Sudan University of Science
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Production and Characterization of the oil Biodiesel and Biodiesel blends from *Moringa oleifera* seeds

إنتاج وتوصيف الزيت والوقود الحيوي ومخاليطه من بذور المورينجا اوليفيرا

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry

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إستهلال

بسم الله الرحمن الرحيم

قال تعالى:

{يَرْفَعِ **اللَّهُ** الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ}

[سورة المجادلة: الآية 11]

Dedication

To my

Parents,

Brothers and Sisters

Acknowledgement

Thanks Allah before and after

Special appreciation and deep gratitude to my supervisor. Prof. Mohammed Elmubark Osman for his encouragement, close and valuable supervision as well as precious advice.

I ask God to bless him for all that he has done for me to complete this study. I cannot repay his worthy services for me.

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Abstract

This study aims to identify a sustainable source of energy from natural and environmentally friendly resources. The oil from *Moringa oleifera* seeds was extracted using the solvent extraction method. Therefore, the solvent extraction proved to be suitable for the extraction of Moringa oleifera oil, *Moringa oleifera* crude oil was converted to Biodiesel, sample of *Moringa oleifera* seeds were collected from National Tree Seed Center – Forest Research, located in Khartoum, Sudan.

the chemical composition of the seeds was investigated, and the percent of each component was calculated. The investigation showed that *Moringa oleifera* seeds contained approximately (7.0%) moisture, (31.6%) protein, (38%) oil, (7.8%) fiber, (6.3%) ash and (19.9%) carbohydrates.

The chemical composition indicated that *Moringa oleifera* seeds contained appreciable amount of oil that encouraged its extraction and characterization.

The oil extracted from *Moringa oleifera* was then evaluated for its physicochemical properties, the values obtained for pH (5.42), kinematic viscosity at 40 C (35.75 cSt), refractive index (1.4791), density at 40 C (0.9022 g/ml), flash point (158.0 °C), Acid Value (9.89 mg KOH/g), free fatty acid (9.22 %), peroxide value (6.00 mEq/Kg), saponification value (210.13 mg KOH/g), iodine value (66.5 I_2/100g).

The produced biodiesel was identified using (GC-MS, FT-IR) techniques, the results show that the total ester content in the biodiesel was 100% of the total components of produced *Moringa oleifera* biodiesel.

The physical and chemical properties of *Moringa oleifera* biodiesel were compared with the ASTM D 7467 requirements. B20 blend of biodiesel with Diesel were all within the level of ASTM D 7467. Biodiesel-Diesel-Ethanol blends were investigated and compared also with standard method ASTM D 7467 and all the results were found with the standard requirements. The flash point of pure biodiesel was 137 ^{o}C, reduced to 87.00 ^{o}C in the B20 blend and reduced to 15 C in D60 B20 E20.

The findings of the present investigation proved that the oil extracted from *Moringa oleifera* seeds has acceptable physicochemical properties. Hence, *Moringa oleifera* can be considered a potential source to produce a high-quality oil.

المستخلص

تهدف هذه الدراسة الى تحديد مصدر مستدام للطاقة من الموارد الطبيعية والصديقة للبيئة، تم استخلاص الزيت من بذور الموينجا اوليفيرا بإستخدام طريقة الإستخلاص بالمذيبات. لذلك، اثبت الاستخلاص بالمذيب انه مناسب لاستخراج زيت المورينجا اوليفيرا، تم تحويل زيت المورينجا اوليفيرا الخام الى وقود الديزل الحيوي، تم جمع عينة من بذور المورينجا اوليفيرا من مركز بذور الاشجار القومي – بحوث الغابات، الموجود في الخرطوم السودان.

تم اختبار التركيب الكيميائي لمكونات بذور المورينجا اوليفيرا وحساب نسبها المئوية. أظهرت الدراسة ان المحتوى التقريبي لبذور المورينجا اوليفيرا هو 7.0 % رطوبة، 31.6 % بروتين، 38% زيت، 7.8% الياف، 6.3% رماد، 19.9% كربوهيدرات.

دلّ التركيب الكيميائي على ان بذور المورينجا اوليفيرا تحتوي على كمية مقدرة من الزيت الذي شجع استخلاصه وتوصيفه.

وقد تم تقييم الخصائص الفيزيائية والكيميائية للزيت المستخلص لبذور المورينجا اوليفيرا، القيم التي تم الحصول عليها هي: الاس الهيدروجيني (5.42)، اللزوجة الحركية عند 40 درجة مئوية (35.75 cSt)، معامل الانكسار (1.4791)، الكثافة عند 40 درجة مئوية (0.9022)، نقطة الوميض (158.0)، درجة

الحموضة (9.89 mg KOH/g)، الاحماض الدهنية الحرة (9.22%)، رقم البيروكسيد (6.00 mEg/Kg)، رقم التصبن (210.13 mgKOH/g)، رقم اليود ($66.5\ I_2/100g$).

تم تحديد وقود الديزل الحيوي المنتج بإستخدام تقنيات GC-MS و FT-IR واظهرت النتائج ان محتوى الإستر الكلي في وقود الديزل الحيوي كان 100% من اجمالي مكونات الديزل الحيوي المنتج من زيت المونجا اوليفيرا.

تمت مقارنة الخصائص الكيميائية والفيزيائية لوقود الديزل الحيوي من المورينجا اوليفيرا مع متطلبات ASTM D 7467.

مزيج 20% من وقود الديزل الحيوي مع الديزل كانت جميعها ضمن المتطلبات القياسية، تم فحص خليط من وقود الديزل الحيوي مع الديزل والايثانول ومقارنته مع الطريقة القياسية ASTM D 7467 وكانت النتائج ضمن المتطلبات القياسية.

كانت نقطة الوميض لوقود الديزل الحيوي النقي 137 درجة مئوية، وانخفضت الى 78.00 درجة مئوية في مزيج 20% من وقود الديزل الحيوي، وانخفضت الى 15 درجة مئوية في خليط من 20% وقود الديزل الحيوي و 60% من الديزل و20% من الايثانول.

أثبتت نتائج هذه الدراسة ان الزيت المستخرج من بذور المورينجا اوليفيرا له خصائص فيزوكيميائية مقبولة. وبالتالي، يمكن اعتبار مورينجا اوليفيرا مصدرا محتملا لإنتاج زيت عالي الجودة.

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Chapter one

Introduction and literature review

1.1 Introduction

1.1.1 Oils

Plants are capable of synthesizing two kinds of oils: fixed oils and essential oils (volatile oils). Fixed oils consist of esters of glycerol and fatty acids (triglycerides or triacylglycerols), while essential oils (EOs) are complex mixtures of volatile and semi volatile organic compounds originating from a single botanical source that determines the specific aroma of plants and the flavor and fragrance of the plants(Tisserand and Young, 2013; Sirousmehr *et al*., 2014).

1.1.2 Chemistry of fixed oils

Fixed oils are mixtures of long chain saturated and unsaturated fatty acids.

Fatty acids consist of a hydrocarbon chain that is bonded to a carboxyl group (COOH). Saturated fatty acids contain straight chains of carbon atoms with only single bond and they conform to the general molecular formular $CH_3(CH_2)nCOOH$ However, unsaturated fatty acids contain carbon chains with one or more double bonds in the hydrocarbon tail. The monounsaturated fatty acid contains only one double bond that is attached to the CH group Saturated fatty acids are solids at room temperature with high melting point while unsaturated ones are liquids at room temperature with lower melting point.

Saturated fatty acids have perfectly straight chain structure whereas the unsaturated ones are typically bent unless they have a trans configuration(Folayan *et al*.,2019).

1.1.3 Uses of fixed oils

The use of vegetable oils as raw materials for industrial applications has awakened the interest of many researchers from all walks of life hence, identification, characterization development, applicability and cost-effective production methods of such useful vegetable oils have been on the increase. A good number of oil seeds have been, successfully, developed and are in, current, use for domestic and oil processing applications with many, more, products still underdevelopment. Others still, have been identified but lack of information on their chemical composition limits their applications(Adejumo *et al*., 2013).

Due to the increasing world demand it became very imperative to take advantage of more and more vegetable oils being explored to meet global needs for food and energy(Nielsen, 1994).

Vegetable oils are becoming a promising alternative to diesel fuel because they are renewable, natural, resources and can be produced locally and environmental friendly as well. Edible vegetable oils like canola, soybean and corn have been used for biodiesel production and found to be as good substitute for diesel(Lang *et al*., 2001; Freedman *et al*., 2003). Nonedible vegetable oils such as *Madhuca indica, Jatropha curcas* and *Pong-amia pinnata* are found to be suitable for biodiesel production(Pramanik, 2003; Senthil *et al*., 2003 and Maher *et al*., 2006). In view of the several advantages, vegetable oils have a great potential to replace diminishing fossil, petroleum-based fuels in the long run.

