Sudan University of Science and Technology

College of Graduate Studies

Physicochemical Properties and Anti-Microbial Activity of *Moringa oleifra* Seeds Oil

الخواص الفيزيوكيميائية والفعاليه الميكروبية لزيت بذور المورينجا

A Thesis Submitted in Partial Fulfillment of the Requirements of the Degree of Master in Chemistry

By

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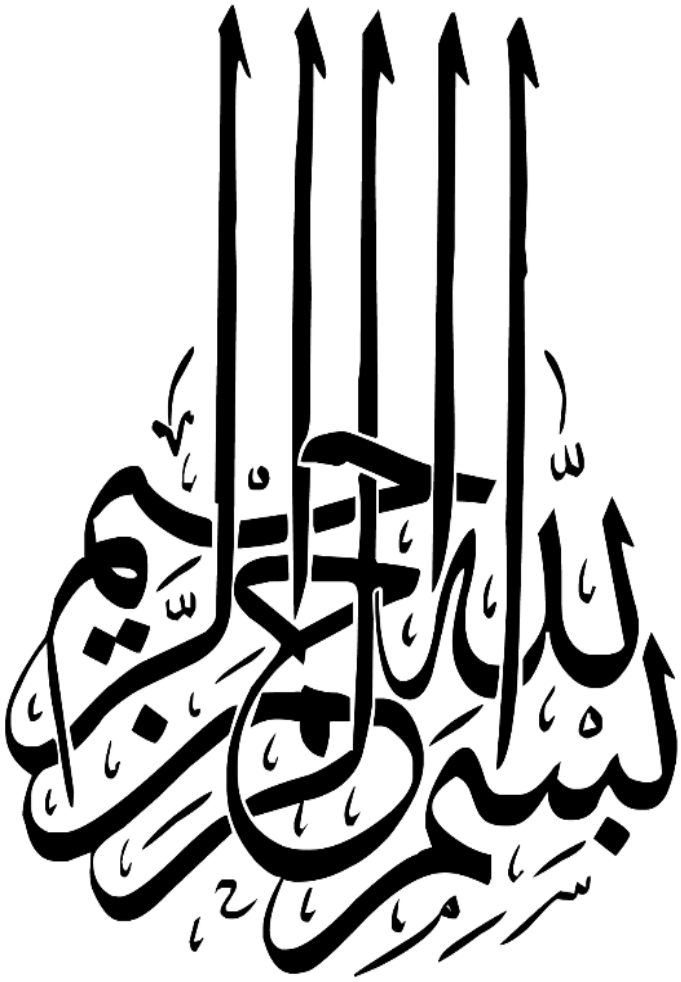
بسم الله الرحمن الرحيم

ا لآيــة

قـــال تعالــى:

بسم الله الرحمن الرحيم

هُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً ۖ لَكُمْ مِنْهُ شَرَابٌ وَمِنْهُ شَجَرٌ فِيهِ تُسِيمُونَ ﴿10﴾ يُنْبِتُ لَكُمْ بِهِ الزَّرْعَ وَالزَّيْتُونَ وَالنَّخِيلَ وَالْأَعْنَابَ وَمِنْ كُلِّ الثَّمَرَاتِ ۗ إِنَّ فِي ذَٰلِكَ لَآيَةً لِقَوْمٍ يَتَفَكَّرُونَ ﴿11﴾

صدق الله العظيم

سورة النحل، الآيات 10-11

Dedication

To my,,

Parents

Husband

Brothers and Sisters

Acknowledgements

Praise to Allah, Almighty, for providing me with this opportunity and granting me the capacity to proceed successfully.

I would like to thank my parents for their continued support, advice and love they have always been there for me through the good times and the bad they always instilled in me the importance of an education and hard work and without them I would never have been able to achieve my dreams. I would like also thank my supervisor **Prof. Mohamed Almubarak** who has guided, support and challenged me to break in this field of research and deserves much of the credit for providing much needed insights of direction. I would like to express my deep thanks to staff members of the Department of **Sudan University of Science and Technology** for their helpful and good advice during the work.

Thanks and appreciations are expressed to all my family members, my Husband, friends, and to my colleagues who encouraged me during the course of the study. Thank are also extended to the people in the department of Industrial Research and Consultancy center and **UMST University of Medical Sciences and Technology**.

Abstract

Moringa oleifera seeds contains highly valuable substances with an impressive range of medicinal, cosmetic and food uses. The purpose of the study is to test the physical, chemical and biological properties of the pressed oil of *Moringa oleifera* .

Titration experiments were done to investigate the physical and chemicals results of the oil as follows viscosity (84cp) ,refractive index (1.4685) ,the density(0.909g/cm3), acid value (1.295mg KOH/g oil), peroxide value (2.75meq KOH/g oil),saponification value (211.775mgKOH/g oil),ester value (210.478 mg KOH/g oil) free fatty acid (0.651%). Fatty acids constituents were determined by GC-MS.

The analysis showed that *Moringa oleifera* seeds oil contains greater a mounts of unsaturated fatty acids especially oleic acid (47.1%)and Docosanoic acid(11.14%) , The oil contains a high percentage of oleic acid47.1%, Docosanoic acid11.14, methyl stearate (9.51%) and ecaonic acid(8.55%) .

The FT-IR wave showed the absorbance functional groups of the *Moringa oleifera* seeds oil. *Moringa oleifera* seeds oil exhibits moderate activity aginst all of the four species of positive and negative bacteria and no activity in aqueous extract of *Moringa* seeds,and showed anti fungal activity by inhibiting zone of growth.

المستخلص

تحتوي بذور المورينجا أوليفيرا على مواد ذات قيمة عالية مع مجموعة رائعة من الاستخدامات الطبية والتجميلية والغذائية.

الغرض من الدراسة هو اختبار الخصائص الفيزيائية والكيميائية والبيولوجية للزيت المضغوط من المورينجا أوليفيرا

اجريت التجارب المعياريه للتحري عن الخصائص الفيريائيه والكيميائيه التي ظهرت كالاتي: (84),معامل الانكسار (1.4685),الكثافة (0.909جم/سم^3)

رقم الحمض(1.295) ملغرام هيدروكسيد البوتاسيوم/جم,رقم البيروكسيد (2.75)وقيمة التصبن (211,775) ملغرام هيدروكسيد البوتاسيوم/جم, رقم الاستر(210,478) ملغرام هيدروكسيد البوتاسيوم/جم,الاحماض الدهنية الحره (0.651%).

مكونات الاحماض الحرة قيست بواسطة جهاز كرومتغرافيا الغاز مع القياس الطيفي ,اوجد التحليل ان زيت المورينجا اوليفرا يحتوي على نسبه عالية من حمض الاوليك (47.1%),حمض الديزناويك (11.14%),ميثيل الستريت (9.51%) وحمض الايسانويك (8.55%). مطيافية الاشعه تحت الحمراء اظهرت امتصاص المجموعات الوظيفية لزيت بذور المورينجا.

أظهر زيت بذور المورينجا نشاطًا معتدلًا ضد جميع الأنواع الأربعة من البكتيريا الموجبة والسالبة ولا يوجد نشاط في المستخلص المائي لبذور المورينجا ، وأظهر نشاطًا مضادًا للفطريات عن طريق تثبيط منطقة النمو.

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1. Introduction

1.1 Vegetable oils

Vegetable oils are a group of fats that are derived from some seeds, nuts, cereal grains, and fruits. It is important to understand that not all of these vegetable oils are liquid oils at ambient temperatures. In addition, not all of the vegetable oils are produced in commercial quantities, and of those that are, not all are considered to be edible as in the sense of being a typical dietary component. (Hammond, 2003). The physical and chemical characteristics of oils depend upon the degree of unsaturation and the length of the carbon chains. Fats which include large amount of saturated fatty acids are solid at room temperature whereas the ones which contain high amount of unsaturated fatty acids are liquid at room temperature. As the chain length of a saturated fatty acid increases the melting point increases. Thus a short chain saturated fatty acid such as butyric acid has a lower melting point. (Gunstone, 2008).

1.2 Major and minor components of vegetable oils

The main components of fats and oils which are called major component are triglycerides (triacylglycerols), accompanied by lower levels of diacylglycerols (diglycerides), monoacylglycerols (monoglycerides) and free fatty acids, and by other minor components [Gunstone, 2013].

