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Analysis of Total Flavonoid and Antioxidant Activity of Ethanol Fraction Watermelon Rind (*Citrullus lanatus*)

Analisis Flavonoid Total dan Aktivitas Antioksidan Fraksi Etanol Kulit Semangka (*Citrullus lanatus*)

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ABSTRAK

Pemilihan kulit semangka sebagai bahan penelitian adalah salah satu upaya pengelolaan limbah rumah tangga menjadi sebuah produk, namun sebelum sampai tahap pengembangan produk menggunakan kulit semangka perlu dilakukan studi pendahuluan potensi dari kulit buah semangka salah satunya aktivitasnya sebagai antioksidan. Tujuan dari penelitian adalah menentukan kadar flavonoid total dan aktivitas antioksidan pada fraksi etanol ekstrak kulit semangka (*Citrullus lanatus*). Ekstraksi dilakukan menggunakan metode maserasi menggunakan pelarut etanol 96%. Fraksi dilarutkan dengan melarutkan ekstrak etanol dengan air kemudian dipartisi dengan n-heksan, dan semi etil asetat. Metode kolorimetri digunakan sebagai penetapan kadar flavonoid sedangkan metode DPPH (*1,1-difenil-2-pikrilhidrazil*) digunakan dalam pengujian aktivitas antioksidan. Hasil ekstraksi diperoleh rendemen sebesar 32,94%. Kadar flavonoid total ekstrak kulit semangka sebesar 49,6 mgQE/g pada fraksi etil asetat, 48,6 mgQE/g pada fraksi air, dan 12,05 mgQE/g pada fraksi heksan. Nilai IC50 terhadap ekstrak kulit semangka sebesar 55,36 µg/mL pada fraksi etil asetat, 71,25 µg/mL pada fraksi air, dan 225,59 µg/mL pada fraksi n-heksan. Berdasarkan peneltian dapat disimpulkan bahwa fraksi etil asetat pada kadar flavonoid total dan uji aktivitas antioksidan memiliki nilai tertinggi.

ABSTRACT

The selection of watermelon rind as research material is one of the efforts to manage household waste into a product, but before reaching the product development stage using watermelon rind, it is necessary to conduct a preliminary study of the potential of watermelon rind, one of which is its activity as an antioxidant. The aim of the study was to determine the total flavonoid content and antioxidant activity in the ethanol fraction of watermelon rind (*Citrullus lanatus*) extract. Extraction was performed using the maceration method using a 96% ethanol solvent. Fractions were dissolved by dissolving the ethanol extract with water, then partitioned with n-hexane and semi-ethyl acetate. The colorimetric method was used to determine flavonoid content while the DPPH (1,1-diphenyl-2-picrylhydrazyl) method was used to test antioxidant activity. The extraction result obtained a yield of 32.94%. The total flavonoid content of watermelon rind extract was 49.6 mgQE/g in the ethyl acetate fraction, 48.6 mgQE/g in the water fraction, and 12.05 mgQE/g in the hexane fraction. The IC_{50} value of watermelon rind extract was 55.36 µg/mL in the ethyl acetate fraction, 71.25 µg/mL in the water fraction, and 225.59 µg/mL in the n-hexane fraction. Based on the research, it can be concluded that the ethyl acetate fraction in the total flavonoid content and antioxidant activity test has the highest value.

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1. INTRODUCTION

Compounds or molecules with one or more unpaired electrons in their highest orbitals have been identified as free radicals. Based on the unpaired electron that exists, the compound becomes highly reactive when it is looking for a partner by attacking and binding to the electrons of the molecules surrounding it. Free radicals are constantly produced in the body due to normal cellular metabolism, inflammation, nutritional deficiencies, and response to external influences such as environmental pollution, ultraviolet light, and cigarette smoke (1). Large concentrations of free radicals lead to oxidative stress causing a number of isomers, including diabetes, stroke, arteriosclerosis, cancer, cardiovascular disease, and the aging process, in living cells. Antioxidants are compounds that slow down or inhibit stress by giving an electron mechanism (2). The plants and vegetables that contribute to a healthy diet and help prevent disease are sources of exogenous antioxidants (3). Antioxidants are necessary to keep the body's natural defenses against free radicals and aging processes intact (4). Watermelon rind (*Citrullus lanatus*) is included in household waste because it is usually discarded. Watermelon rind has a hard texture and has no taste (5). What is rarely known is that watermelon rind is actually rich in vitamins, minerals, enzymes, and chlorophyll. While the flavonoid-group compounds from phenolic substances found in watermelon rind (*Citrullus lanatus*) can act as antioxidants. The compound acts as a peroxyl radical scavenger because it has important molecules, namely aromatic rings and hydrogen groups, that can move places. In addition, it has the ability to reduce free radicals by forming chelates with valenced ions such as Cu, Fe, Zn, and Mn that cause lipid peroxidation (6).

