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A Two-Step Transesterification of *Citrullus lanatus* Seed Oil

Gloria Ihuoma Ndukwe*, Anselem Tochukwu Ugboaja, Nkiru Aghaeze

Department of Chemistry, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria

Email address

gloria.ndukwe@ust.edu.ng (G. I. Ndukwe)

*Corresponding author

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Abstract: This paper presents the transesterification of seed oil from *Citrullus lanatus* (Thunberg) Matsumura & Nakai also known as watermelon; while examining the potential of *C. lanatus* as an alternative feedstock in the production of biodiesel which has become one of the mitigating sources to cushion the depleting nature as well as the environmental effects of combustible petrol fuel. Maceration method was used for the extraction of oil while physicochemical parameters were determined using standard procedures. Biodiesel was synthesized via two-step acid-base catalyzed transesterification while gas chromatography with flame ionization detection and Fourier transform-infra red were used for the characterization of biodiesel. Physicochemical analyses carried out on the synthesized biodiesel showed that *C. lanatus* seed has the potential to serve as an alternative feedstock. The flash points of 115°C and 118°C of the synthesized biodiesels were comparable to American Society of Testing Materials (ASTM) and European Committee for Standardization (EN 14214: 2008) standards; though kinematic viscosity measurements (7.99 and 7.98 mm^2/s) showed biodiesel products of relatively high viscosity which has the tendency to leave deposits on combustion. Spectroscopic analyses indicated successful transesterification with 80-85% biodiesel yield. The seeds of *Citrullus lanatus* which are considered as waste products can serve as alternative feedstock for biodiesel.

Keywords: *Citrullus lanatus*, Watermelon, Transesterification, Biodiesel, *Ethyl oleate*, *Ethyl palmitate*

1. Introduction

Carbon dioxide which is a combustion by-product of fossil fuel is a major and important human-produced, climate-altering greenhouse gas [1]. Due to these huge carbon footprints, there has been an increased search for alternative sources of fuel to replace these traditional but harmful sources. Efforts in renewable energy presents a huge opportunity to mitigating these effects. These efforts include but not limited to solar, wind, hydro and biomass, with biodiesel being on the forefront of such efforts as it can be made from varied sources.

The widespread availability of biomass resources makes biomass-based fuel technology a process that can potentially employ more people than fossil fuel-based technology [2]. Among the different types of fuels that can be produced from biomass resources are liquid fuels, such as ethanol and methanol, biodiesel and gaseous fuels, such as hydrogen and methane. The use of biofuels is varied but can primarily be used in vehicles and engines or fuel cells for electricity generation.

Biodiesel has been described as diesel fuel based from animal or vegetable sources [3]. It is a biofuel that contains long chain mono-alkyl esters which can serve as alternative to conventional or fossil diesel. However, the major problem of biodiesel is its cold flow properties which is represented by the pour point [4].

Transesterification is generally defined as a catalyzed chemical reaction involving the conversion of a carboxylic acid ester into a different carboxylic acid ester. Vandkata *et al*. described it as a process of exchanging the organic alkyl groups of plant oil – an ester with the methyl group of methyl alcohol [5].

Citrullus lanatus seed (Figure 1) can be a potential source of biodiesel. *Citrullus lanatus* (Thunberg) Matsumura & Nakai also known as watermelon, belonging to the Cucurbitaceae family is a vine-like flowering plant with origin in West Africa. The sweet dessert watermelons (Figure

2) features non-bitter, tender, well-coloured flesh, have a narrow genetic base, suggesting that they originated from a series of selection events in a single ancestral population [6]. The average length, width, thickness and mass of 100 seeds are 10·60 mm, 6·18 mm, 2·37 mm and 0·099 g respectively with a moisture content of 7·35% [7].

The antioxidant, anti-inflammatory and analgesic potentials of *C. lanatus* have been reported [8]. Its juice and pulp are used for human consumption, while the rind and seeds are major solid wastes [9]. Conjugated fatty acids in some Cucurbitaceae oils make them incredibly useful as drying oils [10]. This paper investigates the seed of *Citrullus lanatus* as an alternative feedstock in biodiesel production.

