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Research Article

Assessment Of Free Radical In Ethyl Acetate Extract Of Pandanus Julianettii Fruit Using The DPPH Method

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ABSTRACT

Free radicals be able enter the body, attack healthy cells, and cause these cells to lose their function and structure. The community has been long using Pandanus julianettii Marteli as a food reserve, anesthetic, to maintain body health, and boost stamina or endurance. This plant has in height of vitamin C, which is useful as an antioxidant. Aims: This study aims to determine the percent inhibition and AAI (Antioxidant activity Index) of ethyl acetate extract. Methods: The methods used were collecting samples, simplicial making, extraction, phytochemical screening, determining percent inhibition, and AAI. Results: The results obtained from this study stated that the ethyl acetate extract of P. julianettii fruit pulp has DPPH free radical scavenger activity, where the IC50 value is 224 ppm and the AAI value is 0.17, which is classified as having weak antioxidant properties. Conclusion: The ethyl acetate extract of P. julianettii has potential material that used as antioxidant.

INTRODUCTION

A free radical is an atom or molecule that has an unpaired electron. Free radicals can enter the body and attack healthy cells causing these cells to lose their function and structure. The accumulation of this damage contributes to several diseases and causes a condition commonly called premature aging (1). Compounds that can ward off free radicals are antioxidants. Antioxidants work by stopping the process of cell damage by giving electrons to free radicals which will then neutralize the free radicals so that they no longer can steal

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electrons from other cells (2,3). In the Papua region, one of the provinces in Indonesia, namely the Central Mountains, has a plant that is often consumed and according to research results, this plant has high antioxidant activity. Pandanus julianettii Marteli is a type of plant endemic to Papua, especially in the Central Mountains (Wamena, Tolikara, Lanny Jaya, Paniai, etc.). The people call this plant Koka (Paniai), Gawen (Tolikara), Tuke (In other central mountains). P. julianettii has long been used traditionally by the community as a food reserve to prevent famine, anesthesia, maintain body health, and increase stamina/endurance (4,5). Based on data from the analysis of the nutritional content of P. julianettii fruit carried out by Lekitoo et al (2017), the flesh of P. julianettii fruit contains a water content of 10.00 % and an ash content of 3.42%, a fat content of 37.37 grams. Total protein 12.50 grams, crude fiber 23.79 grams, and Vitamin C 102.54 milligrams (6). Chrystomo et al (2016) informed that the flesh of P. julianettii contains double bonded fats or omega 3-6, tocopherol, or vitamin E which is quite high as an antioxidant to prevent degenerative diseases such as hypertension, and cholesterol and increases body stamina. The high content of vitamin C and vitamin E from the pulp of P. julianettii indicates that the pulp of P. julianettii has the potential to have antioxidants so it is necessary to carry out antioxidant tests (7). Sources of natural antioxidants used by the body are vitamin C, vitamin E, and beta-carotene which are contained in many fruits and can be consumed directly by humans to prevent the occurrence of free radicals (2,8). The high level of antioxidant compounds contained in this plant makes it very good for development. So far research on P. julianettii is still limited to testing antioxidants from its oil. Therefore, this research will examine antioxidant data from P. julianettii, especially those contained in the ethyl acetate extract of P. julianettii fruit.

MATERIAL AND METHODS

1. Tools and materials

a. Tool

The tools used consisted of analytical scales, blenders, UV-Vis spectrophotometers, evaporators, measuring flasks, measuring flasks, aluminum foil, stir rods, glass funnels, jars, micropipettes, reaction tubes, vials, hotplates, ovens, and glasses that are generally used in laboratories.

b. Material

The samples used were P. julianettii fruit flesh obtained from the Wamena area, ethyl acetate, pro-analysis ethanol, distilled water, DPPH, vitamin C, and filter paper.

