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Research Article

Antioxidant Activity of Red Fruit Juice (*Pandanus conoideus* L.) Using the CUPRAC Method

Marina Arifin[1], Mamat Pratama[1], Muammar Fawwaz[1,2*], Aminah[1]

[1] Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia
[2] Molecular Probes Discovery Group (MoleGro), Universitas Muslim Indonesia, Makassar, Indonesia
[*] Corresponding Author. E-mail: muammar.fawwaz@umi.ac.id

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ABSTRACT: Red fruit (*Pandanus conoideus* Lam.), which belongs to the *Pandanaceae* family, is widely used by local Papuan people as a source of food, natural dyes, and traditional medicinal ingredients. Red fruit contains several compounds such as tannins, vitamin C, beta carotene, and flavonoids. This research aims to determine the presence of antioxidant activity using the *Cupric Ion Reducing Antioxidant Capacity* (CUPRAC) method. Antioxidant capacity using the CUPRAC method is expressed as mg of ascorbic acid equivalent (AAE) per g of juice (mgAAE/g). The method is measured using a UV-Vis spectrophotometer with the maximum wavelength. Ascorbic acid was used as a comparison to assess the antioxidant ability of red fruit juice. The results showed that the antioxidant activity capacity of red fruit juice was 100.4 mgAAE/g. Thus, red fruit can be developed as a natural antioxidant.

KEYWORDS: Antioxidants; ascorbic acid; CUPRAC; red fruit; UV-Vis Spectrophotometer.

1. INTRODUCTION

Red fruit (*Pandanus conoideus* Lam.) is a species of pandanaceae and is a type of plant found endemic in the provinces of Papua and West Papua. This fruit has the potential to be developed as a source of phytopharmaceuticals in Indonesia. Red fruit contains active compounds that are important for health, including anticancer, energy enhancer, calcium, fiber, protein, vitamin B1, vitamin C, myristic acid, linoleic acid, deconic acid, omega 3, omega 6, and omega 9 (Ayomi, 2015).

Free radicals are atoms or molecules that have unstable properties, so they are very reactive and must destroy cells to gain access to these electron pairs. This free radical formation reaction is temporary and can be quickly converted into harmless substances. Free radicals are formed by donating or taking electrons from other molecules and can combine with other non-radical molecules. This can cause a chain reaction to occur, giving rise to new free radicals. The reaction of combining free radicals with non-radical molecules is an example of a chain reaction. This chain reaction will continue until the antioxidant system can fight it (Pambudi et al., 2017). Free radical reactions in general can be inhibited by certain antioxidants, both natural and synthetic. Antioxidants can also be obtained from consuming foods that contain lots of vitamin C, vitamin E, beta-carotene, and phenolic compounds. Red fruit is a natural ingredient that can function as an antioxidant, this is because red fruit also contains vitamin C, vitamin E, flavonoids, beta-carotene, and other compounds which are antioxidant compounds (Sangkala et al., 2014).

Antioxidants are substances that ward off or neutralize free radicals, thereby allowing atoms with unpaired electrons to obtain electron pairs. Antioxidants work by donating electrons or hydrogen atoms to prevent oxidation reactions or neutralize compounds that have undergone oxidation (Pambudi et al., 2017). One of the antioxidants testing methods is the CUPRAC method. In this method, the reagent used is Cu(II)-neocuproine (Cu(II)-(Nc)2) which functions as an oxidizing chromogen so that the reduction of Cu(II) ions can be measured. This method is widely used because the reagent used in the form of CUPRAC is selective and has a low reduction potential value so it is easy to apply and cost-effective (Sayakti et al., 2022).

Previous research conducted by the Bogor Agricultural Institute (IPB) showed that red fruit ethanol extract contains high amounts of carotenoids and tocopherols. All carotenoids, both provitamin A and non-provitamin A, can act as antioxidants. Antioxidants are compounds that can prevent free radical oxidation processes, prevent cancer and premature aging, as well as treat various degenerative diseases and metabolic disorders caused by diet, such as cancer, hepatitis, diabetes, tumors, coronary heart disease (CHD), eye disorders, prostate disorders, stroke, high blood pressure, gout, osteoporosis, cholesterol and even HIV (Mozes, G S, Nugroho. K P, Puspita, 2018).

