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Antioxidant Activity from The Combination Ethanol Extract Secang Wood (*Caesalpinia sappan* L.) And Red Ginger Rhizome *(Zingiber officinale Roxb.)*

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ABSTRACT
The antioxidant activity test combination of the ethanol extract secang wood (Caesalpinia sappan L) and red ginger rhizome (Zingiber officinale Roxb.) was carried out using the DPPH method. Where the principle of the DPPH method is a decrease in the intensity of the absorbance value of the DPPH solution which is directly proportional to the increase in the concentration of antioxidant compounds called IC50 or Concentration Inhibition 50. The results have shown that the value of the IC50 or Inhibition Concentration 50 of the ethanol extract of Secang wood is 54,53 which is a strong antioxidant, the IC50 or Inhibition Concentration 50 value from the ethanol extract of red ginger rhizome (Zingiber officinale Roxb.) is 197,74 which is a weak antioxidant, and the IC50 or Inhibition Concentration 50 value combination of the ethanol extract Secang wood (Caesalpinia sappan L) and red ginger rhizome (Zingiber officinale Roxb.) for F1 with a ratio of 1:1 is 109,72 which is a moderate antioxidant, F2 with a ratio of 1:2 is 140,96 which is a moderate antioxidant and F3 with a ratio of 2:1 is 90,14 which is a strong antioxidant.

Keywords: *antioxidant, DPPH, ethanol extract of secang wood, ethanol extract of red ginger rhizome*

1. INTRODUCTION

Antioxidants are compounds that have an activity that can delay, prevent and counteract the process of lipid oxidation which can cause damage to cells in the body [1]. The working principle of antioxidant compounds is by donating one electron so that atoms or molecules that have unpaired electrons will get electron pairs. Antioxidants also function as compounds that can bind free radical compounds in the body [2]

Free radicals are molecules that have unpaired electrons, so they are very reactive. Free radicals are also a by-product of the body's normal metabolism that can cause oxidation such as DNA damage, membrane damage and can cause cell death [3].

Based on the source, antioxidants can be divided into two groups, namely synthetic antioxidants, and natural antioxidants. A synthetic antioxidant is an antioxidant compound obtained through the synthesis of a chemical reaction. Examples of synthetic antioxidants that are widely known by the public are Butylated HydroxyAnisole (BHA) and Butylated HydroxyToluene (BHT). Synthetic antioxidants have negative effects on the body when compared to natural antioxidants, including those that can cause liver damage and more severe can cause death because of their carcinogenic nature [4].

While natural antioxidants are antioxidant compounds obtained through the extraction of natural ingredients [5]. Because at this time, antioxidant compounds derived from natural ingredients have received great attention both in the food and medical fields because their use is much safer when compared to synthetic antioxidants. Natural antioxidants, in general, can usually be found in plants such as vegetables, seeds, stems, and fruits.

One of the sources of natural antioxidants can be obtained from the wood plant and on the rhizome of red ginger because these two plants have several bioactive compounds that have potential as antioxidants. Secang wood contains several phytochemical compounds including xanthones, coumarins, chalcones, flavones, and brazilin. The red dye contained in sappan wood is known as a brazilin group compound where brazilin is the main active compound that belongs to the flavonoid group. Brazilin is also an antioxidant compound that has catechol in its chemical structure that can protect the body from poisoning caused by free radicals. While the

red ginger rhizome contains bioactive compounds in the form of gingerols which have antioxidant, antibacterial, anti-inflammatory, anticarcinogenic, antimutagenic, and antitumor activities.

In this study, testing the antioxidant activity of ethanolic extract of secang wood, red ginger rhizome ethanol extract, and the combination of ethanol extract of secang wood and red ginger rhizome (F1, F2, and F3) was carried out using the DPPH method. In the antioxidant activity test, DPPH acts as a free radical compound that will react with antioxidant compounds so that DPPH will turn into *diphenyl-2-picrylhydrazyl* non-radical. The increase in *diphenyl-2-picrylhydrazyl was* characterized by a color change from purple to pink or pale yellow which could be observed using UV-Vis spectrophotometry so that the free radical scavenging activity in the sample could be determined [6]. Based on the description above, the two types of plants must develop their potential and beneficial properties by testing the antioxidant activity of ethanol extract of secang wood, ethanol extract of red ginger rhizome, and a combination ethanol extract of secang wood and red ginger rhizome using the DPPH method.

