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Phytochemical analysis of bioactive compounds in ethanol leaf extract of *Moringa oleifera* [Lam.]

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Abstract

Moringa oleifera is called a miracle plant due to its diverse uses and several medicinal benefits all over the world. There are several bioactive secondary metabolites yet to be discovered. This study was conducted to actuate the biochemical content of ethanol leaf extract of *Moringa oleifera* (ELEMO) using Agilent Gas Chromatography–Mass Spectrometry (GC-MS). Quantitative phytochemical screening of the ethanolic extracts of the leaf revealed the presence of flavonoids, tannins, alkaloids, terpenoids, steroids and phenols. The GC-MS results of the extract were relevant to the National Institute of Standards and Technology (NIST) library. GC-MS analysis of ELEMO showed the presence of n-hexadecanoic acid, methyl ester (33.42), Pentadecanoic acid, 14-methyl-methyl ester (36.23), Ethyl 9,12,15-octadecatrienoate (33.12 and 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1 H-1,2,3-triazole (31.24). Results from this study may potentiate the discovery of more valuable bioactive components of industrial and pharmaceutical importance.

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Introduction

Moringa oleifera Lam. (commonly known as drumstick or horse radish tree) is a small to medium-sized tree (10 to 15 m in height) belonging to the family *Moringa*ceae. It is indigenous to many countries in Africa, Arabia, South East Asia, North West India, the Caribbean Islands, the Pacific, and South America. *Moringa oleifera* is widely cultivated throughout the tropics, subtropics and semi-arid tropics (Raman *et al.,* 2018). Lucky *et al.* (2018) reported that almost all parts of *M. oleifera* (root, gum, bark, leaves, fruits [pods], seeds, flowers) and seed oil are being marketed as herbal remedies for various diseases in Nigeria. All parts of the plant are edible with matured seeds containing approximately 40% edible oil. After oil extraction, seed residues are used as feed supplements for livestock. In arid regions, *M. oleifera* is cultivated as a nutritional crop for the rural populace (Sanchez-Machado *et al.,* 2010). Though 12 varieties of *Moringa* spp. exist, *M. oleifera* is likely the most widely known (Ramachandran *et al.,* 1980). *Moringa oleifera* has been reported to possess various medicinal

properties including high antioxidant activity, neuroprotective, hepatoprotective, anti-inflammatory, antidiarrheal, nephroprotective, antidiabetic and anticancer, among others (Sreelatha & Padma, 2009; Singh *et al.,* 2014; Al-Asmari *et al.,* 2015; Giacoppo *et al.,* 2017; Ekong *et al.,* 2017; Kou *et al.,* 2018; Hagoel *et al.,* 2019). Several reports have shown *M. oleifera* leaves, seeds and pods to contain high-quality protein, vitamins (Vit. A [Beta- carotene] and Vit. C), important minerals (K, Ca, Fe & Cu), amino acids and fatty acids such as behenic, linoleic and linolenic acid (Makkar & Becker, 1996; Adegbenro, 2015; Maizuwo *et al.,* 2017). It has been pharmacologically characterized in some regions of the world such as Thailand (Mokkhasmit *et al.,* 1971), India (Nadkarni*,* 1976), Pakistan (Iqbal & Bhanger, 2006) and Jamaica (Wright *et al.,* 2017). In the present study, quantitative determination of the bioactive secondary metabolites was carried out in the ELEMO followed by the Gas Chromatogram Mass Spectrometric method (GC-MS). This work will brighten the pharmacological profile of *M. oleifera* in the arena of phytomedicine in Nigeria and the world at large.

Materials and Methods

Collection, identification and preparation of Moringa oleifera leaves

Fresh young leaves of *M. oleifera* were obtained from the Pioneer Sustainable Agriculture Limited, KM 36 Kaduna-Zaria Expressway, Jaji, Kaduna State, Nigeria, in October 2019. Authentication of the plant with voucher number, ABU0517, was carried out by Mr Sanusi Namadi (a taxonomist) at the herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria. Fine homogenous powder of the leaf was prepared as described by Saleh *et al.* (2020).

