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FORMULATION AND ANTIOXIDANT ACTIVITY TEST OF EXTRACT AND SERUM ANTIOXIDANT CAPACITY TEST OF ROSELLE CALYX (*HIBISCUS SABDARIFFA* L.) WITH DPPH (2,2-DIPHENYL-1PIKRILHIDRAZYL)

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Abstract

In everyday life, the skin cannot be avoided from exposure to UV rays, air pollution, radiation from electronic goods, to the use of chemicals that can trigger premature aging due to free radicals. Free radicals are chemical compounds that are unstable and highly reactive because they contain one or more unpaired electrons. The effects of free radicals can be prevented by using antioxidants. Antioxidants are compounds that can prevent cell damage caused by free radicals by inhibiting oxidative mechanisms. Roselle calyx (Hibiscus sabdariffa L.) is a plant that contains anthocyanins and has antioxidant activity. This study aims to determine whether there are differences in antioxidant activity at equivalent concentrations of extract and roselle calyx serum preparations using the DPPH (2,2-Diphenyl-1-pikrilhidrazyl) UV-Vis spectrophotometric method. Roselle calyx extract was used as an active ingredient in the manufacture of serum with a concentration of 10% and tested for antioxidant activity with an equivalent concentration of pure roselle calyx extract at a wavelength of 515 nm. The IC50 value can be determined using the obtained linear regression equation. Ascorbic acid was used as a comparison in this study. From the research that has been done, it was found that roselle calyx extract and serum have very strong antioxidant activity with IC50 values of 11.480 and 9.743 ppm respectively.

Keywords: Antioxidants, DPPH, Roselle Calyx, Serum.

INTRODUCTION

The skin is an organ that covers all parts of the human body and has protection against external influences. In everyday life, the skin cannot be spared from exposure to ultraviolet light, air pollution, radiation from electronic products, and the use of chemicals that can trigger premature aging due to free radicals, which is characterized by loss of skin elasticity and decreased collagen production in the skin and makes the skin becomes dry (1). Free radicals are unstable and highly reactive compounds because they contain one or more unpaired electrons (2). The effects of free radicals can be prevented by using antioxidants (3).

Roselle (Hibiscus sabdariffa L.) is a plant that contains a lot of antioxidants. Roselle calyx extract has the highest antioxidant activity value when compared to other plant parts. (4). The content of roselle calyx that act as antioxidants are anthocyanins which can inhibit free radical oxidation in the body and have soluble properties in polar solutions. Anthocyanins are a class of flavonoid compounds which are broadly divided into plant polyphenols and act as natural antioxidants capable of counteracting free radicals (5). Anthocyanins have the largest content when compared to beta carotene, alpha-tocopherol and ascorbic acid (6). The body does not have a high oxidative defense system, so if there is exposure to excess radicals it requires external antioxidants (7). Antioxidant intake can be obtained orally or topically, but the use of roselle as a skin protector topically is still rarely used by the public. Lately one type of

cosmetic preparation that is being developed is serum. Serum is a preparation that contains a high concentration of active substances and low viscosity so that it is light when applied to the skin, has a transparent or semi-transparent color with a higher active ingredient content than other topical preparations (8).

Based on research roselle calyx extract using the soxhletation method of extraction has a strong antioxidant activity with an IC50 value of 67.3 ppm (9). Roselle petal extract which is used as an active ingredient in preparations with a concentration of 0.5% has a relatively strong antioxidant activity (10). Antioxidant levels of roselle flower calyx can be reduced when subjected to heating and drying processes. Based on the description above, the antioxidant activity of extracts and serum preparations of rosella calyx (Hibiscus sabdariffa L.) will be tested using the DPPH (2,2-diphenyl-1-pikrilhidrazyl) method (11).

MATERIALS AND METHODS

Materials

The main material used in this study was rosella flower calyx obtained from the Kertalangu area, Denpasar City, Bali. Roselle calyx extract, pro-analytical methanol (supelco), 96% ethanol, ascorbic acid, xanthan gum, glycerin, potassium sorbate, distilled water, and DPPH (2,2-diphenyl-1-picrylhydrazyl).

Methods

Plant Determination. Rosella flowers used for determination are intact plants. The determination was carried out at the Bedugul "Eka Karya" Botanical Garden Characterization Laboratory, Bali-BRIN (National Research and Innovation Agency).

Simple Setup. Rosella flower calyx are collected from farmers in the village of Kertalangu, rosella flower calyx are washed in running water until clean, then dried in the sun and covered with a black cloth, after drying they are then mashed in a blender.