1. METHODS

This research belongs to the type of experimental research, which is to find the optimal antioxidant activity of the ethanol extract of secang wood (*Caesalpinia sappan L.*) and red ginger rhizome.

1.1. Sample Preparation

5 kg of fresh sappan wood and 4 kg of red ginger rhizome were cleaned and cut into small pieces, then dried at room temperature for 3 days. After that, it is mashed in a blender, until a fine powder of secang wood and red ginger is obtained. The obtained powder will then be extracted by the maceration method.

1.2. Extraction of Secang Wood and Red Ginger Rhizome

The method used was maceration. Secang wood and red ginger rhizome that has been powdered then macerated in 5 L 96% ethanol then allowed to stand for 3 days and placed in a dark place. The obtained macerate was accommodated, then re-macerated 3 times. Then the macerate is evaporated using a rotary evaporator. And the extraction results are then weighed and the % yield is calculated. Furthermore, the process is carried out freeze dry.

1.3. Total Phenolic Content Test

Determination of total phenolic content was carried out by spectrophotometric method using Folin-Ciocalteu reagent. As much as 10 mg of secang wood and red ginger ethanol extract were dissolved to a volume of 10 ml with a mixture of ethanol: aqua dest (1:1). The extract solution was taken 0.3 ml and added with 10% Folin Ciocelteau reagent and allowed to stand for 3 minutes. Each solution was added 1.2 ml of 7.5% Na_2CO_3 then vortexed for 3 seconds and incubated for 30 minutes at room temperature. The absorbance was read by UV-vis spectroscopy at 760 nm. The absorbance of the sample was interpolated into a linear regression equation on the standard curve for gallic acid concentrations of 10, 20, 30, 40, 50 ppm.

1.4. Antioxidant Activity Test

Determination of total phenolic content was carried out by spectrophotometric method using Folin-Ciocalteu reagent. As much as 10 mg of secang wood and red ginger ethanol extract were dissolved to a volume of 10 ml with a mixture of ethanol: aqua dest (1:1). The extract solution was taken 0.3 ml and added with 10% Folin Ciocelteau reagent and allowed to stand for 3 minutes. Each solution was added 1.2 ml of 7.5% Na_2CO_3 then vortexed for 3 seconds and incubated for 30 minutes at room temperature. The absorbance was read by UV-vis spectroscopy at 760 nm. The absorbance of the sample was interpolated into a linear regression equation on the standard curve for gallic acid concentrations of 10, 20, 30, 40, 50 ppm

1.4.1. Preparation of DPPH Solution DPPH

The solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol pa to obtain a DPPH solution with a concentration of 0,004%.

1.4.2. Optimization of DPPH Wavelength The DPPH

The solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol pa to obtain a DPPH solution with a concentration of 0,004%.

1.4.3. Control Absorbance Measurement

To measure the absorbance of the blank, it was done by adding 2 mL of methanol pa to 2 mL of 0.004 % DPPH solution. Then shaken until homogeneous and allowed to stand for 30 minutes in a dark room. Then the absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer to obtain the absorbance value of the blank.

1.4.4. Testing the Antioxidant Activity of the Ethanol Extract of Secang Wood and Red Ginger Rhizome

The testing phase of the antioxidant activity of the ethanol extract of Secang wood and red ginger rhizome was carried out in several stages, namely:

1.4.4.1. Making test solutions with various concentrations of 10,25,50,75 and 100 ppm for ethanol extract of sappanwood and ethanolic extract of red ginger rhizome and F1 (1:1 ratio)

1.4.4.2. Make a test solution with a concentration variation of 9,27,54,81 and 108 ppm for F2 (1:2) and F3 (2:1 ratio)

1.4.4.3. Make a vitamin C test solution as a comparison with variations in concentrations of 5,10,15,20 and 25 ppm.

1.4.4.4. The test solution was pipetted as much as 2 mL and transferred into a vial bottle that had been wrapped in aluminum foil and added 2 mL of DPPH solution, then the solution was shaken and incubated for 30 minutes. Then the absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 514,5 nm. Then the % inhibition value and IC_{50} value are determined. The % inhibition value can be determined by the following equation:

$$\%\ inhibition = \frac{Absorbance\ blank - sample\ absorbance}{Absorbance\ blank} X\ 100 \qquad (1)$$

The % inhibition value is then made a linear equation curve and the obtained equation is used to calculate the IC_{50} value. The IC_{50} value is obtained by replacing y in the linear equation with a value of 50 and the x obtained is the $ICvalue_{50}$.

