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Antioxidant Potential of Red Okra Pods (*Abelmoschus esculentus* Moench)

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Abstract

Okra red (*Abelmoschus esculentus* Moench) grows a lot in Indonesia and its antioxidant ability is unknown. This study was performed to evaluate the antioxidant activities of red okra pods extracts. The powder of red okra was extracted by reflux. The first extraction used n-hexane (three times). Ethyl acetate was used to extract the residue (three times). Then, the residue was extracted three times using ethanol. Antioxidant activities were tested using DPPH and FRAP assays. This study showed that ethanol extract showed the highest antioxidant activity, which had the lowest IC_{50} DPPH (39.8 µg/mL) and the highest FRAP (89.15 µmol Fe2+/g). The result of DPPH and FRAP methods indicates that ethanol extract of red okra pods is classified as a very strong antioxidant.

1 Introduction

The Antioxidants are molecules that are capable of slowing down or preventing the oxidation process of easily oxidized substances. Antioxidants are also defined as compounds that protect cells from the harmful effects of reactive oxygen free radicals. These free radicals can come from the body's metabolism and other external factors (Halliwel et al., 1995). Free radicals are unstable molecules. They have unpaired electrons and look for electron pairs in biological macromolecules (Lobo et al., 2010). Proteins, lipids, and DNA from cells are a good source of electron pairs. Oxidation conditions can cause damage to DNA and proteins that induce cancer, aging, and other diseases (Young and Woodside, 2001).

Red okra (*Abelmoschus esculentus* Moench) is an annual herbaceous plant originating from Ethiopia and Sudan but is already popular in Indonesia (Wahyuningsih et al., 2018). Water extract from okra pods contains polysaccharides which are antidiabetic, anti-ulcer, and can enhance the immune response due to bacteria (Chen et al, 2016; Sheu and Lai, 2012; Wahyuningsih et al., 2018). Leaves, flowers, fruit, and okra pods contain phenolic and flavonoids compounds as antioxidants

(Khomsug et al., 2010). Okra pods contain epicatechin, procyanidin B2, catechin, and rutin. While the seed contains quercetin, procyanidin B1, procyanidin B2, and rutin (Arapitsas, 2008).

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) methods could be used to determine antioxidant activity in many plants extracts (Thaipong et al., 2006). A test method using DPPH free radicals is the method most often used to test the antioxidant activity of medicinal plants. DPPH can react with compounds that can donate hydrogen atoms. The absorbance decreases stoichiometrically according to the number of electrons taken when the electrons become paired up by the presence of free radical scavengers. The presence of antioxidant compounds can change the color of DPPH solutions from purple to yellow (Dehpour et al., 2009). Changes in absorbance due to this reaction have been widely used to test the ability of some molecules as free radical scavengers.

Another method for testing the ability of antioxidants is ferric reducing antioxidant power (FRAP). The FRAP method measures changes in absorbance of the Fe-2,4,6-tripyridyls-triazine (TPTZ) complex at 593 nm with a UV-Vis spectrophotometer. The absorbance of the Fe-TPTZ complex is proportional to the number of Fe-TPTZ complexes (an oxidizer) reduced by antioxidant samples. FRAP testing is based on the electron transfer reaction of antioxidants to Fe3+-TPTZ compounds. Fe3+-TPTZ compounds themselves represent oxidizing compounds that may be present in the body and can damage body cells (Ou et al., 2002).

There is no research regarding the antioxidant activity of pods from red okra (*A. esculentus* Moench) which are extracted using increasing polarity solvents (n-hexane, ethyl acetate, and ethanol), and tested by DPPH and FRAP assays. The goals of this study are to observe antioxidant potential in various polarity extracts (n-hexane, ethyl acetate, and ethanol) of red pods okra grown in Jember, East Java, Indonesia uses DPPH and FRAP assays.

2 Materials and Methods

2.1 Chemicals and Reagents

2,2-Diphenyl-1-picryl-hydrazine (DPPH) from Sigma Life Science, ferric chloride, ferrous sulfate, 2,4,6-tripyridyl-s-triazine (TPTZ) from Sigma Life Science, n-hexane, ethyl acetate, ethanol, methanol, sodium acetate.

2.2 Sample Collection and Extraction

The red okra used was produced in Jember City, East Java, in April 2019. Powdered crude okra (300 g) was extracted by reflux. The first extraction used n-hexane (three times). Ethyl acetate was used to extract the residue (three times). Then the residue was extracted three times using ethanol. Three extract concentrate were evaporated at 30ºC by rotary evaporator and it was freeze-dried. Hence, there were three extracts: n-hexane extracts, ethyl acetate extracts, and ethanolic extracts.

