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Biofuel Production from *Balanite Aegyptiaca* and *Azadirachta Indica* Seeds

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Abstract— The current study highlights biodiesel and ethanol production from *Balanite aegyptiaca* and *Azadirachta indica* plants. Both plants are capable of producing the parent oil as long-chain fatty acid triglycerides showing thirty per cent yield, followed by biodiesel synthesis with high yield. *Balanite aegyptiaca* showed a higher biodiesel yield (75%) compared to *Azadirachta indica* (49%). In addition, *Balanite aegyptiaca* is lighter, showing specific gravity of 0.83 and lower viscosity (4.98 mm^2/s) relative to that of *Azadirachta indica* seed, which has viscosity and specific gravity values of 5.25 mm^2/s and 0.85, respectively. The biomass from the seeds is further used to generate bioethanol to optimize the utility of the seeds in biofuel production to maximize carbon recycling.

Index Terms— Biodiesel, Transesterification, Fermentation, *Balanite aegyptiaca*, *Azadirachta indica*

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1 Introduction

New carbon sources from biomaterials are continuously required for future energy as viable substitutes for fossil fuels. The current energy demand has resulted in the production of biodiesel and bioethanol from renewable sources that include maize. Biodiesels refer to long-chain alkyl esters of fatty acids which are synthesized by transesterification of vegetable oil with alcohol. The properties of biodiesel make it suitable to be used as an alternative source of diesel; therefore, biodiesel can be used directly or as blends in a diesel engine. Biodiesel has advantages that include properties such as biodegradability, renewability, less toxicity, and less emission of gaseous and particulate pollutants with higher cetane number than normal fossil fuels. It contributes little carbon dioxide to the atmosphere during production, and it is reported to have good fuel properties, including high flash point and good lubricity [1]. In addition, it is observed that biodiesel meets the currently increasing demand of world energy that, to a large degree, is dependent on petroleum-based fuel resources, which will be depleted in the near future if the present pattern of energy consumption continues [2].

Transesterification uses alcohols in the presence of a catalyst, which could be an acid, a base or enzyme depending on the free fatty acid content of the starting materials. The process chemically breaks the molecules of triglycerides into alkyl esters as biodiesel fuels and glycerol as a byproduct [3]. In order to push the reaction past equilibrium towards completion, excess alcohol needs to be present. For the existent biodiesel production process, vegetable oil is transesterified with homogenous base catalysis of alkali hydroxide dissolved in methanol.

The use of edible vegetable oils for the production is controversial due to a competition between food and fuel. It is also noted that with increasing demand for vegetable oils for food in recent years, it is highly unlikely to justify the utilization of edible vegetable oils for the production of biofuel [4-6].

Here we hypothesize that non-edible oils of *Balanite aegyptiaca* and *Azadirachta indica* seeds as non-edible plant oil sources can be used for biodiesel and bioethanol production. The current study aims to determine the catalytic conversion of non-edible oil sources for the production of biodiesel and for the maximizing the use of biomass.

2 Materials and Methods

2.1 Materials

Azadirachta indica trees grow wildly around Yola, North Eastern Nigeria, and the seeds were collected from trees around the town. The seeds were washed and dried in an oven (37 °C) for the oil extraction.

Balanite aegyptiaca seeds were purchased from a local market in Yola, Nigeria. The external seed pods were washed and crushed to recover the internal seed pits that were used for oil extraction.

Sodium hydroxide (99% purity, analytical grade, Acros Organics) and methanol (99.8%, analytical grade, Fluka) were purchased. Yeast strains were cultured in the Biology Department laboratory of the American University of Nigeria.

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2.2 Methods

2.2.1 Oil Extraction

A laboratory blender was used to crush the seeds into small pieces. The *Balanite aegyptiaca* and *Azadirachta indica* seeds were mechanically pressed while adding warm water (10-20 ml) to press out the oil. Solvent extraction using n-hexane was performed to enhance the recovery of oil from both types of seeds and the n-hexane was evaporated using a rotary evaporator. The collected oils from both seeds were stored for the transesterification process.

