Introduction and literature Review

1.1 Introduction

Biodiesel is an alternative to petroleum-based fuels derived from vegetable oils, animal fats, and used waste cooking oil including triglycerides. Since the petroleum crises in 1970s, the rapidly increasing prices and uncertainties concerning petroleum availability, a growing concern of the environment and the effect of greenhouse gases during the last decades, has revived more and more interests in the use of vegetable oils as a substitute of fossil fuel [1].

The production and use of biodiesel creates 78% less carbon dioxide emissions than conventional diesel fuel. Carbon dioxide is a greenhouse gas that contributes to global warming by preventing some of the sun's radiation from escaping the Earth. Burning biodiesel fuel also effectively eliminates sulfur oxide and sulfate emissions, which are major contributors to acid rain. That's because, unlike petroleum-based diesel fuel, biodiesel is free of sulfur impurities. Combustion of biodiesel additionally provides a 56% reduction in hydrocarbon emissions and yield significant reductions in carbon monoxide and soot particles compared to petroleum-based diesel fuel. Also, biodiesel can reduce the carcinogenic properties of diesel fuel by 94% [2].

Biodiesel in its pure form is known as "neat biodiesel" or B100, but it can also be blended with conventional diesel, most commonly as B5 (5 percent biodiesel and 95 percent diesel) and B20 (20 percent biodiesel and 80 percent diesel). Biodiesel is registered with the U.S. Environment Protection Agency (EPA) and is legal for use at any blend level in both highway and non road diesel vehicles.

Most diesel engines can run on biodiesel without needing any special equipment. If it is interesting to use biodiesel in a vehicle or equipment, manufacturer recommendations and information regarding engine warranties must be consulted. In addition, once proper blend the vehicle has been determined, fuel must be purchase from a reputable dealer selling commercial grade biodiesel [3].

1.2 Advantages

1. B100can be produced from renewable, domestic resources.
2. B100 is energy efficient. (The total fossil fuel energy efficiency of biodiesel is 320% vs. 83% for petroleum diesel) (National biodiesel board, 1998)
3. B100 can be used directly in most diesel engine applications.
4. B100 can reduce global warming and tailpipe emissions (-41%) (Hill, Nelson, Tilman, Polasky, &Tiffany, 2006).
5. B100 is nontoxic and biodegradable.

6. B100 is a good solvent and may clean out fuel line and tank sediments. (Note that this may result in fuel filter clogging during initial use.)

1.3 Limitations

1. B100 contains approximately 8% less energy per gallon.
2. B100 generally has a higher cloud and pour point (will freeze at a higher temp) than conventional diesel.
3. B100 is not compatible with some hose and gasket materials, which may cause them to soften, degrade, and rupture.
4. B100 is not compatible with some metals and plastics.
5. B100 may increase nitrogen oxide emission.

The most common method used to overcome the limitations of B100 is called “blending”. Here biodiesel is mixed with petroleum diesel in varying proportion starting from 5% and reaching 20% mixture [4].

1.4 Making Biodiesel: Transesterification

Transesterification of natural glycerides with methanol to methylesters is a technically important reaction that has been used extensively in the soap and detergent manufacturing industry worldwide for many years. Almost all biodiesel is produced in a similar chemical process using base catalyzed transesterification as it is the most economical process, requiring only low temperatures and pressures while producing a 98% conversion yield. The transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. A triglyceride has a glycerine molecule as its base with three long chain fatty acids attached. The characteristics of the fat are determined by the nature of the fatty acids attached to the glycerine. The nature of the fatty acids can, in turn, affect the characteristics of the biodiesel.

During the esterificiation process, the triglyceride is reacted with alcohol in the presence of a catalyst, usually a strong alkaline like sodium hydroxide. The alcohol reacts with the fatty acids to form the mono-alkyl ester, or biodiesel, and crude glycerol. In most production, methanol or ethanol is the alcohol used (methanol produces methyl esters, ethanol produces ethyl esters) and is base catalyzed by either potassium or sodium hydroxide. Potassium hydroxide has been found more suitable for the ethyl ester biodiesel production, but either base can be used for methyl ester production.

The figure below shows the chemical process for methyl ester biodiesel. The reaction between the fat or oil and the alcohol is a reversible reaction, so the alcohol must be added in excess to drive the reaction towards the right and ensure complete conversion [5].

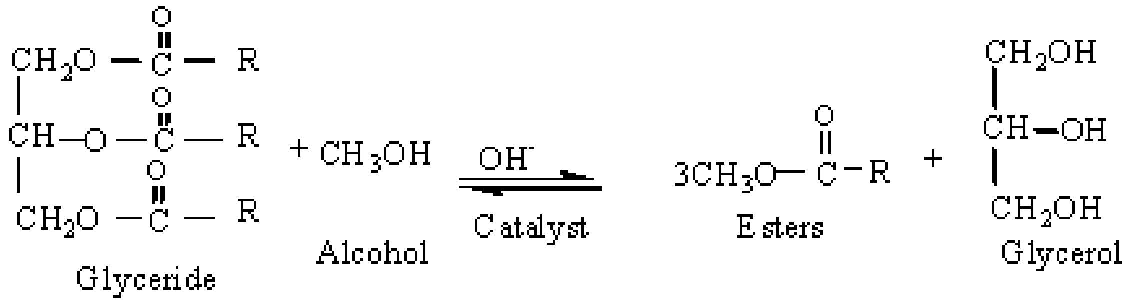

Figure1. 1: Transesterification reaction

1.5 *Balanites aegyptiaca*

1.5.1 Taxonomical classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Zygophyllaceae

Genus: *Balanites* Delile

Species: *Balanites aegyptiaca* (L.) Delile [6].

