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Antioxidant Activity Test Combination Sarang Semut Extract (*Myrmecodia Pendans*) And Rosella Flower Extract (*Hibiscus Sabdariffa L.*) With Dpph (1,1-Difenil-2-Pikrilhidrazil) Method

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ABSTRACT: Antioxidants are compounds that can inhibit oxidation by free radicals by reacting with free radicals to form more stable compounds and are less reactive. Sarang semut (Mymercodia pendans) and rosella flower (Hibiscus sabdariffa L.) contain several active ingredients that can act as antioxidants. The purpose of this research was to determine the antioxidant activity combination of sarang semut extract with rosella flower extract against free radical DPPH (1,1-diphenyl-2-picrylhidrazyl). Withdrawal of active compounds from the sarang semut is done by the extraction process soxhletasi method using ethanol solvent. While the withdrawal of active compounds from rosella flowers carried by the digestion method using solvent of water. The combination of sarang semut extract and rosella flower extract is made in a variety of comparison namely 2:0, 2:1, 2:2, 1:2, and 0:2 are made with a concentration of 0.01%, 0.02%, 0.03% ; 0.04% and 0.05%. Then tested for antioxidant activity by DPPH method is UV-Vis spectrophotometry. From the absorbance data was calculated percent antioxidant activity of control expressed by EC_{50} ANAVA then tested statistically to determine differences antioxidant activity. From the results showed that the EC_{50} value in each comparison as follows: 2:0 = 5.4078 μg/ml; 2:1 = 3.2189 μg/ml; 2:2 = 2.7017 μg/ml; 1:2 = 3.3464 μg/ml and 0:2 = 7.5439 μg/ml. So it can be concluded that the combination of sarang semut extract with rosella flower extract at a ratio of 2:2 has the greatest antioxidant activity. The test results showed the price F ANAVA count (202.221)> F table (2.87). At α (0.05) which means that there are significant differences between the antioxidant activity of sarang semut extract, rosella flower extract and the combination of sarang semut extract with rosella flowers extract.

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KEYWORDS: sarang semut, rosella flowers, antioxidant activity, DPPH, EC_{50}

INTRODUCTION

Free radicals are very reactive molecules because they have one or more unpaired electrons, and to their balance, free radicals try to gain electrons from other molecules or release the unpaired electrons (Praptiwi et al, 2006:33)

Antioxidants are molecules that have the ability to slow down or prevent the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one substance to an oxidizing agent. Oxidation reactions can generate free radicals and trigger chain reactions, causing damage to body cells. Antioxidants stop chain reactions by complementing the electron deficiency of free radicals and inhibit other oxidation reactions by themselves being oxidized. Therefore, antioxidants are often reducing agents such as thiol compounds, ascorbic acid, or polyphenols (http://en.wikipedia.org/wiki/Antioxidants).

Sarang semut is one of the epiphytic plants originating from Papua which has traditionally been used by indigenous Papuans to treat various diseases from generation to generation. The results of the research found that the tubers of this plant contain important active compounds such as flavonoids, tocopherols (vitamin E), phenolics and are rich in various minerals. From the content of tocopherols and flavonoids in sarang semut tubers, it is suspected that sarang semut tubers have antioxidant activity (Subroto, 2008: 28-30).

Roselle plant also functions as an antioxidant. High concentrations of antioxidants from roselle plants can inhibit free radicals. The active substances that most play a role in Rosella flower petals include tannins, polyphenols, gossypetin, anthocyanins,

vitamin C and glucoside hibiscin. Anthocyanins are natural pigments that give the red color to the roselle petals, and are antioxidants.

Maryani and Kristiana (2005) stated that rosella flower petals boiled with water are efficacious as a urinary laxative and stimulate the release of bile from the liver (chloretic), lower blood pressure (hypotensive), reduce blood viscosity, and increase intestinal peristalsis. which have been known from the roselle plant include anticonvulsant (antispasmodic), treat intestinal worms (anthelmitic) and as an anti-bacterial.

Suwandi (2012) stated that rosella flower petal extract contains high amounts of vitamin C as well as succinic acid and oxalic acid which are the two dominant organic acids. Rosella flower petal extract also contains higher ascorbic acid than oranges and mangoes.

The withdrawal of the active compounds from the sarang semut, namely tocopherols, flavonoids, and tannins was carried out by the extraction process used the soxhletation method used ethanol as a solvent. Meanwhile, the withdrawal of active compounds from roselle flowers, namely vitamin C, flavonoids and tannins, was carried out by the extraction method of the digestion method used water as a solvent.

