

# Investigation of Constituents of Oil from the Seeds of *Moringa oleifera* Plant and Its Biological Activity

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## ABSTRACT

Oil of the dry seeds of *Moringa oleifera* was obtained by maceration using n- Hexane. GC/MS analysis was performed using a shimadzu QP2010 plus series gas chromatography coupled with shimadzu mass spectroscopy detector. Characterization of constituents was done by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match. The most common major constituents of the oil include: 10-Octadecenoic acid methyl ester (48.78%), Octadecanoic acid methyl ester (7.97%), Methyl ricinolate (14.59%).

The antimicrobial screening of the oil was also performed: Strong antimicrobial potency was shown against *Staphylococcus aureus*. The oil was also active against the fungal species *Aspergillus niger*. Significant antifungal potency against *Candida albicans* was also observed. Minimum inhibition concentration and minimum bactericidal/ fungicidal activities were also evaluated for the sample: minimum inhibition concentration was recorded at 12.50mg/ml for all the test organisms except *Bacillius cereus* and *Escherachia coli*. Minimum bactericidal potential against test organisms at 50mg/ml was observed for *moringa oleifera* seed oil.

Keywords : Seeds oil constituents, *Moringa oleifera*, Biological Activity

## I. INTRODUCTION

Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases that are generally considered to be harmful to humans (Anselem, 2004). These plants are either wild plant species growing spontaneously in self-maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human actions or domestic. Domesticated plants species are those that have arisen through human actions such as selection or breeding and depend on management for their existence, for example *Aloe barbadensis* (Cowley, 2002). It has been reported that infectious diseases account for one-half of all deaths in the tropical

countries (WHO, 1977). As a result, people of all continents have long applied poultice and imbibed infusions of indigenous plants dating back to prehistory for health purposes and is still in use today (Sofowora, 1993; Iwu, *et al.* 1983). The study of medicinal plants starts with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. Traditional methods such as maceration and Soxhlet extraction are commonly used at the small research setting or at Small Manufacturing Enterprise (SME) level. Significance advances have been made in the processing of medicinal plants such as the modern extraction methods; microwave-assisted (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid

extraction (SFE), in which these advances are aimed to increase yield at lower cost. Moreover, modifications on the methods are continuously developed. With such variety of methods present, selection of proper extraction method needs meticulous evaluation. The exploitation of plants by man for the treatment of diseases has been in practice for a very long time. Herbal drugs constitute a major part in the entire traditional system of medicines (Higa et al., 1994). Screening of medicinal plants oils for antimicrobial agents has gained much importance because lately World Health Organization (WHO) is keenly interested in the development and utilization of medicinal plant resources in the traditional system of medicine in the developing countries so as to extend the health care to maximum number of population in these countries (Goud et al., 2005)

#### **Antimicrobial activity of oils**

Essential (volatile) oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, notably antibacterial, antifungal, and antioxidant properties (Baratta *et al.* 1998; Cosentino *et al.* 1999; Bounatirou *et al.* 2007). Biological activity of essential oils depends on their chemical composition, which is determined by the plant genotype and is greatly influenced by several factors such as geographical origin and environmental and agronomic conditions (Rota *et al.* 2004; Yesil Celiktas *et al.* 2007). However, plants possess antioxidants, which have certain degree of resistance to oxidation (Marinova *et al.*, 2002). Plant oils are used for the prevention of some human diseases such as atherosclerotic cardiovascular diseases, cancer and degenerative eye diseases (Bendini *et al.*, 2002; Kaur and Kapoor, 2002; Marinova *et al.*, 2002; Alamni and Cossu, 2003; Bonaclorisis *et al.*, 2003

#### **Botany and health benefits of *Moringa oleifera***

The beneficial uses of seed oil plants have been known since time immemorial. Apart from their uses as food items, oils extracted from seeds are also used for different purposes ranging from medicinal to

biofuels. Their chemical compositions, physical and chemical properties generally determine their applications for different purposes. Many microbial diseases worldwide have become a serious threat to human health because of the emergence of drug resistant or multi-drug resistant (MDR) microbial strains (Tabassum et al., 2013). Emergence of resistance to the existing drugs (Rios and Recio, 2005) led the scientists to search for the new alternatives including seed oil producing plants and their oil, which are known for their antimicrobial properties.

