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Antioxidant Activity of Roselle Flower Extract (*Hibiscus sabdariffa* L.) at Different Storage Durations Using the DPPH Method

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Abstract: Free radicals can cause cellular damage that contributes to various degenerative diseases, making antioxidant compounds necessary to neutralize them. One plant with potential as a natural source of antioxidants is the rosella flower, which is known to contain bioactive compounds such as flavonoids, phenolics, and anthocyanins. This study aims to determine the antioxidant activity of rosella flower petal extract using the DPPH method based on the IC_{50} value and to compare it with vitamin C as a positive control. This study is a laboratory experiment using a quantitative approach. The sample used was rosella flower petals extracted via maceration using 96% ethanol as the solvent. The antioxidant activity of the extract was tested using the DPPH method, with the IC_{50} value determined via UV-Vis spectrophotometry. The results of the study showed that the IC_{50} value for vitamin C was 96.64 µg/mL, while those for rosella flower petal extract stored for 3 days and 5 days were 106.47 µg/mL and 104.12 µg/mL, respectively. The 5-day storage period showed better antioxidant activity compared to the 3-day period, although it was still lower than that of vitamin C. Thus, the duration of storage affects the antioxidant activity of rosella flower petal extract.

Keywords: Antioxidants, DPPH, IC_{50}, Rosella, Vitamin C

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Introduction

Exposure to free radicals caused by pollution, stress, unhealthy lifestyles, and the consumption of high-fat, low-fiber foods contributes to the rise in degenerative diseases such as diabetes, hypertension, cancer, and cardiovascular disease. The World Health Organization (WHO) reports that noncommunicable diseases (NCDs) caused approximately 43 million deaths or 75% of total global deaths in 2021, with cardiovascular disease being the leading cause. In 2024, the International Diabetes Federation (IDF) recorded that the number of people with diabetes aged 20–79 in Indonesia reached 20.4 million, placing Indonesia fifth-highest in the world. This condition is closely related to oxidative stress resulting from an imbalance between free radicals and the body's antioxidant system (Halliwell, 2024; International Diabetes Federation (IDF), 2024; WHO, 2024).

Free radicals are reactive molecules with unpaired electrons that can damage cells through lipid peroxidation, protein damage, and DNA mutations. Therefore, the body requires antioxidants



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capable of neutralizing free radicals through electron or hydrogen atom donation mechanisms. One potential source of natural antioxidants is the rosella flower (*Hibiscus sabdariffa* L.) from the Malvaceae family, which is widely cultivated in Indonesia. Roselle flower petals are known to contain anthocyanins, flavonoids, phenolic compounds, and ascorbic acid, which play an important role in antioxidant activity (Li et al., 2024; Riaz & Chopra, 2018; Yusoff et al., 2024).

The antioxidant activity of rosella is typically tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, a simple and sensitive method for assessing a compound's ability to scavenge free radicals. The primary parameter used is the IC_{50} value, which is the sample concentration required to inhibit 50% of free radicals; the lower the IC_{50} value, the stronger the antioxidant activity. Research shows that rosella petal extract has good antioxidant activity, but the results vary depending on the extraction method, type of solvent, and storage conditions of the material (Fardani et al., 2023; Syahara et al., 2023; Yusoff et al., 2024).

Storage duration is one of the factors that can affect the stability of active compounds such as anthocyanins and flavonoids, as these compounds are sensitive to oxidation, light, temperature, and humidity. A decrease in the levels of active compounds during storage can reduce the antioxidant activity of the extract (Helilusiatiningsih et al., 2024; Mahfudh & Amelia, 2020). However, research on the effect of storage duration on the antioxidant activity of rosella flower petals remains limited. Therefore, this study was conducted to analyze the antioxidant activity of rosella flower petal extracts at varying storage durations using the DPPH method, measured by inhibition percentage and IC_{50} values, and to compare them with vitamin C as a positive control.

Methods

Tools

The equipment used in this study included an analytical balance (Ohaus Pioneer PA214C), a blender (Philips HR2116), a drying oven (Memmert UN55), a rotary evaporator (Heidolph Laborota 4000), a UV-Vis spectrophotometer, and a desiccator (Duran). In addition, laboratory glassware such as beakers, volumetric flasks, volumetric pipettes, glass funnels, and filter paper (Whatman No. 1), maceration bottles, measuring cups and flasks, Erlenmeyer flasks, 40-mesh sieves, droppers and micropipettes, test tubes, vortex mixers, aluminum foil, test tube racks, spatulas, and water baths.

