Original Research Article

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

Abstract

The present study aimed at screening the different crude extracts of *Moringaoleifera* seeds for their bioactive constituents and exploring the metabolites present using GC-MS (Gas chromatography-Mass spectrometry). The phytochemical screening of the different crude extracts revealed the presence of tannins, 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 methanolextracts.GC-MS analysis confirmed the occurrence of forty-one (41) compounds in n-hexane extract, where major compounds includecis-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 werecis-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, includingcis-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 phyto-constituents; and thetherapeutically valued metabolites obtained will serve as a guide on further drug discovery process.

Keywords:*Moringaoleifera*seeds;phytochemical screening;GC-MS analysis; metabolite profiling.

1. Introduction

"Moringaoleifera (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 ethno-medicinal plant species occupying the food-medicine interface" [2,3]. The plant (*M.oleifera)* parts such as the leaves, seeds, stem, roots, flowers and bark are considered medicinal. The seeds which have been tagged as 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 *M.oleifera* bioactive compounds in functional foods and several commercial food uses. The plant may be used for various food technology applications, such as anti-microbial agents, antioxidants, and food fortification, includingnutritional and technological applications, because of the high amount and 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 the phytochemical screening, the medicinal value of the plant lies in the phytochemical (bioactive) constituents of the plant which shows various physiological effects on the 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 the 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 solvents 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 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 *Moringaoleifera*were collected from the open market, Bauchi,North Eastern Nigeria and identified by a taxonomist in the Department of Biological Sciences, AbubakarTafawaBalewa University, Bauchi, North Eastern Nigeria. The matured seeds were cracked (exerting manual 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 solventsviz., methanol, ethyl acetate and n-hexane were used in the sequential cold extraction methodwith increasing polarity for solvent-extraction of the grounded*Moringaoleifera*seed kernels. 100 g of the pulverized seed kernels were separately soaked in 300 $cm^3$of solvent for 72 hrs in an enclosed glass jar and filtered by the Whatman filter paper.The procedure with each solvent was repeated, evaporated to dryness and stored at 4^{O}Cin a refrigerator for further studies.

2.4.Qualitative PhytochemcialScreening of Different Solvent Extracts of *MoringaOleifera*Seeds

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

Test for Tannins

2 g of each sample was weighed into the beaker and 100cm^3 of water wasadded and allowed to soak for two hrs 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

2g of each sample was soaked with 100cm^3 of distilled water and allowed to stay for 48hrs, and filtered thereafter. The filtrate was kept in a conical flask with free drops of magnesium powder; and concentrated sulphuric acid (H_2SO_4) was 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 glacialacetic acid with a few drops of 5% ferric chloride were mixed together. This was under-layered with 1 ml ofconcentrated sulphuric acid.
Observation: The formation of a brown ring at the interface indicates the presence of cardiacglycosides.

Test for Saponins

2g of each sample was weighed and added to 2cm^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

2g 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 color indicates the presence of steroids.

Test for Terpenoids

(Salkowski test): Driedextract (50mg) was taken and soaked in 5 mL of ethanol.The extract was mixed in 2 mL of chloroform. It was slightlywarmed, and then cooled. 3 ml of concentrated H_2SO_4 wasadded slowly along the sides of the 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 was weighed into a 200ml flask and 95% ethanol was added and left for 4 hrs. The sample was filtered and few drops of Wagner's reagent (iodine crystals and potassium iodide) were added to the filtrate.
Observation: A yellowish coloration indicates the presence of alkaloids

2.5.Quantitative Phytochemcial Analysis of Different Solvent Extracts of *MoringaOleifera*Seeds

The presence of the Phytochemicals in different crude extractsof *Moringaoleifera* seeds wasquantified using the procedures as described by [15,16].

Estimation of Tannins

1g of eachsample was extracted with 25 ml 80:20 acetone: 10% glacial acetic acid for 4 hr. 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, 50mg/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 eachsample was extracted with 10 ml of 80 % methanol and left to stand for 2 hrs. It was filtered through the Whatman filter paper into a petri-dish, evaporated to dryness in an oven at40^0C and weighed.

Estimation of Cardiac Glycosides

1g of eachsample with 40ml of water was extractedand placed in an oven at 100^0Cfor 15 mins. Then, to 1ml of the extract dissolved in 5ml of waterwas added 2ml of glacial acetic acid followedby one drop of iron chloride ($FeCl_3$) and 1ml ofH_2SO_4. The absorbance was then measured at410nm.

