



Extraction of Ethanol from *Nypa fruticans* (Nipa) Palm Fruit

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KMRCL, FMSD and CIU are the principal investigators who designed the study. Authors MJMB, MGMC and JLE performed the sample collection. Authors JAHB, MRC and HGP performed the extraction and purification of ethanol from the nipa palm fruit. Authors FMC, TJLA and MJMB performed the quantitative analysis of the ethanol from nipa palm fruit. All authors read and approved the final manuscript.

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ABSTRACT

Northern Samar is abundant when it comes to nipa resource which has a big potential as a raw material for ethanol. Utilization of nipa in Northern Samar is not optimal due to low interest and ability to process into useful products. The main aim of the study is to extract ethanol from nipa fruit

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to control the shortage of ethyl alcohol in the province. Fermentation was carried out after collection, washing, and cutting of nipa fruit through mixing 15 g of baker's yeast to convert the sugar into alcohol for 12 days. Then, the distillation process followed to remove the excess water from the alcohol. Lastly, purification was done by adding lime (Calcium oxide) into the distillate in order to obtain ethanol. Result showed that the presence of ethanol was not observed after the 24 hours of fermentation since nipa fruit takes longer time to yield concentrated ethanol. But after 72 hours after fermentation it showed positive result from Iodoform test, Ester test, Litmus test, and Flammability test. It was concluded that nipa palm fruit could be a viable source of ethanol as compared to other plant-producing ethanol.

Keywords: Ethanol; nipa palm fruit extract.

1. INTRODUCTION

Nypa fruticans is a species adapted to mangrove ecosystem. This species is the only member of the genus *Nypa*. *Nypa* is structurally unique, lacking an upright trunk and instead having a horizontal stem with dichotomous branching that grows underground [1,2]. *Nypa* is widely distributed in the tropics, it is considered ecologically important species mangal forests and important economically for the community around the nature reserved area [3].

Ethanol is commonly produced by fermentation of carbohydrate. Biomass such as cellulose, animal fat, etc are used for production of ethanol while the main sources of biomass which is converted into ethanol are sugarcane, corn, wheat bran, cassava, sweet potato, etc. These are used for ethanol production but they are mainly used for food source and if these sources will be used for the ethanol production, the whole world is going to face food crisis as world population is increasing rapidly [4], thus this makes nipa palm a potential source of bioethanol among others. However, the general understanding on nipa palm and its utilization has so far been very limited before but there are few progress in recent years that have been made about the utilization of nipa palm [5].

Ethyl alcohol made from nipa palm is a colorless liquid, it is water-soluble, and widely used ever since. Producing ethyl alcohol from nipa is not a new idea, even before the before the Spaniards dominated the Philippines, there was a booming business of alcohol production in Luzon. Even Malaysia produced fuel from nipa that was used in automobiles. Unfortunately, it was cut short with the popularity of fossil fuel abound in the Philippines, which was considered as destructive source of energy [6].

The evolution of new ethanol production technologies could help alleviate some of the

concerns regarding the use of food for fuel by facilitating the use of non-food feedstock's, and could alleviate some of the environmental concerns associated with grain ethanol production [5]. It is expected that this bioethanol extraction from nipa palm promises to be more sustainable in the future compared to other feedstocks being used to produce ethanol since it does not need to be replanted after harvest. This is more economically and environmentally sustainable due to its lesser resource utilization.

The potential and utilization of *N. fruticans* currently faced with the problems of land use changes that threaten the existence and its sustainability. Mangrove ecosystem which becoming a major habitat of nipa gradually depleted or damaged [7]. Exploitation of mangrove areas continuously occurred will potentially reduce the diversity of plant species. Therefore, it needs a habitat conservation strategy to be able for collaborating ecological interests and socio-economic benefits for the surrounding community [8].