To test the presence of antioxidant activity can use the DPPH method. Observation of DPPH radical scavenging can be done by observing the decrease in absorbance. This can occur due to radical reduction by antioxidants (AH) or react with other radical compounds (Yu et al., 2002: 1620).

Based on the problems above, a research was carried out on the antioxidant activity of the combination of sarang semut (Myrmecodia pendans) and roselle flowers (Hibiscus sabdariffa L.), because they both have flavonoids that have antioxidant activity, so they are compared with pure sarang semut to find out the difference, in terms of capacity of free radical scavenging using a combination of ant nest extract and roselle flower extract against DPPH by visible spectrophotometry.

METHODS

Material and Tools

The test materials used were sarang semut and roselle flowers. Chemicals used include 0.1 mM DPPH and ethanol 96%. The tools used include a micropipette, glassware, a vortex mixer and a Shimadzhu 1240 UV-Vis spectrophotometer.

Sarang semut are extracted by the method:

Soxhletasi. 20.0 grams were put into a bag of filter paper arranged in such a way that it could be inserted into a Soxhlet tube. Soxhletation was carried out used 96% ethanol with a volume of 2 times the circulation and at a temperature of 70°-80°C until the solvent was colorless. The extract obtained was concentrated with a vacuum rotary evaporator at a temperature of 65°C until a thick extract was obtained which was considered to have a concentration of 100%.

Roselle flowers are extracted by the method:

Digestion. 20 grams of roselle flower powder plus 150 ml of aquadest, then the simplicia immersion is heated on a water bath at a temperature of 40^0-50^0C for 30 minutes while continuously stirring. The results were filtered used a kola cloth. The filtrate obtained was collected together and then filtered used filter paper. The filtrate obtained was then evaporated using a vacuum rotary evaporator to obtain a thick extract.

Qualitative test of antioxidant activity

Examination of antioxidant compounds by TLC, used a stationary phase of silica gel GF 254 and eluent buthanol : acetyc acid : water (4:1:5), was detected using a DPPH solution. If its look at the stain under UV light, it will form a purple stain. After drying, the plate was sprayed with a DPPH solution. The formation of a yellowish white stain on a purple background indicates the presence of antioxidant compounds.

Quantitative testing of antioxidant activity

Determination of antioxidant activity was carried out by inserting 4.0 ml of 0.1 mM DPPH into a test tube, adding 50.0 µl of sarang semut extract solution (*Myrmecodia pendans*), roselle flower extract (*Hibiscus sabdariffa L.*) or a combination of sarang semut extract with roselle flower extract for each concentration. Then the mixture was homogenized with a vortex for 1 minute and allowed to stand according to the operating time of each test solution. The absorbance of the solution is read at the maximum wavelength. The absorbance of the control solution was also read, namely 0.1 mM DPPH solution.

Data analysis

The absorbance obtained from sarang semut extract (*Myrmecodia pendans*), roselle flower extract (*Hibiscus sabdariffa* L.) and the combination of sarang semut extract and roselle flower extract were compared with their antioxidant activities. Percentage of antioxidant activity can be used the following formula:

$$\% \text{ antioxidant activity} = \frac{\text{Abs Kontrol - Abs Sampel}}{\text{Abs kontrol}} \times 100\%$$

The data on the antioxidant activity used the DPPH method was calculated for the EC_{50} value using linear regression. The EC_{50} values of sarang semut extract, roselle flower extract and the combination of sarang semut extract with roselle flower extract were tested for differences using the ANOVA test used the SPSS 23 method.

RESULT AND DISCUSSION

To take the active compound from the sarang semut (*Myrmecodia pendans*) an extraction process was carried out used a suitable solvent and extraction method. One of the factors that affect the quality of chemical extracts is the extraction method (Depkes RI, 2000: 7). Sarang semut were extracted used the soxhletation method with 96% ethanol as solvent. The soxhletation method is a good method because it use a solvent that is always new, so that continuous extraction occurs with a relatively constant amount of solvent in one circulation. Meanwhile, the extraction of roselle flowers was carried out by the digestion method. Digestion is a maceration method with continuous stirring and a heating temperature of 40° – 50°C for 30 minutes.

After obtaining the thick extract, a preliminary test was carried out on each extract to ensure the compounds contained in the sarang semut extract and roselle flower extract. This preliminary test includes tests for phenolic compounds, polyphenols, flavonoids, tannins, and tocopherols. The tests carried out included color reaction tests and thin layer chromatography (TLC).