Materials

Dry roselle calyx powder (*Hibiscus sabdariffa*) 1 kg, 96% ethanol 2 L (as solvent), DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) 15 g, distilled water 1 L, and vitamin C (ascorbic acid) 20 mg as a comparator or positive control. Materials for phytochemical identification included Dragendorff reagent, Mayer reagent, 2 M HCl, magnesium (Mg), concentrated HCl, methanol, and 1% $FeCl_3$.

Preparation of Rosella Petal Powder

Rosella plants that have undergone determination, 1 kg of flower petals is used. The flower petals are washed under running water, then drained and dried in an oven at a temperature of ≤40°C until the minimum moisture content is achieved. After that, the rosella flower petals are ground into a crude drug powder using a blender.

Roselle Flower Extraction

A total of 1 kg of rosella petal powder was macerated with 96% ethanol at a 1:10 (w/v) ratio in a sealed container at room temperature for 72 hours, with occasional stirring. The mixture was then filtered to obtain the filtrate, and the maceration process was repeated three times to maximize extraction. All filtrates were combined and evaporated using a rotary evaporator at 40–

50°C until a concentrated extract was obtained. The extract was then stored in dark glass bottles at 4–8°C to maintain the stability of the active compounds.

Phytochemical Screening

Phytochemical screening of the roselle calyx extract was conducted to identify the presence of alkaloids, flavonoids, tannins, and saponins using standard qualitative methods (Kumalasari, D., Yuliana, S., Firmansyah, 2020). For alkaloid detection, 0.1 g of extract was treated with 1 mL of 2 M HCl and 10 mL of distilled water, then heated for approximately 5 minutes. The solution was subsequently reacted with Dragendorff's and Mayer's reagents. The formation of an orange precipitate with Dragendorff's reagent and a white precipitate with Mayer's reagent indicated the presence of alkaloids. For flavonoid identification, 0.1 g of extract was dissolved in 10 mL of hot methanol, followed by the addition of 0.1 g magnesium powder and 5 drops of concentrated HCl. The appearance of orange, pink, or red coloration persisting for at least 3 minutes indicated the presence of flavonoids. For tannin detection, 0.1 g of extract was dissolved in 4 mL of water, heated for approximately 5 minutes, cooled, and filtered. The filtrate was then treated with 10 drops of 1% $FeCl_3$ solution. The formation of dark blue, purple, red, or green coloration indicated the presence of tannins. For saponin detection, 0.1 g of extract was dissolved in 4 mL of hot water, and the filtrate was shaken vigorously and allowed to stand for 10 seconds. The formation of stable foam indicated the presence of saponins.

Antioxidant Activity Assay

The antioxidant activity of rosella flower extract (*Hibiscus sabdariffa* L.) was determined using the DPPH method. Extract solutions at concentrations of 20, 40, 60, 80, and 100 µg/mL were reacted with a 0.1 mM DPPH solution and incubated in the dark for 30 minutes. The decrease in the intensity of the DPPH purple color was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The percentage of inhibition was calculated and used to determine the extract's ability to scavenge free radicals.

Determination of the IC_{50} Value

The IC_{50} value is determined based on the relationship between sample concentration (X) and percentage of inhibition (Y) using a linear regression equation Y = bx + a. The value of Y is then substituted with 50 to obtain the concentration value expressed as IC_{50}.

Result and Discussion

Phytochemical Screening Results

Phytochemical screening was conducted to qualitatively identify the classes of secondary metabolites present in roselle flower extract (*Hibiscus sabdariffa* L.) through color reactions and precipitation using specific reagents. The results of the study indicate that roselle flower extract is positive for flavonoids, alkaloids, tannins, and saponins. The presence of these secondary metabolites suggests that roselle has potential as a source of natural antioxidants with a fairly complex profile.

Table 1. Results of Phytochemical Screening of Rosella Flowers

No	Class of Compounds	Test Results	Description
1	Alkaloid	+	A precipitate forms
2	Flavonoid	+	Orange-red color
3	Tannin	+	Blackish-green color
4	Saponin	+	Stable foam forms

The results of this study are consistent with previous research reporting that *Hibiscus sabdariffa* extract contains various bioactive compounds such as flavonoids, tannins, alkaloids, and saponins, which contribute to the plant's biological activity (Fitrotunnisa et al., 2019). Other studies have also shown that the rosella calyx contains higher levels of phytochemicals compared to other parts of the plant, particularly phenolic compounds and flavonoids that contribute to antioxidant activity (Fraga-corral et al., 2021). Additionally, a study by Purba et al., (2020) reported that rosella extract contains flavonoids, tannins, and saponins that contribute to antibacterial and antioxidant activity.