Determination of percentage loss of weight on drying

Percentage loss of weight on drying (% LOD) of *Moringa oleifera* leaf was carried out gravimetrically as described by Saleh *et al.* (2020). Five grams (5 g) of fresh leaf were weighed, air–dried and placed in a dried and tared flat weighing bottle. The sample was air-dried under shade until a constant weight was obtained. The % LOD was calculated using the following equation:

$$\text{Percentage LOD} = \left(\frac{\text{Loss in Weight}}{\text{Weight of Dried Leaf}}\right) \times 100$$

Determination of ethanol soluble extractive value

Five grams of oven–dried leaf powder of *M. oleifera* was transferred to a conical flask. One hundred millilitres of ethanol 70% (v/v) was added to the flask and covered with aluminum foil. They were frequently shaken during the first 6 h and allowed to stand for 18 h separately. They were filtered carefully to minimize the loss of ethanol. The filtrates were collected and transferred to a weighed thin porcelain and evaporated to dryness in a water bath. The sample was dried completely in an oven at 45 ^{o}C until a constant weight was obtained. It was kept in a desiccator to cool and the percentage of ethanol-soluble extractive yield was calculated with reference to oven–dried leaf (Upreti *et al.,* 2013; Saleh *et al.,* 2020) by using the following equation:

$$\text{Percentage Extractive Value} = \left(\frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}}\right) \times 100$$

Preparation of crude ethanol leaf-extracts of Moringa oleifera

The crude extract was prepared according to Saleh *et al.* (2020). Five hundred grams of powdered leaf sample of *M. oleifera* was placed in a 5 L conical flask. It was soaked in 1.5 L of 70% v/v ethanol and covered with aluminium foil. The mixtures were allowed to stand in the laboratory at room temperature for 72 h with frequent agitation. The mixture was then strained using a muslin cloth to remove solid material (residue). The extraction was repeated twice by soaking the solid material using 2/3rd of the initial volume of the ethanol for 24 h. The strained liquid was clarified by gravitational filtration using Whatman filter paper (size 1). The filtrate was then concentrated into semi-solid crude extract in a water bath maintained at 50^{o}C and was packaged in labelled airtight extract bottles and then stored in a desiccator prior to use.

Quantitative determination of the chemical constituents

Determination of total flavonoid content: The total flavonoid content (TFC) of ELEMO was determined by Aluminum calorimetric assays (Zhishen *et al.,* 1999). Then, 0.5 mL aliquot of the ELEMO and standard solution (0.1 mg/mL) of rutin was added with 2 mL of distilled water and, subsequently, 0.15 mL of sodium nitrite (5% $NaNO_2$ w/v) solution was added. After 6 min, 0.15 mL of 5% $AlCl_3$ (w/v) solution was added. The solution was allowed to stand for 6 min and after that 2 mL of 4% NaOH (w/v) solution was added to the

mixture. The final volume was adjusted to 5 mL with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm against the same mixture. The TFC was determined as rutin equivalent per gram of sample with the help calibration curve of rutin. All determinations were performed in triplicate.

Determination of total alkaloid content: Total alkaloid (TA) was quantified by a spectrophotometric method according to Sherif *et al.* (2014). This method is based on the reaction between alkaloid and bromocresol green (BCG). The ELEMO and fractions (1 mg/mL) were dissolved in 2 N HCl and then filtered. The pH of the phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One milliliter (1 mL) of this solution was transferred to a separating funnel and then 5 mL of BCG solution along with 5 mL of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The ELEMO was collected in a 10 mL volumetric flask and diluted with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The whole experiment was conducted in 3 replicates.