Triglycerides are chemical compounds resulting from the combination of one unit of glycerol and three units of fatty acids. They are non-polar, water insoluble substances. When all of the fatty acids in a

triglyceride are identical, it is termed a simple triglyceride. The more common forms, however, are the “mixed” triglycerides in which two or three kinds of fatty acids are present in the molecule. (Johnson and Saikia, 2009; Retief, 2011).

The minor compounds consist about (2-5%) of the vegetable oils. These include mono- and diglycerides, free fatty acids, phosphatides, sterols, phenolic compounds, fat soluble vitamins, tocopherols and tocotrienols, pigments, wax esters, and fatty alcohols (Nielsen, 2010).

Fatty acid in most vegetable oils are straight-chain homologous series of saturated and unsaturated carboxylic acids of even-numbered chain lengths from C_8 to C_{24}. The unsaturated series contains one to three methylene-interrupted, *cis* double bonds. Some vegetable oils contain non typical Fats, but their presence in nutritious edible oils is usually undesirable. (Hammond, 2003)

1.3 Vegetables Oil Production and Processing

Vegetable oils are, principally, recovered from oilseeds, with oil-rich fruit such as the fruit of the oil palm and of the olive tree providing important additional sources. Solvent extraction is used for oil recovery from oilseeds, but in the case of palm and olive oil, the oil is recovered by separating it from the aqueous phase present in the fruit after crushing. Vegetable oils are refined before consumption, refining comprising a series of steps designed to produce bland, stable oil. Refined oils may be modified in order to change their physical properties.

Both refining and oil modification processes are, increasingly, being improved in order to conform to modern standards of a healthy food product. This entails avoiding the formation of undesirable artifacts' as well as minimizing the removal of valuable minor components.

(Hammand, 2003).

All animals produce fat, and marine sources also provide oils. The combined largest source of vegetable oils is the seeds of annual plants grown in relatively temperate climates. A second source of vegetable oil is oil-bearing trees. Most of the oil-bearing tree fruits and kernels provide the highest oil yield (O'Brien, 2004).

1.4 Origin and distribution of *Moringa oleifera*

Moringa Oleifera is native to the Indian subcontinent and has become naturalized in the tropical and subtropical areas around the world, The tree is known by such regional names as Benz olive, Drumstick tree, Horseradish tree, Kelor, Marango, Mlonge, Mulangay, Saijihan and Sajna (Fahy, 2005).

The plant thrives best under the tropical insular climate, it can grow well in the humid tropics or hot dry lands and can survive in less fertile soils and it is also little affected by drought (Anwar *et al*.,2007).

Moringa oleifera belongs to the *Moringaceae* family,its origin in the north-west region of India, south of the Himalaya and Mountains. (Markkar, H, B, S and Becker, 1996).

It is now widely cultivated and has become naturalized in many locations in the tropics. There are thirteen species of *Moringa* trees in

The family *Moringaceae* and *Moringa oleifera* is the most widely cultivated species. It was further stated that they are native to India, the Red Sea area and/or parts of Africa including Madagascar.

Moringa oleifera is naturalized in many African countries. This Rapidly growing tree also known as horsef radish tree or drumstick tree was utilized by the ancient Romans, Greeks and Egyptians. (Bosh, C.H. (2004).

1.5 Uses of *Moringa Oleifera*

Moringa oleifera is esteemed as a versatile plant due to its multiple uses. The leaves, fruits, flowers and immature pods of this tree are edible and they form a part of traditional diets in many countries of the tropics and sub-tropics. (Siddhuraju and Becker, 2003; Anhwange *et al*., 2004) *Moringa oleifera* is one of the World's most useful trees, as almost every part of Moringa tree can be used for food, medication and industrial purposes.(Khalafalla,*et al* (2010).

The leaves of *M. oleifera* are good source of protein, vitamin A, B and C and minerals such as calcium and iron. In addition to its substantial uses and nutritional benefits, *M. oleifera* also has a great potential as a medicinal plant. The flowers, leaves and roots are used for the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulants in folk remedies. The roots of the young tree and also root bark are rubefacient and vesicant. (Hartwell, 1995; Anwar and Bhanger, 2003; Anwar *et al*., 2007).

The seeds from this plant contain active coagulating agents characterized as dimeric cationic proteins, having molecular weight of 13 kDa and an isoelectric point between 10 and 11. The seeds also have antimicrobial activity and are utilized for waste water treatment. In some developing countries, the powdered seeds of *Moringa oleifera* are traditionally utilized as a natural coagulant for water purification because of their strong coagulating properties for sedimentation of suspended undesired particles (Kalogo et al., 2000; Anwar *et al*., 2007).

Moringa seed kernels contain a significant amount of oil that is commercially known as "Ben oil" or "Behen oil". The Ben oil was

erroneously reported to be resistant to rancidity and used extensively in the "enfleurage" process (Ndabigengeser and Narasiah, 1998).

Moringa seeds oil content and its properties show a wide variation depending mainly on the species and environmental conditions. (Ibrahim *et al*,1974).

Leaves could serve as a valuable source of nutrients for all age groups. For example, in Haiti and Senegal, health workers have been treating malnutrition in small children, pregnant and nursing women with *Moringa* leaf powder could be used as food or for medicinal and therapeutic purposes. It is used for improved wound healing, gastric ulcer, diarrhea, sore throat and cancer. (Grever,2001).

This tree has the potential to improve nutrition, most people in South Africa, however, are not aware of the potential benefits of *Moringa* , but recently, a high degree of renewed interest was placed on the nutritional properties of *Moringa* in most countries where it was not native. (Reyes, *et al* 2006).

The nutritional values of *Moringa*, which depend on factors like genetic background, environment and cultivation methods .The nutritional composition *of Moringa* of the South African ecotype has not previously been evaluated; the profile of chemical composition, fatty acids, amino acids and vitamins. Amino acids, fatty acids, minerals and vitamins are essential in animal feed.

These nutrients are used for osmotic adjustment; activate enzymes, hormones and other organic molecules that enhance growth, function and maintenance of life process Nutritional composition of the plant plays a significant role in nutritional value.(Anjorin *et al* ,2010) .

Lead remediation of contaminated water using *Moringa stenopetala* and *Moringa oliefera* seed powder. Many parts of *Moringa* species trees are deemed useful; the seeds are especially prized for their medicinal powers. The seeds have valuable properties that enable them to treat a wide array of illnesses and conditions. The National Charity for Organic Growing has studied the efficacy of *Moringa* species seeds as a medical treatment and found that they provide legitimate relief for many medical problems. These include rheumatism, gout, sexually transmitted diseases, urinary infections, boils, and even epilepsy. (Verginie *et al*, 2010). *Moringa* seeds are also used as primary coagulate in drinking water classification and waste water treatment due to the presence of water soluble cationic coagulant protein able to reduce turbidity of the water treated. (Tarrago,*et al* 2006).

1.6Cultivation for seed production

There are two main ways of obtaining *Moringa oleifera* plants sowing and the use cuttings for seed production, sowing is preferred as improved varieties can be selected for cultivation ensuring proper and profitable production .Seed production, according to the harvest and management practices, requires a low density plantation with triangular pattern.

Normally, *Moringa* seeds are sown during the rainy season and can germinate and grow without irrigation, but for commend, allowing seed production during the dry season as well.

Should irrigation be employed, its conditions depend on the cultivation area. Although *Moringa oleifera* can produce a large quantity of seeds when Fertilization is adequate, there has been no exhaustive research on this issue. Fertilization must be done during soil preparation

before sowing, and when the trees are the onset of the growth period, just before the rainy season .Manure or compost can be used instead of chemical fertilizer. The seeds of the *Moringa* species plant are among the most nutritious and useful botanical and herbal remedies, as nutritional supplements and for industrial and agricultural purposes. *Moringa* seeds are edible in both fresh and dried forms and, along with the seed pods that contain them, can be prepared in numerous ways as both food and medicinal therapeutic purposes. (Mataka *et al*, 2006).

Traditionally, its cultivation is almost exclusively for fresh production where there is low population density. This reliability is not the case for other crops in countries where people are often faced with famine due to crop losses. Its properties make it suitable for both human and commercial purpose.(Rajangam *et al* ,2014).

