EXECUTIVE SUMMARY

HIBAH BERSAING*

PEMANFAATAN DAUN KOPI ARABIKA (*Coffea arabica*) DAN KELOPAK BUNGA ROSELLA (*Hibiscus angustifolia*) SEBAGAI TEH HERBAL TERSTANDAR ANTIDIABETES MELLITUS

Tahun ke 2 dari rencana 2 tahun

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Study of antioxidant activity combination of Arabica coffee leaf ethanol extract and roselle flower petal water extract

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Abstrak

Free radical induced oxidative stress that influences the occurrence of various degenerative diseases. Antioxidant protect the body from damage caused by free radical. Arabica coffee and rosella are plants that have potential as antioxidants which can be used to treat various diseases. This study is focused on antioxidant activity combination of ethanolic extract of Arabica coffee leaves and water extract of rosella flower petal by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. The purpose of this method is to know the equivalent concentration parameter gives 50% effect of antioxidant activity (IC_{50}). In the antioxidant activity test of single extract the greatest antioxidant activity is ethanol extract of leaves of Arabica coffee with IC_{50} value of 19.856 ± 0.126 µg / ml followed by water extract of roselle flower petals with IC_{50} value of 107.683 ± 0.434 µg / ml. In testing of combination extract the greatest activity of the total of the combination of coffee: e(2: 1), the combination of coffe (1: 1), and the combination of coffee: roselle (1: 2) with IC50 values respectively 61.781 ± 0.726 µg / ml; 69.087 ± 0.773µg / ml; and 73.742 ± 1.138 µg / ml.

Keywords: Antioxidant, Arabica coffee, Roselle, DPPH

INTRODUCTION

Free radicals are molecules that have one or more unpaired electrons. As we know that some cardiovascular diseases such as heart disease, diabetes mellitus, and cancer are diseases that triggered by oxidative damage in the presence of free radicals. Excessive oxidation of the nucleic acids, proteins, fats and DNA cells can initiate the occurrence of degenerative diseases such as coronary heart disease, cataracts, cognitive disorders and cancer (Leong and Shui, 2001; Pietta 1999). Humans have had a defense system against oxidants that come from within or from outside the body in the form of diet. Defense of the body such as enzymes peroxidase, catalase, glutathione, histidine, peptidin often still lacking due to environmental influences and poor diet (Pietta, 1999).

Antioxidants are molecules that can inhibit the oxidation of molecules that can produce free radicals. Antioxidant is a compound that has the ability to react with free radicals to produce a stable free radical by accepting or donating electrons. Based on the source of, antioxidant divided into two kinds, namely natural antioxidants and antioxidants synthetic (artificial). Synthetic antioxidants are most often used such as Propyl Error (PG), Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and Tertbutylhydroquinone (TBHQ). The synthetic antioxidants can give side effects that harmful to human health because it's carcinogenic. Various studies on BHA and BHT indicate that this can cause tumors in laboratory animals at long-term use (Kikuzaki, 1993). Natural antioxidant is an antioxidant that is obtained naturally in plants. Natural antioxidants are usually more desirable, because the level of better security and broader benefits in the food, health and cosmetics. Natural antioxidants can be found in food of vegetables and fruits. As an example of natural antioxidants such as Vitamin A, Vitamin C, Vitamin E, and Polyphenols. Their concerns about the potential side effects of synthetic antioxidants used as motivation for the study of natural antioxidants as one alternative.

According to previous studies, plants with high phenol levels have high antioxidant activity as well, this is because most of the antioxidant compounds are derivatives of phenol. Coffee is one agricultural commodity in Indonesia. In the Indonesian market encountered two types of coffee, the Arabica coffee (Coffea arabica) and Robusta coffee (Coffea canephora). Previous research showed that the leaves of the Arabica coffee (Coffea arabica) contains a lot of polyphenol compounds are efficacious as an antioxidant. Research conducted shows the results of a very high antioxidant activity (Pellegrini *et al*., 2003).

Nowdays roselle (Hibiscus sabdariffa L.) become so popular. Almost in every exhibition of medicinal plants, roselle is always introduced. This is due to almost all parts of this plant can be used for treatment,

particularly for alternative medicine. Roselle contain chemical compounds that can provide many benefits. According to the results of previous studies showing that the anthocyanin content in the plant is effective in counteracting free radicals cause cancer and other diseases (Mardiah et al., 2009).

