



Production of Biodiesel from *Citrullus colocynthis* Seeds Oil

Faroug Bakheit Mohamed Ahmed, Samah Fath El-rahman Ahmed Mohamed

Faculty of Science and Technology, Shendi University, Shendi, Sudan

Email address:

farougmhmd@gmail.com (F. B. M. Ahmed)

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Abstract: The research on the production of biodiesel has increased significantly in recent years because of the need for an alternative fuel which with biodegradability, low toxicity and renewability. The present study aimed to production biodiesel from *Citrullus colocynthis* seeds oil and determines its physical and chemical proprieties. The dry seeds of *Citrullus Colonythis* plant were obtained from Elmatama locality. *Citrullus Colonythis* seeds were minced and its oil was extracted by using a soxhlet (hexane as solvent. The oil content percent of the seeds was 18.91% and then was subjected to transesterification reaction until the biodiesel (*C. Colonythis* oil ethyl ester - CCOEE) was produced. Biodiesel properties were investigate which were; kinematic viscosity 9cSt, the calorific value 43.82 MJ/ Kg, the density 0.906 g/m³, the specific gravity 0.913, the pour point -33°C, micro carbon residue 0%, Acid value 0.6 mg KOH/g and API number 23.29. The density of CCOEE was found to be closed to the limits specified ASTM standard for biodiesel and diesel fuel which were 0.88 g/m³ and 0.823 g/m³ respectively. The kinematic viscosity of CCOEE measured at 50°C was higher when it compared with ASTM standard, (1.6 - 6.0) for biodiesel and (1.9 - 4.1) for diesel fuel. While calorific was well within the range of diesel standard 45.5 MJ/kg and the calorific value of biodiesel is 37.27 MJ/kg. Micro Carbone residue was determinate according to ASTM standard method the CCOEE biodiesel (0%) was found in the range of ASTM, the (0.05%) is maximum limit. The biodiesel produced from CCOEE had a flash point of 55°C, this result it close to flash point of diesel fuel (52°C) and so far of flash point of biodiesel it was 130°C that made biodiesel is safe for transportation purpose.

Keywords: Biodiesel, Biofuel, Caloric Value, Pour Point, Acid Value and Flash Point

1. Introduction

Citrullus colocynthis is a desert vine plant that grows in sandy arid soils. It is native to the Mediterranean Basin and Asia and is distributed among the west coast of northern Africa, eastward through the Sahara, Egypt until India and reaches also the north coast of the Mediterranean and the Caspian seas. It grows also in southern European countries as in Spain and on the islands of the Grecian archipelago. *Citrullus colocynthis* known as bitter cucumber, is a fruit-bearing plant, seed oil is used for frying and cooking in some African and Middle Eastern American countries owing to its unique flavor [1]. The seeds are around 6 mm in size (5 mm long and 3 mm wide), smooth, compressed and ovoid-shaped, (figures 1-3) They are located on the parietal placenta. The seeds are light yellowish-orange to dark brown in color [2].

The main chemical contains of fruit pulp colocynthin (the bitter principle up to 14%), colocynthin (resin), colocynthetin, pectin gum. Seed contain a fixed oil (17%) and albuminoids (6%). The seeds are rich in fatty acids such as myristic, palmitic, stearic, oleic, linoleic, linolenic acid. It is reported that the de-oiled cake can be incorporated in the cattle feed of milking cows up to 25% and it did not exhibit significant effect on the milk yield [3]. The protein content of *Citrullus colocynthis* seeds was found to be 8.25% and rich of lysine leucine and sulfo-amino acids as, methionine. Colonythis kernel contains oil 52%, protein 28.4%, fiber 2.7%, ash 3.6% and carbohydrate 8.2%. These are good sources of essential of amino acids (such as arginine, treptophan and methionine) and vitamins (B₁, B₂ and niacin) and minerals (Ca, Mn, Fe, Mg, p, K, and Zn) [4]. Flavonoids quercetin was isolated from in vivo (leaves, stem, root and fruits). Flavone c. glucosides were identified from the fruits

and aerial parts of *Citrullus colocynthis*, fruit contains isovitexine, iso-orientien and iso-orientien 3, methyl ether, while the aerials parts contains three c-p-hydroxy benzyl derivatives as, 8 c- p - hydroxy benzyl livsotexin 6-c-p-hydroxylivtixin and 8 c- p-hydroxy benzyl livsotexin 4- o-golocosides [5].