Non-edible vegetable oils are not suitable for human food due to the presence of some toxic components in the oils(Ahmad *et al.*, 2011). The selection of non-edible vegetable oils as feed stocks for biodiesel production requires reviewing the existing works. Recent comprehensive reviews on biodiesel production from various feed stocks show the advantages of non-edible oils over edible oils. Production of biodiesel from non-edible oils feed stocks can overcome the problems of food versus fuel, environmental and economic issues related to edible vegetable(Gui *et al.*, 2008).

Non-edible biodiesel crops are expected to use lands that are largely unproductive and those that are located in poverty- stricken areas and in degraded forests. They can also be planted on cultivators' field boundaries, fallow lands, and in public land such as along railways, roads, and irrigation canals. Non-edible biodiesel development could become a major poverty alleviation program for the rural poor apart from providing energy security in general and to rural areas in particular and upgrading the rural non-farming sector. All these issues have great impact on the sustainability of biodiesel production. Many researchers have concluded that non-edible feed stocks of biodiesel should be considered as sustainable and alternative fuels(Syers *et al.*, 2007; Ravi *et al.*, 2009 and Ahmad *et al.*, 2011).

1.1.4 *Moringa oleifera*

1.1.4.1 Botanical classification

Scientific Classification of *Moringa oleifera*

Moringa oleifera is one of the 14 species of the *Moringaceae* family.

It can be classified as follows:

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Brassicales*
Family	*Moringaceae*
Genus	*Moringa*
Species	*Oleifera*

1.1.4.2 Description

Moringa oleifera is a small tree up to 7m high. Bark pale grey smooth corky roots of young plants swollen. Leaves bi or more often tri pinnate 30-60 cm long. Leaflets 1.25-2cm long, 0.7-1.25 wide, they lateral and somewhat elliptic, the terminal leaflets are obovate and slightly larger than the lateral ones, generally, with 6 pairs of pinnae, with two pairs of opposite laterals and one terminal.

Flowers are along branches, paniculate, sweet scented, cream colored. Sepals 5 unequal in size Petal’s unequal and slightly larger than sepals, white with yellow dots at the base. Fruit 3-angled, elongate-linear tapering at both ends, 9-ribbed, up to 30cm long. Seeds round with 3 papery wings(El Amin, 1990).

1.1.4.3 Distribution

Moringa oleifera is cultivated in many areas in Sudanese states on the Red sea Hills (Khor langeb and Jabel Elba, Jabel martab) Blue Nile (Sennar, Roseires) Kordofan and Darfur, Equatoria(El Amin, 1990). In Khartoum state the tree is cultivated in many individually owned farms and agricultural companies, The tree is known as Rawag tree due to its capability to purify turbid water (Kuete, 2017). Commercially it called ‘‘ben oil’’ or ‘‘behen oil.’’ *Moringa oleifera* contains sbehenic(docosanoic) acid which, significantly, prevents oxidative degradation of the oil(Rashid *et al.*, 2008).

In some parts of the world, *M. oleifera* is referred to as the “drumstick tree” or the “horseradish tree,” whereas in others it is known as the kelor tree(Anwar and Bhanger, 2003).

The tree parts (leaves, seeds, bark, pods) are of economic importance and available in local markets. Meanwhile, cultivation of *Moringa oleifera* in Sudan as a perennial crop by small-scale farmers is promising for sustainable development. This is because, it has the, added, advantage of not requiring annual clearance of new forest lands(Mohammed *et al.*, 2014).

Moringa oleifera tree can grow, best, on dry sandy soil and withstand drought conditions. It grows quickly from seeds or cuttings, can reach a height of twelve feet within the first year and regenerates itself even after severe pruning(Mustaf *et al* 1999). *Moringa oleifera* leaves are also considered to be a rich source of minerals and vitamins(Gupta *et al.*, 1989).

1.1.4.4 Harvesting: -

Fruit or other part of the plant are, usually, harvested as desired according to some authors but in India fruiting may peak between March and April and again in September and October. Seeds were gathered in March and April to be used for oil extraction(Burkill, 1966). **Fig 1.1** show *Moringa oleifera* seeds.

Fig 1.1: *Moringa oleifera* seeds.

1.1.4.5 Uses

Almost all parts of the tree are used, either for nutrition or in traditional medicines(Fahey, 2005). *Moringa oleifera* is an important component of traditional medicines throughout South Asia and many parts of Africa. It has been used for pain relief, treatment for headaches, fevers, and rheumatism and treatment for bug bites(Goyal *et al*., 2007). More recently, studies on various parts of *Moringa oleifera* have been undertaken to determine the feasibility of *Moringa oleifera* in the treatment of cancer (Jung, 2014). In addition to nutritional and medicinal benefits, *Moringa oleifera* is used in fertilizer, pesticides, contraceptives, perfume, animal food, and as a cleaning agent (Fahey, 2005). *Moringa Oleifera* seeds contain a protein which inhibits coliform bacteria(Kwaambwa *et al*., 2010). This protein is the *Moringa oleifera* cationic protein (MOCP), and it kills bacteria by fusing the bacteria's cell membranes(Shebek *et al*., 2015), *Moringa oleifera* seeds are used to create a powder that can eliminate harmful bacteria in water, making it safe for human consumption(Ndabigengesere *et al*., 1995). Seeds form a coagulant which reduces the turbidity of water(Lea, 2010). The use of *Moringa oleifera* seeds in water purification was first reported by(Jahn, 1977), when he discussed the traditional methods of water purification in Sudan. In Sudan, the *Moringa oleifera* tree is referred to as a 'clarifying tree'(Jahn, 1977). Based on Jahn's work, scientists began to study the mechanisms by which *Moringa oleifera* seeds purify water, as well as how to optimize this water treatment method, the *Moringa oleifera* seed powder also possesses antimicrobial properties(Olsen, 1987). Seeds are able to remove 90-99% of *E. coli* and other fecal coliforms, which are waterborne and can cause diarrheal diseases(Schwarz, 2000).

1.1.4.6 Chemical composition

The chemical and organic composition of *Moringa Oleifera* trees have been studied in order to better understand and utilize the *Moringa oleifera* plant. *Moringa oleifera* is rich in alkaloids, tannins, flavonoids, anthocyanins, proanthocyanidins, cinnamates and cardiac glycosides(Goyal *et al*., 2007; Alhakmani, *et al*., 2013). These are all phytochemicals which have many medicinal applications. *Moringa Oleifera* is also rich in Vitamin A, Vitamin C, B-complex vitamins, potassium, magnesium, selenium and zinc(Fuglie, 2001).

Moringa oleifera is rich in nutrition owing to the presence of a variety of essential phytochemicals present in its leaves, pods, and seeds. In fact, *Moringa oleifera* is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk,9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach(Rockwood *et al*, 2013).

In addition, the oil's fatty acid composition does not seem to be particularly affected by the extraction method. Only one study reported a small increment of the stearic and myristic acid content in solvent-extracted oil compared to oil obtained by cold pressure(Orgunsina *et al*.,2014). On the other hand, the agro-climatic characteristics of the cultivation area and the *Moringa oleifera* variety cultivated could be the reason for some differences in the fatty acid composition of the oil. Never the less, the present fatty acid composition shows that *Moringa oleifera* seed oil falls in the category of high-oleic oils, and contains a high mono unsaturated to saturated fatty acids ratio (MUFA/SFA). The MUFA/SFA ratio is characteristic of several oils, particularly olive oil, and has been associated with a reduced risk of all-cause mortality, cardiovascular mortality, cardiovascular events, and stroke(Schwingshackm *et al*.,2014). Therefore, *Moringa oleifera* seeds oil could be an acceptable substitute for olive oil as the main dietary fat in countries where the tree grows. *Moringa oleifera* seeds oil has a monounsaturated fatty acid content similar to that of olive oil(Boskou,2011; Gnagnarella, *et al*.,2016), but from a nutritional point of view, a lower content of polyunsaturated fatty acids is a limiting factor, which needs to be offset by the consumption of alternative sources rich in polyunsaturated fatty acids. However, from a technological point of view, the low content of polyunsaturated fatty acids ensures greater resistance and stability to oxygen. The chemical properties of different parts of *Moringa oleifera* are shown on Table (1.1).

Table (1.1) Chemical properties of the different parts of the *Moringa oleifera*.