Determination of saponin content: Total saponin was determined according to the method described by Makka *et al*. (2007). One gram (1 g) of freeze-dried ELEMO was dissolved in 200 mL of aqueous 50% methanol to make a suitable aliquot (5 mg/mL). Vanillin reagent (0.25 mL; 8%) was added followed by H_2SO_3 (2.5 mL; 72% v/v). The reaction mixture was mixed well and incubated at 60 ^oC in a water bath for 10 min. After incubation, the reaction mixtures were cooled in ice and absorbance at 544 nm (UV visible spectrophotometer) was obtained from suitable aliquots of diosgenin (0.5 mg/mL in 50% aqueous methanol). The total saponins concentration was expressed as mg diosgenin equivalents (DE) per g of dry weight;

$$\text{Percentage of saponin} = \left(\frac{\text{Weight of saponin}}{\text{Weight of sample}}\right) \times 100$$

Determination of total phenolic content: The total phenol contents (TPC) were determined according to a standard method by Alhakmani *et al.* (2013). The TPC in ELEMO and fractions were measured spectrophotometrically by Folin-Ciocalteu (FC) colourimetric method, with Gallic acid as the standard and the results expressed as GAE per gram of sample. Different concentrations (0.01-0.1 mg/mL) of Gallic acid were prepared in methanol. Aliquots of 0.5 mL of the test sample and each sample of the standard solution were taken, mixed with 2 mL of FC reagent (1:10 in deionized water) and 4 mL of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubated at room temperature for 30 min with intermittent shaking. The absorbance was taken at 765 nm using methanol as a blank. All the samples were analysed in 3 replications. The TPC was determined with the help of a standard curve prepared from pure phenolic standard (Gallic acid).

Determination of tannin content: The tannins were determined by the FC method (AfifyAel-M *et al.,* 2012). About 0.1 mL of the ELEMO was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of FC phenol reagent, 1 mL of 35% of $NaCO_3$ solution and diluted to 10 mL with distilled water. The mixture was well shaken and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with a UV spectrophotometer. The tannin content was expressed in mg of Gallic acid equivalent (GAE)/g of ELEMO.

Gas chromatography-mass spectrometry characterization

Five hundred milligram (500 mg) of ELEMO was dissolved in 10 mL of ethanol to make an aliquot of the extract. Quantitative characterization of the secondary metabolites present in ELEMO was analyzed using GC-MS (Agilent Technology; GC7890B - 5977A MSD, USA) system, and electron-impact source, according to Ademiluyi *et al.* (2016) with slight modification. 5% Phenyl Methyl Siloxane was used to coat the HP-5 capillary column, with 30 m length x 0.32 mm diameter x 0.25 µm film thickness used in the stationary phase of separation of the chemical compounds present. The sample was analyzed in replicates for validation in order to observe the consistency of the respective retention time, constituent compound name, Quality ion (Q-Ion), molecular weight (amu) and percentage total (%Total).

$$\%\text{Total} = \frac{\text{abundance of individual constituents in ELEMO}}{\text{Total abundance of all constituents in ELEMO}} \times 100$$

Results

The mean percentage loss on drying (% LOD) of *M. oleifera* leaf was 78.96 ± 1.220 with a range of 77.60 to 80.40 (Table 1)

The mean ethanol–soluble extractive value for *M. oleifera* leaves was 22.00 ± 0.707 with a range of 21.00 to 23.00% (Table 2).

The quantitative phytochemicals screening showed the presence of 29.33 ± 0.982 mg of TFC; 1.287 ± 0.114 mg of alkaloids; 12.67 ± 0.333 mg of saponin and 56.257 ± 0.311 mg of TPC for every 1 g of the ELEMO. However, tannins appeared to be unquantifiable or absent in the ELEMO (Table 3). The standard calibration curves for the quantification are shown in Figure 1.

The GC-MS characterization of the ELEMO detected a number of bioactive secondary metabolites (Table 4, Figure 2). The active principles and their retention time (RT), molecular formula, molecular weight (MW) and peak area in percentage. GC-MS analysis of ELEMO revealed the existence of n-Hexadecenoic acid, methyl ester (13.805), Methyl 9-cis,11-trans-octadecadienoate, 9,12-Octadecadienoic acid, methyl ester (16.085), Phytol (16.476), 9,12-Octadecadienoic acid (Z, Z)- (16.549), 16-methyl-, methyl ester (16.626), Linoleic acid ethyl ester (17.070), Dodecane, 1,1'-oxybis- (23.299), Squalene (25.487), Eicosane (33.412), Palmitoleic acid, 9-Octadecenoic acid (31.509), Octadec-9-enoic acid, Dodecyl propyl ether, Octyl tetracosyl ether (34.054) were present in ELEMO.

