



## Proximate Analysis and Bioethanol Production Potential of Neem Leaves (*Azadirachta indica*)

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### ABSTRACT

This paper was aimed at investigating the potential of Neem tree (*Azadirachta indica*) leaves as feedstock for bioethanol production. Dried powdered leaves of Neem tree were hydrolyzed using different concentration of H<sub>2</sub>SO<sub>4</sub> and levels of inoculums (*Bacillus firmus*). The hydrolyzed samples were fermented using yeast (*Sacchromyces cerevisiae*) and cultured bacteria (*Bacillus firmus*) respectively. The quantity of bioethanol generated after distillation was determined using U.V Spectroscopy. The amount of bioethanol produced from the leaves using 10%, 5% and 2% H<sub>2</sub>SO<sub>4</sub> and 2.0 ml, 1.5 ml 1.0 ml of inoculums levels (*Bacillus firmus*) were 3.30%, 6.52%, 5.34%, 1.72% 1.85% and 1.42% respectively. The bioethanol obtained was subjected to FTIR Spectroscopy, and the samples showed strong broad peak at 3450 cm<sup>-1</sup> – 2850 cm<sup>-1</sup> indicating the presence of exchangeable protons, typically of aliphatic alcohol. This showed that Neem tree leaves contains some appreciable percentage of sugar that can be fermented into ethanol.

**Keywords:** Neem leaves, Bioethanol, Fermentation, *Sacchromyces cerevisiae*, *Bacillus firmus*, Proximate Analysis

### 1.0 INTRODUCTION

The increase in greenhouse emissions from industries and transportation has been linked to changes in the climate. This concern, coupled with limited fossil fuel reserves, has initiated a justifiable global refocus on research and development of other energy resources (Looz *et al.*, 2009). It is a firmly established reality that bioethanol can be produced from various sources, like starch crops, sugar crops, fruit juices etc. Most of today's bioethanol production is derived from edible sources. However, perusal of literatures at our disposal exposed a dearth of research for the production of bioethanol from non-edible raw materials. Balat *et al.* (2008) suggested that the quest for non-edible raw materials is a useful and sustainable option that should be pursued, with all vigor as a good alternative to fossil fuel since crops can grow renewably in almost all climates around the world with considerable lower emissions of

poisonous gasses to our environment (Brooks 2008; Rabah *et al.*, 2011).

Utilization of non-edible plants for bioethanol production is one of the best options for the fact that the fuel production will not pose food security issues or threats. Bioethanol has become one of the most promising biofuels today and is considered as the only feasible, sustainable alternative to fossil fuel all over the world. With advanced energy saving production technology, bioethanol can considerably reduce the climate distorting greenhouse gas emissions from transport and other industries (Jayant, 2012).

The use of edible plants for biofuels production has recently been of great concern because they compete with food materials. As the demand for these plants and animals for food has increased tremendously in recent years, it is difficult to justify the use of these edible plants for fuel use purposes such as bioethanol production. Moreover, these edible plants could be more expensive to use as fuel. Hence the contribution

of plants such as Neem leaves that is not consume as a food will be significant as a non-edible plant sources raw material for bioethanol production. Bioethanol is eco-friendly and such an alternative fuel that can be used in unmodified petrol engines with current fueling infrastructure and it is easily applicable in present day combustion engine as mixture with gasoline (Hasen *et al.*, 2005). Combustion of ethanol results in low emission of volatile organic compounds, Carbon monoxide and nitrogen oxides and the emission and toxicity of ethanol are lower than those of fossil fuels such as petrol and diesel (Wyman and Hinman,1990). Production of ethanol from lignocellulosic materials has received extensive interest due to their availability, abundance and relatively low cost (Possoa *et al.*, 1997).

Biofuels production has potential advantage in improving rural and agricultural economies, benefiting agricultural and industrial sectors by using by-products such as wood washes, oil, waste products and municipal solid waste, as well as a decrease in dependence on oil imports and therefore providing some energy security (Demirbas, 2008).