1.6 Botanical description

It is multibranched, spiny shrub or tree up to 10 m tall. Crown spherical, in one or several distinct masses. Trunk short and often branching from near the base. Bark dark brown to grey, deeply fissured. Branches armed with stout yellow or green thorns up to 8 cm long. Leaves with two separate leaflets; leaflets obovate, asymmetric, 2.5 to 6 cm long, bright green, leathery, with fine hairs when young. Flowers in fascicles in the leaf axils, and are fragrant, yellowish-green.

1.7 Fruit and seed description

Fruit is a rather long, narrow drupe, 2.5 to 7 cm long, 1.5 to 4 cm in diameter. Young fruits are green and tormentose, turning yellow and glabrous when mature. Pulp is bitter-sweet and edible. Seed is the pyrene (stone), 1.5 to 3 cm long, light brown, fibrous, and extremely hard. It makes up 50 to 60% of the fruit. There are 500 to 1500 dry, clean seeds per kg.

1.8 Flowering and fruiting habit

Flowers are small, inconspicuous, hermaphroditic, and pollinated by insects. Seeds are dispersed by ingestion by birds and animals. The tree begins to flower and fruit at 5 to 7 years of age and maximum seed production is when the trees are 15 to 25 years old.

1.9 Distribution and habitat

Natural distribution is obscured by cultivation and naturalization. It is believed indigenous to all dry lands south of the Sahara, extending southward to Malawi in the Rift Valley, and to the Arabian Peninsula, introduced into cultivation in Latin America and India. It has wide ecological distribution, but is mainly found on level alluvial sites with deep sandy loam and free access to water. After the seedling stage, it is intolerant to shade and prefers open woodland or savannah for natural regeneration. It is a lowland species, growing up to 1000 m altitude in areas with mean annual temperature of 20 to 30°C and mean annual rainfall of 250 to 400 mm [7-8].

1.10 Traditional Uses

Aqueous extract of fruits showed spermicidal activity without local vaginal irritation in human being, up to 4% sperms becoming sluggish on contact with the plant extract and then immobile within 30 s; the effect was concentration-related. Protracted administration of the fruit pulp extract produced hyperglycemia-induced testicular dysfunction in dogs. Seed is used as expectorant, antibacterial, and antifungal. Fruit is used in whooping cough, also in leucoderma and other skin diseases. Bark is used as spasmolytic [9].

The seed is used as a febrifuge.[10] Root extracts have proved 'slightly effective' against experimental malaria.[11-12] In Kenya, a root infusion is used as an emetic.[13] In asthma, about 10 gm of seed powder is taken with glass of water in the morning for 10 days.[14] Tablets are prepared from roots mixed with 'Hing' powder (*Ferula asafoetida*); by adding *Piper betle* leaf, juices are taken once with water for 9 days, soon after the menstruation to avoid unwanted pregnancy [15]. In Egyptian folk medicine, the fruits are used as an oral hypoglycemic [16] and an antidiabetic; an aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice [17]. Used in food preparations and herbal medicine, especially in Africa and some developing Countries [18]. The fresh leaf of the plant *Acalypha* is pounded with small amount of root of *B. aegyptiaca and Cissus quadrangularis*, and then soaked in water for an hour or two. It is decanted and administered intranasally and orally. Latex of the plant is used in epilepsy, administered through intranasal route [19].Used as tooth brush [20]. Fruits are used to treat dysentery and constipation. The seed oil is used to treat tumors and wounds [21]. Used as laxative, also used in treatment of hemorrhoid, stomach aches, jaundice, yellow fever, syphilis, and epilepsy [22]. A fruit is used to treat liver disease and as a purgative, and sucked by school children as a confectionary in some countries [23-24]. The bark is used in the treatment of syphilis, round worm infections, and as a fish poison. The aqueous leaf extract and saponins isolated from its kernel cakes have antibacterial activity [25-26]. Seeds are used as anthelmintic and purgative. Ground seeds are given to camels to cure impaction and colic [27].

In Chifra District, the root of plant is used for the treatment of render pest and anthrax. In East Africa, it is widely used as anthelmintic. Root is used in various folk medicines for the treatment of abdominal pain and as purgative, while the bark is employed as a fish poison and also as a remedy for malaria and syphilis. The root, bark, kernel, and fruit have been shown to be lethal to mollusks [28]. In Sudanese folk medicine, it is used to treat jaundice [29]. Its antimalarial and molluscidal activity is well studied [30-33]. *In vitro* antiplasmodial test of the dichloromethane and methanol (ME) extract of stem bark of the plant showed antimalarial activity.

