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Antioxidant and Cytotoxicity Studies of *Nypa fruticans* (Nypa Palm Sugar) Extract

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Abstract

Nypa fruticans Wurmb which belongs to the family of Arecaceae is one of the most widely distributed and useful palm in the mangrove forests in the Southeast Asia. Nypa palm sugar or commonly known as "Gula Apong" was processed using nypa palm sap as raw material. The nypa palm sugar was expected to benefit wider community as source of alternative sweetener. In this study, 2, 2-diphenyl-1-picryhydrazyl (DPPH) scavenging activity and cytotoxicity test of methanol extract of nypa palm sugar were conducted. The antioxidant activity was determined based on DPPH scavenging activity of extract. The cytotoxicity test was conducted against brine shrimp *Artemia salina*, and the LC_{50} value of the extract was calculated. The antioxidant activity showed that the EC_{50} of nypa palm sugar was 1304 mg/mL while the value of EC_{50} for ascorbic acid was 0.6112 mg/mL. The results of the cytotoxicity showed that the methanol extract of nypa palm sugar was interpreted as non-toxic as the value of LC_{50} was 184.0 mg/mL.

Keywords: 2,2-diphenyl-1-picryhydrazyl, antioxidant, cytotoxicity, *Nypa fruticans*, nypa palm sugar

Studi Antioksidan dan Sitotoksisitas dari Ekstrak *Nypa fruticans* (Gula Nipah)

Abstrak

Nypa fruticans Wurmb yang termasuk keluarga Arecaceae adalah sejenis palem yang paling banyak dimanfaatkan dan tersebar luas di hutan bakau di Asia Tenggara. Gula nipah atau yang biasa dikenal dengan "Gula Apong" diproses menggunakan getah pohon nipah sebagai bahan baku. Gula nipah diharapkan dapat bermanfaat bagi masyarakat luas sebagai sumber pemanis alternatif. Dalam penelitian ini, dilakukan uji aktivitas penangkal 2, 2-difenil-1-picryhydrazyl (DPPH) dan uji sitotoksisitas ekstrak metanol gula nipah. Aktivitas antioksidan ditentukan berdasarkan aktivitas penangkal DPPH oleh ekstrak. Uji sitotoksisitas dilakukan terhadap udang air asin *Artemia salina*, dan nilai LC_{50} dari ekstrak dihitung. Aktivitas antioksidan menunjukkan bahwa EC_{50} gula nipah adalah 1304 mg/mL sedangkan nilai EC_{50} untuk asam askorbat adalah 0,6112 mg/mL. Hasil dari sitotoksisitas menunjukkan bahwa ekstrak metanol gula nipah ditafsirkan sebagai tidak beracun karena nilai LC_{50} adalah 184,0 mg / mL.

Kata kunci: 2,2-difenil-1-pikrilhidrazil, antioksidan, gula nipah, *Nypa fruticans*, sitotoksisitas

1. Introduction

N. fruticans belongs to the family of Arecaceae and its species possessed the bitter range that frequently shapes a wide outskirt past the edge of adjoining mangrove or swamp forest. The generic name of Nypa is a derivative of "Nipah" which is the native name which used in the Philippines, while for "fruticans" is Latin word for shrubby which referred to its stemless appearance.[1] *N. fruticans* can be found in the upstream estuarine zone which shaping broad stands along harsh to tidal freshwater creeks and waterways. Customarily, leaves, stem are utilized to treat asthma, tuberculosis, sore throat, liver infection, wind nibble, as a pain reliever, and can likewise be utilized as soothing and carminative.[2] Based on the recent studies, the studies of antidiabetic and antioxidant activities of *N. fruticans* vinegar from Malaysia was conducted and it showed that it was rich in antioxidant and antidiabetic properties.[3] Hence, the epidemiological studies showed that the incessant utilization of fruits and vegetables which high in natural antioxidant can lower the risk of certain diseases such as cancer, cardiovascular and diabetes.[4] However, there is lack of scientific study of nypa palm sugar of *N. fruticans*. Therefore, the aims of this study were to investigate the antioxidant properties and the cytotoxicity of nypa palm sugar.