Part of Moringa	Medicinal Compound	Uses	References
seeds	Crude protein, crude fat, carbohydrate, methionine, cysteine, 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate, benzylglucosinolate, moringyne, mono-palmitic and dioleic triglyceride, Vitamin A, beta carotene, terygospermin, Moringa oleifera cationic protein	Decreasing liver lipid peroxides, water treatment, treating rheumatism.	Dahot and Memon, 1985; Bennett et al., 2003; Shebek et al., 2015
Flowers	D-mannose, D-glucose, protein, ascorbic acid, polysaccharide.	Abortifacient, cholagogue, aphrodisiac, cures inflammations, muscle diseases, tumors, hysteria, and spleen enlargement. Lowers serum cholesterol and decreases lipid profile of liver and heart	Pramanik and Islam 1998 ; Anwar et al., 2007
Roots	4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate	Laxative, circulatory tonic, antifertility, rubefacient, treating rheumatism and arthritis, as well as back and kidney pain.	Bennett et al.,2003; Anwar et al., 2007, Goyal et al., 2007;
Leaves	Glycoside niazirin, niazirinin and three mustard oil glycosides, 4-[4'-O-acetyl- α -L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B, quercetin-3-O-glucoside, quercetin-3-O-(6''-malonylglucoside), kaempferol-3-O-glucoside,3caffeoylquinic.	Nutrition, headache relief, treats fevers, piles, sore throats, eye and ear infections, and scurvy. Controls glucose levels and reduces swelling.	Faizi et al., 1994; Faizi et al., 1995; Anwar et al., 2007; Goyal et al., 2007;
Bark	4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate	Rubefacient, treats delirium, eye diseases, relieves earaches, painkiller.	Bennett et al., 2003; Anwar et al., 2007;

1.2 Literature review

1.2.1 Fossil fuel

fossil fuels are non-renewable energy sources and thus limited in supply. It also poses a lot of health and environmental problems. These energy resources are not evenly distributed around the world; it is more concentrated in some countries than others. Therefore, countries not having these resources are forced to import crude oil, thereby encountering challenges accompanied by importation such as foreign exchange problems. These countries will seek alternative fuels resources that can easily be produced from indigenous materials available within their country(Singh, *et al.*, 2010).

Biofuels are considered as renewable energy, because the CO_2 released from the burning of biomass is available to produce a new biomass in a closed carbon cycle with no net increase in an atmospheric CO_2 level. On the other hand, the conversion of biomass to fossil fuels takes millions of years. Burning fossil fuels uses old biomass and convert it into a new CO_2, which contributes to the greenhouse effect(Shalby, 2013).

Biofuel is produced from any renewable biomass material. It is commonly used as an alternative, cleaner fuel source to burning fossil fuels(Demirbas,2008). Biofuel can be produced from various materials which are in contrast with conventional diesel that can be produced from only a specific type of material(Vera *etal.*, 2007). Liquid biofuel is a type of biofuel which is essentially employed in fueling vehicles and sometimes engines(Domínguez *et al.*, 2019).

Sudan is a tropical country which is very rich in biomass resources. Exploitation of these resources, efficiently, in a profitable and sustainable manner, is emerging as an urgent necessity for the sake of human health, climate change abatement and to compensate the shortage of energy(Omer, 2005).

1.2.2 Biodiesel

Biodiesel is defined as a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fat(Vicente et al., 2007). It has similar physico-chemical properties of conventional fossil fuel and can consequently, entirely or partially substitute fossil diesel fuel in compression ignition engines(Pasqualinoa et al., 2006).

Biodiesel is among the most promising alternative fuel to reduce world dependence on fossil fuels. It is derived from virgin and waste cooking vegetable oils or animal fats(Balat, 2009). Biodiesel has numerous advantages over normal diesel due to its renewability, biodegradability, low emission profile, nontoxic and high flash point. In addition, biodiesel increases the operational lifetime of the compression engine and reduces the consumption of the engine spare parts due to the high lubricity of biodiesel. Therefore, biodiesel is an alternative fuel with a high potential to compete with petroleum fuel from environmental and economical points of view(Lotero et al., 2005; Guo et al., 2010).

Because every seed oil does not have the potential for biodiesel production, discovering and identifying feed stocks with acceptable properties for use as biodiesel is of research concern to scientists and engineers(Eloka-Eboka., 2010). Other sources of commercial biodiesel include canola oil, animal fat, palm oil, corn oil, waste cooking oil(Van Gerpen etal., 2004) have a limited potential because the sources are edible and are required for human consumption. In recent years, a lot of emphasis has been put on the search for the potential biodiesel feed stocks which can be converted to liquid as well as gas fuels for energy generation.

Various feed stocks have been identified as alternative sources of energy fuels. They range from various kinds of oil-based bio-wastes, energy crops (edible and non-edible oil seeds); and recently various aquatic plants identified as bio-oil sources(Vicente et al., 2007).

Biodiesel sources should be of low production cost and have large-scale production. Certain factors that can influence the quality of biodiesel fuel include the type of raw feedstock used, the production process, and upstream production processes. Fuel quality issues are usually reflected in the contaminants or other minor components of biodiesel(Knothe, 2006).

Biodiesel can be used in its pure forms or as blends with diesel fuels without engine modification but ensures better performance(Bajpai et al., 2006).

The first commercial production of biodiesel took place in Austria in 1988 and since then the Austrian Biofuels Institute has played a pioneering role in establishing the European market for biodiesel. By 1996, production in Europe peaked at 570 MLpa, but in the last few years, it has grown to nearly 2 300 MLpa and is geometrical(Hamilton, et al., 2006).

The International Energy Agency reported in 1998 that 21 countries around the world had implemented biodiesel projects but mostly at relatively small capacities. The North American market has only now just started to grow. In all, world biodiesel production in 2003 has reached 3200 MLpa with the expectation of high growth rates for the future(Koerbitz, et al., 2003). Africa’s contribution to biodiesel production has just begun with research at different developmental stages. At present, there is an emphasis on biofuel/bioethanol production in Nigeria with the federal government’s establishment of its plants and ethanol crop plantations across the country. In fact, within the last few years, the Nigerian government has seen the need to de-emphasize the total dependence on petroleum-based fuels especially with the uneasy calm of the Niger Delta (the major source of petroleum in Nigeria) and focus on alternative and renewable energy for sustainable development(Ajiwe, et al., 2003).

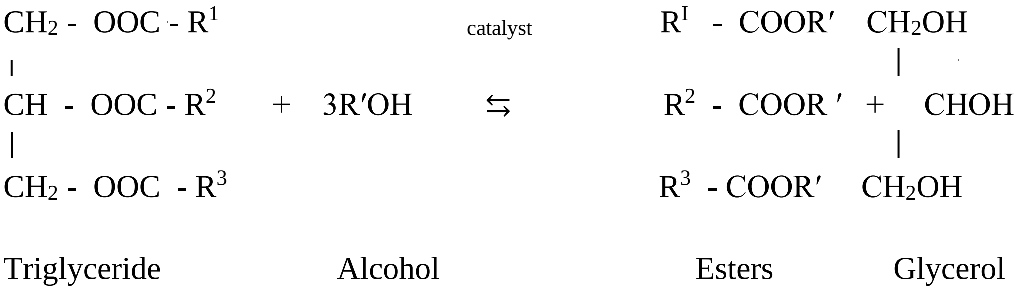
1.2.3 Transesterification reaction

Trans esterification reaction is used to modify one ester to another by changing its alkoxy part(Silva et al., 2011). This process is similar to hydrolysis however, it is performed by using alcohol instead of water. So, it is called alcoholysis(Meher et al., 2006).

The trans esterification reaction mainly depends on the nature of the feedstock, catalyst concentration, the molar ratio alcohol-oil, temperature, agitation rate, acids(Ma, 1999; Canakci, 2001; Dorado, 2002; Mittelbach, 2004;).

The major components of lipids (oil/fats) are triglycerides. Triglycerides are esters of three fatty acids and glycerol. Thus, alcoholysis of lipids produces mono alkyl esters, commonly named biodiesel, and glycerol as a by-product. Fig 1.2 shows the general mechanism of trans esterification of triglycerides, which is a reversible reaction. Thus, an excess amount of alcohol is usually required to shift the equilibrium towards the products side. The stoichiometry of the reaction is 3:1 alcohol to the lipids. However, to get satisfactory yields, the ratio is usually, increased to 6:1. The overall process of trans esterification is a sequence of three consecutive and reversible reactions in which di and monoglycerides are formed as intermediates(Singh and Singh, 2010). Beside, the molar ratio of alcohol to oil, the trans esterification process is affected by other factors such as the type of alcohol, type and amount of the catalyst, reaction time and temperature, mixing intensity and purity of reactants(Meher et al., 2006).