1.7 Chemical characteristics of seed and oil

Moringa olifera seeds are globular ,about 1 cm in diameter .they are three angled with an average weight of about 0.3 gm with wings produced at the base of the seed to the apex 2-2.5cm long 0.4-0.7cm wide, the kernel is responsible for 70%-75% of the weight .

Oil is the main component of the seed and represent 36.7% of the seed weight . Table 1.1the oil can be extracted, using n-hexane, where as less yield is obtained by cold press .

Moringa oleifra has high content of *methionine* and *cysteine* close to that reported for milk and eggs. (Anwar and Asharf, 1998).

Table 1.1 Chemical composition of *Moringa oliefra* seed (g/100g of dry weight) (Oliefra,*et al* 1999).

Nutrients	Mean values	Standard deviation
Fat	36.7	2.8
Proteins	31.4	1.3
Carbohydrates	18.4	1.4
Fiber	7.3	0.5
Ash	6.2	0.9
Moisture	7.0	1.2

Table 1.2 Physicochemical properties of *Moringa oliefra* seeds oil (Pak. J, 2011).

Parameter	*M.olifera* oil
Oil yield	41.47%
Refractive index	1.4713±0.00
Melting point	28.0±0.00
Saponfication value	171.9±0.56
Peroxide value	8.1±0.07
Iodine value	85.3±0.25
Acid value	3.8±0.28

1.8Antimicrobial activity of seeds oil

Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess anti-microbial functions and could serve as a source of antimicrobial agents against bacteria and fungi. In recent years, a large number of oils and their constituents have been investigated for their antimicrobial properties

against bacteria and fungi. (Kim *et al*, 1995). Skin infections are widely encountered in the tropics with lots of orthodox remedies involving the use of systemic antibiotics, the problems of drug resistance and reported allergies also abound. In the present investigation, coconut oil and castor seed oil hexane extract shows maximum activity against *E.coli* and *C. albicans* . (Amit *et al* ,2012) . Where they studied the antifungal activity of lemongrass oil, coconut oil, almond oil and clove oil against *Candida* species isolated from bloodstream infection and they revealed that lemongrass showed highest anticandidal activity against *C. tropicalis* followed by *C.tropicalis* and Clove oil showed maximum activity against *C. albicans*, *C. tropicalis* and *C. guilliermondii.* In the case of almond oil maximum activity was reported against *C. guilliermondii* and Coconut oil showed maximum activity against *C. albicans*. The antimicrobial activity of coconut oil had been attributed to the carboxylic acid – monolaurin metabolized to lauric acid in the body. Coconut oil has been confirmed to possess antimicrobial, antiviral, and antiprotozoal activities. (Enig MG, 2003). Photochemical studies indicated that lauric acid, which is its major fatty acid component, was highly responsible for the activities of the oil. The castor oil have been used in small doses in clinical setting for numerous medical conditions such as, liver and gallbladder disturbances, abscesses, headaches, appendicitis, epilepsy, hemorrhoids, constipation, diarrhea, intestinal obstructions, skin diseases, hyperactivity in children and to avert threatened abortion in pregnant women**. (** Shirley, 2003).

The active compound in the oil previously identified to be monolaurin could have had an enhanced penetration due to the presence of surface-active emulsifying agents used in formulating the cream since emulsification of oils generally increases their *absorpti* Peat Coconut oil has health and nutritional benefits. The choice of an anionic (sodium

lauryl sulfate) and cationic (*cetrimide*) emulsifying agents were to avoid incompatibility with the selected preservatives notably phenolics and carboxylic acids. (Christopher, 1996). Hexane and methanol extracts of *Ricinus communis* seed oil showed maximum antimicrobial activity against *E. coli, C. albicans,* and *T. rubrum* as compared to aqueous extract Kushwah. Antimicrobial activity of *Ricinus communis* seed against *E. coli, B. subtilis, B. cereus, S. aureus, C. glabrata,* and *C. albicans* and revealed methanolic seed extract with strongest antibacterial activity against *E. coli* (15mm). Similar studies reported by(Abhishek,2006) , from roots of *Ricinus communis,* where hexane and methanol extracts showed maximum antimicrobial activity against *E. coli, S. aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus vulgaris, B. subtilis, C. albicans,* and *Aspergillus niger*. Interestingly, the aqueous extract has shown no significant antimicrobial properties, unlike the present finding and the findings of kushwah (Betancur *etal*, 2009). Activity index of the extracts validate the possibilities of using the oil as a therapeutic agent. The continuous research for more reliable antibiotics becomes a worthwhile and noble mission. *Moringa oleifera* seed contains highly valuable substances with an impressive range of medicinal, cosmetic and food uses. *Moringa Oleifra* seeds, seeds oil and its residue were investigated for antioxidant and antimicrobial activities against selected food borne microorganisms using disc diffusion and minimum inhibitory concentration. In addition, the physico-chemical properties, fatty acid compositions and oxidative stability of cold pressed *Moringa Oleifra* seeds oil in comparison with those of extra virgin olive (Ruttarattan *et al*, 2015).

Chapter Two

Chapter Two

2. Materials and Methods

2.1 Objectives

To investigate physical,chemical and antimmicrobial activity of *Moringa oleifera* seeds oil

2.2 Materials

M*oringa oleifera* seeds were procured from local market; the seeds were cleaned from any extraneous materials and stored at room temperature.

2.3 Chemicals

All the chemicals and reagents of technical grade:-

a) Ethyl alcohol
b) Potassium hydroxide (minimum assay 85%).
c) c- Hydraulic acid pure (wt per ml at 20^0C about 1.18g),(assay35-38%)
d) Glacial acetic acid (b.p= 118^0C, minimum assay = 99.7%) 4-
e) Potassium iodide (assay = 99%)
f) Sodium thio sulphate (minimum assay 99%)
g) Chloform (density = 1.474-1.480g/mL at 20^0C, assay = 99.5%)*
h) Starch indicator
i) Phenolphthalein indicator
j) Methanol
k) N-hexane (density = 0.66 g/mL at 20^0C, b.p=65-70^0C, minimum assay= 95%)

2.4 Instruments

- Jiawnshun 1500w 220v.
- Lovibond Tintometer.
- Thermo scientific viscometer .
- Abb refract meter .
- Ultra Violet Visible spectrophotometer.
- Gas chromatography-Mass spectrometry Instruments .
- Fourier Transforms Infrared Spectroscopy.

2.5 Methods of analysis

2.5.1 *Moringa oleifra oil* press

is newly designed with updated feeding system for stable and efficient materials feeding (JIAWNSHUN 1500w 220V).

2.5.2 Determination of acid value

(1.0) g of oil was placed into a 250mL flask and 25mL of alcohol

Previously neutralized by adding 2 drops phenolphthalein solution and enough 0.1 N NaOH to produce faint permanent pink was added to the flask. The contents were titrated against 0.25 N NaOH, with vigorous shaken until permanent faint pink color appears and persists 1min.

The experiment was repeated tow times and the mean and standard deviation were calculated and the acid value and free fatty acids were

Determined using the following equations:

$$\text{Acid value (AV)} = (N.V.56.1)/S$$

$$\% \text{ free fatty acid} = AV\ 0.503$$

Where:

V is ML of KOH required by sample,

N is Normality of KOH and S is the weight of the sample.

2.5.3 Determination of peroxide value

(2.06)g of oil was weighed into a 250mL flask and 15 mL of a mixture of glacial acetic acid and chloroform (3:2) were added and stirred well for complete dissolution. 0.3mL of saturated KI solution was added to the contents of the flask and shacked well for 1min. 15 mL of distilled water and 0.5 mL of 1% starch were added to flask and titrated against 0.001N KOH with vigorous shaking until the blue color disappears (addition of starch was done immediately because the color of the liberated iodine was already Pale yellow color). The experiment was repeated three times and the mean and standard deviations were calculated and the peroxide value was determined using the following equation:

Peroxide value (milliequivelent peroxide/Kg sample) = $(V.N.1000)/S$

Where

V is mL of KOH required by the sample

N is normality of solution and S is weight of the sample.

