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CHARACTERIZATION AND EVALUATION OF SECONDARY METABOLITES FROM *IPOMOEA BATATAS VAR. CILEMBU* BARK: EXTRACTION, ANTIOXIDANT ACTIVITY, PHYTOCHEMICAL SCREENING

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ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi potensi antioksidan dan kandungan metabolit sekunder dari kulit ubi Cilembu (*Ipomoea batatas var. cilembu*) menggunakan metode ekstraksi maserasi dan refluks. Proses ekstraksi menggunakan etanol 70% sebagai pelarut, diikuti dengan analisis fitokimia, aktivitas antioksidan dengan metode DPPH, serta karakterisasi menggunakan KLT dan FTIR. Hasil penelitian menunjukkan bahwa ekstrak maserasi dan refluks mengandung flavonoid, fenolik, terpenoid, dan saponin. Aktivitas antioksidan dinyatakan dalam nilai IC_{50}, di mana ekstrak refluks memiliki IC_{50} sebesar 244,5 ppm, lebih rendah dibandingkan dengan maserasi dengan IC_{50} 86,776 ppm, menunjukkan aktivitas antioksidan yang lebih kuat pada ekstrak maserasi. Analisis FTIR mengidentifikasi gugus fungsi yang konsisten pada senyawa bioaktif utama. Penelitian ini mengkonfirmasi potensi kulit ubi Cilembu sebagai sumber antioksidan alami yang efektif dengan efisiensi yang lebih tinggi melalui metode refluks dibandingkan maserasi

ABSTRACT

This study aimed to evaluate the antioxidant potential and secondary metabolite content of Cilembu sweet potato (Ipomoea batatas var. cilembu) peel using maceration and reflux extraction methods. The extraction process used 70% ethanol as a solvent, followed by phytochemical analysis, antioxidant activity using the DPPH method, and characterization using TLC and FTIR. The results showed that the maceration and reflux extracts contained flavonoids, phenolics, terpenoids, and saponins. Antioxidant activity was expressed as an IC_{50} value, where the reflux extract had an IC50 of 244,5 ppm, lower than the maceration extract with an IC_{50} of 86,776 ppm, indicating stronger antioxidant activity. FTIR analysis identified functional groups consistent with the main bioactive compounds. This study confirmed the potential of Cilembu sweet potato peel as an effective source of natural antioxidants with higher efficiency through the reflux method compared to maceration.

How to Cite

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INTRODUCTION

Indonesia boasts vast forests with a diverse range of flora and fauna. Spices and many other fruit plants in Indonesia can be used medicinally. Indonesia is a vast archipelago with over 13,700 islands, large and small, boasting an extraordinary diversity of flora and fauna. Indonesia boasts an estimated 100-150 plant families, most of which can be used as industrial crops, fruits, spices, and medicines (Fadhilah et al., 2023).

Tubers are a source of carbohydrates and have potential as a food substitute for rice (a raw material for both the food and non-food industries). Due to their orange-yellow color, Cilembu sweet potatoes are considered to have varying antioxidant content across varieties. Cilembu sweet potatoes are widely consumed both as processed products and as raw materials for production, particularly roasted Cilembu sweet potatoes and Cilembu sweet potato flour (Setyawati, 2015).

Antioxidants are compounds that inhibit the formation of free radicals in the body. Antioxidants are substances the body needs to neutralize and prevent damage to normal cells caused by free radicals. Antioxidants can stabilize free radicals by supplementing their electron deficiency and inhibiting the ongoing reaction of free radical formation (Purwandari et al., 2018).

Extraction is the removal of active substances from plant parts. The purpose of extraction is to isolate the chemical components present in herbal medicine (Chahyadi & Elfahmi, 2020). Solid-liquid extraction, both traditional (maceration and percolation) and more recent (e.g., supercritical fluid extraction (SFE) and accelerated solvent extraction), is based on two principles: diffusion and/or osmosis (Naviglio et al., 2019).