Commonly, trans esterification of lipids is applied using short chain alcohol (C1 to C4). However, methanol is the most common alcohol used because it is cheaper, and the recovery of un-reacted methanol is easier. On the other hand, ethyl esters are more renewable because ethanol is produced from agricultural resources(Mendow et al., 2011). In addition, ethyl esters proved to have lower emissions of green gases and more biodegradability in the aquatic environment(Makareviciene, 2003). However, production of ethyl esters suffers from practical difficulties due to fast saponification and solubility of ethyl esters in glycerol which increases at high oil/methanol molar ratio(Mendow et al., 2011).



$$\begin{array}{lcccll} CH_2 - OOC - R^1 & & & & R^I - COOR' & CH_2OH \\ | & & & & & | \\ CH - OOC - R^2 & + & 3R'OH & \overset{catalyst}{\leftrightarrows} & R^2 - COOR' \; + & CHOH \\ | & & & & & | \\ CH_2 - OOC - R^3 & & & & R^3 - COOR' & CH_2OH \end{array}$$

Figure 1.2 General mechanisms of trans esterification of triglycerides.

1.2.4 Biodiesel blend

Biodiesel can reduce some of the exhaust emissions compared to petroleum-based diesel when burned, whether used in pure form or blended with petroleum diesel. These reductions depend on the type of biofuel and its percentage in blends with petroleum diesel fuel (Ghazali *et al.*, 2015). Furthermore, it does not contribute to a net rise in the level of carbon dioxide in the atmosphere and minimizes the intensity of the greenhouse effect (Rodrigues *et al.*, 2012). Biodiesel is also better than diesel in terms of aromatic content and biodegradability (Kumar and Chauhan, 2013).

Biodiesel can be utilized neat or as a blend with diesel fuel to reduce exhaust gas emissions (Atabani *et al*., 2012). The high viscosity can lead to larger droplet size, poor vaporization and narrow injection spray angle. The fuel density is also an important parameter, as fuel injection systems measures fuel by volume and thus the engine output power is influenced by changes in density due to the different injected fuel masses (Imtenan *et al*., 2014). On the other hand, the energy content of the fuel has a direct influence on the engine power output and the usage of fuel with a lower energy content causes lower engine speed and power (Devaraj *et al*., 2015). Furthermore, many researchers have focused on diesel-biodiesel blends which are considered as a viable fuel at low blending ratios (Moon *et al*., 2010). At present, concern about environmental regulations has been the major reason to look for alternative fuels. The use of blended biodiesel-diesel fuel has presented a promising alternative in the world which is limited to low blending ratio (up to 20%).

1.2.5 Oil extraction methods

There is a variety method used for extraction of plant oil, with each method exhibiting certain advantages as determining the biological and physiochemical properties of the extracted oil. Oils are extracted only from the parts of seeds, fruits, kernels, or germs.

1.2.5.1 Mechanical pressing extraction

The extraction of oil from seeds is accomplished by mechanical pressing which is the best technology with low cost compared to the other methods. Mechanical extraction of the oil is accomplished by exerting sufficient force on confined seed. Under this condition pressure is high enough to rupture the force oil from the seed to escape. Extraction is accomplished by compressing the material in container that has small perforations (Sukhdev *et al*., 2008).

1.2.5.2 Soxhlet extraction

The plant seed which may contain many benefit compounds is extracted by using the Soxhlet extractor. The round-bottom distillation flask initially contained an extraction solvent and it heated up. As the solvent vapour goes up to condenser, it condenses and accumulates inside the extractor (William, 2007).

1.2.6 Physicochemical properties of oil

1.2.6.1 Kinematic viscosity

The viscosity is important for the estimation of optimum storage, handling, and operational conditions. Thus, the accurate determination of viscosity is essential to many product specifications. So, it is mandatory to determine the viscosity of vegetable oil especially when it will used as fuel (Stephen, 2013).

1.2.6.1.2 Density

Density is a fundamental physical property that can be used in conjunction with other properties to characterize both the light and heavy fractions of petroleum and petroleum products. Determination of the density or relative density of petroleum and its products is necessary for the conversion of measured volumes to volumes at 15**C** (Stephen, 2013).

1.2.6.1.3 Acid value

The acid number is used to determine the level of free fatty acids in oil or processing acids that may be present in the biodiesel or diesel fuel oil when produced, or those which form upon aging. Biodiesel blends with a high acid number have been shown to increase fueling system deposits and may increase the likelihood of corrosion (Stephen, 2013).

1.2.6.1.4 Iodine value

Is a degree of unsaturated bonds of fats and oils. If the iodine number is high that is the indicator of more C=C in the oil which will affect the engines performance (Stephen, 2013).

Pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of test.

1.2.6.1.5 Water content

A Knowledge of water content of the oil or any raw materials is important in manufacturing, purchase, or transfer for taking more precaution also it will help to determine the product quality and performance. Presence of water in the engines will causes corrosion for the internal parts (Stephen, 2013).

1.2.6.1.6 Free fatty acids

This is the most important test of crude oil which will be used as a raw material for biodiesel production, and regarding the result of Free Fatty Acid we can decide either we will go for one step production or two step production. If the FFA is more than 2% we have to reduce the FFA then we can go for the transesterification process (Stephen, 2013).

1.2.6.1.7 Flash point

The lowest temperature corrected to a barometric pressure of 101.3 kPa (760 mm Hg), at which application of an ignition source causes the vapors of a specimen of the biodiesel and blends sample to ignite under specified conditions of test. This test is important parameter for transporting, shipping and storing to provide the perfect condition for the cargo (Stephen, 2013).

1.2.6.1.8 Saponification value

It expresses the amount of base that will react with 1 g of sample, it gives an estimate of the amount of acid present in the oil sample, high saponification value indicates high acid numbers and high possibility of soap formation (Stephen, 2013).

1.2.6.2 Physicochemical properties of biodiesel and blends

1.2.6.2.1 Cloud point and pour point

Those are basic tests for fuels in winter and cold countries that can keep the engine running in cold weather (Stephen, 2013).

Cloud point is the temperature of a liquid sample when the smallest observable cluster of wax crystals first appears upon cooling under prescribed conditions.

Pour points is the lowest temperature at which movement of the test specimen is observe under prescribed conditions of test.

1.2.6.2.2 Copper strip corrosion

The copper strip corrosion test is designed to assess the relative degree of corrosivity of biodiesel and blends in petroleum products(Stephen, 2013).

1.2.6.2.3 Fatty acids profile of biodiesel

All fuel properties of biodiesel are determined by the structure of, the individual, fatty acids that comprise the oil. Chain length, degree of unsaturation and branching of the chain are the structural features that influence the physical properties of the fatty esters(Knothe, 2005b). The fatty acid composition of the feedstock is the main factor that determines the biodiesel quality, as trans esterification reaction does not alter the fatty acid composition of the feedstock(Moser et al., 2012). The important properties of biodiesel that are, directly, influenced by the fatty acid ester composition are kinematic viscosity, energy content, cold flow properties, oxidative stability cetane number and exhaust emissions(knothe et al., 2005b; Ramose et al., 2009).

1.2.6.2.4 Fourier-transform infrared spectroscopy

FT-IR is being employed as a modern analytical technique for detecting the conversion of biodiesel because it is a fast and easy detection method. Raw oils and the methyl esters are noted as fairly strong absorbers in the infrared region of the electromagnetic spectrum (400–4000 cm^{-1}). The wavelength of the carbonyl peak is a sign at (~ 1744 cm^{-1}) to monitor the progress of the reaction. (Lin-vien *et al.*,1991)studied this method to be effective in a known system but noted its limitation because it is not specific for the end product and there could be a number of interferences. This method is however, being used because spectral resolution is not needed for more specific analysis. They pointed out that the peak typical of the methyl ester (OCH3) at 1436 cm-1 is very narrow and moves along the raw oil peak. These characteristics were noted to make the peak unattractive for monitoring the progress of the biodiesel reaction(Lin-vien *et al.*, 1991). However, they observed the peak measurement to give a direct indication of the attachment of the alkyl group of the alcohol with the fatty acids of the triglycerides but the peak is not influenced by the alkyl group (-CH3) (Lewis *et al.*, 2004)

Ivanoiu and others (2011) performed a comparative study on biodiesel synthesis from different vegetable oils and used infrared spectroscopy using KBr plates in the range of 4000-400 cm^{-1}.

The ester carbonyl group stretching vibration at 1740 cm-1 is shown by strong bands, esteric –COC vibration at 1171 and 1207 cm-1 reveals medium intensity bands, and the presence of the (CH2) n group vibration band is seen at 724 cm-1. They observed the absence of a broad band at the 2500-3300 cm-1 region which confirms the low moisture and free fatty acid content of the sample(Ivanoiu *et al*., 2011).

1.2.6.2.5 Gas chromatography

Gas chromatography is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of (GC) include testing the purity of a particular substance or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to isolate pure compounds(Robert and Eugene, 2004; Pavia *et al*., 2006).

In gas chromatography, the mobile phase (or "moving phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (a homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a gas chromatograph (or "aerograph", "gas separator").