Discussion

In the present study, the phytochemical components, the quantitative analyses and the percentage crude yield of bioactive constituents of the *M. oleifera* leaf revealed the presence of flavonoids, alkaloids, saponins and phenols in the ELEMO, as earlier reported in many studies (Atawodi *et al.,* 2010; Maizuwo *et al.,* 2017; Atta *et al.,* 2018; Atta *et al.,* 2019). Quantitative analysis

Table 1: Percentage loss on drying of *Moringa oleifera* leaf (n = 5)

Fresh weight (g)	Dry weight (g)	Loss in weight (g)	Loss in weight (%)	Mean % LOD ± SD
5.00	1.06	3.94	78.80	78.96 ± 1.220
5.00	1.12	3.88	77.60	
5.00	1.10	3.90	78.00	
5.00	0.98	4.02	80.40	
5.00	1.00	4.00	80.00	

*SD = standard deviation, n = sample size

Table 2: Ethanol soluble extractive value of the dried leaf of *M. oleifera* (n=5)

Dried leaf (g)	Weight of extract (g)	Extract value (%)	% Mean extractive value ± SD
5.00	1.05	21.00	22.00 ± 0.707
5.00	1.10	22.00	
5.00	1.10	22.00	
5.00	1.10	22.00	
5.00	1.15	23.00	

*SD = standard deviation, n = Sample size

Table 3: Quantitative values of some phytochemical constituents in ELEMO

Products	ELEMO (mgGAE/g)
Total flavonoid content (TFC)	29.33 ± 0.982
Alkaloids	1.287 ± 0.114
Saponins	12.67 ± 0.333
Total phenolic content (TPC)	56.257 ± 0.311
Tannins	-

*ELEMO = ethanol leaf-extracts of *Moringa oleifera*

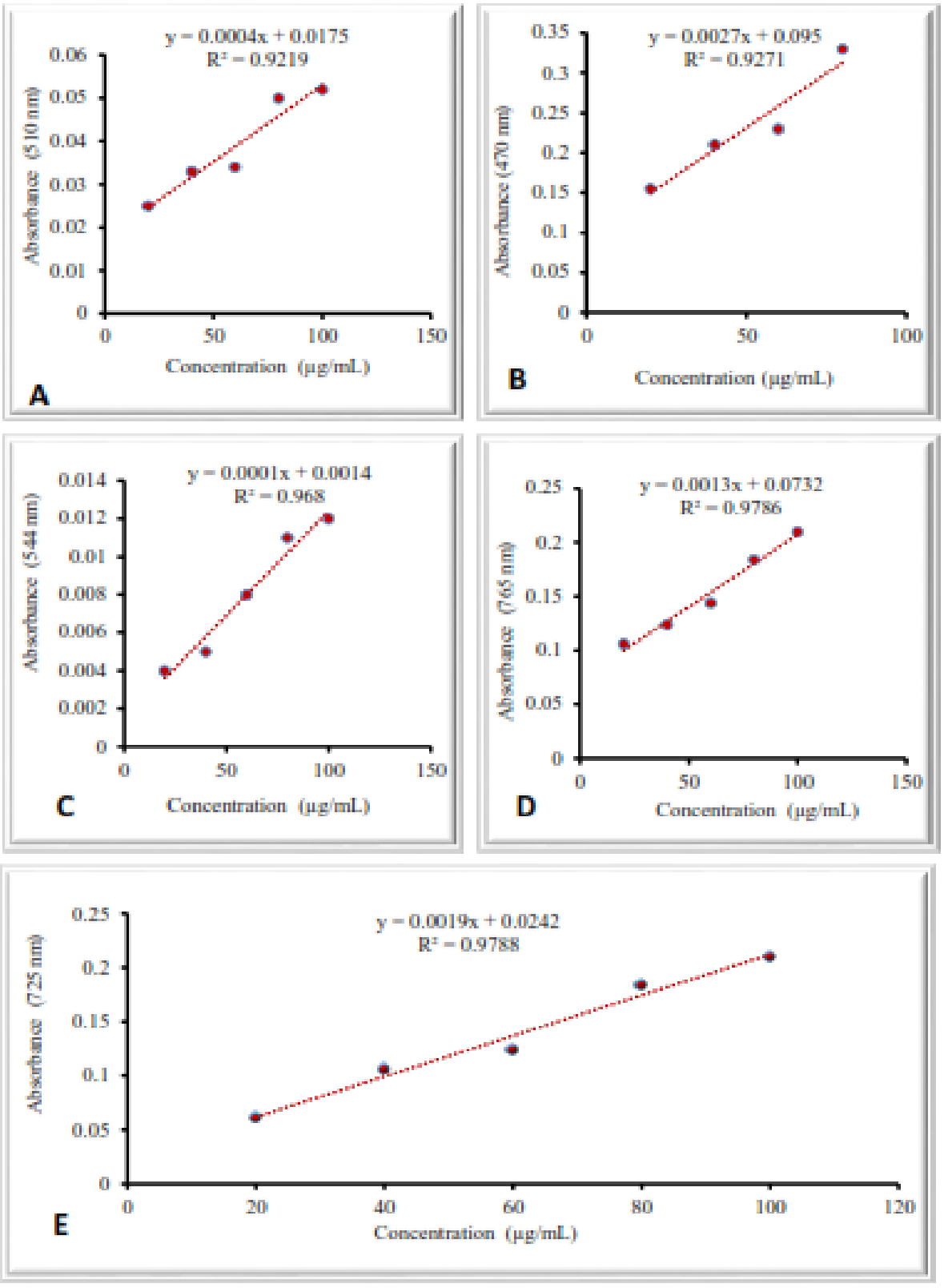
y = 0.0004x + 0.0175
R² = 0.9219
Absorbance (510 nm)
Concentration (µg/mL)
A
y = 0.0027x + 0.095
R² = 0.9271
Absorbance (470 nm)
Concentration (µg/mL)
B
y = 0.0001x + 0.0014
R² = 0.968
Absorbance (544 nm)
Concentration (µg/mL)
C
y = 0.0013x + 0.0732
R² = 0.9786
Absorbance (765 nm)
Concentration (µg/mL)
D
y = 0.0019x + 0.0242
R² = 0.9788
Absorbance (725 nm)
Concentration (µg/mL)
E