This research method utilizes maceration, reflux, distillation, secondary metabolite testing, and an ELISA-based antioxidant activity test of the skin of Cilembu sweet potato (*Ipomoea batatas var. Cilembu*), a technique never used before. Therefore, the objective of this study was to examine the antioxidant activity of Cilembu sweet potato (*Ipomoea batatas var. cilembu*) peel.

Several previous studies have reported antioxidant activity in various sweet potato varieties, but research on the use of Cilembu sweet potato peel waste as a source of bioactive compounds remains limited. Furthermore, information on how extraction methods affect the secondary metabolite profile and antioxidant activity of Cilembu sweet potato peel remains scarce. Comparing maceration and reflux methods is important because they use different extraction conditions that can affect the stability of the resulting bioactive compounds.

The objective of this study was to identify secondary metabolites in the maceration and reflux extractions of Cilembu sweet potato (*Ipomoea batatas var. cilembu*) peel. The antioxidant activity of the maceration and reflux extracts of Cilembu sweet potato (*Ipomoea batatas var. cilembu*) peel was determined using the DPPH method. The extracts were characterized using TLC and FTIR to determine the compound profile.

METHOD

Sample Preparation

90 grams of Cilembu sweet potato peel were weighed and air-dried for 3 days. The air-dried Cilembu sweet potato peel was oven-dried for 20 hours at 400°C. The dried Cilembu sweet potato peel was ground using an oven-dried grinder, yielding a 60-gram sample (Ekaputra & Pramitasari, 2020).

Maceration Process

20 grams of Cilembu sweet potato peel was soaked in 100 mL of 70% ethanol, stirred for 30 minutes, and left to stand for 24 hours in a glass bottle at room temperature. The extracted cilembu sweet potato peel was then re-extracted with 70% ethanol and left to stand for 24 hours. The first and second extracts were

mixed to obtain a liquid extract (Ekaputra & Pramitasari, 2020).

Reflux Process

Twenty grams of cilembu sweet potato peel was placed in a 250 mL Erlenmeyer flask equipped with a condenser. 70% ethanol solvent was added to the Erlenmeyer flask, and the condenser was placed on top of the Erlenmeyer flask. The Erlenmeyer flask was placed on a hot plate. Extraction was carried out at 78°C for 3 hours, resulting in a liquid extract (Ekaputra & Pramitasari, 2020).

Phytochemical Testing

Phytochemical testing was performed using two treatments: the extract before separation with the solvent and the concentrated extract. Phytochemical tests included steroids, terpenoids, phenolics, alkaloids, flavonoids, and saponins (Settaluri et al., 2023).

Thin Layer Chromatography (TLC) Testing

TLC analysis was performed using a GF254 silica gel plate. 5 µL of the sample was spotted using a capillary tube at the starting line 1 cm from the bottom edge of the plate. Elution was performed until a distance of approximately 6 cm was reached from the starting line. The spots were observed under a 254 nm UV lamp. The Rf value was calculated based on the ratio of the spot migration distance to the solvent migration distance.

Fourier Transform Infrared Spectroscopy (FTIR)

100 mg of dry KBr powder was mixed with 2 mg of cilembu sweet potato peel extract until homogeneous. Using a hydraulic pump, the mixture and KBr were compacted into a mold in the form of pellets or thin flakes. Characterization was performed using an FTIR spectrophotometer at wavenumbers in the IR region of approximately 4000- 600 cm^{-1}. The results are displayed graphically (Handayani et al., 2020).

Antioxidant Activity

Test solutions were prepared using different concentrations: 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, and 31.25 ppm. Plate 5 in rows A to E was supplemented with 70 µL of ethanol. A total of 70 µL of 500 ppm test solution was added to row A, and then serial dilution was performed. The solution was then homogenized up to row E. The antioxidant activity test was carried out on plate 7; 50 µL of the prepared test solution was pipetted into each well, and 195 µL of 0.2 mM DPPH solution was added to each well. Leave the solution in the dark for 1 hour, then measure the absorbance at 517 nm using an ELISA reader (Settaluri et al., 2023). The absorbance data obtained were then converted to percent inhibition for each solution concentration. The test was carried out twice or in duplicate.

$$\%\, Inhibisi = \frac{A_{blanko} - A_{sampel}}{A_{blanko}} \times 100$$

The IC_{50} value is determined from a linear regression of sample concentration (x) versus percent inhibition (y). The IC_{50} value is obtained by substituting y=50 into the regression equation. The regression equation is derived from a graph of percent inhibition (y-axis) versus concentration (x-axis). Next, the value 50 is substituted for y in the regression equation to obtain the IC_{50} value (Cahyaningsih et al., 2021).

