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# ANTIOXIDANT CAPACITY OF NYPA FRUTICANS WURMB. FRUIT

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**Abstract:** *Purpose:* *Nypa fruticans* Wurmb. is one of the underutilized fruit of Malaysia. Antioxidant capacity of immature and mature fruits was evaluated.

*Findings:* Total phenolic and flavonoid content of immature fruits were higher ( $6.08 \pm 0.1$  mg GAE/g and  $3.3 \pm 0.5$  RE/g respectively), than mature fruits. Also, immature fruit showed high DPPH radical scavenging activity of  $75.7 \pm 1.1\%$  and antioxidant capacity ( $A=1.3$ ), even higher compared with BHT and mature fruits.

*Practical implications:* Both immature and mature fruits exhibited excellent inhibition of RBC hemolysis and moderate hemoglobin oxidation.

*Value:* Hence, this fruit has the potential to be promoted as a natural source of antioxidant.

**Keywords:** Antioxidant; Fruit; *Nypa Fruticans*; Maturity; Underutilised



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## INTRODUCTION

Antioxidant  
capacity of *Nypa*  
*fruticans* Wurnb.  
Fruit

Antioxidants are the compounds that when added to lipids and lipid-containing foods, can extend the shelf-life by preventing lipid peroxidation during processing and storage. Synthetic antioxidants such as butylated hydroxy toluene (BHT) have restricted usage in foods, because it is reported to be carcinogenic (Namiki, 1992). Hence, the significance for exploiting antioxidants, from underutilized fruits has received much attention recently. Various underutilized fruits have been recognized to possess beneficial effects against free radicals in biological systems as natural antioxidants (Ikram et al., 2009; Prasad et al., 2011). Many studies have shown positive correlation of the increased dietary intake of natural phenolic antioxidants with the reduced coronary heart disease and cancer mortality, as well as with longer life expectancy (Halliwell, 2007).

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*Nypa fruticans* Wurnb. or Nipa palm, belongs to the family *Areaceae* and is classified as ‘underutilised’ fruit, relatively unknown, even though it has been used for ages in the tropics (Joshi et al., 2006). It is commonly found in India, Mynamar, Malaysia, Indonesia, Phillipines and Northern Australia. This palm provided useful products to indigenous peoples in the form of sap from the inflorescence stalk, and the fruit (Tang et al., 2010). The sap or the nira is a good source of sugar and used for making sweets, candies, toffees, vinegar and beverage. The nira is also considered very promising for sugar and alcohol production (Joshi et al., 2006).

To date, no information on the antioxidants from *Nypa fruticans* are documented. Hence, the objective of the present work is to evaluate the antioxidant capacity of *Nypa fruticans* and also to compare their antioxidant potential of immature and mature fruits.

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## MATERIALS AND METHODS

### Plant material

Immature and mature fruits of three and six month's maturity of *Nypa fruticans* (NF) were collected from Alor Setar, Kedah, with the assistance of Muda Agricultural Development Authority (MADA), Malaysia. The fruits were immediately transported to Universiti Putra Malaysia. Upon arrival, the fruits were washed carefully with tap water and air dried. The fruit was manually separated to get non-edible portion (fibrous peel) and the edible portion (juicy pulp).

### Extraction

Edible portion (25 g) of immature and mature of NF was homogenized for 2 min with 50 mL ethanol using a blender. The extract was then filtered (Whatman No 1), and centrifuged at 1000 rpm for 10 min. The supernatant obtained was collected and stored at -20°C until further analysis.

### Determination of total phenolic and flavonoid content

Total phenolic content of the supernatant (diluted 10 folds) was determined according to the method of Singleton & Rossi (1965) and then expressed as milligram gallic acid equivalents/gram. Total flavonoid content was measured following the aluminum chloride colorimetric assay described by Liu et al., (2008) and the results were expressed as rutin equivalents/gram.