This research is also an effort to process waste into products, so preliminary studies need to be carried out before they are developed into pharmaceutical products. According to research (7) using an ethanol extract of watermelon rind (*Citrullus lanatus*) with the DPPH method, the silencing activity of watermelon rind (*Citrullus lanatus*) is very strong, with an IC_{50} value of 14.729 mg/L. The DPPH method was not used in this study to investigate the antioxidant activity of polar, semi-polar, and non-polar antioxidants. So, in this study, we will investigate the antioxidant activity of watermelon rind extract (*Citrullus lanatus*), which is polar, semi-polar, and non-polar.

2. METHODS

Material

Watermelon rind (*Citrullus lanatus*) (Bekasi), spectrophotometer UV-Vis (PG Instrument T80+), rotary evaporator (IKA RV 05), DPPH (1,1-diphenyl-2-picrylhydrazyl) (MERCK), quercetin (SIGMA), ethanol 96% (MERCK), n-hexane (MERCK), ethyl acetate (MERCK), aquadest (MERCK).

Preparation method

The rind of a watermelon (*Citrullus lanatus*) was removed, cleansed in clean water, then baked at 50 ^{o}C till it dried to a constant weight. The dried components were powdered. Ethanol was combined with 300 g of powdered watermelon rind (*Citrullus lanatus*) and let it stand at room temperature for 72 hours. Three different solvents-water, ethyl acetate, and n-hexane-were used to fractionate the crude extracts of watermelon rind (*Citrullus lanatus*). After that, Whatman no. 41 was used to filter the crude extract and fraction supernatants. Concentration of the fraction was done with a rotary evaporator set to 40 ^{o}C and reduced pressure.

Phytochemical screening of plants material

The phytochemical screening of the plant material was examined using several types of methods, and Table 1 describes the results of the study.

Table 1. Screening test for phytochemicals on watermelon rind (*Citrullus lanatus*)

Phytochemical	Test performed	Reference
Alkaloids	Mayer's test	(8)
Flavonoids	Shinoda's test	(9)
Tannin	$FeCl_3$ test	(10)
Steroids	$CH_3COOH + H_2SO_4$ test	(11)
Saponins	Boiled water test	(9)

Determination of total flavonoid

The method for determining the total flavonoid (12). A spectrophotometer UV-Vis was used to measure quercetin at 457.50 nm using a solution that served as a reference. A calibration curve was used to determine the quercetin concentration (mg/g) and the absorbance (x), yielding the total flavonoid content.

Antioxidant activity

This examination's antioxidant activity has been tested using DPPH, a method used in several study approaches (13) (12) (14). The DPPH solution was combined with the component at several concentrations. After half an hour at room temperature and without light. The absorbance of the solution was determined at 517 nm. These formulas were used to calculate the concentration of antioxidant activity:

$$\text{Inhibition (\%)} = (A_0 - A_1)/ A_0 \times 100 \quad \text{..... (eq 1)}$$

Where:

A_0 = the control absorbance

A_1 = the sample concentration

3. RESULT

The ethanol extract was dissolved in water to reconstitute the fraction, which was then separated using n-hexane and ethyl acetate.

Table 2. Results of watermelon rind ethanol extraction

Simplisia	Weight (g)	Extract	
		Weight (g)	Result (%)
Watermelon rind	300	98.83	32.94

The antioxidant activity of the fraction was then assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) technique.

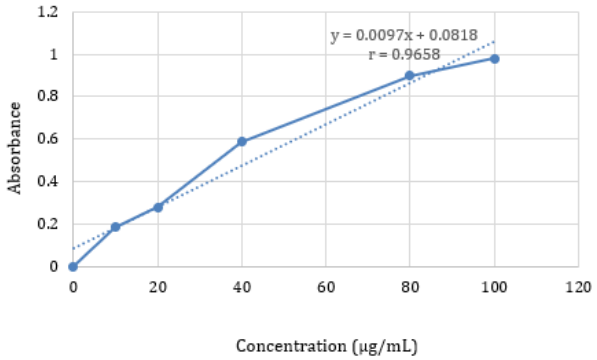

Figure 1. Calibration curve of quercetin

Based on the total flavonoid test results, figure 2 shows that the ethyl acetate fraction had the greatest total flavonoid value of 49.6 mgQE/g, followed by the water fraction with 48.6 mgQE/g and the n-hexane fraction with 12.05 mgQE/g.