Ethanol, also called ethyl alcohol is a volatile, flammable liquid. It is a straight chain alcohol, and has molecular formula of  $C_2H_5OH$ . The fermentation of sugar into ethanol was one of the earlier organic reactions. In 1808 Nicolas Theodore de Saussure determined ethanol's chemical formula (Hugh, 1989). Fifty years later Archibald Scott Cooper published the structural formula of ethanol, which placed ethanol among the first compounds whose chemical formula has been determine (Cooper, 2007). Ethanol was first prepared synthetically in 1826 through the independent efforts of Henry Henkel in Great Britain and S.G Serullas in France, in 1928; Michael Faraday prepared ethanol by acid-catalyzed hydration of ethylene, a process similar to that which is used today for industrial ethanol synthesis (Shilpa *et al.*, 2013).

Several usage of biomass feedstock for bioethanol production has been reported Fatima et al (2010) reported 5.1% ethanol yield via enzymatic saccharification from rice straw. Also in a study conducted by Pramanik (2005) with grape waste using *S. Cerevisiae* at pH 4.5 temperature 30°C ethanol productivity of

73.7g/dl was recorded. Teetrapt and Lerdluku (2006) reported yield of 3.5% ethanol from cassava waste.

Oyeleke and Jibrin (2009) also reported 25.30  $cm^3$  ethanol for guinea corn husk. Wilbur (2010) in his comparative studies of ethanol production using *K. marxianus* and *S. Cerevisiae* in orange processing waste pretreated with steam expansion demonstrated that *K. Marxianus* was capable of producing ethanol titers similar to that of *S. Cerevisiae*. However, *K. Marxianus* required a higher inoculation level than *S. Cerevisiae* to produce comparable amount of ethanol.

Similarly, Uduak *et al.* (2008) showed production of 0.86 litre of 95% bio-ethanol from 2500g of old newspapers and maize substrates by fermentation process using *S. Cerevisiae*. Nimbkar *et al.*, 2009) studied the effect of different inoculums levels on the ethanol production from unspecialized juice of sweet sorghum obtained maximum alcohol concentration of 12.45% (v/v). Rajendran and Saravana (2013) reported 4.0% yield of bioethanol obtained from agave leaves using two different enzymes (amylase and cellulase) for hydrolysis. Anbuselvi and Balamunugan (2013) also reported 5.89% yield bioethanol from cassava leaves using *S. Cereviasiae*.

This research was aimed at assessing the proximate composition and bioethanol production potential of Neem leaves.

## 2.0 MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

Neem tree leaves samples were collected under Neem trees at Usman Danfodiyo University Sokoto main campus and Kantin Daji area of Sokoto Metropolis in polythene bags and taken to the laboratory for further analysis. Sand samples were also collected from decayed Neem tree leaves from the same sites.

The leaves were air dried for two (2) days and grinded to powder using pestle and mortar and then sieved with mesh size (0.4-0.7 mm).

### 2.2 Isolation of Bacteria Species from The Decayed Sands

Exactly 1g of each of the decayed sand samples was dissolved in 9 $cm^3$  of distilled water and serial dilution were made as  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,

and  $10^{-5}$  respectively. Two lowest dilution factors were carried out  $10^{-4}$  and  $10^{-5}$  and inoculated in different sterilized petridishes containing nutrients agar medium by spreading methanol using bend glass rod and incubated at  $37^{\circ}\text{C}$  for 24hr.

After 20 hr, different colony was observed and subcultured into another sterilized petridishes by streaking methanol to obtain pure culture and then incubated for 24 hr. The pure culture was used for microscopy and biochemical characterization.

## 2.3 Proximate Analysis

### 2.3.1 Determination of Ash Content

Exactly 2g of the Neem leaves sample was placed into a clean dry crucible and heated in a muffle furnace at  $550^{\circ}\text{C}$  for 8hr and cooled in a desiccator, weighed and the ash content was calculated using equation 1 (AOAC, 1991).

$$\text{Ash\%} = \frac{W_a}{W_s} \times 100 \quad (1)$$

Where,  $W_a$  is the weight of ash and  $W_s$  is the weight of the sample

### 2.3.2 Determination of Moisture Content

Exactly 2g of the Neem leaves sample was weighed in a covered, flat, aluminium dish and dried to constant weight in a hot air oven fitted with controlled ventilation at  $110^{\circ}\text{C}$  for 24 hr and then cooled in a desiccator. The moisture content was calculated (AOAC, 1991).

$$\text{Moisture\%} = \frac{W_{fs} - W_{ds}}{W_{fs}} \times 100 \quad (2)$$

Where,  $W_{fs}$  is the weight of the fresh sample and  $W_{ds}$  is the weight of the dry sample