#### ***Moringa oleifera***



**Figure A.** *Moringa oleifera* pods



**Figure B.** *Moringa oleifera* seeds

*Moringa Oleifera* belongs to an onogeneric family of trees and shrubs Moringaceae. A single genus with known species, *M. Oleifera* is the most widely known and utilized of these (Morton, 1991). It is a fast growing, aesthetically pleasing small tree; it can grow up to four meter (Ramahandra et al, 1980) and can bear fruit within the same first year (Olivera et al

1997). *Moringa Oleifera* is referred to as “Moringa”, it is considered one of the world’s most useful trees. Almost every part of moringa tree can be used for food or other beneficial applications (Quattrocchi and Umberto, 2000). It is known and called by different names among different people of the world, among the Yoruba people of south west Nigeria, it is called “Ewe Ile”, among the south eastern Igbo people, it is called “Okwe Oyibo”, “Gawara”, among the Fulani’s, “Zogale” among the Hausa’s “Nugyekai”, in Canada, Muringai in Tamil, *M.Oleifera* is also called the “Miracle tree”, “Mother’s best friend”, “Never Die” and “Benzolive tree” (Ramahandra et al 1980). The seeds of this wonderful tree have a semi-permeable seed hull and are round in shape. Descriptively, the hull has three white wings that run from top-bottom at 120 degree intervals, the seed production is between 15,000 and 25,000 seed per year for each tree. The average weight per seed is 0.3g (Makkar and Becker, 1997). The dry seeds can be grounded into powder and used for seasoning sauces. The root from the young plant can also be dried and grounded for use as a hot seasoning base (Deleveau and Boiteau, 1980). A tasty hot sauce from the roots can also be prepared by cooking them in vinegar (Deleveau and Boiteau, 1980). The content of the de-hulled seed (Kernel) is approximately 42%, the oil is brilliant yellow. It is used as a lubricant for fine machinery such as time pieces because it has little tendency for it to deteriorate and become rancid and sticky (Ferrao and Ferrao, 1970). It is also useful as vegetable cooking oil. The oil is known for its capacity to absorb and retain volatile substances and is therefore valuable in the perfumery industry for stabilizing scents; the free fatty acid content varies from 0.5-3%. The extract obtained from the leaves of Moringa in 80% ethanol contains growth enhancing principles (i.e., hormones of cytokine type). The extract can be used in the form of a foliar spray to accelerate the growth of young plants: use of the growth hormone spray will also cause the plants to be firmer and more resistant to pest and disease and will produce more and larger fruit which have a higher yield at harvest

time (Makkah and Becker, 1996). *M. Oleifera* seed oil is commercially known as “Ben Oil” or “Behen Oil”. The oil content is ranging from 25-40%. (Lalas and Tsaksins, 2002) and characterized by high amounts of oleic acid up to 75% (Anwar et al 2006) which makes it suitable for edible purpose due to good oxidative stability.

## II. METHODS AND MATERIAL

*Moringa oleifera* seeds were collected from around Katsina, Nigeria and authenticated by direct comparison with authentic samples.

A Shimadzu QP2010 plus series gas chromatography coupled with Shimadzu QP2010 plus mass spectroscopy detector (GC-MS) system was used.

### GC-MS analysis

Oil from Moringa was studied. The oil was extracted by maceration (Handa et al., 2008), In GC-MS analysis, the temperature program was set up from 70°C to 280°C. Helium gas was used as carrier gas. The injection volume was 2 µL with injection temperature of 250°C and a column flow of 1.80 ml/min for the GC. For the mass spectroscopy ACQ mode scanner with scan range of 30-700 amu at the speed of 1478 was used. The mass spectra were then compared with the NIST05 mass spectral library (NIST, 2012)

### Antimicrobial screening

In cup plate agar diffusion bioassay, seed oil from *Moringa oleifera* plant was assessed for antimicrobial activity (Hammer et al., 1999), the antimicrobial activity was assessed against six standard pathogenic microbes. The microbes were obtained from the Department of Medical Microbiology, ABU Teaching Hospital, Shika, Zaria.

0.5g of the oil was weighed and dissolved in 10ml of DMSO to obtain a concentration of 50mg/ml. This was the initial concentration of the oil used to check the antimicrobial activities. Diffusion method was the

method used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to the manufacturer's instructions, sterilized at 121°C for 15 minutes, poured into sterile Petri dishes and were allowed to cool and solidify. The sterilized media were sealed with 0.1ml of the standard inoculums of the test microbe (Mueller Hinton agar was sealed with the bacteria and Sabouraud dextrose agar sealed with the fungus). The inoculums were spread over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6mm in diameters, a well was cut at the centre of each inoculated medium. (0.1ml) of the oil (concentration of 50mg/ml) was then introduced into the well on the inoculated medium. Incubation of the inoculated medium was made at 37°C for 24 hours for the bacteria and at 30°C and for 4 days for the fungus. After incubation each plate of the medium was observed for the growth inhibition zone. The zone was measured with a transparent ruler and the results were recorded in millimeters.

