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Bioethanol Production from Seeds and Leaves of Drumstick Tree (*Moringa Oleifera*)

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Abstract

This study undertakes the comparative analysis of bioethanol production from *Moringa oleifera* seeds and leaves at pH 6, 7, and 8 by the action of *Aspergillus niger* and *Zymomonas mobilis*. The *Moringaceae* is a single genus family of oilseed tree with 10 known species which is fast growing, drought tolerant; tolerates poor soil and a wide rainfall range. It was well known for its multipurpose attributes and ease of establishment. Its leave, pods, and flower are parked with nutrients important to both human and animals.

The samples were collected and milled to powdered form, pretreatment with dilute hydrochloric acid, enzyme hydrolysis with fungi *Aspergillus niger*, reducing sugar with dinitrosalicyclic acid, fermentation with bacteria *Zymomonas mobilis*, distillation of fermented broth at 78°C. The sugar content of *M. oleifera* leave (1.8%) at p^H 6 was higher than the sugar content in *M. oleifera* seed (1.6%) at p^H 6. The average concentration of bioethanol produced from M. oleifera leave (3.885 mg/l) at p^H 6 was higher than the average concentration of *M. oleifera* seed (3.774 mg/l) at p^H 6.

The quantity yielded from *M. oleifera* leave (57.0 g/l) at p^H 6 produced higher than *M. oleifera* seed (32.1 g/l) at p^H 6. Maximum yield of bioethanol could be produced through *M. oleifera* leaves at p^H 6 (57.0 g/l) and with average concentration of bioethanol produced from *M. oleifera* leave (3.885 mg/l) at p^H 6.

Key words: *Bioethanol; Comparative Analysis Moringa oleifera seeds; Moringa oleifera leaves*

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Introduction

Biofuels, simply, are fuels that are derived from agricultural products. Biofuels are a renewable source of energy that can be used in many applications, from fueling your vehicle, to generating electricity and heating your home. Recently, biofuels have been attracting attention from various sectors. Biofuels are considered to be carbon-neutral, which simply means that the amount of carbon dioxide which is created when burned is equal to the amount of carbon dioxide used during growing (Admin, 2009).

It is hoped to be a green alternative to petroleum, which has generated a lot of interest from investors and different countries around the World. In 2007, investment into biofuels production capacity exceeded $4 billion worldwide, while in 2008, they provided 1.8% of the world's transport fuel and is growing (UNEP, 2009). According to the International Energy Agency (IEA), biofuels have the potential to meet more than a quarter of world demand for transportation fuels by 2050 (Meghan, 2011).

At the beginning of the 20th century, Henry Ford considered ethanol the most efficient means to fuel his Otto engines. However, gasoline soon edged out ethanol as the top consumer choice for automotive fuel, mainly due to its abundance and lower cost. When crude oil started to be used as a political weapon with dramatic rises in costs, several economies dependent upon crude oil started to suffer with negative trade balances, and the old idea to use ethanol as a gasoline substitute reemerged as one possible solution to the recovery of the Brazilian economy.

The idea of producing ethanol to replace oil imports is an old one; in 1931 the government created legislation that allowed blends of ethanol in gasoline (gasohol) of up to 40% (E-40) and encouraged the sugarcane industry to produce as much ethanol as it could (Kovarik, 2007). Bioethanol is not only cheap and environmentally friendly, but can also help the country to improve their economic gains by producing their own fuel from renewable source.

Even though many fast growing trees and shrubs have been considered as ethanol producers, *Moringa oleifera* is considered a better candidate because of its easy production biologically using microorganisms' such as bacteria and fungi (Meghan, 2011). Traditional ethanol industries utilize crops such as maize and sugar cane as a substrate in the production of ethanol; however this raises the cost of production and renders the resulting bioethanol uncompetitive. The use of these crops will lead to competition with their use as food.

Many investigations have been performed to find low cost as well as high efficient substrate such as waste product for bio-ethanol production but research into the use of tree such as drumstick tree has not been investigated. The main aim of the work is to produce ethanol from *moringa oleifera* seeds and leaves and to compare their main yield.

- To produce bio-ethanol by fermentation and hydrolysis using Zymmonas mobilis and Aspergillus niger from drumstick tree.
- To determine the physio-chemical quantities of the ethanol such as concentration, density, and viscosity.
- To determination of percentage yield of the samples under specific variables.

