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Crystallization and Biological Studies of *Nypa fruticans* Wurmb Sap

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Abstract

Nypa fruticans is a standout amongst the most broadly appropriated and helpful palm in the mangrove backwoods in the Southeast Asia. The sap of this plant is produced at the fruit stalks or called as flower stalks or inflorescence. Nipa sap contains a lot of chemical constituents that can be used other alternative to produce sugar that give a lot benefits. The crystallization process of this sap was conducted in controlled temperature, pressure and humidity. As the crystal formed, remaining liquid was tested for biological studies which are antidiabetic, antioxidant and toxicity test. The antidiabetic properties were determined by inhibition of α-amylase and the result was compared to the synthetic drug which is acarbose. The sample showed that a high property of antidiabetic (50 mg/mL= 78.50% inhibition) compared to the acarbose (50 mg/mL= 80.90% inhibition). Meanwhile, the antioxidant properties of the sample were determined by2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The sample also showed a positive result which is EC_{50} 112.90 mg/mL. Lastly, the toxicity test was tested to brine shrimp (*Artemia salina*) to get the LC_{50}. The result (LC_{50}=271.7 mg/mL) showed low toxicity at different concentration of the sample. From this study, the sample had potential antioxidant and antidiabetic properties, with low toxicity.

Key words: α-amylase, antioxidant, *Artemia salina, Nypa fruticans*, toxicity test

Kristalisasi dan Tinjauan Biologis dari Getah *Nypa fruticans* Wurmb

Abstrak

Nypa fruticans merupakan tanaman palm yang paling banyak tersebar dihutan mangrove Asia Tenggara dan kaya akan manfaat. Getah tanaman ini dihasilkan pada tangkai buah atau disebut tangkai bunga atau perbungaan. Getah nipa dapat dijadikan alternatif lain untuk menghasilkan gula. Proses kristalisasi getah ini dilakukan pada suhu, tekanan dan kelembaban yang terkendali. Kristal yang terbentuk diuji aktivitas antidiabetes, antioksidan dan uji toksisitasnya. Aktivitas antidiabetes ditentukan oleh penghambatan α-amilase dan hasilnya dibandingkan dengan acarbose. Sampel menunjukkan bahwa aktivitas penghambatan antidiabetes (50 mg/mL = 78,50%) dibandingkan dengan inhibisi acarbose (50 mg/mL = 80,90%). Sementara itu, aktivitas antioksidan sampel ditentukan oleh radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH), menghasilkan nilai EC_{50} 112,90 mg/mL. Sedangkan, uji toksisitas diuji terhadap udang *Artemia salina* didapatkan nilai LC_{50}. Nilai LC_{50} = 271,7 mg / mL) menunjukkan toksisitas rendah pada konsentrasi sampel yang berbeda. Berdasarkan penelitian, sampel memiliki potensi antioksidan kuat dan antidiabetes, dengan toksisitas rendah.

Kata Kunci: α-amilase, antioksidan, *Artemia salina, Nypa fruticans*, uji toksisitas

1. Introduction

Nypa fruticans is a monoecious palm with special characteristics. Contrast to usual palms like coconut and oil palm it thrives in river sites and brackish water environment in which salt and fresh water mingle. Nipa differs from most palms in the lack of an upright stem, trunkless and develops its inflorescence at a height of about 1 meter.[1] Nipa sap that obtained from fruit stalks is used as a drink by the local peoples, while young fruits are eaten. The sap is a good source of sugar and used for making sweets, vinegar, beverage, and alcohol production. Recent studies showed that methanol extract of stem and leaves of this plan have antidiabetic and analgesic properties.[2] Thus, these studies focused on the sap of this plant. Diabetes mellitus is a complicated metabolic disorder of the endocrine system, characterized by hyperglycaemia due to defects in insulin secretion, insulin action, or both.[3] The conventional therapeutic approaches mainly involve drugs that enhance insulin secretion or signalling, as well as inhibitors of endogenous glucose production.[4] Previous researchers more focused on leaves, stem and fruit for this plant. The information of medicinal value of *N. fruticans* sap was still not wide in literature. Thus, the main objective was investigates antioxidant and antidiabetics properties of this plant sap because the useful phytochemical in this sap have potential in food and drug industries.

2. Method

2.1. Instruments

Ultraviolet-Visible (UV) Spectro photometer at wavelength 517 nm (Jasco V-630) was used to determine the absorbance value of sample for DPPH radical scavenging activity.