Production of Rosella Petal Extract. Extraction of 500 grams of roselle flower calyx was carried out using the ultrasonic method at room temperature (300C) with the help of stirring using ethanol:water (50:50) with a ratio of ingredients and solvent (1:10). Then filtered with a Buchner funnel, the filtrate obtained was then evaporated with a rotary evaporator until a thick extract was obtained. The extract obtained was weighed and the yield was calculated.

$$\% \text{ yield} = \frac{\textit{Jumlah ekstrak yang dihasilkan}}{\textit{Jumlah bahan sebelum di ekstrak}} \times 100\%$$

Rosella Flower Petal Serum Formulation. In the formulation of roselle calyx serum preparations, there are several additional ingredients used, namely xanthan gum, glycerin, potassium sorbate, and aquadest. Serum preparation formulas are presented in Table 1.

Table 1: Serum Formulas

Material Name	Concentration (%b/v)
Roselle petal extract	10
Xanthan gum	0.5
Glycerin	10
Potassium sorbate	0.1
Aquadest	ad 100

Procedure for Making Roselle Petal Serum. Prepare the tools and materials needed. Then weigh the ingredients used according to the calculation. The serum formulation begins by mixing xanthan gum and water 20 times the weight of xanthan gum and then stirring it to form mucilago. Then added glycerin little by little while continuing to stir. Grind and add potassium sorbate, add roselle petal extract into mucilago and stir until homogeneous. Then added distilled water up to 100 ml, stirred until homogeneous, then stored in a container.

Antioxidant Activity Testing

Preparation of 500 ppm DPPH Main Standard Solution. Dissolve up to 25 mg of DPPH powder in a 50 ml volumetric flask with 50 ml of methanol pa to obtain a concentration of 100 ppm.

Preparation of 40 ppm DPPH Working Standard Solution. Put 20 mL of 500 ppm DPPh mother standard solution into a 250 mL volumetric flask, dissolve it to the mark with methanol, and get a DPPH working standard concentration of 40 ppm.

Preparation of Extract Sample Solution. A master sample solution with a concentration of 1000 ppm was prepared by weighing 50 mg of roselle calyx extract and dissolved with methanol pa and homogenized and then the volume was made up to 50 ml. Furthermore, variations in the concentration of 10 ppm, 30 ppm, 50 ppm, 70 ppm, and 90 ppm were made. Pipette 0.1 ml, 0.3 ml, 0.5 ml, 0.7 ml and 0.9 ml from the mother liquor of 1000 ppm, each put into a 10 ml volumetric flask, add methanol pa to the mark then shaken until homogeneous.

Preparation of Serum Sample Solution. A mother sample solution with a concentration of 10,000 ppm was prepared by weighing 500 mg of roselle calyx serum and dissolved with methanol pa and homogenized and then the volume was made up to 50 ml. Furthermore, variations in the concentration of 100 ppm, 300 ppm, 500 ppm, 700 ppm, and 900 ppm were made. From the main solution of 10,000 ppm, 0.1 ml, 0.3 ml, 0.5 ml, 0.7 ml, and 0.9 ml were pipetted each into a 10 ml volumetric flask, methanol was added until the mark was marked. shaken until homogeneous.

Preparation of Ascorbic Acid Sample Solution as a Comparison. A master sample solution with a concentration of 100 ppm was prepared by weighing as much as 5 mg of ascorbic acid and dissolved with methanol pa and homogenized and then the volume was made up to 50 ml. Furthermore, variations in the concentration of 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm were made. Pipette 0.4 ml, 0.6 ml, 0.8 ml, 1 ml, and 1.2 ml from the mother liquor of 1000 ppm in a 10 ml volumetric flask, add methanol pa to the mark, then shake until homogeneous.

Determination of the Maximum Wavelength of the DPPH Standard Solution. 40 ppm DPPH working standard solution was pipetted as much as 4 ml into a cuvette, then the absorption spectrum was observed at a wavelength of 400-800 nm with a UV-Vis spectrophotometer. For the blank solution, 4 ml of methanol pa was used. From the absorption curve, the maximum wavelength can be determined.

Measurement of the Antioxidant Power of Extract Samples. Pipette the 1 ml test sample solution in different concentrations (10 ppm, 30 ppm, 50 ppm, 70 ppm, and 90 ppm) working standard solution plus 4 ml of 40 ppm DPPH, set aside for 30 minutes, then observe the absorbance at the maximum wavelength. Repeat three times.