Determination of antioxidant activity was carried out using the CUPRAC method. The comparator used in the CUPRAC method of antioxidant testing is ascorbic acid. To reduce CUPRAC free radicals, it can be determined by calculating the antioxidant capacity in percent. Identification of the chemical components of red fruit juice was carried out using the Gas Chromatography Mass Spectrometry (GC-MS) method.

2. EXPERIMENTAL SECTION

2.1. Sample collection

The sample used in this research was the flesh of the red fruit of the red fruit plant obtained from Timika Papua, Mimika Baru District, Mimika Regency, Central Papua Province.

2.2. Sampel Processing

Sample preparation was carried out by preparing and cleaning the red fruit flesh samples from adhering dirt, and then cutting them into small pieces. Next, grind the red fruit flesh sample using a blender. Then filter it using a cloth

2.3. Preparation of CUPRAC Reagent

Preparation of 0.01 M CuCl2.2H2O solution was prepared by dissolving 0.4262 g of CuCl2.2H2O dissolved in water and diluted to 250 mL. Preparation of 0.0075 M neocuproine ethanolic solution was prepared by dissolving 0.039 g of Nc dissolved in 96% ethanol and diluted to 25 mL. Preparation of 1 M ammonium acetate buffer solution pH 7 was prepared by dissolving 19.27 g of NH4Ac in water and diluting it to 250 mL.

2.4. Quantitative analysis

2.4.1. Determination of maximum wavelength

The test was carried out by mixing 1 mL of 0.01 M $CuCl_2.2H_2O$, adding 1 mL of 0.0075 M neocuproine ethanolic, 1 mL of 1 M ammonium acetate buffer, then adding 1 mL of ethanol p.a. and adding 0.1 mL of distilled water to the vial, then measuring absorption at a wavelength of 400-600 nm using UV-Vis spectrophotometry. From the running results, the maximum wavelength value was obtained, namely 450 nm (Haeria et al., 2018).

2.4.2. Measurement of absorbance of ascorbic acid antioxidant activity

Weighed 10 mg and dissolved in 10 mL ethanol p.a. Then several concentration series were made, namely 2.5 ppm; 5 ppm; 7.5 ppm; 10 ppm; 12.5 ppm; 15 ppm, and 17.5 ppm. A solution was made in a vial by pipetting 1 mL of $CuCl_2$. $2H_2O$ 0.01 M, 1mL neocuproine ethanolic 0.0075 M, 1 mL 1 M ammonium acetate buffer, 1 mL 96% ethanol then added 1 mL of each concentration of ascorbic acid and 0.1 mL of distilled water. Incubate for 30 minutes at room temperature. Absorbance was determined using the UV-Vis spectrophotometric method at a maximum wavelength of 450 nm (Fawwaz et al., 2023).

2.4.3. Measurement of absorbance of antioxidant activity of the sample

The sample was weighed as 10 mg dissolved in 5 mL of 96% ethanol, to obtain a concentration of 2000 ppm. Next, it is diluted to 100 ppm. From the 100 ppm concentration then add 1 mL of 0.01 M $CuCl_2$ solution, 1 mL of neocuproine (7.5 x 10-3) M, 1 mL of ammonium acetate (NH4Ac) pH 7 buffer, 0.1 mg of sample, and 0. 1 mL distilled water. Samples were incubated for 30 minutes at 37 ºC. Absorbance was determined using the UV-Vis spectrophotometric method at a wavelength of 450 nm. Samples were made in 6 replications.

2.5. Data analysis

Data analysis used to determine the antioxidant activity of red fruit (*Pandanus conoideus* L.) ethanol extract was carried out at the maximum wavelength using a UV-Vis spectrophotometer to obtain absorbance values. After obtaining the absorbance value of the sample and the standard ascorbic acid solution, the antioxidant power of the sample was then calculated. Antioxidant power is calculated by finding the AAE value based on absorbance and concentration data from the linear regression equation y = a + bx obtained from the standard curve.