Estimation ofSaponins

1g of each sample was dispersed in15ml of 20% ethanol. The suspension was putinside the water bath at 55^0C for 4 hrs. Themixture was filtered and the residue was re-extracted with another 15ml of 20% ethanol twice. Theextract was reduced to about 5ml in the oven.
The concentrate was transferred into a 250mlseparating funnel and 5ml of petroleum ether was added and mixed vigorously. The petroleumether layer was discarded and 3ml of butanol was added to the aqueous layer. The extract waswashed twice with 5ml of 5% sodium chloride. The remaining solution was poured into aweighed petri-dish, evaporated to dryness in theoven and the residue was weighed.

Estimation of Steroids

1ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) 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

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

Estimation of Alkaloids

1g of each sample (W) was extracted with20 ml of 10% acetic acid in ethanol, mixed and

allowed to stand for 4 hrs. The extract was filtered through the 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 (W1) was weighed and filtered through Whatman filter paper. The filter paper was dried in the oven at 60 ^{0}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 (Gas chromatography-Mass spectrometry) analysis on the hexane, ethyl acetate and methanol extracts of *Moringaoleifera* seeds was carried out using Agilent technologies 7890A GC and 5977B MSD with experimental conditions of GC-MS system as follows: Hp 5-MS capillary standard non-polar column, dimension: 30 M, ID: 0.25 mm, film thickness: 0.25µm. Flow rate of the mobile phase (carrier gas: Helium) was set at 1.0 ml/min. In the gas chromatography part, the temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and the injection volume was 1 µl. Samples were dissolved in methanol and 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. Statistical analysis

In each experiment, the datawerereplicated threetimeswith results expressed as mean ± standard deviation (SD).Data were compared using one-way analysis of variance (One-way ANOVA) and Tukey's test was performed to determine the statistically significant differences at $p < 0.05$ levelusing GraphPad Prism (version 5.0) statistical software.

3. Results and Discussion

3.1. Solvent Yield

The yields of methanol, ethyl acetate andn-hexaneobtained from the *Moringaoleifera* seed are given as8.15 ± 0.52, 18.44 ± 0.03 and 17.25 ± 0.84.The yields varied greatly between the different solvents used for the extraction.

3.2. Phytochemical Screening

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

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

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

Key: - MeOH = Methanol Extract
EtOAc = Ethylacetate 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 *MoringaOleifera*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

The 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.

3.3. Metabolite Profiling of Different Crude Extracts

The Gas Chromatogram Mass Spectrometry (GCMS) analysis shows the distinct chromatogram of *Moringaoleifera* 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 Tables**3a-3c**.

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 Figures**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 offatty acids (67 %), esters (26 %), aldehyde (2 %), alkane (2 %) and steroids (3 %).

