

Nipah (*Nypa fruticans* Wurmb.) fruit as a potential natural antioxidant source

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Abstract. Nipah is one of mangrove plant that grows in coastal areas. South Sumatra province has a region with a watershed which is overgrown with nipah plants. Until now, nipah fruit has a low economic value because of the lacks of knowledge about the processing techniques of nipah fruit and the lacks of scientific attention which cover up the potential of nipah fruit as a functional food. This study aims to reveal the potential of nipah fruit, especially as natural antioxidants source. Total phenolics content (TPC) and antioxidant activities of *Nypa fruticans* fruit extracts (ripe and unripe) have been analyzed. Fruit extract from unripe nipah fruits (FEUN) got the highest phenolics content (121.3 ± 3.3 mg GAE/g). Radical scavenging activity of FEUN, assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals showed inhibitory activity about $81 \pm 3.1\%$ RSA. Hopefully, this research could reveal the potential of nipah fruit as a potential source of natural antioxidant

1. Introduction

Natural antioxidant used as an exogenous antioxidant in food system [1]. Many natural antioxidants come from various parts of plants such as fruits, root, flowers, and even stems [2,3]. Food antioxidants that exist in food products, both added (fortified), and which are naturally present in these foods, have many health benefits, including: smooth bowel movements, prevent constipation, so as to reduce the risk of cancer, heart disease, so that it can reduce cholesterol levels in the blood [4]. One of the local food products in South Sumatra that has the potential to be functional food as a source of food fiber and natural antioxidants is the fruit of the nipah plant (*Nypa fruticans*)

Nipah is monocot plant and growth wildly in the land near river area in Banyuasin district, in South Sumatera, Indonesia, which the family is Palmae alias Areaceae and subfamily Nypoideae. Nipah is the only plant in the Nypoideae subfamily, nothing else. This plant is not branched, lives in brackish water with straight stems soaring upwards and has fruit that grows above the water's surface (figure 1). Almost all parts of nipah plants can be utilized to meet the needs of riverine communities, the most commonly used nipah plant parts are sweetened tap water from mayang (nira), leaves, and fruit. Palm sap is generally tapped only for drinking and as a basic ingredient in making brown sugar, while palm leaves are used as a material for making roofs, walls, various wicker baskets and for cigarette wrapping leaves,



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while the fruit is only eaten fresh (without processing), and the sap can be used as bioethanol source [5]. The portion of nipah plants that has the potential to be used as a fulfillment of food needs for people who live in coastal areas of the river is the fruit [6]. Nipah is known to be beneficial as food that has many benefits, especially as a source of food fiber and natural antioxidants even until now this fruit is still not familiar as food natural antioxidant source due to the lacks of scientific research [7]. In this research, we want to reveal the potential of nipah fruit as a potential source of natural antioxidant

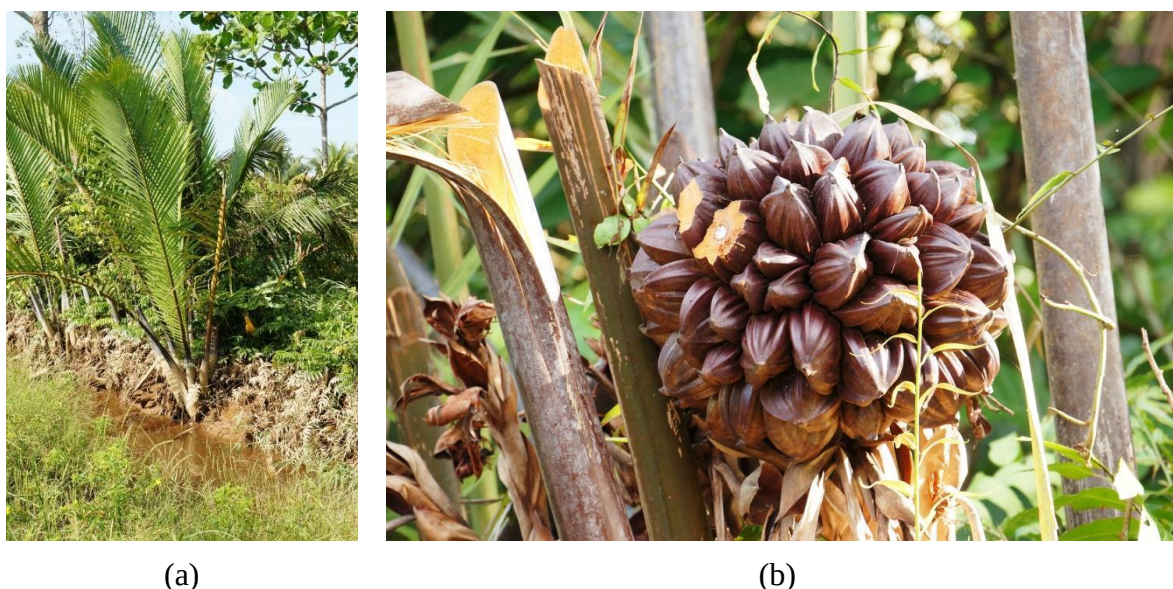


Figure 1. (a) Nipah plants and (b) nipah fruits.

