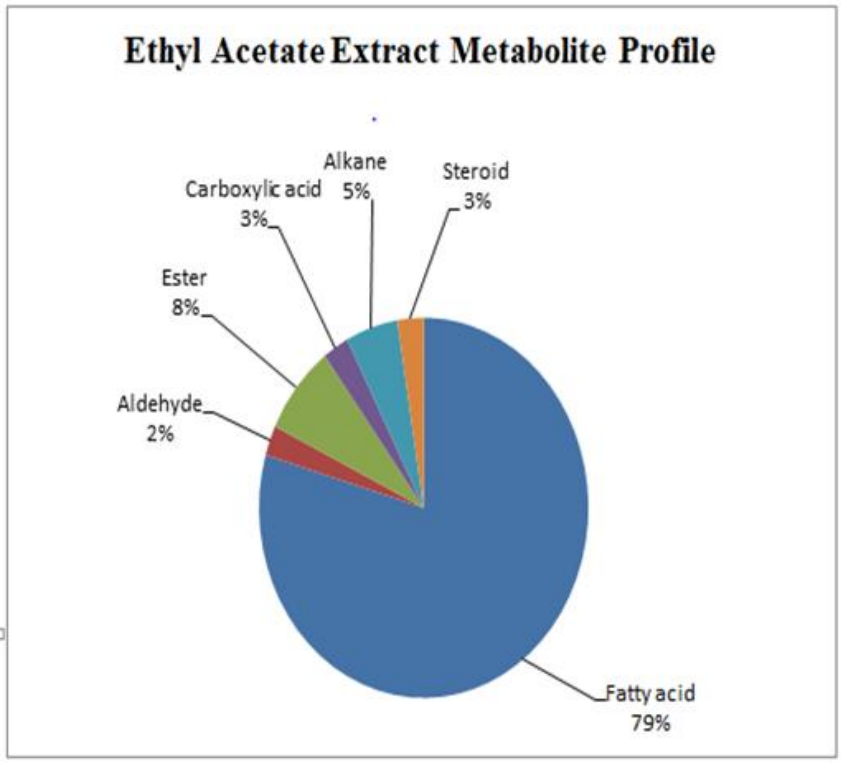

FIG.3: THE ABUNDANCE OF EACH PRIMARY AND SECONDARY METABOLITES OF ETHYL ACETATE EXTRACTS OF *MORINGA OLEIFERA* SEEDS

Class of Metabolite	Metabolite number
Fatty acid	28
Aldehyde	3
Ester	5
Benzene derivative	1
Alkane	2
Alkene	1
Pentose sugar	1