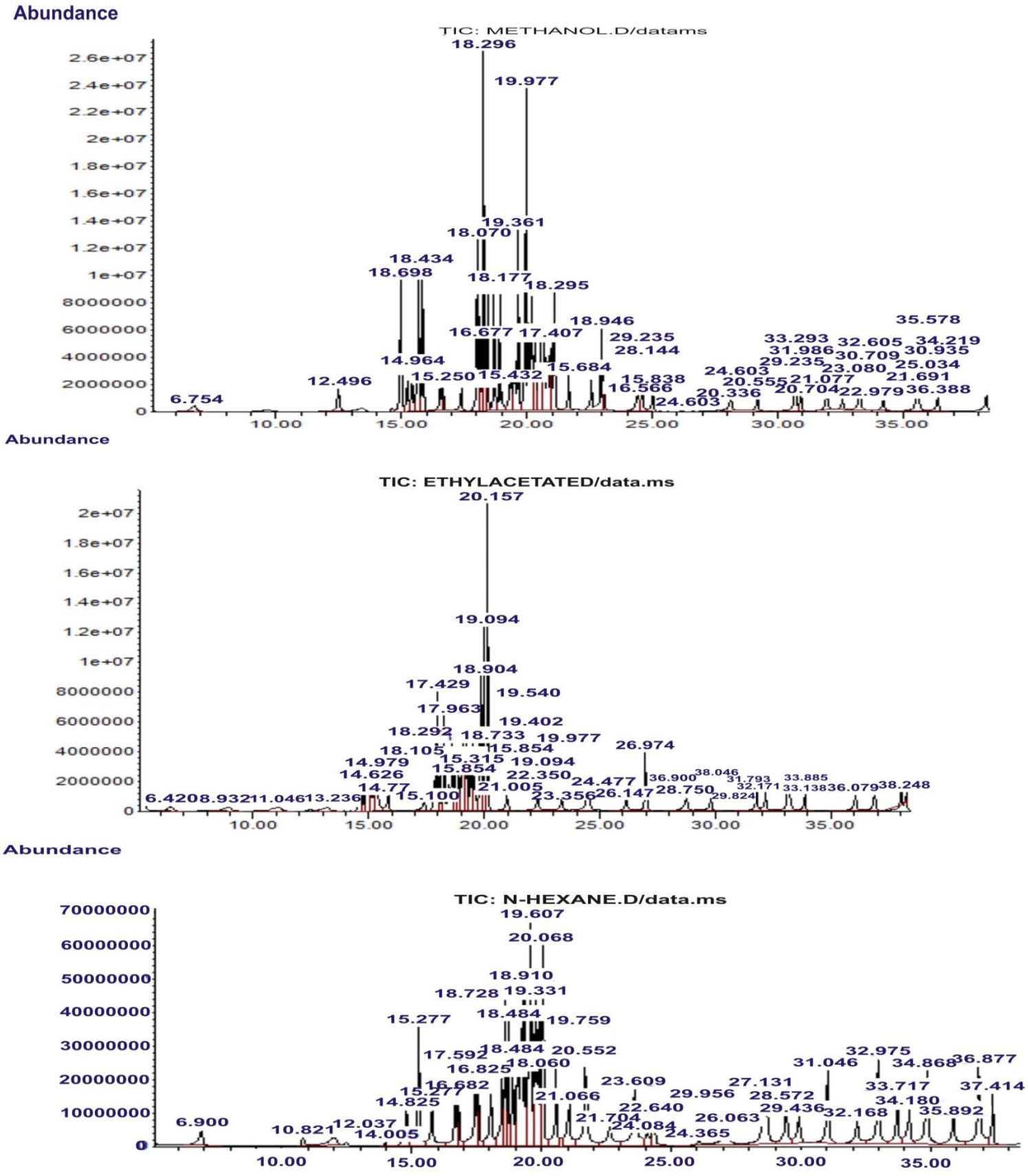

Fig 1: GC-MS total ion chromatogram (TIC) of analysis of different solvent extract of *M. oleifera seeds* (a) methanol (b) ethyl acetate (c) n-hexane extracts.

TABLE 3a: GC-MS profile of Methanol extract of *M.Oleifera*seeds.

S/No.	Name of the compound	Peak number	R. time	Peak area (%)
	Methanol 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-Octaecenoic 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

TABLE 3b: GC-MS profile of Ethyl acetate extract of *M.Oleifera*seeds.

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 3c: GC-MS profile of n-Hexane extract of *M.Oleifera*seeds.