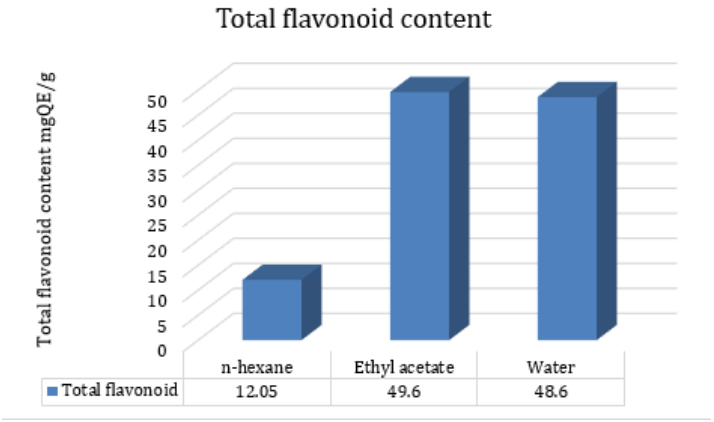

Figure 2. Total flavonoid content

The fractionation results shown in figure 3 that watermelon rind extract had strong antioxidant activity in the ethyl acetate fraction, which was 55.36 µg/mL, then in the water fraction which was 71.25 µg/mL, and the results were not strong in then n-hexane fraction, which was 225.59 µg/mL.

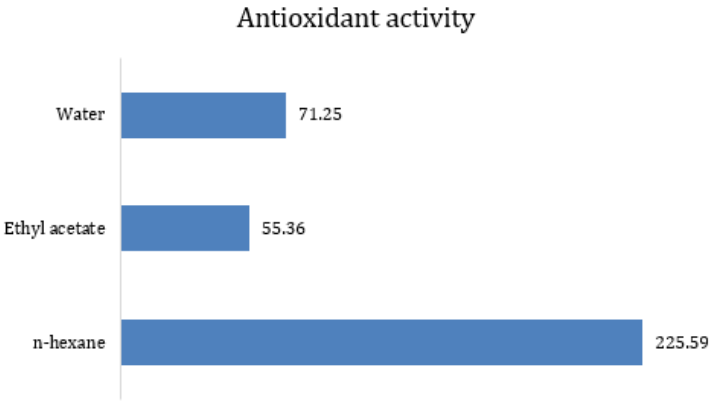

Figure 3. Antioxidant activity watermelon rind

4. DISCUSSION

Phytochemical screening

The maceration method was used to carry out the extraction process. The maceration method is used to avoid potential damage to the sample's chemical composition since there is no heating involved in the extraction process, which means that temperature cannot impact the extract's chemical properties or accelerate processes. Simplisia is macerated using 96% ethanol solvent because flavonoid compounds are more soluble in 96% ethanol and more compounds are drawn so that the cell walls in plants are not susceptible to degradation and polar phenolic compounds are easily released from plant cells. The extraction process was remacerated to maximize the process that had been carried out at the maceration stage, then concentrated with a rotary evaporator at 50°C. The extraction results produced a yield of 32.94% in accordance with the requirements of the yield is declared good if > 10%.

The watermelon rind (*Citrullus lanatus*) phytochemical screening analysis (Table 2) identified the presence of alkaloid, flavonoid, and tannins As opposed to that, the fraction showed an absence of steroids and saponin. The fraction yields of watermelon rind (*Citrullus lanatus*) extract show the ethyl acetate fraction produced the highest yield which was 50%, followed by the water fractions yield of 43.3% and n-hexane fractions yield of 22%.

