Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.24, 2014
www.iiste.org

Comparative Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Chemical Compounds of Moringa oleifera Leaves and Seeds from Abakaliki, Nigeria

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Abstract
Moringa oleifera is a medicinal plant widely used in forkloric medicine in Africa and Asia for the treatment of ailments such as ulcer, wound, inflammation, heart problem, cancer, stroke, obesity, anemia and liver damage. *Moringa oleifera* leaf and seed samples from Abakaliki, Nigeria were used for chemical constituents' analysis. The chemical constituents of the methanol extract of *Moringa oleifera* leaves and seeds were investigated using Gas chromatography-mass spectrometry. Sixteen chemical constituents were identified in the leaf methanol extract with 9-octadecenoic acid (20.89%), L-(+)-ascorbic acid- 2,6-dihexadecanoate(19.66%), 14 –methyl -8-Hexadecenal (8.11%) , 4- hydroxyl-4-methyl-2-pentanone (7.01%), 3-ethyl-2, 4-dimethyl-pentane (6.14%) and phytol (4.25%), octadeamethyl-cyclononasiloxane (1.23%), 1, 2-benzenedicarboxylic acid (2.46%), 3, 4-epoxy-ethanone comprising (1.78%), N-(-1-methylethyllidene)-benzene ethanamine (1.54%), 4, 8, 12, 16-tetramethylheptadecan-4-olide (2.77%), 3-5-bis (1, 1-dimethylethyl)-Phenol (2.55%), 1-Hexadecanol (1.23%), 3, 7, 11, 15-tetramethyl-2 hexadecene-1ol (1.17%), hexadecanoic acid (2.03%) and 1, 2, 3-propanetriyl ester-9 octadecenoic acid(1.23%) as the chemical constituents while five chemical constituents were identified in methanolic seed extract with oleic acid (84%), L-(+)- ascorbic acid- 2,6-dihexadecanoate (9.80%), 9-octadecenoic acid (1.88%), methyl ester-hexadecanoic acid (1.31%) and 9-octadecenamide (0.78%) as the chemical constituents. The compounds identified through the GC-MS analysis were used in various applications as anti-microbial, anti-inflammatory, antioxidant, cardio protective, cancer preventive, flavour and anti-infertility agents.
Keywords: GC-MS analysis, chemical constituents, *Moringa oleifera,* methanol extract, leaves and seeds

Introduction

Medicinal plants have been used by all civilizations as a source of medicines since ancient times. In the recent times, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects (Naik *et al*., 2003). Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fuelled by rising costs of prescription drugs and the bioprospecting of new plant-derived drugs (Sharma *et al*., 2010). Generally, plants which produce constituents having medicinal values are called medicinal plants. These substances differ from plant to plant, thus the plant kingdom provides a large store of various chemical substances with potential therapeutic properties which have been utilized in treatment and cure of human and other animal diseases including relieving of pains, convulsion and cardiovascular diseases (Oyenuga and Fetuga, 2003).

Drugs of natural origin are considered to be less toxic and free from adverse effects than synthetic ones. Even though active compounds of many herbal drugs were unknown, they have been widely prescribed by the practitioners of the traditional medicines due to their minimal adverse effects and low cost (Valiathan, 1998). World Health Organisation (WHO) has estimated that 4 billion people (80% of the world population) use herbal medicines for some aspect of primary health care.

Moringa oleifera commonly known as drumstick-tree or horse radish-tree is generally considered as vegetable and also used in Indian folk medicine for the treatment of various illnesses (Ranjan *et al.,* 2009). *Moringa oleifera* is a small graceful tree with sparse foliage often planted in compounds or used in fencing in Northern Nigeria. It resembles a leguminous species at a distance especially when flowering, but immediately recognized during fruiting. *Moringa oleifera* has the following local names: "Zogallagandi" (Hausa), "Ewe-igbale" (Yoruba) and "Okwe Oyibo" (Igbo) (Keay, 1989). *Moringa oleifera* is a common vegetable in Nigeria especially in the Eastern Nigeria.

