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Research Article

Antioxidant Activity Test of Combination Extract from Roselle Flower (Hibiscus sabdariffa L.) and Basil Leaf (Ocimum sanctum L.) in Vitro Using the DPPH Method

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Abstract: Antioxidants are substances that can provide protection against endogenous and exogenous oxidative stress by capturing free radicals. Antioxidants are molecules that can inhibit the oxidation of other molecules. Antioxidants are generally phenolic or flavonoid compounds that are spread throughout the medicinal plant parts, including rosela and basil. Roselle petals contain anthocyanins that act as antioxidants by reducing free radicals that attack body molecules. Flavonoids from basil leaves have antioxidant effects by scavenging free radicals. This study aims to determine the antioxidant activity of a combination of rosela flower extract and basil leaf extract. The stages of the research method include 500 g of roselle flowers and basil leaves each extracted using 70% ethanol by maceration method and the combination of roselle flower extract and basil leaf extract was tested for antioxidant activity in vitro using 1,1-diphenyl-2-picrylhydrazine (DPPH) receptor. The results showed that the yield of roselle flower extract was 13.28% and basil leaf extract was 12.52%. The results of the in vitro antioxidant activity test showed that the combination of roselle flower and basil leaf extracts with a ratio of 1:1, 1:2, and 2:1 had strong antioxidant activity with IC50 values of 70.33 µg/ml, 56.92 µg/ml, and 57.90 µg/ml, respectively.

Keywords: Antioxidant Activity; Basil Leaf; Diphenyl Picrylhydrazyl; Flavonoids; Roselle Flower.

1. Introduction

The body needs antioxidants to reduce reactive free radicals to prevent oxidative stress that causes various degenerative diseases. Among degenerative diseases, the most prevalent are DM, dyslipidemia, cardiovascular disease, and cerebrovascular disease [1]. Antioxidants are substances that can provide endogenous protection and exogenous oxidative stress by capturing free radicals. Antioxidants are molecules that can inhibit the oxidation of other molecules. Antioxidant sources can come from plants around us [2].

Rosela (*Hibiscus sabdariffa* L.) is one of the plants as a source of antioxidants. People utilize rosela flowers as tea called red tea. Rosela is a plant from the hibiscus family. Its flowers and seeds can be utilized as raw materials for health drinks. Antioxidants contained in rosela flowers can be utilized by processing them into a product [3]. Flavonoids in rosela flowers are useful for preventing cancer, especially those caused by free radicals, such as gastric cancer and leukemia. Based on the research of Iqbal et al. (2023) stated that 70% ethanol extract of red rosela petals has antioxidant activity, and based on Amriani and Tuahatu (2021) stated that ethanol: water extract of rosella petals extracted by maceration method produces antioxidant activity of 43µg/mL.

The basil plant (*Ocimum sanctum* L.) often called sweet basil belongs to the Lamiaceae family, a plant native to the Indo-Malay region. In general, basil has antibacterial, antifungal and antioxidant activities. Basil leaves are known to have high phenolic content. This phenolic compound has been known to have the ability as an antioxidant so that it can be an alternative and natural antioxidant candidate in maintaining the health of the human body [4]. The results of research by Iqbal et al, (2024) showed that 70% ethanol extract of basil leaves has strong

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antioxidant activity against 1,1-diphenyl-2-picrylhydrazine DPPH, with an IC50 value of 31.63 μg/ml.

Based on the description above, it can be seen that there is a synergy between roselle flowers and basil leaves, which has antioxidant activity, but so far the test is still in a single extract. Therefore, the thought arose to combine roselle flower and basil leaf extracts, expected to have better antioxidant activity. The objective to be achieved in this study is to determine the antioxidant activity of a combination of roselle flower extract and basil leaf extract tested in-vitro using the DPPH method.

2. Preliminaries or Related Work or Literature Review

2.1. Antioxidants and Free Radicals

Free radicals are reactive molecules with one or more unpaired electrons that can cause oxidative damage to cells and tissues. Oxidative stress resulting from the accumulation of free radicals has been linked to various degenerative diseases such as cancer, atherosclerosis, and premature aging [5]. Antioxidants function by neutralizing free radicals through electron or hydrogen donation mechanisms, thereby inhibiting the oxidation of vital biomolecules.