Table 2. Screening of watermelon rind (*Citrullus lanatus* (Thunb.) Matsun & Nakai) and its fraction on preliminary phytochemicals

Phytoconstituents	Ethanol watermelon rind extract fraction		
	n-hexane	Ethyl acetate	Water
Alkaloids	-	+	+
Flavonoids	+	+	+
Tannin	+	+	+
Steroids	-	-	-
Saponins	-	-	-

(+ = presence; - = absence)

Total flavonoid content

Watermelon rind extract's total flavonoid concentration is ascertained using the colorimetric method (aluminum chloride). This method is based on the idea that flavonoids, which contain conjugated aromatics and exhibit strong absorption bands in the UV and visible light spectrum, form complexes with aluminum chloride and keto groups of C-4 atoms and hydroxyl groups of C-3 or C-5 atoms adjacent to flavone and flavonol groups of total flavonoid concentrations. Wavelength determination was carried out at 350-500 nm. The purpose of determining the wavelength determination is to determine the maximum absorbance of the sample. The measurement obtained a maximum wavelength of 457.50 nm. After determining the wavelength, a calibration curve was made where the quercetin standard was connected between absorbance and quercetin concentration to obtain a calibration curve equation with the form of the equation y = 0.0097x + 0.0818 with a correlation coefficient of 0.965. There is a linear calibration curve when the value of r is closer to 1. There is a connection between the absorbance value and the quercetin solution concentration.

The total flavonoid content of the various fractions was determined in order to conduct the test. The ethyl acetate fraction contained larger amounts of flavonoids than water and n-hexanes, according to the results of the analysis of the flavonoid content of different fractions. The ethyl acetate fraction has a larger flavonoid content because it is semipolar, which allows it to draw in both polar and nonpolar flavonoid molecules. Due to the plant's presence of free flavonoids that dissolve quickly in semi-polar chemicals, this may be the cause, in semi-polar molecules such flavones, flavonoids, and flavonols, which are easily soluble. The n-hexane fraction produced the lowest flavonoid content which is non-polar. Flavonoids can dissolve with non-polar solvents from flavonoid types such as polymethoxy aglycones or isoflavones whose sugar groups or glycoside forms have been released so that they can only dissolve in non-polar solvents, namely n-hexanes. Table 3 shows the total flavonoid concentration of the water, ethyl acetate, and n-hexane fractions.

Tabel 3. Total Flavonoid Content

Fraction	Total flavonoid content
n-hexane	12.05 mgQE/g
Ethyl acetate	49.6 mgQE/g
Water	48.6 mgQE/g

The results showed that the antioxidant potential to scavenge free radicals to inhibit the production of free radicals increased with total flavonoid content (15).

DPPH radical scavenging activity

Analyze the antioxidant activity of the water, n-hexane, and ethyl acetate fractions using the DPPH method. The reduction of free radical compounds from antioxidant compounds results in a color shift from purple to yellow. This process requires the attachment of hydrogen atoms from antioxidant compounds to the free electrons of radical compounds. This process is necessary to convert free radicals (diphenylpicrylhydrazyl) into non-

radical compounds (diphenylhydrazine). This is how the DPPH method runs. The absorbance of each fraction shows that with increasing concentration of extract, the absorbance of the sample will decrease and the value of the inhibition level increases. The absorbance of the sample decreases because the electrons in DPPH become paired with the electrons of the sample.

The ethyl acetate fraction produced the highest result of 55.36 μg/mL among the tested extracts of n-hexane, water, and ethyl acetate, which were all categorized as strong antioxidants, while the water fraction obtained 71.25 μg/mL classified as strong antioxidants and n-hexane fraction 225.59 μg/mL classified as weak antioxidants. Semi-polar solvents such as ethyl acetate have the ability to counteract strong free radicals. The antioxidant ability of the n-hexane solvent is classified as less active due to the presence of interfering substances such as proteins, fats, and other compounds that dissolve in non-polar compounds, thus blocking the capture of free radicals.

Based on the extraction results, the watermelon rind has antioxidant activity from flavonoid compounds that can be useful for maintaining the balance of the body from free radicals. Watermelon white skin is thought to have a marker compound in the form of lycopene. Lycopene compounds are known to have the greatest antioxidant potential, twice that of β-carotene and ten times that of vitamin E, but to confirm this, further testing can be done. confirmed, further testing can be done.

5. CONCLUSION

Based on research, the watermelon rind (*Citrullus lanatus*) ethyl acetate fraction exhibited the highest level of flavonoid component concentration and antioxidant activity in the DPPH study being conducted. The result shows watermelon rind (*Citrullus lanatus*) has potential sources as an antioxidant to prevent free radicals. Flavonoids are reducing substances that can stop multiple oxidation processes in the path. Flavonoids could indeed transfer an electron to a free radical compound, making it potential to act as antioxidants.

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