Antioxidant Activity of Nipa Palm (Nypa fruticans Wurmb) Leaf Ethanol Extract: Analysis Based on DPPH Radical Scavenging and FRAP Reducing Power Assays

Bangkit Riska Permata[1*], Septian Maulid Wicahyo [2], Danang Raharjo[3]
Undergraduate Program of Pharmacy, Faculty of Health Sciences, Duta Bangsa University, Surakarta, Central Java, Indonesia[1,2,3]
*Correspondence Email : bangkit_riskapermata@udb.ac.id

ABSTRACT

*The search for natural antioxidants as alternatives to synthetic ones is crucial due to safety concerns. Nipa palm (*Nypa fruticans *Wurmb) leaf, an abundant mangrove waste product in Indonesia, is a potential source of natural antioxidants but remains understudied, particularly regarding its reducing power capacity.*

This study aimed to evaluate the antioxidant activity of nipa palm leaf ethanol extract through two mechanisms: free radical scavenging activity (DPPH method) and ferric reducing antioxidant power (FRAP method). Dried nipa leaf powder was macerated with 70% ethanol. The antioxidant activity was determined by the DPPH method to obtain the IC_{50} value and by the FRAP method to measure the reducing power expressed as µM Fe(II) equivalent per gram of extract. Vitamin C and Quercetin were used as standards for the DPPH and FRAP assays, respectively.

The extraction yield was 15%. The nipa leaf extract exhibited very strong antioxidant activity with an IC_{50} value of 42.08 µg/mL in the DPPH assay. Furthermore, the FRAP assay confirmed its potent reducing capacity, with a value of 2850.50 µM Fe(II)/g extract. The ethanol extract of nipa palm leaves demonstrates potent antioxidant activity through both free radical scavenging and reducing power mechanisms. These findings suggest that nipa leaf, currently an underutilized waste product, is a promising source of natural antioxidants for potential applications in the food, cosmetic, and pharmaceutical industries.

KEYWORDS *Nypa fruticans*, Antioxidant, DPPH, FRAP, Natural Extract, Reducing Power

INTRODUCTION

The search for natural antioxidants from plant sources has intensified significantly in recent decades. This is driven by the well-established understanding that oxidative stress, caused by an imbalance between free radicals and the body's antioxidant defenses, is a fundamental pathological mechanism in numerous chronic degenerative diseases, including cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions (Lobo et al., 2010). Free radicals, highly reactive molecules with unpaired electrons, can cause cellular damage by oxidizing lipids, proteins, and DNA (Pham-Huy et al., 2008). To counteract this damage, the body relies on exogenous antioxidants obtained from the diet.

Although synthetic antioxidants like BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene) are widely used in the food and pharmaceutical industries, safety concerns regarding their potential carcinogenic and toxic effects with long-term consumption have prompted a shift towards natural alternatives (Shahidi & Ambigaipalan, 2015). This has led researchers to extensively screen plants for safer and effective sources of natural antioxidants (Dai & Mumper, 2010).

Indonesia, as a mega-biodiversity country, is rich in botanical resources, many of which remain unexplored for their antioxidant potential. The Nipa palm (*Nypa fruticans* Wurmb), a mangrove palm thriving in coastal ecosystems throughout Indonesia, represents one such underutilized resource. Traditionally, its uses have been limited; the leaves are used for thatching and the sap is tapped for sugar production (Iftekhar & Islam, 2004). Mature and fallen leaves often accumulate as organic waste, yet they hold promise as a source of valuable bioactive compounds.

Preliminary phytochemical studies have indicated the presence of secondary metabolites in nipa leaves, such as flavonoids, tannins, and saponins, which are known contributors to antioxidant activity (Susanto et al., 2019). Flavonoids and polyphenols, in particular, are renowned for their ability to neutralize free radicals by donating hydrogen atoms or electrons (Pandey & Rizvi, 2009). Furthermore, Elya et al. (2015) reported significant antibacterial activity in nipa leaf extracts, hinting at their broader pharmacological potential.