However, apart from this traditional, medicinal and nutritional uses, there are several reports on biological activities of *Moringa oleifera* in literature. These include hypotensive activities (Nikkon *et al.,* 2003), hypoglycemic and hypocholesterolemic effects (Dangi *et al*., 2002; Ghosi *et al.*, 2000; Naznin *et al*., 2008), anti- inflammatory and anti hepatotoxic activities (Rao and Mishra, 1998) and anti-helmic, analgesic, management of heart diseases, dyspepsia and ulcers (Nikkon *et al.,* 2003). Despite the popular use of *Moringa*

oleifera leaves and seeds for treating various disorders, there is limited or no scientific data available regarding Gas chromatography–mass spectrometry (GC/MS) analysis of chemical constituents of locally grown *Moringa oleifera* leaves and seeds in Abakaliki, Nigeria climatic condition. The study therefore evaluates the Gas chromatography–mass spectrometry (GC/MS) analysis of chemical constituents of *Moringa oleifera* leaves and seeds grown in Abakaliki, Nigeria in albino rats. In this study the methanol leaf extract of *Moringa oleifera* leaf was aimed to identify the chemical constituents through Gas chromatography-mass spectrometry technique

MATERIALS AND METHODS

Plant Collection: The fresh leaves and seeds of *Moringa oleifera* were collected from Abakaliki in Ivo Local Government Area of Ebonyi State, Nigeria and were identified by taxonomy in the department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.

Preparation of Samples: The leaves were destalked, washed and dried at ambient temperature by turning the leaves to avert fungal growth. The seeds were dehusked and dried through the same process. The leaves and seeds were later milled to obtain the vegetable leaf meals (VLMs) and seed meals (SMs) using an electric blender and both were stored in refrigerator in well labeled air-tight containers for analysis.

Preparation of Extract: 40gms of dried powdered leaves of *Moringa oleifera* was extracted successively with methanol in an orbital shaker for 24 hrs at room temperature. The extracts were filtered using Whatman No.1 filter paper to remove extractable substances, at every 3 hrs interval. The combined extracts were then evaporated at 40°C on water bath and the dried extract was stored at 4°C in a sterile container.

GC-MS Analysis

Principle: GC/MS is a combination of two different analytical techniques, Gas Chromatography and Mass Spectrometry.

Procedure: GC-MS analysis of the methanol extract of *M. oleifera* leaves and seeds were performed using SHIMADZU JAPAN Gas Chromatography QP2010PLUS with a fused GC column (2010) coated with polymethyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80–200^{o}C held at 80^{o}C for 1 min, rate 5^{o}C/min and at 200^{o}c for 20 min. Field Ionization Detector (FID)Temperature 300^{o}C, injection temperature 220^{o}c, carrier gas nitrogen at a flow rate of 1 ml/min, split ratio 1:75. Gas Chromatography Mass Spectrum analysis was conducted using GCMS –QP 2010 Plus Shimadu Japan with injector temperature of 220^{o}c and carrier gas pressure of 116.9 kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used.

Component Identification: Chemical constituent components of the extracts were identified by matching the peaks with Computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature (Okwu and Ighodaro, 2010)