2.2.2 Transesterification of *Azadirachta indica* oil

One hundred milliliters of the *Azadirachta indica* oil was transferred into a three-necked round bottom flask. A reflux condenser and a thermometer were attached to two necks while the third neck was closed with a glass stopper. The round bottom flask containing oil and a magnetic stirrer was placed in a water bath to ensure uniform temperature. The oil was allowed to heat at a temperature of 65 °C for 30 minutes. NaOH catalyst (2% of the mass of the oil) was mixed with methanol (25 ml) in a separate beaker to form sodium methoxide base catalyst. The methoxide solution was added to the oil at 65 °C. The reaction was left for completion with continuous stirring for 1 hour while maintaining the temperature between 60 and 65 °C. The reaction products were transferred into a separating funnel and allowed to separate into two layers by adding 10 milliliters of water to wash the biodiesel. The obtained biodiesel was dried and stored for analysis. *Balanite aegyptiaca* oil (100 ml) was transesterified using the same conditions.

2.2.3 Ethanol fermentation from biomass

Thirty grams each of *Azadirachta indica* and *Balanite aegyptiaca* biomass was mixed with 100 milliliters of distilled water. The mixtures were filtered using a filter paper and a separating funnel. The biomass was sterilized in an autoclave and cultured yeast (3.5 g) was added in 50 milliliters of water. The mixture of the biomass filtrate and yeast solution were then put inside a fermentation shaker for 72 hours. Ethanol was collected through distillation (1-3% yield).

3 Results and Discussion

The percentage yield illustrates the effectiveness of the procedure in generating the desired biodiesel and ethanol product.

Azadirachta indica seed (677 g) produced the parent oil (205 g) to give 30 per cent yield.

Previous reports indicated 25 to 45 percent oil content in *Azadirachta indica* seed [7-8]. *Azadirachta indica* seed (88.7 g) produced the parent oil (43.5 g) to give 49 per cent yield.

Azadirachta indica oil consists of triglycerides and a large amount of triterpenoids, which are responsible for its bitter taste. It also contains steroids like campesterol, beta-sitosterol, stigmasterol, and a plethora triterpenoids of which azadirachtin is the most well known. The azadirachtin content varies between 300 ppm to 2000 ppm depending on the quality of the seeds. After separating the azadirachtin, which is used as insect repellant, most of the oil could well be used for biodiesel production [9].

The parent oil extraction (409.9 g) from *Balanite aegyptiaca* seeds (1404g) gave 29.2 per cent yield. The large shells of the *Balanite aegyptiaca* seeds account for the huge mass which gives a low yield of recovered oil. However, when the shells are excluded from the calculations, the oil recovery is about 47 per cent.

Biodiesel from *Balanite aegyptiaca* oil (91.5 g) produced 68.2 grams of long-chain fatty ester showing 74.5 per cent yield.

Viscosity is an intensive property that offers resistance to flow and depends on the content of the fatty acid of the oil source. Transesterification lowers the viscosity of the oils to a level comparable to standard biodiesel and petroleum diesel. The viscosities of the standards, parent oils, and biodiesels are illustrated in Table 1. A comparison of the fuels shows that the parent oil has higher viscosity compared to the biodiesels as well as the standard oil. However, biodiesel viscosity is within the acceptable range for the standard biodiesel, suggesting the suitability of using these biodiesel products in diesel engines.

TABLE 1

Comparison of Viscometric and Specific Gravity Values of Biodiesels and ASTM Standards

Fuel	Viscosity at 40 °C (mm^2/s)	Specific Gravity at 25 °C
Standard for Diesel Fuel (ASTM Standard D 975)	1.3-4.1	0.85
Standard for Biodiesel Fuel (ASTM D 6751)	1.9-6.0	0.88
Azadirachta Indica Oil	15.29	0.93
Balanite Aegyptiaca Oil	11.86	0.89
Azadirachta Indica Biodiesel	5.246	0.85
Balanite Aegyptiaca Biodiesel	4.984	0.83

Infrared spectroscopy (Nicolet IR 100 FT-IR) was used to identify functional groups in biodiesel obtained from *Azadirachta indica* and *Balanite aegyptiaca* oils. The spectra of the biodiesel obtained from both seeds showed two major peaks, at 3000- 2850 (cm^{-1}) and 1800-1650 (cm^{-1}). The first peak indicates the C-H bond of an ester alkyl group, and the second peak represents the presence of a carbonyl functional group (C=O).

