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Evaluation of the Antioxidant Activity of Red Fruit (*Pandanus conoideus* L.) Juice Using the DPPH Radical Scavenging Assay

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ABSTRACT: Red fruit (*Pandanus conoideus* Lam.), a member of the Pandanaceae family, is a traditional plant widely found in Papua, Indonesia, and has long been utilized for its nutritional and medicinal properties. Empirical evidence suggests that red fruit possesses therapeutic potential against various degenerative diseases, including cancer, cardiovascular diseases, tuberculosis, respiratory disorders, as well as certain eye and skin conditions. These health benefits are closely associated with its rich content of bioactive compounds, particularly antioxidants. This study aimed to evaluate the antioxidant activity of red fruit juice using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The DPPH assay is a widely used method for assessing antioxidant capacity, based on the reduction of the DPPH radical, which exhibits maximum absorbance in ethanol or methanol, making it suitable for analyzing polar compounds. The antioxidant activity of red fruit juice was assessed both qualitatively and quantitatively. Qualitative analysis showed a visible color change from purple to yellow upon the addition of DPPH reagent, indicating the presence of antioxidant compounds. Quantitative analysis was performed using a UV-Visible spectrophotometer at a maximum wavelength of 519 nm, with ascorbic acid used as a standard reference. The antioxidant activity was determined by IC_{50} (Inhibition concentration 50%) of the antioxidant compound. The results revealed that red fruit juice exhibited notable antioxidant activity, with IC_{50} of 47.68 µg/mL. In conclusion, red fruit juice demonstrates significant antioxidant potential and may serve as a natural source of antioxidants for the prevention of oxidative stress-related diseases. These findings support the further development and utilization of *P. conoideus* as a functional food and natural therapeutic agent.

KEYWORDS: Absorbance; antioxidants; DPPH; red fruit; UV-Vis spectrophotometry.

1. INTRODUCTION

Red fruit (*Pandanus conoideus* Lam.) is one of Papua's indigenous plants belonging to the Pandanaceae family. This species grows widely across Papua, both in lowland and highland regions, and has long been utilized by local communities as a functional food. Traditionally, red fruit is consumed to enhance stamina and overall body fitness, and it is also used in the treatment of helminthiasis and various skin diseases. Moreover, it has been reported to possess therapeutic potential against a wide range of degenerative and infectious diseases, including cancer, diabetes mellitus, gout, osteoporosis, hypertension, cardiovascular diseases, hypercholesterolemia, stroke, as well as infections caused by bacteria, fungi, and viruses (Asrianto et al., 2023). These diverse pharmacological effects are closely associated with its rich content of bioactive compounds, particularly those with antioxidant properties.

Free radicals are molecules or atoms that contain one or more unpaired electrons in their outer orbitals, making them highly reactive. These unstable species can initiate chain reactions by capturing electrons from surrounding molecules, thereby generating new radicals and inducing oxidative stress. Free radicals are produced both endogenously, as by-products of normal metabolic processes such as the oxidation of proteins, carbohydrates, and lipids, and exogenously from environmental sources including air pollution, vehicle emissions, chemicals, and thermally processed foods (Salim, 2018; Pratama et al., 2015). Excessive accumulation of free radicals in the body can lead to cellular damage affecting lipids, proteins, carbohydrates, and DNA, ultimately contributing to the development of various chronic and degenerative diseases (Fawwaz et al., 2025).

Antioxidants play a crucial role in mitigating oxidative stress by neutralizing free radicals and inhibiting oxidation reactions. They can be classified into intracellular antioxidants, such as superoxide dismutase (SOD), and extracellular antioxidants, including various dietary compounds such as flavonoids and anthocyanins. Natural antioxidants have gained increasing attention as safer alternatives to synthetic compounds. However, under conditions of excessive oxidative stress, endogenous antioxidant defenses may be insufficient, necessitating the intake of exogenous antioxidants from dietary or natural sources (Pratiwi et al., 2022).

The antioxidant activity of a compound is commonly evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. This method measures the ability of antioxidants to donate hydrogen atoms or electrons to stabilize DPPH radicals, resulting in a color change that can be quantified spectrophotometrically. Antioxidant capacity is often expressed as the percentage of inhibition or as the IC_{50} value, which represents the concentration required to inhibit 50% of DPPH radicals. A lower IC_{50} value indicates stronger antioxidant activity. According to Molyneux (2004),

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antioxidant strength is categorized as very strong (IC_{50} < 50 µg/mL), strong (50–100 µg/mL), moderate (100–150 µg/mL), weak (150–200 µg/mL), and very weak (>200 µg/mL).

Previous studies have demonstrated that red fruit extracts exhibit potent antioxidant activity. For instance, Sangkala et al. (2014) reported that red fruit extract from the Poso region of Central Sulawesi showed very strong antioxidant activity with an IC_{50} value of 14.45 µg/mL using the DPPH method. Despite these promising findings, studies focusing specifically on the antioxidant activity of red fruit juice remain limited.

