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Secondary Metabolite Screening of Extracted Oil from *Nypa fruticans* Wurmb. (Nipa Palm)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Nypa fruticans Wurmb. (Nipa palm) oil was extracted from its ripe, semi-matured, fruits. Nipa palm possesses a diverse array of biological characteristics that can be a huge advantage to many fields and industries. The objective of the study is to characterize nipa palm oil extract through physicochemical analysis and secondary metabolite screening. The physicochemical properties determined were density, pH level, viscosity, solubility, and rancidity. The secondary metabolites such as tannin, saponin, alkaloid, flavonoid, and glycoside were also determined. Characteristic data for physicochemical analysis of nipa palm oil extract exhibited the following; a density of 1.34g/mL, acidic (6 pH level), more viscous (3.16/second), immiscible to hexane at room temperature, miscible to ethanol, water-soluble, and not rancid. Qualitative data for secondary metabolites showed the presence of only saponin and alkaloids among the test used. Overall, nipa palm oil extract has superior characteristics and can be a potential source of therapeutically and industrially important compounds.

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1. INTRODUCTION

Nypa fruticans Wurmb (Nipa palm) also known as mangrove palm is considered one of the most important Southeast Asian products since it has a variety of uses agriculturally and ecologically. Previous studies revealed that the chemical constitution in its various parts plays a crucial role in the development of new products but nipa palm fruit was given less attention. It has been widely used for livelihood purposes and its capabilities in other fields were neglected. Nipa palm fruit has the potential as a sustainable oil production system without sacrificing the massive production of palm trees due to high economic demands thus this study will aim to prove it.

Nipa palm, a species of palm that grows along the coastlines and habitats can be one possible solution to the problem of the food crisis that the country will soon be facing, according to the World Agroforestry Center Philippines [1]. *Nypa fruticans* belong to the family Arecaceae Bercht & J. Presl. The genus *Nypa* Steck has been placed in its subfamily, the Nypoideae, and is the sole species in the genus Nypa, forming monotypic taxa [2]. It is commonly known as the nipa palm, which is a species of palm native to the coastlines and estuarine habitats of the Indian and Pacific Oceans. It is the only palm considered adapted to the mangrove biome [3,4].

The multi-uses of *Nypa fruticans* include food, raw material for roofing, decorative household items, and ethanol from neera as bio-fuel should turn this palm into a special plant species with a great economic contribution. Nypa palm is considered a mangrove plant species and normally does not compete with other types of plants, as it grows mostly in clusters in areas that are unsuitable for most other plant types. Since the natural environment of this palm is quite challenging, strategic efforts must be made to successfully domesticate this palm. Apart from its multi-uses, the most critical requirement for this palm is the current lack of scientific basis behind the traditional practices in producing the neera. Thus, research and development are very much in need for this palm in both upstream and downstream activities such as nursery management for the production of quality Nypa seedlings, optimum planting density, agronomic practices, the tapping technique, harvesting methods, storage, processing, and product development. Effective technologies would ensure Nypa palm could be turned into a source of economic growth for Malaysia [5].

Oil has been a crucial household need as it plays a vital role in food preparation, modification, flavoring, cooking, and even in the health care system. Health care professionals proposed that nipa palm oil appeared to be composed of a combination of saturated fatty acids and conjugated, unsaturated fatty acids, but its chain of unsaturation is not high enough to be classified as drying or semi-drying oil [6]. It follows that nipa palm oil has relatively higher proportions of saturated to unsaturated fatty acid contents. Moreover, the need for a more health-friendly oil became an urgent call for dietary and nutritional recommendations. Nipa palm oil can be a potential alternative source of oil considering its continuous and sustainable growth and chemical composition compared to other types of oil. A kind of palm that people see but often neglect can be one possible answer to the crisis the world will soon be facing.