Materials and Methods

Sample of moringa oleifera seeds were obtained from Sokoto central market, Sokoto state. Young leaves of moringa oleifera were collected from botany garden, botany department Usmanu Dan Fodiyo, Sokoto. The collection of the fruits of moringa and drying involves stimulation and opening of the fruits to obtain seeds embedded inside. The seeds were separated from the chaffs and other impurities. This preparation was important, since any impurity in the seed will eventually reflect on the oil during extraction (Oyeleke., *et al.* 2012).

Sample Preparation

This involves the drying, reduction in size and weighing. Drying of the seeds involves thorough cleaning; dried in an electric oven at 45°C to reduce the moisture content of the seeds. Moringa oleifera seeds were than milled into a paste using the local miller. This operation ruptures the cell wall and releases the solutes for direct contact with the solvent during equilibrium process. The weight was done before and after the seed was dried using an electronic weighing balance (Handa, 2008).

Harvested young leaves of moringa oleifera were washed and dried for seven days under the shed at room temperature to avoid loss of active compounds. The dry leaves were milled using local hand milling machine and weighed on electronic weighing balance. The powdered sample was stored in a glass bottle (Handa, 2008).

Isolation of *Aspergillus niger* used for Enzyme Hydrolysis

Aspergillus Niger was isolated from a soil sample that was collected in Usmanu Dan Folio University Complex, 9 ml of distilled water each was dispensed into three test tubes labeled 10^{-1}, 10^{-2} and 10^{-3} and was autoclaved at 121°C for 30 minutes, after autoclaving, it was then allowed to cool. One gram of the soil sample was measured and transferred into the test tube labeled 10^{-1} and shacked vigorously and serial dilution was carried out using a sterile syringe. It was then transferred directly into petri plates containing PDA using a sterile syringe, this was done aseptically and the petri plates were kept for 5-7 days for growth of the fungi to occur before it was subcultured to obtain pure isolate (Cheesbrough, 2003).

Isolation of *Zymomonas mobilis* used in Fermentation

The isolation of *zymomonas mobilis* from Roselle juice (*Habiscus subdariffa*) is carried out according to the method described by Obire (2005). The sample was washed and then cooked to obtain the juice. The juice is serially diluted from test tubes $1x10^{-1}$ - $1x10^{-5}$. Then 0.1 ml aliquots from the tubes are plated onto the MYPGA (Malt, Yeast, Peptone, and Glucose Agar) medium using spread plate techniques. Each medium is treated with Nyastin to inhibit yeast growth; the plate are inoculated in an anaerobic jar in which gas pack sachet is placed to exhaust the oxygen in the jar and incubated at 37°C for one day.

Colonies suspected to be those of zymomonas mobilis is characterized on the basis of their cultural and morphological characteristics. The isolation were purified by streaking on freshly prepared media of MYPGA (Malt, Yeast, Peptone, Glucose Agar) and inoculated for one day at 37°C in an anaerobic jar (Obire, 2005).

Bioethanol Production Process

The methods used for bioethanol production involves; pretreatment, enzyme hydrolysis, determination of reducing sugar, fermentation and distillation process.

Dilute Acid Pre-treatment

Fifty gram (50g) each of the substrate are taken individually and treated with 600 ml of 1% dilute hydrochloric acid separately. Both the samples are kept at room temperature overnight. The samples were thoroughly washed with distilled water to remove traces of alkali and acids present, (if any). The samples are then placed in hot air oven and dried at 45°C for about 2-3 days in order to remove the moisture (Mohit., *et al*. 2011).

Enzyme Hydrolysis

Aspergillus niger is cultured and used to hydrolyze the moringa oleifera seed and leaves powder. Ten gram (10g) each seed and leave powder is weighed and mixed with 100 ml of distilled water into three sterile conical flasks to dissolve the substrate and adjusted using p^H meter to acidic, neutral and basic. A sterilized wire loop is used to pick the *A. Niger* growth and is aseptically inoculated into each of the solution of the conical flask respectively and stirred thoroughly with a sterile glass rod, so as to enable the *A. Niger* get access to the substrate. The flask are well covered with the aid of a cotton wool and aluminum foil and left on the bench for seven [7] days and shaken well on daily basis. The hydrolysis solution is filtered using muslin cloth after seven days (Damaso., *et al*. 2004).