Figure 1. *Citrullus lanatus seed.*

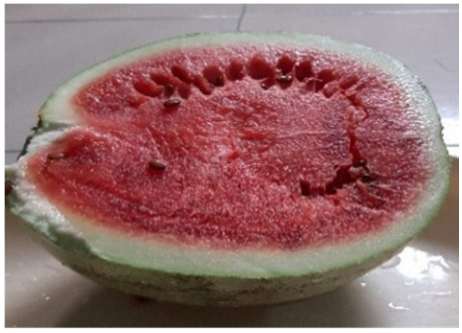

Figure 2. *Citrullus lanatus fruit.*

2. Materials and Methods

2.1. Reagents

Chemicals used during this research include absolute ethanol, absolute methanol, conc. sulphuric acid, potassium hydroxide, glacial acetic acid, iodine, potassium iodide, sodium thiosulphate, chloroform, isopropyl alcohol (all Sigma Aldrich) and re-distilled n-hexane.

2.2. Instrumentation

The following instruments were employed for analyses in this work; Perkin-Elmer Fourier transform-infra red (FT-IR) spectrum 2 with serial number 97529, viscometer bath (Stanhope SETA) maintained at 40°C, Pensky Martens closed cup flash point tester, gas chromatograph-flame ionization detection (GC-FID) (HP6890) column type HP INNOWax with carrier gas being nitrogen gas, Hinotek SYD-510D pour and cloud point tester, water bath, rotary evaporator, and analytical balance. The infrared spectra of biodiesels produced from *Citrullus lanatus* seed oil were measured using a PerkinElmer® Spectrum™ Two FT-IR spectrometer. To obtain the best contact time with the crystal and the background spectrum, the samples were injected into the empty accessory. Approximately 5 minutes was required for spectral collection which were recorded within a range of 4000-550 cm^{-1} with a 4 cm^{-1} resolution. Gas chromatographic analyses were performed on the biodiesel in accordance with European Union (EU) norm EN 14103, using a GC–FID HP6890 apparatus (Agilent Technologies, USA) equipped with data acquisition software HP Chemstation Rev. A. 09.01 (1206). A 15 m long HP-INNOWAX capillary column, (30 m X 0.25 mm and 0.25 μm film thickness) at a constant hydrogen flow of 22 psi were used to accomplish separation. With an injector temperature of 250°C, samples (1 μL) were injected in a split ratio of 20:1. The oven started with an initial temperature of 12°C and was followed by an increase in temperature up to 15°C.

2.3. Plant Material

C. lanatus fruits were purchased from a fruit market in Port Harcourt, Rivers State, Nigeria. The seeds were extracted from the fruits and air dried. The dried seeds were subsequently pulverized to increase the surface area for subsequent extraction of oil.

2.4. Extraction of Oil

Pulverized *C. lanatus* seeds (3.012 kg) was macerated with 3 L of n-hexane in an aspirator bottle for 24 hours. It was then filtered and concentrated using a rotary evaporator. The extraction process was repeated 6 times with fresh n-hexane each time [11, 12]. Extracted oil was weighed, and percentage yield calculated.

2.5. *Citrullus lanatus* Seed Oil Characterization

2.5.1. Acid Value and Percentage Free Fatty Acid (%FFA)

C. lanatus seed oil (0.5 g) was weighed into a dry conical flask and 20 ml of isopropyl alcohol (IPA) added to it. 3 drops of phenolphthalein indicator were added and mixed properly. The solution was titrated with 0.1 N potassium hydroxide until a pink colour that stayed for 15 sec was observed. The volume of KOH used was recorded as A. The procedure was repeated for the blank starting with IPA and volume of KOH used recorded as B. Acid value and percentage free fatty acid were obtained using equations 1 and 2 respectively [13].

$$Acid\ value = \frac{(A-B)\times Normality\ of\ the\ base \times 56.1\ g}{Weight\ of\ oil\ used\ (g)} \quad (1)$$

$$\%\ FFA = \frac{(A-B)\times Normality\ of\ the\ base \times 28.2\ g}{Weight\ of\ oil\ used\ (g)} \quad (2)$$