### Analyses of antioxidant activities

#### DPPH radical scavenging activity

DPPH radical scavenging activities were determined following the method of Blois (1958) with some modifications. Four

different dilutions (1, 1/10, 0.5/10 and 0.1/10) of the samples were prepared in ethanol. An aliquot of 100 µl of the sample were placed in different test tubes and were mixed with 1 mL of DPPH (200 mM, dissolved in methanol). The reaction mixture was shaken vigorously and incubated at 37 °C in a dark room for 30 min. The control was prepared as above without any extract, and methanol was used for the baseline correction. The changes in absorbance were measured at 517 nm using a spectrophotometer. The inhibition of DPPH<sup>•</sup> radicals was calculated as scavenging activity (%) = (Control OD – sample OD / control OD) × 100. BHT (200 µg/ml) was used for comparison.

### **Antioxidant capacity by phosphomolybdenum method**

Antioxidant capacity was determined by the method of Prieto et al., (1999). A higher absorbance value indicates higher antioxidant capacity. Gallic acid (200 µg/mL) was used for comparison.

### **Hemoglobin oxidation assay**

Fasting venous blood (10 ml) from healthy volunteers (aged 20–30 years) were collected in EDTA tubes (0.4 g/L). Hemoglobin oxidation was performed as previously described by Rodríguez et al., (2006). The percentage inhibition of the samples against hemoglobin oxidation was also calculated using the following equation (%) = (OD of H<sub>2</sub>O<sub>2</sub> induced haemoglobin – sample OD / OD of H<sub>2</sub>O<sub>2</sub> induced haemoglobin) × 100.

### **Inhibition of RBC hemolysis**

RBC hemolysis was carried out according to the method of Huang et al., (2009). The percentage of RBC hemolysis was calculated by the following equation. Inhibition of hemolysis

### Statistical analysis

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Data were expressed as means ± standard deviations (SD) of three replicate determinations and then analyzed by SPSS V.13 (SPSS Inc., Chicago, USA). One way analysis of variance (ANOVA) and Duncan's New Multiple-range test were used to determine the differences among the means. *P* values < 0.05 were considered to be significantly different.

## RESULTS AND DISCUSSION

### Total phenolic and flavonoid contents

Total phenolic content of immature fruit was higher (6.08±0.1 mg GAE/g), when compared to mature fruit (0.22±0.1 mg GAE/g). Immature fruit also had higher flavonoid content of 3.3±0.5 mg RE/g, compared to mature fruit 0.07mg RE/g.

### DPPH radical scavenging activity

Immature fruit exhibited excellent DPPH radical scavenging activity in a concentration dependent manner. As the concentration decreases, the activity also decreased (Table 1). The highest inhibition of 75.7% was noticed in immature fruit, even higher compared to BHT (27%). However, mature fruit exhibited moderated activity. At lower dilutions, mature fruit did not exhibit any scavenging activity. It has been found that phenolics, flavonoids and tocopherols scavenge DPPH radicals by their hydrogen donating ability (Huang, 2005). The results obtained in this investigation reveals that the samples of NF could act as free radical scavengers, which might be attributed to their electron donating ability.

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## Antioxidant capacity

Antioxidant  
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The antioxidant capacities of NF samples were measured spectrophotometrically through phosphomolybdenum method. A high absorbance value indicates high antioxidant activity. Immature fruit exhibited highest absorbance of 1.30, compared to mature fruit having the absorbance of 0.30. The absorbance gradually decreased with increase in dilution (Table 1). The absorbance of immature fruit was very high, compared to mature fruit and gallic acid. Previously, Jayaprakasha et al., (2008) indicated that the antioxidant activity of citrus was due to the presence of phenolics and flavonoids. The antioxidant capacity in the present investigation may be attributed to total phenolic and flavonoid contents.

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## Hemoglobin oxidation assay

The result showed that mature and immature fruits had a good protective effect against hemoglobin oxidation (Table 1). The percentage inhibition of hemoglobin oxidation varied significantly with the highest activity ( $30 \pm 1.1\%$ ) observed in immature fruit, while the lowest ( $4.3 \pm 0.5$ ) was in mature fruit. Interestingly, lower dilution of both samples exhibited high activity. A similar finding was reported recently by Khoo et al., (2010) and Prasad et al., (2011) where in carotenoids had pro-oxidation effect at higher concentrations.

