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Research Article

Antioxidant Test of Ethanol Extract of Purple Passion Fruit Leaves (*Passiflora edulis Sims var edulis*) from Tenggarong City, Kutai Kartanegara Regency

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Abstract (in English)

Antioxidants are compounds that have the ability to reduce free radicals. Antioxidants are obtained from secondary metabolites such as flavonoids. One of the plants that contain flavonoids is purple passion fruit leaves (*Passiflora edulis* Sims *var edulis*). This study aims to determine the percentage of yield obtained from the soklet extraction of purple passion fruit leaves using 96% ethanol solvent, and to determine the IC50 of the extract. Percent yield done by calculating the weight of extract divided by the weight of the simplisia and multiplied by one hundred percent, obtained a percent yield of 18.6% show a good yield. Antioxidant activity test using DPPH (*2,2- diphenyl-1-picrylhydrazyl*) method, calculate the IC50 value, where ascorbic acid is used as a positive control. The results of the antioxidant activity test of extract ethanol purple passion fruit leaves showed very strong activity with IC50 value of 26,37 ppm and ascorbic acid IC50 value of 6,03 ppm.

Keywords: Antioxidant, Purple passion fruit, DPPH

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1 Introduction

Passiflora edulis Sims *var edulis* or purple passion fruit, is the plant that will be tested and analyzed in this study. There are two types of passion fruit in Indonesia, namely purple passion fruit (Passiflora *edulis* Sims) which grows in the highlands, and yellow passion fruit (*Passiflora flavicarva*) which grows in the lowlands. Purple passion fruit can be used as an analagetic, especially in its leaves. Passion fruit leaves contain alkaloid, saponin, and flavonoid compounds. These leaves are then used by the community as medicines to relieve joint pain (analgesics), muscle spasms, sprains, rheumatism, inflammation and wound healing and antioxidants [1]. The extraction method chosen to be used in this study is the soklet extraction method. The advantage of this extraction method is that the extraction process is continuous, the sample is extracted by pure solvent from condensation so it does not require a lot of solvent and does not take much time [2].

Biological antioxidants are compounds that can counteract or reduce the negative effects of oxidants. Chemical antioxidants are compounds that can donate one electron to oxidant compounds so that the activity of these oxidant compounds can be inhibited. Antioxidants are needed by the body to protect the body from free radical attacks. Phenolic compounds have various biological effects such as antioxidant activity through mechanisms as reducers, free radical catchers, metal chelators, absorbers of singlet oxygen formation and electron donors. Antioxidants are a group of compounds that neutralize free radicals and reactive oxygen species (ROS) in cells [3].

Antioxidant activity test can be measured by looking at its ability to inactivate free radicals. One of the most commonly used stable free radicals is DPPH (*2,2-diphenyl-1-picrylhydrazyl*). DPPH solution is dark purple in color with maximum absorption at a wavelength of 517 nm. Through reaction with substances that release hydrogen atoms, a reduced form of DPPH *2,2-diphenyl-1-pikrylhidrazyl* is formed, and then the purple color of the solution turns yellow as the absorption decreases. DPPH free radical capture activity is generally expressed in terms of the percentage of free radical inhibition by the antioxidants examined [4]. Compounds that have antioxidant activity are very strong category if the IC50 value is <50 ppm, strong category if the IC50 value is between 50-100 ppm, moderate category if the IC50 value is between 101-150 ppm, and weak category if the IC50 value is between 151-200 ppm [5]. DPPH free radicals will be captured by flavonoid compounds. Flavonoids will be oxidized by DPPH free radicals to produce a more stable form of radicals, namely radicals with low reactivity. Flavonoids donate hydrogen radicals (H-) from their aromatic rings to reduce toxic free radicals resulting in resonance-stabilized flavonoid radicals and making them non-toxic [6]. This study aims to indentify and calculate the percent yield and antioxidant activity of extract of purple passion fruit leaves (*Passiflora edullis* Sims) using the DPPH method.

2 Method

The equipment used in this study includes a rotary evaporator, a series of soxhlet extraction tools, and UV-Visible spectrophotometer, incubator, freezer, refrigerator, and the necessary glassware.

The materials used in this study include purple passion fruit plants (*Passiflora edulis* Sims *var edulis*) obtained from Kutai Kartanegara Regency, East Kalimantan; ascorbic acid; DPPH; extracts from test samples; 96% ethanol; and ethanol p.a.

2.1 Sample Extraction Process

Purple passion fruit leaves (*Passiflora edulis* Sims *var edulis*) that have been taken are then washed and wet sorted, dried without using sunlight, then chopped into smaller parts to maximize the extraction process. Extraction was carried out by the sokhletation method, using 96% ethanol solvent. The

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extraction results were then concentrated using a rotary evaporator. The goal is to avoid solvent saturation and maximize the process of withdrawing compounds contained in simplisia [5]. The concentrated extract will be used for testing.