Measurement of the Anti-Oscdian Power of Serum Samples. Pipette 1 ml of the test sample solution at different concentrations (100 ppm, 300 ppm, 500 ppm, 700 ppm, and 900 ppm) plus 4 ml of DPPH 40 ppm working standard solution, let stand for 30 minutes, then observe the absorbance at the maximum wavelength . Repeat three times.

Measurement of the antioxidant power of ascorbic acid as a comparison. Pipette 1 ml of test sample solution at different concentrations (4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm) working standard solution plus 4 ml of 40 ppm DPPH, set aside for 30 minutes, then observe the absorbance at the maximum wavelength. Repeat three times.

IC50 Value Determination. The parameter used in the determination of antioxidant activity using the DPPH method is IC50, namely the concentration of the sample required to scavenge 50% of the DPPH radicals. The smaller the IC50 value, the higher the antioxidant activity of a sample (12). The IC50 value was obtained from the linear regression equation between the percentage of attenuation and the concentration of free radical inhibition. Analysis of antioxidant testing using the DPPH method was carried out to measure the absorbance using a UV-Vis spectrophotometer at a wavelength of 515 nm. Then the absorbance obtained is calculated by the formula:

$$\%\ peredaman = \frac{absorbansi\ kontrol - absorbansi\ sampel}{absorbansi\ kontrol} x100\%$$

After getting the percentage of attenuation of each concentration. Then a regression curve is made, so that the equation y = bx + a is obtained. This equation is used to calculate the IC50 value of each sample.

Data Analysis.

The percentage attenuation value obtained was then subjected to statistical analysis using SPSS version 26. The normality test was performed using the Shapiro-Wilk test because the number of samples was <50. If the sig value is obtained. > 0.05, the data can be said to be normally distributed. Furthermore, if normally distributed data is obtained, an unpaired T test is performed. However, if the data is not normally distributed, the statistical test is carried out using the Mann-Whitney test.

RESULTS AND DISCUSSION

Determination Test Results

In this study, rosella (Hibsicus sabdariffa L.) was used which was determined to be intact and fresh. Identification was carried out at the Bali-BRIN "Eka Karya" Botanical Garden Characterization Laboratory (Badan Riset dan Inovasi Nasional). Roselle plants belong to the Hibiscus clan and the Malvaceae tribe.

Extract Yield Value Calculation

The extraction was carried out using the ultrasonic method because this method can help obtain ± 12 times more anthocyanin levels compared to using the conventional method (maceration). The ultrasonic method is an extraction method using a tool (elmasonic) to extract anthocyanins by utilizing ultrasonic waves (13). Roselle calyx powder was extracted using ethanol:water (50:50) with a ratio of the amount of ingredients and solvent (1:10). The weight of the extract obtained was 109.5 grams.

The weight of roselle calyx powder = 500 grams, Roselle calyx extract weight = 109.5 grams

$$\% \text{ yield} = \frac{109{,}5\ gram}{500\ gram} \times 100\% = 21.9\%$$

The yield calculation was carried out to determine the ratio of the amount of extract obtained to the weight of the simplicia material and to find out the amount of bioactive compounds contained in the extracted material (14).

DPPH Maximum Wavelength Determination Result.

The maximum wavelength is determined by measuring the absorption at a wavelength of 400 – 800 nm using a 40 ppm DPPH solution. Based on the results of measurements of the maximum wavelength of the DPPH working standard solution with a concentration of 40 ppm with a UV-Vis spectrophotometer, namely 515 nm.
The DPPH radical has unpaired electrons, complementary colors and can produce maximum absorption at a wavelength of 515-520 nm (15).

Antioxidant Activity Test Results

The Results of Testing the Antioxidant Activity of the Samples

Testing the antioxidant activity of the three samples, namely roselle calyx extract, roselle calyx serum and ascorbic acid used the DPPH method by making five variations in the concentration of each sample. From the absorbance results obtained, the percentage of damping is calculated using the following formula:

$$\% \text{ attenuation} = \frac{absorbansi\ kontrol - absorbansi\ sampel}{absorbansi\ kontrol} \times 100\%$$

Information:

The absorbance of the control was measured by dissolving 1 ml of methanol pa with 4 ml of 40 ppm DPPH solution and the absorbance of the samples was measured by dissolving 1 ml of each concentration variation with 4 ml of 40 ppm DPPH solution.