In Senegal, Nigeria, Morocco, and Ethiopia, *B. aegyptiaca* is taken a purgative for colic and stomach ache. In Chad, fresh twigs are put on the fire in order to keep insects away. For intestinal worm, the fruits are dried and mashed in millet porridge and eaten [34]. In Libya and Eritrea, the leaves are used for cleaning infected wounds. In Sudan and Chad the bark of *B. aegyptiaca* is component of soap [35]. The use of the kernel oil for treatment of wounds has been reported from Nigeria [36]. For contraception, in Nigeria, a mixture of dried leaves powder of *B. aegyptiaca* and *Ricinus communis* in water and in Somalia, the bark of root is crushed and mixed with two glasses of water, which is then filtered. This preparation is repeated for three days and one glass is drunk three times daily for three days [37].

1.11 Aim of the study

- Extraction of *Balanites aegyptiaca* seeds oil using solvent extraction techniques and test some of its physicochemical properties such as free fatty acid content, density, viscosity, color, moisture content and refractive index.
- Convert the extracted oil into biodiesel by transesterification reaction.
- Subject the product biodiesel to IR and GC-MS analysis.

Materials and methods

2.1 Materials

2.1.1 Sample

Fruits of *Balanites aegyptiaca* were collected from Khartoum. The seed kernels were released and crushed, it was 446.50g.

2.2 Methods

2.2.1 Oil extraction

The ground seeds kernels was soxhlet extracted with normal hexane for a period of 8 hours. The solvent removed by using a rotary evaporator.

2.2.2 Oil percentage

The weight of oil extracted from 446.50g of seeds was weighted to determine percentage of oil in the seed (W/W). According to the formula

$$\%\text{oil} = \frac{w1}{w2} \times 100\%$$

Where w1 = weight of oil

w2 =weight of ousted seeds pulp

2.2.3 Determination Free fatty acid

0.9g of oil sample was weighted into a conical flask. 100ml of isopropanol was added and warmed until the oil dissolved. 3drobs of phenolphthalein indicator were added and titrated with standard sodium hydroxide until a pink color appearance persists for half a minute.

$$\%\text{free fatty acid as oleic acid} = \frac{28.2*N*V}{W}$$

Where V = volume of sodium hydroxide solution used (ml)

N = normality of sodium hydroxide solution used

W = weight of sample taken (g)

2.2.4 Density

The density of the oil was determined using density bottle 50ml. The density bottle was filled with oil and weight. The density was calculated as:

$$d=\frac{m}{v}$$

Where d is density, m is mass of the oil, and v is the volume of the density bottle.

2.2.5 Viscosity

The viscosity of the oil was measured by using HAAKE Viscotester 6 plus (Thermo ELECTRON CORPORATION).

2.2.6 Color

The color of the oil was determined by using Lovibond comparator colorimeter.

2.2.7 Moisture content

The moisture content of oil is expressed as percentage weight loss when the oil is dried to a constant weight at 110°C. A dry crucible was weighed and the dried oil (5 g) was poured into it. The crucible and content were dried in an oven at 110°C and cooled in a dessicator and weighed.

2.2.8 Refractive index

The refractive index of oil is a function of molecular structure and impurity. The refractometer was used. Placed drops of the oil on the prism, and closed by another one after cleaned the both prisms. The prisms were tightened firmly with the screwhead. The instrument was adjusted and lighted and the refractive index was determined.

2.3 Biodiesel preparation

Transestarificatin reaction is a typical substitution reaction. The process involves substituting the alkyl group of the esters with the alkyl group of the alcohol. In the case of fatty acid methyl esters (FAME) using *Balanites aegyptiaca* seed oil, the alkyl group on the triglyceride (oil) is substituted with the methyl group of the alcohol. The base (NaOH) catalyst was dissolved in the alcohol to make it convenient for dispersing the solid catalyst into the oil. The methoxide produced ($NaOCH_3$) was then mixed with the oil and the substitution reaction proceeds in a series of steps.

2.4 Testing of biodiesel

2.4.1 Infra red analysis

The sample was put directly in the KBr windows, introduced into the FT IR and scanned at 400-4000 cm^{-1}.The IR spectrum was recorded.

2.4.2 Gas chromatography-mass spectrometric analysis

Gas chromatography mass spectrometry (GC-MS) is a technique for the analysis and quantification of organic volatile and semi-volatile compounds.

(GC) is used to separate mixtures into individual components using a temperature-controlled capillary column.

(MS) is used to identify the various components from their mass spectra. GC-MS analysis can work on liquids, gases and solids. For liquids, the sample is directly injected into the GC.

The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

The type of system is used in this study was VARIAN 4000 GC/MS/MS spectrometer.

Results and discussion

3.1 *Balanites aegyptiaca* seed oil analysis

3.1.1 Physicochemical analysis

Using soxhlet extraction, oil from the kernel of *Balanites aegyptiaca* was obtained.