2.2 Percent Yield of Test Samples

The thick extract obtained from the concentrating process using a rotary evaporator is then used to calculate the percentage yield. The weighing process begins with weighing an empty glass container that will be used to hold the extract. After that, the container that has been filled with thick extract is weighed again. The difference in weight between the container containing the extract and the empty container indicates the weight of the extract obtained. The value is then divided by the weight of the purple passion fruit leaf sample used at the beginning of the extraction. The yield of a good viscous extract should not be less than 10% [7].

2.3 Preparation of DPPH Solution (40 ppm)

DPPH solution was made by weighing 4 mg of DPPH powder, then dissolved in 100 mL of ethanol p.a in a 100 mL volumetric flask. The volumetric flask was tightly closed with a lid, then the mixture was homogenized and violet in color. The mixing process is carried out in a place protected from light. Then filtered solution that has been homogenized, and stored in a brown bottle.

2.4 Determination of Maximum Wavelength (λ)

DPPH solution was pipetted as much as 1.5 mL, then added with 1 mL ethanol p.a and then left for 30 minutes in a place protected from light. The solution that has been made is put into a cuvette and tested for absorption using a visible spectrophotometry device at a wavelength of 500-530 nm [8].

2.5 Preparation of Ascorbic Acid Comparative Standard

Ascorbic acid was weighed as much as 10 mg and then dissolved in 100 mL of ethanol p.a. The standard comparison solution obtained had a concentration of 1000 ppm. Ascorbic acid comparison standard solution was pipetted as much as 20 µL, 40 µL, 60 µL, 80 µL and 100 µL to make exciting concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm and dissolved with ethanol to the limit of a 10 mL volumetric flask. 1 mL of each solution was pipetted into the cuvette and 1.5 mL of 200 ppm DPPH solution was added. The solution was incubated for ± 30 minutes.

2.6 Preparation of Test Solution

Samples of ethanol extract of purple passion fruit leaves (*Passiflora edulis* Sims *var edulis*) were weighed as much as 10 mg and dissolved in 10 mL of ethanol p.a. The standard solution obtained had a concentration of 1000 ppm. The extract standard mother solution was pipetted as much as 20 µL, 40 µL, 60 µL, 80 µL and 100 µL and then put into a 10 mL volumetric flask, so as to obtain a mixture concentration of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm. Then added ethanol p.a until the limit of the 10 mL volumetric flask. A 1 mL pipette of each solution was put into the cuvette and 1.5 mL of 200 ppm DPPH solution was added. The solution was incubated for ± 30 minutes.

2.7 Antioxidant Activity Test

Antioxidant activity test was measured using DPPH method. Each concentration series solution was pipetted as much as 1 mL and 1.5 mL DPPH solution was added. The mixture was incubated for 30 minutes at room temperature in a cuvette. Then the absorbance was measured at the maximum wavelength of DPPH, the whole reaction was carried out in a dark room. Furthermore, the %inhibition was determined. The absorbance value of DPPH solution before and after sampling was calculated as

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percent inhibition (% inhibition). Then the IC50 value was calculated, which is the value used to describe the amount of antioxidant concentration of the test sample extract that can capture radicals by 50%. The IC50 value can be calculated using a regression equation. The regression equation can be obtained by entering the concentration of the test sample as the abscissa (x-axis) and the percent inhibition value of DPPH as the ordinate (y-axis). The regression equation will produce an r value (correlation coefficient). Then the results of the calculation of the regression equation are calculated and the results are obtained for the IC50 value [8].

3 Result and Discussion

Yield is the ratio between the amount of extract obtained and the amount of simplisia used. The unit used to express the yield is percent (%). The greater the yield value, the more the content of active compounds in the sample. The process of calculating the yield is done by comparing the final weight (weight of the resulting thick extract) with the initial weight (weight of the simplisia used), then the result is multiplied by 100 percent. This yield value is directly related to the amount of bioactive compounds contained in the extract. The higher the yield produced, the better the quality of the extraction carried out [9]. The active compounds contained have various important benefits including as antioxidants, antibacterial, anti-inflammatory, and anticancer [10]. Based on Table 6.1, the extract yield obtained was 18.6%. The requirement for a good thick extract yield is that the value is not less than 10%, so it is said that the yield results have met the requirements [7].

Antioxidant activity testing is done to measure the potential value of an extract as an antioxidant. This test was conducted using the DPPH method. DPPH (2,2-Dyphenyl-1-picrylhydrazyl) is a free radical compound that will be reduced to yellow color when reacting with antioxidant compounds. The yellow color is formed after adding DPPH, this can be caused by the presence of antioxidant compounds that donate hindrogen atoms which can result in the formation of reduced DPPH-H. The DPPH method is a simple, fast, and easy method for screening the radical-capturing activity of several compounds, besides that this method has proven to be accurate, effective and practical. The DPPH method measures the percent inhibition of DPPH uptake of antioxidant compounds and calculates the IC50 value compared to the positive control.