Phytochemicals are natural plant compounds and edible disease fighters. It gives the plant color, aroma, and flavor, but when eaten, they work with other phytochemicals and nutrients to fend off cancer, heart disease, age-related eye disease, and more. Some phytochemicals stimulate the immune system [7]. Others slow the growth of cancer cells or prevent DNA damage. Still, others help prevent plaque build-up in the arteries. Safeguard human health by eating ample plant foods to get all types of phytochemicals that can be found in fruits, vegetables, whole grains, nuts, beans, and even tea and coffee and herbs and spices. Identifying these active constituents through phytochemical screening will provide additional information on the genetic diversity of the nipa palm [8].

This study was initiated to characterize the properties of nipa palm fruit oil extract through physicochemical analysis, determine the active constituent present in the extracted oil through phytochemical screening, and evaluate its potential in a sustainable production system. It aims to provide alternative cooking oil that is safer and healthier compared to commercial oils. It will serve as an aid to equip the public with knowledge that will lead to understanding its nutritional value and provide importance for the utilization of abundant plant resources on a local scale.

2. METHODOLOGY

The mature fruit of *Nypa fruticans* was harvested a week before the actual experiment. Fruit meat or nipa palm kernel was collected with its shell and core removed to prevent the early formation of molds, they were collected in the river sides of Catarman, Northern Samar. The sample was washed thoroughly with tap water and rinsed with distilled water. The rinsed sample was air-dried under shade for about 24 hours and oven-dried for 12-16 hours until it reached a crunchy state. The dried sample was crushed using a grinder to obtain powder and stored in an airtight container protected from sunlight until use. Name of the plant species, family, date of collection, locality, and conditions of the plant parts that were used are noted and labeled.

Extraction of oil from *Nypa fruticans.* In a lightweight container, powdered nipa palm fruit was gathered and weighed using an analytical balance. For every 100 g of the sample, it was percolated with 1 L of pure ethanol. It was soaked for 3 days and subjected to manual extraction using filter paper. The fruit residue was discarded. The organic solvent filtrates were concentrated at a temperature of 70ºC in a rotary evaporator for about 12-16 hours. Aqueous extracts were dried using incubation until ethanol is completely evaporated. Speed-vacuum can also be used as an alternative treatment for incubation. Samples were collected and stored for further analysis.

2.1 Determination of Physicochemical Properties

2.1.1 Density

About 2 mL of *Nypa fruticans* oil was weighed using an analytical balance; the weight in gram (g) of the nipa palm oil was recorded and divided by the volume of extract used in mL. The procedure was repeated thrice [9].

2.1.2 pH level

pH level was tested using a pH paper. Cut the pH paper into three equal long strips. Dip the pH paper strips into the oil extract and compare it to the pH level color indicator. The procedure was repeated thrice. Note down the result.

2.1.3 Solubility

For testing the solubility of the nipa palm oil, three solvents were used: ethanol, hexane, and water. About 2 mL of *Nypa fruticans* (Nipa palm) oil was put into 9 test tubes. Three test tubes were poured with 8 mL of each sample. The 9 test tubes were shaken and observed for their solubility. The result was recorded as miscible and immiscible if more than one phase is formed [9].

2.1.4 Viscosity

The oil of nipa palm was subjected to a viscosity test. About 5 mL of Nipa palm oil was put into a graduated cylinder and another cylinder was added with 5 mL of water. A metal ball was dropped into each liquid. Using a timer, the researcher determined how fast or how slow the ball falls to the bottom of the cylinder in the *Nypa fruticans* oil extract and the water. Repeat thrice and record the time results [9].

2.1.5 Rancidity

The Kreis Test was used to determine the rancidity of the nipa palm oil extract. Thoroughly mix 1 mL of nipa palm oil extract and 1 mL concentrated HCl in a test tube. Add 1 mL of 1% solution phloroglucinol in diethyl ether (freshly prepared), and again mix thoroughly. If a pink color develops, the fat is slightly oxidized; if a red color develops, the fat is oxidized [10].

2.2 Determination of Secondary Metabolites

2.2.1 Test for the presence of Tannin

The Ferric Chloride Test was used to determine the presence of tannin. Mix 2 mL extract and mix with 1 mL 5% $FeCl_3$. The appearance of a green-black color indicates the presence of tannin. Repeat thrice [11].