This research will be conducted to determine the antioxidant activity of ethanol extract of the leaves of the Arabica coffee (Coffea arabica) and water extract of roselle calyx (Hibiscus sabdairffa) by DPPH (1,1-diphenyl-2-picrylhydrazyl) because the method this is the simplest method, fast, easy, accurate, inexpensive, and capable of measuring the various components that act as free radical or hydrogen donor. In addition DPPH method does not require a lot of reagents such as another method of measuring antioxidant activity. Comparative compounds used in measuring the activity of these antioxidants are Vitamin C or ascorbic acid. Used as a comparison compound for the preparation Vitamin C is widely used in the market and has been shown to have antioxidant activity and relatively cheap. According to the study, Vitamin C is used by many researchers as a benchmark test for the antioxidant activity have the ability to reduce free radicals.

MATERIAL AND METHOD

Making Extract

Arabica coffee leaves and roselle flower petal each weighed 2 kg. Leaves and petals washed with water, sorted, cut into small pieces and dried with aerated indoors, not exposed to direct sunlight for 4 days. After aerated,put in a 50 ° C oven for final drying. The dried simplicia subsequently comminuted by means of a blender.

The extraction process is done by maceration. A total of 100 grams of powder coffee leaves in the dry state macerated in 1 liter of ethanol maserator with a technical for 24 hours. Every 3 hours for soaking stirring with a stirring bar. After the liquid extract was filtered with filter paper and the result of maceration evaporated with a rotary evaporator temperature of 50 ° C and the results are concentrated in a water bath to obtain a thick extract. The process of boiling water roselle done by boiling crude powder roselle flower petals as much as 3 grams (included in tea bag) in 200 ml water with a temperature of 70 - 80 ° C for 15 minutes. This process is done 5 times obtain to get much extract.

Combination of Extract

This research will test the antioxidant activity of the combination of the ethanol extract of the leaves of arabica coffee and water extract of roselle flower petals in 3 comparisons of different combinations. The combination is done by mixing the extract of leaves of coffee and boiling water roselle flower petals

with various proportions in glass beaker. After the combination, the solvent evaporated using a water bath at 70^{o}C.

Antioxidant Activity Test

Preparation of Standard Solution Vitamin C

Vitamin C is weighed as much as 25 mg put in a 25 ml flask and dissolved with ethanol p.a to limit the volume so that the concentration of vitamin C of 1000 ppm. Pipette solution, put flask was added ethanol to limit the volume to obtain solution concentrations of the main vitamin C. Made two main liquor Vitamin C which is a concentration of 50 µg / ml and 100 µg / ml. Pipette solution, put flask was added ethanol to the volume limit in order to obtain the concentration of vitamin C final test solution.

Preparation of Test Solution

Each combination results obtained weighed as much as 25 mg and 50 mg put in a 25 ml flask and dissolved with ethanol p.a to obtain solution concentrations of test extracts of 1000 µg / ml and 2000 µg / ml. Pipette solution, put 10 ml flask was added ethanol to obtain the final extract concentration of test solutions for each combination.

Preparation of DPPH Solution

DPPH powder weighed amount of 2 mg, dissolved in 50 ml of ethanol in order to obtain a concentration of 0.1 mM. The solution is then stored in dark bottles and for each test created new DPPH solution.

Determination of Maximum Wavelength

Before testing the antioxidant activity, determination of the maximum wavelength is done by pipette 1.2 ml of 0.1 mM DPPH. Then add 0.3 ml of ethanol. The mixture was shaken until homogeneous and incubated at room temperature for 30 minutes in a dark place. Absorption was measured by UV-Vis spectrophotometer that has been set wavelengths of 400-600 nm.

Optimization of Incubation Time

Optimization of incubation time is done with pipette 1.2 ml of 0.1 mM DPPH solution was added 0.3 ml of the test solution then measuring the absorbance every 5 minutes starting at minute 0 to minute 100.

Antioxidant Activity Test

Measurement of antioxidant activity is done by 0.3 ml of each test solution extracts and vitamin C solution and 1.2 ml of 0.1 mM DPPH was added in test tube. The mixture was then shaken until

homogeneous test solution and vitamin C solution was incubated at room temperature for incubation time optimizations. Measured absorbance at the maximum wavelength.

RESULT AND DISCUSSION

Extraction of sample

Extract obtained from the leaves of Arabica coffee are 15.460 grams, early simplicia that used are 100 grams, so the % yield obtained is 15.460%. While the extract obtained from roselle flower petal are 9.529 grams, early simplicia that used are 15 grams, so the% yield is 63.527%.