2.3 DPPH Free Radical Scavenging Assay and IC_{50}

The reaction mixture consisted of 100 µL of red okra pods extracts, 100 µL of pure methanol, and 100 µL of DPPH radical in methanol solution (50 µg/mL), which was incubated at room temperature for 30 min. Several red okra extract concentrations (200; 150; 100; 75; 50; 35; 25; 15; 12.5; 10; 6.25; 3.125 µg/mL) were used, and readings were monitored at 517 nm, using a UV/Vis microplate spectrophotometer (Thermo Scientific™ Multiskan™GO). The antioxidant activity measured by DPPH free radical method can be expressed as IC_{50}, i.e., the antioxidant concentration required to

reduce the initial DPPH radical by 50%. The concentration of red okra pods samples required to reduce the initial DPPH radical by 50% was expressed in µg/mL.

2.4 Ferric Reducing Antioxidant Power Assay (FRAP)

To determine the antioxidant activity by iron reduction, using the ferric reducing antioxidant power (FRAP) assay, we followed the methodology described by Benzie and Strain (1996) with some modifications. FRAP measures the ferric reducing ability of the samples, in acidic medium (pH 3.6), forming an intense blue color as the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous (Fe^{2+}) form. FRAP reagent was prepared immediately before analysis by mixing 20 mL of acetate buffer (300 mM, pH 3.6), 2 mL of TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2 mL of $FeCl_3$ (20 mM) in aqueous solution. An aliquot of 50 µL of the red okra pods extracts was added to 1 mL of FRAP reagent and 450 µL H_20. It incubated in a water bath at 37 °C for 30 min. After this time, the absorbance was measured using a UV/Vis microplate spectrophotometer (Thermo Scientific™ Multiskan™GO) that was reset with FRAP solution. The calibration curve was constructed using ferrous sulfate (100–2000 µM) and the results were expressed in µmol Fe2+/g.

3 Results and Discussion

3.1 Antioxidant activity by DPPH assay

Free radicals of DPPH dissolve in methanol and show absorption at wavelength 517 nm. The hydrogen will be transferred antioxidant to DPPH, which will be stable. If the free radicals are scavenged by an antioxidant, then the colors of DPPH would be changed from purple to yellow. Percent of DPPH inhibition showed radical scavenging activity of each sample or red okra pods extract (Table 1). An ethanol extract of red okra pods has a relatively greater percent inhibition at a concentration of 150 µg/mL compared to other extracts. IC_{50} values are also known as the inhibitory concentration at 50% inhibition. It is a measure of the effectiveness of a substance in inhibiting a biochemical function or specific biological.

Extraction Type	% Inhibition of red okra pods						Linear Regression Equation
	10 µg/mL	25 µg/mL	35 µg/mL	50 µg/mL	75 µg/mL	150 µg/mL	
N-Hexane	20.37	23.76	28.71	32.52	40.35	59.67	y = 0.2823x + 17.997 (R^2 = 0.996)
Ethyl acetate	38.12	41.24	42.92	46.36	47.36	57.96	y = 0.1355x + 37.870 (R^2 = 0.980)
Ethanol	19.19	33.15	40.98	56.16	93.96	184.93	y = 1.2105x + 1.7938 (R^2 = 0.996)

Table 1: Percent inhibition and linear regression equation of various extractions from red okra pods

The IC_{50} value was calculated by plotting an x-y linear regression for all the various concentrations prepared. Based on the results using the DPPH method, the IC_{50} of extract red okra pods were calculated and are shown in Figure 1.

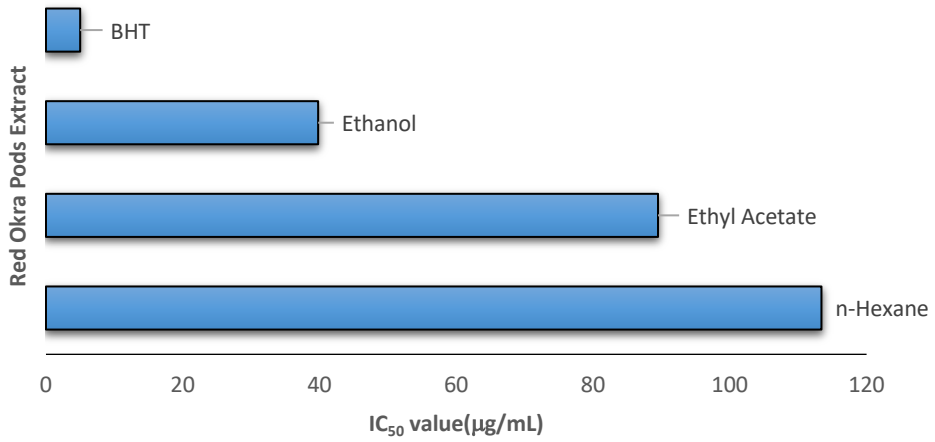

Figure 1: Concentration of red okra pods extract required to reduce the initial DPPH radical by 50%.