Some of the physicochemical parameters of the *Balanites aegyptiaca* seed oil were shown in Table 3.1 and compared with those of oil extracted from *Jatropha*. Free fatty acid % in *B. aegyptiaca* was only 1.53% and it was quite dry i.e. low moisture content compared to *Jatropha* oil in which the moisture content was 0.8%. High free fatty acid and high moisture content are disadvantages as their high amounts in the oil reduce the efficiency of biodiesel production, since more soap is produce and more consumption of catalysts is required. Although the densities of the two oils i.e. *Balanites aegyptiaca* seed oil and *Jatropha* oil are comparable their viscosities are very different, the viscosity of *Jatropha* oil is almost five folds that of *B. aegyptiaca*. This difference in viscosity may be related to the type of fatty acids involved in each oil, and the degree of unsaturation of the fatty acids. Low viscosity oil usually facilitate the processing and mixing with catalysts and accelerate transesterification reaction. The results show the superiority of *B. aegyptiaca* compared to Jatropha oil. This is an encouraging result for further exploration of economical viability of converting *B. aegyptiaca* seed oil into biodiesel.

Table 3.1: comparison between physicochemical properties of *Balanites aegyptiaca* seed oil and *Jatropha* oil used for biodiesel production

Physicochemical property	**Oil studies**	
	Balanites aegyptiaca	***Jatropha***
Free fatty acid %(w/w)	1.53%	5% max
Density (g/cm^3)	0.945	0.920
Viscosity (cps)	11	50.73
Color	3	4
Moisture %(w/w)	0%	0.8 max
Refractive index	1.47	1.47

3.1.2 IR analysis

IR spectrum of *Balanites aegyptiaca* seed oil is shown in figure 3.1, shows (-C-H) stretching peaks were found at 2843.17, 2874.03, 2889.46, 2928.04, 2970.48 Cm^{-1}, bending peak of (-C-H2) at 1464.02 Cm^{-1}, bending (-CH3) at 1377.22Cm^{-1}, stretch peak of (C=O) at 1753.35 $^{-1}$Cm, (C=C) presence at 1629.90, 1649.19 Cm^{-1}, stretch peak of (=C-H) at 3007.12Cm^{-1}, and the peak at 723.33 Cm^{-1}. This feature indicates a long chain structure.