Combination of Extract

This research will test the antioxidant activity of the combination of the ethanol extract of the leaves of arabica coffee and water extract of roselle flower petals in 3 comparisons of different combinations. The combination is done by mixing the extract of leaves of coffee and boiling water roselle flower petals with various proportions of the beaker glass. The combination can be seen in Table 1.

Table 1. Comparison of extract combination

Comparison of Combination	Arabica coffee extract (gram)	Roselle extract (gram)
1 : 0	1.0	-
2 : 1	2.0	1.0
1 : 1	1.5	1.5
1 : 2	1.0	2.0
0 : 1	-	1.0

Optimization of Incubation Time

The optimum incubation time indicates that each sample extract or comparison has reacted with DPPH solution completely. The optimization of incubation time can be seen in Table 2.

Table 2. Result of incubation time

Sample	Optimum Time (minute)
Arabica cofee extract	30
Roselle extract	45
Vitamin C	30

Antioxidant Activity Test

IC_{50} is an effective concentration to reduce free radicals as much as 50 percent. In the relationship between the concentration and the IC_{50}, the greater the value of the concentration of the test solution, the IC_{50} values are getting smaller. The results of measuring the antioxidant activity obtained can be seen in Table 3.

Table 3. Result of Antioxidan Activity Test

Sample	IC50 ± SD (µg /ml , n=3)
Arabica cofee extract	19.856 ± 0.126
Roselle extract	107.683 ± 0.434
Combination Arabica coffee : Roselle (1 : 2)	73.742 ± 1.138
Combination Arabica coffee : Roselle (1 : 1)	69.087 ± 0.773
Combination Arabica coffee : Roselle (2 : 1)	61.781 ± 0.726
Vitamin C	3.265 ± 0.003

Based on data from the results showed that in general the whole extract samples of single and combination of each faction has antioxidant activity. The average of all samples tested had a power level

of antioxidant activity are active because they have IC_{50} values between 50-100 µg / ml and only the ethanol extract of leaves of Arabica coffee are classified as antioxidants which are very active as vitamin C because it has a IC_{50} value of less than 50 µg / ml, while the water extract of roselle flower petals are classified as antioxidants with the power level was moderate because it has IC_{50} values between 100-250 µg / ml. The smaller of IC_{50} value become the greater the antioxidant activity because only with a small concentration is able to reduce free radicals by 50%. An antioxidant compound can be very active if IC_{50} < 50 µg / ml, active for IC_{50} values between 50-100 µg / ml, moderate for the IC_{50} value of between 100-250 µg / ml, and weak if the IC_{50} value above 250 µg / ml (Jun *et al*., 2006).

In the antioxidant activity test of single extract the greatest antioxidant activity is ethanol extract of leaves of Arabica coffee with IC_{50} value of 19.856 ± 0.126 µg / ml followed by water extract of roselle flower petals with IC50 value of 107.683 ± 0.434 µg / ml. In testing of combination extract the greatest activity of the total of the combination of coffee: roselle (2: 1), the combination of coffe (1: 1), and the combination of coffee: roselle(1: 2) with IC_{50} values respectively 61.781 ± 0.726 µg / ml; 69.087 ± 0.773 µg / ml; and 73.742 ± 1.138 µg / ml. Based on the result of the combination with the increasing amount of the composition of the ethanol extract of leaves of Arabica coffee can lower the IC_{50} values so it increase antioxidant activity. Phenolic compounds contained in Arabica coffee leaves suspected to contribute antioxidant activity sufficiently large so as to reduce the IC_{50} value of roselle flower petals.

Vitamin C in this method is used as a comparison because these compounds are powerful antioxidants that are widely used in the community. Marinova and Batchvarov (2011) stated that there are five compounds were used as a comparison in measuring the antioxidant activity, the α-tocopherol (vitamin E), ascorbic acid (Vitamin C), BHA, BHT, and trolox. The comparison of the five, the most widely used is Vitamin C. Vitamin C can directly capture free radicals of oxygen, either with or without the enzyme catalyst. The reaction to the reactive oxygen compounds more rapidly than other components, besides Vitamin C also protects important macromolecules of oxidants (Levine et al., 1995).

CONCLUSION

In the antioxidant activity test of single extract the greatest antioxidant activity is ethanol extract of leaves of Arabica coffee with IC_{50} value of 19.856 ± 0.126 µg / ml followed by water extract of roselle flower petals with IC_{50} value of 107.683 ± 0.434 µg / ml. In testing of combination extract the greatest activity of the total of the combination of coffee: e(2: 1), the combination of coffe (1: 1), and the

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