Potential Flavonoid Fractions of Purple Eggplant Skin (Solanum melongena var. serpentinum L.) As an antioxidant

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Abstrak

Antioksidan merupakan senyawa yang dapat digunakan untuk mengatasi radikal bebas dengan cara menetralisirnya sehingga dampak negatif yang ditimbulkannya dapat dihindari. Antioksidan dapat diperoleh dari dalam tubuh maupun dari luar, misalnya senyawa aktif yang terdapat pada tumbuhan seperti terong ungu. Kulit terong ungu diketahui mengandung senyawa aktif fenolik, flavonoid, antosianin, saponin, dan alkaloid. Tujuan penelitian ini adalah untuk mengetahui potensi antioksidan fraksi flavonoid kulit terong ungu. Metode ekstraksi dilakukan dengan cara remaserasi; fraksinasi dilakukan dengan metode kromatografi kolom vakum dengan pelarut n-heksana, etil asetat, dan metanol dengan berbagai perbandingan. Metode DPPH digunakan untuk menguji antioksidan dari sampel. Hasil fraksinasi kulit terong ungu mengandung senyawa flavonoid, dan aktivitas antioksidan fraksi flavonoid kulit terong ungu sebesar 275,218 ppm.

***Kata kunci**: fraksi, flavonoid, kulit terong ungu, antioksidan*

Abstract

Antioxidants are compounds that can be used to deal with free radicals by neutralizing them so that the negative effects they cause can be avoided. Antioxidants can be obtained from inside the body or from outside, for example active compounds found in plants such as purple eggplant. Purple eggplant skin is known to contain active phenolic compounds, flavonoids, anthocyanins, saponins, and alkaloids. The aim of this research was to determine the antioxidant potential of the flavonoid fraction of purple eggplant skin. The extraction method is carried out by remaceration; fractionation is carried out by vacuum column chromatography method with n-hexane, ethyl acetate, and methanol as solvents in various proportions. The DPPH method is used to test antioxidants from samples. The results of purple eggplant skin fractionation contain flavonoid compounds, and the antioxidant activity of the purple eggplant skin flavonoid fraction is 275.218 ppm.

Key Words: fractions, flavonoids, purple eggplant skin, antioxidants

INTRODUCTION

Free radicals are reactive compounds because they have free electrons and are unstable. This compound can react with proteins, fatty acids, and DNA, causing damage to cells and body tissues as well as premature aging and even cancer (Lobo et al., 2010). Antioxidants are compounds that can be used to stabilize free radicals so that damage to body cells due to free radicals can be avoided (Ibroham et al., 2022). Antioxidant compounds can be obtained naturally from plants, one of which is the purple eggplant plant. Purple eggplant (Solanum melongena var. serpentinum L.) is a plant that is widely planted by Indonesian people because of its easy planting and maintenance process. Purple eggplant skin contains secondary metabolite compounds such as phenolics, flavonoids, anthocyanins, saponins, and alkaloids (Wulandari et al., 2022). These compounds can be used as a source of natural antioxidants. Flavonoids are secondary metabolites from the group of phenolic compounds. Flavonoids are found in many plants and produce purple, red, yellow, blue, and orange pigments or dyes in plants (Harborne, 1996). Flavonoids in tamarillo seed extract can be used as antioxidants in blood plasma fat (Dewi et al., 2014). Fractionation is a method of separating an active substance or secondary metabolite based on the polarity of the substance to be separated. The solvents used in this method are solvents that have different polarities, namely polar, semi-polar, and non-polar. Separation of compounds based on polarity can be done by liquid-liquid extraction method using a separating funnel or by Vacuum Liquid Chromatography and Column (Aprilia et al., 2015). The aim of this research was to determine the antioxidant potential of the flavonoid fraction of purple eggplant skin.

METHOD

Extraction

The extract was made by maceration from purple eggplant skin powder plus 96% ethanol solvent in a ratio of 1:10. Soaking is carried out for 3x24. The solvent was evaporated with the help of a rotary evaporator and water bath at a temperature of 50°C until a thick extract was obtained.

Fraksinasi

The flavonoid fraction was obtained by vacuum liquid chromatography technique using 100% n-hexane solvent, followed by n-hexane: ethyl acetate 90:10, n-hexane: ethyl acetate 80:20, n-hexane: ethyl acetate 70:30, n-hexane: ethyl acetate 60:40, n-hexane: ethyl acetate 50:50, n-hexane: ethyl acetate 40-60, n-hexane: ethyl acetate 30:70, n-hexane: ethyl acetate 20:80, n-hexane: ethyl acetate 10:90, ethyl acetate 100% and methanol 100%.

