Chemical Constituents of *Moringa oleifera* Leaves and Seeds from Abakaliki, Nigeria

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**ABSTRACT**

*Moringa oleifera* is a medicinal plant widely used in folkloric medicine of Africa and Asia for the treatment of ailments such as ulcer, wound, inflammation, heart problem, cancer, stroke, obesity, anaemia and liver damage. The chemical constituents of the methanolic extract of *Moringa oleifera* leaves and seeds were investigated using Gas chromatography-mass spectrometry. Sixteen chemical constituents were identified in the leaf methanolic extract; they 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-dimethylpentane (6.14%), phytol (4.24%), octadecamethyl-cyclononasiloxane (1.23%), 1, 2-benzene dicarboxylic 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-dimethylpentyl) -phenol (2.55%), 1-hexadecanol (1.23%), 3, 7, 11, 15-tetramethyl-2 hexadecene-1-ol (1.17%), hexadecanoic acid (2.03%) and 1, 2, 3-propanetriyl ester-9 octadecenoic acid(1.23%). Five chemical constituents were identified in methanolic seed extract and they are 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%). Results obtained showed that the methanolic leaf extract of *Moringa oleifera* has more chemical constituents than the seed with 9-octadecenoic acid (20.8%) as the highest in the leaf and oleic acid (84%) in the seed. These relatively diverse chemical constituents may be responsible for the medicinal properties of *Moringa oleifera* leaves and seeds.

**Keywords:** GC-MS analysis, Chemical constituents, Methanol extract, *Moringa oleifera*.
INTRODUCTION

Medicinal plants have been used by all civilizations as a source of medicines since ancient times. In 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. 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 bio-prospecting of new plant-derived drugs. Generally, plants which produce constituents mostly as secondary metabolites 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.

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. 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* is commonly known as drumstick-tree or horse radish-tree. It is used as vegetable and also in Indian folk medicine for the treatment of various illnesses. *Moringa oleifera* is a small graceful tree with sparse foliage often planted in compounds or used in fencing in Nigeria. It resembles a leguminous species at a distance especially when flowering. *Moringa oleifera* is known with the following local names: “Zogallagandi” (Hausa), “Ewe-igbale” (Yoruba) and “Okwe Oyibo” (Igbo). *Moringa oleifera* is a common vegetable in Nigeria especially in the Eastern Nigeria. The pictures of *Moringa oleifera* leaves and seeds are shown in figure 1 and 2 below.

However, apart from its traditional, medicinal and nutritional uses, there are several reports on the biological and physiological activities of *Moringa oleifera*. These include hypotensive properties, hypoglycemic and hypocholesterolemic effects, anti-inflammatory and anti hepatotoxic activities, anti-helmic, analgesic, dyspepsia and in the management of heart diseases, and ulcers. According to Aja *et al.*, the seed of *Moringa oleifera* revealed that administration of aqueous, ethanolic and methanolic seed extracts of *Moringa oleifera* significantly reduced the levels of some liver enzymes in albino rats. They also observed that the liver pathology showed that no significant lesions were observed and this may point to the fact that the seed of *Moringa oleifera* is relatively safe for use medicinally.

Despite the popular use of *Moringa oleifera* leaves and seeds for treating various disorders, there is limited data available regarding Gas chromatography–mass spectrometry (GC/MS) analysis of chemical constituents of locally grown *Moringa oleifera* leaves and seeds in Abakaliki, Nigeria. This study therefore intends to evaluate the Gas chromatography–mass spectrometry (GC/MS) analysis of the chemical constituents of the methanolic extract of *Moringa oleifera* leaves and seeds grown in Abakaliki, Nigeria.
MATERIALS AND METHODS

Plant Collection
The fresh leaves and seeds of Moringa oleifera were collected from Abakaliki Area of Ebonyi State, Nigeria and were identified by taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. A part was also deposited in the hebarium for reference purposes.

Preparation of Samples
The leaves were destalked, washed and shade dried at ambient temperature with constant turning averts 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 4°C temperature in refrigerator in well labeled airtight containers for analysis.

Preparation of Extract
40gms of dried powdered leaves and seeds of Moringa oleifera were extracted successively with 300 ml of 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 with rotary evaporator and the dried extracts were stored at 4°C in two different sterile containers.