2. RESULT AND DISCUSSION

2.1. Extraction Results of Secang Wood and Red Ginger Rhizome

The purpose of extracting natural ingredients is to extract the chemical components contained in a sample. The maceration method was chosen because the process is easy and does not use high temperatures in the work process which may damage the chemical compounds contained in the sample which may potentially have antioxidant activity.

The solvent used in the extraction with the maceration method is ethanol 96%. The use of ethanol as a solvent, because ethanol is a universal solvent that can attract most components of chemical compounds in plants so that it can dissolve a polar component. Polar solvents tend to attract polar compounds and vice versa. In addition, ethanol also has the advantage that it is not harmful to the environment.

Then extracted from the wooden cup and red ginger rhizome is filtered and then evaporated by using *a rotary* evaporator aims to thicken the extract so that an ethanol extract of secang wood and red ginger rhizome is obtained which is then weighed and the % yield is calculated. The % yield of the ethanol extract of secang wood was 10,62116% and the % yield of the red ginger rhizome ethanol extract was 13,48645%.

2.2. Total Phenolic Content Test Result

A total phenolic test on an ethanol extract of secang wood and red ginger rhizome was carried out using the Folin-Ciocalteu method. The principle of this method is based on the reducing power of the phenolic hydroxy group which reacts with Folin-Ciocalteu reagent and gallic acid is used as standard. In determining the levels of phenolic compounds used is gallic acid is a standard solution because gallic acid is a comparison compound and is one of the phenolic acids found in plants and is often used to determine phenol in plants through reagents. Folin – Ciocalteu and is a derivative of hydroxybenzoic acid which is classified as a simple phenolic acid and as a standard with stable and pure substance availability.

Table 1. Standart Absorbance Data For gallic Acid

Concentration (ppm)	Absorbance	Linier Regression
10 22 30 40 50	0.1897 0.3447 0.4810 0.6627 0.7957	y = 0.153x + 0.0357 R = 0.9981

Table 2. Data Of Total Phenolic Content

Sample	Extract Weight (mg)	Dilution factor	Absorbance	Total phenolic content (%)
Sappan wood	10	100	0,1983	10,67
Red Ginger Rhizome	10	10	0,4256	2,53

The presence of an aromatic core in phenolic compounds can reduce the phosphomolybdate phosphotung state to molybdenum tungsten. Phenolic compounds only react with the Folin-Ciocalteu reagent in

an alkaline environment so that proton dissociation occurs in phenolic compounds into phenolic ions [7]. The results of the absorbance measurement of gallic acid standards are presented in table 1.

Based on table 2, it is known that the ethanol extract of Secang wood has a total phenol content of 10.67% while the red ginger rhizome ethanol extract has a total phenol content value of 2.55%.

2.3. Antioxidant Activity Test Results

Activity test of ethanol extract of Secang wood and red ginger rhizome was carried out using the DPPH method and using a UV-Vis Spectrophotometer instrument at a wavelength of 514,5 nm. The DPPH method was chosen because the DPPH method is a fast and simple method. The principle of the DPPH method is that there is a decrease in the intensity of the absorbance value of the DPPH solution which is directly proportional to the increase in the concentration of antioxidant compounds which can be called IC_{50} or *Inhibition Concentration* 50.