2.5.2. Iodine Value

C. lanatus oil (0.5 g) was weighed and dissolved in a conical flask with 20 ml of chloroform. 20 ml of KI solution was added followed by 100 ml of distilled water. The resulting solution was placed in the dark for 30 minutes. The solution was titrated with standardized 0.1 M sodium thiosulphate using 1 ml of starch as indicator and introducing 1ml of starch test solution, stirred continuously till the end point was achieved (V_1). A blank (V_2) was carried out starting with 20 ml of chloroform. Solution turned to blue black precipitate and then to colourless [14]. Iodine value was calculated using equation 3.

$$Iodine\ value = \frac{(V_2 - V_1) \times Normality\ of\ Na_2S_2O_3 \times 12.69\ g}{Weight\ of\ oil\ used\ (g)} \quad (3)$$

2.5.3. Saponification Value

The seed oil of *C. lanatus* (0.25 g) was weighed into a round bottomed flask and dissolved with 10 ml of 0.5 N ethanolic solution of potassium hydroxide and the solution was refluxed to ensure perfect dissolution. The solution was allowed to cool. Phenolphthalein (3 drops) was added. The solution was titrated with 0.5 N HCl (V_1). A blank (V_2) was carried out as well [14]. Thus, saponification value was calculated using equation 4.

$$Saponification\ value = \frac{(V_2 - V_1) \times Normality\ of\ HCl \times 56.1\ g}{Weight\ of\ oil\ used\ (g)} \quad (4)$$

2.5.4. Peroxide Value

The oil of *C. lanatus* seeds (0.5 g) was weighed into a conical flask and dissolved with 25 ml mixture of acetic acid and chloroform (2 volume of glacial acetic acid and 1 volume of chloroform). 1 ml of saturated solution of potassium iodide was added, followed closely by the addition of 7.5 ml of distilled water. The solution was titrated with 0.1 N sodium thiosulphate until the yellow colour disappeared. Starch indicator (0.5 ml) was added to the solution and the titration continued to end point (V_1). A blank was carried out as well [14]. Peroxide value was calculated using equation 5.

$$Peroxide\ value\ (Milleqv.peroxide/kg = \frac{(V_2 - V_1) \times Normality\ of\ Na_2S_2O_3 \times 1000}{Weight\ of\ oil\ used\ (g)} \quad (5)$$

2.6. Two step Acid-base Catalyzed Transesterification

2.6.1. Acid Pretreatment (Acid Catalyzed Esterification)

C. lanatus seed oil (250 ml) was heated on a heating mantle at 55°C. A mixture of 5 ml of concentrated sulfuric acid and 25 ml of methanol was introduced into a round bottomed flask containing the oil. The mixture was stoppered, placed on a thermostatic magnetic stirrer maintained at 400 rpm and refluxed for 1 hour. The mixture was then poured into a separatory funnel and allowed to stand for 2 hours. The lower layer consisting of aqueous phase was tapped off. The same procedure was repeated using ethanol [3, 12].

2.6.2. Base Catalyzed Transesterification

The pretreated *C. lanatus* seed oil (200 ml of oil pretreated with methanol) was measured and poured into a round bottomed flask which was immersed in a water bath set at 55°C and allowed to heat up until the temperature of the water bath was attained. KOH (1.73 g) was weighed and added to a conical flask containing 20 ml of methanol and swirled gently until all the KOH pellets were dissolved, thereby forming methoxide solution. The methoxide solution was added to the heated oil and refluxed for 1 hour at a temperature maintained at 55°C. The mixture was poured into a separatory funnel and allowed to stand for 1 hour for separation to take place. The lower layer (glycerol) was tapped off and the upper layer (biodiesel) was washed 6 times with warm distilled water. This same process was repeated for the oil that was pretreated with ethanol; in this case, ethanol was used instead of methanol. The volume and weight of obtained biodiesel were measured and recorded [12].