1.2 Procedures

1.2.1 Preparation of plant material

Sample preparation was carried out by preparing samples of ripe P. julianettii fruit, cutting or spliting, taking the flesh, cutting again into small pieces, then drying it in the oven for 6 hours at a temperature of 50 0 C. The dried samples were ground by using a blender. Samples of crushed P. julianettii fruit flesh are ready to be extracted.

1.2.2 Extraction

P. julianettii fruit flesh sample powder, then the sample was put in a maceration container (glass jar) and added with 2 L of ethyl acetate then covered in the glass jar using aluminum foil and stored for 3 x 24 hours in a place protected from direct sunlight while stirring occasionally. The extract was filtered, and separated into dregs and filtrate. The dregs were extracted again with a new solvent in the same amount. The ethyl acetate extract that has been obtained then collected and the filter liquid was evaporated until a thick ethyl acetate extract was obtained. Then calculate the yield using the formula:

$$\%\ \text{Extract yield} = \frac{\text{total weight of extract (g)}}{\text{total weight of simplicia powder (g)}} \text{ x } 100\%$$

1.2.3 Phytochemical Screening

Phytochemical screening was tested qualitatively to determine secondary metabolites (9)contained in the ethyl acetate extract of P. julianettii fruit flesh.

a. Alkaloid test

A total of 15 mg of extract was dissolved in 6 ml of 1% HCl and then filtered. The filtrate was divided into 2 tubes, the first tube was given a few drops of Mayer's reagent (potassium mercury iodide). If a yellow precipitate forms, it indicated the presence of alkaloids. The second tube was given a few drops of Dragendroff's reagent (potassium bismuth iodide solution). If a red or orange precipitate forms, it indicateds the presence of alkaloids. The third tube was dripped with Wagner's reagent. If a brownish-red precipitate forms, it indicates the presence of alkaloids (10)

b. Flavonoid test

A total of 15 mg of extract was dissolved in 2 ml of 70% ethanol and added 3 drops of NaOH solution. The presence of a yellow color that becomes colorless when H2SO 4 was added that indicated the presence of flavonoid compounds (11–13).

c. Saponin test

A total of 15 mg of extract was dissolved in 20 ml of distilled water, then the solution was shaken in a volumetric flask for 15 minutes. The formation of foam as high as 1 cm designated the presence of saponin (13,14).

d. **Phenol test**

A total of 15 mg of extract was dissolved in 2 ml of 70% ethanol and 3 drops of FeCl 3 solution were added. The formation of a bluish-black color showed the presence of phenolic compounds (9,15).

e. Tannin test

A total of 15 mg of extract was dissolved in 2 ml of 70% ethanol, filtered, and then added with 3 drops of 10% FeCl 3 solution. The formation of a brownish-green or blackish-blue color directed the presence of tannin compounds (14–17).

1.2.4 0.1 mM DPPH solution

DPPH powder 3.94 mg was dissolved with 15 ml of ethanol pro analysis in a 100 ml volumetric flask, then the volume was filled with ethanol (DPPH 0.1 mM) (18).

1.2.5 Preparation of blank solution

A total of 3 ml of 0.1 mM DPPH solution was put into a vial, 3 ml of ethanol pro analysis was added and vortexed to homogenize, then incubated in the dark for 30 minutes, the wavelength was measured at 517 nm (19).

1.2.6 Preparation of comparator solution (Vitamin C)

a. Preparation of stock solution with a concentration of 10 ppm

1 mg of vitamin C was weighed and dissolved in ethanol with a 100 ml measuring flask.

b. Preparation of series test solutions with concentrations of 2, 4, 6, 8, and 10 ppm

The vitamin C mother liquor was prepared in a concentration series of 2, 4, 6, 8, and 10 ppm. From the 1000 ppm stock solution, 2000 µL, 4000 µL, 6000 µL, 8000 µL and 10,000 µL were pipetted, then filled up to the mark with ethanol pro analysis.