2. Materials and methods

2.1. Materials

Nipah fruit used in this study was obtained from Dusun 4 of Teluk Betuk Village, Pulau Rimau District, Banyuasin Regency, South Sumatra Province, Indonesia. Harvested nipah is a nipah with two levels of maturity, namely unripe nipah (3 months maturity) and ripe nipah (6 months maturity) which collected on the 23 rd of April 2019. Unripe nipah fruit is characterized by a soft fruit texture and has a transparent appearance of fruit flesh (can be penetrated by light), while ripe nipah fruit is characterized by a hard fruit texture (resembling coconut) and has a white flesh appearance that is impermeable (figure 2). Chemical reagent for antioxidant activity assay and phenolic content like 2,2-diphenyl-picrilhydrazil (DPPH), ethanol, ascorbic acid, gallic acid, were obtained from Sigma Co., St. Louis, MO, USA. Folin-Ciocalteu was obtained from Merck (Darmstadt, Germany)

2.2. Preparations of nipah fruit extract

Preparations of nipah fruit extract using Prasad, *et al* method [8] with a minor change. The endosperm of nipah fruit (150 g) dried in an oven at 60°C (constant temperature) until constant weight obtained. The dried nipah fruit was crushed by Philips HR 2118 food processor to make it powder size and sieved at 20 mesh particle size to make uniform powder. The dry powder was used to make nipah fruit extract by weighing 10 g dry powder from ripe and unripe endosperms and then extracted separately with 50% ethanol (100 mL) using maceration method with a water bath shaker at 30°C for 12 h duration. The extracts were then filtered using Whatman filter paper (no. 4), concentrated, freeze-dried, and stored at -5°C until further analysis. Total phenolics content (TPC) and antioxidant activities of *Nypa fruticans* fruit extracts (ripe and unripe) have been analyzed.



Figure 2. (a) Unripe nipah fruits and (b) ripe nipah fruits.

2.3. DPPH radical scavenging activity

Determination of 2,2-diphenyl-picrylhydrazil (DPPH) radical scavenging activity based on the method developed by Prasad *et al* [8] with a minor change. An aliquot of 0.1 mL of nipah extract at different concentrations (10, 50, 100, and 200 $\mu\text{g/mL}$) mixed with 1 mL of DPPH (200 mM, dissolved in methanol). The reaction mixture was vortexed and then incubated at the dark room with 37°C temperature for 30 min. The absorbance of each sample was measured by spectrophotometer at 517 nm wavelength. The inhibition of DPPH $\cdot$ radicals was calculated as scavenging activity (%) = (Control OD – sample OD/control OD) $\times$ 100. Ascorbic acid was used for comparison.

2.4. Determination of total phenolic content of nipah extract

Determination of total phenolic content based on the Folin-Ciocalteu method Ebrahimzadeh *et al* [9]. Analysis of phenolic content in nipah extract uses gallic acid standards, so the units are expressed in gallic acid equivalent (mg GAE / 10 g sample). The steps include making a gallic acid stock solution, diluting it, making a 2% sodium carbonate (Na_2CO_3) solution, making a standard curve, and testing the total phenolic of nipah extract.

3. Result and discussion

3.1. Physical parameters of Indonesian nipah fruit

Teluk Betung village, Pulau Rimau District, Banyuasin Regency is one of the villages that is overgrown with wild nipah plants with an area of ± 40 ha, nipah in this area has not been maximally utilized because the potential of nipah fruit is not widely known by the public. Many researchers have tried to reveal the antioxidant content in nipah, especially nipah from Malaysia [8]. Because of different places of growing, nipah plants can have different fruit sizes, antioxidant content, and also polyphenol compounds. Compared in size of nipah fruit, both individual fruit and endosperm fruit, nipah fruit that grows in Indonesia have slightly smaller in size and weight when compared to nipah fruit which grows in Malaysia (table 1).

Tabel 1. The difference in physical parameters of nipah fruit from Indonesia and Malaysia.