After it was proven that the test results contained antioxidant compounds, then the antioxidant activity was measured spectrophotometrically used the DPPH method. The principle is to measure the amount of color reduction that occurs due to radical reduction by antioxidants to form diphenyl-picryl hydrazine (DPPH-H). The color changes that occur are measured by visible spectrophotometer at each maximum wavelength.

To determine the level of color reduction as a result of the presence of antioxidant compounds that can reduce the intensity of the purple color of DPPH, the measurement of the color reaction was carried out at different concentrations of the extract. The higher the concentration of the extract, the greater the reduction, which is indicated by the formation of a yellow color. Due to the high concentration of compounds contained there will be more and cause the greater the antioxidant activity.

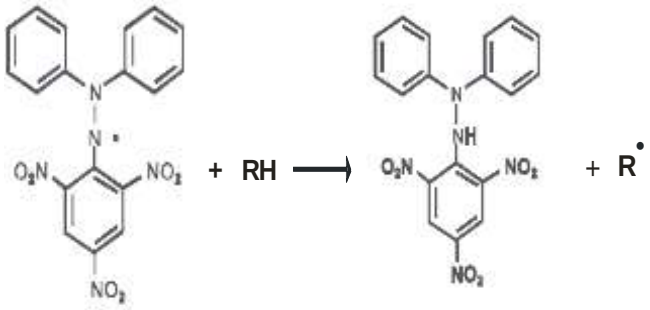

Figure 1. Radical Capture Reaction by DPPH (Molyneux, 2004)

The % antioxidant activity data obtained, calculated the EC_{50} value used a linear regression equation. The EC_{50} value is inversely proportional to the antioxidant ability of a compound contained in the test material. The smaller the EC_{50} value, the greater the antioxidant ability.

Table 1. EC_{50} Value Calculation Result Data

Replication	Value EC_{50} (µg/ml) Combination of Sarang semut Extract : Roselle flower extract				
	2 : 0	2 : 1	2 : 2	1 : 2	0 : 2
1	5,2812	3,6563	2,9185	3,6531	7,9570
2	5,6182	2,9625	2,9083	3,5969	7,9391
3	5,3130	3,3478	2,6894	3,2049	6,8433
4	5,4181	2,8171	2,5173	3,2741	7,1662
5	5,4085	3,3108	2,4848	3,0029	7,8539
Average	5,4078	3,2189	2,7017	3,3464	7,5439

From the average EC_{50} in each combination of sarang semut extract and roselle flower extract, the lowest EC_{50} value was obtained at a ratio of 0:2, which was 7.5439 µg/ml, followed by a 2:0 ratio of 5.4078 µg/ml. , a 1:2 ratio of 3.3464 µg/ml, a 2:1

ratio of 3.2189 µg/ml and a 2:2 ratio of 2.7017µg/ml. This means that at this concentration the test solution can reduce DPPH by 50%.

From the results of the ANOVA test calculations show that there are differences in the concentration groups. This can be seen by looking at the significance value of 0.004 which is smaller than the 0.05 value and the Fcount value of 202.221 which is greater than the Ftable which is 2.87.

Judging from the statistical data obtained, namely sarang semut extract, roselle flower extract and sarang semut extract combined with roselle flower extract in different comparisons, in general there were differences indicated by a significance value smaller than α=5%, which means Ho is rejected or there are differences in antioxidant activity in the extract of sarang semut, roselle flower extract and sarang semut extract combined with roselle flower extract. So that the initial hypothesis which states that there is a difference in antioxidant activity between sarang semut extract, roselle flower extract and the combination of sarang semut extract with roselle flower extract is proven.

CONCLUSION

Based on the results of the study, the following conclusions can be drawn:

1. The EC50 value of the combination of sarang semut extract and roselle flower extract in a ratio of 2:0, 2:1, 2:2, 1:2, and 0:2, respectively, was 5.4078 µg/ml, 3.2189 µg /ml, 2.7017 µg/ml, 3.3464 µg/ml and 7.5439 µg/ml.
2. The optimal EC50 value was obtained from the combination of sarang semut extract and roselle flower extract with a ratio of 2:2, which was 2.7017 µg/ml.
3. There are differences in antioxidant activity between sarang semut extract, roselle flower extract and the combination of sarang semut extract and roselle flower extract.

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