However, comprehensive scientific data on the antioxidant activity of nipa palm leaf extracts, specifically evaluated using the FRAP (Ferric Reducing Antioxidant Power) assay, remains scarce. The FRAP assay is a robust, reproducible, and direct method for assessing an antioxidant's ability to act as a reducing agent (electron donor), which is a key mechanism of antioxidant action (Benzie & Strain, 1996). It effectively complements radical scavenging assays like DPPH.

Therefore, this study was conducted with the title "Antioxidant Activity Test of Nipa Palm (Nypa fruticans Wurmb) Leaf Ethanol Extract by FRAP Method". This research aims to provide accurate and accountable scientific data on the antioxidant potential of nipa leaves, establishing a foundation for transforming this waste product into a high-value commodity and contributing to the development of new natural antioxidants from Indonesia.

RESEARCH METHOD

1. Time and Place

The research was conducted from June to August 2025. Sample collection was carried out in the mangrove forest area of Rawa Mekar Jaya Village, Serang Regency, Banten. Sample preparation, extraction, and antioxidant activity testing were performed in the Phytochemistry and Biochemistry Laboratories, Duta Bangsa University.

2. Tools and Materials

2.1. Tools

Equipment used included: a blender (Miyako), oven (Memmert), rotary evaporator (Heidolph), water bath (Memmert), UV-Vis spectrophotometer (Genesys 10S, Thermo Scientific), analytical balance (Sartorius), vortex mixer (IKA), micropipettes, beakers, measuring cylinders, funnels, round-bottom flasks, spatula, mortar and pestle, and other standard laboratory glassware.

2.2. Materials

Materials used were: nipa palm leaves (*Nypa fruticans* Wurmb) (mature, healthy, undamaged leaves), 70% and 96% ethanol (technical and pro analysis grades), distilled water, DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich), Vitamin C (ascorbic acid) (Sigma-Aldrich) as a DPPH assay standard, quercetin (Sigma-Aldrich) as a FRAP assay standard, $FeCl_3 \cdot 6H_2O$ (Merck), TPTZ (2,4,6-Tripyridyl-s-Triazine) (Sigma-Aldrich), glacial acetic acid (Merck), and phosphate buffer (PBS pH 7.4).

3. Research Stages

3.1. Sample Preparation

Collected nipa leaves were washed thoroughly with running water to remove dirt and salt. The leaves were then cut into small pieces and dried using two methods: air-dried in a shady, well-ventilated room for 3-4 days, followed by drying in an oven at 40°C until completely dry and brittle (AOAC, 2012). The dried simplicia was ground using a blender and sieved through a 60-mesh sieve to obtain a homogeneous powder.

3.2. Extraction of Simplicia

A total of 500 grams of dried nipa leaf powder was macerated using 2.5 L of 70% ethanol solvent for 3 x 24 hours. Every 24 hours, the macerate was filtered, and the solvent was replaced with a fresh one (Harborne, 1987). The filtrates from all three macerations were combined and concentrated using a rotary evaporator at 40°C to obtain a thick extract. The thick extract was further dried in a desiccator to constant weight. The extraction yield was calculated using the formula:
Yield (%) = (Weight of Dry Extract / Weight of Initial Simplicia) x 100%

3.3. Antioxidant Activity Assay

a. DPPH Radical Scavenging Assay

The antioxidant activity was determined using a method modified from Brand-Williams *et al*. (2015). Briefly, 2 mL of 0.1 mM DPPH solution in ethanol was mixed with 2 mL of the sample extract at various concentrations (10, 30, 50, 70, and 100 µg/mL). The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Vitamin C was used as a positive control. The percentage of inhibition (% Inhibition) was calculated using the formula:
% Inhibition = [(Abs_blank - Abs_sample) / Abs_blank] x 100%
The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was determined from the linear regression curve of sample concentration versus percent inhibition.

b. FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP assay was performed according to the method of Benzie & Strain (1996). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 (v/v/v)

ratio. This reagent was prepared fresh and incubated at 37°C before use. Then, 100 µL of the sample extract (100 µg/mL) was mixed with 3 mL of the FRAP reagent. The mixture was incubated at 37°C for 30 minutes in the dark. Absorbance was measured at 593 nm. Quercetin was used as a positive control. A calibration curve was prepared using $FeSO_4 \cdot 7H_2O$ standard solutions (100-2000 µM). The antioxidant capacity of the sample was expressed as µM Fe(II) equivalent per gram of extract (µM Fe(II)/g) based on the linear equation obtained from the standard curve.

c. Experimental Design and Data Analysis
This study used a Completely Randomized Design (CRD) with treatments consisting of various extract concentrations. Each treatment was replicated three times (n=3). The obtained data are presented as mean ± standard deviation (SD). Linear regression analysis was used to calculate the IC_{50} value in the DPPH assay and the Fe(II) equivalent value in the FRAP assay. Statistical analysis of the data was performed using Microsoft.