## Inhibition of RBC hemolysis

Immature and mature fruits showed excellent protection against RBC hemolysis in a concentration dependent manner (Table 1). Immature fruit had the highest inhibition of 82%, compare to mature fruit (61.8%). Also, the protective effects of both the samples were much higher than BHT.

Several studies exhibited a close relationship between antioxidant activities and total phenolic content (Liu et al., 2008, Jayaprakasha et al., 2008). Our results in the present study are in good agreement with several researchers, where in immature fruit are more potent in having high phenolic, flavonoid content and antioxidant activity (Gruz et al., 2011; Herrera-Hernandez et al., 2011; Menichini et al., 2011). Synthesis of phenolic compounds takes place, when the fruits are immature or at early stage of maturity. Once the fruits have attained maturity, the concentration of phenolics decreases due to dilution caused by cell growth (Castillo et al., 1992). Decline in phenolics during maturation also occurs due to polymerisation, oxidation and conjugation of bound phenolics. Decrease in phenolics is also related to the reduction of primary metabolism in the mature fruit, resulting in a lack of substrates necessary for the biosynthesis of phenolic compounds (Gruz et al., 2011).

## CONCLUSIONS

The antioxidant capacity of *Nyba fruticans* was evaluated. Immature fruits showed high phenolic, flavonoid and antioxidant capacity as assessed by various *in-vitro* methods. Further investigations into the identification of phenolic are needed to better elucidate their antioxidant activities.

## BIOGRAPHY

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**Zulfiki Bin Romli** helped us in getting fruits of *Nypa* and currently working with MUDA Agricultural Development Authority (MADA), Alor Setar 05990, Kedah, Malaysia.

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Samples	DPPH scavenging activity (%)	Total Antioxidant Capacity (OD at 750 nm)	Inhibition of RBC hemolysis (%)	Inhibition of hemoglobin oxidation (%)
Immature Fruit				
Dilutions				
1	75.7±1.1 <sup>a</sup>	1.30±0.36 <sup>a</sup>	82.4±0.0 <sup>a</sup>	20.1±0 <sup>b</sup>
1/10	72.1±0.4 <sup>a</sup>	0.11±0.001 <sup>b</sup>	61.8±4.2 <sup>b</sup>	21.4±0 <sup>b</sup>
0.5/10	57.5±0.9 <sup>b</sup>	0.07±0.004 <sup>c</sup>	53.3±7.0 <sup>b</sup>	21.7±3.8 <sup>b</sup>
0.1/10	NA	0.04±0.001 <sup>d</sup>	27.2±6.5 <sup>c</sup>	30.0±1.1 <sup>a</sup>
Mature Fruit				
1	28±4.6 <sup>a</sup>	0.30±0.02 <sup>a</sup>	61.8±4.2 <sup>a</sup>	4.3±0.5 <sup>c</sup>
1/10	NA	0.05±0.006 <sup>b</sup>	61.3±0.0 <sup>a</sup>	8.7±1.0 <sup>b</sup>
0.5/10	NA	0.04±0.0 <sup>c</sup>	37.3±4.2 <sup>b</sup>	9.1±1.2 <sup>b</sup>
0.1/10	NA	0.04±0.0 <sup>c</sup>	31.2±0.4 <sup>b</sup>	25.5±1.5 <sup>a</sup>
BHT (µg/mL)				
200	27.2±0.6	0.04±0.0 <sup>*</sup>	32±6.6	13.6±2.2

**Table 1:**  
Antioxidant Capacity of  
Immature and Mature  
Fruits of *Nypa fruticans*.

NA-No Activity; \*Gallic acid. Values are mean ± standard deviation of three replicate analyses. For each treatment, the means in a column followed by different small letters were significantly different at  $P < 0.05$ .