RESULT AND DISCUSSION

The sample of Cilembu sweet potato skin obtained from 3 kg of Cilembu sweet potato was 90 grams. After drying, a 60-gram powder was obtained. 20 grams of the sample was used for maceration and 20 grams for reflux. Filtrates 1 and 2 were obtained in a total of 137 ml from the multistage maceration. The first filtrate generally contains compounds that are more soluble and located on the surface of the matrix, while the second filtrate usually

contains compounds that are more bound or hidden in the matrix. This method is used to extract active compounds that are sensitive to high temperatures.

Table 1. Percentage of Extract Yield

Methods	Sample Mass (g)	Extract Mass (g)	Yield (%)
Maceration	20	2,02	10,10
Reflux	20	1,40	7,00

A 93 ml liquid extract of the Cilembu sweet potato peel was obtained by reflux extraction. Extraction was carried out at 780°C, which corresponds to the boiling point of ethanol (Ariff et al., 2020). The reflux method places the crude extract in a solvent with continuous circulation, ensuring maximum contact between the sample and the solvent. The resulting maceration extract was then distilled to re-evaporate the solvent and reduce the solvent volume for further identification of the sample's constituents. The volume of the maceration extract obtained from the distillation process was 30 ml at 780°C, corresponding to the ethanol boiling point. The same procedure was performed on the liquid extract obtained by reflux distillation at 780°C, yielding 15 ml of liquid extract (Alara et al., 2018).

The distillation results were then thickened using a water bath to obtain 2.02 g of maceration extract and 1.40 g of reflux extract. This study did not test the water content of the resulting extract. Several phytochemical extraction studies also report extraction results based on the mass of concentrated extract without specifically characterizing the water content in the initial stages of the study, particularly when the focus is on identifying secondary metabolites and the extract's biological activity (Alara et al., 2018) (Settaluri et al., 2023). However, the lack of water content measurement is a limitation of this study, as water content can affect the concentration of active compounds, the stability of the extract during storage, and the reproducibility of antioxidant activity test results. Therefore, further research is needed to analyze the water content to obtain a more comprehensive characterization of the extract and support a more accurate interpretation of biological activity.

Phytochemical content testing of the Cilembu sweet potato extract was conducted twice: before and after distillation. The maceration and reflux extracts were each tested with appropriate reagents. The phytochemical tests performed revealed differences in the content before and after distillation, as shown in Table 1. The secondary metabolites lost after the distillation process were alkaloid compounds.

Table 2. Phytochemical test results

Test	Before solvent separation		After solvent separation	
	Maceration	Reflux	Maceration	Reflux
Steroids	-	-	-	-
Terpenoids	+	+	+	+
Phenolic	++	+	++	+
Flavonoids	++	++	++	+
Alkaloids (Wagner)	+	+	-	-
Alkaloids (Meyer)	++	+	-	-
Saponin	Fresh Sample (+)	Fresh Sample (+)	-	-

Alkaloids have unique solubility in organic solvents. They are readily soluble in alcohol and sparingly soluble in water. Loss of alkaloid compounds after distillation is thought to be due to their resistance to heat, which degrades them during the process. Alkaloids can also be hydrolyzed by water at high temperatures, resulting in inactive compounds (Lidyawati et al., 2021).