### 2.3.3 Determination of Crude Protein

Total nitrogen was determined by the Kjeldahl method and the result multiplied by 6.25 to give crude protein (Hames *et al.*, 2008).

$$\text{Nitrogen\%} = \frac{ml_{acid} \times N_{acid}}{W_s} \times 0.014 \times 100 \quad (3)$$

Where,  $ml_{acid}$  is the volume of the acid;  $N_{acid}$  is the Normality of the standard acid.

$$\text{Crude protein content (\%)} = \text{nitrogen content} \times 6.25 \quad (4)$$

### 2.3.4 Determination of Crude Fiber

Exactly 2g of the Neem sample was put into 1-litre conical flask and charged with  $\text{H}_2\text{SO}_4$  (200  $\text{cm}^3$ , 1.25%). The content was boiled for 30 minutes, filtered with poplin material, and rinsed with hot distilled water. The content was dried and scraped back into flask with spatula. To the flask,  $\text{NaOH}$  (200  $\text{cm}^3$ , 1.25%) was added, and boiled for 30 minutes, filtered through poplin cloth. The residue washed thoroughly with hot distilled water, before rinsed once with  $\text{HCl}$  (10%) and twice with industrial methylated spirit. The sample was rinsed finally three times with petroleum ether and allows to drain dry, and then the residue was scraped into a crucible. The sample was allowed to dry in the oven overnight at  $105^{\circ}\text{C}$ , and then cooled in the desiccator and weighed. The sample was ashes at  $550^{\circ}\text{C}$  for 90 minutes in a muffle furnace and cooled in a desiccator and weighed again.

$$\text{Crude fibre\%} = \frac{W_{Dresidue} - W_{Aresidue}}{W_s} \times 100 \quad (5)$$

Where,  $W_{Dresidue}$  is the weight of the crucible plus dried residue;  $W_{Aresidue}$  is the weight of crucible plus ashed residue; and  $W_s$  is the weight of the sample.

### 2.3.5 Determination of Crude Lipid

The fat content in the plant tissues are generally extracted with petroleum ether using Soxhlet extraction method. Dry extraction flask (250  $\text{cm}^3$ ) was heated in an oven at  $110^{\circ}\text{C}$ , cooled in the desiccator and weighed. 2g of the sample was weighed into a porous thimble and covered with clean white cotton wool; petroleum ether (200  $\text{cm}^3$ ) was added into the dry extraction flask (250  $\text{cm}^3$ ). The covered porous thimble was placed into the condenser of the extraction flask for about 6hr.

$$\text{Crude lipid\%} = \frac{W_{lipid}}{W_{sample}} \times 100 \quad (6)$$

Where, *W<sub>lipid</sub>* is the weight of the lipid and *W<sub>sample</sub>* is the weight of the sample.

### 2.3.6 Determination of Carbohydrate Composition

Carbohydrate composition of lignocellulosic biomass is one of the main factors for production of biofuel from the plant biomass. Various methods like Thermogravimetric analysis (TGA), near infrared spectroscopy (NIR) often used in determination of polysaccharide component of the plant biomass. However, used of such tools is based on the prediction and very costly. In this paper, the carbohydrate content was obtained by subtracting the sum of ash, moisture, crude fiber, crude protein, and crude fat from 100.

$$\text{Carbohydrate\%} = (\text{Ash} + \text{Moisture} + \text{Fibre} + \text{Protein} + \text{Lipid}) - 100 \quad (7)$$

## 2.4 HYDROLYSIS

### 2.4.1 Chemical Hydrolysis

Exactly 30g of the powdered leaves of Neem sample were put into three conical flasks (500 cm<sup>3</sup>) and H<sub>2</sub>SO<sub>4</sub> (300 cm<sup>3</sup>, 10%) was added. The flasks were plugged with cotton wool and wrapped in aluminium foil and sterilized at 121°C for 15 minutes and allowed to cool. The procedure was repeated using 5% and 2% H<sub>2</sub>SO<sub>4</sub>. The content of each flask was filtered and pH was then adjusted to 4.5 using NaOH for fermentation (Akpınar *et al.*, 2009).