Figure 1: The standard curve for the quantification of A: Flavonoids; B: Alkaloids; C: Saponins; D: Total phenolic compound; E: Tannins in ethanol leaf extract of *Moringa oleifera*

Table 4: GC-MS characterization of ethanol leaf extract of *Moringa oleifera*

S/No	RT	Pk Area (%)	Name of the compound	MW (amu)	CAS Number	Qual
1	9.231	0.03	9-Octadecenal	126818	005090-41-5	58
			9,12-Octadecadienoyl chloride, (Z, Z)-	157778	007459-33-8	52
2	9.542	0.13	n-Nonadecanol-1	144418	001454-84-8	30
			Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	128537	1000131-33-2	30
3	9.563	0.14	1-Eicosanol	158029	000629-96-9	38
			n-Tetracosanol-1	209169	000506-51-4	38
			2- Bromopropionic acid, pentadecylester	214638	1000292-44-2	30
4	9.720	0.45	Hexadecane	89843	000544-76-3	64
5	9.739	0.52	Tetracontane, 3,5,24-trimethyl-	273207	055162-61-3	68
			Hexatriacontane	267213	000630-06-8	64
6	10.599	2.71	Methyl 18-methylnonadecanoate	184595	1000352-20-6	97
			Cyclopentanetridecanoic acid	155765	024828-61-3	95
			Eicosanoic acid	184598	001120-28-1	94
7	10.840	0.06	Isobutyl tetratriacontyl ether	270903	1000406-33-7	49
8	11.698	0.11	Eicosanoic acid, isobutyl ester	219428	1000405-16-9	43
			Trichloroacetic acid, 3-tetradecylester	211619	1000282-06-7	35
			2-Tetradecanol	78258	004706-81-4	35
9	11.810	0.15	1-Hexacosanol	228711	000506-52-5	46
10	12.276	0.13	Isopulegol	27461	000089-79-2	64
			4-Heptafluorobutyroxy pentadecane	248206	1000245-50-2	58
11	12.453	0.12	Oxirane, [(dodecyloxy)methyl]-	104311	002461-18-9	55
12	12.704	0.30	17.alpha. -Methyltestosterone	161825	000058-18-4	53
			Z-2-Octadecen-1-ol	161825	000058-18-4	53
			Hexadecane, 1-chloro-	121204	004860-03-1	52
13	12.774	0.15	9-Oxabicyclo [6.1.0] nonane	11611	000286-62-4	50
14	12.803	0.28	7,11-Hexadecadienal	98680	1000130-85-7	78
			Cyclohexaneethanol, 4-methyl-. beta-methylene-, trans-	27794	015714-12-2	46
			Androst-5-en-3-ol, 4,4-dimethyl-, (3. beta.)-	161928	007673-17-8	46
15	13.047	0.07	n-Propyl 9-hexadecenoate	155711	1000336-64-8	46
16	13.107	0.82	Palmitoleic acid	115311	000373-49-9	95
			Hexadecenoic acid, Z-11-	115321	002416-20-8	83
			Oxiraneundecanoic acid, 3-pentyl-, cis-	171283	038520-30-8	68
17	13.568	3.05	n-Hexadecanoic acid	117416	000057-10-3	98
28	13.730	0.41	Methyl pentadactyl ether	104447	007307-52-0	50
			Hexadecyl octyl ether	209173	1000406-38-6	49
19	15.012	1.01	Nonadecane, 1-chloro-	161769	062016-76-6	87
			prop-1-en-2-yl trid ecyl ester	144155	1000382-90-8	86
			Tetracosane	195674	000646-31-1	83
20	15.305	0.47	Aspidospermidin-17-ol,1-acetyl-19	244774	002122-26-1	45
21	15.719	0.33	9,12-Octadecadienal	124999	026537-70-2	55
22	15.861	0.41	3-Octyne, 6-methyl-	10827	062108-34-3	60
23	15.976	0.51	tridecyl-	89784	018633-25-5	64
			6-Octadecenoic acid, methyl ester, (Z)-	155752	002777-58-4	59
24	16.228	0.08	9-Tetradecenal, (Z)-	74488	053939-27-8	70
25	16.256	0.11	9-Oxabicyclo [6.1.0] nonane, cis-	11674	004925-71-7	78
26	16.383	2.09	cis-Vaccenic acid	142073	000506-17-2	96