Figure 1. *Citrullus Colocynthis* fruit [6].



Figure 2. *Citrullus Colocynthis* seeds (Researchers).

Citrullus colocynthis known as bitter cucumber, is a fruit-bearing plant, seed oil is used for frying and cooking in some African and Middle Eastern American countries owing to its unique flavor [1]. The desert Bedouin are said to make a type of bread from the ground seeds. There is some confusion between this species and the closely related watermelon, whose seeds may be used in much the same way. The seed flour is rich in micronutrients (vitamins and minerals), calcium and niacin and could therefore be used in food formulations especially in regions with low milk consumption like West Africa [7].

Colocynth has been widely used in folk medicine for centuries. More recent pharmacological studies showed that *Citrullus colocynthis* is used widely in different part of world for the treatment of a number of diseases such as intestinal disorder, constipation, hypertension, antidiabetic medication in many tropical and subtropical countries, as a remedy for sore throat and skin infection. The simply leaves of *Citrullus colocynthis* showed considerable antimicrobial, and antioxidant, regional pain-killer and anti-inflammatory action [8]. Aqueous and methanol extracts of colocynth showed high antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and other bacteria. Extracts of fruits, leaves, roots and stems were also found to be potentially usable against many gram positive bacilli and fungi as *Aspergillus fumigatus*, *Aspergillus flavus* and *Mucorsp* [9]. In addition, some of these extracts were found to have an insulin tropic effect and therefore an anti-diabetic effect,

which may make them relevant to the treatment of diabetes mellitus [10, 11].

Biodiesel is a liquid biofuel obtained by chemical processes from vegetable oils or animal fats and an alcohol that can be used in diesel engines, alone or blended with diesel oil. American Society for Testing and Materials (ASTM) defines biodiesel as a mixture of long-chain monoalkylic esters from fatty acids obtained from renewable resources [12]. Biofuels can be derived directly from plants, or indirectly from agricultural, commercial, domestic, and/or industrial wastes. Biodiesel is an alternative diesel fuel that is produced from vegetable oils and animal fats and it is used in diesel engines and heating systems [13].

Biodiesel is a mixture of fatty acid methyl esters (FAMES) which is produced from renewable resources that can be either in a solid state (fats) or a liquid state (oils). The raw materials for biodiesel production are vegetable oils, animal fats and short chain alcohols. Besides its lower cost, another undeniable advantage of non-edible oils for biodiesel production lies in the fact that no foodstuffs are spent to produce fuel. These and other reasons have led to medium- and large-scale biodiesel production trials in several countries, using non-edible oils such as castor oil, cotton, jojoba and jatropa. Animal fats are also an interesting option, especially in countries with plenty of livestock resources, although it is necessary to carry out preliminary treatment since they are solid; furthermore, highly acidic grease from cattle, pork, poultry, and fish can be used. Microalgae appear to be a very important alternative for future biodiesel production due to their very high oil yield; however, it must be taken into account that only some species are useful for biofuel production [14]. However, fats and oils are often used interchangeably referring to the feedstock employed in biodiesel production. The raw materials used for production of biodiesel can be crude, refined or waste oil such as frying oils/fats [15].

Chemically, biodiesels are produced from the transesterification of triglycerides found in vegetables oils and fats [16], formed by a catalyzed reaction of the triglycerides in the oil or fat with a simple monohydric alcohol [13]. Transesterification is one of the classic organic reactions, the step wise reversible reactions of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. Little excess of alcohol is used to shift the equilibrium towards the formation of esters [17]. Transesterification has been demonstrated as the simplest and most efficient route for biodiesel production in large quantities, against less eco friendly, costly and eventual low yield methods of pyrolysis and microemulsification. Therefore, transesterification has become popular and the production method of choice [18, 19]. The transesterification is the reaction between oil and fat, with a short chain alcohol (methanol, ethanol, and propanol) in the presence of suitable catalysts in the transesterification reaction [20]. Oils of high free fatty acids content can be converted into biodiesel via dual step transesterification process. In the first step, the oil is treated by an acid dissolved in methanol to reduce FFA content,

whereas in the second step the preheated oil is transesterified with methanol in the presence of base catalyst to form ester and glycerol [21].