Thin-layer chromatography is used to

identify and separate components using dyes, fluorescence, and ultraviolet radiation (Kowalska & Sajewicz, 2022). The stationary phase used in TLC is a silica gel GF 624 nm plate because it fluoresces well under UV light and contains chromophore groups that produce colored spots. The TLC results are as follows:

Figure 1. TLC spots on n-hexane:methanol 9:1 eluent (left) and diluted extract (right)

The rf values obtained (Table 2) from chromatography with two different solvents were tested on the sample to determine the effective eluent ratio for the extract. The first eluent used was n-hexane:methanol in a ratio of 9:1. This eluent was used due to the non-polar nature of n-hexane, which allows it to separate non-polar compounds contained in the extract. Methanol was used because it is more polar, so the eluent used provided a combination of non-polar and slightly polar compounds, allowing for effective node separation.

Variations in the eluent as the mobile phase were carried out to determine which eluent was effective in separating the spots. A 3:7 mixture of chloroform:ethyl acetate provided polar, non-polar, and semi-polar properties to the movement of the sample spots. Ethyl acetate, being polar, will attract both polar and semi-polar compounds. Meanwhile, chloroform, being non-polar, will attract both semi-polar and non-polar compounds.

Table 3. Rf values of maceration and reflux extracts of Cilembu sweet potato skin

Eluent	Maceration	Reflux
n-hexana methanol (9:1)	Rf_1 0.072	Rf_1 0.072
	Rf_2 0.24	Rf_2 0.25
	Rf_3 0.40	
Cloroform:ethyl acetate (3:7)	Rf_1 0.21	Rf_1 0.18

The elution results of the compounds contained in the maceration extract had higher rf values than the reflux extract using n-hexane:methanol as the eluent. This indicates that the compounds separated better were non-polar compounds because the non-polar solvent dominated over the polar solvent, namely methanol. Meanwhile, the 3:7 chloroform:ethyl acetate variation only produced 1 rf value for each sample spot (Saleh, 2022).

From the FTIR test results conducted on both Cilembu sweet potato peel extracts, functional group peaks were obtained as shown in Figures 1 and 2. The identification of functional groups for each sample is presented in Table 2.