The flavonoids detected in this study act as the primary antioxidants through mechanisms involving the donation of electrons or hydrogen atoms to neutralize free radicals (Khuanekkaphan et al., 2020; Panche et al., 2016; Sies et al., 2022). Tannins also possess antioxidant properties through mechanisms such as free radical scavenging, inhibition of lipid peroxidation, and the ability to chelate metal ions that play a role in free radical formation (Fraga-corral et al., 2021). This antioxidant activity is related to the chemical structure of tannins, which is rich in phenolic groups, enabling them to act as electron donors and radical scavengers (Melo et al., 2023). Meanwhile, saponins and alkaloids also contribute to biological activity, including antioxidant activity, although their role is relatively minor (Syahara et al., 2023; Yusoff et al., 2024). Thus, the presence of these compounds is believed to contribute to the antioxidant activity of the rosella extract, which was subsequently analyzed using the DPPH method based on the percentage of inhibition and the IC_{50} value.

Antioxidant Activity of Rosella Flower Extract

The antioxidant activity of rosella flower extract was analyzed using the DPPH method based on the sample's ability to scavenge free radicals, as indicated by a decrease in absorbance at a wavelength of 517 nm. Antioxidant capacity is expressed as a percentage of inhibition (% inhibition), where a higher percentage of inhibition indicates a greater ability of the sample to scavenge DPPH free radicals. The results of the antioxidant activity testing of vitamin C as a positive control and rosella flower extract at various concentrations are shown in Table 2.

Table 2. Results of Antioxidant Activity Tests on Vitamin C and Rosella Flower Extract

Sample	Concentration (µg/mL)	Absorbance (A)	% Inhibition
Vitamin C	20	0.605	6.27
	40	0.449	30.44
	60	0.425	34.16
	80	0.389	39.74
	100	0.321	50.27
Rosela, 3-day storage	20	0.862	-33.62
	40	0.783	-21.25
	60	0.739	-14.43
	80	0.519	19.56
	100	0.307	52.4
Rosela, 5-day storage	20	1.225	-89.41
	40	1.189	-84.21
	60	1.036	-60.66
	80	0.598	7.33
	100	0.287	55.52

The results of the antioxidant activity test showed that increasing the concentration of rosella flower extract led to an increase in the percentage of inhibition against DPPH free radicals. Vitamin C, used as a positive control, exhibited more stable antioxidant activity, with the percentage of inhibition increasing from 6.27% at a concentration of 20 µg/mL to 50.27% at a concentration of 100 µg/mL. These results indicate that the higher the sample concentration, the greater the ability of antioxidant compounds to donate electrons or hydrogen atoms to neutralize DPPH free radicals. This phenomenon is consistent with previous studies reporting that antioxidant activity increases with increasing extract concentration because the number of bioactive compounds reacting with free radicals becomes greater (Sies et al., 2022; Yusoff et al., 2024).

Roselle extracts stored for 3 days and 5 days showed negative inhibition percentages at low concentrations, but these increased significantly at high concentrations. At a concentration of 100 µg/mL, the 3-day and 5-day stored rosella extracts showed inhibition percentages of 52.40% and 55.52%, respectively. The negative inhibition values at low concentrations are thought to be caused by color interference from the anthocyanin-rich rosella extract, which increases the solution's absorbance at a wavelength of 517 nm. Anthocyanins are natural red pigments that can affect spectrophotometer readings if blank correction is not performed optimally (Khoo et al., 2017; Olszowy & Dawidowicz, 2018; Tena & Asuero, 2020). Additionally, the low concentration of active compounds also results in suboptimal free radical scavenging capacity, causing the sample's absorbance to remain higher than that of the control.

The increase in the percentage of inhibition at higher concentrations indicates that rosella extract retains good antioxidant activity. This activity is believed to stem from the flavonoids, tannins, and phenolic compounds identified in the phytochemical screening. Flavonoids are known to stabilize free radicals through electron and hydrogen atom donation mechanisms, while tannins play a role in inhibiting oxidation by binding metal ions and scavenging free radicals (Camarda et al., 2026; Panche et al., 2016). Thus, rosella flower extract has potential as a source of natural antioxidants, although its activity remains lower than that of vitamin C, the standard antioxidant.