2. Materials and Methods

2.1. Instruments

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

2.2. Materials

The chemical used for the biological activities of N. fruticans sugar were methanol (HmBG Chemicals), dimethyl sulfoxide (R&M Chemicals), 2, 2-diphenyl-1-picryhydrazyl (Sigma-Aldrich) and ascorbic acid (Sigma-Aldrich). The brine shrimp (Artemia salina) was used in the cytotoxicity assay.

2.3. Plant materials

Nypa palm sugar was supplied by a local producer from Kampung Pinggan Jaya, Kota Samarahan, Sarawak, Malaysia.

2.4. DPPH Radical Scavenging Assay

The DPPH was used to determine the antioxidant activity of the extract as described earlier with some adjustments.[5] An amount of 10 mg of sample was dissolved in 10 mL of methanol and 20 mg of DPPH was dissolved in 100 mL of methanol. Thus, the stock solution was prepared with different concentrations by transferring 10, 30, 50, 100, 300 and 500 µL of sample into vials. 1 mL of methanol was added into diluted solutions of stock solutions (1 mL for each vial) were mixed with 1 mL of DPPH solution and incubated in the dark room for 1 hour. The control solution was prepared by mixing 3.5 mL of methanol and 0.3 mL of DPPH solution. The absorbance of the mixture was recorded at 517 nm. Ascorbic acid was used as a positive control. The DPPH radical scavenging activity is often expressed as percentage of inhibition (%) and it can be calculated as the equation below:

$$\text{Percentage of inihibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100\%$$

2.5. Cytotoxicity Assay

The toxicity of methanol extract against brine shrimp (*Artemia salina*) was conducted with some modifications.[6] The eggs of *A. salina* were hatched in the beaker which contained seawater and continuous oxygen supply for 48 hours. The stock solution was prepared by dissolving 10 mg of methanol extract into 10 mL of methanol. The different concentrations were prepared by transferring 10, 20, 100, 200, 600 and 1000 µL of stock solution into multiwell for three replicate and incubated for 24 hours. 50 µL of dimethyl sulfoxide (DMSO) and 1000 µL of seawater was added into each well. The matured shrimp was collected and 10 nauplii were used for each well. After 24 hours, the dead brine shrimp were counted using magnifying glass. The mortality percentage of brine shrimp was calculated and was analysed to determine the LC_{50} values.

Tabel 1. The percentage inhibition and EC_{50} value for methanol extract of nypa palm sugar and ascorbic acid.

Compound concentration (ppm)	Percentage Inhibition (%)		Control	EC_{50} (mg/mL)	
	Methanol Extract	Ascorbic Acid		Methanol Extract	Ascorbic acid
10	0.121	95.900	1.652	0.6112	1304
30	3.027	96.355			
50	3.340	96.811			
100	3.450	96.128			
300	24.516	95.444			
500	24.637	95.216			

3. Result

3.1. DPPH Radical Scavenging Activity

Antioxidant test was performed for methanol crude extract of the nypa palm sugar. The percentage of the inhibition of the crude extract was calculated for determining its scavenging free radical activity which can be observed when DPPH reacts with the antioxidant compound. Hence, it resulting the hydrogen to be reduced. The colour changed from deep purple to light yellow when the methanol extract was added with the DPPH solution. Table 1 shows the results of DPPH radical scavenging activity.

According to the table 1, it can be observed that the methanol extract of nypa palm sugar with the concentration of 500 ppm was the highest antioxidant properties with the value of 24.637 %. Hence, it can be indicated that it has higher antioxidant properties and higher hydrogen donating capability compare to others concentration. The effective concentration (EC_{50}) of methanol extract of nypa palm sugar was 1304 mg/mL while the EC_{50} for ascorbic acid was 0.6112 mg/mL. Figure 1 shows the percentage inhibition of ascorbic acid and methanol extract against log concentration.