2.2. In Vitro Antioxidant Assays

One of the most widely used methods for measuring antioxidant activity in vitro is the DPPH (1,1-diphenyl-2-picrylhydrazine) radical scavenging assay. DPPH is a stable free radical that appears purple in solution and changes to yellow upon reduction by an antioxidant. This color change is quantitatively measured at a wavelength of 515–517 nm using a UV-Vis spectrophotometer [6]. Antioxidant activity is often expressed in terms of IC_{50}, the concentration of the test substance that scavenges 50% of DPPH radicals. A lower IC_{50} value indicates stronger antioxidant activity [7].

2.3. Roselle Flower (*Hibiscus sabdariffa* L.)

Roselle is a widely known medicinal plant, and its calyces are rich in phenolic compounds such as anthocyanins, flavonoids, and ascorbic acid, which contribute significantly to its antioxidant properties [8]. Previous studies have demonstrated that ethanol extracts of roselle exhibit strong DPPH radical scavenging activity, supporting its potential as a natural antioxidant agent [9].

2.4. Basil Leaf (*Ocimum sanctum* L.)

Basil, scientifically known as Ocimum sanctum L., is a traditional herb known for its therapeutic effects. Its leaves contain bioactive compounds including eugenol, apigenin, rosmarinic acid, and flavonoids that exhibit notable antioxidant properties by stabilizing free radicals and inhibiting lipid peroxidation [10]. Zulfajri et al. (2020) found that ethanol extracts of basil leaves demonstrated moderate to strong antioxidant activity using the DPPH assay.

2.5. Combined Herbal Extracts for Antioxidant Synergy

Combining herbal extracts is a promising strategy to achieve synergistic or additive effects and reduce possible toxicity. The combination of roselle and basil offers complementary antioxidant phytochemicals such as polyphenols, flavonoids, and anthocyanins that may work together to enhance free radical scavenging [11],[9]. Investigating such combinations is crucial to developing more effective and safer antioxidant therapies derived from natural sources.

3. Proposed Method

3.1. Equipment and Materials

The equipment used included standard laboratory glassware, a rotary evaporator (IKA-RV 10), a UV-Visible spectrophotometer (Agilent 8453), an analytical balance (Dragon 303), a gram balance (O'Hauss), and a vortex mixer (Mixer-Hwashin).

The materials used in this study were roselle flowers (*Hibiscus sabdariffa* L.), basil leaves (*Ocimum sanctum* L.), distilled water, ascorbic acid (vitamin C), 1,1-diphenyl-2-picrylhydrazine (DPPH), 70% ethanol, and analytical grade methanol (methanol p.a.).

3.2. Population and Sample

Roselle flowers were obtained from Tombolo Pao Subdistrict, Gowa Regency, and basil leaves were collected from Sulili Village, Pinrang Regency, South Sulawesi Province. The roselle flower calyces and basil leaves were sorted, cleaned, washed, drained, and then chopped. A total of 500 g of fresh chopped plant material was macerated in 1500 mL of 70% ethanol for five days with occasional stirring. After five days, the mixture was filtered. The residue was then re-macerated with another 1500 mL of 70% ethanol until the solvent became colorless. The filtrates were collected and concentrated using a rotary evaporator to obtain a thick extract.

3.3. Experimental Procedure

Total of 0.394 mg of DPPH crystals was weighed and placed into a 25 mL volumetric flask, dissolved, and the volume was adjusted to the mark using analytical grade methanol (methanol p.a.). The solution was vortexed until homogeneous and stored in a dark bottle. Then, 1 mL of the 0.4 mM DPPH solution was transferred into a 5 mL volumetric flask, diluted with methanol p.a. to the mark, vortexed until homogeneous, and incubated in a dark room for 30 minutes. The solution was then poured into a cuvette and the absorbance was measured at a wavelength of 515.5 nm using a UV-Visible spectrophotometer [5],[6].

100 ppm ascorbic acid stock solution was prepared by weighing 10 mg of ascorbic acid powder and dissolving it with methanol p.a. in a 100 mL volumetric flask. The stock solution was then diluted to prepare a series of concentrations: 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. Each 5 mL volumetric flask contained 1 mL of 0.4 mM DPPH solution and was then diluted to the mark with methanol p.a. Each concentration was vortexed to homogenize the mixture, incubated in a dark room for 30 minutes, poured into a cuvette, and the absorbance was measured at 515.5 nm using a UV-Visible spectrophotometer [5].