The [22] US Department of Energy 2004 indicates that a perfect biodiesel should only comprise mono-unsaturated fatty acids. For instance, the pour point and cloud point temperatures, cetane number and the iodine index depend on the number of unsaturation and the length of the fatty acid chains. A higher content of double-covalent bonds gives a lower solidification point and a higher iodine index [23]. Alcohols that can be used in biodiesel production are those with short chains, including methanol, ethanol, butanol, and amylic alcohol. The most widely used alcohols are methanol (CH₃OH) and ethanol (C₂H₅OH) because of their low cost and properties. Methanol is often preferred to ethanol in spite of its high toxicity because its use in biodiesel production requires simpler technology; excess alcohol may be recovered at a low cost and higher reaction speeds are reached. The alcohol-to-oil volume ratio, R (ratio), is another key variable of the transesterification process. 1 mol of oil to react requires 3 mol of alcohol, to obtain 3 molecules of fatty acids methyl esters (FAME) and 1 mol of glycerin. However, since the reaction is reversible, excess alcohol as a reactant will shift the equilibrium to the right side of the equation, increasing the amount of products. Although a high alcohol-to-oil ratio does not alter the properties of FAME, it will make the separation of biodiesel from glycerin more difficult, since it will increase the solubility of the former in the latter [14].

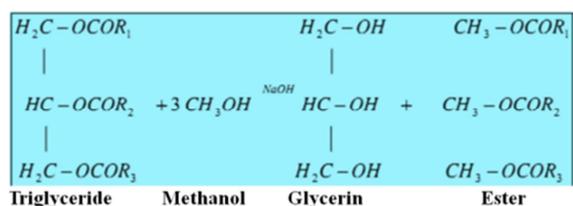


Figure 3. Equation of esterification reaction.

In summary the efficiency of the reaction diminishes with the increase of the acidity of the oil; basic transesterification is viable if the content of free fatty acids (FFAs) is less than 2%. In the case of highly acidic raw materials an acid transesterification is necessary as a preliminary stage, to reduce the level of FFAs to the above-mentioned value [24]. Besides having low humidity and acid content, it is important that the oil presents a low level of non saponification substances. If the latter were to be present in significant amounts and soluble in biodiesel, it would reduce the level of esters in the product, making it difficult to comply with the minimum ester content required by the standards [25]. The AOCS standards list the required properties of oils. Anyway, the properties required by the oils are finally determined by the biodiesel industry in each country [26].

The separation of reaction products takes place by decantation: the mixture of fatty acids methyl esters (FAME) separates from glycerin forming two phases, since they have different densities; the two phases begin to form immediately

after the stirring of the mixture is stopped. Due to their different chemical affinities, most of the catalyst and excess alcohol will concentrate in the lower phase (glycerin), while most of the mono-, di-, and triglycerides will concentrate in the upper phase (FAME). The mixture of fatty acids methyl esters (FAME) obtained from the transesterification reaction must be purified in order to comply with established quality standards for biodiesel. Therefore, FAME must be washed, neutralized and dried. Successive washing steps with water remove the remains of methanol, catalyst and glycerin, since these contaminants are water-soluble. Care must be taken to avoid the formation of emulsions during the washing steps, since they would reduce the efficiency of the process. The first washing step is carried out with acidified water, to neutralize the mixture of esters. Then, two additional washing steps are made with water only. Finally the traces of water must be eliminated by a drying step. After drying, the purified product is ready for characterization as biodiesel according to international standards [14].

Objective of the study

The present study aim to produce the biodiesel from *Citrullus colocynthis* oil by transesterification reaction, detect the physicochemical properties of produced biodiesel and compare them with standard values of biodiesel.

2. Materials and Methods

2.1. Sample

Citrullus Colonythis seeds oil

2.2. Reagents and Solutions

Sulfuric acid, potassium iodide, sodium thiosulfate, glacial acetic acid, ethanol, sodium hydroxide, phenolphthalein solution, potassium thiosulphate, iodine trichloride, tetrachloride, starch, potassium hydroxide, normal saline, diethyl ether, hydrochloric acid, methanol, normal hexane and acetone.

2.3. Equipments

Conical flask, burette, magnetic stirrer, beaker, pipette, separation funnel, Bottles, sylender, metal dishes and air condenser.

2.4. Apparatus

Gas chromatography mass spectroscopy, refractometer, Livobond tintometer, viscometer, soxhlite, heater, pour and cloud point instrument, density meter, Pensky-Martens Closed Cup Apparatus (automated), Colorimeter and MCR instrument.