2.5.4 Saponfication value:

(1.0)g of the oil was weighed into a 250 mL conical flask and 25mL of alcoholic potassium hydroxide solution (0.5 N) were added and the flask was connected with air condenser. Heating was conducted on a boiling water bath for 30 min with occasional shaking and after completion of the heating process the flask was left to cool and 3 drops of phenolphthalein indicator were added and titrated against a 0.5 N hydrochloric acid until the pink color was disappeared. A blank solution was treated using the same procedure above but without oil. The experiment was repeated three times and the mean and standard deviation were calculated and the saponification value was determined using the following equation:

$$SV = 56.1\ (B-S)\ N/Wt\ of\ sample$$

Where

B is mL of HCI required by Blank,

S is mL of HCI required by Sample and

N is normality of KOH solution

2.5.5 Ester value

It is calculated from saponfication value S.V and the acid value A.V

Ester value= (S.V-A.V) mgKOH/g

2.5.6 Determination of relative density

The relative density of the oil was determined using a clean, dried and pre-weighed empty pycnometer. The pycnometer was filled with distilled water and weighed again. Dry pycnometer was refilled by oil and

weighed. The experiment was repeated double times and the results were recorded and the mean and standard deviation were calculated. Density was determined

Using the following equation**:**

$$\text{Relative density of oil} = (B - A)/(C - A)$$

Where

A is weight of pycnometer

B is weight of pycnometer with sample and

C is weight of pycnometer with water.

2.5.7 Determination of Color

The color of oil was measured using Lovibond Tintometer .The oil was filtered through a filter paper to remove any impurities and traces of moisture.

The glass cell was cleaned, dried, filled with the oil and placed in the Tntometer. The color of the oil was matched with the standard slides red, yellow and blue colors. The results were recorded.

2.5.8 Determination of viscosity

The viscosity of the oil was measured using a Thermo Scientific HAAKE Viscometer 6 plus model. A rotor rotating at a constant speed (200 round /min) was immersed in 60 mL of oil to be tested .The viscosity was recorded.

2.5.9 *D*etermination of refractive index

The refractive index was determined using an Abbe refract meter.

A drop of oil was placed on a surface of the lower prism. The prisms were closed and the mirror and light were adjusted until a dark border line was observed on the cross wire. The refractive index was determined.

2.6 UV-Visible Spectrophotometer analysis of *moringa oleifra* seeds oil:

Based on a standard solution of cyclohexane, 0.2 mL of sample transferred to the cell and then diluted by cyclohexane and put in instrument.

2.7 Antifungal assay

Anti fungal activity of *moringa oleifra* seeds oil aqueous extract and was performed by the gar medium assay with different concentration of *moringa oleifra* seeds oil (0.5, 1.0, 1.5) were prepared by adding appropriate quantity of *moringa oleifra* seeds oil to melted medium. About 20 ml of the medium were poured into glass Petri dishes (9cm*1.5cm). A 6mm diameter agar disk bearing hyphase of *fusarium oxysporum, fusarium solani, Rhizoctonia solani* and *alteraria altranate.*

2.8 Anti bacterial assay

The oil were screened against strains of bacteria including *Pasturella multocida* , *Escherchia coli*, *Basillus* subtilies and *Staphocous aureus* using the disk diffusion method according to Parez et al.1990 using 200 µL of oil after 24h of incubation at 37**C** all platea were observed for zones of growth inhibition and the diameter of these zones were measured in millimeters .

2.9 GC-MS Analysis

2.9.1 Sample preparation

2ml of the sample was mixed thoroughly with 7ml of alcoholic 1sodium hydroxide that was prepared by dissolving 2 g in 100 ml methanol .7 ml from alcoholic sulfuric acid (1ml H2SO4 to 100 ml methanol) was then added .The mixture was then shaked for 5 minutes The content of the test tube was left to stand overnight .1 ml of super saturated sodium chloride (NaCl) was then added and the contents beinttg shaken .2 ml of normal hexane was added and the contents were shaked throughlyfor three minutes .Then the n-hexane layer (the upper layer of the test tube) was taken using disposable syringe .5(microlietr) from the n-hexane extract was diluted with 5 ml of diethyl ether. Then the mixture was filtered through syringe filter 0.45 micro meter and dried with 1g of anhydrous sodium sulphate as drying agent and 1 microliter of diluted sample was injected in GC-MS instrument.

2.9.2 GC-MS Conditions

The qualitative and quantitative analysis of the sample was carried out by using at GC/MS technique model (GC/MS-QP2010-Ultra) from japans, with serial number 020525101565SA and capillary column (Rtx-5ms-30*0.25mm *0.25micro m).

The sample was injected by using split mode ,helium as carrier gas passed with flow rate 1.61ml/min ,the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 5 minute hold time the injection port temperature was 300c ,the ion source temperature was 200c and the interface temperature was 250c .

The sample was analyzed by using scan mode of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library ,the National Institute of Standards and Technology (NIST) ,results were recorded.

2.9 Fourier transforms infrared spectroscopy (FT-IR):

FT-IR spectrum of the oil was obtained using an IR 300 model spectrometer (Thermo Nicolet).A drop of oil was placed between a pair of NaBr salt plates. The plates were inserted into a holder that fits into the Infrared spectrophotometer. The scanning was done in the range between 4000 and 500 cm^{-1} with resolution Of 4 cm^{-1}.

Chapter Three

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3. Results and discussion

Table 3.1 Chemical properties of *Moringa olifera seeds* oil

Characteristic	Value obtained	Units
Acid value	1.295	(mg KOH/g oil)
Peroxide value	2.753	(meq KOH/g oil)
Saponification value	211.775	(mgKOH/g oil)
Ester value	210.478	(mg KOH/g oil)
Free fatty acid	0.651	%

Table 3.1 shows that the acid value of *Moringa olifera* seed oil is 1.295 this value agree with international standard for edible oil FAO/WHO (2009).The saponification values in (meqKOH/g oil) of Moringa oil have an average value of 211.775.which have an inverse relationship with molecular weight of lipids which in the range of the international standard for edible oil 5.58-249.90 mgKOH/g. Saponification values gives information concerning the character of the fatty acid present in the oil and the solubility of the soap derived from it in water.nA high saponification value indicates that the oil contains low portion of fatty acids. This is an indication that *Moringa* seed oil is not suitable for soap manufacture.

The peroxide value of *Moringa oleifra* seeds oil determined was 2.753 which are in the range of FAO/WHO (2009) for fresh edible oil. Peroxide value is a measure of its oxygen content, which is used to monitor the development of rancidity through the evaluation of the quantity of peroxide generated in the product .The lower peroxide value

of *Moringa oleifra* seeds oil indicates that it will not easily go rancid which is related to its longer shelf life and its stability conforms to the FAO/WHO (2009) . The free fatty acid of *Moringa oleifra* seeds oil is 0.651% which is within the range of the FAO/WHO (2009) standard. Ester value is 210.478 this value indicate high ester content of the oil.

Table 3.2 Physical properties of *Moringa olifera* seeds oil

Properties	**values**
Viscosity	84cp at 26c 85cp at 26c
Refractive index	1.469at 24c 1.468
Colour Yellow Red blue	11.2 11.3 0.7 0.7 0.1 0.1
The relative density	0.909g/cm3 0.909g/cm3

Table3.2 shows the physical properties of *Moringa oleifra* seeds oil, which covers: refractive index, viscosity, relative density and color. Physical characteristics of *Moringa olifera* seeds oil, it is liquid at room temperature. The color is yellow is expressed as the sum total of yellow and red used to match the colur of oil. The viscosity of *Moringa oleifra* seeds oil was 84.5cp. The refractive index of *Moringa oleifra* seeds oil determined

Shows little or no differences from the international standard for edible oil presented by FAO/WHO (2009) which is 1.4685.

The relative density is mass of oil relative to volume which is 0.909.

The physical properties of the oil extracted from *moringa oleifera* seeds were in conformity with the FAO/WHO (2009).