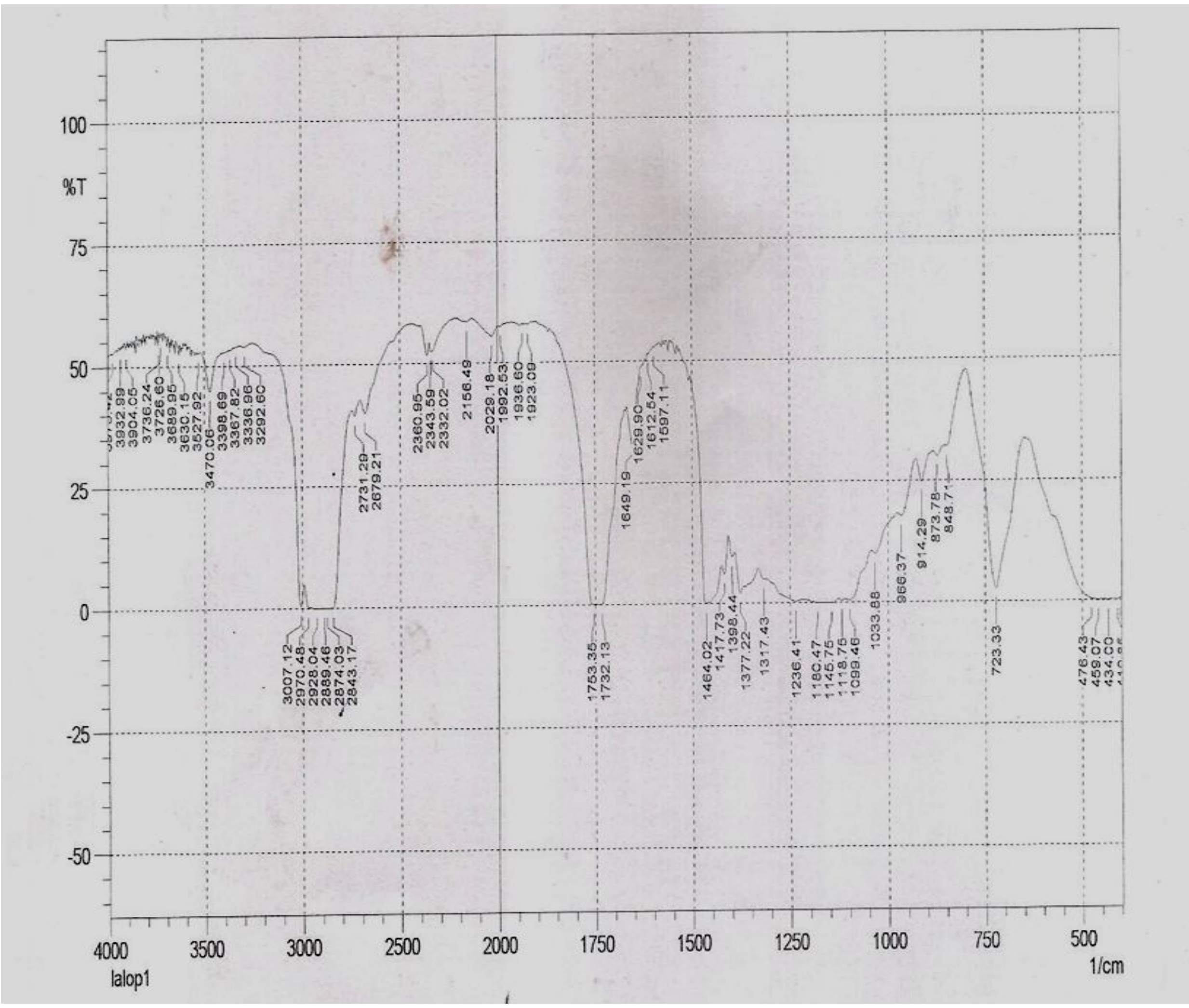

Figure 3.1: FT IR Spectrum of balanites aegyptiaca seed oil

3.2 Biodiesel analysis

3.2.1 IR analysis

Result of infrared analysis of biodiesel produced from *Balanites aegyptiaca* seed oil shown in figure 3.2. In the IR spectrum of biodiesel, peaks of esters are observed. These beaks are stretch (C=O) at 1739.85 Cm^{-1}, stretch (C-O) at 1195.91, 1170.83, 1246.06Cm^{-1}, saturated (-C-H) stretching were found at 2895.25, 2870.17, 2845.10 Cm^{-1}, bending peak of (-C-H2-) at 1437.02, 1452.45 Cm^{-1} and bending methyl group (-CH3) at 1365.65 Cm^{-1}, stretch (C=C) at 1656.91 Cm^{-1}. Also there is peak at 723.33 Cm^{-1} indicates the long chain structure, and stretch peak at 3009.05 Cm^{-1} indicates presence of (=C-H).

