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Comparison of Antioxidant Activity of Ethanol Extract of Fruit and Okra Leaves (*Abelmoschus esculentus L. Moench*) by DPPH and ABTS Methods

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Abstract. Excessive free radical activity in the human body can be characterized by pathological conditions such as cardiovascular disease, pre-natal complications, arthritis, cataracts, parkinsonism, alzheimer's, and aging. One way to prevent the formation of free radicals is to use nutrients that act as antioxidants. Natural antioxidants found in fruits and vegetables are effective free radicals and are considered non-toxic when compared with synthetic antioxidants, one of which is the okra plant Abelmoschus esculentus L.The purpose of this study is to determine differences in the antioxidant activity ethanol extract of fruit and okra leaves with DPPH method and ABTS method. The methods used in this study were the maceration extraction method with 70% ethanol solvent to obtain fruit extracts and okra leaves. The antioxidant activity test was carried out by the DPPH (1,1-diphenyl-2-picrylhydrazi) method by measuring the absorption of a mixture of sample extracts and DPPH by UV-Visible spectrophotometry at a wavelength of 520 nm and by the ABTS method ((2,2-azinobis- (3 -Ethylbenzothiazoline-6-Sulfonic Acid) by measuring the absorption of a mixture of sample extracts and ABTS by UV-Visible spectrophotometry at a wavelength of 734 nm. measurement of antioxidant activity of okra fruit ethanol extract by ABTS method has very strong antioxidant activity (IC_{50} 24.50 ppm).

Keyword: Antioxidants, DPPH, ABTS, Abelmoschus esculentus (L.) Moench.

Abstrak. *Aktifitas radikal bebas yang berlebihan pada tubuh manusia dapat ditandai dari keadaan patologis seperti: penyakit pembuluh jantung, komplikasi pre-natal, arthritis, katarak, parkinson, alzheimer, dan penyakit penuaan. Salah satu cara pencegahan pembentukan radikal bebas adalah dengan menggunakan nutrisi yang berperan sebagai antioksidan. Anti oksidan alami terdapat dalam buah-buahan dan sayuran yang merupakan anti radikal bebas yang efektif dan dianggap tidak beracun jika dibandingkan dengan anti oksidan sintesis ,salah satunya adalah tanaman okra* Abelmoschus esculentus *L.Tujuan penelitian ini adalah untuk mengetahui perbedaan uji aktivitas antioksidan ekstrak etanol buah dan daun okra dengan metode DPPH dan metode ABTS. Metode yang digunakan dalam penelitian ini adalah metode ekstraksi maserasi dengan pelarut etanol 70% untuk memperoleh ekstrak buah dan daun okra. Uji aktivitas antioksidan dilakukan dengan metode DPPH (*1,1–diphenyl-2-picrylhydrazi*) dengan mengukur serapan campuran ekstrak sampel dan DPPH secara spektrofotometri UV-Visible pada panjang gelombang 520 nm dan dengan metode ABTS (*(2,2-azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid) *dengan mengukur serapan campuran ekstrak sampel dan ABTS secara spektrofotometri UV-Visible pada panjang gelombang 734 nm.Hasil pengukuran ekstrak etanol buah okra dengan metode DPPH memiliki aktivitas antioksidan sangat kuat (IC_{50}*

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27,15 ppm) dan hasil pengukuran aktivitas antioksidan ekstrak etanol buah okra dengan metode ABTS memiliki aktivitas antioksidan sangat kuat (IC_{50} 24,50 ppm.

Kata Kunci: *Antioksidan, DPPH, ABTS, Abelmoschus esculentus (L.)Moench.*

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1. Introduction

Free radicals are a form of reactive oxygen compounds, compounds that have unpaired electrons. Free radicals are atoms, molecules or independent compounds that have unpaired electrons, therefore are highly reactive and unstable. Unpaired electrons always try to find a new partner, so it is easy to react with other substances (protein, fat or DNA) in the body. Free radicals are more dangerous than non-radical oxidant compounds because the high reactivity of free radical compounds that cause the formation of new radical compounds. When new radical compounds meet with other molecules, new radicals will form again so that chain reactions will occur. This like reaction will stop if the reactivity is quenched by antioxidant compounds [1], [2].

Antioxidants are compounds that can slow down the oxidation process of free radicals. The mechanism of action of antioxidant compounds, one of which is by donating hydrogen atoms or protons to radical compounds so as to supplement the lack of electrons needed by free radicals and inhibit chain reactions from forming free radicals. Its makes the radical compound more stable [3], [4].

Natural antioxidants can be obtained from fruits and vegetables that contain antioxidant compounds. The compounds contained in plants that have antioxidant activity are vitamins C, E, A, carotenoids, polyphenols, phenolic acids, flavonoids, tannins, and lignans, one of which is the okra plant (*Abelmoschus esculentus L.*) [5].

Methods for testing the presence of antioxidant activity are the DPPH method and the ABTS method. The DPPH method (1,1-diphenyl-2-picrylhydrazyl) measures the reducing power of the sample (extract) against DPPH free radicals. DPPH will react with hydrogen atoms from free radical reduction compounds to form a more stable DPPH. Free radical reduction compounds that react with DPPH will become new radicals that are more stable or non-radical compounds [6].

