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Research Article

Determination of Antioxidant Activity of Stem Bark (*Manihot esculenta* Crantz) by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Method

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ABSTRACT: Cassava bark is a part of the cassava plant which is still underutilized besides being used as animal feed and wasted as waste. Each cassava can usually produce 10-15% cassava bark. The amount of waste produced can cause accumulation, resulting in environmental damage if not appropriately utilized. This research aims to determine the antioxidant activity of cassava bark (*Manihot esculenta* Crantz) using the DPPH (1,1-diphenyl-2-pikrihidrazil) method, measured using a UV-Vis spectrophotometer. Extraction used the maceration method of 96% ethanol as solvent. The determination of antioxidants in ethanol extract of cassava bark by the DPPH method and using vitamin C was used as a reference. Vitamin C was made in four series of concentrations, namely 2, 4, 6, and 8 ppm, while the ethanol extract of cassava bark was 20, 40, 60, and 80 ppm. From several concentration series, 2 ml was taken, and 1 ml of 50 ppm DPPH was added; then, the mixture obtained was incubated for 30 minutes in a place protected from light. The absorbance was measured at a wavelength of 515 nm. The results showed that the ethanol extract of cassava bark has a potent antioxidant activity based on the IC_{50} value obtained, which is 3.847 mg/L. Therefore, the ethanol extract of cassava bark can be developed as a natural antioxidant.

KEYWORDS: Cassava bark; antioxidants; DPPH; UV- spectrophotometer.

1. INTRODUCTION

Cassava can still grow well if there is sufficient rainfall because this plant has a wide range of adaptability, both to unfavorable climatic conditions and unfavorable soil conditions. Cassava is often referred to as a food ingredient that comes from villages or villages. Cassava is still classified as a traditional food ingredient, however, now that various cassava-based food businesses have begun to mushroom, on average these businesses are motivated to elevate cassava to make it more prestigious (Laksita, 2019). Cassava peel is part of the cassava plant which has been underutilized so far apart from being used as animal feed and wasted as waste. Each cassava can usually produce 10-15% cassava skin. The large amount of waste produced can be a cause that results in environmental damage if not utilized properly (Asia, 2019).

The parts of cassava that are used are leaves, tubers, and tuber skin. Cassava leaves contain the amino acid methionine, vitamins A, B1, C, calcium, calories, phosphorus, protein, fat, carbohydrates, and iron. Cassava tubers contain calories, protein, fat, carbohydrate, calcium, phosphorus, iron, vitamins B and C, and starch. The bark contains tannins, peroxidase enzymes, glycosides, and calcium oxalate (Maulidina, 2019). The chemical and nutritional content of cassava peel is 8.11 grams of protein, 15.2 grams of crude fiber, 0.22 grams of pectin, 1.29 grams of fat, and 0.63 grams of calcium. Cassava peel is one of the solid wastes produced in the manufacture of processed cassava chips from the home industry. This waste contains a fairly high carbon element of 59.31% (Maulidina, 2019).

Antioxidants are substances that can fight the harmful effects of free radicals or Reactive Oxygen Species (ROS) which are formed as a result of oxidative metabolism, namely the result of chemical reactions and metabolic processes that occur in the body (Haki, 2021). Antioxidants have the main function of minimizing the occurrence of spoilage processes in food, minimizing the occurrence of the oxidation process of fats and oils, extending the shelf life in industrial foods, increasing the stability of fats in foods, and preventing loss of sensory and nutritional qualities (Haki, 2021).

Half-inhibitor concentration (IC_{50}) measures the effectiveness of antioxidants. The IC_{50} concentration of a chemical that can capture free radicals is 50%. The lower the IC_{50} value, the greater the antioxidant capacity (Febryanto, 2017). The purpose of this study was to determine the antioxidant activity of ethanol extract on cassava (*Manihot esculenta* Crantz) stem bark using the DPPH method and to determine the IC_{50} value of the antioxidant activity of ethanol extract on cassava bark. The benefits of this research are expected to be able to provide scientific information about the antioxidant activity of the ethanol extract of cassava stem bark so that cassava peel can be used more broadly in the pharmaceutical field to be used as an alternative treatment and later it can be used as a reference for further research.

Based on the description above, the researchers chose to research subscribing to the antioxidant activity of the ethanol extract of cassava stem bark because it was proven to have powerful antioxidant content using the DPPH method.

2. EXPERIMENTAL SECTION

This research is an experimental study to determine the antioxidant activity of the ethanol extract of cassava stem bark (*Manihot esculenta* Crantz) using the DPPH method. This research was conducted at the Laboratory of Phytochemistry, Analytical Chemistry, and Pharmaceutical Instruments at Mega Rezky University Makassar from August to completion.

2.1. General

Stir bar, blender, vial, porcelain cup, separating funnel, beaker (Iwaki, Japan), filter paper, cuvette, volumetric flask (Iwaki, Japan), dropping pipette, volume pipette (Iwaki, Japan), rotary vacuum evaporator (IKA RV 3V), spectrophotometer UV-Vis, test tube (Pyrex, USA), analytical balance, maceration container (glass jar). The materials used in this study were cassava (*Manihot esculenta Crantz*) bark, aluminum foil, distilled water, ethanol, DPPH, and vitamin C (ascorbic acid).