Table 3. Data Of Anioxidant Activity Test Result

Sample	concentration (ppm)	Absorbance	% inhibition (%)	Linier Regression
Blank	-	0,9690	-	-
Secang	10 25 50 75 100	0,8414 0,6782 0,5331 0,2934 0,1709	13,1687 30,0137 44,9807 69,7246 82,3583	y = 0.7699x + 8.0156 R^2 = 0.9878
Ginger	10 25 50 75 100	0,9290 0,8747 0,8187 0,7684 0,7144	4,1281 9,7324 15,5150 20,7056 26,2773	y = 0.2383x + 2.878 R^2 = 0.9908
F1	10 25 50 75 100	0,9382 0,8754 0,7344 0,6546 0,5272	3,1785 9,6594 24,2105 32,4458 45,5934	y = 0.4675x - 1.2929 R^2 = 0.9941
F2	9 27 54 81 108	0,9367 0,8787 0,7771 0,6903 0,5991	3,3333 9,3189 19,8039 28,7616 38,1734	y = 0.3537x + 0.1434 R^2 = 0.9994
F3	9 27 54 81 108	0,9314 0,8388 0,6852 0,5377 0,3829	3,8803 13,4365 29,2879 44,5098 60,4850	y = 0.5731x - 1.6564 R^2 = 0.9998
Vitamin C	5 10 15 20 25	0,9640 0,7584 0,6667 0,5293 0,3673	0,5172 21,7344 31,2004 45,3813 62,1001	y = 2.9363x - 11.857 R^2 = 0.9878

In the antioxidant activity test, the ethanol extract of secang wood and red ginger rhizome has used a concentration of 10, 25,50,75, and 100 ppm for ethanol extract of sappan wood and ethanolic extract of red ginger rhizome and F1 (ratio 1:1) and concentrations of 9,27,54,81 and 108 ppm for F2 (comparison 1:2) and F3 (comparison 2:1).

Each concentration was added with 0.004% DPPH solution and incubated for 30 minutes so that the sample could reduce free radicals optimally. Then measurements were made using UV-Vis spectrophotometry to determine the absorbance value of each of these concentrations and calculate the % attenuation or % inhibition and the $ICvalue_{50}$.

Based on table 3, shows that the concentration of the test solution affects the absorbance value. According to Gordon (1990) which states that the amount of an added concentration of antioxidants can affect the rate of oxidation. The greater the concentration of the sample extract, the absorbance of the sample decreases, and the percentage (%) of inhibition increases. DPPH free radicals will be captured by antioxidant compounds which will release hydrogen radicals, thus forming reduced DPPH-H. The antioxidant capacity is determined by the amounts of hydrogen donors of a substance. The interaction of the sample which acts as an antioxidant compound will neutralize free radicals from DPPH. The neutralization reaction can cause a change in the color of the DPPH solution from purple to yellow because the free electrons from the unpaired DPPH become paired.

Table 4. IC_{50} Value

Sample	IC_{50}	Antioxidant Activity Level ($ICValue_{50}$) With DPPH Method			
		Very Strong (< 50)	Strong (50-100)	Medium (101-150)	Weak (> 150)
Secang Wood	54,5323		√		
Red Ginger Rhizome	197,7423				√
F1	109,7174			√	
F2	140,9573			√	
F3	90,1351		√		
Vitamin C	21,0663	√			

The value of % attenuation or % inhibition is used to determine the value of IC_{50}. The $IC_{50\ value}$ can be obtained from the results of the linear regression equation with the x-axis as the sample concentration and the y-axis as the % attenuation or % inhibition. The linear regression curve generated from the sample can be seen as follows:

From the linear equation above, it is then used to determine the IC_{50} value of each sample where 50% of the sample concentration can reduce the absorbance of the DPPH solution.

Vitamin C is used as a comparison because vitamin C is a natural antioxidant compound that has a very strong antioxidant activity when compared to vitamin E and vitamin A. In addition, vitamin C in its use of safe, practical and can be dissolved in water. The comparison function is to determine whether the test substance can have the same effect as the standard antioxidant source used.

3. CONCLUSION

Based on the results of the research that has been carried out, it shows that the total phenolic ethanol extract of sappan wood is 10.67% while the total phenolic ethanol extract of red ginger is 2.55%. Antioxidant activity test of the ethanol extract of secang wood and red ginger rhizome using the DPPH method, it can be concluded that the ethanol extract of secang wood has an antioxidant activity value or IC50 of 54.5323which is a strong antioxidant. The antioxidant activity value or IC50 of the red ginger rhizome ethanol extract is 197.7423 which is a weak antioxidant. The antioxidant activity values or IC50 of F1, F2, and F3 are 109.7174, 140.9573 which is a medium antioxidant, and 90.1351 which is a strong antioxidant.

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