2.5. Methods

2.5.1. Preparation of the Sample

The seeds were isolated from *Citrullus colocynthis* fruits and then it was dried in shade, 1500g from *Citrullus colocynthis* seeds were weighted and crushed by moulinex

grinder into powder.

2.5.2. Extraction of the Oil

1500g of the crushed seeds were placed in a soxhlet apparatus using 500 mL of hexane, heated at 40°C for to 5 hours. Then mixture was evaporated, the solvent was condensed and returned to the sample tube, and with repeated evaporation and condensation the oil was extracted, at the end of extraction hexane was removed by air at room temperature, and the oil was collected and yield percent was calculated as follows:

$$\text{Yield\%} = \frac{\text{weight of oil}}{\text{weight of seeds}} \times 100$$

2.5.3. Biodiesel Production

The oil was filtered to remove suspended particles, and then 100ml of oil poured in bottle with cover heated in heater with magnetic stirrer in 135c° to remove water for 15 minutes. In 50ml beaker a solution of sodium ethoxide was prepared using 0.7g of sodium hydroxide pellet with the molar ratio 1: 6 ethanol (20ml). The solution was stirred until the sodium hydroxide pellet was completely dissolved (the mixture was called sodium ethoxide solution). The solution was then heated low to 60 c° and slowly poured into preheated oil. The mixture was stirred (650rpm) vigorously for 60min, then the mixture was allowed to settle for 24 hours in a separating funnel thereafter, upper layer biodiesel was decanted into a separate beaker while the lower layer which comprised glycerol and soap was collected from the bottom of separating funnel. Was washed to remove any excess glycerol and soap from the biodiesel with hot acidic water, and then allowed it to remain in separating funnel until clear water was seen below the biodiesel in the separating funnel. The washed biodiesel sample was dried by placing it on a hot plate to remove excess water.

2.5.4. Methods of Standard Tests for Biodiesel

i. Flash Point

A brass test cup of specified dimensions was filled to the inside mark with test specimen and fitted with a cover of specified dimensions then was heated and the specimen stirred at specified rates. The temperature of the test cup and test specimen was being 18°C below the expected flash. The heat was applied at such a rate that the temperature, as indicated by the temperature measuring device, increases 5 to 6 °C/min. The test flame was lighted, and adjust it to a diameter of 3.2 to 4.8 mm directed into the test cup at regular intervals with simultaneous interruption of the stirring, until a flash was detected. The observed flash point was recorded on the temperature measuring device at the time ignition source application causes a distinct flash in the interior of the test [27].

ii. The color

The sample container was filled to a depth of at least 50 mm with distilled in the compartments of the colorimeter through which the standard glasses were observed. Then the sample placed in its container in the other compartment and the containers was covered to exclude all exterior light. The

light source switched on and the color of the sample compared with that of the standard glasses [27].

iii. The density

A small amount (0.7mL) of sample was introduced into the clean, dry sample tube of the instrument using a suitable syringe. The external TFE-fluorocarbon capillary tube was plugged into the lower entry port of the sample tube while the other end of the capillary immersed in the sample and applied suction to the upper entry port using a syringe until the sample tube was properly filled. The illumination light was turned on and examined the sample tube carefully. Surely there was no bubbles are trapped in the tube, and that it was filled to just beyond the suspension point on the right-hand side and the sample became homogeneous. Then the illumination light was turned off immediately after sample introduction, because the heat generated can affect the measurement temperature, after that the density was read to four significant figures [28].

iv. Pour Point

The test sample was poured into the test jar to the level mark, immediately the test jar closed with the cork carrying the high cloud and poured thermometer, Adjust the position of the cork was adjusted and thermometer so the cork fitted tightly, the thermometer and the jar were coaxial, and the thermometer bulb was immersed to a depth that places the beginning of the capillary 3 mm below the surface of the test specimen. The test jar was kept with the test specimen at room temperature (between 18 and 24°C) for least 24 h. As soon as the test specimen was reached the required temperature, the cork carrying the thermometer was removed and stir the test specimen was stirred gently with a spatula. The cork carrying the thermometer was putted back in place. The disk placed in the bottom of the jacket and the disk and jacket were placed in the cooling medium a minimum of 10 min before the test jar was inserted. The test jar was removed from the water bath and dried with a tissue. The gasket was placed around the test jar, 25 mm from the bottom and the test jar was inserted into the jacket of the first bath maintained at 21°C then observations for pour point was commenced [29].