2.2.2 Test for the presence of Saponin

The Foam Test was employed to identify the presence of saponin. About 2 mL extract was mixed with 4 mL distilled water and was agitated in a test tube for 15 minutes. The formation of 1 cm foam indicates the presence of saponin. Repeat thrice [7].

2.2.3 Test for the presence of Alkaloids

In this test, Dragendorff's reagent and Mayer's reagent test was used in determining the presence of alkaloid. A positive result indicates the presence of orange precipitate in Dragendorff's reagent and white precipitate in Mayer's reagent. First, add 1 mL HCl and 6 drops of Mayer's reagent and Dragendorff's reagent for every mL of the extract. Any organic precipitate indicates the presence of alkaloids in the sample. Repeat thrice [7].

2.2.4 Test for the presence of Flavonoids

The Shinoda Test was used to test the presence of flavonoids. Mix pieces of ribbon magnesium ribbon and HCl concentrate with oil extract. After a few minutes. The appearance of a pink color shows the presence of flavonoids. Repeat thrice [7]. The Alkaline Reagent Test is the second test that was used to identify the presence of flavonoid. Mix 2 mL of 2.0 % NaOH mixture with oil extract; a concentrated yellow color is produced. Add 2 drops of diluted acid to the mixture, and it becomes colorless [11].

2.2.5 Test for the presence of glycoside

The test that was used to identify the presence of glycoside is the Salkowski Test. Add 2 mL of H_2SO_4 concentrate on the whole oil extract. The formation of reddish-brown color indicates the presence of the steroidal aglycone part of the glycoside [11].

3. RESULTS AND DISCUSSION

This section presents the results of the data gathered in this study. Oil extract from *Nypa fruticans* fruit was isolated, from white solid nipa palm meat to an extract with a paste-like texture. It also underwent various oil extraction methods, physicochemical analysis, and phytochemical screening. A 29.5% yield was calculated after extracting the nipa palm oil from its fruits of 100g. The results of this study were further illustrated through tables in the next sections.

The physicochemical properties in chemistry deal with the analysis of both physical and chemical characteristics of a substance. In this study, density, pH level, viscosity, solubility, and rancidity were tested in the *Nypa fruticans* fruit oil extract. These are the important parameters in oil characterization as such it assesses qualitatively the comparability of nipa palm oil properties from commercially available kinds of oil.

Density, in Table 1, indicates the compactness, of how tightly mass is squeezed into a given volume. In this, the researchers sought to determine if the sample has comparable results to commercial oil products. The density of the oil extract was consistent in the three trials performed having an average mean of 1.34 g/mL in a 1 mL sample. It implies that this type of oil has a greater density compared to commercially available oil products ranging from 0.91 – 0.93 g/mL as a standard density rate.

pH, in Table 2, is a measure of hydrogen ion concentration, a measure of the acidity or alkalinity of a solution. In oil characterization, it is essential to have a neutral pH level to ensure that the oil is health-friendly. The *Nypa fruticans* fruit itself is alkaline but becomes acidic when oil extraction is heat-based. The pH paper method, a traditional method of determining the pH level in samples was used. The three trials performed showed consistent results with a pH level of 6, an acidic – weak acid. It implies that it is still safe for human consumption since most likely it is less acidic compared to other commercially available oil products [12].

Viscosity, in Table 3, corresponds to the informal notion of thickness. In this research, the measurement of kinematic viscosity using gravity was used to determine the viscosity of the *Nypa fruticans* oil extract. The metal ball settled at the bottom of the test tube at an average time rate of 3.16 per second in the three trials performed an indication that it has higher viscosity since it took a couple of seconds to settle at the bottom of the container.