2.7. Characterization of Biodiesel Synthesized from *C. lanatus* Seed Oil

2.7.1. Density Measurement

An empty beaker was weighed using an analytical weighing balance. To the weighed empty beaker was added 30 ml of synthesized biodiesel and the combined weight measured. The difference between the weight of the beaker plus biodiesel and that of the empty beaker was obtained and recorded as the weight of the oil [13]. The density was obtained by taking the ratio of the weight of the biodiesel and its volume (Equation 6).

$$Density\ of\ biodiesel = \frac{Weight\ of\ measured\ biodiesel}{Volume\ of\ measured\ biodiesel} \quad (6)$$

2.7.2. Kinematic Viscosity Measurement

The biodiesel synthesized from *C. lanatus* (10 ml) was poured into a viscometer tube. The tube was then immersed into a viscometer bath maintained at 40°C. The oil in the tube was sucked up to the upper limit mark using a suction pump and allowed to drop under gravity. A stopwatch was started and the set up monitored till the oil got to the lower limit of the tube and the watch stopped. The time was recorded, and the procedure repeated twice [13]. The kinematic viscosity was calculated using equation 7.

$$KV\ @\ 40\ ℃\ (mm^2/s) = Time\ (s) \times Tube\ constant \quad (7)$$

2.7.3. Flash Point

Pensky-Martens closed cup apparatus was used to obtain the flash points of synthesized biodiesel. Synthesized biodiesel was poured into the brass cup to touch the prescribed mark inside the cup and then gently placed into its position until it locked. A thermometer was placed inside through the provided opening. At intervals, lighted flame was passed through the sample in the cup to check for flash, while temperature was being monitored. The temperature at the first distinct flash was taken and recorded. This gave the closed flash point of the biodiesel [13].

2.7.4. Pour Point

Biodiesel in a test jar with a thermometer clamped to it was cooled inside a cooling bath, as it cooled it formed wax crystals. The test jar was removed at every degree drop in temperature and tilted to check the surface movement. When the surface did not flow for 5 seconds, the temperature was recorded [13].

3. Results and Discussion

Physicochemical properties of *C. lanatus* seed oil are summarized in Table 1. The oil yield of *C. lanatus* seed was 13.52% with acid value of 38.64 mgKOH/g and %FFA of 19.43. The oil was pre-treated to reduce the acid value to 3.784 mgKOH/g and %FFA to 1.892 which is within the permissible limit. High FFA can cause emulsification and hence present difficulty during separation of biodiesel from glycerol and during washing [3].

Table 1. *Some Physicochemical properties of C. lanatus seed oil.*

Property	Unit	Value
Colour		Orange
Appearance		Liquid
% Yield	%	13.52
Saponification value	mgKOH/g	192.20
Peroxide value	Millieqv peroxide/kg	5.00
Iodine value	g I_2/100g of oil	9.91
Density @ 15°C	g/cm^3	0.867
Acid Value	mgKOH/g	38.64
%FFA	%	19.43

In Table 2, the physicochemical properties of the synthesized biodiesel are outlined and compared with European biodiesel standard EN 14214: 2008 [15] and American biodiesel standard as contained in ASTM D6751-07b [13]. The pretreated *C. lanatus* seed oil, when transesterified, recorded high conversion percentages of 85% and 80% with potassium ethoxide and potassium methoxide respectively. This implies that most of the starting material (*C. lanatus* seed oil) were transesterified.

The European standards for biodiesel EN 14214: 2008 [15], outlined the standard density of biodiesel to range from 0.86-0.9 g/cm^3 and that for petroleum diesel is 0.82-0.845 g/cm^3. From the foregoing, it is evident that the density of the biodiesels synthesized with ethoxide and methoxide which are 0.934 g/cm^3 and 0.983 g/cm^3 respectively, are within specifications. Ibeto *et al*. posited that density and other gravities are important parameters for diesel fuel injection systems. According to them, the values must be maintained within tolerable limits to allow optimal air to fuel ratios for complete combustion [4].