GC-MS Analysis
Procedure
GC-MS analysis of the methanolic 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°C held at 80°C for 1 min, rate 5°C/min and at 200°C for 20 min. Field ionization detector (FID) temperature 300°C, injection temperature 220°C, carrier gas nitrogen at a flow rate of 1 ml/min, split ratio 1:75. Gas chromatography mass spectrum was conducted using GCMS –QP 2010 Plus Shimadzu Japan with injector temperature of 220°C and carrier gas pressure of 116.9 kpa. The column length is 30 m with a diameter of 0.25 mm and flow rate of 50 ml/min. The elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermlez 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\(^\text{11}\).

RESULTS
Sixteen peaks were identified from the chromatogram of the methanolic leaf extract of Moringa oleifera (Figure 3). These peaks (1-16) indicate the presence of sixteen compounds (1-16) in the extract (Figure 1). The molecular formula, percentage content and molecular mass of the compounds are 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-hydroxy-4-methyl-2-pentanone and has molecular formula of C₈H₁₂O₂ (m/z 116) with base peak at m/z 43 which was due to loss of propanone group ((CH₃)₂C=O) from the parent molecule. The fragmentation peak at m/z =101 was due to loss of methyl radical while the loss of H₂O molecule gave weak peak at m/z=83. It constitutes 7.01% of the extract. Compound 2 constitutes 6.14% of the extract with molecular formula C₂₆H₅₀O₂ (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 C₁₀H₁₆O₂ (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 constituting 1.78% of the extract. Compound 4 is N-(1-methylethylidene)-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 C₁₄H₂₅O (m/z 206) and base peak at m/z 57. The base peak occurred as a result of the detachment of C₆H₉ (m/z 57) fragment from the compound. It constitutes 2.55% of the extract. Compound 6 is 1-hexadecanol with molecular formula C₁₆H₃₄O (m/z 242) and base peak at m/z 55 which was due to loss of propyl group (C₃H₇). It constitutes 1.23% of the extract. Compound 7 was identified as 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol and with molecular formula C₂₀H₄₀O (m/z 296). It constitutes 1.17% of the extract. Compound 8 was identified as hexadecanoic acid and with molecular formula C₁₇H₃₄O₂ (m/z 270) and it constitutes 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 rearrangement. 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 constitutes 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 constitutes 20.89% of the extract. Compound 12 was identified as 4, 8, 12, 16-tetramethyl heptadecan-4-olide with molecular formula C₂₁H₄₀O₂ (m/z 324) and it constitutes 2.77% of the extract. Compound 13 has molecular formula C₁₈H₃₆O₂ (m/z 284) and constitutes 1.23% of the extract. It was identified as 1, 2, 3-propanenitril 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 C₃₄H₃₈O₄ (m/z 390) and was identified as 1, 2-benzenedicarboxylic acid. The base peak occurred at C₄H₉ (m/z 57). It constitutes 2.46% of the extract. Compound 16 has molecular formula C₁₈H₃₄O₈Si₉ (m/z 666) and was identified as octadec methylcyclononasiloxane. The base peak occurred at Si₂O (m/z 73). It constitutes 1.23% of the extract.

The chromatogram of the methanolic extract of the seeds of Moringa oleifera showed five peaks (Figure 4). These peaks (1-5) indicate the presence of five compounds (1-5) in the extract (Figure 2). The molecular formula, percentage composition and molecular mass of the compounds are shown in Table 2. These compounds comprise
mainly hydrocarbons, fatty acids, alcohols and esters. The constituent 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%) (Table 2).

Compound 1 was identified as methyl ester-hexadecanoic acid with molecular formula of C17H34O2 (m/z 270) and base peak at m/z 74 which was due to loss of benzylic group ((C6H13) from the parent molecule. It constitutes 1.31% of the extract. Compound 2 has molecular formula C38H68O8 (m/z 652) and constitutes 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 constituting 1.88% of the extract. Compound 4 is oleic acid with molecular formula C18H34O2 (m/z 282) and base peak at m/z 55. This constituent made up of 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 constitutes 0.78% of the extract.