			cis-13-Octadecenoic acid	142083	013126-39-1	95
27	16.489	0.60	Oleyl alcohol, trifluoroacetate	216236	1000352-68-4	43
28	16.941	0.21	6-Nitroundec-5-ene	64169 1	000192-40-3	64
			Hexadecanal	102564	000629-80-1	64
			7-Hexadecenal, (Z)-	100565	056797-40-1	64
29	17.429	0.11	Erucic acid	195586	000112-86-7	84
			14-Pentadecenoic acid	102343	017351-34-7	78
			Octadecanoic acid, octadecyl ester	269882	002778-96-3	58
30	17.623	0.25	Z-8-Methyl-9-tetradecenoic acid	102375	1000130-84-5	52
			Cyclopentadecanone, 2-hydroxy-	102369	004727-18-8	51
31	18.447	4.74	Di-n-octyl phthalate	233367	000117-84-0	47
			Diisooctyl phthalate	233366	000131-20-4	43
			Undecane, 4,6-dimethyl-	51429	017312-82-2	42
32	18.728	1.33	Docosanoic acid	209118	000929-77-1	50
			Methyl cyclohexane propionate	39580	020681-51-0	49
33	21.057	1.50	Eicosyl vinyl ester	219345	1000382-54-3	81
			hexadecyl prop-1-en-2-yl ester	184482	1000382-90-3	74
34	23.299	1.28	Dodecane, 1,1'-oxybis-	209175	004542-57-8	89
			Tetradecane, 2,6,10-trimethyl-	102610	014905-56-7	76
			2-methyl-	155892	001560-84-5	76
35	23.599	0.98	Hexacosanal	227463	026627-85-0	62
			Cyanoacetic acid, tetradecyl ester	140969	1000406-23-1	52
36	24.868	0.16	Octadecanal	128800	000638-66-4	92
			13-Octadecenal, (Z)-	126830	058594-45-9	83
37	25.487	57.67	Squalene	243219	000111-02-4	94
38	26.962	0.82	Supraene	243217	007683-64-9	93
			6,11-Dimethyl-2,6,10-dodecatrien-1-ol	72632	1000196-53-3	91
39	27.043	0.80	N- [4-bromo-n-butyl]-	95915	195194-80-0	78
			Dodecyl nonyl ether	171543	1000406-37-5	68
40	27.467	0.39	1-Decanol, 2-hexyl-	104437	002425-77-6	49
			Oxalic acid, cyclobutyl heptadecyl ester	228451	1000309-70-7	49
			Trihexadecyl borate	275455	002665-11-4	49
41	27.515	0.22	Oxirane, tetradecyl-	102574	007320-37-8	64
			cis-11-Hexadecenal	100562	053939-28-9	60
42	27.618	0.34	Octadecane, 1-(ethenyloxy)-	155862	000930-02-9	86
			Tetradecanal	76506	000124-25-4	64
			2-Piperidinone, N-[4-bromo-n-butyl]-	95915	195194-80-0	87
43	27.967	0.48	Corynan-17-ol, 18,19-didehydro-10- methoxy-, acetate	219043	056053-13-5	81
			Cyclohexanol, 2-(2-propynyloxy)-, trans-	28602	007229-32-5	43
44	28.246	0.20	Pentadecyl nonanoate	219403	278181-44-5	86
			Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	244774	002122-26-1	81
45	28.866	0.63	octadecyl prop-1-en-2-yl ester	209053	1000383-11-5	83
			Pentadecane, 1-bromo-	149688	000629-72-1	74
46	30.301	1.49	26-Nor-5-cholesten-3. beta. -ol-25-one	231232	007494-34-0	93
			Cholesterol	231246	000057-88-5	90
			Cholest-5-en-3-ol, (3. alpha.)-	231287	000474-77-1	62
47	30.782	0.12	3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	139548	1000305-61-3	32
48	30.942	0.27	pentadecyl prop-1-en-2-yl ester	171278	1000382-91-0	68
49	30.963	0.33	Eicosyl nonyl ether	248659	1000406-37-8	87

			Carbonic acid, dodecyl vinyl ester	117251	1000382-54-8	83
50	31.486	0.08	9-Octadecenoic acid	142074	002027-47-6	93
			Oleic Acid	142069	000112-80-1	93
			trans-13-Octadecenoic acid	142094	000693-71-0	83
51	33.412	0.44	Eicosane	142240	000112-95-8	90
			1-Pentadecene	74570	013360-61-7	80
			Cetene	87834	000629-73-2	80
52	34.054	0.15	Octadec-9-enoic acid	142076	1000190-13-7	83
			Dodecyl propyl ether	91582	1000406-27-7	64
			Octyl tetracosyl ether	260621	1000406-39-0	58

*RT (min) = Retention time (min); MW (amu) = Molecular Weight (atomic mass unit); % Area = Percentage Total of all compounds