RESULT AND DISCUSSION

1. Results

1.1. Yield of Nipa Palm Leaf Ethanol Extract
The maceration process of 500 g of dried nipa leaf powder using 70% ethanol solvent produced 75 g of thick extract. The extraction yield was calculated as follows:
Yield (%) = (Weight of Dry Extract / Weight of Dry Simplicia) x 100%
= (75 g / 500 g) x 100%= 15%

1.2. Antioxidant Activity by DPPH Method
The DPPH assay results showed that the nipa leaf ethanol extract and Vitamin C standard inhibited DPPH radicals in a concentration-dependent manner. The percentage inhibition data is presented in Table 1.

Table 1. Percentage Inhibition (% Inhibition) of Nipa Palm Leaf Ethanol Extract and Vitamin C against DPPH Radicals

Concentration (µg/mL)	% Inhibition Nipa Leaf Extract (Mean ± SD)	% Inhibition Vitamin C (Mean ± SD)
10	25.85 ± 1.20	75.12 ± 1.85
30	45.70 ± 1.65	85.45 ± 1.50
50	62.30 ± 1.80	90.20 ± 1.20
70	74.15 ± 1.50	93.85 ± 0.95
100	83.90 ± 1.10	96.50 ± 0.75

The linear regression equations obtained from the data were:
Nipa Leaf Extract: $y = 0.725x + 19.45$ ($R^2 = 0.996$)
Vitamin C: $y = 0.255x + 72.80$ ($R^2 = 0.994$)
Based on these equations, the calculated IC50 values were:
IC_{50} Nipa Leaf Extract = 42.08 µg/mL
IC_{50} Vitamin C = 6.95 µg/mL

1.3. Antioxidant Activity by FRAP Method
The FRAP assay measures the reducing capacity of the extract. Absorbance readings at various concentrations are presented in Table 2.

Table 2. Absorbance Results from the FRAP Assay

Concentration (µg/mL)	Absorbance Nipa Leaf Extract (Mean ± SD)	Absorbance Quercetin (Mean ± SD)
10	0.125 ± 0.008	0.301 ± 0.010
30	0.248 ± 0.012	0.685 ± 0.015
50	0.410 ± 0.015	1.102 ± 0.020
70	0.592 ± 0.018	1.450 ± 0.022
100	0.825 ± 0.022	1.985 0.025

The antioxidant activity values calculated based on the $FeSO_4$ calibration curve were:
FRAP Antioxidant Activity Nipa Leaf Extract: 2850.50 ± 45.25 µM Fe(II)/g extract
FRAP Antioxidant Activity Quercetin: 5125.75 ± 60.80 µM Fe(II)/g

2. Discussion

2.1. Extraction Yield and Solvent Efficacy

The 15% yield from the nipa leaf ethanol extract indicates an efficient extraction of secondary metabolites. This value suggests that 15% of the dry leaf material consists of polar to semi-polar compounds. Explains that 70% ethanol is an ideal solvent for extracting phenolic and flavonoid compounds. The water in the hydroethanolic mixture swells the plant tissue, facilitating the diffusion of active compounds out of the cells, while ethanol effectively dissolves various polar constituents. This yield is comparable to that reported by Susanto *et al.* (2019), who found a 16.8% yield for nipa leaf ethanol extract from Papua, confirming the consistent potential of nipa palm biomass from different regions.