1.2.7 **Comparative antioxidant activity test (Vitamin C) Using UV–Vis Spectrophotometry**

A total of 3 ml of vitamin C solution was put into a vial, then 3 ml of 0.1 mM DPPH solution was added and homogenized, incubated for 30 minutes in a dark room so that it could react with DPPH (19), then the absorption was measured at a wavelength of 517 nm. and replicated three times so that the results obtained were more accurate.

1.2.8 P. Julianettii fruit flesh extract solution

a. Preparation of stock solution with a concentration of 1000 ppm

Weighed 100 mg of P. julianettii fruit pulp extract, then dissolved it in pro-analysis ethanol, and then put it in a 100 ml measuring flask, the volume was

filled with pro-analysis ethanol solution to the limit mark.

b. Preparation of a series of test solutions with concentrations of 100, 150, 200, 250 and 300 ppm

From the mother liquor of ethyl acetate extract of P. julianettii fruit flesh, pipet 1000 µL, 1500 µL, 2000 µL, 2500 µL, and 3000 µL respectively, then make up the volume to the mark with ethanol pro analysis.

1.2.9 Antioxidant activity test using UV – Vis Spectrophotometry

P. julianettii fruit flesh was put into a vial, 3 mL of 0.1 mM DPPH solution was added and homogenized, incubated for 30 minutes in a dark room so that it could react with DPPH then the absorption was measured at a wavelength of 517 nm. and replicated three times so that the results obtained were more accurate (19).

1.2.10 Determination of percent inhibition (20)

Percent inhibition of DPPH radicals can be calculated using the formula:

% Radical inhibition of DPPH=(Blank Absorbance-Sample absorbance)/(Blank absorbance) X 100%

1.2.11 **Determination of IC_{50}**

Determining the IC50 value from the ethyl acetate extract of P. julianettii fruit pulp used a linear regression equation, namely on the x-axis and y-axis, where the y value is equal to 50 and the x value will be obtained as the IC50 (21).

1.2.12 Determination of AAI (Antioxidant Activity Index) Values

The AAI value was determined by dividing the DPPH concentration used in the test (ppm) by the IC50 value obtained (ppm). If the AAI value is < 0.5, the antioxidant activity is weak, the AAI value is 0.5 – 1, the antioxidant activity is moderate, the AAI value is 1 – 2, the antioxidant activity is strong and the AAI value is > 2, the antioxidant activity is very strong (22,23).

1.2.13 Data Collection and Data Analysis

Data was obtained from the absorbance measurements of P. julianettii fruit ethyl acetate extract and vitamin C using UV–Vis and statistical data analysis using linear regression equations.

RESULTS AND DISCUSSION

P. julianettii plant is a monocot plant that has morphological characteristics, densely gathered at the end of the stem, in 3 rows in a spiral shape, sitting, with the base hugging the stem. The leaves are incomplete, the leaf shape is like a ribbon or line and the leaf bones are parallel, 5-10 cm wide, up to 1 m long. The base of the leaf is rounded and the tip is pointed (Figure 1). The edges of the leaves are serrated/spiky. The texture of the leaves is smooth, and when they fall they leave ring-shaped marks on the stem. The leaves are arranged in a circle following a spiral line that appears circular on the stem, so the leaf layout formula is difficult to determine. So the location of the leaves on the stem follows the orthostic which has turned into a spiral line which is called spirostic. The pandan plant itself shows 3 spirostic. The stem shows circular lines where leaves sit which fall off when they are old. The roots are large and have shorter supporting roots than Pandanus coideus (red fruit) which supports this plant. P. julianettii fruit is arranged in round bouquets, like durian fruit. The size of this plant varies, from a height of 50 cm to 5 m, even in Papua there are many pandan plants up to 15 meters high (Figure 1). Its habitat and distribution grows in areas with high humidity and wet soil with high rainfall, especially in highland areas in the central mountainous areas of Papua such as in Lanny Jaya, Paniai, Yalimo, Tolikara, and Jayawijaya Wamena (7).