Determination of Reducing Sugar

The reducing sugar content of hydrolyzed *moringa oleifera* seed and leave will be determined using the dinitrosalicyclic acid colorimeter method of Miller (1959) with glucose as standard. It will be assayed by adding 2 ml of 3, 5-DNS reagent to 1 ml of sample. The mixture will be heated in boiling water for 5 minute to develop the red-brown color.

Then 1 ml of 40% potassium sodium titrate solution will be added to stabilize the color, it will then be cooled to room temperature under running tap water. The absorbance of the samples will be measured at 540 nm using ultraviolet (UV-VIS) spectrophotometer. The reducing sugar content will be determined by making reference to a standard curve of known glucose (Miller, 1959).

Absorbance of sample

$$\text{Concentration of reducing sugar} = \frac{\text{Concentration standard}}{\text{Absorbance of glucose}}$$

Where concentration standard is = 0.02 mg/ml

Absorbance of glucose is = 0.183

Fermentation Medium

The supernatant from the above hydrolysis process is transferred into another sets of conical flasks correctly labeled. A sterile wire loop is used to pick fresh growth of zymomonas mobilisis aseptically inoculated into the set of flask. The flasks are corked using cotton wool, shake and incubate at temperature 45°C for five days. The flasks are shaken at interval to produce a homogenous solution and even distribution of the organisms in the substrates' mixture (Oyeleke and Manga, 2008).

Distillation

This was done using the procedure described by Oyeleke and jibrin (2009). The fermented samples are poured into two separate Macrokjelder flasks. They are appropriately labeled to reflect the samples. The whole contents of the fermented are emptied into the flask. The flasks containing the samples are placed on the heating coils. Two conical flasks containing 250 mls are directly placed under heating coils.

The samples are heated to 78°C, glass and rubber tubes are fitted to the mouth of the Macrokjelder flask to the empty conical flask. This is done to collect the evaporated samples. This process is followed in collecting the ethanol. The whole process is arrested when samples in the flasks begin to bubble. It is further confirmed when the ethanol stop to drop into empty flasks (Oyeleke and jibrin, 2009).

Determination of Density of Ethanol

Measured 10 ml from each sample, weighed it and divide by the volume measured.

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

Determination of Quantity of Bioethanol Production

The distillate collected is measured using a measuring cylinder and expressed as quantity of ethanol produced in g/l by multiplying the volume of distillate by the density of the ethanol (0.8033 g/cm^3) (Oyeleke and Jibrin, 2009).

Determination of Bioethanol Concentration

This was carried out using ultra violet-visible quantitative analysis of alcohols using chromium VI reagent according to the methods described by Oyeleke and Jibrin (2009). 1 ml of standard ethanol is diluted with 100 ml of distilled water to give a concentration 1%. Then 0,2,4,6 and 8ml each of 1% ethanol is diluted to 10 ml with distilled water to produce 0, 0.2, 0.4, 0.6 and 0.8ml of the ethanol. To each varying ethanol concentration 2 ml of chromium reagent is added and allowed to stand for an hour for color development.

The absorbance of each concentration is measured at 540 nm using an ultra violet-visible spectrophotometer and the readings used to develop standard ethanol curve. Then 5 ml of each bioethanol sample are put in test tubes and treated with two drops of chromium reagent. The mixture is allowed to stand for an hour and the absorbance is measured at 540 nm using the ultra violet-visible spectrophotometer (Oyeleke and Jibrin, 2009).

$$\text{Average concentration} = \frac{\text{Total concentration}}{\text{Number of replicate}}$$

$$\text{Standard deviation} = \frac{\sqrt{\sum x - (\sum x)^2}}{x}$$

$\sum x$ = total concentration

x = number of replicates

Data Analyses

Statistical analysis was done using Microsoft Excel and SPSS 21 for windows (SPPS Inc., Technologies) The SPSS statistical package (Version 21.0) was used for all statistical analysis. Statistical analysis of frequency of percentage yield of the two samples, standard curve of concentration and standard reducing sugar curve.