The extract that showed the lowest extract concentration to reduce the initial amount of DPPH radical by 50% was ethanol extract (39.8 µg/mL), followed by ethyl acetate extract (89.5 µg/mL) and n-hexane (113.4 µg/mL). Red okra pods extract presenting the lowest IC_{50} values can be considered better in terms of antioxidant activity, since a lower concentration to reduce the DPPH free radical by 50% is required. Graham et al. (2017) reported that five different okra seed genotypes (Asha, Asontem, Agbagoma, Sengevi, and Penkruma) in Ghana had IC_{50} values for the sample infusions ranged from 127.800-405.667 µg/mL and the defatted samples ranging from 28.714-338.333 µg/mL. While this study showed that IC50 values had ranged from 39.8-113.4 µg/mL.

In general, the free radical scavenging potentials of extracts of the red okra pods accessions were variable but extraction type dependent. However, the ethanol extracts of the accessions were better able to reduce or inhibit the free DPPH radicals than the other extracts. This also suggests that compounds present in the red okra pods accessions responsible for scavenging the free DPPH radicals have a higher solubility in ethanol than in n-hexane and ethyl acetate.

The scavenging activity against DPPH free radical has been widely used to determine the antioxidant activity of plant extract (Vaziri, 2008). Sample with IC_{50} lower than 50 µg/mL can be classified as a very strong antioxidant, 50-100 µg/mL as strong, 101-150 µg/mL as medium and greater than 150 µg/mL as a weak antioxidant (Sakai, 2000). The results of the DPPH assay in this study showed that red okra ethanolic extract the lowest IC_{50} value (<50µg/mL) which indicated the best free radical scavenging activity and very strong antioxidant activity. While red okra ethyl acetate extract has strong antioxidant activity (89.5 µg/mL), but red okra n-hexane extract has medium antioxidant activity (113.4 µg/mL).

3.2 Antioxidant activity by FRAP assay

The FRAP assay is the only assay directly that measures antioxidants (or reductants) in a sample. While the other test is to measure the inhibition of free radicals. The values expressed from the FRAP assay represent the corresponding concentration of electron-donating antioxidants with the reduction

in the ferric iron (Fe3+) to the ferrous ion (Fe2+). FRAP is a suitable assessment for total antioxidants in plants that are consumed by humans (Halvorsen et al., 2002). The highest ability to reduce Fe3+ to Fe2+ was found in ethanol extract (89.15 µmol Fe2+/g) compared with ethyl acetate extract (36.52 µmol Fe2+/g) and n-hexane extract (18.38 µmol Fe2+/g) (Figure 2). Xia's study (2015) reported that the FRAP value of okra pods extract in China was 76.7±5.32 µmol Fe2+/g.

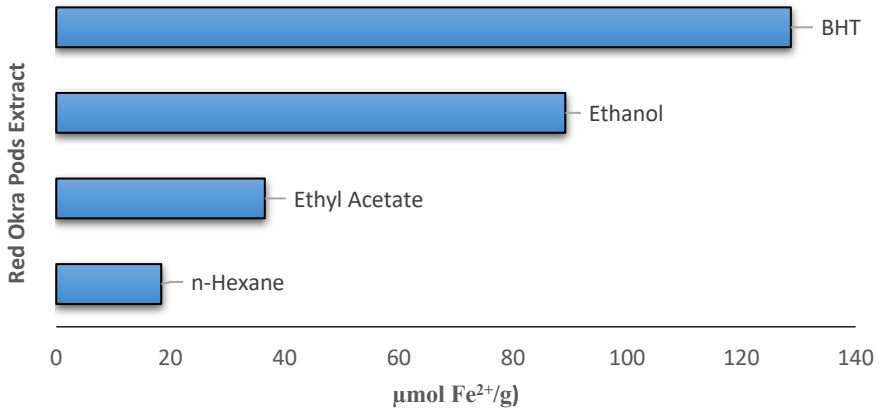

Figure 2: Concentration of red okra pods extract required to reduce Fe^{3+} to Fe^{2+} by FRAP assay.

4 Conclusions

We conclude that ethanol extract from okra pods has very strong antioxidant activity via DPPH and FRAP assays.

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