Table 1. Density of nipa palm oil

Trials	Wt. of the sample in grams (g)	Results (g/mL)
T_1	1.34 g	1.34 g/mL
T_2	1.34 g	1.34 g/mL
T_3	1.34 g	1.34 g/mL
Mean	1.34 g	1.34 g/mL

Table 2. pH level of nipa palm oil

Trials	Results
T_1	6
T_2	6
T_3	6
Mean	6
Interpretation	Acidic

Table 3. Viscosity of nipa palm oil

Trials	Time result per second
T_1	3.06/ second
T_2	3.16/ second
T_3	3.25/ second
Mean	3.16/ second

Solubility is a property referring to the ability of a given substance, the solute, to dissolve in a solvent. This parameter is very essential in oil characterization as it increases the bioavailability of the oil. The solubility of the *Nypa fruticans* oil extract was determined using three solvents namely ethanol, hexane, and water all shown in Table 4. The three trials performed in hexane were immiscible in the oil extract since two phases were formed. On the other hand, a homogeneous mixture was observed in both ethanol and water solvents, an indication that it is miscible and water-soluble. Given that, it is safe to assume that the nipa palm oil extract is a polar substance since it mixes well with the polar solvents used, as anchored by the like dissolve like the principle of solubility where a polar substance is soluble to only polar substances and non-polar to non-polar.

Rancidity refers to the development of unpleasant smells or tastes usually from chemical change or decomposition of a substance. The rancidity of the *Nypa fruticans* oil extract for both three trials was observed to have negative results since no formation of pink color in the acid layer was observed shown in Table 5. It implies that *Nypa fruticans* oil extract is not rancid and has also great potential as a food additive that can prolong a product's shelf life and extend food storage if used as an active ingredient in food preparation. It has antioxidant properties and oxidation-preventing effects as mentioned in the research study of Bae [13] that affects food stability and prevents food oxidation.

Secondary metabolite screening conducted on the *Nypa fruticans* fruit oil extract revealed the presence of active constituents that are known to exhibit medicinal as well as physiological activities. Tannin, flavonoid, and glycoside exhibit negative results, a positive result on saponin, and a positive-negative result in alkaloids. These compounds are known to be biologically active that exert through different mechanisms.

Tannins are the providers of astringent and hemostatic properties to a compound. The researchers used the Ferric chloride test to determine the presence of tannin in the *Nypa fruticans* fruit oil extract. Upon addition of the aqueous Ferric chloride solution, a brownish color was observed in the three test trials performed, an indication of a negative result shown in Table 6. The possible variables affecting this alteration in research data can either be an error in the methodological procedure used or environmental factors as such previous research studies on this plant's secondary metabolites revealed a promising positive result for tannin and other phenolic compounds.

Saponins are chemical compounds abundant in different plant species. In this research, the foam test method was used to determine the presence of saponin in the *Nypa fruticans* fruit oil extract. A centimeter height soap-like foam was observed when the extract was agitated in the water solution shown in Table 7. It was observed that the saponin content is minimal in the nipa palm fruit as it showed less persistent foam in the three trials performed. This chemical change occurred because saponin is composed of one or more hydrophilic glycoside moieties held by a lipophilic triterpene derivative. Furthermore, they are also classified as a surface-active agent and can be used as a cleansing agent because of their detergent properties.

Table 4. Solubility of nipa palm oil

Solvent	Trial 1	Trial 2	Trial 3
Ethanol	Immiscible	Immiscible	Immiscible
Hexane	Immiscible	Immiscible	Immiscible
Water	Miscible	Miscible	Miscible

Table 5. Rancidity of nipa palm oil

Trials	Results/ Appearance
T_1	Negative result and no formation of pink color in the acid layer
T_2	
T_3	

Table 6. Test for the presence of Tannin

Trial	Method	Observation	Inference
T_1		Brownish Color	Tannin not present
T_2	Ferric Chloride Test	Brownish Color	Tannin not present
T_3		Brownish Color	Tannin not present

Table 7. Test for the presence of Saponin

Trial	Method	Observation	Inference
T_1		Foam formation	Saponins present
T_2	Foam Test	Foam formation	Saponins present
T_3		Foam formation	Saponins present