The main purpose of this study is to extract ethanol from nipa palm fruit that could be a source of energy and livelihood that could also boost the local industry. Hopefully, if the potentiality of nipa was proven, conservation activities will be encouraged to prevent this resource from depletion.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Nipa Palm Fruit

The *nypa fruticans* (nipa) was obtained from different municipalities in Northern Samar, namely Gamay, Mapanas, Palapag, and Catarman, located on swampy area and with a moderate tidal influence and flooding in the rainy seasons (June – December). The palm fruit

collected early in the morning (before 7:00 a.m.) was immediately brought to the laboratory. The fruits were cracked and dried in an oven drier at 70°C and then milled. 50 grams of dry palm fruit powder were mixed with 200 ml of distilled water. Whole mixture was heated at 90°C for 45 min in hot water bath to liquefy.

2.2 Fermentation Process

The actual production of ethanol took place during fermentation. These process is often defined as the process of the breakdown of carbohydrates and amino acids anaerobically, that is without the need of oxygen. Compounds that can be broken down in the fermentation process is mainly carbohydrates, while amino acids can only be fermented by some certain types of microbes [9]. The enzymes, invertase (maltase), and zymase contained in the used yeast, as well as diastase contained in the malt acted on monosaccharide and disaccharide produced during mashing and in the process degrades the saccharides to ethanol and CO₂. An amount of 15 g of baker's yeast was measured and added to the yeast nutrient (Ammonia); this will then be added to the mixtures and were confined in a flask blocked with a rubber cork and allowed to ferment for a period of 12 days.

2.3 Distillation Process

Fractional distillation was carried out after fermentation. This is done because the ethanol that will be collected during fermentation contains a significant quantity of water, which must be removed. This is achieved by using the fractional distillation process. The fermented broth was dispensed into a round-bottom flask fixed to a distillation column enclosed in running tap water. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heating mantle with the temperature adjusted to 78°C was used to heat the round-bottomed flask containing the fermented broth. The distillate collected was measured using a measuring cylinder, and expressed as the quantity of ethanol produced in g/L by multiplying the volume of distillate collected at 78°C by the density of ethanol (0.8033 g/ml). g/L is equivalent to the yield of 100 g of dried substrate [10,9].

2.4 Purification Process

Distillation process results just have not fully pure ethanol alone, the process is still entrained water

even in small quantities. Bioethanol is used as the fuel mixture for a vehicle that does not cause corrosion on the machine, then it must be completely dry or anhydrous. Therefore, it needs to be purified to remove water that is carried in bioethanol [11,9]. The alcohol produced from the fermentation process usually still contain gases such as CO₂ and aldehydes that need to be cleaned according to Nurdyastuti [11]. The distillate was further purified by the use of lime (calcium oxide). Lime, a basic oxide, was added to the ethanol, an alkaline solution. The calcium hydroxide formed will be separated from the ethanol by further distillation, which leaves absolute ethanol. Limestone finely ground before use in order to faster water absorption. Comparison usage is 7 liters of bioethanol required for 2-3 kg of limestone. Mixture allowed to stand for 24 hours while stirring occasionally. After that the mixture is evaporated and condensed into a liquid again as ethanol 99% or more [9].

2.5 Confirmatory Test

2.5.1 Iodoform (Triiodomethane test)

Transfer 10 drops of ethanol to a clean and dry test tube, add 25 drops of iodine solution and enough sodium hydroxide solution to remove the color of the iodine and mix gently for a few minutes. If nothing happens in the cold, it may be necessary to warm the mixture very gently. A positive result formed a very pale yellow precipitate of triiodomethane (previously known as iodoform) is indicated [12].

Note: This test gives positive results for other alcohols containing a methyl group and a hydrogen atom attached to carbon bearing the hydroxyl group. Methanol gives negative result.

2.5.2 Litmus test

Litmus test was determined by dipping the piece of litmus paper into the small amount of the sample. No change in the color of the litmus paper indicates positive result.

Note: Ethanol is a neutral compound and will not change the color of the litmus paper [12].

2.5.3 Ester test

Ester test was determined by taking 1 ml of the sample into the clean dry test tube, adding 1 mL of glacial acetic acid and 2-3 drops of conc. sulfuric acid into the sample. Heat the mixture in

Table 1. Confirmation test for ethanol

Time (hrs)	Ester	Iodoform	litmus test	flammability
24	negative	negative	negative	non-flammable
72	positive	positive	positive	flammable
144	positive	positive	positive	flammable
216	positive	positive	positive	flammable
288	positive	positive	positive	flammable

a water bath for 10 minutes. Then poured the mixture into a beaker containing cold water. A fruity smell indicates positive result.