v. Carbone Residue

Determine the mass of each clean sample vial being used in the sample analysis, and record the mass to nearest 0.1mg, was take representative sample of the stock. Stir the sample to be tested. Samples that were homogeneous liquids could be transferred directly to vials using a rod, syringe, or eyedropper, transfer an appropriate mass of the sample into a tarred sample vial, reweigh to nearest 0.1 mg, and record. The loaded sample vials was placed into vial holder, noting position of each sample with respect to index mark processing of samples, with the oven at less than 100°C, the vial holder placed into the oven chamber and secure lid, then was purged with nitrogen for at least 10 min at 600 ml/min. The purge was decreased to 150mL/min and the oven heated slowly to 500°C at 10°–15°C/min, the oven held at 500±2°C for 15 min. Then furnace power was shut off and allowed oven to cool freely while under nitrogen

purge of 600 ml/min. When oven temperature is less than 250°C, the vial holder was removed for further cooling in desiccators. After the samples were removed from the oven, the nitrogen purge was shut off. The oven opened only after oven temperature falls below 250°C during the cooling step. Maintain nitrogen was flown until after the vial holder had been removed from the oven. Sample vials were transferred (maintained in place in the vial holder) to desiccators and allowed the vials to cool at room temperature. The cooled vials were weighed to the nearest 0.1 mg and record [29].

vi. The Viscosity

The viscometer bath was adjusted and maintained at the required test temperature within the 15-100°C taking account of the conditions and of the corrections supplied on the certificates of calibration for the thermometers. Thermometers was held in an upright position under the same conditions of immersion as when calibrated, In order to obtain the most reliable temperature measurement, it was recommended that two thermometers with valid calibration certificates be used, They viewed with a lens assembly giving approximately five times magnification and was arranged to eliminate parallax errors, Select a clean, dry, calibrated viscometer having a range covering the estimated kinematic, the flow time was less than 200s, When the test temperature was below the dew point, fill the viscometer in the normal manner. To ensure that moisture did not condense or freeze on the walls of the capillary, draw the test portion into the working capillary and timing bulb, place rubber stoppers into

the tubes to hold the test portion in placed, and inserted the viscometer into the bath. After insertion, allowed to the viscometer reach to bath temperature, and then removed the stoppers. When performing manual viscosity determinations did not use those viscometers which cannot be removed from the constant temperature bath for charging the sample portion. The use of loosely packed drying tubes affixed to the open ends of the viscometer is permitted, but not required [30].

3. Results and Discussion

3.1. Percent of *Citrullus Colocynthis* Seeds Oil Extract

The *C. Colocynthis* oil was extracted by using soxhlet apparatus through solvent extraction process (hexane). Percentage of oil yield was 18.915%, it so close and somewhat close to some international studies results and relatively different from others. These differences in the extract percentages could be attributed to using different condition of extraction such as soil, weather, water bath instead of directing heating and a longer period of time about 7 hours.

3.2. GC-MS result of *C. Colocynthis* Oil Ethyl Ester (CCOEE)

GC-MS analysis of *Citrullus colocynthis* oil ethyl ester (biodiesel) showed 20 compounds, illustrated in Table 1.

Table 1. Chemical compounds of *Citrullus colocynthis* biodiesel.

No	Name	Ret. Time	Area	Area%
1.	Hexadecanoic acid, methyl ester	15.298	800917	1.39
2.	Hexadecanoic acid, ethyl ester	15.957	2297573	4.00
3.	9, 12-Octadecadienoic acid (Z, Z)-, methyl ester	16.945	1040495	1.81
4.	9-Octadecenoic acid (Z)-, methyl ester	16.990	621088	1.08
5.	Methyl stearate	17.209	651528	1.13
6.	Linoleic acid ethyl ester	17.551	9656002	16.79
7.	Ethyl Oleate	17.590	2219349	3.86
8.	Octadecanoic acid, ethyl ester	17.807	2022783	3.52
9.	Octadecanoic acid, 2-hydroxy-1, 3-propanediyl ester	18.744	1204736	2.10
10.	9, 12-Octadecadienoic acid (Z, Z)-	19.127	203776	0.35
11.	Eicosanoic acid, ethyl ester	19.509	69241	0.12
12.	Trilinolein	19.817	1352118	2.35
13.	12-Methyl-E, E-2, 13-octadecadien-1-ol	19.901	196759	0.34
14.	9-Octadecenoic acid, 1, 2, 3-propanetriyl ester, (E, E, E)-	20.032	488253	0.85
15.	9, 12-Octadecadienoic acid, methyl ester, (E, E)-	20.206	12975094	22.56
16.	Glycidol stearate	20.408	1314627	2.29
17.	Cyclopropane, 1, 1-dichloro-2, 2, 3, 3-tetramethyl-	21.568	14549166	25.31
18.	Glycine, N-butoxycarbonyl-, isohexyl ester	21.746	1535268	2.67
19.	9, 12-Octadecadienoic acid (Z, Z)-, 2-hydroxy-1-ethyl ester	22.489	2796769	4.86
20.	Squalene	22.817	1507312	2.62