### 2.4.2 Enzymatic Hydrolysis

Exactly 30g of the powdered leaves of Neem sample were put into three conical flasks (500 cm<sup>3</sup>) and distilled water (300 cm<sup>3</sup>) was added. The flasks were plugged with cotton wool and wrapped in aluminium foil and sterilized at 121°C for 15 minutes and allowed to cool. Each flask was inoculated with suspension of *Bacillus firmus* (1.0 cm<sup>3</sup>). The procedure was repeated using 1.5cm<sup>3</sup> and 2.0 cm<sup>3</sup> of *Bacillus firmus* suspension. The flasks were inoculated at 37°C for five (5) days. After the five (5) days, the content of each flask was filtered and pH was then adjusted to 4.5 using H<sub>2</sub>SO<sub>4</sub> for fermentation (Zhao *et al.*, 2012).

## 2.5 Determination of Reducing Sugar

The glucose standard was prepared by dissolving 1g of glucose in distilled water (100 cm<sup>3</sup>) and the portion (10 cm<sup>3</sup>) was pipetted and transferred into volumetric flask (100 cm<sup>3</sup>) and made to the mark using distilled water. Series of standards were then prepared by pipetting 0, 0.3, 0.9, and 1.2 cm<sup>3</sup> from second standard glucose into five clean test tubes. The volume was then diluted by adding 3, 2.7, 2.4, 2.1, and 1.8 cm<sup>3</sup> distilled water to each test tube. To each test tube Dinitrosalicylic acid (DNS) (3 cm<sup>3</sup>) reagent was added. Each of the test tubes was placed in boiling water bath for 10min to develop red brown color. Then potassium sodium titrate (1 cm<sup>3</sup>, 40%) solution was added to stabilize the color. The mixture was cooled at room temperature and the absorbance was measured at 540 nm with UV-Vis spectrophotometer.

Determination of sugar was done by adding Dinitrosalicylic acid (DNS) (3 cm<sup>3</sup>) solution to each filtrate sample (3 cm<sup>3</sup>). The mixture was heated in a boiling water bath for 10 minutes to develop color. The mixture was allowed to cool at room temperature. The absorbance of the portion of the mixture from each test tube was measured at 540 nm using UV-VIS Spectrophotometer. The reducing sugar content was subsequently determined by referring to standard curve of known glucose concentration (Miller, 1959).

## 2.6 Fermentation

The fermentation was conducted as described by Ohgren *et al.* (2006). The hydrolyzed filtrate (250 cm<sup>3</sup>) from each flask was covered with cotton wool wrapped in aluminium foil, the sterilized at 121°C. The flasks were then cooled and the pH of each flask was adjusted to 4.5 with NaOH. The samples were inoculated with Beaker's yeast (*Sacchromyces cerevisiae*) (3g) and incubated at 37°C for five days. The procedure was repeated using suspension of culture bacteria (*Bacillus firmus*) (3cm<sup>3</sup>).

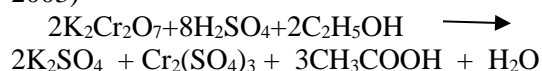


## 2.7 Distillation

Each conical flask containing the fermented liquor was dispensed into round bottom flask, which was fixed to a distillation column enclosed in running tap water. A heating mantle with temperature adjusted to 78.3°C was used to heat the flask and the distillate collected in a clean conical flask as the bioethanol.

## 2.8 Qualitative Test for Ethanol

The distillate (2 cm<sup>3</sup>) was put into a test tube, 2 drops of acidified K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (0.1M) was added, heated for 30 minutes on a water bath. The content of the test tube changed to green color indicating the presence of ethanol. The equation of the reaction is represented below (Raymond, 2003)



## 2.9 Determination of Ethanol Concentration

This was carried out using UV-VIS quantitative analysis of alcohol using chromium (vi) reagent according to method described by Oyeleke and Jibrin (2009). Absolute ethanol (1 cm<sup>3</sup>, 98% v/v) was diluted to 100 cm<sup>3</sup> using distilled water to give a concentration of 1% (ethanol stock solution). Series of standards were prepared by pipetting 0, 2, 4, 6 and 8 cm<sup>3</sup> of the 1% stock solution and diluted to 10 cm<sup>3</sup> with distilled to produce 0.0, 0.2, 0.4, 0.6 and 0.8% of the ethanol. To each of the varying ethanol concentration, chromium reagent solution (2 cm<sup>3</sup>) was added. The content of each test tube was then heated in a boiling water bath for five (5) minutes, for full color development. The absorbance of each concentration was measured at 585 nm using UV-VIS Spectrophotometer and the reading was used to plot Standard ethanol curve.