When fuel viscosity increases, the fuel flow rate decreases, and this can be attributed to higher flow resistance encountered by highly viscous fluid [16]. If fuel viscosity is low, the outflow will correspond to a power loss for the engine and if it is high, the injection pump will be unable to supply enough fuel to fill the pumping chamber. Again, the effect will be power loss [17]. Thus, from the kinematic viscosity at 40°C results as presented in table 2 showing 7.99 mm^2/s and 7.98 mm^2/s for biodiesels synthesized with ethoxide and methoxide respectively, it can be seen that the biodiesels synthesized from *C. lanatus* were above the EN 14214: 2008 and ASTM D6751-07b ranges of 3.5 - 5 mm^2/s and 1.9 - 6 mm^2/s respectively, and may have the tendency to cause damage to fuel injector and subsequently, incomplete combustion.

The flash point values of the biodiesels were 115°C for biodiesel synthesized with ethoxide and 118°C for biodiesel synthesized with methoxide (Table 2), which are within the standard range for biodiesel cited. The values were higher than that of petrodiesel (55°C) and this makes them more stable to fire. The United Nations classification of flammable liquids is based on flashpoint determination and is helpful in categorizing the flammability of different substances [18, 19]. The obtained flash points values suggest that the biodiesels synthesized from *C. lanatus* seed oil are not highly flammable but would require safety precautions like any fuel during usage, storage and transportation [20].

Table 2. *Physicochemical properties of synthesized biodiesels from C. lanatus seed oil.*

Property	Unit	Biodiesel synthesized with ethoxide	Biodiesel synthesized with methoxide	Standards		
				Biodiesel EN14214: 2008	ASTM D6751-07b	Petrodiesel EN 590: 1999
Colour		Golden brown	Wine red			
Appearance		Liquid	Liquid			
% Yield	%	85	80			
Flash point	°C	115	118	≥ 101	≥ 93	55
KV @ 40°C	mm^2/s	7.99	7.98	3.5 - 5	1.9 – 6.0	2.0 - 4.5
Density @ 15°C	g/cm^3	0.934	0.983	0.86 - 0.9		0.82 - 0.845
Pour Point	°C	8	8		-5 - 10	5.6 - 11.1

Table 3 outlines major fatty acid ethyl ester composition of the biodiesel synthesized using ethoxide. This result was obtained from GC-FID analysis (Figure 4) of the biodiesel. The result (Table 3 and Figure 3) indicates a remarkably high percentage (above 70%) of ethyl oleate (2), a mono-unsaturated fatty acid ethyl ester; followed by ethyl palmitate ((1), 10.3%). Altun observed that the degree of unsaturation of biodiesel fuels has effects on engine emissions via its effect on the cetane number and adiabatic flame temperature; while engine performance is not significantly affected by the type of biodiesel fuel nor its degree of unsaturation [21].

Table 3. *Major components of biodiesel synthesized from C. lanatus seed oil using ethoxide.*

Retention time (min)	Compound	Concentration (%)	No. of C-atoms: double bonds
16.24	Ethyl palmitate (1)	10.30	C 16:0
18.16	Ethyl stearate	7.20	C 18:0
19.02	Ethyl oleate (2)	72.32	C 18:1
19.75	Ethyl linoleate	4.50	C 18:2

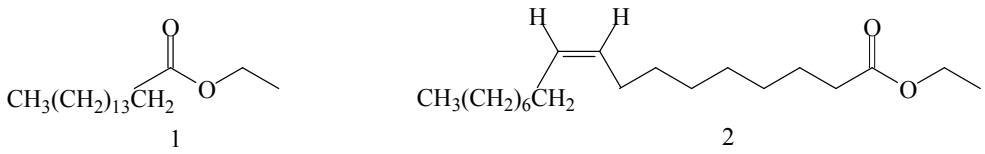

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Figure 3. *Structures of ethyl palmitate (1) and ethyl oleate (2).*