Table 2: Percentage of Soaking of Rosella Petal Extract

Extract Concentration (ppm)	abs Control	Average abs. Sample	Attenuation (%)
2	0.828	0.702	15,257
6	0.828	0.595	28,180
10	0.828	0.451	45,571
14	0.828	0.326	60,628
18	0.828	0.224	72,987

Table 3: Percentage of Reduced Serum of Rosella Calyx

Extract Equivalent Concentration (ppm)	abs Control	Average abs. Sample	Attenuation (%)
2	0.847	0.847	32,349
6	0.847	0.573	41,086
10	0.847	0.499	49,744
14	0.847	0.425	59,897
18	0.847	0.339	69,933

Table 4: Ascorbic Acid Reduction Percentage

Ascorbic Acid Concentration (ppm)	abs Control	Average abs. Sample	Attenuation (%)
0.8	0.816	0.687	15,849
1,2	0.816	0.586	28,186
1,6	0.816	0.463	43,218
2	0.816	0.370	54,656
2,4	0.816	0.291	64,297

In the table above the concentration used is the concentration of the final sample solution after reacting with DPPH. Antioxidant activity test in this study used ascorbic acid as a comparison because it is included in the class of secondary antioxidants which have high antioxidant activity and are able to reduce free radicals from outside and prevent chain reactions from occurring. Ascorbic acid has a free hydroxy group which acts as an antidote to free radicals and has a polyhydroxy group which can increase antioxidant activity (16). Based on testing the antioxidant activity of five concentrations in each sample at a wavelength of 515 nm, it shows that each concentration undergoes a change in absorbance, that is, the higher the concentration of the test sample solution, the lower the absorbance value.

IC50 Value Calculation

From the average percentage of the three samples in each variation of the concentration of the test sample solution is plotted to form a linear regression curve so that the regression equation is obtained as follows:

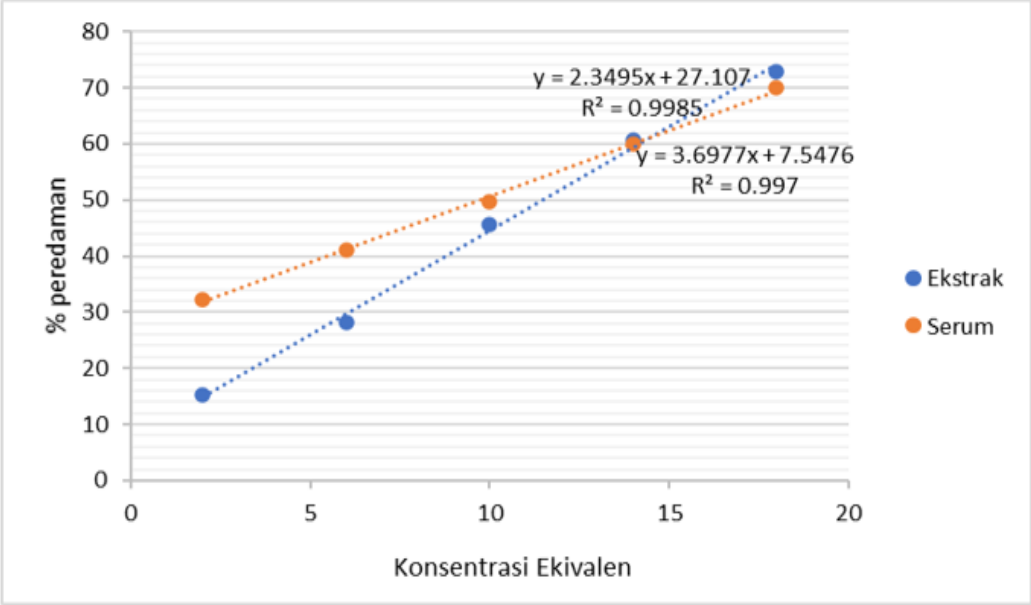

Figure 1: Curve of Relationship between Extract and Serum Concentrations of Rosella Calyx to the Attenuation Percentage