The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness(Pavia *et al*., 2006).

Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences. Firstly, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, whereas in column chromatography the stationary phase is a solid and the mobile phase is a liquid. (Hence the full name of the procedure is "Gas–liquid chromatography", referring to the mobile and stationary phases, respectively.) Secondly, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) has no such temperature control. Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas(Pavia *et al*., 2006).

Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure) differences. However, fractional distillation is typically used to separate components of a mixture on a large scale, whereas GC can be used on a much smaller scale (i.e. micro scale) (Pavia *et al*., 2006).

Gas chromatography is also sometimes known as vapor-phase chromatography (VPC), or gas–liquid partition chromatography (GLPC). These alternative names, as well as their respective abbreviations, are frequently used in scientific literature. Strictly speaking, GLPC is the most correct terminology, and is thus preferred by many authors (Robert and Eugene, 2004).

1.2.7 Objective of the study

1.2.7.1 The general objective of this study is

to produce an environmentally friendly biodiesel from *Moringa oleifera* seeds oil.

1.2.7.2 Specific objectives

- To extract *Moringa oleifera* oil using the technique which ensures a high yield.
- To study the chemical compositions of *Moringa oleifera* seeds.
- To convert oil to biodiesel by transesterification reaction.
- To blend the produced biodiesel with conventional diesel and ethanol in different portions.
- To determine the physiochemical properties of diesel-biodiesel and diesel-biodiesel-ethanol blends using ASTM standard.

Chapter Two

Materials and methods

2.1 Materials

Dried seeds of *Moringa oleifera* were collected (December,2017), from National Seed Center – Forest Research, Khartoum, Sudan. The seeds were cleaned to remove damaged seeds, wood, leaves, stones, dust, and any other foreign materials Cleaned seeds were stored in plastic bags.

2.2 Methods

2.2.1 Chemical extraction (Soxhlet) method

n-Hexane was used to extract oil from 1000 g of *Moringa oleifera* seeds at 60°C for 8 hours. The remaining solvent was removed by rotary evaporation.

2.2.2 Proximate analysis of *Moringa Oleifera* seeds

The moisture content, ash, crude fiber, crude protein, and fat contents were analyzed by AOAC methods

2.2.2.1 Determination of moisture content

2 grams of sample were placed into a pre-dried aluminum dish, covered with a lid and placed in a temperature-controlled oven at 103**C** ± 2 overnight (about 8 hours). The moisture content was calculated as percentage of the original weight of the sample using the formula below (AOAC, 2008):

Moisture content (%) = $\frac{W1 - W2}{W3} \times 100$

Where: W1 = weight of dish + lid, W2 = weight of dish +lid + sample, and W3 = weight of dish +lid + sample after drying.

2.2.2.2 Determination of ash content

Ash content was measured by weighting two grams and placed in muffle furnace at 600ºC for 6hours. The sample was transferred to cooling room and then weighted and calculated using the formula below (AOAC, 2008):

Ash content (%) = $\frac{W1 - W2}{Weight\ of\ sample}$ x 100

Where: W1= weight of crucible with ash, W2= weight of empty crucible.

2.2.2.3 Determination of crude fiber

Crude fiber was measured by weighting two grams of fat free meals was treated successively with a boiling solution of H_2SO_4 and KOH. The residue was separated by filtration, washed, dried, weighed and ashed at 500ºC. The loss of the weight resulting from ashing corresponded the crude fiber in the sample (AOAC, 2008).

2.2.2.4 Determination of protein content

Protein content was determined by using 0.2 g of defatted sample and weighed accurately into a micro-kjeldahl flask, 0.4g of catalyst mixture and 3.5 ml of concentrated sulphuric acid were added, the flask was then placed into the kjeldahl digestion unit for about 2 hours until a colorless digest solution was obtained. The flask was left to cool to room temperature. 20 ml of 40% sodium hydroxide solution were added to the digested solution and the mixture was heated.

The ammonia evolved was trapped into 10 ml of 2% boric acid solution, then titrated against 0.02 N hydrochloric acid using universal indicator. The total nitrogen and protein were calculated using the following formula (AOAC, 2008):

Crude nitrogen (%) = $\frac{Volume\ of\ HCl\ x\ N\ x\ 14\ x\ 100}{Weight\ of\ sample\ x\ 1000}$

Crude nitrogen (%) = Nitrogen (%) x 6.25

Where: Nitrogen (%) = crude nitrogen, Protein (%) = crude protein, and N = normality of HCl.

14 = the equivalent weight of nitrogen.

2.2.2.5 Determination of fat content

2 grams of seeds which were extracted with hexane for 8hours using Soxhlet apparatus. The solvent was evaporated, and the remaining crude fat was determined using the following formula (AOAC, 2008):

Fat (%) = $\frac{W2 - W1}{Weight\ of\ sample} \times 100$

Where: W1 = weight of empty flask, W2 = weight of flask with oil.

2.2.3 Physicochemical characteristics of *Moringa oleifera* oil, biodiesel, and blends.

(American Society for Testing and Materials) ASTM and Standard methods for the analysis of oils, fats, and derivatives.

2.2.3.1 Oil yield percentage

The yield percentage of seeds was determined according to AOAC (2008).

Five grams of finely ground seeds samples were accurately weighted in an empty thimble and was then plugged with a piece of cotton, n. hexane was poured into a pre-weighed round bottom flask. The Soxhlet apparatus was assembled and the sample was extracted for 8 hours on a steam bath. After, carefully, dismantling the apparatus. The solvent was evaporated to dryness using a rotary evaporator.

The oil content was calculated as percentage according to the following equation.

$$\text{Oil content \%} = \frac{weight\ of\ oil\ extracted}{weight\ of\ sample} \times 100$$

2.2.3.2 Kinematic viscosity at 40C

The time was measured for a fixed volume of liquid to flow under gravity through the capillary of a calibrated viscometer under a reproducible driving head and at a closely controlled and known temperature. The kinematic viscosity was the product of the measured flow time and the calibration constant of the viscometer (ASTM, 2004).

2.2.3.3 Density at 15C

A small volume (approximately 0.7 ml) of liquid sample was introduced into an oscillating sample tube and the change in oscillating frequency caused by the change in the mass of the tube was used in conjunction with calibration data to determine the density of the sample (ASTM, 2002).

2.2.3.4 pH value

2 g of the sample was placed In a dry clean 25 mls flask followed by 13 mls of hot distilled water and the mixture was stirred slowly. The mixture was then cooled in a cold-water bath to 25C. The pHmeter electrodes was standardized with buffer solution (pH 4 and 7) and the electrodes immersed into the sample and an average pH of three readings per sample were recorded (Isah, 2006).

2.2.3.5 Refractive index

Refractive index was determined by Abbe-60 refractometer as described by (AOAC, 2008) where a double prism was opened by means of screw head and few drops of sample were placed on the prism. The prism was closed firmly by lighting screw head. The instrument was left to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument. The prism was cleaned between readings wiping off oil with soft cloth, then with cotton moistened with petroleum ether and left to dry. The refractive indices of all samples were determined at 39-40 C.

2.2.3.6 Acid value

The sample was dissolved in a mixture of toluene and propan-2-ol containing a small amount of water and titrated potentiometrically with alcoholic potassium hydroxide using a glass indicating electrode and a reference electrode or a combination electrode. The meter readings were plotted manually or automatically against the respective volumes of titrating solution and the end points are taken only at well-defined inflections in the resulting curve. When no definite inflections were obtained and for used oils, end points were taken at meter readings corresponding to those found for aqueous acidic and basic buffer solutions (ASTM- D 664–04, 2004).

2.2.3.7 Iodine value

0.2 grams of oil were accurately weighed and placed in a dry clean flask and dissolved in10 mls of chloroform. 25 mls of pyridine sulphate dibromide were added and finally 20 mls of KI (0.1N) were added. The flask was then stoppered and the mixture was allowed to stand for 10 minutes in the dark. The stopper and the side of the flask were rinsed with enough amount of distilled water, the contents of the flask were then shaken and titrated against 0.1N sodium thiosulphate solution using starch as an indicator. A blank determination was carried out simultaneously (Paquot and Hautfenne, 1987).

$$\text{Iodine value} = \frac{(b-a)\ x\ 0.01269\ x\ 100}{\text{S}}$$

Where;

b: Volume (ml) of sodium thiosulphate in blank solution

a: Volume (ml) of sodium thiosulphate in test active solution

S: Weight (gm) of the oil sample

0.01269: Iodine factor.