	Indonesian Nipah Fruit		Malaysian Nipah Fruit [8]	
	Unripe	Ripe	Unripe	Ripe
Individual Fruit	121.4 ± 2.4 ^b	143.4 ± 3.3 ^a	138.1 ± 5 ^b	159.6 ± 7.7 ^a
Weight (g)	8.6 ± 2.5 ^b	10.4 ± 1.2 ^a	11.1 ± 0.5 ^b	12.9 ± 0.7 ^a
Length (cm)	7.1 ± 0.3	8.3 ± 1.4	7.8 ± 0.5	8.1 ± 0.3
Breadth (cm)				
Endosperm	2.9 ± 1.7 ^b	16.6 ± 1.1 ^a	3.6 ± 1.5 ^b	19.6 ± 0.8 ^a
Weight (g)	2.7 ± 0.8 ^b	3.4 ± 0.2 ^a	3.0 ± 0.2 ^b	4.5 ± 0.5 ^a
Length (cm)	4.3 ± 1.2 ^b	8.2 ± 2.7 ^a	5.8 ± 0.7 ^b	10.1 ± 0.5 ^a
Perimeter (cm)				

For each treatment means in a row followed by different letters are significantly different at $P < 0.05$

This difference in size and weight can occur due to the different places of growth so that the nutrients obtained by the nipah tree are different and produce different sizes and weights of the nipah fruit. The effect of this size is also a cause of differences in activity and antioxidant content of nipah extract which has a different location to grow, this data according to Hainida *et al* (2009) research [7].

3.2. DPPH radical scavenging activity and total phenolic content

Increased ability to neutralize 2,2-diphenyl-picrilhydrazil (DPPH) as a radical occurs along with increased concentrations of nipah extract and ascorbic acid as a comparable antioxidant compound. The increase occurred in both types of nipah extract both nipah fruit extract from unripe nipah (FEUN) and fruit extract from ripe nipah (FERN). DPPH is a stable free radical compound and can accept electrons and hydrogen radicals to become stable diamagnetic molecules that are widely used to investigate the activity of radical scavenging in plant extract compounds [8]. In the DPPH method, the antioxidant will react with DPPH and change the color of the solution from dark purple to yellow due to the formation of α -diphenyl- β -picryl hydrazine compounds [10]. The degree of purple decay indicates the ability and activity of an antioxidant compound. FEUN has significantly higher antioxidant activity ($81 \pm 3.1\%$) compared to FERN ($45 \pm 2.2\%$) at the same concentration ($200 \mu\text{g/mL}$) and was comparable to the scavenging activity of ascorbic acid ($90 \pm 3.2\%$). The differences between FEUN and FERN was because of the differences in total phenolic content. Total phenolic content of FEUN had significantly ($P < 0.05$) higher ($121.3 \pm 3.3 \text{ mg GAE/g}$) than FERN ($10.2 \pm 2.5 \text{ mg GAE/g}$). Phenolic compounds are synthesized rapidly during the early stages of fruit maturity. After the fruit is ripe, the decrease in the concentration of phenolic compounds is caused by cells undergoing a growth process [12]. The decrease in primary activity in mature/ripe fruit occurs due to the reduction in the substrate needed for biosynthesis of phenolic composition. In addition, the process of polymerization, oxidation, and conjugation of phenolic compounds during the fruit ripening process can also result in a decrease in phenolic composition [11].

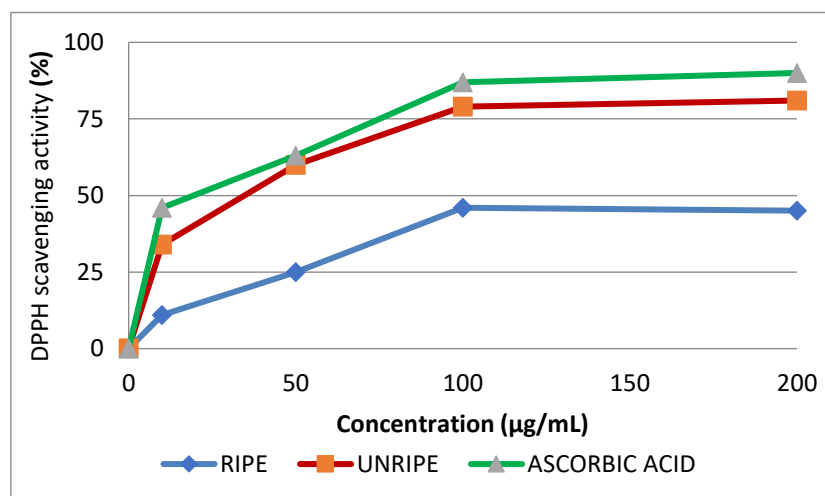


Figure 3. DPPH scavenging activity over concentration of phenolic compounds.

4. Conclusion

The antioxidant activity of fruit extract from unripe nipah (FEUN) is higher when compared to the antioxidant activity of fruit extract from ripe nipah (FERN) because in FEUN there are still many phenolic compounds that have not changed much in line with the ripening process of nipah. The difference in the content of total phenolic compounds was also seen due to differences in the age of nipah fruit, where FEUN had a higher total phenolic compound content compared to FERN. Palm fruit has the potential to be a source of natural antioxidants, especially to be applied to the food system. Need further research on the analysis of the types of antioxidants and antioxidant extraction techniques in palm fruit

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