Result

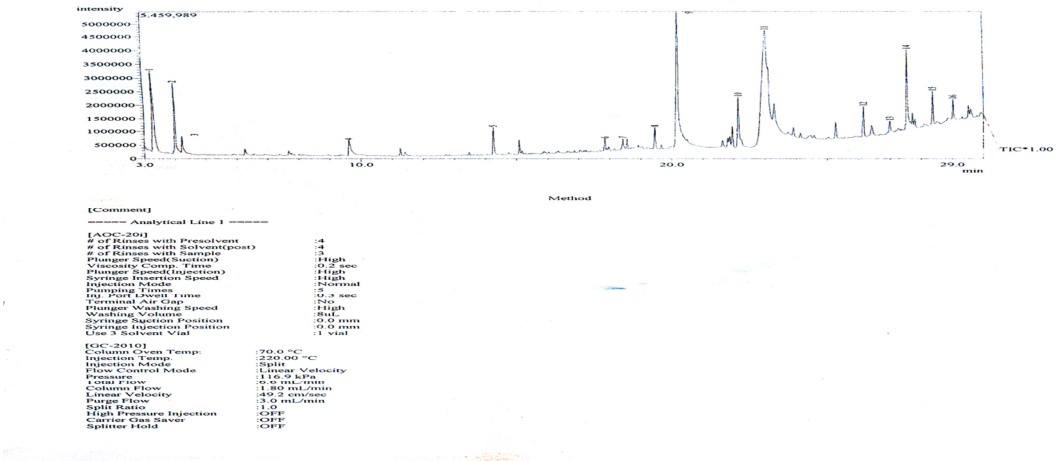

Figure 1: GC/MS Chromatogram of *Moringa oleifera* Methanol Leaf Extract

Table 1: GC-MS Analysis and Mass Spectral Data of Methanol Fractions from the Leaves of *Moringa oleifera* Showing Molecular formula, Molecular weight, Percentage content, Retention time and Mass Peak.

Peak	Compound	Molecular Formular	Molecular Weight	Retention Time	Percentage content	Mass Peaks
1	4-hydroxy-4-methyl-2-Pentane	$C_6H_{12}O_2$	116	3.292	7.01%	42
2	3-ethyl-2,4-methyl	$C_6H_{12}O$	100	4.008	6.14%	49
3	3-4-epoxy-ethanone	C_9H_20	128	4.233	1.78%	35
4	N-(1-methylethyllidene)-Benzene ethanamine	$C_{11}H_{15}N$	161	9.635	1.54%	50
5	3,5-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$	206	14.250	2.55%	94
6	1-Hexadecanol	$C_{16}H_{34}O$	242	17.850	1.23%	64
7	3,7,11,15-Tetramethyl-2 hexadecene-1ol	$C_{16}H_{32}O$	240	18.425	1.17%	67
8	Hexadecanoic acid	$C_{17}H_{34}O_2$	270	19.458	2.03%	90
9	L-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	20.183	19.66%	136
10	Phytol	$C_{20}H_{40}O$	296	22.142	4.24%	83
11	9-Otadecenoic acid	$C_{18}H_{34}O_2$	282	23.000	20.89%	129
12	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	26.133	2.77%	172
13	9-Octadecenoic acid-1,2,3-propanetrieyl ester	$C_{57}H_{104}O_6$	884	26.983	1.23%	123
14	14-methyl-8-Hexadecenal	$C_{17}H_{32}O$	252	27.533	8.11%	222
15	1,2-Benzenedicarb oxylic acid,	$C_{24}H_{38}O_4$	390	28.358	2.46%	144
16	Octadecamethyl – cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	666	9.017	1.23%	199

The methanol extract of the leaves of Moringa oleiferai showed sixteen peaks from the chromatogram of the extract (Figure 1). These peaks indicate the presence of sixteen compounds (1-16) in the extract (Figure 1). The molecular formula, percentage constituents and molecular mass of the compounds is shown in Table 1. These compounds comprise mainly hydrocarbons, fatty acids, alcohols, esters and phenols. The composition of the extract comprises; 9-Octadecenoic acid (20.89%), L-(+)- Ascorbic acid- 2,6-dihexadecanoate(19.66%), 14 – methyl -8-Hexadecenal (8.11%) , 4- hydroxyl-4-methyl-2-pentanone (7.01%), 3-ethyl-2, 4-dimethyl-pentane (6.14%) and phytol (4.25%) as the major chemical constituents.

