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Antioxidant Activity of Extract and Fraction from *Boesenbergia pandurata* Rhizome by FRAP Method

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Abstract

Boesenbergia pandurata is one of the Zingiberaceae families found in Southeast Asia. In Indonesia, this plant which is known as "temu kunci" is widely cultivated by people everywhere included as home plant and used as family medicinal plant. *B. pandurata* contains flavonoid compounds which have the efficacy of being an antioxidant. Antioxidant is useful to overcome the negative effects of free radicals. Antioxidant test using the FRAP (*Ferric Reducing Antioxidant Power*) method began with *B. pandurata* extract using ethanol 96%, then continued with fractionation process of n-hexane, ethyl acetate, and ethanol. Free radical activity using spectrophotometry was seen with a wavelength of 712 nm and the total antioxidant value was calculated based on the absorbance of each samples. The result of study of antioxidant activity from ethanol extract, non-polar, semi-polar, and polar fraction were 37.59; 27.43; 32.93; and 35.68 mgAAE/g.

Key words : *Boesenbergia pandurata, Antioxidant*, FRAP, extract, fraction.

INTRODUCTION

Free radicals are products of metabolism in the form of atoms or molecules containing one or more unpaired electrons and very reactive (Indarti *et al*., 2019). Free radicals will form in the body continuously and can be one factor of several degenerative disease processes (Halliwell, 2012). Radical compounds can damage cells, which can cause several diseases such as liver, cancer, and Alzheimer's (Hernani & Raharjo, 2006). So we need a compound that can overcome the negative effects of these free radicals, namely antioxidants. Antioxidants are compounds that are able to reduce, delay and avoid lipid oxidation. In another mean, antioxidants are substances that can request or cancel the revision of free radicals in lipid oxidation. Antioxidants can reduce, restrain and prevent the oxidation process by donating one or more electrons to free radicals that free radicals can be bemuted (Ahmad, *et al*., 2012).

Indonesia rich in herbs that have medicinal properties. One of them is from the Zingiberaceae family, namely *Boesenbergia pandurata* or what is known as "temu kunci". This plant is widely cultivated in home yards and its rhizomes are commonly used as a food flavoring because it provides an aromatic flavor that can increase appetite (Chatsumpun *et al*., 2017). Besides having a pleasant aroma, *B. pandurata* rhizome also has many benefits and becomes one of the traditional medicines that are widely used by people. *B. pandurata* rhizomes made in the form of herbal medicine and believed to be efficacious to overcome various diseases such as gout, gastrointestinal disorders, and dyspepsia (Chaudhury & Rafei, 2001). The results of screening conducted in the study of Chahyadi, *et al*., 2014, showed that key rhizomes are known to contain flavonoid compounds that can be efficacious as antibacterial, antifungal, and antioxidant.

Some methods that can be used to determine antioxidant activity include 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis- (3-ethyl-benzothiazolin-6-sulfonic acid) method (ABTS), 2-thiobarbituric acid (thiobarbituric acid, TBA), ferric reducing ability (FRAP), cupric ion reducing antioxidant capacity (CUPRAC), and oxygen radical absorbance capacity (ORAC)). This

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study aims to measure the antioxidant activity of extracts and key Intersect Rhizome fractions. The method used is the FRAP (Ferric Reducing Antioxidant Power) method, because this method is simple, easy to do and fast, cheap, and the reagents used are easily prepared (Maryam *et al.*, 2015).

METHODS

Research Design

This research was carried out experimentally by using the FRAP method to find out the antioxidant activity in rhizomes of *B. pandurata* or *temu kunci* rhizomes originating from the Batu-Malang. This research was conducted at the Laboratory of Pharmaceutical Chemistry, University of Muhammadiyah Surakarta.

Tools

Stirring rods, measuring flasks, watch glasses, beakers, micropipets, pH meters, measuring pipettes, centrifuges, test tubes, centrifugation tubes, analytical scales, rotary evaporators, ovens, and UV-Vis spectrophotometers.