**DISCUSSION**

The methanolic extract of the leaves of *Moringa oleifera* showed sixteen peaks from the GC-ms chromatogram. These peaks indicate the presence of sixteen compounds (1-16) in the extract (Figure 3). 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 (Table 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. Organosulphur compounds (OSCs) prevent or slow down the carcinogenic process induced by a variety of chemical carcinogens. OSCs offer protection against cancer. These include inhibition of the carcinogens, dermatitis and other minor wounds. 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. Ascorbic acid in the body helps in absorption from the intestine. It is required for connective metabolism especially the tissues, bones and teeth. 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. The hypothesis also supports the use of *M. oleifera* in treating wounds by the native communities in Nigeria. Ascorbic acid and OSCs act as antioxidants in the skin by scavenging and quenching free radicals generated by ultraviolet (UV) radiation from stabilization. Ascorbic acid and other phenolic compounds identified are important antioxidants. They act as electron donors to stabilize peroxides and other free radicals.

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donors for eight important enzymes in humans\(^2\). Ascorbic acid may protect against the oxidative damage of light in the eye and may also play an important role in sperm maturation\(^1\). It helps in stabilizing plasma components and has been shown to be an effective scavenger of superoxide radical anion (H\(_2\)O\(_2\)), the hydroxyl radical (OH\(_-\)), singlet oxygen (O\(_2\)) and reactive nitrogen oxide (NO)\(^{11}\).

The methanolic extract of the seeds of *Moringa oleifera* showed five peaks from the chromatogram (Figure 4). These peaks indicate the presence of five compounds (1-5). These compounds comprise mainly hydrocarbons, fatty acids, alcohols and esters. The composition of the extract comprises; oleic acid (84\%) L- (+) - Ascorbic acid- 2, 6-dihexadecananoate (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 dictates the pharmacological properties of the plant. Fatty acids and alcohols in the plant undergo esterification reaction to form esters\(^2\). 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 breakdown 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\(^1\).

The constituent compounds in methanolic extract 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 uncommon (secondary metabolite) fatty acids are known to have antibacterial and antifungal properties\(^{21}\). Dodecanoic, tetradecanoic, hexadecanoic, octadecanoic and oleic acids are among the fatty acids known to have potential antibacterial and antifungal properties\(^2\). 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\(^{21}\). It has also been proposed that these fatty acids have potential antibacterial and antifungal principles for clinical application\(^{11}\). 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\(^{21}\).

CONCLUSION

GC-MS analysis showed that there are 9-octadecenoic acid (20.89\%), L- (+) - ascorbic acid- 2, 6-dihexadecananoate(19.66\%), 14 - methyl -8-Hexadecenal (8.11\%) , 4-hydroxy-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-methylthyllidene)-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 composition 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%) constitutes the major of the leaf extract while oleic acid (84%) is the major component of the seed extract. GC-MS analysis showed the presence of many chemical constituents of Moringa oleifera leaves and seeds locally grown in Abakaliki, Nigeria. The presence of various bioactive compounds confirms the application of Moringa oleifera for various ailments by traditional practitioners.

ACKNOWLEDGEMENT

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REFERENCES


![Figure 1. Dried seeds of *Moringa oleifera*](image-url)
**Figure 2.** *Moringa oleifera* leaves

**Figure 3.** GC/MS chromatogram of *Moringa oleifera* methanolic leaf extract
Figure 4. GC/MS chromatogram of *Moringa oleifera* methanolic seed extract
Table 1. GC-MS analysis and mass spectral data of methanolic fraction from the leaves of *Moringa oleifera* showing molecular formula, molecular weight, percentage content, retention time and base peak.

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<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Retention time</th>
<th>Percentage content</th>
<th>Mass peaks</th>
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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 base peak

<table>
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<tr>
<th>Compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Retention time</th>
<th>Percentage content</th>
<th>Base peak</th>
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<tr>
<td>Methyl ester-hexadecanoic acid</td>
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<td>270</td>
<td>19.458</td>
<td>1.31%</td>
<td>74</td>
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<td>L-(+)-ascorbic acid 2,6 dihexa-decanoate</td>
<td>C₃₈H₆₈O</td>
<td>242</td>
<td>20.23</td>
<td>9.80%</td>
<td>73.05</td>
</tr>
<tr>
<td>Methyl ester-9-octadecenoic acid</td>
<td>C₂₈H₄₄O₂</td>
<td>296</td>
<td>21.875</td>
<td>1.88%</td>
<td>55.05</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>C₁₅H₂₈O₂</td>
<td>240</td>
<td>23.233</td>
<td>84%</td>
<td>55.05</td>
</tr>
<tr>
<td>9-octadecanamide</td>
<td>C₁₈H₃₅NO</td>
<td>281</td>
<td>26.417</td>
<td>0.78%</td>
<td>59</td>
</tr>
</tbody>
</table>