DPPH solution was prepared by weighing 5 mg of DPPH. Dissolve it with 50 mL of ethanol and then put it in a 50 ml volumetric flask and add enough ethanol p.a to the boundary mark, then homogenize and store the solution at a low temperature protected from light (Rahma P et al, 2019).

2.2. Sample preparation

The cassava skin is cleaned of adhering dirt using running water until it is completely clean. Then the skin of cassava (*Manihot esculenta* Crantz) is dried by aerating at room temperature until completely dry (dried Simplisia). Samples of cassava bark were obtained from as much as 500 g and soaked using a 96% ethanol solution. The extraction method was done by soaking the samples 3 times 24 hours at room temperature protected from light and occasionally stirring. The extract results were filtered using filter paper, the filtrate was taken, and the residue was discarded. After that, the filtrate was concentrated using a rotary evaporator until a thick extract was obtained (Amanda, 2019).

Drying the cassava bark by aerating, machine size reduction, sieving, then extraction by maceration method. Maceration extraction was carried out for 5 days, maceration was carried out for 5 days due to achieving a balance between the extracted materials. With 96% ethanol solvent, 96% ethanol was used because ethanol is a polar solvent that can attract secondary metabolites, which are contained in green apple peel samples. Then maceration was carried out 2 times. The extraction results were concentrated with a rotary evaporator (Kurniawati SD et al, 2919).

2.3. Antioxidant activity test

Antioxidant activity by DPPH method was performed following the previous study report (Fawwaz et al., 2023).

a. Preparation of blank solutions and optimization of wavelengths

Take 1 mL of 0.5 mM DPPH solution and put it into a 50 mL volumetric flask then add methanol solution up to the mark and then leave it for 30 minutes at 37 °C. then the absorption was measured at a wavelength of 515 nm (Rahma P et al, 2019).

b. Making a comparison solution (positive control) Vitamin C

Weighed 10 mg of vitamin C (positive control). Then it was dissolved with enough CO_2-free water, then put into a 100 mL volumetric flask and made up to the mark as the mother liquor. Then a 2:4 dilution series was made; 6; and 8 then the absorption is measured at certain wavelengths (Rahma P et al, 2019).

3. RESULTS

Extraction results of 500 gram of cassava (*Manihot esculenta* Crantz) bark by maceration method using 96% ethanol solvent. The results obtained can be seen in the following table.

Tabel 1. Hasil rendamen ekstrak etanol kulit singkong *(Manihot esculenta Crantz)*

Sampel	Jenis Pelarut	Berat Sampel Kering (g)	Berat Ekstrak Kental (g)	Rendamen (%)
Kulit singkon	Etanol 96%	500	15,20	3,04%

Furthermore, the measurement of antioxidant activity was carried out with several concentration series and using vitamin C as a comparison. The absorbance obtained was used to calculate the percent inhibition of free radicals and then a regression was carried out between the percent inhibition of the samples and vitamin C. The results of the percent inhibition, linear equations and IC_{50} values can be seen in **Table 2**.

Tabel 2. Hasil Pengukuran Aktivitas Antioksidan

Sampel	Konsentrasi (ppm)	Absorbansi	% Inhibisi	Persamaan regresi linear	IC_{50}	Ranges
Vitamin C	2 ppm	0,1148	44,567	y = 3.4331x +37.059	3,772 (Sangat kuat)	≥50 ppm (Sangat Kuat)
	4 ppm	0,105	49,332			50-100 ppm (Kuat)
	6 ppm	0,0862	58,651			100-150 ppm (Sedang)
	8 ppm	0,0737	64,348			150-200 ppm (Lemah)
kulit singkong *(Manihot esculenta Crantz)*	20 ppm	0,1207	41,735	y = 0.4546x-32.512	3,847 (sangat Kuat)	200-1000 ppm (Sangat Lemah)
	40 ppm	0,1006	51,456			
	60 ppm	0,0875	57,878			
	80 ppm	0,0626	69,901			

Based on **Table 2**, the results of measuring vitamin C between concentration and percent inhibition were obtained with a value of y = 3.4331x + 37.059, the value of R2 = 0.9851.

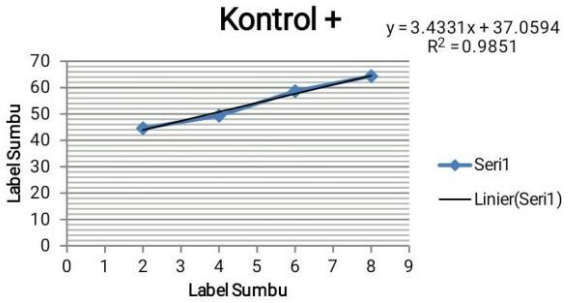

Figure 3. Effect of concentration on inhibition percentage of standard vitamin C solution (comparison).