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

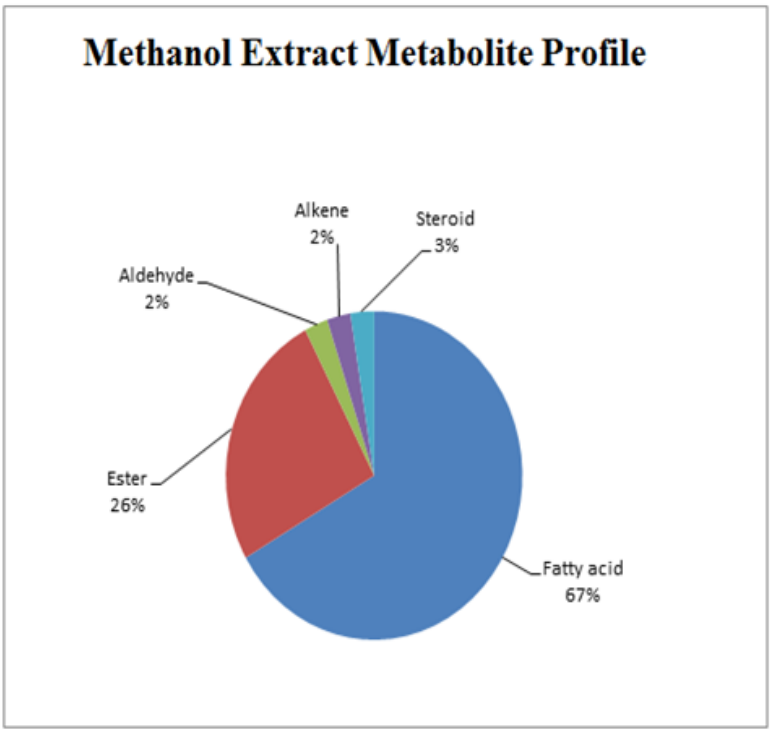

FIG.2: THE ABUNDANCE OF EACH PRIMARY AND SECONDARY METABOLITE OF METHANOL EXTRACT OF *MORINGA OLEIFERA* SEEDS

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

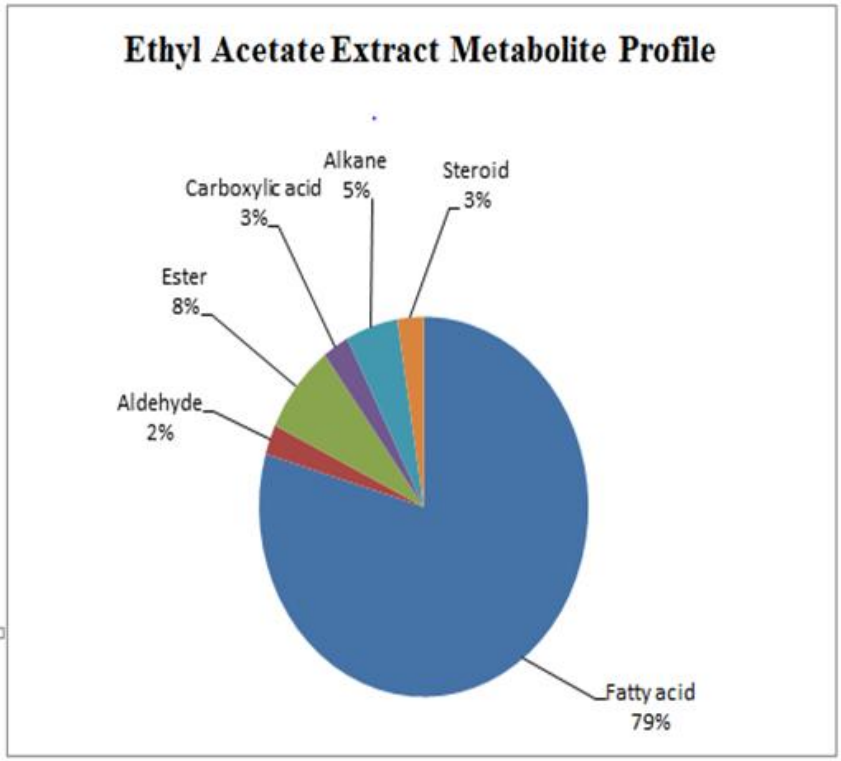

FIG.3: THE ABUNDANCE OF EACH PRIMARY AND SECONDARY METABOLITE OF ETHYL ACETATE EXTRACT OF *MORINGA OLEIFERA* SEEDS

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

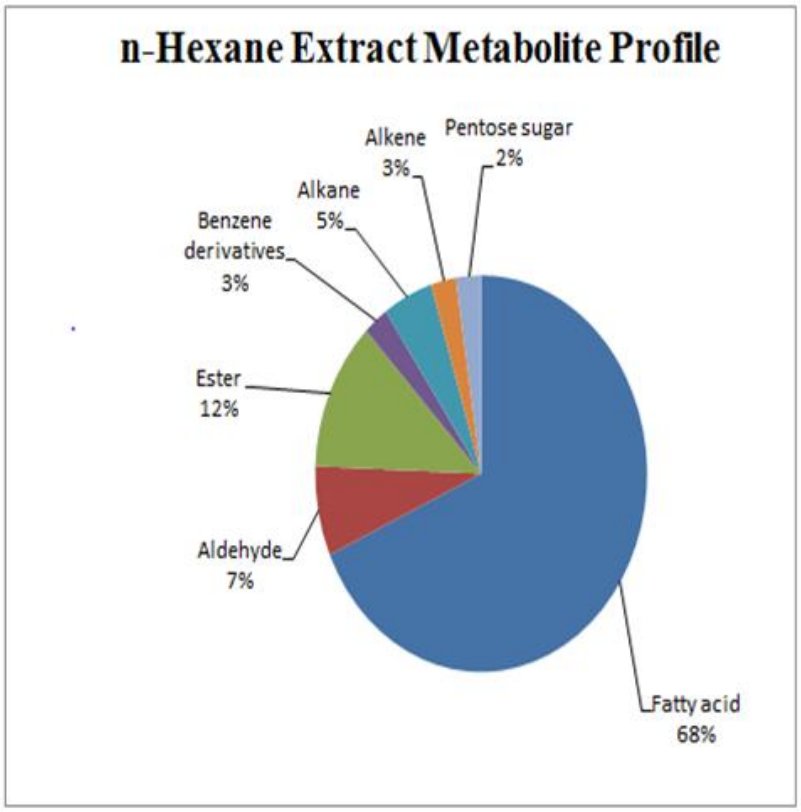

FIG.4: THE ABUNDANCE OF EACH PRIMARY AND SECONDARY METABOLITE OF n-HEXANE EXTRACT OF *MORINGA OLEIFERA* SEEDS

4.0.Discussion

Phytochemical screening usually aids in revealing bioactive constituents of the plant extracts as well as to discover the prominent ones. These bioactive constituents can be used in the synthesis of drugs to treat various metabolic, immunological and neurological disorders in humans.

This study established that *Moringaoleifera* 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 for the treatment of varicose ulcers, hemorrhoids, minor burns, frostbite, as well as inflammation of the gums. Internally tannins are administered in cases of diarrhea, 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 disease. In addition, flavonoids protect against ulcer development by initiating a gastric mucosa cover, increasing capillary resistance, and improving 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 leads 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], Saponinsare used for antimicrobial activity and inhibit mouldas they havehaemolytic activities, cholesterolsbinding usage, also in treatment of yeast andfungal infections. Saponins also prevent cancercell multiplication, thus inhibiting unwantedcancerous cell generation in the body [23]. The saponin content of the sample may bethe reason for its usage as a natural antibiotic and aids in the fight of infection and microbialinvasion [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].Thepresence of terpenoid found in the seed of *M.oleifera* is suggestive of its antifungal and antibacterialactivities and this can be attributed to their membrane disruption and inhibitory action on bacterial cells orfungi.

Alkaloids are significant for protection against microbial and pesticide activities as they are 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].