High Performance Liquid Chromatography (HPLC) was used to compare the purity of the bioethanol obtained from the fermentation of the biomass of *Balanite aegyptiaca* and *Azadirachta indica* seeds. The bioethanol product was compared to pure standard ethanol, which was used as a positive control. The HPLC showed one major peak which appeared to be strong, and it eluted at a retention time of 1.345 minutes for all the bioethanol as well as the control. Bioethanol from *Balanite aegyptiaca* biomass showed a higher concentration compared to the bioethanol from *Azadirachta indica* seeds. Our data suggest that the *Balanite aegyptiaca* seed is a more viable source for the production of ethanol compared to the *Azadirachta indica* seed. Other minor peaks were observed that indicated impurities in the ethanol fermentation.

Balanite aegyptiaca belongs to the family Zygophyllaceae and is considered an underutilized species for fuel production.

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The tree can be found in Africa and in some regions of western Asia. It adapts to a variety of soil types and conditions. The tree has a narrow shape and has a height of about 10 meters. Recent studies claimed that the finest of the trees can yield a maximum of 52 kilogram/tree of the fruits [10]. *Balanite aegyptiaca* kernel oil constitutes about 46.7 per cent based on dry weight. Previous studies highlighted that the oil is comprised of four main fatty acids, including palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2), constituting 98-100 per cent of the total fatty acids. Among the four major fatty acids, linoleic acid was the most predominant, constituting 31 to 51 per cent of the fatty acids, similar to soybean oil profile [11].

The *Azadirachta indica* tree grows naturally in southern Asia and can grow in dry subtropical and tropical areas of the world. It grows where there is limited rainfall and can survive extreme heat. It is classified under the order Sapindales, from the family Meliaceae, with the genus Azadirachta. It can grow to 30 meters, and the seed oil content varies depending on the nature of the seed. The percentage of oil content in *Azadirachta indica* seed ranges from 25 to 45 per cent. It comprises mainly triglycerides and large amounts of triterpenoid. On average, the composition of *Azadirachta indica* oil fatty acids is omega-6 linoleic acid (6-16%), omega-9 oleic acid (25-54%), palmitic acid, hexadecanoic acid (16-33%), stearic acid, octadecanoic acid (9-24%), omega-3 alpha-linoleic acid, palmitoleic acid, and 9-hexadecenoic acid. Previous reports used thermal pyrolysis as a method of producing biofuels from neem seed which showed the presence of methyl esters and other products [12]. The production of biodiesel from neem seed can be performed through extraction and transesterification of the oil. However, there is a disadvantage of biodiesel production from high free fatty acid in neem oil [13].

4 Conclusion

Biodiesel can be produced from both edible and non-edible plant seeds. *Balanite aegyptiaca* and *Azadirachta indica* seeds are inedible seeds for which the current study tested the hypothesis as to whether or not inedible seeds can generate biodiesel and bioethanol as new energy sources. Our data highlights the oil recovery capacity, percentage yield of biodiesel, and biodiesel viscosity from each of the plants. Oil recovery capacity levels are similar for both parent oils, showing *Azadirachta indica* seed at 30 per cent and *Balanite aegyptiaca* at 29 per cent. Despite its low oil content, *Balanite aegyptiaca* oil proved to have a higher biodiesel yield (74.5%) compared to a lower yield of 49 per cent by *Azadirachta indica*. In addition, *Balanite aegyptiaca* is lighter, showing specific gravity of 0.83 and has a lower viscosity (4.983 mm^2/s) compared to that of *Azadirachta indica* seed, which has viscosity and specific gravity values of 5.246 mm^2/s and 0.85, respectively. Although its oil retrieval capacity is low, *Balanite aegyptiaca* oil yields a lighter biodiesel and also a higher production potential compared to *Azadirachta indica*. Our data suggest that *Balanite aegyptiaca* is a better alternative starting material for biodiesel production relative to *Azadirachta indica*. Finally, our study demonstrates that ethanol fermentation of biomass after biodiesel production will maximize the carbon recycling as a new energy source.

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