Free radical reduction method 2,2-azinobis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) is a testing method to measure the amount of free radicals that have quite high sensitivity, the excellence of ABTS is its simple, effective, fast testing , and easy to repeat [1].

Literature studies show that the antioxidant activity of okra fruit ethanol extract by DPPH method have relatively weak antioxidant activity with an IC_{50} value of 172.92 µg /ml [7]. The

results of antioxidant activity testing on okra leaves using DPPH method have a relatively strong antioxidant activity with an IC_{50} value of 75.68 µg /ml [6].

Based on the data above, this study carried out, the comparison of antioxidant activity of ethanol extract of fruit and okra leaves (*abelmoschus esculentus l. moench*) by DPPH and ABTS methods using spectrofotometer UV-Vis.

2. Method

This research was a laboratory experimental study to determine the antioxidant activity of ethanol extracts of fruit and ocra leaves with various concentrations of 10, 20, 40, 60 and 80 µg/ ml. Then the antioxidant activity was tested using free radical trapping (1,1-diphenyl-2-picrylhydrazyl) (DPPH) and 2,2-Azinobis (3-ethylbenzothiazoline) -6-sulfonicacid (ABTS) methods using UV-Visible Spectrophotometry.

2.1. Extraction

Dried simplicia are soaked with 70% ethanol as much as 7.5 parts of the weight of the sample for 5 days while occasionally stirring, then let stand for 24 hours. Separated extract by filtering. Repeat the process of extracting at least once the same type of solvent and the volume of the solvent amounting to half the amount of the volume of the solvent on the first search. Collect all extract, then concentrated with a rotary evaporator until a thick extract is obtained [8].

2.2. Test Antioxidant Activity with DPPH Method

The testing procedure is carried out based on the Burits and Bucar method. 1 ml of ethanol extract solution of okra fruit (10,20,40,60 and 80 µg / mL) mixed with 1 ml of DPPH solution of 40 µg / mL. The mixture was incubated in a dark room for 30 minutes, then its absorbance was measured at a wavelength of 520 nm with UV-Visible spectrophotometer. Measurements were made three times with vitamin C as a comparison. The same procedure was carried out for ethanol extract of okra leaves. Percent value of radical scavenging represented by IC_{50} value is calculated by the following formula [9]:

$$\%\ \text{radical scavenging} = \frac{(\text{A Blank} - \text{A extract})}{\text{A Blank}} \text{x}100\%$$

Where: A Blank = Absorbance of ethanolic DPPH Solution

A Extract = Absorbance of Sample [9].

2.3. Test Antioxidant Activity with ABTS Method

The testing procedure is carried out based on the Emad A. Shalaby method. ABTS solution is made by mixing 5 ml of ABTS stock solution 7 mM and 5 ml of potassium persulfate solution 2.45 mM , the mixture is incubated for 12-16 hours. ABTS solution was added with 70%

ethanol until was obtained an absorbance value of 0.7 ± 0.02 at a wavelength of 734 nm. 0.1 ml of ethanol extract solution of okra fruit (10,20,40,60 and 80 µg/mL) mixed with 0.9 ml of ABTS solution. The mixture was incubated in a dark room for 6 minutes, then the absorbance was measured at a wavelength of 734 nm with a UV-Visible spectrophotometer. Measurements were made three times with vitamin C as a comparison. The same procedure was carried out for ethanol extract of okra leaves. Percent value of radical scavening represented by IC_{50} value is calculated by the following formula [10]:

$$\%\ \text{radical scavenging} = \frac{(\text{A Blank} - \text{A extract})}{\text{A Blank}} \text{x}100\%$$

Where: A Blank = Absorbance of ethanolic ABTS Solution
A Extract = Absorbance of Sample [10].

2.4. Data Analysis

The results of the calculation of % radical scavening were entered into the regression equation with the extract concentration (µg/ mL) as abscissa (x axis) and the value of% antioxidant inhibition as the ordinate (y axis). IC_{50} values are calculated when the % radical scavenging value is 50% using the equation: y = ax + b. Comparison of the antioxidant activity value of ethanol extract of okra fruit with DPPH and ABTS methods was statistically tested (T test to compare two averages).

3. Result and Discussion

3.1 Antioxidant activity test results using the DPPH method

Ethanol extract of fruit and okra leaves has antioxidant activity obtained from the measurement of DPPH absorbance at the 60th minute by adding test solutions at concentrations of 10 ppm, 20 ppm, 40 ppm, 60 ppm and 80 ppm. The ability of ethanol extract of fruit and leaves okra to capture DPPH free radicals is evidence that the test sample has antioxidant activity which can be proven by decreased the absorbance of DPPH. The presence of antioxidants in the ethanol extract of fruit and okra leaves will neutralize DPPH radicals by giving electrons to DPPH, resulting in a change in color from purple to yellow or the intensity of the purple color of the solution was reduced [11]. Determination of antioxidant activity used is IC_{50} parameters that is the sample concentration needed to capture DPPH radical by 50% where the smaller the IC_{50} value then the stronger the antioxidant activity [4]. The results of the antioxidant activity of ethanol extract of fruit and leaves okra can be seen in tables 1 and 2, with vitamin C as a comparison.