Therefore, this study aims to evaluate the antioxidant activity of red fruit (*Pandanus conoideus* Lam.) juice using the DPPH method, with vitamin C (ascorbic acid) as a reference standard. The findings of this study are expected to provide scientific evidence supporting the potential of red fruit juice as a natural source of antioxidants for the prevention of oxidative stress-related diseases.

2. EXPERIMENTAL SECTION

2.1. Sample collection

This study was conducted at the Pharmaceutical Chemistry Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, from March to July 2024. The study material consisted of red fruit (*Pandanus conoideus* Lam.), with samples specifically obtained from the seeds collected in Sorong City, Southwest Papua Province. An experimental laboratory design was employed to evaluate the antioxidant activity of red fruit juice using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method.

2.2. Qualitative analysis

Pipetted as much as 1 mL of sample solution into a test tube, then added 4 mL of DPPH solution little by little and observed the color change. The presence of antioxidants is indicated by the color formed from each test sample is yellow (Muflihunna & Sarif, M., 2015).

2.3. Preparation of DPPH

A total of 5 mg of DPPH was accurately weighed and dissolved in 96% ethanol in a 100 mL volumetric flask to obtain a 50 µg/mL stock solution. A working solution of 25 µg/mL was then prepared by pipetting 25 mL of the 50 µg/mL DPPH solution into a 50 mL volumetric flask and diluting to volume with 96% ethanol.

2.4. Quantitative analysis

2.4.1. Determination of maximum wavelength

The test was carried out by pipetting as much as 4 mL of 25 µg/mL DPPH, after which it was left for 30 minutes in a dark place. The absorbance of the solution was measured with a UV-Vis spectrophotometer at a wavelength of 400 - 800 nm. Then the maximum wavelength obtained is a wavelength of 519 nm (Fawwaz et al., 2018).

2.4.2. Antioxidant activity test

Ascorbic acid was used as a standard comparison. Preparation of 1000 µg/mL ascorbic acid stock solution was carried out by weighing as much as 10 mg of sample and then solution with 10 mL of 96% ethanol. The dilution of the comparison stock solution was divided into five concentrations (5, 10, 15, 20, and 25 µg/mL), then pipetted as much as 0.5 mL of samples of various concentrations and put into a test tube, then add 2.5 ml of DPPH reagent solution and homogenize leave for 30 minutes at room temperature in a dark place. The absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 519 nm. A sample of 10 mg was dissolved in 10 mL of 96% ethanol to obtain a concentration stock solution for 1000 µg/mL. Dilution of the sample stock solution was divided into five concentrations (5, 10, 15, 20, and 25 µg/mL), the same procedure was carried out on the ascorbic acid comparison solution.

2.5. Data analysis

The antioxidant activity was determined by IC_{50} (Inhibition concentration 50%) of the antioxidant compound. IC_{50} value is the concentration required to reduce free radical activity by 50%. The lower the IC_{50} value, the higher the antioxidant activity. The IC_{50} value of each sample concentration can be calculated using the linear regression equation formula using the Microsoft Excel application. Linear regression equation with (x) as sample concentration and (y) as percent antioxidant activity, and obtained equation: $y = ax + b$.

3. RESULTS AND DISCUSSION

Antioxidant activity testing using the DPPH method aims to measure the ability of a compound to capture free radicals. This method is often used in research due to its simplicity and speed in providing results. The testing process involves measuring the color change of the DPPH solution which relates to the number of free radicals neutralized by the antioxidants in the sample.

The samples of red fruit (*Pandanus conoideus* Lam.) that have been obtained are cleaned, and then cut into small pieces, and separate the seeds from the red fruit. Then the sample is mashed with a juicer and filtered until juice is obtained from the red fruit. Next, the antioxidant test begins with a qualitative test of red fruit juice. The qualitative test results showed that the red fruit juice samples were positive for antioxidant activity, which was indicated by a change in color from purple to yellow. This color change indicates that DPPH has been reduced by donating hydrogen or electrons from antioxidant compounds (Fawwaz et al., 2023).