#### **Minimum Inhibition Concentration**

The minimum inhibition concentration of the oil was determined using the broth dilution method. Mueller Hinton and Sabouraud dextrose broth were prepared, 10mls of each broth was dispensed into test tubes and was sterilized at 121°C for 15 minutes, and the broth was allowed to cool. Mc-Farlands turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared. 10mls solutions were dispensed into sterile test tubes and the test microbes inoculated and incubated at 37°C for 6 hours. Dilution of the test microbe was done in the manual saline until the turbidity matched that of Mc-Farland's scale by visual comparison, at this point the test microbe concentration was about  $1.05 \times 10^8$  cfu/ml.

Two-fold serial dilution of the oil was in the sterile broth to obtain the concentrations at 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.13mg/ml and

1.56mg/ml. The initial concentration was obtained by dissolving 0.5g of the oil in 10mls of the sterile broth. Having obtained the different concentrations of the oil in the sterile broth, 0.1ml of the test microbe in the normal saline was then introduced into the different concentration. Incubation was made at 37°C for 24 hours for the bacteria and at 30°C for the fungus, then the test tubes was observed for turbidity (growth). The lowest concentration of the oil in the broth which shows no turbidity was regarded as the minimum inhibition concentration.

#### **Minimum bactericidal and fungicidal concentrations**

MBC/MFC was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton and Sabouraud dextrose agars were prepared, sterilized at 121°C for 15 minutes, poured into sterile Petri dishes and were allowed to cool and solidify.

The content of the mixture in the serial dilution were then sub-cultured onto the prepared media. The bacteria were cultured onto the Mueller Hinton agar and the fungus on the Sabouraud dextrose agar. Incubation was made at 37°C for 24 hours for the bacteria and at 30°C for 4 days for the fungus after which the plates of the media were observed for colony growth, MBC/MFC were the plates with lowest concentration of the oil without colony growth.

### **III. RESULTS AND DISCUSSION**

#### **GC-MS analysis of *Moringa oleifera* seeds oil**

Oil from *Moringa oleifera* seeds was studied. The oil was extracted by maceration and then identified and quantified by GC-MS analysis. Characterization of constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra

#### ***Moringa oleifera***

*Moringa oleifera* oil was studied by GC-MS . The total ion chromatogram is displayed in Fig.1, while the constituents of the oil are shown in Table 1.

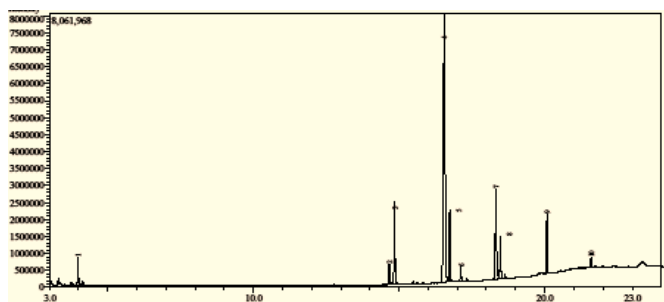


Fig.1: Total ion chromatogram

Table.1: Constituents of the oil

Peak#	R.Time	I.Time	F.Time	Area	Area%
1	3.974	3.942	4.108	2014847	3.45
2	14.663	14.617	14.733	1279236	2.19
3	14.843	14.792	14.950	5515079	9.46
4	16.551	16.450	16.692	28448866	48.78
5	16.738	16.692	16.817	4647266	7.97
6	17.113	17.067	17.150	942786	1.62
7	18.326	18.242	18.433	8510775	14.59
8	18.473	18.433	18.533	2696111	4.62
9	20.074	20.025	20.133	3652755	6.26
10	21.589	21.542	21.642	610362	1.05
				58318083	100.00

Major constituents of the oil are discussed below:

### 10-Octadecenoic acid methyl ester (48.78%)

The EI mass spectrum of 10-octadecenoic acid methyl ester is shown in Fig. 2. The peak at m/z 296, which appeared at R.T. 16.551 in total ion chromatogram, corresponds to  $M^+[C_{19}H_{36}O_2]^+$ .

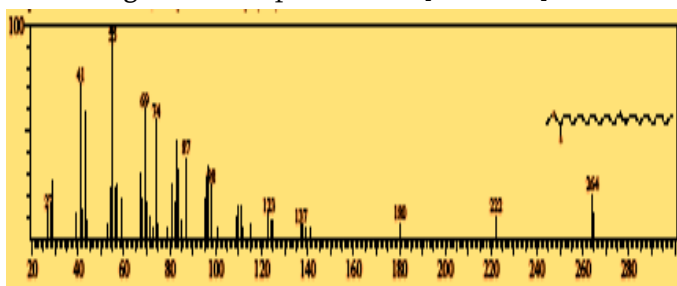


Fig.2: mass spectrum of 10-octadecenoic acid methyl ester

### Octadecanoic acid methyl ester (7.97%)

The EI mass spectrum of octadecanoic acid methyl ester is shown in Fig. 3. The peak at m/z 298, which appeared at R.T. 16.738 in total ion chromatogram, corresponds to  $M^+[C_{19}H_{38}O_2]^+$ .