2.2. Materials

Methanol (HmBG),2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich), ascorbic acid (Sigma Aldrich), α-amylase enzyme (Sigma Aldrich), 3,5-Dinitrosalicylic acid (DNS) (Sigma Aldrich), starch, acarbose (Abacus 50), Artemiasalina (INVE).

2.3. Sample preparation

N. fructicans sap was freshly collected at Kampung Pinggan Jaya, Kota Samarahan, Sarawak. The temperature, pressure and humidity of the sample controlled to perform supersaturation process.After the supersaturation process the sample produced a crystal sugar and nipa syrup. Nipa syrup was tested for biological assay of antidiabetic, antioxidant and toxicity test.

2.4. Antidiabetic assay

Antidiabetic assay was performed using α-amylase enzyme. The assay was done after the standard convention with slight changes.[5] This in-vitro biological evaluation of α-amylase inhibition activity was conducted using 20, 40, 60, 80 and 100 mg/mL of nipa sap syrup. Sample was incubated with 200 µL α-amylase enzyme and 400 µL 1% starch concentration at 36.5 ºC for 20 minutes. After 20 minutes, the reaction was stopped using 800 µL DNS reagent. The sample was placed in boiling water for 5 minutes and immediately cooled using ice bath for 3 minutes in the test tube. After that, the sample was diluted with distilled water and the absorbance was measured using 530 nm UV spectrometer. Percentage of inhibition was calculated and compared with acarbose.

$$\text{Percentage of } \alpha\text{-amylase inhibition } (\%) = \frac{\Delta\text{sample} - \Delta\text{control}}{\Delta\text{control}} \times 100$$

2.5. Antioxidant assay

Antioxidant activity was determined used DPPH solution. The measurement of the DPPH radical scavenging activity was performed by method that standard proposed with some modification.[6] The antioxidant assay was conducted using 20, 40, 60, 80 and 100 mg/mL of nipa sap syrup. 20 mg of DPPH was dissolved in 100 mL of methanol. The sample was placed in the vials and 1 mL of methanol was added into each vial. The antioxidant test was conducted by mixing 1 mL of sample, 0.8 mL of methanol and 0.5 mL of DPPH solution. Methanol was served as the blank. The control solution was

prepared by mixing 3.5 mL methanol and 0.3 mL DPPH radical solution.

$$\text{Percentage of DPPH radical scavenging activity (\%)} = \frac{\Delta\text{sample} - \Delta\text{control}}{\Delta\text{control}} \times 100$$

2.6. Toxicity test

Toxicity test of the sample conducted referred to the method of Moshi MJ.[7] with some adjustment. Brine shrimp (*Artemia salina*) eggs were hatched in artificial salt water at 20°C and constant illumination. The brine shrimp eggs were incubated in a 500 mL beaker with a seawater height of 5 cm. These hatching conditions corresponded to those in the natural environment. After 48 hours from hatching, the shrimp larvae were used for the experimental bioassay. The 3 mg of each sample was measured and dissolved in 0.6 mL of methanol together. From the dilution 10, 20, 100, 200, 600, 1000 mg/mL is placed in seven different well. The volatile solvent were evaporate overnight. After two days, 5 mL of sea water and ten brine shrimp were added to each well and left it for 24 hours of incubation. Then, the each well were observed by using a magnifying glass and the number of survivors in each vial was count and the mortality rate were determined. The resulting data were performed to probit analysis for determination of lethal concentration (LC_{50}) values for the sample of *N. fruticans*. LC_{50} is Lethality Concentration in the chemical compound of nipa sap syrup.

$$\text{Percentage of mortality (\%)} = \frac{\text{total brine shrimp death}}{\text{total brine shrimp}} \times 100$$

3. Result

Table 1 and Figure 1 showed the potent result of the sample toward inhibition of α-amylase enzyme. The result was compared to the acarbose the synthetic drug in market. The highest concentration of the sample (100 mg/ml) had shown the semblance result with acarbose. The value of antidiabetic test was compared based on IC_{50}.

Table 2 and Figure 2 showed the result of antioxidant activity of the sample. The ascorbic acid had the highest antioxidant activity and the nypa sample shown the potent result. While acarbose only showed deficient result. The antioxidant test was compared to the EC_{50}.

The LC_{50} of the sample is shown in Table 3, with the value is 271.7 mg/mL showed a low toxicity content. Thus, the sample have a potential in drug and food industries.