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

"Palmitoleic acid an omega-7 monounsaturatedfatty 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 healthbenefits"[29].

Erucic acid(monounsaturated omega-9 fatty acid)found only in the methanol extract isused 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 canbe 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 anunsaturated fatty acid used as broad spectrum antibiotic in the treatment ofdiarrhea [31]. Docosanoic acid, ethyl esterfound 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 anantimycobacterial agent. It is further involved in the hypocholesterolemic activity [32,33].
n-Hexadecanoic acid found in the n-hexane, ethyl acetate and methanol extracthelps in designing of specific inhibitors of phospholipase A(2) as anti-inflammatory agents [34]. Methyl 6-O-[1-methylpropyl]-.beta.-d-galactopyranosidefound only in methanol extractexhibits hepatoprotective activity.

Dodecanoic acid (Lauric acid) found only in n-hexane extractis a medium-chain saturated fatty acid used forviral 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

In this research,the analysis of bioactive constituentsand GC-MS profiling of*Moringaoleifera*seed crude extracts indicated the presence of biochemical metabolites with important medicinal properties.Phytochemicalcomposition and yield of the extracts varied depending onthe solvent types used for the extraction.Further investigation can be performed on the fractions of solvent other than the crude extracts to isolate a greater yield of additional bioactive compounds.

References

1).Lakshmidevamma, T. N., Ugalat, J., Apoorva, K. A., Suresh, S. P., Doddamani, M., Kadam, S., et al. (2021). Genetic Diversity of Moringa (*MoringaOleifera Lam*.).*The Moringa Genome*, 57–65. Compendium of Plant Genomes, Cham: Springer.

2). Palada, M. C. (2019). *The Miracle Tree: MoringaOleifera*. Philippine: Xlibris Corporation. Google Scholar

3).Jattan, M., Kumari, N., Kumar, R., Kumar, A., Rani, B., Phogat, D. S., et al. (2021).Moringa (*MoringaOleifera L*.): An Underutilized and Traditionally Valued Tree Holding Remarkable Potential. *J. Hortic. Sci.* 16 (1), 1–13. doi:10.24154/jhs.2021.v16i01.001CrossRef Full Text | Google Scholar

4) Makkar H, Becker K. Nutrients and anti-quality factors in different morphological parts of the *Moringaoleifera* tree. J. Agri. Sci. Cambridge. 1997; 128:311-322.