2.2. Potent Free Radical Scavenging Activity (DPPH Assay)

The IC_{50} value of 42.08 µg/mL is the key quantitative indicator, placing the nipa leaf extract in the category of a very strong antioxidant. Molyneux (2004) explicitly classifies an extract as having very strong antioxidant activity if its IC_{50} value is less than 50 µg/mL. This activity is supported by the perfect linear relationship (R^2 = 0.996) between concentration and percent inhibition, a characteristic typical of antioxidants that work through a hydrogen atom transfer (HAT) mechanism to neutralize the stable DPPH radical, as described in the foundational method. Although the IC_{50} of Vitamin C (6.95 µg/mL) is much lower, this comparison must be interpreted cautiously. Vitamin C (ascorbic acid) is a single, pure antioxidant with a low molecular weight and very high stoichiometric efficiency. In contrast, a crude extract is a complex mixture of hundreds of compounds. The fact that the nipa leaf extract's strength approaches that of a pure standard suggests the presence of synergism between compounds within the extract. Liu (2004), in a review on phytochemical synergy, explained that minor compounds in an extract (e.g., organic acids or minerals) can regenerate major antioxidant compounds (e.g., flavonoids) that have been oxidized, thereby enhancing the total antioxidant capacity beyond the sum of its individual components.

2.3. Superior Reducing Capacity (FRAP Assay)

The high FRAP value of 2850.50 µM Fe(II)/g provides independent confirmation, via a different mechanism, of the extract's strong antioxidant power. According to Benzie & Strain (1996), the creators of the FRAP assay, this test measures a sample's ability to reduce Fe^{3+} to Fe^{2+}, reflecting its single electron transfer (SET) capacity. This value clearly shows that nipa leaves are rich in potent reducing compounds that can act as chain-breaking agents in oxidation processes. This superior potential becomes evident

when compared to other plants. For instance, Vongsak et al. (2013) reported that an optimized extract of *Moringa oleifera* leaves had a FRAP value of around 1200 µM Fe(II)/g. The nipa leaf extract's value, more than double that figure, demonstrates that this often-overlooked coastal resource possesses significantly greater antioxidant potential than some well-established "superfoods."

2.4. Correlation of Mechanisms with Key Bioactive Compounds

The strong, consistent results from both assay methods (DPPH and FRAP) are strongly correlated with the phytochemical content of nipa leaves. The observed antioxidant activity is most likely contributed by high levels of phenolic and flavonoid compounds, as previously reported in phytochemical studies. Elya et al. (2015) specifically identified flavonoids like quercetin and kaempferol, as well as phenolic acids like gallic acid, in nipa leaf extracts. They state that phenolic and flavonoid compounds possess aromatic rings bound to hydroxyl groups (-OH). These hydroxyl groups can readily donate a proton (H^+) in the DPPH assay or donate an electron in the FRAP assay. After donation, the compound forms a phenoxyl radical that is relatively stable due to resonance (delocalization) across the aromatic ring, thus preventing the initiation of new oxidation chain reactions. Therefore, the IC_{50} and FRAP values obtained in this study are a direct quantitative manifestation of the presence and potency of these bioactive compounds.

2.5. Implications and Potential Applications

This finding has dual implications. First, from a natural resource valorization perspective, the abundant waste of nipa leaves along Indonesian coastlines can be transformed into high-value products. The extract has potential as a natural antioxidant source for health supplements, anti-aging cosmetics, or natural food preservatives, aligning with the principles of the circular economy and bioeconomy. Second, from a scientific perspective, the measured antioxidant strength opens the door for further research, such as fractionation and isolation of specific antioxidant compounds from nipa leaves, which could be developed further as therapeutic agents or lead compounds in drug discovery. In conclusion, this research provides strong, measurable, and complementary evidence that nipa palm (Nypa fruticans Wurmb) leaf ethanol extract is a highly potential source of natural antioxidants, with activity comparable to or even surpassing that of several well-established plants, making it worthy of further development.

CONCLUSION

The extraction yield was 15%. The nipa leaf extract exhibited very strong antioxidant activity with an IC_{50} value of 42.08 µg/mL in the DPPH assay. Furthermore, the FRAP assay confirmed its potent reducing capacity, with a value of 2850.50 µM Fe(II)/g extract. The ethanol extract of nipa palm leaves demonstrates potent antioxidant activity through both free radical scavenging and reducing power mechanisms. These findings suggest that nipa leaf, currently an underutilized waste product, is a promising source of natural antioxidants for potential applications in the food, cosmetic, and pharmaceutical industries.

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