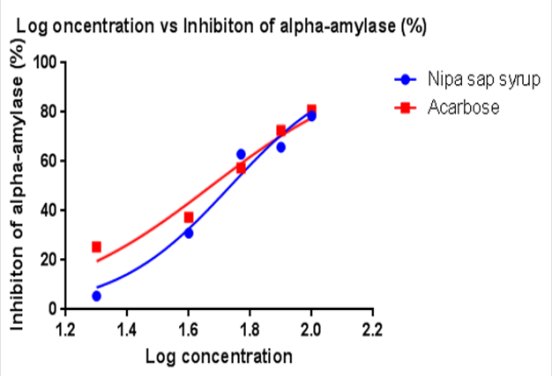

Figure 1 Graph percentage of inhibition between nipa sap syrup and acarbose

4. Discussion

Delaying of digestion oligosaccharide to absorbable monosaccharides in the intestinal will reduced the potential of diabetes. In our body, pancreatic α-amylase help the process of conversion oligosaccharide to monosaccharides.[8]

Table 1 Table percentage inhibition of α-amylase

Concentration of sample (mg/mL)	Inhibiton of α-amylase (%)		IC_{50}(mg/mL)	
	Nipa syrup	Acarbose	Nipa syrup	Acarbose
20	5.50	25.40		
40	31.00	37.47		
60	63.00	57.45	54.18	46.85
80	65.90	72.60		
100	78.50	80.90		

Table 2 Percentage of DPPH radical scavenging activity

Concentration of sample (mg/mL)	Percentage of inhibition (%)			EC_{50}(mg/mL)		
	Nipa Sample	Ascorbic acid	Acarbose	Nipa Sample	Ascorbic acid	Acarbose
10	0.550	95.900	2.07	112.9	0.6112	7398
30	16.050	96.355	0.00			
50	32.032	96.811	3.67			
100	61.684	96.128	8.11			
300	69.070	95.444	4.27			
500	69.620	95.216	4.27			

Thus, inhibition of α-amylase activity in body will reduces the monosaccharides intake in our body.

Phenolic compound, for example, phenolic acids and flavonoids tied covalently to α-amylase enzyme and change its activity because of the capacity to form quinones or lactones that respond with nucleophilic group on the enzyme bind site.[9] Besides that, some of flavonoid compounds effectively inhibited α-amylase enzyme activity based on the ability to form quinone when reacted to the hydroxylgroup at C-3 and C-4 of ring B.[10] From the Table 1, percentage of inhibition α-amylase activity is high for the sample. The sample showed that IC_{50} 54.18 mg/mL while acarbose show IC_{50} 46.85 mg/mL. The lower the IC_{50} indicated the great antidiabetic properties. As shown in Figure 1, the sample reacted to inhibition of the enzyme is high as the acarbose. Furthermore, phenolic compound also related to the antioxidant activity. The oxidizing nature of free radical resulted in enzymes inhibition or causing proteins to denature or degrade.[11] From Table 2 and Figure 2, the antioxidant activity of the sample showed potential result. The result compared to the ascorbic acid which is the best antioxidant compound. Meanwhile, acarbose showed lowest activity due to the low antioxidant level. The acarbose is synthetics drug which designed to bind completely to the bind site of α-amylase enzyme.[12] The antioxidant activity is compared by the EC_{50}, which is the sample (112.9 mg/mL), ascorbic acid (0.6112 mg/mL) and acarbose (7398 mg/mL).

Referring to the Sowndhararajan K,[13] the lower the EC_{50}, the greater the antioxidant activity of the compound. So, the sample showed the mild antioxidant compared to the ascorbic acid. Lastly, brine shrimp lethality bioassay is a straightforward, high throughput cytotoxicity trial of bioactive chemicals.[14] The toxicity test of the sample is shown in the Table 3. The LC_{50} of the sample was determined the toxicity level in every sample. The higher of LC_{50} value showed the low toxicity level while, the lower the LC_{50} showed the toxic compound.[15] The LC_{50} of

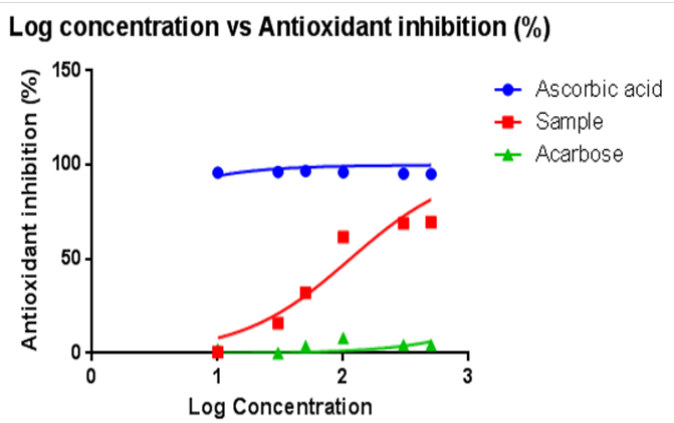

Figure 2 Graph of Log concentration vs Antioxidant inhibition (%)

Table 3 Percentage of Mortality of brine shrimp

Concentration of sample (mg/mL)	Percentage of Mortality (%)			LC_{50} (mg/mL)
10	0	0	0	271.7
20	0	0	0	
100	0	0	0	
200	0	0	0	
600	10	0	0	
1000	0	0	0	

the sample was 271.7 mg/mL which was low toxicity content. This was because the sample was traditionally a food for local people.