5). Radha C, Ogunsina BS, Hebina BKT (2015) Some quality and micro-structural characteristics of soup enriched with debittered*Moringaoleifera* seeds flour. Am J Food SciTechnol 3:145–149

6). Devisetti R, Sreerama YN, Bhattacharya S (2016) Processing effects on bioactive components and functional properties of moringa leaves: development of a snack and quality evaluation. J Food SciTechnol 53:649–657

7).Oyeyinka AT, Oyeyinka SA (2018) *Moringaoleifera* as a food fortificant: recent trends and prospects. J Saudi SocAgricSci 17:127–136

8).Radka Z. Vrancheva, Ivayla N. Dincheva, Ina Y. Aneva and Atanas I. Pavlov. Metabolite profiling by means of GC-MS combined with principal component analyses of natural

populations of *Nectaroscordumsiculum* ssp. *bulgaricum* (Janka) StearnJournal: ZeitschriftfürNaturforschung C, 2020, Volume 75, Number 11-12, Page 451
DOI: 10.1515/znc-2020-0058

9). Sewell R.D.E, and. Kopaei M.R. The history and ups and downs of herbal medicines usage.Journal of Herbmedpharmaology. 3,(2014)

10). Mirzaeian R., Sadoughi F, Tahmasebian S and Mojahedi M. The role of herbal medicines in health care quality and the related challenges Journal of herb med pharmacology. (2021). http://doi.org/10.34172/jhp.2021.17

11).Lapornik B, Prošek M, Wondra AG. Comparison of extracts prepared from plant by-products using different solvents and extraction time. J Food Eng. 2005;71(2):214-222.

12).Debela, A., Manual for phytochemical screening of medicinal plants. Ethiopian. Health and Nutrition Research Institute, AddisAbaba, Ethopia, 2002, 35 – 47.

13). Indumathi, C., G. Durgadevi, S. Nithyavani. and P.K.Gayathri (2014). Estimation of terpenoid contentand its antimicrobial property in *Enicostemmalitorrale*. Int. J. Chem.Tech. Res. 6 (9): 4264 –4267

14). Omokpariola, D., Precious-Egere, S., Omokpariola, P., &Okechukwu, V. (2021). Phytochemical and Anti-Microbial Analysis of Metabolites in seeds of *Moringaoleifera* grown in Nigeria.*Progress in Chemical and Biochemical Research*, *4*(3), 268-277. doi: 10.22034/pcbr.2021.269528.1173

15). Mokogwu, A.T.H., Okorie, U.P., Digban, K.A., Abubakar, A., &Mokogwu, E.E. (2014) Phytochemical and Trace Element Analysis of *VernoniaAmygdalina* (Bitter Leaf) in Different Locations in Nigeria.African Journal of Cellular Pathology 3:44-50.

16). Madhu, M., Sailaja, V.J., Satyadev, T.N., &Satyanarayana, M.V. (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry, 5, 25-29.

17).Chollom, Solomon & A, Agada&Gotep, Jurbe&Mwankon, s.E.& C, Dus& Bot, Yakubu& Y, Nyango&Lagang, Changjul&Fyaktu, Emmanuel John & J, Okwori. (2010). Investigation of aqueous extract of *Moringaoleifera lam* seed for antiviral activity against newcastle disease virus in ovo. Journal of medicinal plant research. 6. 3870-3875. 10.5897/JMPR12.394.
18).Ajibade TO, Arowolo R, Olayemi FO. 2013. Phytochemical screening and toxicity studies on the methanol extract of the seeds of *Moringaoleifera.* J Complement Integr Med 10: 11–16.

19). Iwu, I.C., Onu, U.L Ukaoma, A.A and Oze, R.N. Phytochemical, Antimicrobial and GC/MS analysis of the root of *StachytarphetaCayennensis* (L.Vahl) grown in Eastern Nigeria. International Research Journal of Natural Science6(2), (2018) 1-14.

20). Ahmed, F.A.R., Muhammad, D.I., Saie, B.K., Health Benefits of *Moringaoleifera.* Asian Pacific Journal of Cancer Prevention. 15,(2014) 8571-8576.