2.2.3.8 Free fatty acids content

A Perkin Elmer model Clarus 600 T combined with single quadrupole mass spectrometer was used for GC-MS analysis. The chromatographic column was an

Elite 5-MS column (30 m × 0.25 mm × 0.25 µm film thickness), with high-purity helium as the gas carrier, at a flow rate of 1 mL/min. The injector temperature was 280°C and it was equipped with a split less injector at 20:1. The temperature was set initially to 40°C (held for 2 min), was increased to 150°C at 5° C min−1 (held for 2 min), then increased further to 300°C at 5°C min−1(held for 5 min). The MS ion source temperature was 200°C and inlet line temperature at 220 °C. The scan mass range was wet at 40 to 618 in scan mode and the electron energy 70 eV electron energy. The solvent delay of 4 min. The peaks of the compounds were identified by comparing the spectra with that of the NIST 2005 (National Institute of Standard and Technology library) and Wiley 2006 library. The total time required for analyzing a single sample was 61 minutes.

2.2.3.9 Peroxide value

One gram of oil was accurately weighed into 250 ml conical flask. Thirty mls of a mixture of glacial acetic acid and chloroform (3:2) were added and the solution was swirled gently to dissolve the oil. 0.5 ml of 0.1N KI was added, and then the contents of the flask were left to stand for one minute before adding 30 mls of distilled water. After a while, the contents were titrated with 0.01N sodium thiosulphate until the yellow color almost disappeared. A 0.5 ml of 1% starch solution was added, and the titration continued with vigorous shaking until the blue color completely disappeared. The number of ml of 0.01N sodium thiosulphate required (a) were recorded. The same process was repeated for blanks. The number of ml of 0.01N sodium thiosulphate required by the blank (B) was recorded(Paquot and Hautfenne, 1987).

Peroxide value of the oil = $\frac{(b-a)\ x\ N\ x\ 100}{S}$

Where;

b: Reading of blank (ml).

a: Reading of oil (ml).

S: Original weight of oil sample (gm).

2.2.3.10 Saponification value

The sample was melted and filtered through filter paper to remove any impurities and last traces of moisture. The dry sample weighed about 1.5 to 2g into 250 ml Erlenmeyer flask. A 25 mls of the alcoholic potassium hydroxide solution were pipetted into the flask. The sample flask and blank flask were connected with air condenser, placed in a water bath to boiling within one hour. 10 mls of hot ethyl alcohol, neutral to phenolphthalein, were washed down the inside of the condenser when the flask and condenser have cooled.

The excess of potassium hydroxide was titrated with hydrochloride acid using 1.0 ml of phenolphthalein as indicator (Paquot and Hautfenne, 1987).

Saponification value = $(56.1 – (B – S)N)/W$

Where;

B = Volume in ml of standard hydrochloric acid required for the blank.

S = Volume in ml of standard hydrochloric acid required for the sample.

N = Normality of standard hydrochloric acid.

W = Weight in gm of the oil/fat taken for the test.

W = Weight in gm of the oil/fat taken for the test.

2.2.3.11 Determination of distillation temperature

The distillation test is used to determine the boiling range characteristics of a hydrocarbon sample. A simple batch distillation process was conducted on a sample of the fuel and the initial boiling point was recorded as the distillation temperature (ASTMD6751).

2.2.3.12 The free fatty acids treatment

2% concentrated Sulphuric acid in 100 ml of methanol were added to 500 ml of hot crude *Moringa Oleifera* oil. The mixture was kept at 55°C for two hours with continuous stirring at 3000 rpm. The mixture was transferred to a separating funnel and allowed to settle for 24 hours. The lower layer of glycerol, sulfuric acid and unreacted methanol was separated from the upper layer of treated oil. The oil was washed three times with warm distilled water at 40C to remove impurities and soap (Kombe et al., 2007).

2.2.3.13 Transesterification reaction

500 ml of oil were placed into a 1 L beaker and heated up to 60**C**, 100 ml of fresh methanolic potassium hydroxide were added under stirring at 3000 rpm for two hours. The mixture was transferred to a separating funnel and kept for 24 hours. Then the lower, glycerol layer, was drained and the upper biodiesel layer was washed, three times, with warm distilled water to remove soap, methanol and remaining glycerol (Okullo *et al.*, 2006).

2.2.3.14 Identification of *Moringa Oleifera* methyl ester

The conversion of Moringa Oleifera oils to biodiesel was investigated using Fourier transform infrared spectrophotometer SHIMADZU, IR Tracer-100 (206-30199-18) (JAPAN).

The small drop of the oil was placed on one the KBr plates. The second was placed on top and make a quarter turn on obtain in a nice even film. The plates were placed into the sample holder, equipped with a ZnSe crystal attenuated total reflectance (ATR) cell was used to obtain the IR spectra in the mid region 400 to 700 cm-1 with 24 scans run a spectrum(Mahamuni et al., 2017).

Chapter Three

Results and Discussion

3.1 Proximate composition of *Moringa oleifera* seeds

Table (3.1) shows the proximate compositions of *Moringa oleifera* seeds.
The seeds contained 7.0, 6.3, 38.3, 7.8, 31.6, and 19.9 % of moisture, ash, fat, fiber, protein, and carbohydrates respectively.

The moisture content 7.0 % was higher to that obtained by Salah (2006).

Moisture content is variable even in the same variety depending upon the locality and other environmental factors. Low moisture content is a good indicator for long shelf life which is very useful in storage of food.

Ash content was 6.3 %, was more than3.6% reported by Salah (2006).

The fiber content of *Moringa oleifera* seeds was 7.8 %. It was more than reported by Salah (2006)was 1.2%.

The protein content was 31.6 % was less than those reported by Salah (2006)47.2%.

The carbohydrate content was 19.9 %. It is more than that reported by Salah (2006).

The variation in values may be due to method of analysis and species and climatic conditions. However, the results showed that seeds could serve as an animal feed nutrient because of its relatively high carbohydrate, fat, protein content and good source of energy.

Table (3.1): Proximate composition of *Moringa Oleifera* seeds

Constituent	**Percentage %**
Moisture content	7.0 %
Ash content	6.3%
Fat	38.3%
Fiber	7.8%
Protein	31.6%
Carbohydrate	19.9%

3.2 Physicochemical characterization of *Moringa oleifera* seeds oil

Table (3.2) shows the oil content and physicochemical characterization of *Moringa oleifera*. The oil extract has a yellow color.

The oil content of *Moringa oleifera* seed was 38.0 %, it was less than that reported by Salah (2006). Free fatty acids were 9.22% which is more than 1.128% reported by Salah (2006).

Kinematic viscosity at 40**C** was found to be 35.75% which is less than those reported by Salah (2006)and David *et al*., (2015),they reported Kinematic viscosity of 45.82%, 44.5% respectively.

Acid value of *Moringa oleifera* seeds was 9.89mg KOH g^{-1} less than 13.2 mg KOH g^{-1} reported by David *et al*., (2015).

Peroxide value was found 6.00 mEq kg^{-1} which was less than that reported by David *et al.*, (2015)and Salah (2006)which were 10.4 ,9.0 mEq kg^{-1} respectively.

saponification value was 210.13 mg/KOH/g which is higher that reported by David *et al.*, (2015) and Salah (2006)which were 179, 182 mg/KOH/g respectively.

Iodine value of *Moringa oleifera* seeds was 66.5 mg g^{-1}less than 88 mg g^{-1}reported by Salah (2006).

Refractive Index was 1.479 which is similar to that 1.464 reported by Salah (2006).

pH was 5.42 less than 5.96 reported by Orhevba *et al.*, (2013).

Density at 15C was 0.90 Kg/m^3 more than 0.81Kg/m^3reported by Adedunni (2016).

Flash point was158.0°C less than 162°C reported by Azad *et al.*, (2015).

Table (3.2): Physicochemical characterization of the extracted oil.

Parameter	**Value**
Oil yield	38.0 %
pH	5.42
Kinematic viscosity at 40 °C	35.75
Refractive index	1.4791
Density at 15C	0.9022
Flash point	158.0
Acid value KOH/g	9.89
Free fatty acid %	9.22
Peroxide value mEq/Kg	6.00
Saponification value mg KOH/g oil	210.13
Iodine value I_2 /100g oil	66.5

3.3 Fatty acid composition of *Moringa oleifera* seed oil

Table (3.3) shows the ester content of *Moringa Oleifera* biodiesel. The total ester content was found to be 21 compounds in the sample and 100 % of total components which indicate that the triglycerides were successfully converted to methyl ester to produce high purity biodiesel, however, the identified chemical compounds contain 82.2% probability and 65% area in methyl ester sample, while fatty acid determined in methyl ester sample.

Moringa oleifera biodiesel presented 22.3% of Hexadecanoic fatty acid ester (methyl ester) as the major constituent, followed by Docosanoic acid methyl ester (19.180) and 10-Octadecenoic acid methyl ester (17.23). *Moringa oleifera* biodiesel mainly contains of Oliec, Palmitic, and stearic acid methyl esters with percentages of 81.6, 5.2 and 4.0 respectively as reported by David *et al*., (2015).