The sample (5 cm<sup>3</sup>) was taken in the test tube, and then Dichromate reagent (2 cm<sup>3</sup>) was added. The content of each test tube was then heated in a boiling water bath for five (5) minutes, for the reaction to complete and develop color. The absorbance of each concentration was measured at 585nm, using U-V Visible Spectrophotometer (Rabah *et al.*, 2011).

## 2.10 Fourier Transforms Infrared Spectroscopy (FTIR)

The sample of bioethanol produced was subjected to FTIR Spectroscopy to confirm the presence of alcohol content in the sample. Ethanol sample (3 drops) were put on the plate, and another plate was placed on top and tight to obtained a nice even film. The plate was put in the machine and run.

## 3.0 RESULTS AND DISCUSSION

The results of analysis for proximate composition, the amount of reducing sugar and bioethanol produced are presented in Tables 1 and 2. The results suggest that Neem leaves contain appreciable amount of sugars, which can be used for bioethanol production.

### 3.1 Proximate Analysis

The proximate composition of the sample was analyzed and shown in Table 1. Moisture content (9.0%), ash (16.50%), crude lipid (5.80%), crude fibre (19.13%), crude protein (12.80%) and carbohydrate (45.60%). Esonu *et al.* (2005); Oforjindu (2006); Oyeleke (2009) reported a crude fibre content of 16.60% from Neem leaves meal, which is closed to the results obtained in this research. This suggests that Neem leaves contain appreciable amount of sugar for bioethanol production.

Table 1: Proximate Composition of Neem Leaves

Parameters	Result (%) Dry weight
Ash	16.50 +/- 0.30
Moisture	9.00 +/- 0.60
Crude Lipid	5.80 +/- 0.25
Crude Protein	12.80 +/- 0.15
Crude Fibre	19.31 +/- 0.21
Available Carbohydrates	45.60 +/- 0.30

### 3.2 Reducing Sugar

The amount of reducing sugar produced from Neem leaves indicate that using 5% H<sub>2</sub>SO<sub>4</sub> gives the highest amount of reducing sugar (3.20g/kg), followed by 2% (1.80g/kg) and 10% (1.23g/kg). The significant variation was therefore observed (p < 0.05) to suggest that 5% H<sub>2</sub>SO<sub>4</sub> would be the optimum amount required to breakdown the complex sugar contained in the Neem sample. In the enzyme hydrolysis cultured bacteria (*Bacillus firmus*) of different inoculums

suspension level was used and the result obtained in Table 2 indicate that 1.5 cm<sup>3</sup> suspension gives the highest amount of reducing sugar (2.85g/kg), followed by 2.0 cm<sup>3</sup> (1.97g/kg) and 1.0cm<sup>3</sup> (1.42g/kg). Significant variation (p<0.05) was also observed to suggest that 1.5 cm<sup>3</sup> would be the optimum amount required for hydrolysis of cellulosic biomass in to sugar. There will be no significant change in yield of reducing sugars if either 1.0 cm<sup>3</sup> or 2.0 cm<sup>3</sup> of *B. Firmus* suspension is used.

**Table 2: Reducing Sugar and Bioethanol Produced from Neem Leaves**

Samples	Reducing sugar (g/kg)	Bioethanol Produced (g/kg)	% Yield
2% H <sub>2</sub> SO <sub>4</sub>	1.80+/-0.52 <sup>bc</sup>	53.41+/- 19.26 <sup>ab</sup>	5.34
5% H <sub>2</sub> SO <sub>4</sub>	320+/- 0.24 <sup>a</sup>	65.24+/-0.86 <sup>a</sup>	6.52
10% H <sub>2</sub> SO <sub>4</sub>	1.23+/-0.04 <sup>c</sup>	32.96+/-14.26 <sup>c</sup>	3.30
1.0 cm <sup>3</sup> <i>B. Firmus</i>	1.42+/- 0.05 <sup>bc</sup>	14.22+/-2.02 <sup>c</sup>	1.42
1.5 cm <sup>3</sup> <i>B. Firmus</i>	2.85 +/- 0.14 <sup>a</sup>	18.46+/-1.10 <sup>c</sup>	1.85
2.0 cm <sup>3</sup> <i>B. Firmus</i>	1.97+/- 0.20 <sup>b</sup>	17.16+/- 5.53 <sup>c</sup>	1.72