Based on **Figure 4**, the results of sample measurements between concentration and inhibition percentage were obtained with a value of y = 0.4546x + 32.5125, and the value of R^2 = 0.9874.

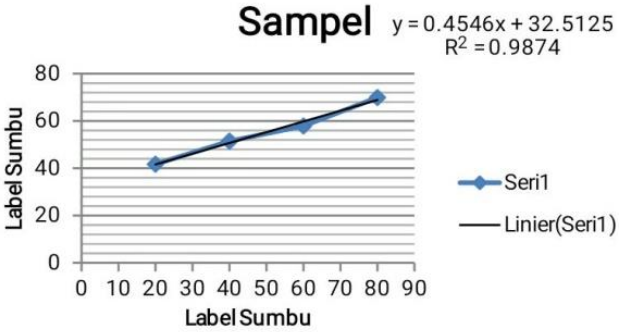

Figure 4. The effect of concentration on the inhibition percentage of the cassava peel extract sample solution.

4. DISCUSSION

This study aims to determine the antioxidant activity of the ethanol extract of cassava stem bark. The sample extraction used is the maceration method. This method was chosen because judging from the nature of the sample to be extracted, the maceration method is a method whose process is effective for withdrawing the desired substance and does not use a heating process, so as to prevent the possibility of decomposition of the active substance contained in the sample due to the influence of temperature and compounds. which cannot stand heating and does not use special tools. Meanwhile, another cold maceration method is the percolation method where the sample percolation method in the percolator is not homogeneous, so it is difficult for the solvent to reach all areas, besides that this method also requires a lot of solvent and takes a lot of time. The dried samples were extracted using 96% ethanol solvent. 96% ethanol was used because ethanol is a polar solvent that can attract secondary metabolites, which are contained in green apple peel samples such as alkaloids, flavonoids, saponins, tannins (Fawwaz et al., 2017). The ability of ethanol to attract these compounds is influenced by the chemical structure of ethanol which contains polar OH, where this group can bind to polar groups, in plant metabolites such as flavonoids, whereas according to Putri (2019) other polar solvents such as water are rarely used because they can cause fermentative meaning resulted in faster destruction of the active ingredients in the sample, the evaporation process took longer and it was easily contaminated. Meanwhile, methanol solvent is toxic to the human body which can cause visual disturbances, impaired consciousness and so on. Moreover, 70% ethanol has more water content, resulting in 70% ethanol taking longer in the evaporation process using a rotary evaporator. Filtra results of cassava stem (*Manihot esculenta* Crantz) yielded a viscous extract obtained in this study as much as 15.20 g so that the percent yield was 3.04%.

Antioxidant activity testing was carried out using the DPPH method, the advantages of this method are that it is simple, easy and fast and requires a small number of samples and is also easy to apply because the DPPH radical compound used is relatively stable compared to other methods (Fawwaz et al., 2020). The initial step in carrying out this test is to first measure the maximum wavelength of DPPH using UV-Vis spectrophotometry. A blank solution was prepared and incubated for 30 minutes, the purpose of incubation was because the reaction was slow and samples containing antioxidants had the optimum in reducing DPPH free radicals at that time and to obtain stable results.

This process was incubated at 37 °C because it is the optimum temperature so that the reaction between DPPH radicals and antioxidant compounds takes place more quickly and optimally and where temperature is one of the factors that can accelerate the rate of reaction. From the measurements taken, a wavelength of 515 nm was obtained with an absorbance value of 0.2076. According to (Putri, 2017), a compound is declared as a very strong antioxidant if the IC_{50} value is <50 ppm, strong if the IC_{50} value is 50-100 ppm, moderate if the IC_{50} value is 100-150 ppm and weak if the IC_{50} value is 150-200 ppm. The smaller the IC_{50} value, the greater the inhibition of free radicals or the inhibition of free radicals.

The sample concentrations used were 20, 40, 60 and 80 ppm. This concentration was chosen in order to know the concentration at which the sample can inhibit 50% of the DPPH radicals or commonly called the IC_{50} value, which is to determine the level of antioxidant activity of a sample. Comparison of vitamin C as a positive control. This is in accordance with the standard, namely <50 ppm. It is used as a comparison because it functions as a secondary antioxidant, namely capturing free radicals, easy to obtain and vitamin C is more polar than other vitamins. The reason for using vitamin C as a comparison is that vitamin C is a very high antioxidant and has a very high polarity compared to other vitamins, and vitamin C is widely used as a comparison according to several previous journals.

5. CONCLUSION

Based on the research that has been done, it can be concluded that the ethanol extract of cassava stem bark (*Manihot esculenta* Crantz) has been shown to have antioxidant content which is categorized as very strong using the DPPH method. The antioxidant test results obtained IC_{50} value from the ethanol extract of cassava stem bark (*Manihot esculenta* Crantz) of 3.847 ppm and included in the category as a very strong antioxidant. It is better to conduct further research on cassava stem bark (*Manihot esculenta* Crantz) using other methods and compare the results obtained from this study.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical Approval: -

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