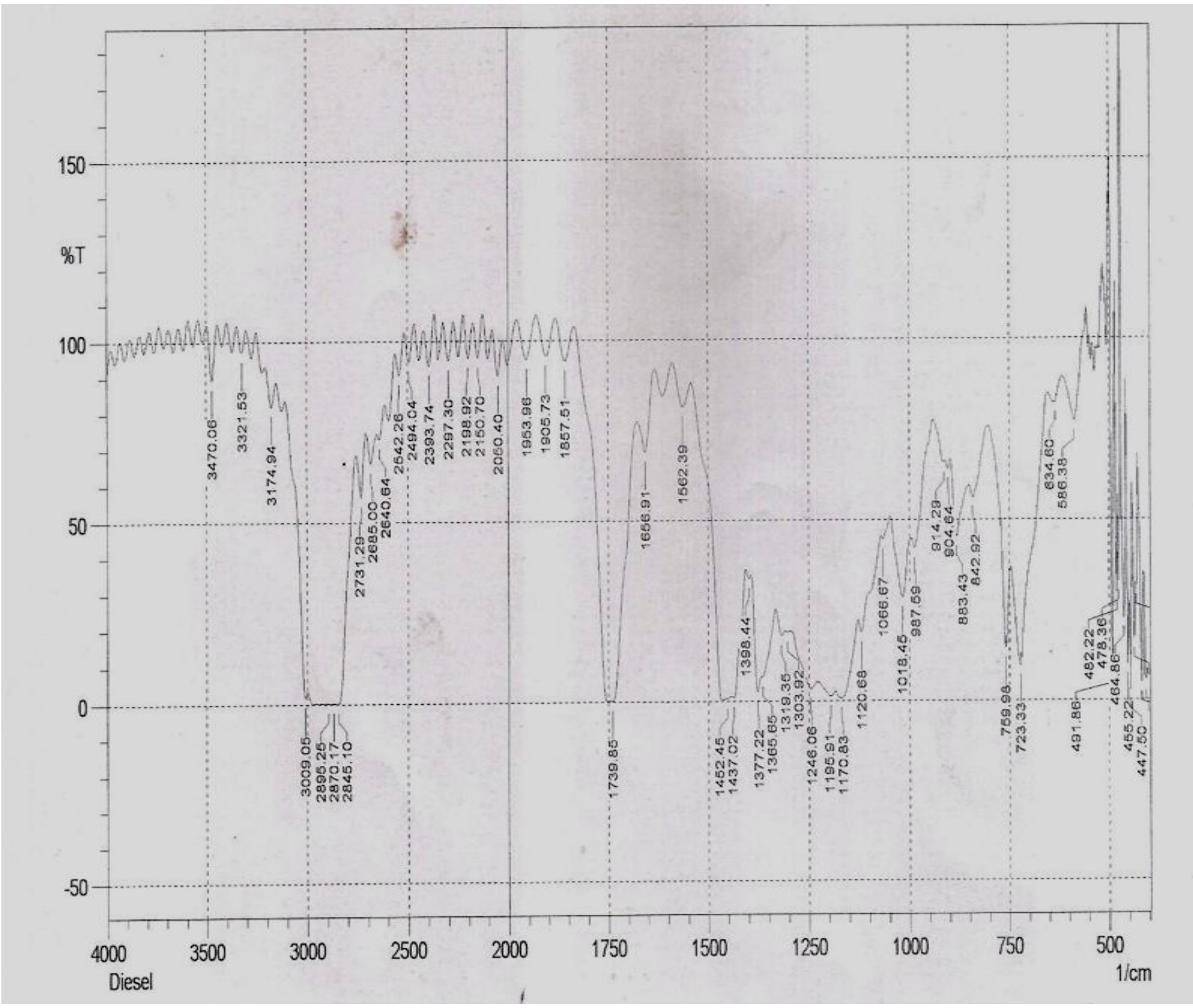

Figure 3.2: FT IR spectrum of biodiesel

3.2.2 GC-MS analysis

Fatty acid profile of the biodiesel prepared from *Balanites aegyptiaca* seed oil was determined by using GC-MS analysis. The individual peaks of the chromatogram shown in figure 3.3 were analyzed and the fatty acids were identified. Relative percentage of fatty acid esters was calculated by computerized integrator and results are presented in table 3.2.

The methyl esters content of the biodiesel were 44.64% 9-octadecenoic acid, 43.33% Linolic acid, 6,581% Hexadecanoic acid and 5.451% Octadecanoic acid, methyl ester.

The mass spectra of biodiesel are shown in figures 3.4, 3.5, 3.6, 3.7. Molecular ion peaks and base peaks of the fatty acid methyl ester (FAME) are also shown in above figures and they are in the expected values.

Table 3.2 Components of biodiesel

MW= Molecular weight

RT=Retention time

No	Name	Formula	RT	MW	Area	Area%
1	Hexadecanoic acid	$C_{16}H_{32}O_2$	31.37	256	28190000	6.581
2	Linolic acid	$C_{18}H_{32}O_2$	36.69	280	185600000	43.33
3	9-octadecenoic acid	$C_{18}H_{34}O_2$	36.87	282	191200000	44.64
4	Octadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	37.56	284	23350000	5.451