Compound 1 was identified as 4- hydroxyl-4-methyl-2-pentanone and has molecular formula of C6H12O2 (m/z 116) with base peak at m/z 43 which was due to loss of propanone group ((CH3)2C=0) from the parent molecule. The fragmentation peak at m/z =101 was due to loss of methyl radical while the loss of H20 molecule gave weak peak at m/z=83. It comprises 7.01% of the extract. Compound 2 contains 6.14% of the extract with molecular formula C9H20 (m/z 128) and base peak at m/z 43 which occurred due to the detachment of a propyl fragment C3H7 (m/z 43) from the compound. It was identified as 3-ethyl-2, 4-dimethyl-pentane. Compound 3 has molecular formula C6H10O2 (m/z 114) and base peak at m/z 43 which was due to the loss of butanone group. The compound was identified as 3, 4-epoxy- ethanone comprising 1.78% of the extract. Compound 4 is N-(-1-methylethyllidene)-Benzene ethanamine with molecular formula C11H15N (m/z 161) and base peak at m/z 70 which was due to the loss of benzene methyl group. The constituent was 1.54% of the extract. Compound 5 was identified as 3-5-bis (1, 1-dimethylethyl)-Phenol with molecular formula C14H22O (m/z 206) and base peak at m/z 57. The base peak occurred as a result of the detachment of C3H9 (m/z 57) fragment from the compound. It comprises 2.55% of the extract. Compound 6 is 1-Hexadecanol with molecular formula C14H34O (m/z 242) and base peak at m/z 55 which was due to loss of propyl group (C3H7). It comprises 1.23% of the extract. Compound 7 was identified as 3, 7, 11, 15-Tetramethyl-2 hexadecene-1ol and with molecular formula C20H40O (m/z 296). It comprises 1.17% of the extract. Compound 8 was identified as Hexadecanoic acid and with molecular formula C17H34O2 (m/z 270) and it comprises 2.03% of the extract. The base peak occurred as a result of the detachment of C2H5COOH (m/z 74) and hydrogen molecule (H2) fragments from the compound. Compound 9 has molecular formula C38H68O8 (m/z 652) and comprises 19.66%

of the extract. The base peak occurred at C3H5O2 (m/z 73). This peak occurred due to McLafferty re-arrangement. Other prominent peaks observed on the compound occurred at m/z 43 (C3H7 +) and m/z 41 (C3H5). These peaks occurred due to proton migration and rearrangement. Compound 9 was identified as L-(+)-Ascorbic acid- 2,6-dihexadecanoate. Compound 10 was identified as phytol with molecular formula C20H40O (m/z 296). It comprises 4.24% of the extract. The base peak occurred due to loss of methyl butyl group at m/z 71. Compound 11 has a molecular formula of C57H104O6 (m/z 884). It was identified as 9-Octadecenoic acid and it comprises 20.89% of the extract. Compound 12 was identified as 4, 8, 12, 16-Tetramethylheptadecan-4-olide with molecular formula C21H40O2 (m/z 324) and it comprises 2.77% of the extract. Compound 13 has molecular formula C18H36O2 (m/z 284) and comprises 1.23% of the extract. It was identified as1, 2, 3-propanetriyl ester-9 Octadecenoic acid. Compound 14 has molecular formula C17H32O (m/z 252) and it was identified as14 –methyl -8-Hexadecenal. It comprises 8.11% of the extract. Compound 15 has molecular formula C24H38O4 (m/z 390) and was identified as 1, 2-Benzenedicarboxylic acid. The base peak occurred at C4H9 (m/z 57). It comprises 2.46% of the extract. Compound 16 has molecular formula C18H54O9Si9 (m/z 666) and was identified as Octadeamethyl-cyclononasiloxane. The base peak occurred at Si2O (m/z 73). It comprises 1.23% of the extract.