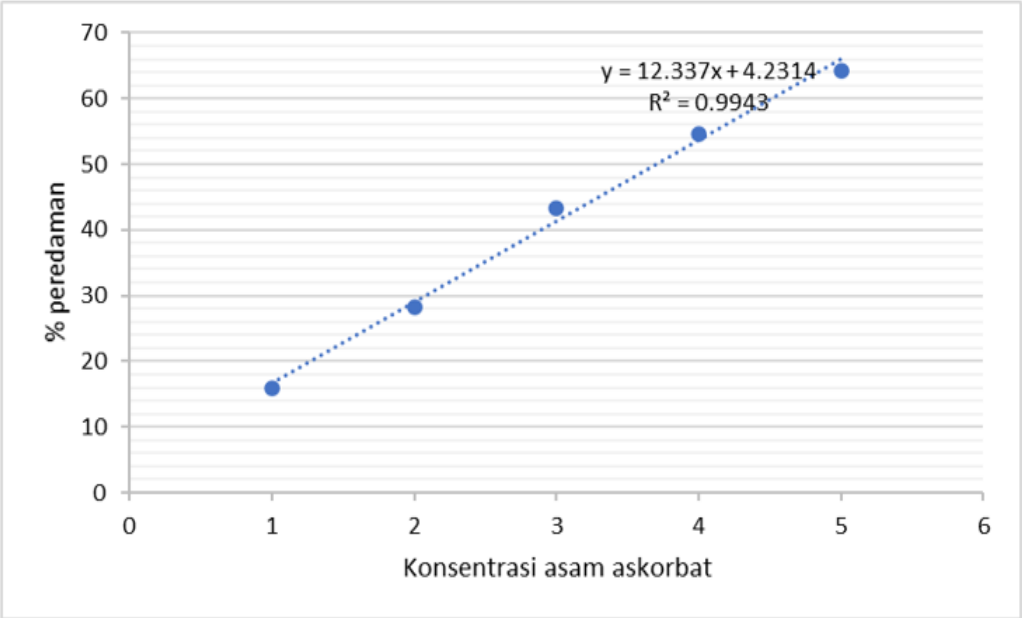

Figure 2: Relationship Curve of Ascorbic Acid Concentration to the Attenuation Percentage

From the curve of the relationship between the concentration of the test solution and the percentage of damping, a linear regression equation was obtained from the extract, serum, and ascorbic acid, respectively y = 3.6977x + 7.5476 with R2 = 0.997; y = 2.3495x + 27.107 with R2 = 0.9985; and y = 12.337x + 4.2314 with R2 = 0.9943.

From this equation, the IC50 value is calculated using the formula y = bx + a, by replacing y = 50. Specifically, a compound is said to be a very strong antioxidant if the IC50 value is less than 50 ppm, it is classified as strong for an IC50 value of 50-100 ppm. classified as moderate if the value is 100-150 ppm, classified as weak if the value is 150-200 ppm, and very weak if the value is more than 200 ppm (17).

The results showed that roselle calyx extract, roselle calyx serum, and ascorbic acid had very strong antioxidant activity, with IC50 values of 11,480 ppm respectively; 9,743 ppm; and 3.709 ppm. These results are different from the research conducted by Ingrid et al. (2018) said that roselle petal extract has a relatively strong antioxidant activity with an IC50 value of 67.3 ppm.

This can be influenced by several factors such as the method used during extraction, the environment where it grows and the time of harvest of the plant so that it is suspected that there are differences in the content of secondary metabolites contained in roselle flower calyx.

The IC50 value of roselle calyx serum preparation was greater than the IC50 value of roselle calyx extract, 10% or 10 grams in 100 ml of preparation. In addition, the use of xanthan gum which functions as a thickener in the manufacture of preparations is known to increase antioxidant activity because it is a polysaccharide consisting of heteropolysaccharide units which contain a lot of hydroxy groups so that they can produce anti-oxidative effects (18).

Data Analysis

Statistical analysis was carried out on the percentage of attenuation of roselle petal extract and serum samples using an unpaired T test because the data tested has a comparative hypothesis type, namely comparing the results of the percentage attenuation values between the two samples consisting of two unpaired groups. The results of data analysis are presented in table 5 .

Table 5: Unpaired T Test Results

Levene's Test		t-test		
F	Sig.	t	df	Sig. (2-tailed)
0.350	0.571	-1.314	8	0.255

The results show a significant value on the Levene's Test > 0.05 which is equal to 0.571, so the variance of the data is assumed to be the same/homogeneous so as to obtain the research hypothesis seen from the Sig value. (2-tailed) Equal variances assumed >0.05, which is equal to 0.225, it can be concluded that the results showed that there was no significant difference in the attenuation value of roselle petal extract and serum at equivalent concentrations.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that there is no significant difference between extract and serum of roselle calyx (Hibiscus sabdariffa L.) in scavenging free radicals at equivalent concentrations.

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