Table 1. The antioxidant activity of ethanol extract of okra fruit and vitamin C by DPPH method

No	Sample	Concentration	% radical scavenging	IC_{50} Value
1	Ethanol Extract of Okra Fruit	10	57.04	27.15 ppm
		20	59.12	
		40	66.77	
		60	73.11	
		80	76.28	
2	Vitamin C	10	83.35	3.38 ppm
		20	85.84	
		40	85.8	
		60	90.32	
		80	91.15	

Table 2. Antioxidant activity of ethanol extract of okra leaves and vitamin C by DPPH method

No	Sample	Concentration	% Radical scavening	IC_{50} Value
1	Ethanol Extract of Okra Leaves	10	50.58	49.54 ppm
		20	52.13	
		40	54.80	
		60	57.52	
		80	62.12	
2	Vitamin C	10	83.35	3.38 ppm
		20	85.84	
		40	85.8	
		60	90.32	
		80	91.15	

Table 1 shows the antioxidant activity of okra fruit ethanol extract has a very strong antioxidant activity with a value of 27.15 ppm where the value is smaller than the antioxidant activity of vitamin C. Table 2 shows the antioxidant activity of ethanol extract of okra leaves has a very strong antioxidant activity with a value of 49, 54 ppm where the value is smaller than the antioxidant activity of vitamin C.

3.2 Antioxidant activity test results using the ABTS method

Test antioxidant activity with the ABTS method based on the ability of antioxidant compounds to stabilize free radical compounds by donating proton radicals. The ability of ethanol extract of fruit and okra leaves to stabilize free radical compounds can be seen from the change in color of the greenish blue test solution to a colorless or intensity reduced . The results of the antioxidant activity of ethanol extract of fruit and okra leaves can be seen in tables 3 and 4, with vitamin C as a comparison.

Table 3. Antioxidant activity of ethanol extract of okra and vitamin C by ABTS method

No	Sample	Concentration	% Radical scavening	IC_{50} Value
1	Ethanol Extract of Okra Fruit	10	58.25	24.50 ppm
		20	63.56	
		40	69.79	
		60	77.55	
		80	81.63	
2	Vitamin C	10	74.8	10.34 ppm
		20	77.28	
		40	83.58	
		60	90.17	
		80	91.84	

Table 4. Antioxidant activity of ethanol extract of okra leaves and vitamin C by ABTS method

No	Sample	Concentration	% Radical scavening	IC_{50} Value
1	Ethanol Extract of Okra Leaves	10	59.86	21.14 ppm
		20	65.23	
		40	77.21	
		60	81.68	
		80	84.71	
2	Vitamin C	10	74.8	10.34 ppm
		20	77.28	
		40	83.58	
		60	90.17	
		80	91.84	

Table 3 shows the results of antioxidant activity testing using the ABTS method, where ethanol extract of okra fruit has an IC_{50} value of 24.50 ppm which value is smaller than the antioxidant activity of vitamic C as a comparison. Table 4 shows the results of testing the antioxidant activity of okra leaf ethanol extract by ABTS method, where the ethanol extract of okra leaf has an IC_{50} value of 21.14 ppm which value is smaller than the antioxidant activity of vitamic C as a comparison. A compound is said to be a very strong antioxidant if it has an IC_{50} value of less than 50 ppm [4], [12]. Based on IC_{50} values obtained from ethanol extracts of okra fruit and leaves with DPPH and ABTS methods, the antioxidant activity of okra fruit and leaves has a very strong category. This IC_{50} value is inversely proportional to antioxidant activity, the higher the antioxidant activity, the lower the IC_{50} value [11]. The strength of the antioxidant activity of ethanol extract of fruit and okra leaves is due to the high content of polyphenol and flavonoid compounds. The higher the total content of polyphenols and flavonoids in a plant extract, the higher the antioxidant activity [1].

3.3. Statistical Test Results

Test Statistically based on the T-test, the average IC_{50} value of ethanol extract of okra fruit obtained the value of t-count (0.852) is smaller than t-table (2.78) at 95% confidence level and the free degree $\Phi = 4$, then there is no significant difference between the DPPH method and the ABTS method or it can be said that the DPPH method is the same as the ABTS method. Likewise for the ethanol extract of okra fruit statistically there was no difference in antioxidant activity with the DPPH and ABTS methods where the t-count value (2.17) was smaller than the t-table value (2.78).

4. Conclusion

Ethanol extract of okra fruit has a strong antioxidant activity where the DPPH method obtained an IC_{50} value of 27.15 ppm and with the ABTS method obtained an IC_{50} value of 24.50 ppm. The antioxidant activity test of ethanol extract of fruit and okra leaves by DPPH and ABTS methods was not significantly different.

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