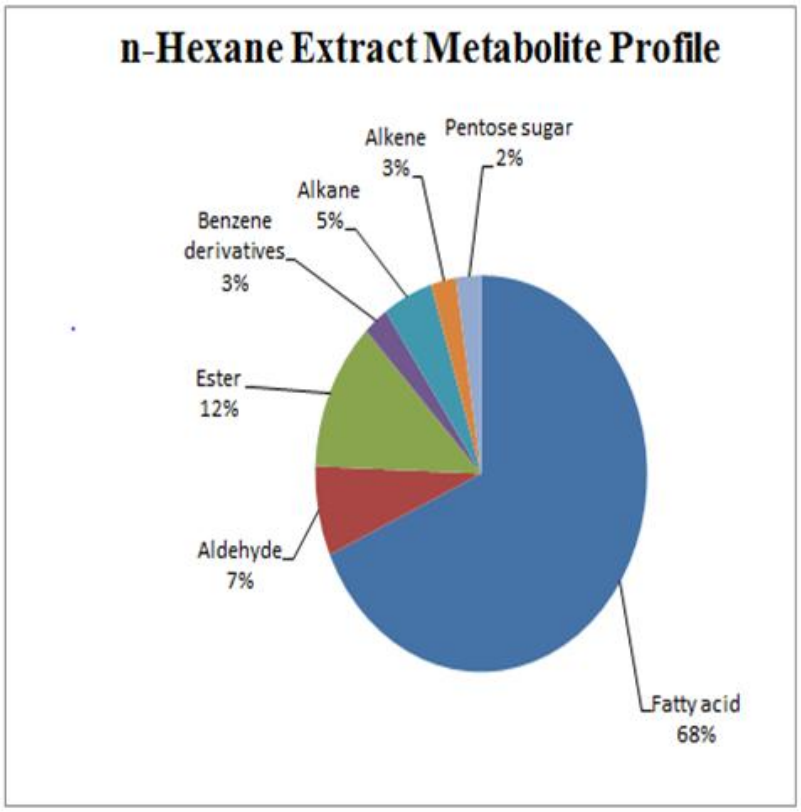

FIG.4: THE ABUNDANCE OF EACH PRIMARY AND SECONDARY METABOLITES OF n-HEXANE EXTRACTS OF *MORINGA OLEIFERA* SEEDS

4.0. Discussion

The compounds in the seed extracts resulted from the GC-MS analysis are reported to exhibit important biological activities. Some of the metabolites in the findings of this study differ according to the type of the extracting solvent and are found to be present in the earlier studies [26,27].

Palmitoleic acid an omega-7 monounsaturated fatty acid found in the n-hexane, ethyl acetate and methanol extract has been reported to have beneficial effects on insulin sensitivity, cholesterol metabolism, and hemostasis. It has been proposed that palmitoleic acid may prevent beta-cell apoptosis induced by glucose or saturated fatty acids [28]. Ethyl oleate found only in the methanol extract is used as a solvent for pharmaceutical drug preparations involving lipophilic substances such as steroids. It is also used as a lubricant and a plasticizer.

Cis-Vaccenic acid found in the n-hexane, ethyl acetate and methanol extract is the only known dietary precursor of cis-9, trans-11 isomers of conjugated linoleic acid (CLA), which is the polyunsaturated fatty acid (PUFA) with putative health benefits [29].

Erucic acid (monounsaturated omega-9 fatty acid) found only in the methanol extract is used to produce emollients, especially for skin and healthcare products. Like other fatty acids, it gets converted into surfactants.

Cis-11-Eicosenoic acid found in both ethyl acetate and methanol extract is a cis11-mono-unsaturated fatty acid that has potential medicinal use for treating diabetes and improving lipid metabolism. 2-0-Mesyl arabinose found only in n-hexane extract can be used in medical and pharmaceutical applications for the treatment of diseases such as diabetes, chronic constipation, mineral absorption disorder and secondary bile acid formation disorder [30].

9-Octadecenoic acid found in the n-hexane, ethyl acetate and methanol extract is an unsaturated fatty acid used as broad spectrum antibiotics in the treatment of diarrhea [31]. Docosanoic acid, ethyl ester found only in methanol extract is usually used in hair conditioners and moisturizers to give their smoothing properties. Bis(2-ethylhexyl) phthalate found only in n-hexane extract has a role as an apoptosis inhibitor, an androstane receptor agonist and a plasticiser. Octadecanoic acid compound found only in methanol extract is used to cure asthma, anti-inflammatory, and antiviral. Thiomorpholine found only in ethylacetate extract is used as an intermediate for blonanserin. It is used in the preparation of pyrrole derivatives, which acts as an antimycobacterial agent. It is further involved in the hypocholesterolemic activity [32,33].

n-Hexadecanoic acid found in the n-hexane, ethyl acetate and methanol extract helps in designing of specific inhibitors of phospholipase A(2) as anti-inflammatory agents [34]. Methyl 6-O-[1-methylpropyl]-.beta.-d-galactopyranoside found only in methanol extract exhibits hepatoprotective activity.

Dodecanoic acid (Lauric acid) found only in n-hexane extract is a medium-chain saturated fatty acid used for viral infections such as the flu, common cold and genital herpes. It is also used in the manufacturing of soaps and other cosmetics.The esters identified in this study are fatty acid esters. The alkanes are biosynthesized from fatty acids by direct decarboxylation whereby unsaturated fatty acids are converted to alkenes by direct decarboxylation and decarbonylation.

Oleic acid found in the n-hexane, ethyl acetate and methanol extract is a monounsaturated omega-9 fatty acid; Oleic acid is most commonly used for preventing heart disease, reducing cholesterol and preventing cancer [35]. Oleic acid may also be responsible for the hypotensive (or blood pressure reducing) effects of *M.oleifera* seeds.

5.0. Conclusion

The analysis of the active constituents and GC-MS profiling of *M. oleifera* seed crude extracts indicated the presence of biochemical metabolites with important medicinal properties. Phytochemical composition and yield of the extracts varied depending on the solvent types used for the extraction. Further investigation can be performed on fractions of solvent order than the crude extracts to isolate a greater yield of additional bioactive compounds.

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