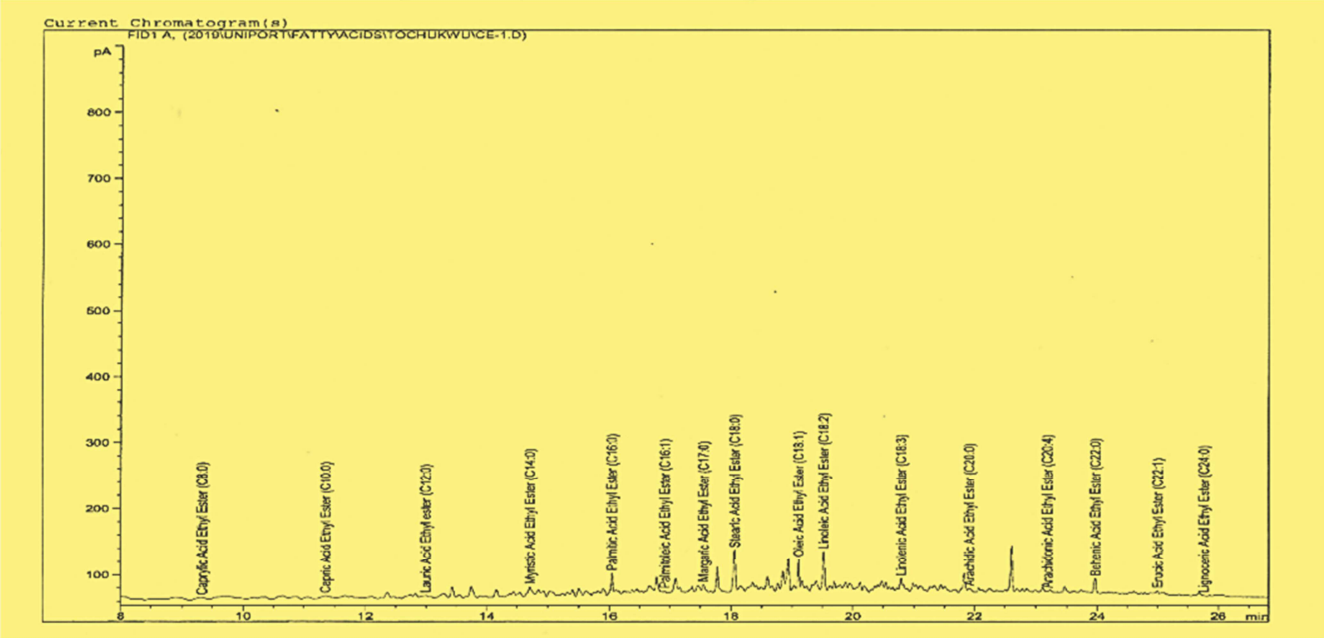

Figure 4. *GC-FID spectrum of biodiesel synthesized from C. lanatus seed oil using ethoxide.*

The FT-IR absorption peaks and their corresponding functional groups for biodiesels synthesized from *C. lanatus* seed oil are presented in Table 4 and Figures 5-6. The biodiesels showed strong absorption peak at 1750 cm^{-1} which agrees with reported absorption attributed to stretching of C=O, characteristic of esters [22]. Another prominent peak was observed at 2900 cm^{-1} attributable to the symmetrical C-H stretching vibration in esters; as well as strong absorption at 1480 cm^{-1} assigned to CH_2 bending vibration [22]. Tariq *et al*. posited that esters have two characteristically strong absorption bands arising from carbonyl (C=O) around 1750 - 1730 cm^{-1} and that of C-O (antisymmetric axial stretching and asymmetric axial stretching) at 1300 - 1000 cm^{-1} [22, 23]. According to Sabrina *et al*., the fingerprint region which lies from 1500 - 900 cm^{-1} is the main spectrum region that allows for distinction between the oil and its respective fatty acid alkyl esters [23, 24]. In the FT-IR spectra of the biodiesels, there are marked absorptions at 900 cm^{-1} assignable to =C-H bending vibration and at 1050 cm^{-1} attributed to C-O asymmetric stretching vibration [23, 24].