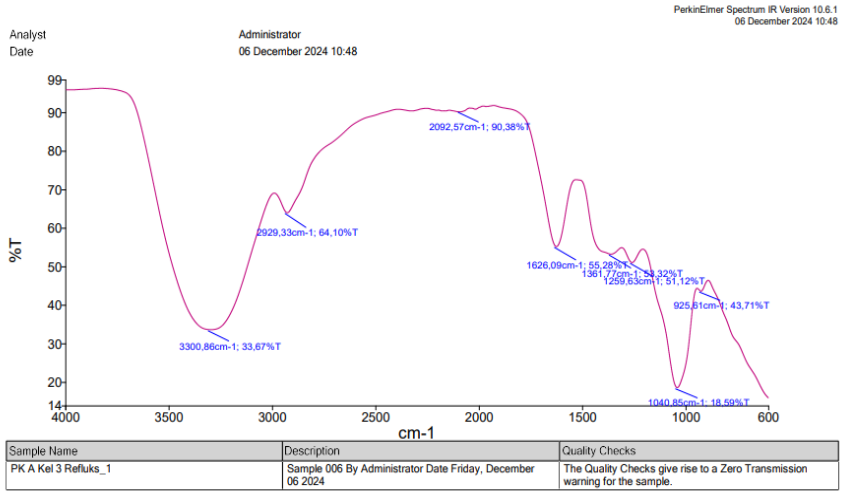

Figure 2. FTIR test results of the reflux extract of Cilembu sweet potato skin

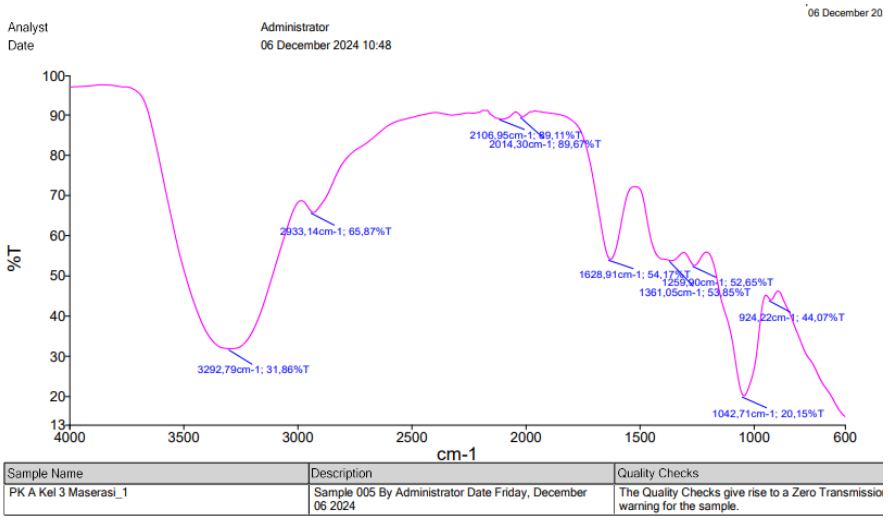

Figure 3. FTIR test results of macerated cilembu sweet potato leaf extract

Based on the functional groups obtained, the maceration and reflux extract of Cilembu sweet potato skin is suspected to contain flavonoids, alkaloids, steroids, terpenoids, and phenolic compounds. A higher %T value indicates a lower absorption intensity, and conversely, a lower %T value indicates a higher absorption intensity. The NCO or CN functional groups have a relatively strong intensity compared to other functional groups due to their low %T (Lidyawati et al., 2021).

Table 4. Peaks in Functional Groups

Peak	X (cm^{-1})	Analysis
1	3292,79	(O-H) Alcohol
2	2033,14	(C-H) Streaching
3	2106,95	(C≡C) Alkyne
4	2014,30	(C=O) Esther
5	1628,91	(C=C) Alkene (cis)
6	1361,05	(C-H) Bending CH_3
7	1259,90	Amines, amides, esters
8	1042,71	C-O sp^3
9	924,22	(C=C) Alkene

The FTIR results also identified compounds such as terpenoids (peaks 1 and 4) with carbon double bonds and hydroxyl groups, phenolics (peaks 1 and 4) with hydroxyl groups and aromatic rings, and flavonoids (peaks 1, 4, and 5) with hydroxyl groups, carbonyl groups, and aromatic structures.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method was used to test the antioxidant activity of guava leaf soxhlet extract. This method is simple, easy, rapid, sensitive, and does not require a large sample. The sample solution was diluted to concentrations of 500, 250, 125, 62.5, and 31.25 ppm. Absorbance was measured at a wavelength of 517 nm using an ELISA reader. Table III shows the results of the DPPH method (Cahyaningsih et al., 2021).

Antioxidant tests were conducted on reflux and maceration extracts of Cilembu sweet potato skin at concentrations of 500, 250, 125, and 62.5 ppm. Ascorbic acid was used as a positive control dissolved in 96% ethanol with varying concentrations of 200, 100, 50, and 25 ppm. Dpph was used as a radical compound for absorbance testing of each sample extract. Antioxidant testing was carried out using an Elisa Reader. The absorbance values of the Cilembu sweet potato skin extract samples are shown in Table V.

Table 5. Antioxidant activity of reflux extract and maceration samples of Cilembu sweet potato skin

Test solution	Concentration (ppm)	Absorbance	AA	%AA
Reflux Extract	500	0,179	0,746	74,664
	250	0,277	0,607	60,782
	125	0,445	0,370	37,023
	62,5	0,545	0,228	22,882
Maceration Extract	500	0,128	0,818	81,833
	250	0,151	0,785	78,597
	125	0,262	0,629	62,965
	62,5	0,480	0,321	32,113
Ascorbic Acid	200	0,203	0.740	74.024
	100	0,592	0.244	24.443
	50	0,607	0.224	22.487
	25	0,674	0.139	13.972