3.1The UV-visible spectroscopy analysis

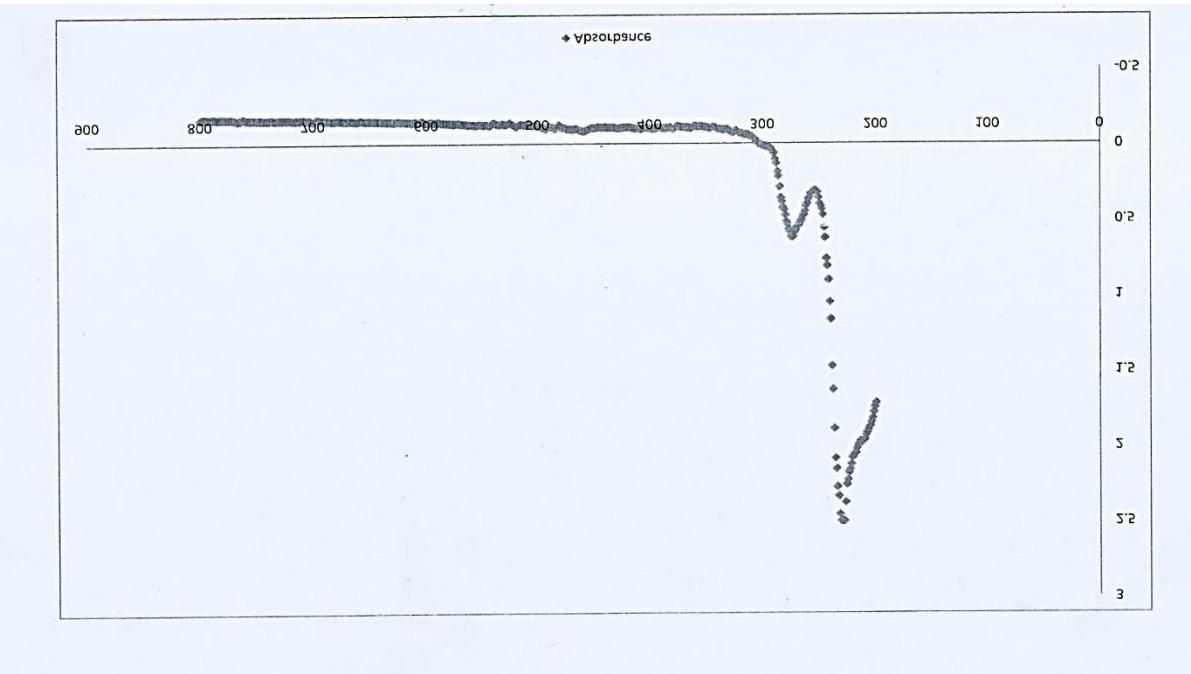
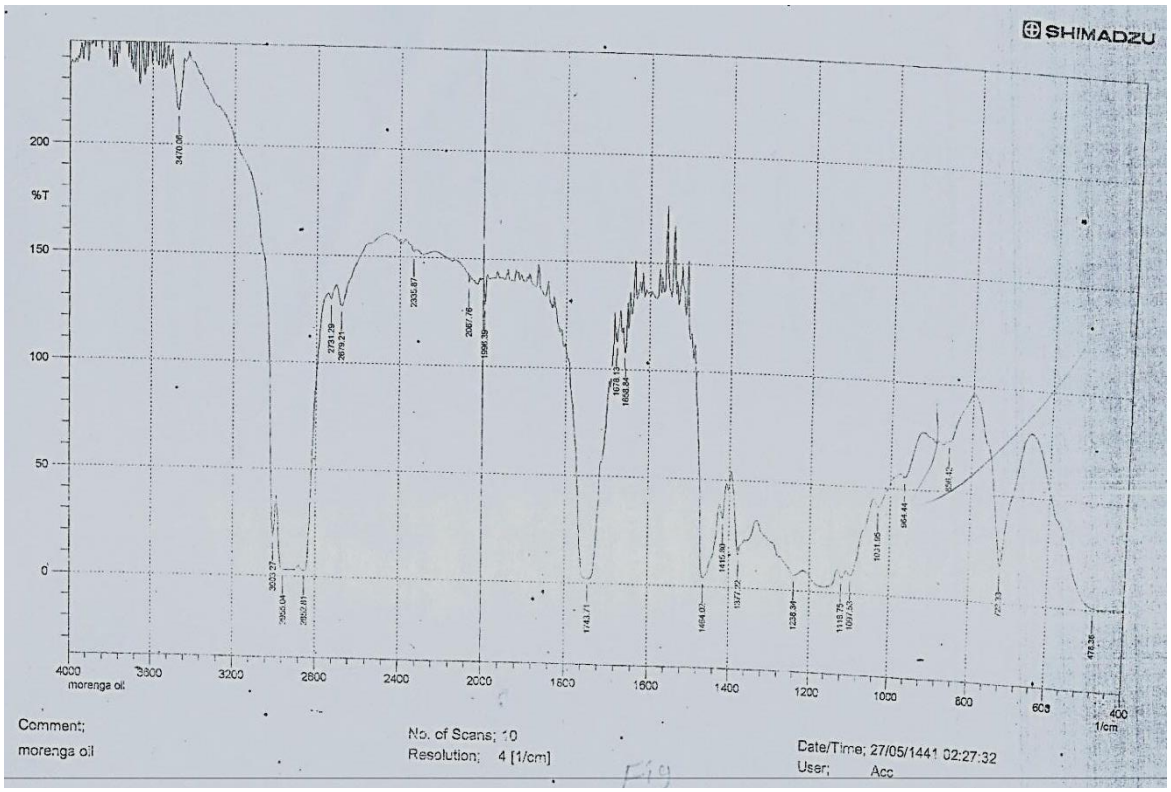


Figure (1) UV-Visible analysis

3.1.1 The FT-IR Analysis:



Figure(2) FT-IR Analysis

The Fourier transform infrared analysis was used to investigate the structural characteristics of the raw sample and the possible changes in the structural characteristics as a result of processing

Table 3.3 displays the FT-IR wave number and the functional group which give rise to absorption band for oil sample .

Table 3.3 functional IR groups of *Moringa oleifra* seeds oil

Wave number(cm^{-1})	**Functional group**
3003	C-H stretching vibration (sp3hybridized)
2955 and 2852	O-H stretching vibration
1743	Double bond stretching of carbonyl group
1678 and 1658	C=C stretching vibration
1464 an1377	C-H bending vibration
1118 and1097	Stretching vibration of the C-O ester group
723	C-N stretching vibration

Table 3.4 The Anti fungal assay result of *Moringa oleifera* seeds oil:

Specific fungi	**Concentration of *Moringa olefira* seeds *oil***					
	0.5%		**1.0%**		**1.5%**	
	Diameter mm	**Inhibition percentage**	**Diameter mm**	**Inhibition percentage**	**Diameter mm**	**Inhibition percentage**
Fusarium oxysporum	38	55.8	28	68.8	16	75.1
Fusarium solani	40	54.0	26	66.6	12	70.2
Alternaria alternate	60	36.0	45	50.0	36	57.6
Rhizoctonia solani	68	28.8	52	43.2	40	54.0

Table 3.5 Anti bacterial assay of Moringa oleifera seeds oil

Bacteria species	Diameter of inhibition zone (mm)	Minimum inhibity of concentration
Pasturella multocida	21	26
Escherchia coli	20	28
Basillus subtilies	36	22
Staphocous aureus	31	24

Table 3.5 shows that the alcoholic extract of *Moringa oleifra* seeds show resistance to all fungal organisms by inhabiting the growth.

Fungal organisms producing zone of growth inhibition decrease inhibity by increasing concentration of oil.

Table 3.5 shows that *Moringa oleifra* seeds oil exhibits moderate activity aginst all of the four species of positive and negative bacteria and no activity in aqueous extract of *Moringa oleifra* seeds.

Minimum inhibity concentration was 20 mg/ml so all four type of bacteria so moderate activity.