3.3. Fuel Properties of *C. Colocynthis* Oil Ethyl Ester (CCOEE)

The fuel properties of the CCOEE were determined and compared with diesel and biodiesel of ASTM standard,

represented in the following table.

Table 2. The comparison of physiochemical of biodiesel.

Propriety	Diesel	Biodiesel	CCOEE
Density (g/m ³)	0.832	0.88	0.906

Propriety	Diesel	Biodiesel	CCOEE
Kinematic viscosity (cSt)	1.9-4.1	1.9-6	9
Calorific value (Mj/Kg)	45.5	37.27	43.82
Micro carbon residue (%)	-	0.05	0%
Flash point (°C)	52	130	55
Pour point (°C)	-	Reported	-33
API	22.3 - 31.1		23.29
Specific gravity	0.82-0.88	0.88-0.90	0.913
Acid value	-	0.5	0.6
Color	-	Reported	2.0

The density of CCOEE was 0.906 g/m^3 this result close to the limits of ASTM standard which it was 0.88 g/m^3 for biodiesel and 0.823 g/m^3 for diesel fuel. The kinematic viscosity of CCOEE measured at 50°C was $9.214.83 \text{ cSt}$, it higher than that of ASTM standard (1.6 - 6.0) and diesel fuel (1.9 - 4.1). As shown in the above table, calorific value of our study was 43.82 MJ/kg , which fall between biodiesel and diesel values (37.27 & 45.5 MJ/kg) respectively. Micro Carbone residue was (0%) it agreement with standard of ASTM (0.05% as maximum limit). The flash point of the present study biodiesel (55°C) was light higher than diesel fuel (52°C), while it was very low than that of biodiesel (130°C) which made biodiesel is safer for transportation purpose than others. The acid value of *Citrullus colocynthis* oil biodiesel produced from this work was 0.6 mg of KOH/g and it very close to the biodiesel value (0.5 mg of KOH/g). The API were 23.29 and it fall in the range of both standards ($22.3 - 31.1^\circ$). The specific gravity in this study was (0.913), it somewhat agreement with upper limit of biodiesel rather than diesel standard which their ranges were $0.88 - 0.90$ and $0.82 - 0.88$ respectively.

3.4. The Lighting Experiment

C. colocynthis biodiesel were subjected to simple practical test by compared its combustion and lighting with that from kerosene. The test was done at one time and under the same circumstances, then the following results were observed:

1. The ignition speed of kerosene was higher than that produced from *C. colocynthis* biodiesel, which make biodiesel is better in hot weather and more save for transportation.
2. The percent of CO_2 emission is higher in kerosene than that of study biodiesel and this indicate to that the biodiesel is eco friendly more than kerosene.
3. The lighting of *C. colocynthis* biodiesel is more clear and pure than kerosene.
4. Ignition period of the biodiesel is longer than kerosene.



Figure 4. Lighting when using kerosene.



Figure 5. Lighting when using produced biodiesel.

4. Conclusion

Biodiesel is a mixture of fatty acid alkyl esters; the scientific name of biodiesel *C. colocynthis* is *C. Colonythis* oil ethyl ester (CCOEE) which was obtained through transesterification reaction of *C. colocynthis* oil with alcohol. Biodiesel fuel properties and qualities of CCOEE confirmed with ASTM standard ASTM D6751 standards. The produced biodiesel has physical and chemical properties similar to that of international standard of diesel and biodiesel. The comparison of *C. colocynthis* biodiesel with kerosene was reflected it had best properties for using as fuel.

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