Fruit Flesh of fruit

Figure 1 Morphology of P. julianettii (Pandanus.julianettii Mart) (6)

Traditionally this plant was used to increase body stamina and endurance as well as to maintain health. In the central mountains of Papua, it functions as a food reserve to prevent famine when the main staple food source of hypere or sweet potato is reduced due to crop failure caused by extreme climate change (5). The way to use it is that the ripe P. julianettii fruit is cut or split then eaten directly or can also be grilled over coals then eaten like sunflower seeds (known as coconut fruit, small with length: width = 2 cm: 0.5 cm) (24,25). Information on chemical compound content and pharmacological activity, P. julianettii fruit contains double-bonded fats or omega 3-6, tocopherol, or vitamin E which is quite high as an antioxidant to prevent degenerative diseases such as hypertension, cholesterol as well as increasing body stamina and increasing immune substances (4,7,24).

1.3 Sample Extraction

In this research, the extraction of simplicia P. julianettii used ethyl acetate solvent using the maceration method has recovery yield as shown in Table 1.

Table 1. Extraction results P. julianettii

Simple weight	Solvent volume	Thick extract weight	% Yield
400 grams	2 Litres	9.99 grams	2.49 %

P. julianettii fruit pulp simplicia because the fruit pulp simplicia contains vitamin C which is not resistant to heating (26)P. julianettii fruit flesh is not yet known, thereby minimizing the occurrence of damage to active compounds due to the heating process and The process is simple and the equipment used is easy to obtain. In the sample extraction process using the maceration method, the working principle is that the solvent will enter the simplicia cells through the cell walls (12). The contents of the simplicia cells will dissolve because there is a difference in concentration between the solution inside the cell and the solution outside the cell through a diffusion process so that there is a balance between the solution outside the cell and inside the cell, so the contents of the cell including the active substance will come out and dissolve in the solvent so that the higher the percentage value of the extract yield. the more active substances will be extracted (9). The samples were powdered to expand the contact area between the ethyl acetate solvent and the P. julianettii fruit pulp simplicia powder so that extraction could occur more effectively. The choice to use ethyl acetate solvent is because ethyl acetate is semi-polar so it can attract active compounds that are both polar and non-polar, has low toxicity, and is easy to evaporate (27).

1.4 P. julianettii Fruit Flesh

Phytochemical screening was tested qualitatively to identify and know the secondary metabolic content contained in the ethyl acetate extract of P. julianettii fruit pulp so that secondary metabolite compounds that have the potential to have antioxidant activity can be identified.

Table 2. Results of phytochemical screening of ethyl acetate extract of P. julianettii fruit pulp

Extract testing	Information	Results
Alkaloid Test	Mayer: No yellow precipitate formed	-
	Dreagendorf: No orange precipitate is formed	-
	Wagner: No brownish-red precipitate was formed	-
Flavonoid Test	3 drops of NaOH: changes color to yellow	+
Saponin Test	+ H2SO4: Yellow color disappears	-
Phenol Test	Shake 10 ml of distilled water: No foam forms	+
Tannin Test	3 drops of FeCl 3: A bluish-black color is formed	+

Based on the results in Table 2 of phytochemical screening carried out on the ethyl acetate extract of P. julianettii fruit flesh, show the presence of secondary metabolite compounds, namely flavonoids, phenols, and tannins. For qualitative analysis of flavonoids, carried out by adding a few drops of NaOH, gave positive results which were marked by a yellow color change because the compound, which is a derivative of the flavon compound, when NaOH was added, was decomposed by alkali into molecules such as acetophenone which was yellow due to the breaking of bonds in the isoprene structure. After being dripped with sulfuric acid (H2SO4) the yellow color disappears and becomes brown because an oxidation-reduction reaction occurs between H2SO4 and flavonoids which causes the formation of complex compounds that cause the loss of the yellow color. For the qualitative analysis of tannins and phenols, the ethyl acetate extract of P. julianettii fruit flesh showed positive results as indicated by a blackish-green color change after adding FeCl 3 . The color change occurs due to the presence of phenol groups contained in the extract which will form complex compounds with Fe 3+ ions (28). The direct mechanism of action of flavonoids as antioxidants is by donating hydrogen ions so that they can stabilize reactive free radicals. Flavonoids as antioxidants indirectly work in the body by increasing endogenous antioxidant enzymes such as SOD (Superoxide dismutase) (29,30). The mechanism of action of phenol as an antioxidant is the ability of phenol compounds to form phenoxide ions which can give one electron to free radicals so that reactive radical compounds are not formed (31–33). The mechanism of action of tannin as an antioxidant is as a donor of hydrogen atoms in DPPH because tannin compounds are composed of polyphenol compounds that have free radical scavenging activity (34–37).