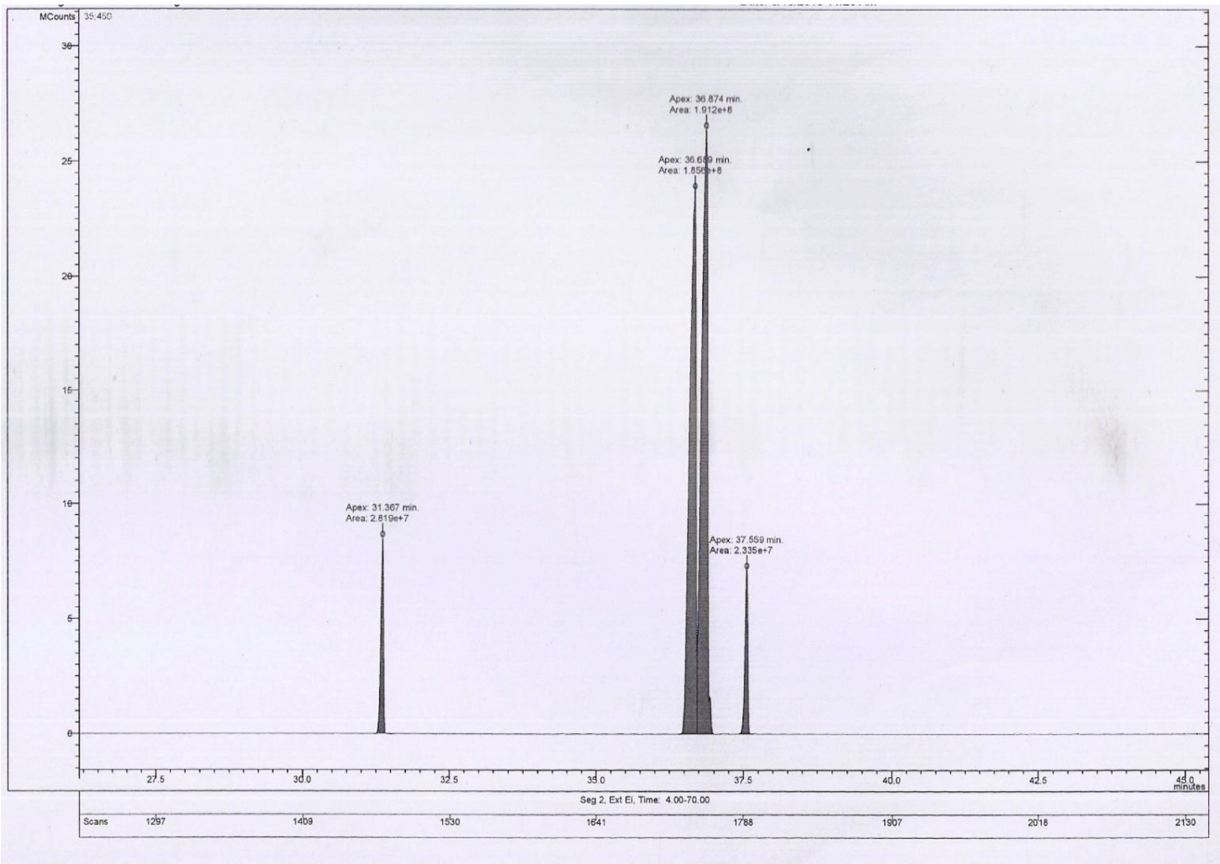

Figure 3.3 chromatogram of biodiesel showing the major components

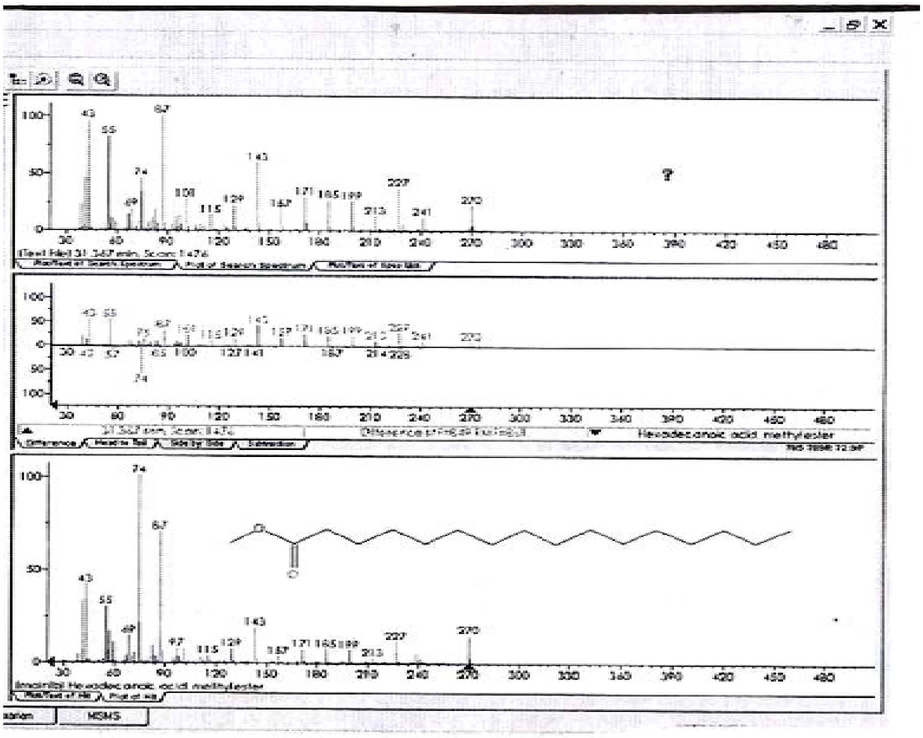

Figure 3.4: MS Spectrum of Hexadecanoic acid

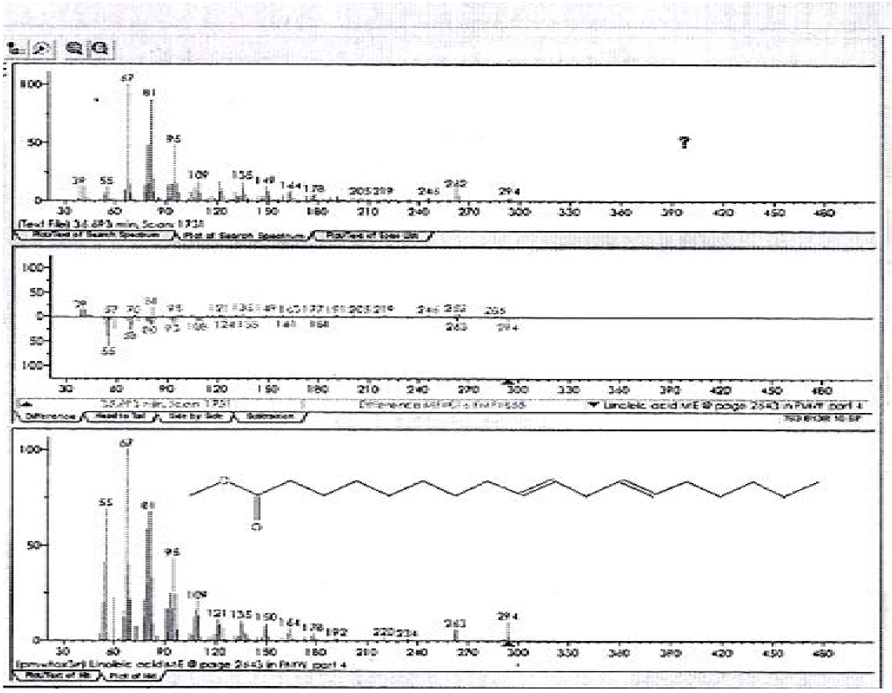

Figure 3.5: MS Spectrum of Linoleic acid

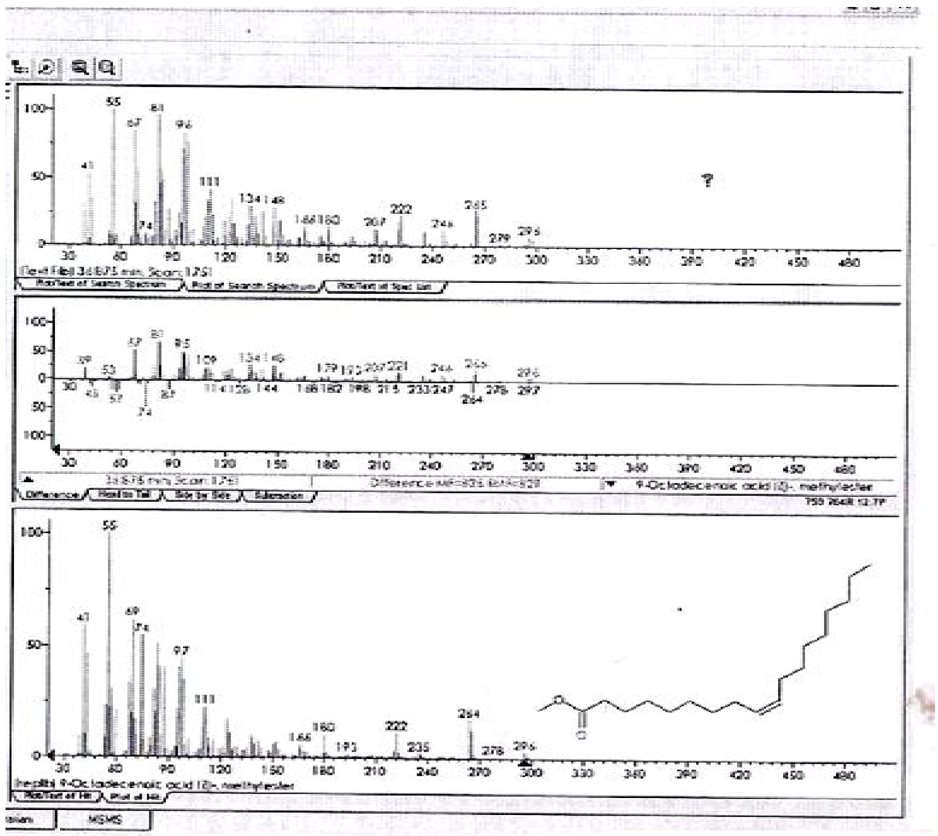

Figure 3.6: MS Spectrum of 9-octadecenoic acid

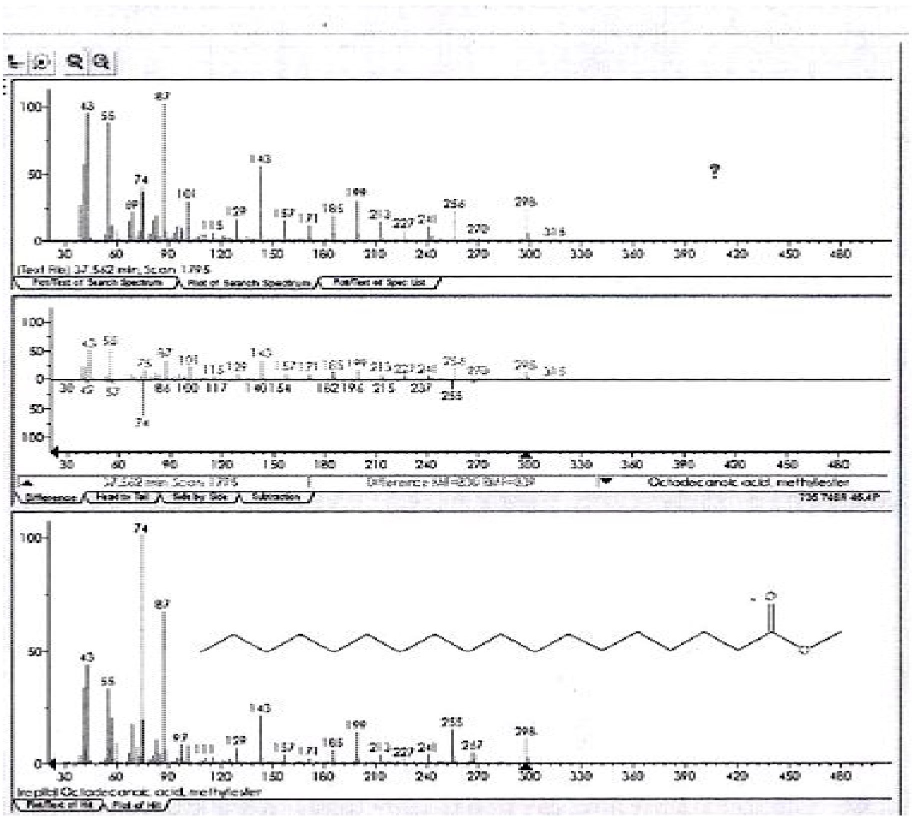

Figure 3.7: MS Spectrum of Octadecenoic acid, methyl ester

3.3 Conclusion

The oil of *Balanites aegyptiaca* seeds used in this study has physicochemical properties that exceeds the range of specification limits of the edible oils used in preparing biodiesel.

GC-MS analysis of biodiesel confirmed the presence of methyl esters and provide evidence of completed transestrificacion of the *Balanites aegyptiaca* seed oil.

3.4 References

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