Results and Discussions

From the experiment, it was observed that the reducing sugar content produced from *M. oleifera* seed and leave after hydrolysis varied, which was carried out using UV visible spectrophotometer at wavelength of 540 nm. Pretreatment was required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monometric sugars can be achieved more rapidly and with greater yields (Sun and Cheng, 2002). The seed and leave were hydrolyzed in three different containers and at varying p^H of 6, 7 and 8. It implies that the reducing sugar content in *M. oleifera* leave at p^H 6 (1.8%) was higher than the content in *M. oleifera* seed at p^H 6 (1.6%) this could be as a result of low carbohydrate in the *M. oleifera* seed.

S/n	pH	Reducing sugar (mg/ml)	Concentration (mg/l)	Quantity (g/l)	Density (g/ml)	Viscosity (mpas)
1	6	0.016	3.774	32.132	0.866	0.5
2	7	0.002	5.390	24.099	0.880	0.6
3	8	0.004	1.382	20.885	0.847	0.6

Table 1: *Physico-chemical features of bioethanol produced by M. oleifera seed.*

S/n	pH	Reducing sugar (mg/ml)	Concentration (mg/l)	Quantity (g/l)	Density (g/ml)	Viscosity (mpas)
1	6	0.018	3.885	57.034	0.841	0.5
2	7	0.006	6.212	44.984	0.871	0.6
3	8	0.004	0.677	63.460	0.887	0.6

Table 2: *Physico-chemical features of bioethanol produced by M. oleifera leave.*

It was observed from the above result at the period of 5-7 days of fermentation the concentration of bioethanol produced from *M. oleifera* seed and leave varies according to the method described by Oyeleke and Jibrin (2009) using the bacteria *zymomonas mobilis*. The average concentrations of bioethanol produced from *M. oleifera* leave (6.212 mg/l) at pH 7 was higher than the average concentration of *M. oleifera* seed (5.390 mg/l) at pH 7.

This could be as a result of the bacteria zymomonas mobilis been resistance to the effect of bioethanol in *M. oleifera* seed than in *M. oleifera* leave. According to Oyeleke and Jubril, who reported percentage of bioethanol concentration of 67.7% and 63.8% as recorded when *A. Niger* and *Z. mobilis* were used simultaneously on guinea corn husk and millet husk respectively.

It revealed that the quantity of ethanol yielded after distillation of fermented *M. oleifera* seeds and leaves as described by (Huili., *et al*. 2009) implies that the quantity yield from *M. oleifera* leave at pH 8 (63.4 g/l) produce relatively higher quantity than the highest quantity produced from *M. oleifera* seed at pH 6 (32.1 g/l), because moringa oleifera seeds contain between 33 and 41% w/w of vegetable oil as obtained by (Rabiu 2006) which was clear that *M. oleifera* leave produced higher ethanol than *M. oleifera* seed.

It was volatile, miscible in both water and non-polar solvents at ordinary conditions and has a density of 0.792 g/cm^3 at 15.5°C (Purwadi, 2006; Thomsen., *et al*. 2008). The closest density of bioethanol produced from *M. oleifera* seeds and leaves in relation to the standard density of bioethanol was in *M. oleifera* leave (0.841 g/ml) at pH 6. This proves that *M. oleifera* leave produce ethanol at a closer density to the standard density than *M. oleifera* seed.It was observed from the result that the viscosity of bioethanol produced from *M. oleifera* seeds and leaves are relatively the same.

Conclusion

The work shows the possibility of producing bioethanol from fermentation of *M. oleifera* seeds and leaves which may serve as cheap alternative source of fuel and energy generation. Maximum yield of bioethanol could be produced through *M. oleifera* leaves at p^H 6 (57.0 g/l) and with an average concentration of bioethanol produced from *M. oleifera* leave (3.885 mg/l) at P^H 6.

Recommendation

1. Implementation of bioethanol policy can be helpful in conserving Nigeria's over stretched fossil fuel, solving some energy crises and in improving biomass feed stock towards bioethanol industries that will serve as income generation and production renewable to overcome the effect of global warming and environmental pollution.
2. Bioethanol utilization may not be the case for lower oil price, but it is hopefully a bridge towards becoming a little bit more dependent on ourselves rather than others.

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