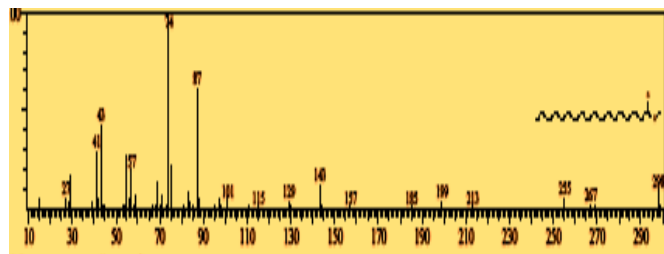


Fig.3.: Mass spectrum of octadecanoic acid methyl ester

### Methyl ricinoleate (14.59%)

The EI mass spectrum of methyl ricinoleate is shown in Fig. 4. The peak at m/z 319, which appeared at R.T. 18.326 in total ion chromatogram, corresponds to  $M^+[C_{19}H_{36}O_2]^+$ . with the database on MS library revealed about 90-95% match

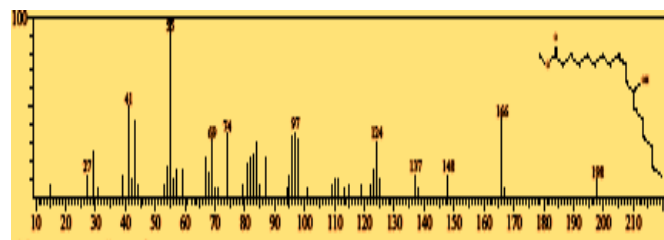


Fig.4: Mass spectrum of methyl ricinoleate

### Antimicrobial activity

In disc diffusion bioassay moringa oleifera seeds oil showed strong antimicrobial potency against *Staphylococcus aureus* (Table.2). Moringa seeds oil showed no activity against the bacterial strain *Escherichia coli*. The oil was inactive against the fungal species *Aspergillus niger*, significant antifungal potency against the yeast *Candida albicans* was also observed for the oil. (Table.2).

Table .2: Antimicrobial activity of target species

Test organism	
Staphylococcus aureus	S
Bacillus careus	R
Escherachia coli	R
Vibro cholareae	S
Salmanella typhi	S
Candida albicans	S
Aspergillus niger	R

Bacillus careus	0
Escherachia coli	0
Vibro cholareae	18
Salmanella typhi	19
Candida albicans	19
Aspergillus niger	0

The minimum inhibition concentration is displayed in Table. 4., *Moringa oleifera* oil showed a minimum inhibition concentration at 12.50 mg/ml.

Table .3: Diameter of inhibition zones (mm)

Test organism	
Staphylococcus aureus	21

Table 4. Minimum inhibition concentration- *Moringa oleifera*

Test organism	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml	1.56mg/ml
Staphylococcus aureus	-	-	0*	+	++	+++
Bacillus careus						
Esgherachia coli						
Vibro cholareae	-	-	0*	+	++	+++
Salmanella typhi	-	-	0*	+	++	+++
Candida albicans	-	-	0*	+	++	+++

The minimum bactericidal activity was evaluated for the oil sample and the results are depicted in

Table. 5. *Moringa oleifera* oils exhibited a minimum bactericidal potential against test organisms at 50mg/ml

Table 5. Minimum bactericidal concentration- *Moringa oleifera*

Test organism	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml	1.56mg/ml
Staphylococcus aureus	0*	+	++	+++	+++	+++
Bacillus careus						
Esgherachia coli						
Vibro cholareae	0*	+	++	+++	+++	+++
Salmanella typhi	0*	+	++	+++	+++	+++
Candida albicans	0*	+	++	+++	+++	+++

oil of the dry seeds of *Moringa oleifera* plant grown in Nigeria was obtained by maceration using n-hexane. GC/MS analysis was performed. Characterization of constituents was done by

#### IV.CONCLUSION

comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. The most common major constituents of the oil were: 10-Octadecenoic acid methyl ester (48.78%) Octadecanoic acid methyl ester (7.97%) Methyl ricinolate (14.59%). The minimum inhibition concentrations and minimum bactericidal/fungicidal activities were evaluated for the oil

## V. RECOMMENDATIONS

1. The target species may also be investigated for other bioconstituents (steroids, alkaloids, saponins etc)
2. The biological activity of the total extracts of target species may also be assessed.
3. The extracted oil may be evaluated for other biological activities (antioxidant, antimalarial, antileishmanial..etc).

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