Table (3.3): The free fatty acid composition of *Moringa oleifera* biodiesel (GC-MS)

S.N	**Fatty Acid**	**Area %**
01	Hexadecanoic acid methyl ester	22.300
02	Docosanoic acid methyl ester	19.180
03	10-Octadecenoic acid methyl ester	17.230
04	Octadecanoic acid methyl ester	15.090
05	Eicosanoic acid methyl ester	14.590
06	Tetracosanoic acid methyl ester	3.880
07	9-Octadecenoic acid (Z) methyl ester	3.490
08	9-Hexadecenoic acid methyl ester	1.880
09	Octadecanoic acid methyl ester	0.560
10	Heptadecanoic acid methyl ester	0.340
11	9-Octadecenal	0.290
12	Tricosanoic acid methyl ester	0.230
13	Ricinoleic acid methyl ester	0.230
14	Triacontanoic acid methyl ester	0.190
15	13-Docosenoic acid methyl ester	0.170
16	Di-(Octadecenoyl)-Glycerol	0.090
17	Cyclopropaneoctanoic acid	0.080
18	2-Methylene-1,5-pentanediol	0.070
19	(E)-9-Octadecenoic acid methyl ester	0.040
20	10-Nonadecenoic acidmethyl ester	0.040
21	Stigmasterol	0.030
Total		100

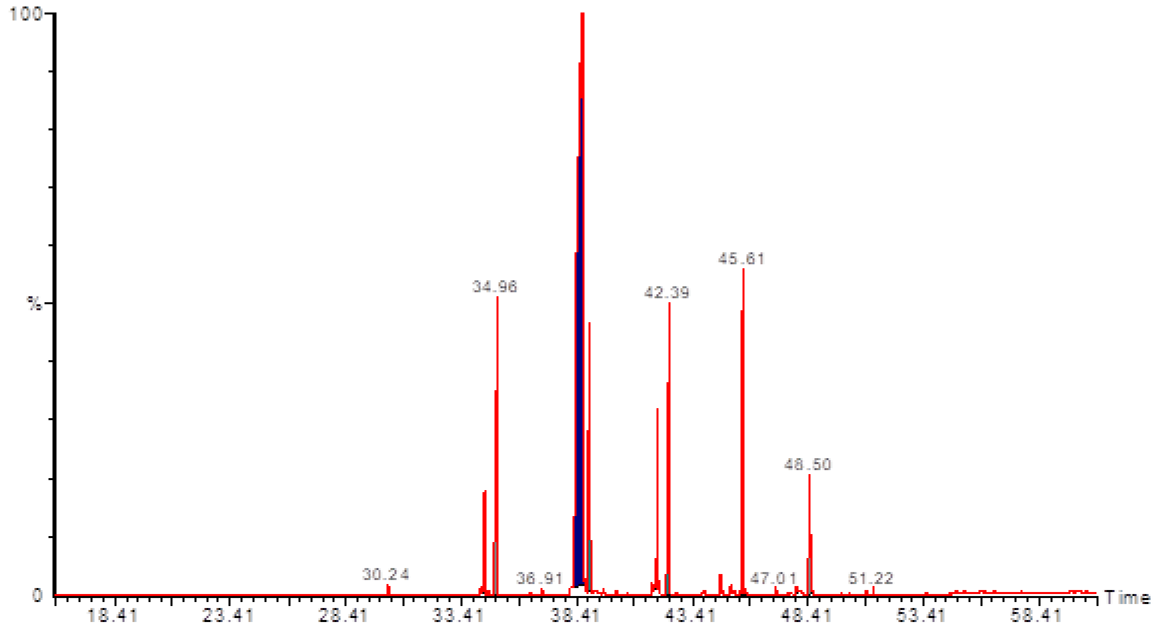


Figure (3.1): GC-MS Spectrum of *Moringa oleifera* seeds oil.

3.4 FTIR spectroscopy characterization of *Moringa oleifera* Biodiesel

Fig 3.2 and 3.3 show the FTIR of *Moringa oleifera* oil and Biodiesel. The spectrums of *Moringa oleifera* oil shows absorbance peaks at, 2924.09 cm^{-1}, 2854.65 cm^{-1} which are similar to that obtained in biodiesel absorbance peaks 2923.88 cm^{-1}, 2852.52 cm^{-1} due to C-H stretching. *Moringa oleifera* oil absorbance at 1747.51 cm^{-1} represent of C=O Carbonyl group of esters was similar to that absorbance obtained in *Moringa oleifera* biodiesel 1745.46 cm^{-1}.

Furthermore, peaks at 1377.17 cm^{-1} and 1377.08 cm^{-1} can be attributed to the glycerol group O–CH2 (mono-, di- and triglycerides) of *Moringa oleifera* oil and *Moringa oleifera* biodiesel respectively. Also, absorbance at 1165.00 cm^{-1} and

1163.00 cm^{-1} of *Moringa oleifera* oil and *Moringa oleifera* biodiesel respectively, due to stretching of O–CH3 which indicates the conversion of oil to methyl ester due to C-O of esters in triglycerides. The range 1500-900 cm^{-1} known as “fingerprint” region. The peak at 1465.80 cm-1 correspond to the asymmetric stretching of –CH3 present in the biodiesel spectrum and should be absent in the refined oil spectrum, *Moringa oleifera* oil and *Moringa oleifera* biodiesel showed peaks at 721.36 cm^{-1} and 721.33 cm^{-1} respectively which represent aromatic C-H (Rabelo et al., 2015).

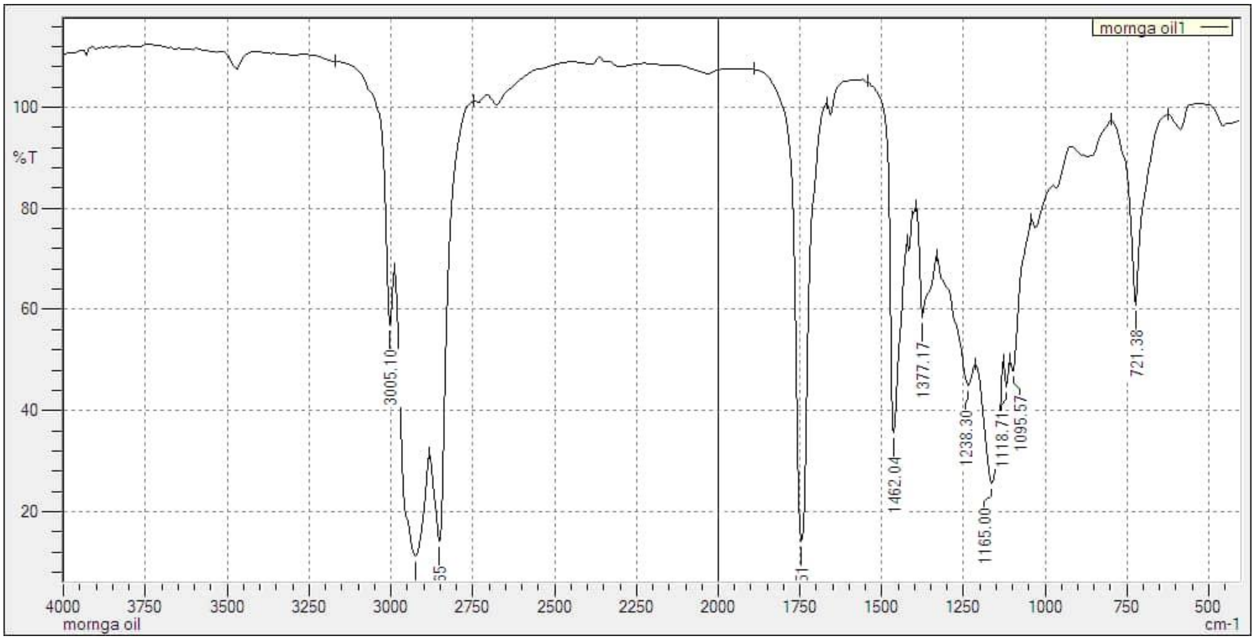


Figure (3.2): FTIR spectrum of *Moringa oleifera* oil.