1.5 P. julianettii Fruit Flesh

The antioxidant activity test was carried out using the DPPH method. The DPPH method was chosen because this method can measure antioxidant activity in both polar and non-polar solvents, it can measure all antioxidant compounds both soluble in fat and water (18,38). The DPPH method is a simple, easy, fast, and sensitive method and only requires a small sample to test antioxidants from natural compounds. The working principle of the antioxidant activity test using the DPPH method is that there is a change in the intensity of the purple-black color of the DPPH free radical which will change color to pale yellow when the DPPH free radical electrons pair. This occurs due to the reduction of free radicals which result from the reaction between the DPPH molecule and the hydrogen atoms of the sample compound, resulting in the formation of the diphenyl picryl hydrazyl compound which causes the color change of DPPH from blackish purple to pale yellow. This color change results in a change in absorbance at the maximum wavelength of DPPH using UV-Vis spectrophotometry (19). The solvent used for antioxidant testing is ethanol pro analysis because

it has a high level of purity and can provide accurate results so it is used in antioxidant activity test analysis. In this antioxidant activity test that it does not support oxidation reactions besides that (Table 2), it is also a natural antioxidant that is easy and cheap to obtain (39,40).

Table 2. IC50 value range (19)

IC value 50	Antioxidant Activity Properties
< 50	Very strong
50 – 100	Strong
100 – 150	Currently
150 – 200	Weak
> 200	Very weak

In this test, a blank is made. A blank is a solution that receives the same treatment as the test sample and comparison sample and does not contain analytes. The purpose of the blank measurement is research, the comparator used as a positive control is vitamin C which is used as a comparator because vitamin C as an antioxidant function to bind O2 so to determine the amount of absorption by non-analyte substances, namely measuring the absorbance of DPPH in pro-analysis ethanol without the addition of extract. From the blank measurement results, an absorbance value of 0.451 was obtained. Measuring the absorbance of the extract, the color changed from dark purple to lighter, which was indicated by a decrease in the absorbance value because the compounds in the extract provided electrons to DPPH, increasing the percent inhibition, but the IC50 value was quite high, indicating that the antioxidant activity was very weak.

Table 3. Results of antioxidant activity test of ethyl acetate extract of P. julianettii fruit flesh

Concentration (ppm)	Average Absorbance	% Inhibition	IC_{50} (ppm)	AAI
100	0.334	25,923	224 (IC50 > 200 ppm means the antioxidant is very weak	0.17 (AAI < 0.5 means the antioxidant is weak)
150	0.284	37,075		
200	0.220	51,329		
250	0.196	56,573		
300	0.186	58,863		
DPPH blank	0.451	0.00	-	-

The absorbance value can be calculated as the percentage of inhibition of DPPH free radicals. From the % inhibition value, the IC 50 value can be calculated. The IC 50 value is defined as the concentration of the test compound that can reduce free radicals by 50% which is obtained from the linear regression equation with concentration as the x-axis and % inhibition as the y-axis. by replacing y with 50 in the equation. The smaller the IC 50 value, the higher the antioxidant activity, namely if the IC 50 value < 50 ppm has very strong antioxidant activity, the IC 50 value is between 50 - 100 ppm has strong antioxidant activity, the IC 50 value is between 100 - 150 ppm has antioxidant activity moderate, the IC 50 value is between 150 - 200 ppm which has weak antioxidant activity and if the IC value is 50 > 200 ppm has very weak antioxidant activity (19), then the antioxidant obtained from the ethyl acetate extract of P. julianettii fruit pulp is classified as very weak, namely 224 ppm which is an extract concentration capable of inhibiting 50% of DPPH free radicals (Table 3).