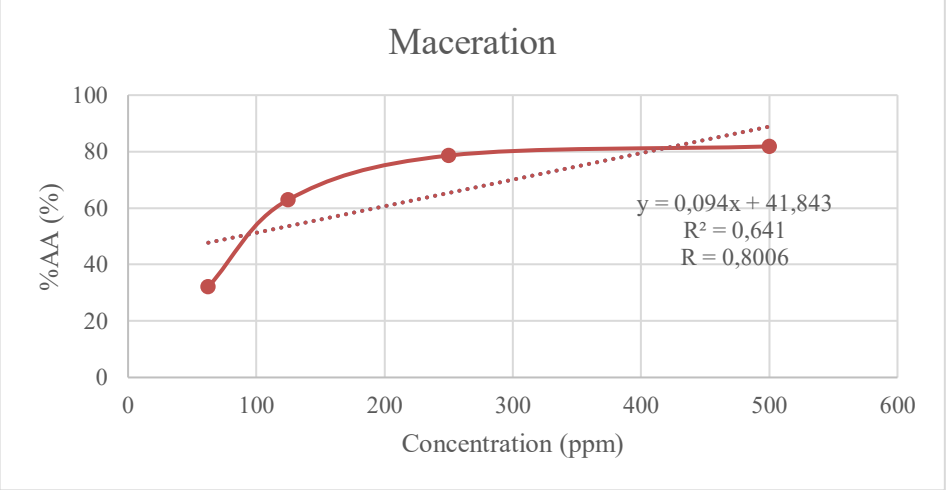

Figure 4. Results of maceration antioxidant test

From the absorbance value of the antioxidant test results on the Cilembu sweet potato skin extract using the maceration method, an increase in extract concentration is seen which is directly proportional to the percentage of antioxidant activity (% AA). The IC_{50} value is the concentration value of an antioxidant substance that provides 50%

inhibition or scavenging of free radicals. From the linear regression equation of the graph, the IC_{50} value of the maceration extract is 86.776 ppm, which is the concentration of the sample solution required to inhibit 50% of the activity of DPPH free radicals. This IC_{50} value indicates that the maceration extract has strong antioxidant activity (Gulcin & Alwasel, 2023). It is proven that the maceration extract contains secondary metabolite compounds in the form of flavonoids, phenolics, and terpenoids which can function as free radical scavengers.

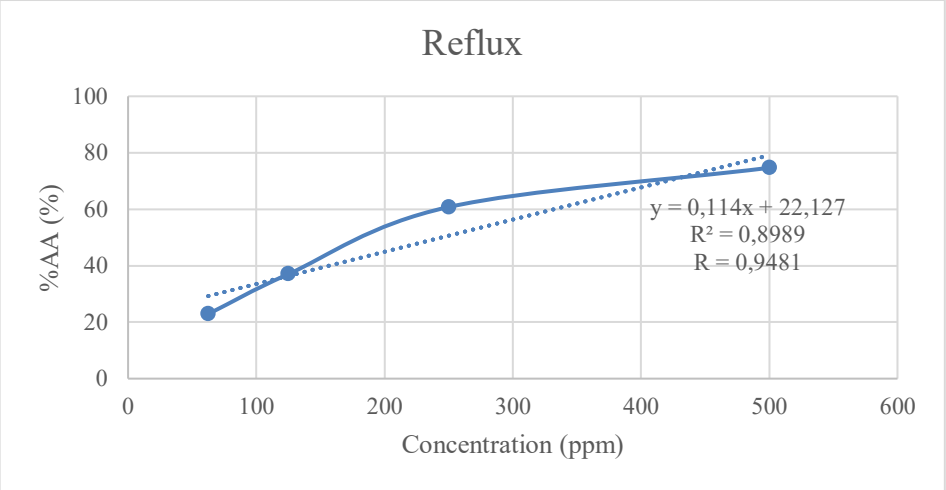

Figure 5. Results of the reflux antioxidant test

The %AA graph obtained from the absorbance value of the refluxed sweet potato peel sample yielded an IC_{50} value of 244.5, indicating low antioxidant activity. Secondary metabolites exhibiting antioxidant properties in the refluxed sweet potato peel extract sample include flavonoids, terpenoids, and phenolics.

Compared with the maceration extract, the concentration of the refluxed extract required to inhibit 50% of free radical activity is higher. This means that using only a small amount of the maceration extract can inhibit 50% of free radical activity. The difference in IC_{50} values between the two extraction methods may be due to differences in extraction conditions, which affect the quantity and type of active compounds extracted. The maceration method, conducted at 300°C, helps preserve bioactive compounds, such as flavonoids, phenols, and terpenoids, without degradation. Meanwhile, the reflux method, which extracts at 780°C, has the potential to cause degradation or structural changes in the active compounds, thereby reducing antioxidant effectiveness.