also showed that *M. oleifera* contain pharmacologically important phytochemicals in varying amounts in their leaf. Flavonoids, a family of phenolic compound has gained wide acceptance because of their medicinal benefits. Flavonoids were reported to exhibited high antioxidant activity (Gao *et al.*, 2002; Mira *et al.,* 2002; Demir *et al.,* 2011; Kalender *et al.,* 2012), hepatoprotective (Janbaz *et al.,* 2002), anti-inflammatory, myocardial protecting (Pozin *et al.,* 1996), anti-allergic, antipyretic, analgesic, spasmolytic, anticancer (Bear & Teel, 2000), immunomodulation (Huang *et al.,* 2010) anti-bacterial, anti-viral, anti-diarrhea (Raymond *et al.,* 2010; Russo *et al.,* 2013; Cushnie *et al.,* 2014), prevention of platelets aggregation (Okwu & Emenike, 2006), vasodilatory, antiarrhythmic, antihyperglycemic and cholinomimetic activities (Qiu *et al.,* 2014). However, some contrary reports showed that it could also be poisonous (Robbers *et al.,* 1996). Okwu (2004) reported that saponins have lipid profile binding properties, whereas, phenols are essential in plant reproduction and growth. They protect against pathogens, preventing chronic illnesses including cardiovascular disease (CVD), neurodegenerative disease, certain types of cancers, diabetes (Scalbert *et al.,* 2005), immune enhancers, anti-inflammatory and hormone modulators (Okwu, 2004; Okwu & Omodamino, 2005). Tannins have been reported to have hemostatic and physiological astringent properties, hastening wounds healing, ameliorating inflamed mucus membranes, and possessed immunostimulant properties (Kumar & Subrahmanyam, 2013). They have important roles such as stable and potent anti-oxidant ability (Tyler *et al.,* 1988; Trease & Evans, 1989; Awosike, 1991; Ogunleye & Ibitoye, 2003). According to Lucky *et al.* (2018), the inhibitory activity of extracts of *M. oleifera* on acetylcholine was inversely correlated to the TPC and TFC, and with ascending inhibitory potency of the various parts; seed < flowers < leaf < bark < root. Hence, the high TFC, saponins and TPC observed in the ELEMO in the present study could be a great contributing factor to its broad pharmacological activities for treating various diseases and prevention of damage due to free radicals in the body.

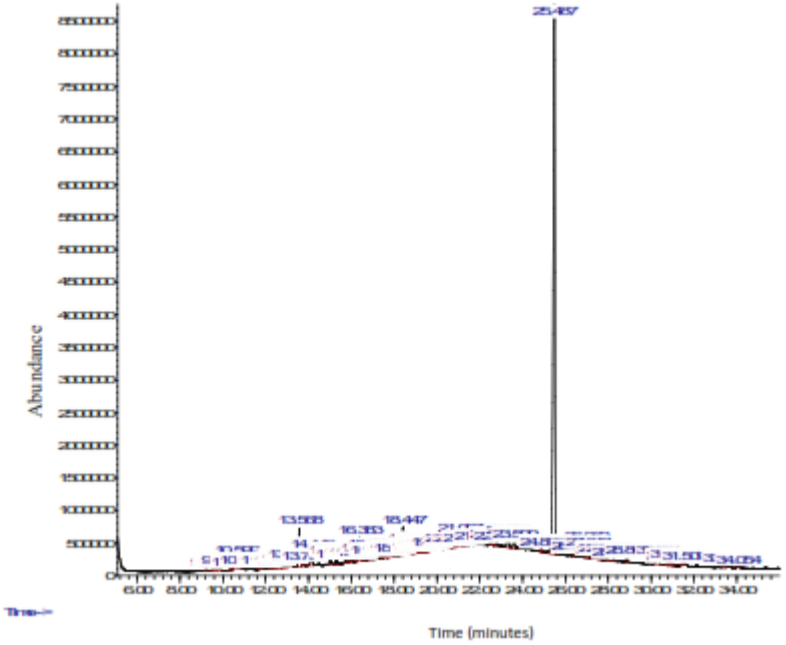

Figure 2: Total ion chromatogram (TIC) for GC-MS Characterization of the compound from ethanol leaf extract of *Moringa oleifera*

In conclusion, quantitative phytochemical analysis of the ELEMO reveals the presence of medicinally valued bioactive components (flavonoids, alkaloids,

saponins, phenols and tannins) at various concentrations, with TFC being the highest followed by saponins, TPC and then total alkaloids. These components have been proven to have high medicinal value. The Gas Chromatography–Mass Spectrometry revealed the presence of n-hexadecanoic acid, methyl ester (33.42), Pentadecanoic acid, 14-methyl-methyl ester (36.23, Ethyl 9,12,15-octadecatrienoate (33.12 and 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1 H-1,2,3-triazole (31.24) among others. Results from this study may potentiate the discovery of more valuable bioactive components of industrial and pharmaceutical importance for managing various ailments. Further studies should be carried out in view of all the medicinal importance associated with each of the identified bioactive components in the ELEMO through isolation, quantification and characterization in order to determine their potential for utilization.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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