Materials

Aquades free of CO2, ascorbic acid, oxalic acid, FeCl3, phosphate buffer pH 6.6, *B. pandurata* rhizomes extract, ethanol 96%, potassium ferrisianide, n-hexane, and ethyl acetate.

Extraction and Fractionation

Simplisia of *B. pandurata* rhizomes in the form of powder macerated using ethanol 96% for 24 hours while occasionally stirring. Then filtered using a buchner funnel and vacuum, and maserat is evaporated using a rotary evaporator and get a dense extract of *B. pandurata* rhizomes. Preparation of *B. pandurata* rhizomes ethanol extract by weighing 5 mg dense extract and dissolved with 5 mL ethanol 96% until homogeneous.

The fractionation process is carried out using the separating funnel method. It starts by weighing 5 mg of the dense extract of *B. pandurata* rhizomes and is dissolved with 50 mL ethanol 96%. After completely dissolved and homogeneous, then fractionated using non-polar, semi-polar, and polar solvents. The solution of extract ethanol 96% was fractionated with 50 mL n-hexane. Then shake it slowly and you will see the separation of the two solutions. Then the n-hexane fraction was separated from the solution of extract ethanol 96% *B. pandurata* rhizome, and followed by the addition of 50 mL of ethyl acetate into the funnel then shaken slowly. There will be a separation of the two solutions, then the ethyl acetate fraction is separated from the *B. pandurata* rhizome extract. The solution of *B. pandurata* rhizome extract which has been fractionated using n-hexane and ethyl acetate then becomes ethanol fraction.

Standard Curve

A standard curve is made by dissolving 25 mg ascorbic acid with oxalic acid to 25 mL and a standard solution concentration of 1000 ppm is obtained. Then dilution is carried out to get the concentration of standard solutions of ascorbic acid 60, 70, 80, 90, and 100 ppm.

Test Solution

1. Phosphate buffer solution pH 6.6

   Phosphate buffer solution pH 6.6 is made by weighing 2 grams of NaOH and dissolving it with distilled water up to 250 mL. Then also weighed 6.8 grams of KH_2PO_4 dissolved with

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distilled water up to 250 mL. Next 8.2 mL NaOH was taken and 25 mL KH_2PO_4 added, then the pH was measured up to 6.6 and distilled water added up to 100 mL.

2. Oxalate Solution

   Oxalate solution is made by weighing 1 gram of oxalic acid and dissolved with distilled water up to 100 mL then homogenized.

3. Potassium Ferrisianide Solution

   The solution is made by weighing 1 gram of potassium ferrisianide and dissolving it with distilled water up to 100 mL then homogenized.

4. FeCl3 Solution

   The solution is made by weighing 0.1 gram of FeCl3 and dissolved with distilled water up to 100 mL then homogenized.

5. Trichloroacetic Acid Solution (TCA)

   The solution is made by weighing 10 grams of TCA and dissolved in distilled water up to 100 mL then homogenized.

Antioxidant Test by FRAP Method

Test of the antioxidant activity by the FRAP method was carried out by dissolving 5 mg of *B. pandurata* rhizome extract with ethanol 96% and pipette 1 mL. Then 1 mL of phosphate buffer pH 6.6 was added and 1 mL of pottasium ferrisianida and incubated for 20 minutes at 50°C. After incubation, 1 mL of TCA was added and then centrifuged at 3000 rpm for 10 minutes. After centrifugation, then pipette 1 mL of the upper layer and 1 mL of distilled water and 0.5 mL $FeCl_3$ added. The solution is allowed to stand for 10 minutes, then the absorbance is measured at λ 712 nm. For the n-hexane fraction, the ethyl acetate fraction and the ethanol fraction were treated the same as the *B. pandurata* rhizome ethanol extract. As a blank, a mixture of oxalate solution is used. Calibration curves are made using ascorbic acid solutions made in various concentrations. The value of antioxidant activity by the FRAP method is expressed in mg ascorbic acid equivalent/ gr (mgAAE / gr).