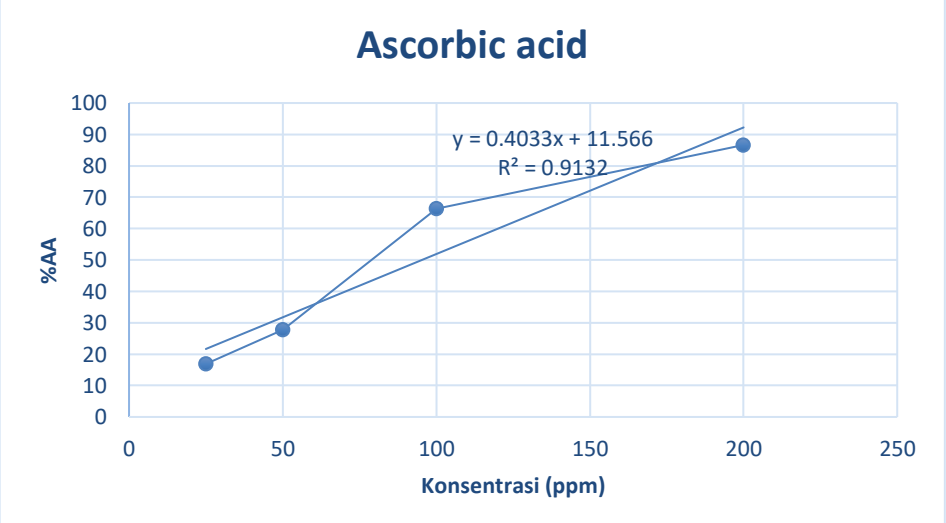

Figure 6. Ascorbic acid antioxidant test

Ascorbic acid was used as a positive control in the antioxidant test because it is a form of vitamin C that has been proven to inhibit free radicals. Ascorbic acid is a pure compound with strong antioxidant activity. Ascorbic acid can donate protons (H^+) and neutralize free radicals. From the positive control test solutions prepared at concentrations of 200, 100, 50, and 25 ppm, the regression coefficient for ascorbic acid was 0.96, indicating that the graph equation can be used due to its high accuracy. The IC_{50} value of ascorbic acid, as a positive control, was 95.296 ppm. This value indicates that ascorbic acid has high antioxidant activity because it only requires a low concentration to inhibit 50% of DPPH free radicals.

Based on the IC_{50} values of the three test solutions, the results showed that the maceration method extract sample was more effective than the reflux method in producing extracts with high antioxidant activity, even better than ascorbic acid. The higher antioxidant activity of the reflux extract than ascorbic acid as a positive control indicates that the metabolite compounds in the extract contribute well to the DPPH free radical reducing activity.

CONCLUSION

Cilembu sweet potato (*Ipomoea batatas var. cilembu*) peel extract contains secondary metabolites such as flavonoids, phenolics, and terpenoids, which were confirmed through phytochemical tests using the reflux extraction and maceration methods. The antioxidant activity of the Cilembu sweet potato peel extract using the DPPH method showed that the macerated extract had higher antioxidant activity with an IC_{50} of 86.776 ppm compared to the reflux extract, which had an IC_{50} of 244.5 ppm. This antioxidant activity originates from secondary metabolites, primarily flavonoids, phenolics, and terpenoids. The polarity of the antioxidant compounds in the Cilembu sweet potato peel extract samples in the TLC test indicated the presence of terpenoids (nonpolar), flavonoids (polar), and phenolics (polar). These results indicate that the maceration method is more effective in retaining bioactive antioxidant compounds than the reflux method.

This study still has limitations because it has not identified specific compounds using advanced instruments such as GC-MS or LC-MS, and has not yet conducted quantitative analysis of total phenolics and total flavonoids. Therefore, further research is needed to identify the active compounds that play a role in the antioxidant activity of Cilembu sweet potato skin extract.

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