Combinations of roselle flower extract and basil leaf extract were prepared in 25 mL volumetric flasks at ratios of 1:1 (12.5 mg:12.5 mg), 1:2 (8.35 mg:16.65 mg), and 2:1 (16.65 mg:8.35 mg). Each combination was dissolved and diluted with methanol p.a. up to 25 mL to obtain a 1000 ppm stock solution. Serial dilutions of the combination extract were then prepared at concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm, each mixed with 1 mL of 0.4 mM DPPH solution. The measurement procedure was conducted in the same manner as that for the ascorbic acid reference solution.

3.4. Data Analysis

The antioxidant activity of the test solution can be determined based on its ability to inhibit DPPH free radicals, as indicated by the absorbance values [7]. The percentage of DPPH radical scavenging activity is calculated using the following equation:

$$\% \textbf{ Inhibition Activity} = \frac{\boldsymbol{A0 - A1}}{\boldsymbol{A0}} \times \mathbf{100\%}$$

Where:

A_0 = Absorbance of the DPPH solution (control)

A_1 = Absorbance of the sample solution

The sample concentration and the corresponding percentage of inhibition are plotted on the X-axis and Y-axis, respectively, to obtain a linear regression equation. This equation is used to determine the IC_{50} value of each sample by setting the y value to 50, with the corresponding x value representing the IC_{50}.

The Antioxidant Activity Index (AAI) indicates the strength of the antioxidant activity. It is calculated by dividing the DPPH concentration (in ppm) used in the assay by the IC_{50} value (in ppm) obtained from the sample [8].

4. Results and Discussion

Table 1. Antioxidant activity test results of extract combinations and reference compound using DPPH reagent, measured with a UV-Vis spectrophotometer at a wavelength of 515.5 nm.

No	Sample	Linear Regression Equation	IC_{50} (ppm)	AAI Value
1	Vitamin C (Ascorbic Acid)	y = 10.42x – 7.604	5.528	7.151
2	Roselle Flower Extract: Basil Leaf Extract (1:1)	y = 0.568x + 10.07	70.33	0.561
3	Roselle Flower Extract: Basil Leaf Extract (2:1)	y = 0.542x + 19.66	57.90	0.692
4	Roselle Flower Extract: Basil Leaf Extract (1:2)	y = 0.692x + 10.40	57.21	0.681

Based on the results obtained, the antioxidant activity of the combined extracts of roselle flower and basil leaf exhibited strong antioxidant activity, as indicated by IC_{50} values ranging between 50–100 μg/mL. The activity is categorized as moderate according to the Antioxidant Activity Index (AAI), which ranged between 0.5–1. In contrast, vitamin C demonstrated very strong antioxidant activity, with an IC_{50} value of 5.528 μg/mL (less than 50 μg/mL) and an AAI value of 7.151 (greater than 2).

This study utilized extracts from roselle flowers and basil leaves, derived from fresh simplicia and macerated using 70% ethanol. The maceration method was chosen due to its simplicity, low cost, and suitability for samples that do not contain substances that readily swell in the solvent, such as flowers and leaves. A 70% ethanol solvent was used because phenolic and flavonoid compounds present in roselle flowers and basil leaves are readily soluble in organic solvents mixed with water (Ministry of Health, Republic of Indonesia, 2000). The maceration of fresh roselle flowers and basil leaves yielded 66.42 g of roselle extract (13.28% yield) and 62.60 g of basil extract (12.52% yield).

Antioxidant activity was assessed using a spectrophotometric method with DPPH reagent. The ability to reduce DPPH radicals is indicated by a decrease in absorbance at a specific wavelength, which occurs due to antioxidant activity. The color change from purple to reddish or yellow corresponds to the number of electrons transferred to stabilize the DPPH radical. This process results in the conversion of DPPH to its reduced form, 1,1-diphenyl-2-picrylhydrazine, caused by antioxidant molecules [9].

The antioxidant parameter used with the DPPH reagent is the IC_{50} value. IC_{50} represents the concentration of the sample required to inhibit 50% of the oxidation process. A lower IC_{50} value indicates higher antioxidant activity. Specifically, an IC_{50} value below 50 μg/mL is classified as very strong antioxidant activity; 50–100 μg/mL is strong; 100–150 μg/mL is moderate; 150–200 μg/mL is weak; and above 200 μg/mL is considered very weak (Blois, 1958). The Antioxidant Activity Index (AAI) is used to categorize the antioxidant potency of the extract. An AAI value below 0.5 is classified as weak; 0.5–1.0 as moderate; 1–2 as strong; and above 2 as very strong [8].