Determination of IC_{50} Value

The IC_{50} value is used to indicate the sample concentration required to inhibit 50% of DPPH free radicals. The lower the IC_{50} value, the stronger the antioxidant activity of a sample, as fewer compounds are needed to neutralize free radicals (Panche et al., 2016; Sies et al., 2022). Based on Table 3, vitamin C, as the positive control, has an IC_{50} value of 96.64 µg/mL and falls into the category of strong antioxidants. This indicates that vitamin C has a good ability to donate electrons or hydrogen atoms to stabilize DPPH free radicals.

Table 3. Determination of IC_{50} Value

Sample	IC_{50} value (µg/mL)	Category
Vitamin C	96.64	Strong
Rosela, 3-day storage	106.47	Moderate
Rosela, 5-day storage	104.12	Moderate

Roselle flower extracts stored for 3 days and 5 days had IC_{50} values of 106.47 µg/mL and 104.12 µg/mL, respectively, which fall into the moderate antioxidant category. These results indicate that roselle flower extracts retain antioxidant activity, although not as potent as vitamin C. This antioxidant activity is believed to stem from the flavonoids, tannins, and phenolic compounds identified in the phytochemical screening. Flavonoids are known to scavenge free radicals by donating electrons or hydrogen atoms, thereby inhibiting oxidative reactions in the body (Panche et al., 2016). Additionally, the phenolic compounds and anthocyanins in rosella also

play a significant role in antioxidant activity through their ability to reduce free radicals and inhibit lipid oxidation (Khoo et al., 2017).

The results of this study are consistent with several previous studies reporting that *Hibiscus sabdariffa* extract exhibits moderate to strong antioxidant activity, depending on the extraction method, type of solvent, and processing conditions. Research by Syahara et al., (2023) showed that rosella extract exhibits antioxidant activity with IC_{50} values influenced by the content of phenolic and flavonoid compounds. Variations in IC_{50} values across studies may be attributed to differences in storage conditions, the stability of active compounds, and the extraction processes employed (Yusoff et al., 2024).

The Effect of Storage Duration on Antioxidant Activity

Storage duration affects the antioxidant activity of rosella flower extract, as evidenced by differences in IC_{50} values between 3-day and 5-day storage. Based on Table 3, rosella extract stored for 5 days had a lower IC_{50} value compared to that stored for 3 days, namely 104.12 µg/mL and 106.47 µg/mL. A lower IC_{50} value indicates better antioxidant activity because the concentration of extract required to inhibit 50% of free radicals is lower (Kartika et al., 2025).

These differences in antioxidant activity are thought to be influenced by the stability of bioactive compounds during storage, particularly flavonoids and anthocyanins, which are known to be sensitive to light, temperature, oxygen, and humidity. Anthocyanins in rosella may undergo oxidative degradation during storage, thereby affecting their free radical scavenging capacity (Enaru et al., 2021; Khoo et al., 2017; Oancea, 2021). However, in this study, storage for 5 days still showed relatively good antioxidant activity, suggesting that the active compounds in the extract remained sufficiently stable during that storage period.

These results indicate that storage duration can affect the antioxidant activity of rosella flower extract, although the differences are not significant. Therefore, controlling storage conditions such as low temperature and protection from light is necessary to maintain the stability of antioxidant compounds in rosella extract. Further research is still needed to evaluate the effect of longer-term storage on the stability of bioactive compounds and the antioxidant activity of rosella extract.

Conclusion

Based on the research results, roselle flower petal extract (*Hibiscus sabdariffa* L.) exhibited antioxidant activity as measured by the DPPH method, with IC_{50} values of 106.47 µg/mL and 104.12 µg/mL for 3-day and 5-day storage, respectively, while vitamin C, used as a positive control, had an IC_{50} value of 96.64 µg/mL. Storage for 5 days yielded better antioxidant activity compared to 3-day storage because it had a lower IC_{50} value. These results indicate that storage duration affects the antioxidant potential of rosella extract. The active compounds identified in the extract are believed to contribute to this antioxidant activity. For future researchers, it is recommended to conduct tests with a wider range of storage durations, perform quantitative analysis of active compound levels, and use other antioxidant assay methods such as ABTS or FRAP to strengthen the research findings.

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