### 3.3 Bioethanol Production

The amount of bioethanol produced by acid hydrolysis was in the order 5% H<sub>2</sub>SO<sub>4</sub> was produced the highest concentration of (65.24g/kg), followed by 2% (53.14g/kg) and 10% (32.96g/kg). No significant (p > 0.05) variation was however observed using either 2% or 10%, even though there are numerical differences.

When the experiment was conducted using *B. Firmus*, 1.5cm<sup>3</sup> of inoculums suspension produced the highest concentration of bioethanol (18.45g/kg) followed by 2.0 cm<sup>3</sup> (17.16g/kg) and 1.0 cm<sup>3</sup> (14.22g/kg). No significant (P> 0.05) variation was observed using 1.0 cm<sup>3</sup> or 2.0 cm<sup>3</sup> concentration of *B. Firmus*.

The amount of bioethanol produced in Table 2 shows a linear relationship between the volume of ethanol produced and the reducing sugar. The amount of ethanol increase with increase in the mass of reducing sugar up to 5% H<sub>2</sub>SO<sub>4</sub> and 1.5 cm<sup>3</sup> enzymes, beyond this level the ethanol become toxic to the microorganism and kills them before the total sugar present in fermented (Graham and John, 2000).

The maximum yield of ethanol produced in this study by acid hydrolysis and yeast fermentation was 8.52% and the value is close to the amount

reported by Fatima *et al.* (2010) for the straw but higher than from cassava waste reported in the work of Teetrapt and Lerdluku, (2006).

When the enzymatic hydrolysis and *B. firmus* and fermentation was examined in 1.5 cm<sup>3</sup> inoculums suspension, (18.46g/kg) of bioethanol was produced and the result is in agreement with what was reported by Oyeleke and Jibrin, (2009), who obtained 25.30cm<sup>3</sup> ethanol from guinea corn husk. Nimbkar *et al.* (2009) also reported 12.45% as ethanol from unspecialized juice of sweet sorghum. Their finding agrees with this study by given maximum yield at 1.5 cm<sup>3</sup> inoculums levels. The highest yield obtained was also in agreement with what was reported by Anbuselvi and Balamurugan (2013) where 4% yield of bioethanol was obtained from Agava Leaves using *S. Cerevisiae*. Anbuselvi and Balamurugan (2013) also reported 5.89% yield of bioethanol from cassava leaves using *S. Cerevisiae*, which is closed to what was obtained in this study.

### 3.4 Fourier Transforms Infrared Spectroscopy (FTIR)

The higher yield of bioethanol obtained from both acid hydrolysis and enzymes hydrolysis were subjected to FTIR Spectroscopy, both samples shows strong broad peak at 3450-2850 cm<sup>-1</sup>, therefore indicating -CH<sub>2</sub> and -CH<sub>3</sub>

stretching vibrations and well resolved peak around  $3416\text{ cm}^{-1}$  can be assigned to alcoholic -OH vibrations. These values are in agreement with the values obtained in reference spectra - that is free O-H stretching normally occurs at  $3550\text{-}3200\text{ cm}^{-1}$ , while C-H stretch occurs at  $3000\text{-}2840\text{ cm}^{-1}$ . Therefore, the production of ethanol was successful.

#### 4. CONCLUSION

It can be concluded that in the present study acid hydrolysis produced more bioethanol than enzyme hydrolysis. The maximum bioethanol yield was recorded at 5%  $\text{H}_2\text{SO}_4$  via acid hydrolysis and fermentation with *Saccharomyces cerevisiae* which suggest that the use of acid and Baker's yeast (*Saccharomyces cerevisiae*) as fermenting agent is more suitable than *Bacillus firmus* in the production of bioethanol using Neem leaves sample.

The study showed acid or enzymes hydrolysis of Neem leaves produces high amount of reducing sugar. Further fermentation using Baker's yeast (*Saccharomyces Cerevisiae*) gives higher yield of bioethanol than the cultured Bacteria (*Bacillus Firmus*).

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