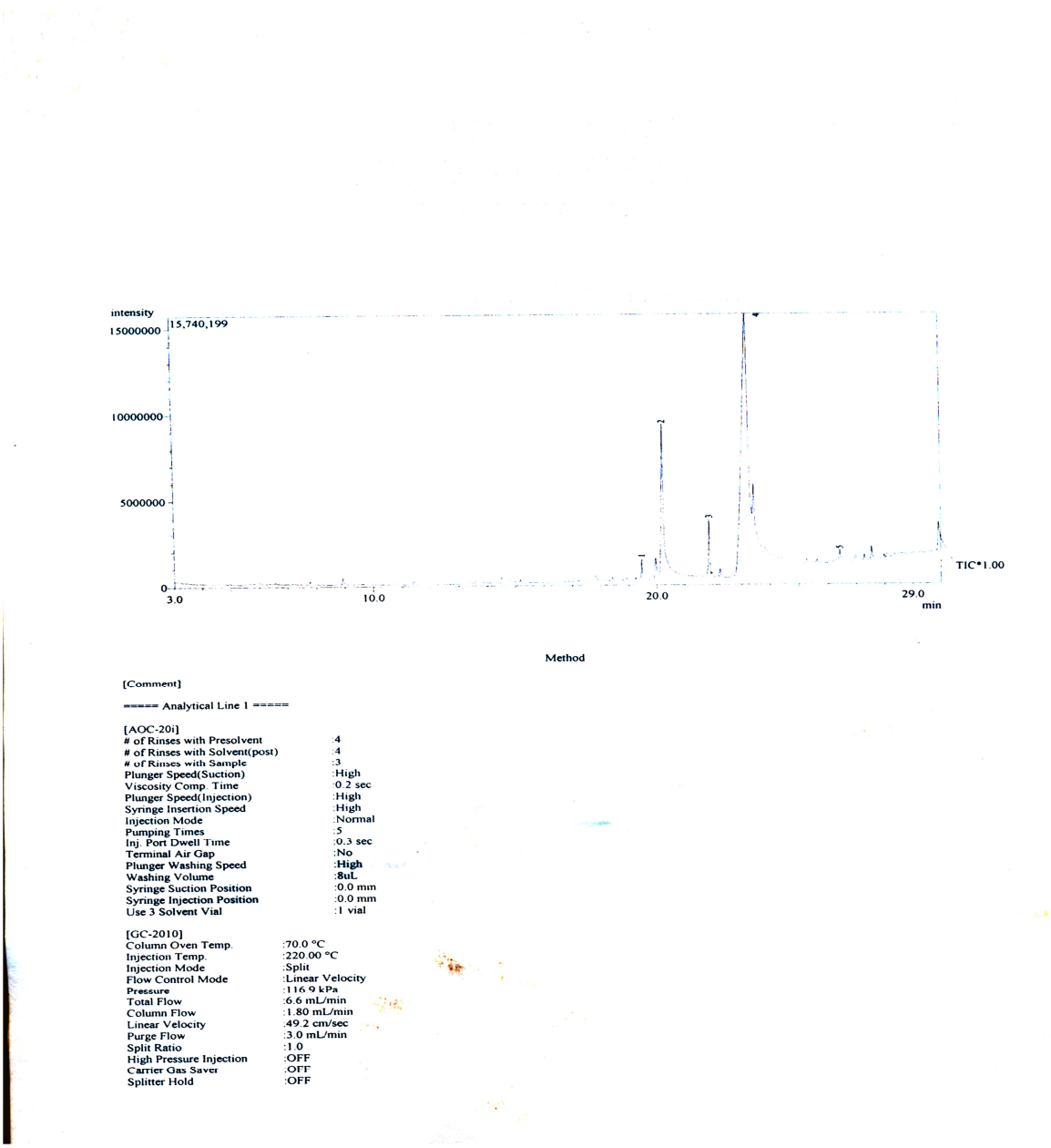

Figure 2: GC/MS Chromatogram of Moringa oleifera Methanol Seed Extract

Table 2: GC-MS Analysis and Mass Spectral Data of Methanol Fractions from the Seeds of Moringa oleifera Showing Molecular formula, Molecular weight, Percentage content, Retention time and Mass Peak.

Compound	Molecular Formula	Molecular Weight	Retention Time	Percentage Content	Base Peak
Methyl ester-hexade Canoic Acid	$C_{17}H_{34}O_2$	270	19.458	1.31%	74
L-(+)-ascorbic acid 2,6-dihexa decanoate	$C_{38}H_{68}O$	242	20.23	9.80%	73.05
Methyl ester-9-octadecenoic acid	$C_{19}H_{34}O_2$	296	21.875	1.88%	55.05
Oleic acid	$C_{15}H_{28}0_2$	240	23.233	84%	55.05
9-octadecenamide	$C_{18}H_{35}NO$	281	26.417	0.78%	59

The methanol extract of the seeds of Moringa oleifera showed five peaks from the chromatogram of the extract (Figure 13). These peaks indicate the presence of five compounds (1-5) in the extract (Figure 2). The molecular formula, percentage constituents and molecular mass of the compounds is shown in Table 2. These compounds comprise mainly hydrocarbons, fatty acids, alcohols and esters. The composition of the extract comprises; oleic acid (84%), L-(+)-ascorbic acid- 2,6-dihexadecanoate (9.80%) 9-octadecenoic acid (1.88%), methyl ester-hexadecanoic acid (1.31%) and 9-octadecenamide (0.78) as the constituents as shown in table 2 above.