Tests conducted on ascorbic acid and EEDMU (ethanol extract of purple passion fruit leaves) using a concentration series of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm. Table 1 and table 2 show that the higher the concentration variation of the test sample, the lower the absorbance value, this can be caused by the activity of the test material that reduces DPPH free radicals which is greater directly proportional to the concentration of the test sample. The absorbance value that can be measured is the remaining part of the DPPH radical that has not reacted with the test sample. Therefore, the higher the concentration variation of the test sample, the higher the percent of free radical silencing obtained. The percent of inhibition can be measured by the IC50 value. IC50 (Inhibition Concentration) value is the concentration of test material that can reduce 50% of free radicals. Compounds that have antioxidant activity are very strong category if the IC50 value is <50 ppm, strong category if the IC50 value is between 50-100 ppm, moderate category if the IC50 value is between 101-150 ppm, and weak category if the IC50 value is between 151-200 ppm [5]. Based on the test results, the IC50 value of EEDMU has an activity that is close to the IC50 value of the positive control of ascorbic acid which is 26.378 ppm which is categorized as a very strong antioxidant. The positive control of ascorbic acid has an IC50 value of 6.033 ppm, this can be due to ascorbic acid being able to strongly inhibit DPPH free radicals at a concentration of 2 ppm.

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Table 1 Antioxidant activity test of EEDMU

Concentration (ppm)	Average Abs	IC_{50} (ppm)	Category
2	0,651		
4	0,640		
6	0,618	26,378	Very strong
8	0,603		
10	0,584		

Table 2 Antioxidant activity test of ascorbic acid

Concentration (ppm)	Average Abs	IC_{50} (ppm)	Category
2	0,638		
4	0,545		
6	0,445	6,033	Very strong
8	0,375		
10	0,224		

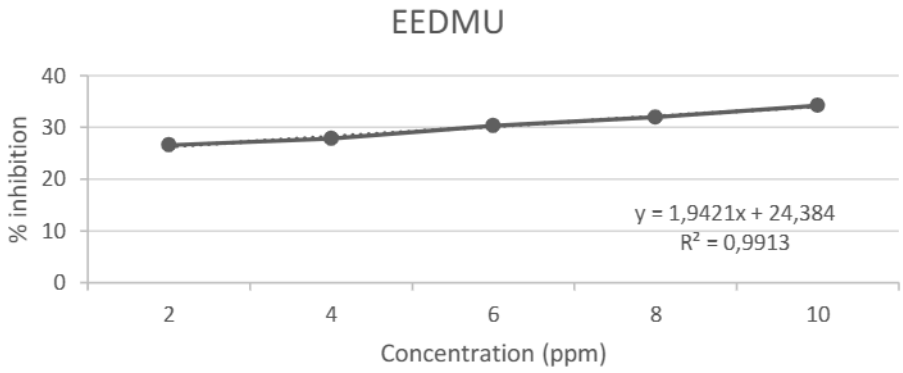

Figure 1 Standard curve of ethanol extract of purple passion fruit leaves

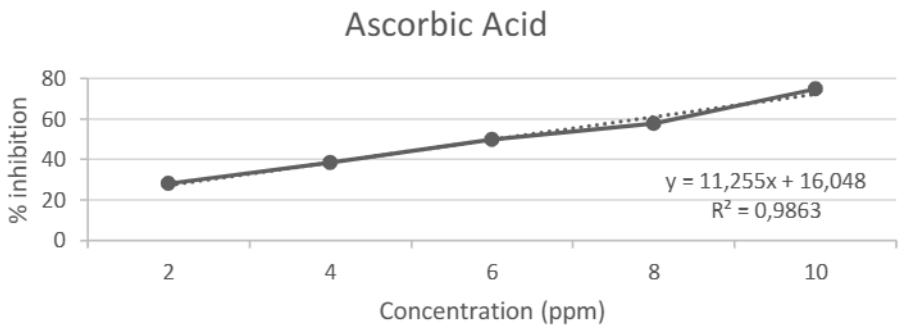

Figure 2 Standard curve of ascorbic acid

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4. Conclusion

Based on the results of experimental research conducted in the laboratory, it can be concluded that the ethanol extract of purple passion fruit leaves (*Passiflora edulis* Sims *var edulis*) extracted using the soklet extraction method obtained a yield of 18.6%. The ethanol extract of purple passion fruit leaves (*Passiflora edulis* Sims *var edulis*) based on the results of antioxidant activity testing has an IC50 value of 26.378 ppm which indicates that the test extract has antioxidant activity categorized as very strong antioxidants.

5. Declarations

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5.2 Author contributions

All authors were involved in the development and writing of this article.

5.3 Ethics

The study did not require ethical approval because it did not involve human participants, animals, or identifiable personal data.

5.4 Conflict of Interest

The authors declare no conflicts of interest during the research and the preparation of this article.

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