Table 4. Vitamin C test result

Concentration (ppm)	Average Absorbance	% Inhibition	IC_{50} (ppm)	AAI
2	0.310	24,554	12	3.25 (AAI > 2 means the antioxidant is very strong
4	0.294	28,554		
6	0.273	33,630		
8	0.245	40,438		
10	0.233	43,436		

DPPH blank	0.411	0.00	-	-

The AAI (Antioxidant Activity Index) value is calculated by comparing the final DPPH concentration used (ppm) with the IC 50 value obtained from the test sample. The AAI value is used to classify the antioxidant properties of the extract, namely if the AAI value is <0.5 the antioxidant is weak, the AAI value is 0.5 -1 the antioxidant is moderate, the AAI value is 1-2 the antioxidant is strong and > 2 the antioxidant is very strong (22), then the antioxidant value obtained from the ethyl acetate extract of P. julianettii fruit pulp is classified as a weak antioxidant, namely 0.17. P. julianettii fruit pulp, which is classified as a weak antioxidant, is thought to be due to several reasons, namely first, the influence of temperature and the length of time the extract was stored before its antioxidant activity was tested because the antioxidant test extract was stored before being tested for six months at room temperature. Storage at a temperature of 4 o C, the AAI value was stable with relatively strong activity compared to extracts at room temperature storage and 35 o C where there was a decrease in antioxidant activity from strong to moderate on the 45th day of storage and namely storage of antioxidant activity test samples for 14 days decreased after two weeks of storage so that the higher the temperature and longer the storage time for the extract, the lower the AAI (Antioxidant Activity Index) value, which indicates a decrease in the strength of the extract's antioxidant activity because the compounds contained in the extract undergo oxidation. Second, it is suspected that the vitamin C contained in the flesh of P. julianettii undergoes oxidation during sample processing. Vitamin C is very sensitive so it can be lost continuously during sample processing, for example when stripping, cutting, and drying, exposure to air in the sample tissues causes loss of vitamin C or oxidization to L-dehydroascorbic acid which is chemically very labile and can undergo changes. Furthermore, it becomes L-diketogulonic acid which no longer has activity as vitamin C (39). Third, choose a few concentration points. The comparative antioxidant activity test for vitamin C is 3.25 which is classified as a very strong antioxidant. In this research, there are similarities and differences with previous research conducted by Zebua and Sari (2017) (25)with the title P. julianettii Pandan Oil: physicochemical properties, total phenol, total carotene, vitamin e and antioxidant activity. What these two studies have in common is the antioxidant activity test using the DPPH method and the pulp test samples of P. julianettii fruit of the Pandanus julianettii Mart type. The difference with previous research is that sample extraction using the wet rendering method uses samples of fresh P. julianettii fruit flesh, the solvent is water and methanol, the result obtained is pure oil, the IC 50 value is 45.83 mg/ml which is classified as having very strong antioxidant properties, and the results The oil obtained was immediately tested for antioxidant activity. Meanwhile, in this study, extraction was carried out using the maceration method using dry simplicia (powder), the solvent was ethyl acetate, the result obtained was a thick extract, and the IC50 value was 224 ppm which was classified as a very weak antioxidant properties and the sample storage time was antioxidant test, namely for 6 months at room temperature due to Covid-19.

CONCLUSION

The conclusion of this research is: the ethyl acetate extract of P. julianettii fruit flesh has DPPH free radical scavenger activity where the inhibitory concentration (IC50) value is 224 ppm and the antioxidant activity index (AAI) value is 0.17 which is classified as a weak antioxidant.

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