Compound 1 was identified as methyl ester-hexadecanoic acid and has molecular formula of C17H34O2 (m/z 270) with base peak at m/z 74 which was due to loss of benzyl group ((C6H13) from the parent molecule. It comprises 1.31% of the extract. Compound 2 has molecular formula C38H68O8 (m/z 652) and comprises 9.80% of the extract. The base peak occurred at C3H5O2 (m/z 73). This peak occurred due to McLafferty re-arrangement. Other prominent peaks observed on the compound occurred at m/z 43 (C3H7 +) and m/z 41 (C3H5). These peaks occurred due to proton migration and rearrangement. Compound 2 was identified as L-(+)- Ascorbic acid- 2,6-dihexadecanoate.Compound 3 has molecular formula C19H36O2 (m/z 296) and base peak at m/z 55. The compound was identified as methyl ester 9-Octadecenoic acid comprising 1.88% of the extract. Compound 4 is Oleic acid with molecular formula C18H34O2 (m/z 282) and base peak at m/z 55. The constituent was 84% of the extract. Compound 5 was identified as 9-Octadecenamide with molecular formula C18H35NO (m/z 281) and base peak at m/z 59. The base peak occurred as a result of the detachment of C3H9 (m/z 57) and hydrogen molecule fragment from the compound. It comprises 0.78% of the extract.

Discussion

The methanol extract of the leaves of Moringa oleiferai showed sixteen peaks from the chromatogram of the extract. These peaks indicate the presence of sixteen compounds (1-16) in the extract (Figure 1). The flavouring phytochemical 2-pentanone reduces prostaglandin production and COX-2 expression in colon cancer cells. Inflammation and subsequent elevation of the enzyme cyclooxygenase-2 (COX-2) are two such factors involved in the development of colon cancer, and inhibition of these processes could be important targets for chemoprevention (Petterson et al., 2008). Organosulphur compounds (OSCs) prevent or slow down the carcinogenic process induced by a variety of chemical carcinogens (Siess et al., 2007). OSCs offer protection against cancer. These include inhibition of the carcinogens, dermatitis and other minor wounds (Brillo and Selvakymari, 2006). The occurrence of thiobenzoic acid and L-(+)-ascorbic acid 2, 6-dihexadecanoate in the

leaves of M. oleifera may be the reason behind the use of the extracts in the treatment of wounds in herbal medicine in Nigeria (Akinmoladum et al., 2007). It possesses anti-scorbutic activity. Ascorbic acid in the body helps in absorption from the intestine (Akinmoladum et al., 2007). It is required for connective metabolism especially the tissue, bones and teeth (Akinmoladum et al., 2007). It is necessary as anti-stress and protects against colds, chills and dumps. It prevents muscle fatigue and scurvy which is characterized by hemorrhages, bleeding gums, fragile bones, anemia and pains in the joints and defects in skeletal calcification. This function of ascorbic acid also accounts for its requirement for normal wound healing (Okwu and Emenike, 2006). This also supported the use of M. oleifera in treating wounds by the natives in Nigeria. Ascorbic acid and OSCs act as antioxidant in the skin by scavenging and quenching free radicals generated by ultraviolet (UV) radiation stabilization. Ascorbic acid and other phenolic compounds identified are important antioxidants. They act as electron donors for eight important enzymes in humans (Akinmoladum et al., 2007). Ascorbic acid may protect against the oxidative damage of light in the eye and may also play an important role in sperm maturation (Okwu and Ighodaro, 2010). It helps in stabilizing plasma components and has been shown to be an effective scavenger of superoxide radical anion (H2O2), the hydroxyl radical (OH.), singlet oxygen (O.) and reactive nitrogen oxide (NO) (Okwu and Ighodaro, 2010).