3.2. Cytotoxicity Assay

The dead of brine shrimp (*A. salina*) was calculated after 24 hours. The test was repeated thrice and the results were used by calculating the average death of *A. salina.* Table 2 shows the percentage average death of *A. salina* against the methanol crude extract of nypa palm sugar. Based on the observation, the value of LC_{50} was 184.0 mg/mL and it indicated that the methanol extract was non-toxic to *A. salina*. Figure 2 shows the mortality percentage of methanol crude of nypa palm sugar against log concentration

4. Discussion

Based on the Table 1 and Figure 1 for DPPH radical scavenging activity, the higher the concentration of nypa palm sugar, the higher the radical scavenging activity.

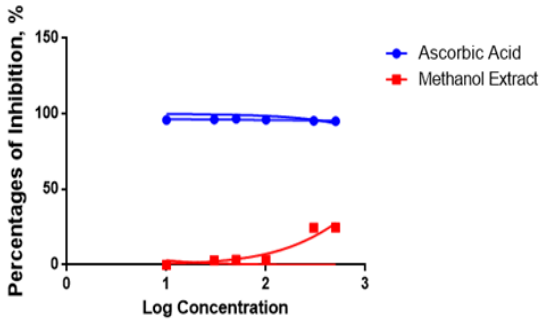

Figure 1. The percentage inhibition against log concentration

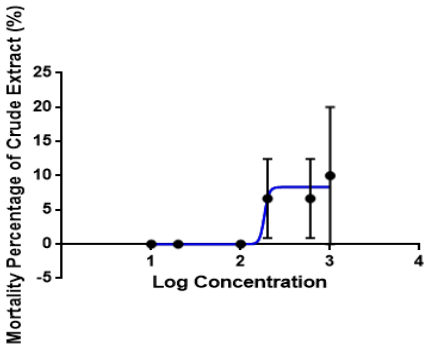

Figure 2. The mortality percentage of crude extract against log concentration.

Tabel 2. The average percentage death of A. salina against methanol extract of nypa palm sugar.

Methanol Extract	Percentage death of A. salina (%)						LC_{50}(mg/mL)
	Concentration (ppm)						
	10	20	100	200	600	1000	
1st replicate	0.0	0.0	0.0	10.0	10.0	10.0	184.0
2nd replicate	0.0	0.0	0.0	10.0	10.0	0.0	
3rd replicate	0.0	0.0	0.0	0.0	0.0	20.0	
Average	0.0	0.0	0.0	6.7	6.7	10.0	

However, methanol crude extract of nypa palm sugar has significantly lower antioxidant properties compare to the ascorbic acid. The EC_{50} of methanol extract of nypa palm sugar was 1304 mg/mL while the EC_{50} value for ascorbic acid was 0.6112 mg/mL. The lower the value of EC_{50} indicated greater scavenging of DPPH. Thus, ascorbic acid was greater scavenging compare to the methanol extract of nypa palm sugar. Based on the previous studies, the ethyl acetate extract of nypa palm vinegar (NPV) exhibited the highest scavenging activity.[7] Other than that, ethyl acetate fractions of young and mature leaves of *N. fruticans* also demonstrated the highest antioxidant activity compare to other crude extracts of *N. fruticans*.[8] The antioxidant activity were influenced by extracting solvent.[9]

The mortality rate of *A. salina* was found to be increased with the increase of the concentration of stock solution of nypa palm sugar. The percentage of mortality of the *A. salina* was calculated or each of the concentration. Therefore, based on the Figure 2, the mortality percentage of methanol extract against the log concentration of the nypa palm sugar showed an approximate linear correlation between both of them. Hence, from the graph the LC_{50} value was 184.0 mg/mL. Thus, it can be indicated that the extract was non-toxic to the brine shrimp (*A. salina*).

5. Conclusion

In a conclusion, *N. fruticans* sugar showed that it is not toxic because the value of LC_{50} was 184.0 mg/mL However, the nypa palm sugar does not exhibited potential antioxidant activity compare to other parts of *N. fruticans*. Hence, the further studies should be conducted to determine other useful potential of nypa palm sugar especially in food industry or pharmaceutical.

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