The antioxidant activity test of the reference compound ascorbic acid and the combination extract of roselle flower and basil leaf using DPPH reagent was carried out with a UV-Vis spectrophotometer at a wavelength of 515.5 nm, as presented in Table 1. The complete data are available in the appendix.

5. Conclusions

Based on the results and discussion, it can be concluded that the in-vitro antioxidant activity test demonstrated that the combination extracts of roselle flower and basil leaf in ratios of 1:1, 1:2, and 2:1 exhibited strong antioxidant activity, with IC_{50} values of 70.33 μg/mL, 56.92 μg/mL, and 57.90 μg/mL, respectively.

Table 2. Absorbance values of the test solution for the combination extract (roselle flower and basil leaf) at a 1:1 ratio

Konsetrasi (ppm)	Absorbansi
DPPH	0,814
20 ppm 1	0,689
20 ppm 2	0,658
20 ppm 3	0,670
40 ppm 1	0,515
40 ppm 2	0,518
40 ppm 3	0,515
60 ppm 1	0,420
60 ppm 2	0,425
60 ppm 3	0,444
80 ppm 1	0,372
80 ppm 2	0,386
80 ppm 3	0,363
100 ppm 1	0,265
100 ppm 2	0,289
100 ppm 3	0,276

Table 3. Percentage of DPPH inhibition by the test solution of the combination extract (roselle flower and basil leaf) at a 1:1 ratio.

Konsetrasi (ppm)	% Inhibisi	Rata-rata
20 ppm 1	15,35	17,40 ± 1,92
20 ppm 2	19,16	
20 ppm 3	17,69	
40 ppm 1	36,73	36,61 ± 0,21
40 ppm 2	36,36	
40 ppm 3	36,73	
60 ppm 1	48,40	47,21 ± 1,55
60 ppm 2	47,79	
60 ppm 3	45,45	
80 ppm 1	54,29	54,09 ± 1,42
80 ppm 2	52,58	
80 ppm 3	55,40	
100 ppm 1	67,44	65,48 ± 1,70
100 ppm 2	64,49	
100 ppm 3	64,49	

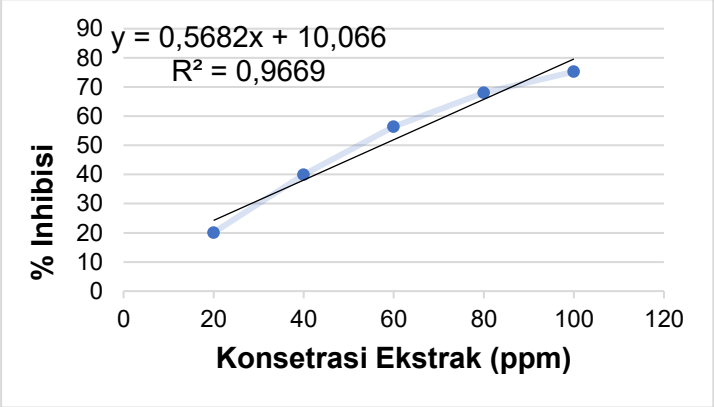

Figure 1. Profile of the relationship between the concentration of the combination extract at a 1:1 ratio and the percentage of DPPH inhibition.

Table 4. Absorbance values of the test solution of the combination extract from roselle flower and basil leaf at a 2:1 ratio.

Konsetrasi (ppm)	Absorbansi
DPPH	0,835
20 ppm 1	0,594
20 ppm 2	0,595
20 ppm 3	0,597
40 ppm 1	0,494
40 ppm 2	0,498
40 ppm 3	0,494
60 ppm 1	0,387
60 ppm 2	0,390
60 ppm 3	0,389
80 ppm 1	0,301
80 ppm 2	0,327
80 ppm 3	0,305
100 ppm 1	0,244
100 ppm 2	0,252
100 ppm 3	0,247

Table 5. Percentage of DPPH inhibition by the test solution of the combination extract from roselle flower and basil leaf at a 2:1 ratio.