The quantitative antioxidant test begins with determining the maximum wavelength of DPPH and the result obtained is 519 nm. In this study, a comparison solution was used, namely ascorbic acid and red fruit juice (*Pandanus conoideus* Lam.) samples. Where for the comparison solution, measurements were made of 5 concentration series, namely (5, 10, 15, 20, and 25 µg/mL), then measured absorbance at a wavelength of 519 nm. The absorbance data obtained was then calculated as % inhibition, which was converted into IC_{50}. Testing of red fruit juice samples was carried out by making 5 concentration series (20, 30, 40, 50, and 60 µg/mL). Furthermore, the same test was carried out as in ascorbic acid. The results of the calibration curves of red fruit juice are presented in **Figure 1.**

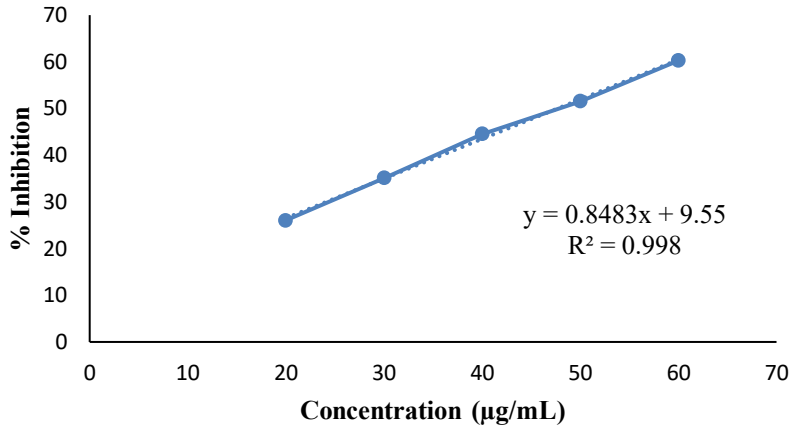


Figure 1. Red fruit juice calibration curve

The results of the calibration standard curve for ascorbic acid at a wavelength of 519 nm obtained a regression line equation, namely y = 0.322x + 42.486 and a correlation coefficient (R^2) value of 0.994. Meanwhile, for the calibration curve for the red fruit juice test sample, the regression equation was obtained, namely y = 0.8483x + 9.55, and the correlation coefficient (R^2) is 0.998. The two R^2 value data obtained show that the probit data for ascorbic acid and red fruit juice is almost close to 1, which means the value is very good. From the curve results of the comparison solution and test sample, the absorbance and %inhibition values were obtained, and the %inhibition results were then analyzed using a linear regression equation to obtain the IC_{50} value. From the data in **Table 1**, it can be concluded that the higher the sample concentration, the lower the absorbance value recorded. This decrease in absorbance value means that the higher the concentration, the higher the % inhibition produced. This data shows that both test solutions (red fruit juice and ascorbic acid) were effective in inhibiting the process measured in this study, namely the free radical process given from DPPH.

Table 1. Antioxidant activity of red fruit juice *(Pandanus conoideus* Lam) and ascorbic acid with DPPH method.

Sample	Concentration (µg/mL)	Absorbance of the sample	Inhibition (%)	IC_{50} value (µg/mL)
Red Fruit Juice	20	0.746	26.06	47.68
	30	0.655	35.08	
	40	0.560	44.49	
	50	0.489	51.53	
	60	0.401	60.25	
Ascorbic acid	5	0.463	44.41	22.21
	10	0.45	45.97	
	15	0.435	47.77	
	20	0.42	49.57	
	25	0.411	50.66	

The IC_{50} antioxidant activity values obtained from red fruit juice (47.68 µg/mL) and ascorbic acid (22.21 µg/mL) indicate that both test solutions have very high antioxidant activity. test solutions have very strong antioxidant activity. This is by literature which says that the IC_{50} value is categorized as very strong if the IC_{50} value is less than 50 µg/mL, strong category if the IC_{50} value is between 50 - 100 µg/mL, moderate category if the IC_{50} value is between 100 - 150 µg/mL, weak category if the IC_{50} value is between 150 - 200 µg/mL and very weak category if the IC_{50} value is between 200 - 250 µg/mL.

When compared with previous research by (Sangkala et al., 2014), these results show similarities in the effectiveness of red fruit as an antioxidant. Thus, testing red fruit in the form of extracts or juice can be used as a natural antioxidant because it is classified as a very strong antioxidant in inhibiting free radicals. The factors that can affect the value of antioxidant activity include the test method, extraction time, temperature, and pressure, leaf powder size, solvent volume, extraction method, and the chemical composition contained in a plant. The composition of a plant is influenced by the habitat conditions of the plant such as light and temperature (Manurung et al., 2023).

4. CONCLUSION

Based on the results of this study, it can be concluded that red fruit juice (*Pandanus conoideus* Lam.) exhibits significant antioxidant activity as demonstrated by qualitative analysis using the DPPH method, indicated by a visible color change from purple to yellow. Quantitative evaluation further confirmed its strong antioxidant potential, with an IC_{50} value of 47.68 ppm, which falls into the category of very strong antioxidant activity. These findings suggest that red fruit juice is a promising natural source of antioxidants and may contribute to the prevention of oxidative stress-related diseases. Therefore, *Pandanus conoideus* Lam. has potential for further development as a functional food or natural therapeutic agent. Future studies are recommended to isolate and characterize the active compounds responsible for this activity, as well as to evaluate its efficacy through in vivo and clinical investigations.

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