Table 3.6 GC.MS results of *Moringa oleifera* seeds oil

Peak No	Name of compound	R.Time	Area%
1	Methyl tetradecanoate	13.015	0.13
2	Pentadecanoic acid ,methyl ester	14.083	0.01
3	7-Hexadecenoic acid , methyl ester	14.865	0.10
4	9-Hexadecenoic acid , methyl ester	14.911	1.90
5	Hexadecanoic acid, methyl ester	15.109	**8.55**
6	Cis -10-Heptadecenoic acid methyl ester ,	15.870	0.05
7	Heptadecanoic acid , methyl ester	16.079	0.12

8	9,12-Octadecadienoic acid(Z,Z) methyl ester	16.753	0.71
9	9-Octadecenoic acid (Z),methyl ester	16.833	**47.18**
10	9-Octadecenoic acid ,methyl ester(E)	16.863	**6.55**
11	Methyl sterate	17.019	**9.51**
12	Cis-11-Eicosanoic acid ,methyl ester	18.565	4.98
13	Eicosanoic acid ,methyl ester	18.768	**6.56**
14	Docosanoic acid ,methyl ester	20.388	**11.14**
15	Tetracosanoic acid ,methyl ester	21.884	2.50

Table 3.6 shows that oleic acid was the major component of oil with 47% and retention time 16.8. Oleic acid is a mono-saturated omega-9-fatty acid with many health's benefits and is safe in present practices for use and concentrations. Protects cell from free radical damage, reduce blood pressure and increase fat burning. Behenic acid or docosanoic acid is the second constituent of oil with area% is 11.1% and retention time 20.3, it is saturated fatty acid. Methyl stearate is saturated methyl ester also *known* kemester 9.5% and retention time 17. The other constituent is hexadecanoic acid or palmatic acid is saturated long chain fatty acid with sixteen carbon backone .It is one of the most abundant and wide spread natural saturated acids present in plants. Other constituents are Eicosanoic acid, cis-11-Eicosenoic acid and other constituents.