Konsetrasi (ppm)	% Inhibisi	Rata-rata
20 ppm 1	28,86	28,70 ± 0,18
20 ppm 2	28,74	
20 ppm 3	28,50	
40 ppm 1	40,84	40,68 ± 0,27
40 ppm 2	40,36	
40 ppm 3	40,84	

60 ppm 1	53,65	53,45 ± 0,18
60 ppm 2	53,29	
60 ppm 3	53,41	
80 ppm 1	63,95	62,75 ± 1,67
80 ppm 2	60,84	
80 ppm 3	63,47	
100 ppm 1	70,78	70,14 ± 0,55
100 ppm 2	69,82	
100 ppm 3	69,82	

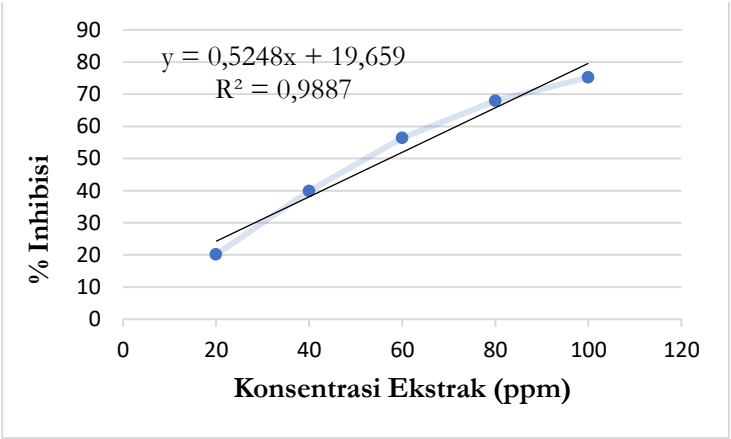

Figure 2. Profile of the relationship between the concentration of the combination extract from roselle flower and basil leaf at a 2:1 ratio and the percentage of DPPH inhibition.

Table 6. Absorbance values of the test solution of the combination extract from roselle flower and basil leaf at a 1:2 ratio.

Konsetrasi (ppm)	Absorbansi
DPPH	0,835
20 ppm 1	0,644
20 ppm 2	0,667
20 ppm 3	0,668
40 ppm 1	0,504
40 ppm 2	0,502
40 ppm 3	0,500
60 ppm 1	0,376
60 ppm 2	0,355
60 ppm 3	0,361
80 ppm 1	0,271
80 ppm 2	0,264
80 ppm 3	0,267
100 ppm 1	0,206

100 ppm 2	0,207
100 ppm 3	0,210

Table 7. Percentage of DPPH inhibition by the test solution of the combination extract from roselle flower and basil leaf at a 1:2 ratio.

Konsetrasi (ppm)	% Inhibisi	Rata-rata
20 ppm 1	22,87	20,10 ± 1,65
20 ppm 2	20,12	
20 ppm 3	20,00	
40 ppm 1	39,64	39,88 ± 0,24
40 ppm 2	39,88	
40 ppm 3	40,12	
60 ppm 1	54,97	56,41 ± 1,29
60 ppm 2	57,48	
60 ppm 3	56,76	
80 ppm 1	67,54	67,98 ± 0,42
80 ppm 2	68,38	
80 ppm 3	68,02	
100 ppm 1	75,33	75,25 ± 0,07
100 ppm 2	75,21	
100 ppm 3	75,22	

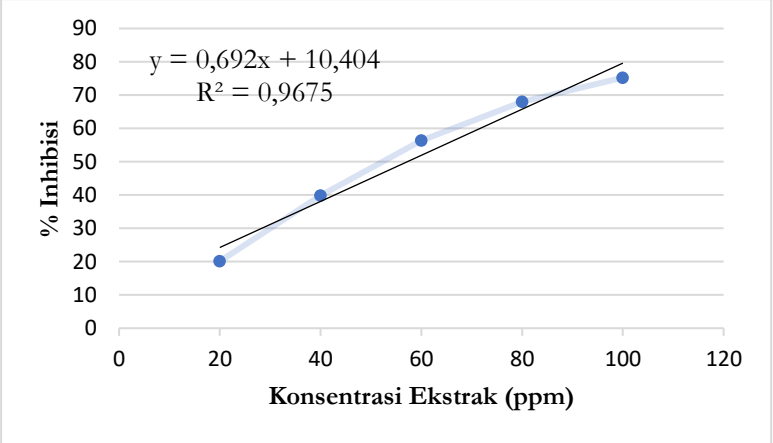

Figure 3. Profile of the relationship between the concentration of the combination extract from roselle flower and basil leaf at a 1:2 ratio and the percentage of DPPH inhibition.

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