21). Aiyelaagbe, Olapeju&Osamudiamen, Paul. (2009). Phytochemical screening for active compounds in *Mangiferaindica* leaf from Ibadan, Oyo State. Plant Sci Res. 2. 11-13.
Ajibade, T.O., Arowolo, R., &Olayemi, F.O. (2013). Phytochemical screening and toxicity studies on the methanol extract of the seeds of moringaoleifera. Journal of complementary & integrative medicine.

22). Iwu, I.C. Onu, I.U. Chijioke-Okere, A. Ukaoma M.A and Azorji, J.N GC-MS, Phytochemical and antimicrobial analysis of *Pentaclethramacrophylla* leaf. Int. J. Sci. Tech. 2016, 4 (7), 151-159

23). Park, B., You, S., Cho, W.C.S., Choi, J-Y., and Lee, M.S. A systematic review of herbal medicines for the treatment of cancer cachexia in animal models. Journal of Zhejiang University-Science B. 20(1), (2019) 9-22.

24) Dash, D. K. ,Tyagi, C. K. , Sahu, A. K. , &Tripathi, V. (2022). Revisiting the Medicinal Value of Terpenes and Terpenoids.In V. S.Meena, H. P. Parewa,& S. K. Meena (Eds.), Revisiting Plant Biostimulants. IntechOpen. https://doi.org/10.5772/intechopen.102612

25). Zanrate, H., Susana, M., Juarez, A., Rosario, M.D., Abel, C.G., Cesar, O.L., Chavez, G., Josue, A., Garfias, S., Nezahualcoyotl J.D.J., and Ramos. A.F. Flavonoids, Phenolic content and antioxidant activity of *propolis*from various areas of Guanajuato. Mexico. Food Science and Technology. 38(2) (2018) 210-215. http://doi.org/10.1590/fst.29916.

26). Chelliah, R.; Ramakrishnan, S.; Antony, U. Nutritional quality of *Moringaoleifera* for its bioactivity and antibacterial properties. Int. Food Res. J. 2017, 24, 825–833.

27). Bhalla, N.; Ingle, N.; Patri, S.V.; Haranath, D. Phytochemical analysis of *Moringaoleifera* leaves extracts by GC-MS and free radical scavenging potency for industrial applications. Saudi J. Biol. Sci. 2021. [CrossRef]

28) Morgan, N. G., &Dhayal, S. (2010). Unsaturated fatty acids as cytoprotective agents in the pancreatic beta-cell.*Prostaglandins, leukotrienes, and essential fatty acids*, *82*(4-6), 231–236. https://doi.org/10.1016/j.plefa.2010.02.018

29). Field, C. J., Blewett, H. H., Proctor, S., & Vine, D. (2009). Human health benefits of vaccenic acid. *Applied physiology, nutrition, and metabolism = Physiologieappliquee, nutrition etmetabolisme*, *34*(5), 979–991. https://doi.org/10.1139/H09-079

30). Hu, B. , Li, H. , Wang, Q. , Tan, Y. , Chen, R. , Li, J. , Ban, W. and Liang, L. (2018) Production and Utilization of L-Arabinose in China. *World Journal of Engineering and Technology*, **6**, 24-36. doi: 10.4236/wjet.2018.63B004.

31). Garba S and Garba I. Anti-Diarrhoeal Properties of Cis-9-Octadecenoic Acid Isolated From *LandolphiaOwariensis* Plant. Organic &Medicinal Chem IJ. 2017; 3(4): 555619. DOI: 10.19080/OMCIJ.2017.03.555619

32). Kacan, M.; Turkyilmaz, M.; Karabulut, F.; Altun, O.; Baran, Y. Complexation, thermal and catalytic studies of N-substituted piperazine, morpholine and thiomorpholine with some metal ions. *Spectrochim.Acta, Part A***2014,***118*, 572-577.

33).Tooulia, K. K.; Theodosis-Nobelos, P.; Rekka, E. A. Thiomorpholine Derivatives with Hypolipidemic and Antioxidant Activity. *Arch. Pharm.***2015,***348* (9), 629-634.

34).Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., &Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical biology & drug design*, *80*(3), 434–439. https://doi.org/10.1111/j.1747-0285.2012.01418.x

35).Voelker R. Oleic Acid Can Make Heart Claim Without Hard Evidence. *JAMA.* 2019;321(1):23. doi:10.1001/jama.2018.19747