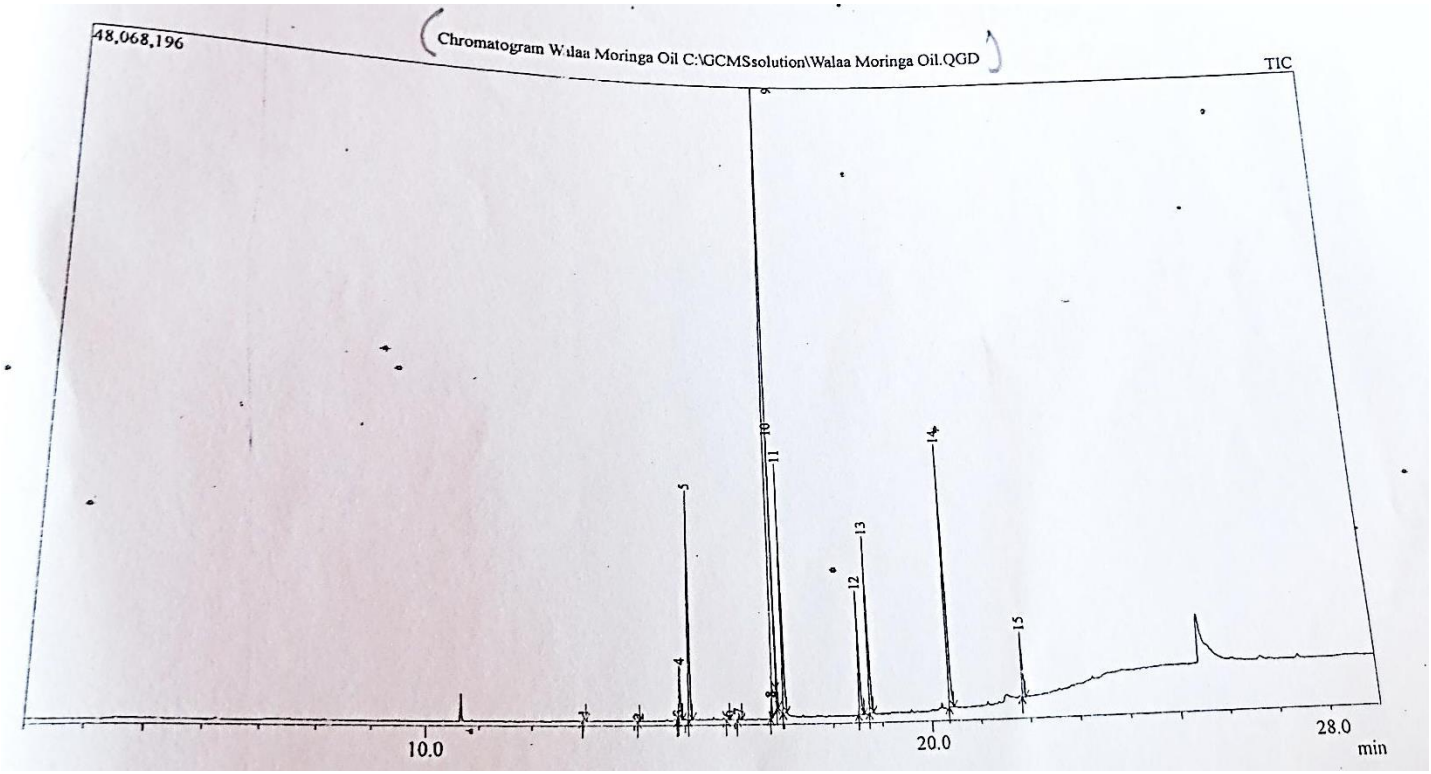


Figure (3) GC-MS chromatogram of *Moringa oleifra* seeds

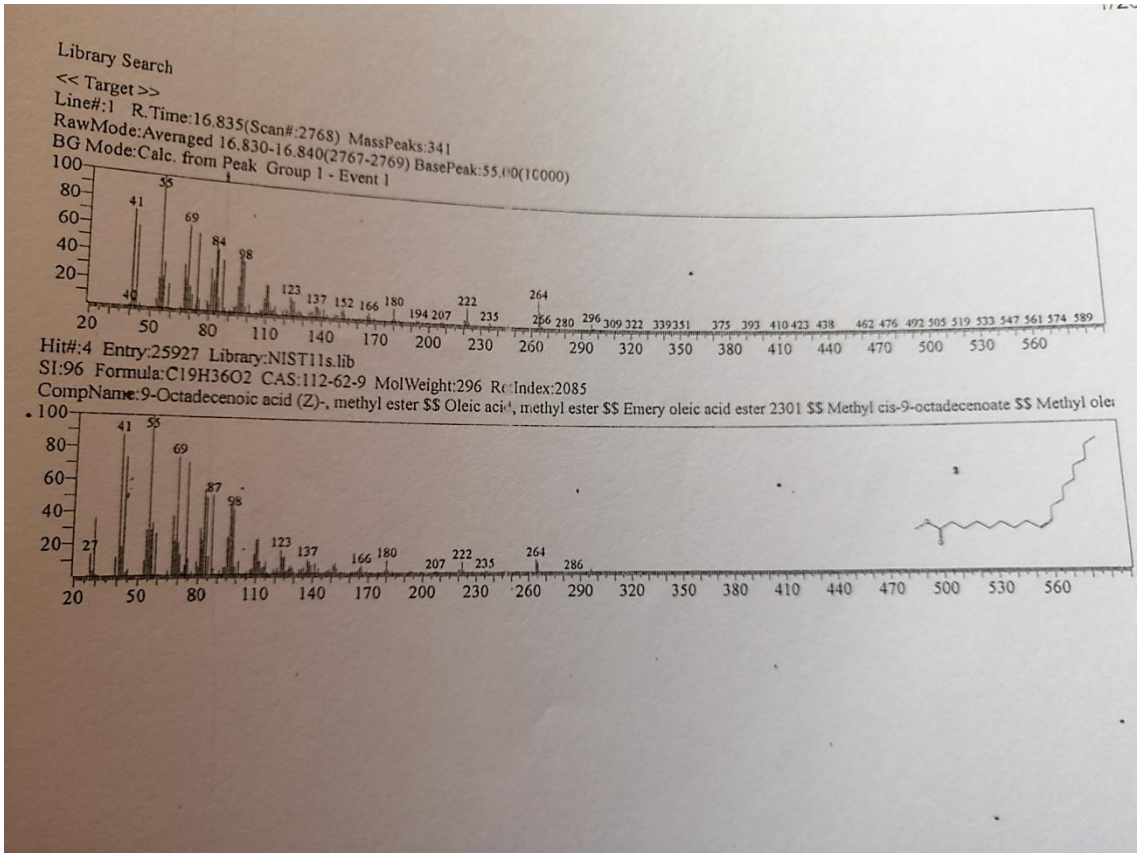


Figure (4) The mass spectrum analysis of 9-Octadecenoic acid , methyl ester

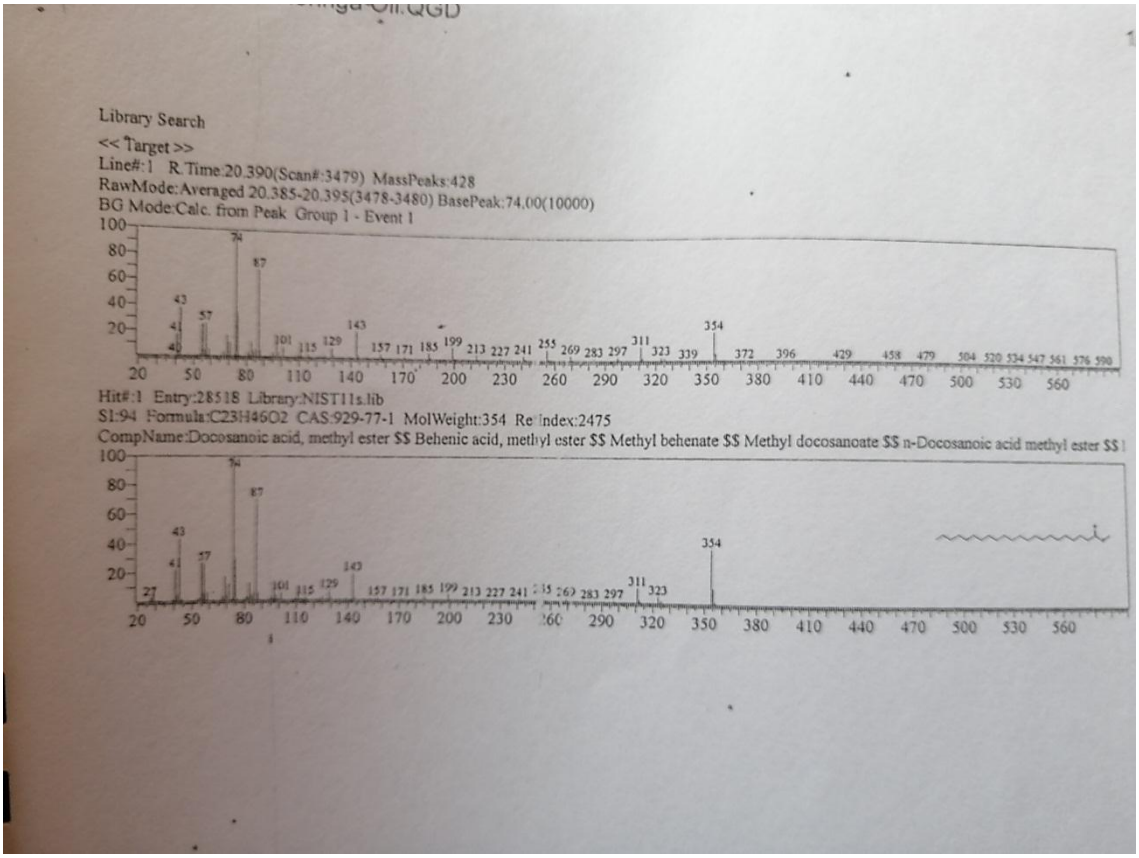


Figure (5) The mass spectrum analysis of Docosanoic acid , methyl ester

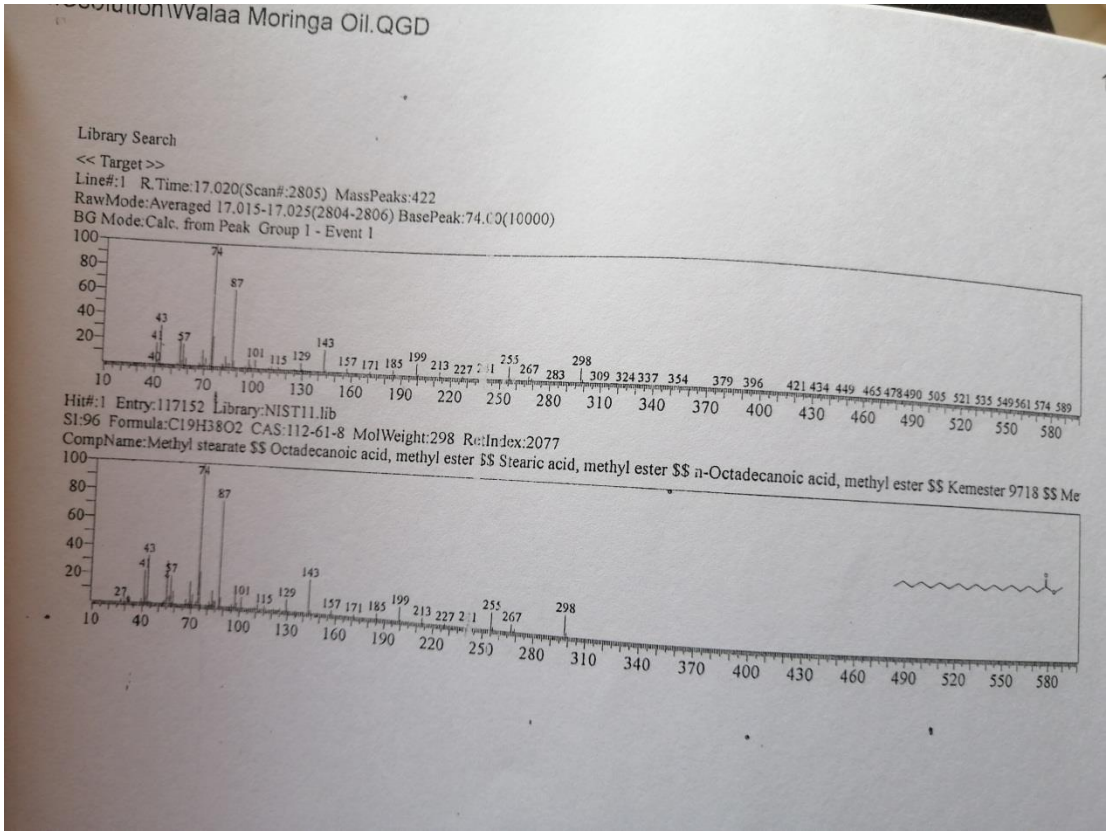


Figure (6) The mass spectrum analysis of Methyl stearate

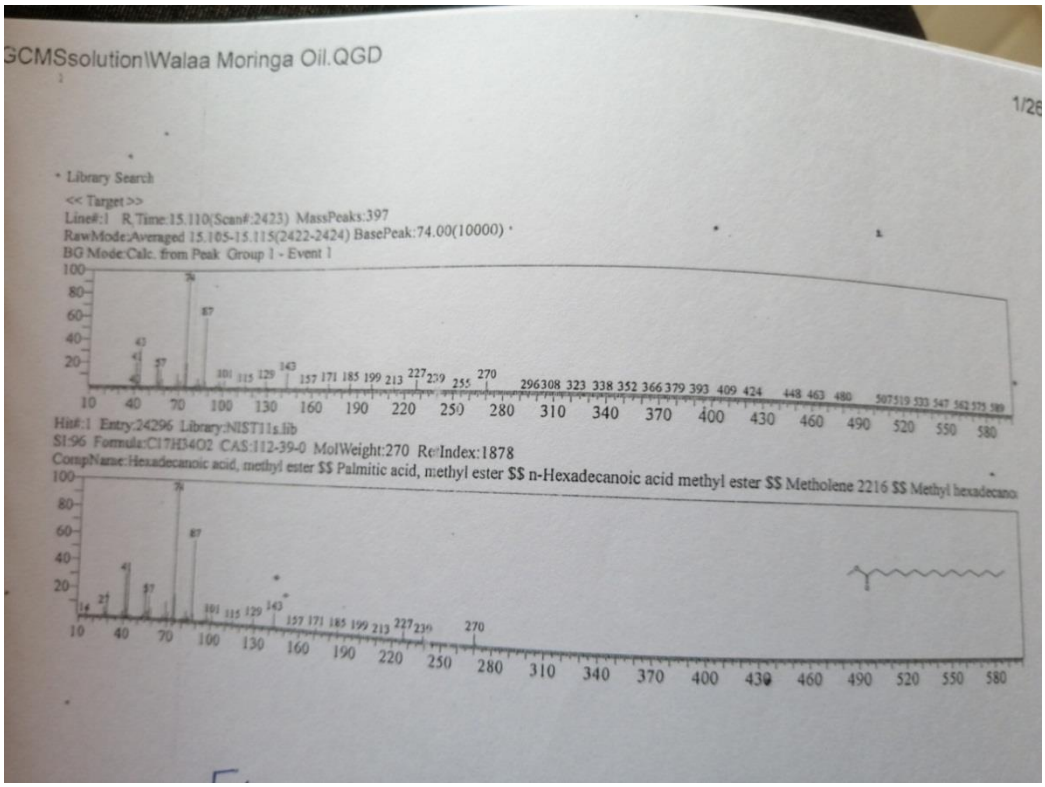


Figure (7) The mass spectrum analysis of Hexadecanoic acid , methyl ester

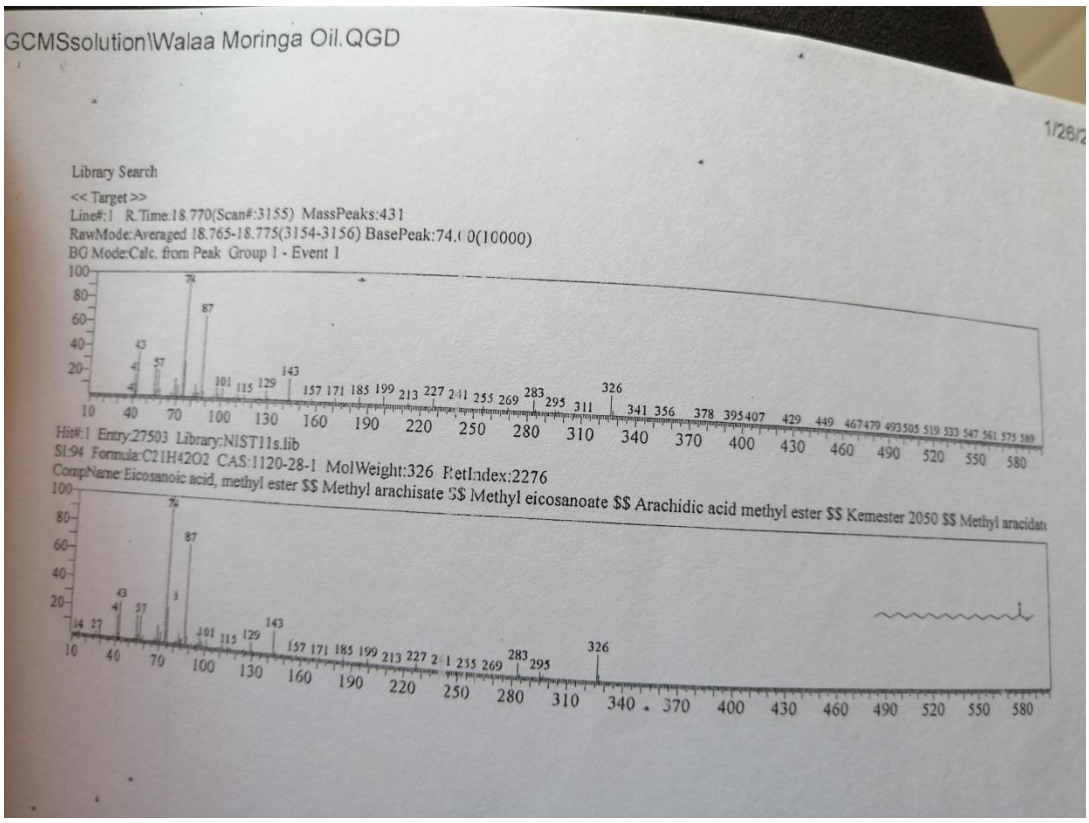
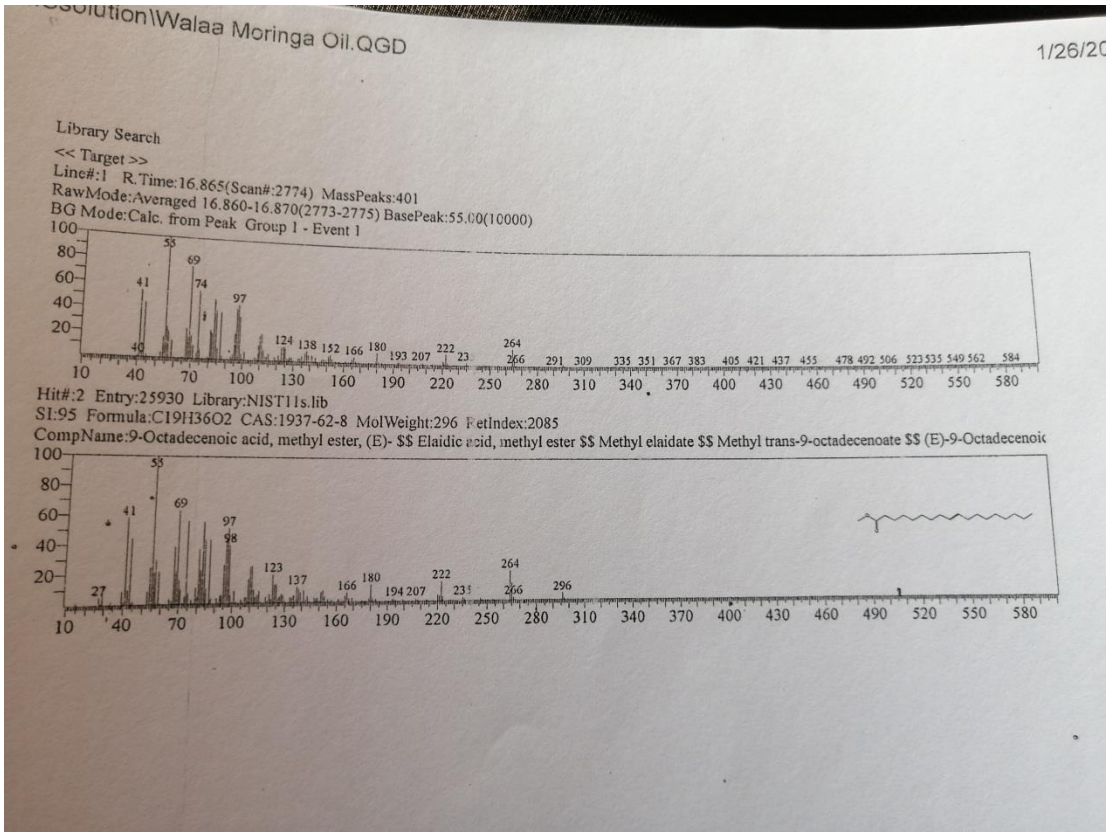


Figure (8) The mass spectrum analysis of Eicosanoic acid , methyl



ester

Figure (9) The mass spectrum analysis of 9 – Octadecenoic acid (z-) methyl ester

Consolation:

Moringa oleifera is a tree growing rabidly even in poor soil and little affected by drought so can be easily grown in wide range of world. The seeds and oil of moringa oleifra are interesting prouducts for their nutritional composition and their content of bioactive compounds.

The physical and chemical properties of the *moringa oleifra* seeds oil indicates that they will not easily go rancid which were related to its longer shelf life and its stability conforms to the FAO/WHO (2009) oil contains low portion of fatty acids. This is an indication that *Moringa oleifra* seeds oil is not suitable for soap manufacture.

This study indicated that the chemical composition of *Moringa oleifra* seeds contained high oil content and this means the seed of *Moringa oleifra* tree is a good source of edible oil.

The oil extracted from *Moringa oleifra* seeds have good physicochemical properties in such a way that no additional processing operations methods will be needed for the oil. The oil has good quantity of oleic acid and behenic acid,therefore it can be used for frying and other food purposes.

Moringa oleifra seeds oil exhibits moderate activity against all of the four species of positive and negative bacteria and also oil show antifungal activity against tested organisms.

It is essential that further studies be aimed at indentifying the best cultivation conditions to maximize plant production and demonstrating the effects of *moringa oleifera* seeds and oil consumption on human health.

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