Note: a fruit smell confirms the presence of alcohol.

2.5.4 Flammability (Combustion test)

Transfer about 5 mL ethanol to a large test tube, add a boiling chip, hold with a test tube holder and heat until the liquid is boiling. Hold the open end of the test tube to the flame and ignite the ethanol vapors. A pale blue color flame indicates positive result.

Note: Ethanol burns with a pale blue flame with no smoke [12].

3. RESULTS AND DISCUSSION

In this study, ethanol was extracted from yeast fermentation of *Nypa fruticans* (nipa) palm fruit and the presence of ethanol was evaluated by confirmatory test such as: litmus, ester, iodoform and flammability test, this suggests that the nipa fruit contains significant amount of ethanol (Table 1).

Table 2 shows that at 24 hours of fermentation the ethanol extract from nipa fruit exhibits negative result for all confirmatory test, this implies that presence of ethanol was not yet concentrated since fruit samples takes longer time to ferment as compared to other plant part such as the leaves which only takes at maximum of 24 hours of fermentation [5]. Presence of ethanol was traced at 72 hours of fermentation which is the maximum time for the fruit to yield enough ethanol which turn out positive.

Table 2. Effect of time

Time (hrs)	Presence of ethanol
24	negative
72	positive
144	positive
216	positive
288	positive

4. CONCLUSIONS

Based on the results of the study, it is concluded that the *Nypa fruticans* (nipa) palm fruit is a good source of ethanol. Proper planning and conservation practices must be conducted for the efficient utilization of nipa palm and to prevent it from exhaustion and depletion.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dransfield J, Uhl NW, Asmussen CB, Baker WJ, Harley MM, Lewis CE. Genera Palmarum: The evolution and classification of palms. Royal Botanic Gardens, Kew. 2008;732.
2. Irawan B, Khabibi J, Agustina A. The potential of nipah (*Nypa fruticans* Wurmb) as bioenergy resources. The 1st International Conference on Green Development–University of Jambi; 2016.
3. Hartini S, Guridno BS, Yulianto M, Suprajaka. Assessing the Used of Remotely Sensed Data for Mapping Mangroves Indonesia. Selected Topics in Power Systems and Remote; 2010.
4. Kumar N, Singh J, Ranjan R, Subathra D and Mohana S. Bioethanol production from weed plant (*Cyperus rotundus*) by enzymatic hydrolysis. Advances in Applied Science Research. 2013;4(4):299-302.
5. Sharmila S, Rebecca LJ, Ray SD, Kowsalya E. Extraction of bioethanol from plant leaves. Der Pharmacia Lettre. 2016;8(8):97-99.
6. Cudas R. Nipa palm: joining the fight against COVID-19 and global warming. Ecoweb; 2020. Available: <https://ecowebph.org> (Retrieved on November 24, 2020).
7. Ridho RM, Sundoko A, Ulqodry TZ. Analisis per ubahanluasan mangrove di

- Muara Sungai Banyuasin, Sungsang dan Upang Provinsi Sumatera Selatan Menggunakan Citra Satelit Landsat-TM. *Jurnal Pengelolaan Lingkungan dan SDA*. 2006;4(2):11-18. [Indonesian].
8. Hidayat IW. Natural production potency of nipa (*Nypa fruticans*) sap as production commodity for bioethanol. *Pros Sem Nas Masy Biodiv Indon*. 2015;1(1):109-113.
 9. El Kiyat W, Yunida M, Rahma E, Mubarak Y. Optimizing utilization of nipa sap as bioethanol basic materials for the rural community in increasing the national economic growth. *Knowledge Publishing Services*. 2015;1:153-159.
 10. Oyeleke SB, Jibrin NM. Production of bioethanol from guinea corn husk and millet husk. *Afri. J. of Microbiol. Res*. 2009;3(4):147-152.
 11. Nurdyastuti I. Teknologi proses produksi bio-ethanol. *Journal prospek Pengembangan Bio-fuel*. 2008;75-81.
 12. American Herbal Product Association. *Ethanol Identity Test Methods*. AHPA: Silver Spring, MD. 2017;1-4.

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