The methanol extract of the seeds of Moringa oleifera showed five peaks from the chromatogram of the extract (Figure 2). These peaks indicate the presence of five compounds (1-5) in the extract. These compounds comprise mainly hydrocarbons, fatty acids, alcohols and esters. The composition of the extract comprises; oleic acid (84%) L-(+) - Ascorbic acid- 2, 6-dihexadecanoate (9.80%) 9-octadecenoic acid (1.88%), methyl ester-Hexadecanoic acid (1.31%) as the major constituents.The presence of fatty acids and their derivatives in M. oleifera seed extract shows the pharmacological properties of the plant. Fatty acids and alcohols in the plants undergo esterification reaction to form esters (Akinmoladum et al., 2007). One or both of the oxygen atoms of carboxylic acid can be replaced by sulphur giving a thio acid or dithio acid respectively. Thio acids react readily with alcohols to form thio-esters. Thio-esters play an important part in the break down and synthesis of lipids and steroids in living tissues. Carboxylic acids are transferred from one enzyme reaction to another as thio-esters of the complex thiol, Co enzyme A (CoA-SH). The thio-ester of benzoic acid with Co-enzyme A is the form in which acetate enters the sequence of enzyme catalyzed reactions which results in the synthesis of fatty acids and glycerides (Okwu and Ighodaro, 2010).

The constituent compounds in the essential oil are long chain aliphatic carboxylic acids, (saturated and unsaturated) and their derivatives including alcohols, aldehydes as well as benzene carboxylic acid esters and a steroidal compounds. It is pertinent to identify the possible roles of these constituent compounds in the curative properties attributed to the plant by herbal medical practitioners. Oleic acid is an unsaturated fatty acid present in several plants and being unsaturated is considered as a healthy source of fat in the diet. Many fatty acids are known to have antibacterial and antifungal properties (Ogunleshi et al., 2010). Dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid and oleic acids are among the fatty acids known to have potential antibacterial and antifungal properties (Ogunleshi et al. ,2010). Oleic acid has been found to be fungistatic against a wide spectrum of moulds and yeasts. For example, it was observed to cause a delay of 6-8 hours in the germination of fungal spores, and was also found to be effective at low concentrations (Ogunleshi et al., 2010). It has also been proposed that these fatty acids have potential antibacterial and antifungal principles for clinical application (Okwu and Ighodaro, 2010). Triterpene-fatty acid esters and free fatty acids including long chain C16-C20 unsaturated fatty acids were suggested to be responsible for the anti-inflammatory activity in the extract from M. oleifera seed ((Ogunleshi et al., 2010).

Conclusion

GC-MS analysis showed that there are 9-octadecenoic acid (20.89%), L-(+)- ascorbic acid- 2,6-dihexadecanoate(19.66%), 14 –methyl -8-Hexadecenal (8.11%) , 4- hydroxyl-4-methyl-2-pentanone (7.01%), 3-ethyl-2, 4-dimethyl-pentane (6.14%), phytol (4.25%) , octadeamethyl-cyclononasiloxane (1.23%), 1, 2-benzenedicarboxylic acid (2.46%), 3, 4-epoxy- ethanone comprising (1.78%), N-(-1-methylethyllidene)-benzene ethanamine (1.54%), 4, 8, 12, 16-tetramethylheptadecan-4-olide (2.77%), 3-5-bis (1, 1-dimethylethyl)-Phenol (2.55%), 1-Hexadecanol (1.23%), 3, 7, 11, 15-tetramethyl-2 hexadecene-1ol (1.17%), hexadecanoic acid (2.03%) and 1, 2, 3-propanetriyl ester-9 octadecenoic acid(1.23%) as the chemical components in the leaf extract and oleic acid (84%) L-(+)-ascorbic acid- 2, 6-dihexadecanoate (9.80%), 9-octadecenoic acid (1.88%), methyl ester-hexadecanoic acid (1.31%) and 9-octadecenamide (0.78) are the chemical components in the seed extract. The study also revealed that 9-octadecenoic acid (20.89%) is the highest in concentration in the leaf extract and oleic acid (84%) is the highest in the seed extract. It is believed that the results of this study have added to the overall value of the medicinal and therapeutic potential of this plant since the global scheme is now changing towards the use of nontoxic plant products having traditional medicinal value to control various diseases.

Acknowledgement
Authors are grateful to Prof. Dr Johnny Ogunji (Director of research, innovation and commercialization (DRIC), EBSU) and TET Fund for financial support.

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