Table 4. *Comparative FT-IR peaks of biodiesels synthesized from C. lanatus.*

Peak (cm^{-1})	Functional group	
	Biodiesel synthesized with methoxide	Biodiesel synthesized with ethoxide
2900	C-H (stretching)	C-H (stretching)
1750	C=O (esters)	C=O (esters)
1485	CH_2 (bending)	CH_2 (bending)
1050	C-O (asymmetric stretching)	C-O (asymmetric stretching)
900	=C-H (bending)	=C-H (bending)

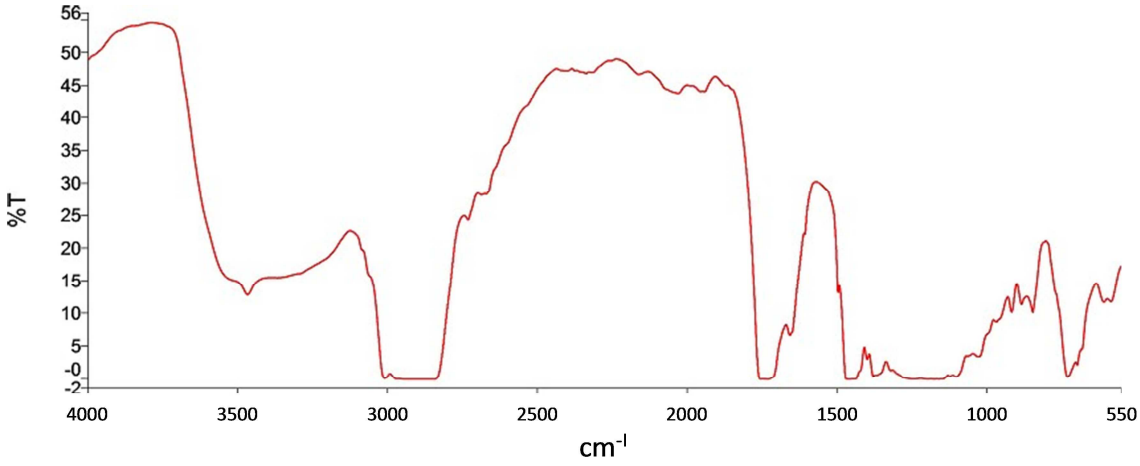

Figure 5. *FT-IR spectrum of biodiesel synthesized from C. lanatus seed oil using ethoxide.*

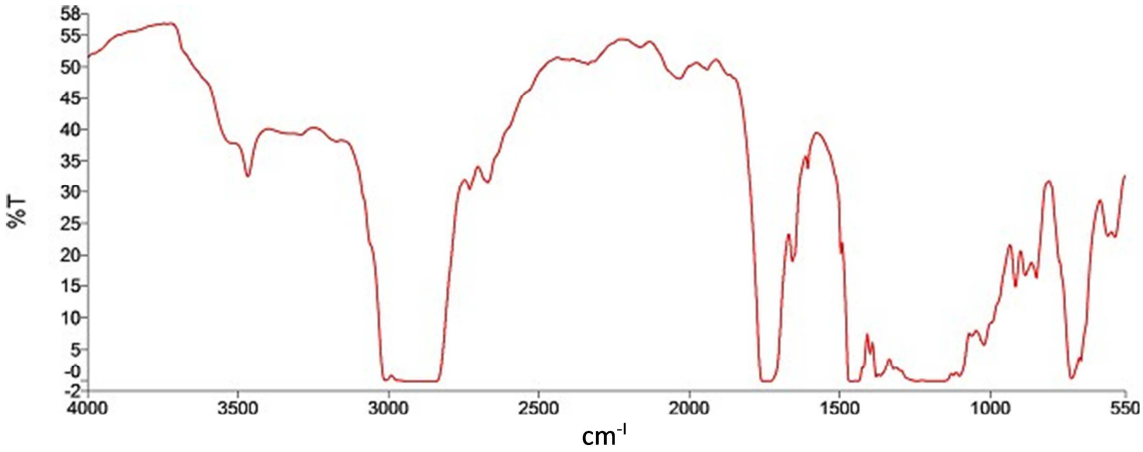

Figure 6. *FT-IR spectrum of biodiesel synthesized from C. lanatus seed oil using methoxide.*

4. Conclusion

Results obtained showed successful transesterification of oil from *Citrullus lanatus* seeds. The seeds which are considered as waste products gave a 13.52% oil yield and as such, can serve as alternative feedstock for biodiesel. The transesterification process yielded over 80% of biodiesel. Other tested parameters on the biodiesels showed successful conversion.

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