Table 8. Test for the presence of Alkaloid

Trial	Method	Observation	Inference
T_1	Dragendorff's Test	Dark Brown Color	Alkaloid not present
	Mayer's Test	White precipitate	Alkaloid present
T_2	Dragendorff's Test	Dark Brown Color	Alkaloid not present
	Mayer's Test	White precipitate	Alkaloid present
T_3	Dragendorff's Test	Dark Brown Color	Alkaloid not present
	Mayer's Test	White precipitate	Alkaloid present

Table 9. Test for the presence of flavonoid

Trial	Method	Observation	Inference
T_1		Orange color	Flavonoids not present
		Yellow color	
T_2	Shinoda Test and	Orange color	Flavonoids not present
	Alkaline Reagent Test	Yellow color	
T_3		Orange color	Flavonoids not present
		Yellow color	

Table 10. Test for the presence of glycoside

Trial	Method	Observation	Inference
T_1		Reddish color	Glycosides not present
T_2	Salkowski Test	Reddish color	Glycosides not present
T_3		Reddish color	Glycosides not present

Alkaloids are colorless, crystalline, non-volatile, solids, and are insoluble in water but soluble in most the organic solvents. The researchers employed two methods to determine the presence of alkaloids in the *Nypa fruticans* oil extract namely the Dragendorff's Test and Mayer's Test. A dark-brown color with no precipitate was observed in the three trials for Dragendorff's Reagent Test indicating that alkaloid is not present, as shown in Table 8. On the other hand with Mayer's Test, a white precipitate or cream color was observed in the latter test as an indication of the presence of alkaloid. The three trials performed showed a consistently positive result as well.

Flavonoids are widely distributed polyphenolic secondary metabolites with diverse biological activities in plants. The Shinoda's Test and Alkaline Reagent Test was used to determine the presence of flavonoid in the *Nypa fruticans* fruit oil extract. Both tests showed no presence of flavonoids in the three trials performed and did not exhibit the standard indicators shown in Table 9. Related studies involving phytochemicals of *Nypa fruticans* showed the presence of flavonoids thus either microbiological contamination during collection, methodological error, or environmental conditions are the possible variables in the modification of research data.

Glycosides are colorless, solid, amorphous, nonvolatile, and water-soluble compounds, insoluble in organic solvents. Salkowski test was employed to determine the presence of glycosides in the *Nypa fruticans* fruit oil extract, shown in Table 10. The formation of a reddish color was observed indicating that the steroidal glycone part of the glycosides is not present. The three trials exhibit a consistent negative result.

In this study, a percent yield of 29.5% was obtained from a 100g of nipa palm fruit sample. In another study of Hamzah [14] nipa kernel oil they yielded a 27,4% (by soxhlet extraction), 23,2% (by dry method), and 25,1% (by wet method). The characteristic data showed that the iodine value is 25.55% and 32,86 mg KOH/g of saponification value.

4. CONCLUSIONS

After several tests, *Nypa fruticans* yielded higher density, slightly acidic, higher viscosity, immiscible to hexane, miscible to ethanol, water-soluble, and not rancid. This oil is not comparable to commercial oils but has promising characteristics of its own that can have a great advantage to different fields or industries. Negative results were observed in the secondary metabolites such as tannin, flavonoid, and glycoside while saponin exhibited a positive result. However, a positive-negative result in alkaloids as such the two methods employed was observed to exhibit varying results. These phytochemicals have properties that can pave the way in the development of new drugs and has the capacity as a market-worthy product of commercial value.

For the improvement of the study, the researcher recommends the following: (1) to identify the maximum properties of *Nypa fruticans*, a different, new, and updated approach in terms of methodological procedures should be done, (2) detection of other active constituents that have not been yet extensively studied should be done to contribute to the additional information on the genetic diversity of *Nypa fruticans*, (3) for oil industries, establish a sustainable oil production system equipped with functional methods for production, harvest, collection, drying, filtration, concentration, extraction, and storage, (4) develop well-designed equipment for the isolation of oil extract in nipa palm palm that excludes heat-based processes to ensure the rawness of natural oil in plant samples, and (5) future researches for further qualitative and quantitative analysis on the agricultural lands of nipa palm palm in Northern Samar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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