Identification of Flavonoid Compounds

Flavonoid compounds were identified using magnesium powder reagents, hydrochloric acid, and amyl alcohol. It is said that the sample contains flavonoids if it is red to violet in color. Further identification can be made by thin-layer chromatography using BAA solvent (4:1:5) and spotting ammonia vapor, which shows a yellow stain on the chromatogram (Hanani, 2015).

Test of Antioxidant Activity of Purple Eggplant Skin Fraction

Preparation of DPPH (2,2 -difenil-1-pikrilhidrazil) solution.

A total of 0.007 grams of DPPH powder was added with 50 mL of methanol and vortexed until dissolved. Take 1 mL of DPPH composition, then add 5 mL of methanol and leave for 30 minutes.

Determination of the maximum absorption wavelength of DPPH.

1 mL of DPPH solution was taken, then 5 mL of methanol was added and left for 30 minutes in a dim place, and retention was estimated at a frequency of 517 nm.

Antioxidant activity testing was carried out using the DPPH method with the reference standard quercetin.

DPPH was made with a concentration of 0.4 mM by weighing 7.8864 mg of DPPH, dissolving it in 50 ml of methanol solvent, and covered with aluminum foil. A blank solution was made by adding 1.0 ml of methanol p.a. to 1.0 ml of 0.4 mM and DPPH and adding up to 5 ml of methanol p.a. The maximum wavelength of 0.4 mM DPPH was determined by placing a blank solution in a cuvette and then reading the absorbance with a visible spectrophotometer at a wavelength of 500-600 nm (Kedare & Singh, 2011).

Data Analysis

Absorbance was obtained by measuring the flavonoid fraction of purple eggplant skin, 0.4 mM DPPH solution, and the reference standard quercetin was used to calculate the percentage of antioxidant potential expressed as percent DPPH reduction.

Percent DPPH reduction is calculated using the following formula:

$$\% \text{ Reduction} = \frac{Blank\ absorbance - sample\ absorbance}{Blank\ Absorbance} \text{ X } 100\ \%$$

Then, the IC50 (inhibition concentration 50) value is calculated using a linear regression equation between actual concentration and percent attenuation. IC50 categories can be grouped as follows: IC50 < 50 ppm is considered very strong, IC50 between 51 – 100 ppm is considered strong, IC50 between 101 – 150 ppm is considered moderate, IC50 between 151 – 200 ppm is considered weak, and IC50 > 200 ppm is considered very weak (Pine et al., 2015).

RESULTS AND DISCUSSION

Extract or essence is the result of the process of filtering active compounds with a suitable solvent (Notoatmodjo, 2018). Eggplant skin extraction was carried out using the remaceration method using 96% ethanol. Ethanol 96% is a polar solvent that can attract compounds such as

flavonoids (Harborne, 1996). Flavonoids are compounds that contain hydroxyl groups and are often found in the form of glycosides, which cause flavonoids to be polar (Robinson, 1995). The extract obtained was identified to determine the presence of flavonoids using a reagent of magnesium powder, hydrochloric acid, and amyl alcohol to produce a red or purple color (Hanani, 2015). This is due to the reduction of the benzopyrone core in flavonoids to form flavylium salts, which are red in color (Ergina et al., 2014). The results of identifying flavonoid compounds in eggplant skin extract are shown in Figure 1.

Figure 1. **Identification of flavonoids produces a red color**

Extracts are still broad in nature and contain many mixtures of compounds. Fractionation is important to isolate mixtures based on their polarity. One method of fractionation is a vacuum column chromatography technique, where the methodology uses several eluents or solvents with different concentrations. In this research, n-hexane 100% was used, followed by n-hexane: ethyl acetate 90:10, n-hexane: ethyl acetate 80:20, n-hexane: ethyl acetate 70:30, n-hexane: ethyl acetate 60:40, n-hexane: ethyl acetate 50:50, n-hexane: ethyl acetate 40-60, n-hexane: ethyl acetate 30:70, n-hexane: ethyl acetate 20:80, n-hexane: ethyl acetate 10:90, ethyl acetate 100% and methanol 100%. Of the 12 fractions obtained, re-identification was carried out using thin layer chromatography using the eluent n-butanol: acetic acid: water (4:1:5) with the appearance of ammonia vapor spots, which showed yellow stains on the chromatogram. Flavonoids are secondary metabolites that are included in the group of phenolic compounds. Identification using ammonia vapor spotting can cause the phenolic hydroxy groups on flavonoids to turn yellow ((Mutiara & Wildan, 2014). Testing with thin layer chromatography using the eluent butanol: glacial acetic acid: water (4: 1: 5) and the appearance of ammonia vapor spots produces a yellow stain, where the stain is characteristic of flavonoid compounds (Mulyani & Laksana, 2011). In Figure 3, the yellow stains are numbers 2, 3, 4, 5, 6, and 7, so the fractions used to test antioxidant activity are stains with these numbers.