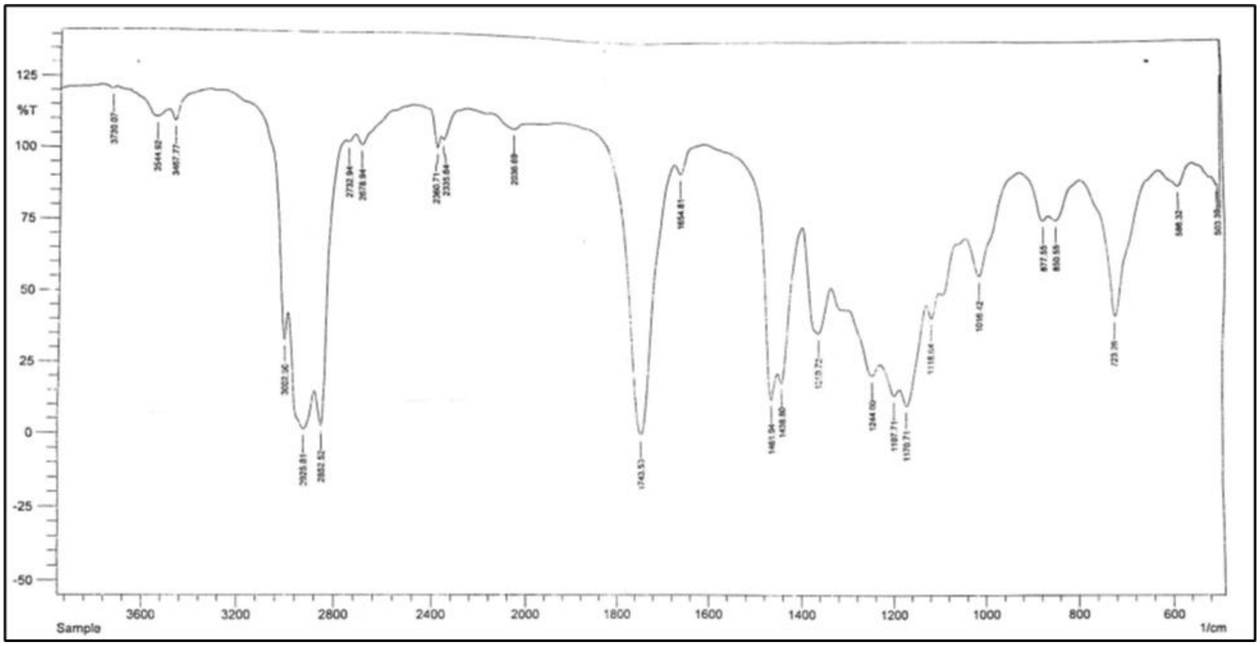


Figure (3.3): FTIR spectrum of *Moringa oleifera* Biodiesel.

3.5 The physicochemical properties of fossil diesel

Table (3.4) shows the physical and chemical properties of Fossil Diesel in comparison with the limits of standard method ASTM D 7467. The results show that all parameters of Fossil diesel within the limit of ASTM D 7467.

The Kinematic viscosity of Diesel was obtained at 3.589 cSt was higher than that determined by Mofijur *et al*., (2015) which were 3.23cSt.

Density at 15 °C was 852 Kg/m3 higher than 827.2 Kg/m3 determined by Mofijur *et al*., (2015).

Flash point was 81.00 °C higher than 68.5, 42°C determined by Muralidharan *et al*., (2011), Mofijur *et al*., (2015).

Cloud point was obtained at+3 °C more than +8 °C reported by Mofijur *et al*., (2015).

Density at 15 °C was obtained 0.852 Kg/cm^{3}which was higher comparable to that determined by Muralidharan *et al*., (2011) which was 0.835 Kg/cm^3.

The distillation temperature at three different rates of recovery, were evaluated using ASTM methods, at 10% recovered was 227.6°C higher than 195°C, and at 50% recovered was 290°C higher than 267°C, and at 90% recovered was 324.8°C lower than 340°C determined by Constantinos *et al*., (2013).

Sulfur Content was obtained 0.012% lower than 0.048% reported by Qi *et al*., (2014).

Table (3.4): The physicochemical properties of Diesel.

Test Name	ASTM D 7467	Fossil Diesel
Kinematic Viscosity at 40 °C, cSt	1.9 – 4.1	3.589
Density at 15 °C, Kg/cm^3	_____	0.852
Specific Gravity	_____	0.8234
Flash Point, °C	Min. 93	81.00
Distillation Temperature		
10% recovered	Max. 343	227.6
20% recovered		247.7
50% recovered		290.9
90% recovered		324.8
95% recovered		340.4
Recovery at 365^{0}C		94.5
Color, ASTM	____	L1.0 ASTM color
Cloud Point, °C	Report	+3
Copper Strip Corrosion (3 Hours @ 50ºC), Rating	Max. 3	1 A
Sulfur Content, % mass	Max. 0.05	0.012
Water Content, wt%	Max. 0.05	<0.03

3.6 The physicochemical properties of *Moringa oleifera* biodiesel and blends

The data presented in Table (3.5) shows the physicochemical characterization of *Moringa oleifera* biodiesel and blends in comparison with ASTM D 7467.

The density of Biodiesel, B20 (biodiesel 20%, diesel 80%), B20E20D60 (Biodiesel 20%, ethanol 20%, diesel 60%) was obtained at 0.8805, 0.8581 and 0.8454 g/cm3 respectively which agree with the studies reported by Buhari *et al*., (2014) and Muralidharan *et al*., (2011) which were 0.86 and 0.89g/cm^3 respectively.

The Kinematic viscosity of Biodiesel, B20 and B20E20D60 were obtained at 12.53, 3.175 and 2.924 cSt respectively.

The Kinematic viscosity value of Biodiesel was higher than that determined by Buhari *et al*., (2014) and Muralidharan *et al*., (2011) which were 0.859 ± 0.0349 and 2.72 cSt respectively.

The cloud point of Biodiesel, B20 and B20E20 was obtained at -7.5, 5 and 9C respectively. The cloud point value was lower than reported by Buhari *et al*., (2014) who was reported that pour point was 10C.

The flash point of Biodiesel, B20 and, B20E20D60 obtained was at 137, 87.00 and 15C respectively which was lower comparable to that determined by Buhari *et al*., (2014) which was 170C.

Sulfur Content of Biodiesel, B20 and, B20E20D60 was obtained 0.025,0.015, and 0.018%respectivelyhigher than 0.014% determined by Qi *et al*., (2014).

Water content is an important parameter, and it is within the limit in Biodiesel blends D80B20, D60E20B20.

Table (3.5): The physicochemical properties of *Moringa Oleifera* biodiesel blends.

Test Name	**ASTM D 7467**	**Biodiesel**	**D80 B20**	**D60 E20 B20**
Kinematic Viscosity at 40 °C, cSt	1.9 – 4.1	12.53	3.175	2.924
Density at 15 °C, g/cm³	_____	0.8805	0.8581	0.8454
Specific Gravity	_____	____	0.8589	0.8462
Flash Point, °C	Min. 52	137	87.00	15
Water Content, Wt%	Max. 0.05	< 0.05	< 0.05	< 0.05
Color, ASTM	____	L 1.5 ASTM color	1.0 ASTM color	L1.0 ASTM color
Cloud Point, °C	REPORT	-7.5	+5	+9
Copper Strip Corrosion (3 Hours @ 100°C), Rating	Max. 3	1A	1A	1A
Sulfur Content, %mass	Max. 0.05	0.025	0.015	0.018

Conclusion

1.The chemical composition of *Moringa oleifera* indicated clearly that, like other oil-bearing seeds, the seeds of the plant contain appreciable amount of oil (38.0%). In addition, appreciable amount of protein (31.6%), was also found, and therefore residue resulting after the separating of the oil from the solid matter normally associated with it could be used for agricultural purposes either for stock feeding or as fertilizer. The seed cake can be used for water purification.

2.The fossil diesel, Biodiesel-Diesel and Biodiesel-Diesel-Ethanol all parameters were within the limit of ASTM D 7467.

3.*Moringa oleifera* biodiesel was produced Successfully and prove that the triglycerides were converted to methyl ester, synthesized using Alkali transesterification and proven using FTIR.

4. High yield of oil was gained from Chemical extraction using Soxhlet system.

Moringa oleifera oil contains 82.2 % of saturated fatty acids and 17.8% of unsaturated fatty acid.

5. The Physical and Chemical properties of *Moringa oleifera* oil show high Kinematic Viscosity (35.75) and low Acid Value (9.89).

Recommendations

- Research on the field of cultivation and propagation of *Moringa Oleifera* is strongly recommended to increase the yield of the oil and other products.
- Solvent method is recommended for the extraction of the oil from *Moringa oleifera* seeds.
- *Moringa oleifera* is recommended for commercial production of oil, as it gave high oil content and good physical and chemical characteristics. i.e., high-grade oil.
- Further research may be required to strengthen the finding of the present investigation, such as establishment of the fatty acids profile, effect of storage, frying quality and stability of the oil and packaging.
- *Moringa oleifera* tree could be considered as a promising plant naturally growing in Sudan. Besides its high oil content, moringa contained substantial amount of protein 31.6%, *Moringa oleifera* is recommended to be used for many agricultural purposes (animal feed).

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