RESULT AND DISCUSSION

The results of the absorbance measurement of the comparative solution of ascorbic acid are shown in Table 1.

Table 1. Absorbance of Ascorbic Acid

Concentration (ppm)	Absorbance
60	0.3752
70	0.5454
80	0.6712
90	0.7631
100	0.8138

The results of absorbance measurements and antioxidant activity values of extracts and fraction of *Boesenbergia pandurata* rhizome are shown in Table 2.

Table 2. Absorbance and Antioxidant Activity of Extracts and Fraction of *Boesenbergia pandurata* Rhizome

Sample of *B. pandurata*	Absorbance in λ 712 nm	Antioxidant Activity (mg AAE/g)
Extract ethanol	3.8735	37.59
n-Hexane fraction	2.7615	27.43
Ethyl acetate fraction	3.3635	32.93
Ethanol fraction	3.6644	35.68

Boesenbergia pandurata Rhizome is one of the plants that has many health properties, one of which is as an antioxidant. In this study, it is intended to determine the antioxidant activity found in extracts and fractions of *B. pandurata* rhizomes by using the FRAP method. Test of antioxidant activity with the FRAP method was chosen because it is a simple procedure, easy, cheap and fast method, and the reagents used were quite simple. Antioxidant testing using the FRAP method has been widely used because it is simple and fast, and the reaction is reproducible and linear related to the molar concentration of antioxidants.

Antioxidants are compounds that provide a single electron or hydrogen atom for reduction (Rabeta & Faraniza, 2013). In this study, ascorbic acid is used as a standard curve because ascorbic acid functions as a secondary antioxidant, which is to capture free radicals. Ascorbic acid or better known as vitamin C is able to ward off extracellular free radicals. That is because vitamin C has a free hydroxy group that acts as a free radical scavenger and if it has a polyhydroxy group, it will increase antioxidant activity (Wahdaningsih, *et al.*, 2011).

Measurement of antioxidant activity using the FRAP method with ascorbic acid solution as standard. The addition of TCA solution aims to precipitate the potassium ferrosianide complex. The addition of FeCl3 is intended to form green to blue complexes. Reducing power is an indicator of the potential of an antioxidant compound. In this case, the reducing power is measured by the ability of an antioxidant to convert Fe^{3+} to Fe^{2+} (Kim, 2005). In this method, the reduction reaction in an acidic atmosphere of Fe^{3+} complexes with yellow becomes Fe^{2+} complexes with bluish green due to electron donors from antioxidant compounds. The antioxidant activity test using the FRAP method can be monitored by measuring the absorption of Fe^{2+} complex compounds formed by spectrophotometer UV-Vis (Panda, 2012).

Based on the results of the antioxidant activity test using the FRAP method, the regression results obtained from the concentration (x) with the absorbance value (y) of the standard solution of ascorbic acid in the equation y = 0.01095x - 0.2422 so that the known value of R^2 = 0.9575. To calculate the value of antioxidant activity, the absorbance value of the sample is entered into the equation. The value of the antioxidant activity test using the FRAP method is expressed in mg ascorbic acid equivalent/ gr sample (AAE). The content of vitamin C in each sample is expressed as the ascorbic acid equivalent (AAE) which is a general reference for measuring the amount of vitamin C contained in a sample. The results of absorbance measurements and the value of the antioxidant activity of the *B. pandurata* rhizome extract were 37.59 mgAAE / g. As for the value of antioxidant activity in the non-polar, semi-polar, and polar rhizomes of *B. pandurata* rhizomes, respectively 27.43 mgAAE/ g; 32.93 mgAAE/ g; and 35.68 mgAAE/ g. Its mean in every gram of sample is equivalent to this value in mg of ascorbic acid.

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CONCLUSION

From the results of study the antioxidant activity of ethanol extract and fraction of *B. pandurata* rhizome using FRAP method and ascorbic acid as a comparison solution, obtained antioxidant activity on ethanol extract of *B. pandurata* which has the greatest value compared to the antioxidant activity in fraction of *B. pandurata* rhizome that is 37.59 mgAAE/ g.

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