5. Conclusion

As conclusion, the nipa sap showed the great potential in the antidiabetics and antioxidant activity which were IC_{50} 54.8 mg/mL and EC_{50} 112.9 mg/mL. The further study is needed to determine the compound that helps in this useful biological assay. Thus, the sample could be designed as new drug or diet in human.

References

1. Tamunaidu P, Saka S. Chemical characterization of various parts of nipa palm (*Nypa fruticans*). Industrial Crops and Products. 2011 Nov 30;34(3):1423-8.
2. Bandaranayake WM. Traditional and medicinal uses of mangroves. Mangroves and salt marshes. 1998 Sep 1;2(3):133-48.
3. Yusoff NA, Yam MF, Beh HK, Razak KN, Widyawati T, Mahmud R, Ahmad M, Asmawi MZ. Antidiabetic and antioxidant activities of *Nypa fruticans* Wurmb. vinegar sample from Malaysia. Asian Pacific journal of tropical medicine. 2015 Aug 31;8(8):595-605.
4. Triastuti A, Park HJ, Choi JW. Phaleriamacrocarpa suppress nephropathy by increasing renal antioxidant enzyme activity in alloxan-induced diabetic rats. Natural Products Sciences. 2009;15(3):167-72.
5. Tamil IG, Dineshkumar B, Nandhakumar M, Senthilkumar M, Mitra A. In vitro study on α-amylase inhibitory activity of an Indian medicinal plant, Phyllanthus amarus. Indian journal of pharmacology. 2010 Oct;42(5):280.
6. Lugemwa FN, Snyder AL, Shaikh K. Determination of radical scavenging activity and total phenols of wine and spices: A randomized study. Antioxidants. 2013 Jul 25;2(3):110-21.
7. Moshi MJ, Innocent E, Magadula JJ, Otieno DF, Weisheit A, Mbabazi PK, Nondo RS. Brine shrimp toxicity of some plants used as traditional medicines in Kagera region, north western Tanzania. Tanzania Journal of Health Research. 2010;12(1):63-7.
8. Sim L, Jayakanthan K, Mohan S, Nasi R, Johnston BD, Pinto BM, Rose DR. New glucosidase inhibitors from an ayurvedic herbal treatment for type 2 diabetes: structures and inhibition of human intestinal maltase-glucoamylase with compounds from *Salacia reticulata*. Biochemistry. 2009 Dec 29;49(3):443-51.
9. Oyedemi S, Koekemoer T, Bradley G, van de Venter M, Afolayan A. In vitro anti-hyperglycemia properties of the aqueous stem bark extract from *Strychnoshenningsii* (Gilg). International Journal of Diabetes in Developing Countries. 2013 Jun 1;33(2):120-7.
10. Kim JS, Kwon CS, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Bioscience, biotechnology, and biochemistry. 2000;64(11):2458-61.
11. Lugemwa FN, Snyder AL, Shaikh K. Determination of radical scavenging activity and total phenols of wine and spices: A randomized study. Antioxidants. 2013 Jul 25;2(3):110-21.

12. Al Kazaz M, Desseaux V, Marchis-Mouren G, Prodanov E, Santimone M. The mechanism of porcine pancreatic alpha-amylase. Inhibition of maltopentaose hydrolysis by acarbose, maltose and maltotriose. European journal of biochemistry. 1998 Feb;252(1):100-7.
13. Sowndhararajan K, Kang SC. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight &Arn. Saudi journal of biological sciences. 2013 Oct 31;20(4):319-25.
14. Wu C. An important player in brine shrimp lethality bioassay: The solvent. Journal of advanced pharmaceutical technology & research. 2014 Jan;5(1):57.
15. Mbwambo ZH, Moshi MJ, Masimba PJ, Kapingu MC, Nondo RS. Antimicrobial activity and brine shrimp toxicity of extracts of Terminaliabrownii roots and stem. BMC complementary and Alternative Medicine. 2007 Mar 30;7(1):9.