3. RESULTS AND DISCUSSION

In testing antioxidant activity, vitamin C is often used as a comparison because vitamin C is a natural antioxidant compound. In addition, natural antioxidants do not cause toxicity and are relatively safe. According to research, vitamin C is more commonly used as a comparison compound than vitamin A and Vitamin E, because it is easier to obtain and the price is cheaper. Vitamin C is also called an electron donor (electron giver) so it is an antioxidant compound. As an electron donor, vitamin C can also be interpreted as a reducing agent, originating from the nature of the double bond between C-2 and C-3 in the 6-carbon lactone ring. Vitamin C prevents other compounds from being oxidized. Vitamin C itself of course undergoes oxidation (Rantung et al., 2021). The antioxidant capacity of ascorbic acid as shown in **Table 1** showed high potential of antioxidant.

Table 1. Measurement of Percent Capacity of Ascorbic Acid

Concentration ppm	Absorbance	Transmittance	% Capacity
17.5	0.954	0.111	88.9
15	0.774	0.168	83.2
12.5	0.655	0.221	77.9
10	0.535	0.291	70.9

7.5	0.474	0.335	66.5
5	0.395	0.402	59.8
2.5	0.321	0.477	52.3

This research uses the bisneocuproine-copper(II) complex method which will oxidize antioxidant compounds in plant extracts and undergo reduction to form a bisneocuproine-copper(I) complex. Visually, this can be seen from the change in the complex color of the solution from blue to yellow, saying that the CUPRAC reagent is a fairly good selective reagent, not sensitive to physical parameters such as temperature, sunlight, pH, and humidity.

CUPRAC was chosen as the antioxidant activity test method because this method is more suitable for determining the antioxidant activity of a plant extract. After all, it can be applied to deal with various sample matrices contained in plants. This method has advantages compared to other antioxidant measurement methods because it is fast enough to oxidize thiol types of antioxidants. CUPRAC reagent is more stable and accessible than other chromogenic reagents.

The percent capacity data obtained was then plotted to obtain a linear regression equation for the effect of ascorbic acid and red fruit juice on bisneocuproin-copper (II). The results of the linear regression equation are y = 2.4024x + 47.297 with a correlation coefficient (r) = 0.9966 (**Figure 1**).

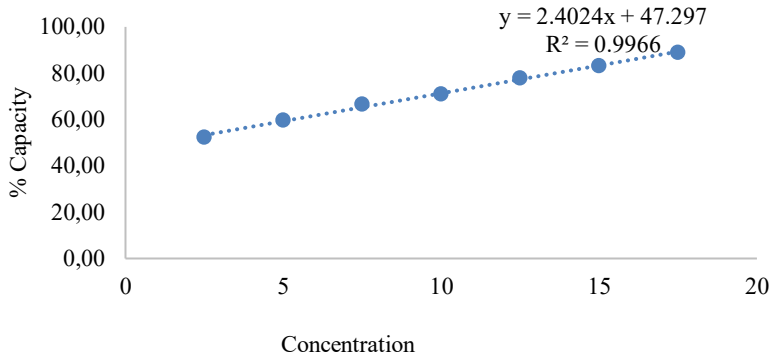

Figure 1. Ascorbic acid calibration curve

Calculating the antioxidant capacity where y is the % capacity, the percent capacity value is 100.4 mgAAE/g (**Table 2**). Based on research conducted, red fruit juice contains ascorbic acid compounds which have the potential to act as antioxidants. The presence of ascorbic acid in red fruit juice has been proven to have antioxidant properties.

Table 2. Calculation of Antioxidant Capacity of Red Fruit Samples

Replication ppm	Absorbance	Transmittance	% Capacity
1	0.533	0.293	70.69
2	0.555	0.279	72.14
3	0.529	0.296	70.42
4	0.546	0.284	71.56
5	0.542	0.287	71.29
6	0.56	0.275	72.46

4. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the results of quantitative analysis of Red Fruit (*Pandanus conoideus* L.) using the CUPRAC method with Ascorbic Acid as a comparison show an antioxidant capacity in the sample of 100.4 mgAAE/g which is classified as a strong antioxidant.

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