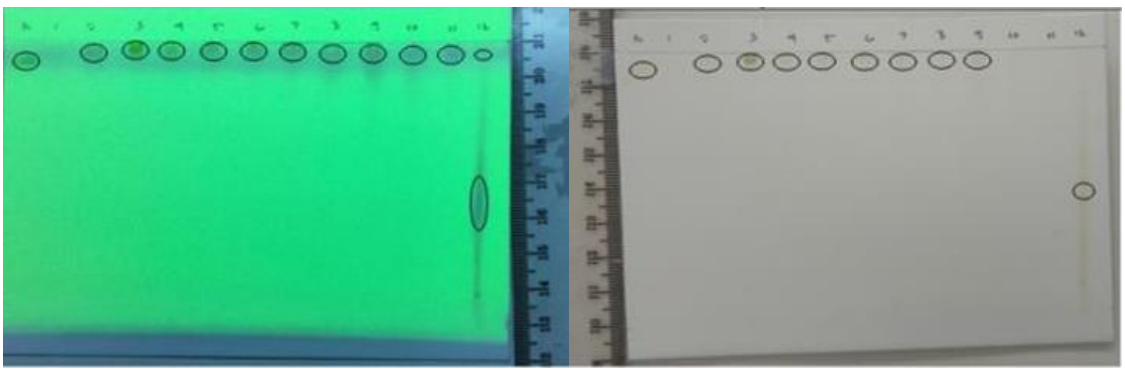

Figure 2. **Results of Thin Layer Chromatography of the Skin Fraction of Purple Eggplant (Solanum melongena var. serpentinum L.)**

The chromatography results of stains with 2,3,4,5,6,7 show a yellow stain, where the stain is the same as the color of the stain from standard quercetin. So, a stain with that number is used to

test antioxidant activity. Antioxidants are compounds that protect the body against the harmful effects of oxidative reactions. Flavonoids can be used to prevent oxidative reactions by capturing free radicals. Flavonoids can be oxidized by radicals, producing more stable radicals. In other words, flavonoids stabilize reactive oxygen species through reactions with radical reactive compounds (Arifin et al., 2022). The results of the antioxidant activity test of the flavonoid fraction of purple eggplant skin are in Table 1.

Table 1. **Antioxidant Activity Test of Purple Eggplant Skin Flavonoid Fractions**

Sample concentration	Absorbance		% Inhibisi	Regresi Linier	IC50 (ppm)
	Blank	Sample			
100	0,865	0,714 ± 0,058	17,46	Y=b X + a	
150	0,865	0,598 ± 0,074	30,79	Y= 0,1768 X + 1,3410	275,218
200	0,865	0,561 ± 0,056	35,11		
250	0,865	0,465 ± 0,077	46,20		
300	0,865	0,398 ± 0,089	53,95		

The potential of the flavonoid fraction as an antioxidant was determined using the DPPH method. This method can be used to determine the antioxidant activity of active compounds in a sample by looking at their ability to overcome free radicals. From Table 1 it can be seen that the flavonoid fraction from purple eggplant skin has the ability to reduce free radicals. The IC50 value (50% inhibitory concentration) is a parameter used to determine the fraction concentration required to inhibit/reduce 50% of free radicals. The smaller the IC50% value, the stronger the antioxidant ability of the active compound; conversely, the greater the IC% value, the weaker the antioxidant activity of a compound (Molyneux, 2003). The flavonoid fraction from purple eggplant skin has an IC50 value of 275.218 ppm. This value is included in the very weak antioxidant category. This can be caused by the flavonoid compounds in the fraction not being yet pure, so an isolation stage is needed to obtain purer compounds. Similar research conducted by Martiningsih et al. (2014), stated that purple eggplant fruit extract had very weak antioxidant activity, namely 385.06 ppm. Flavonoid compounds inhibit free radicals depending on the type of functional group and structural arrangement of the compound. The mechanism of action of flavonoid antioxidants can be carried out by eliminating free radical compounds directly or by inhibiting enzymes that play a role in the formation of free radicals (Dias et al., 2021).

CONCLUSION

The flavonoid fraction of purple eggplant skin has the potential as an antioxidant with an IC50 value of 275.218 ppm.

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