JPPIPA 12(4) (2026)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Optimization of Essence Formulation Containing Purified Extract of Purple Rosella (*Hibiscus sabdariffa* L.) and Evaluation of Its Antioxidant Activity Using DPPH Method

Novena Yety Lindawati[1*], Nastiti Utami[1], Doni Nur Cahyadi[1], Lusia Murtisiwi[1]

[1] Bachelor of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Nasional, Surakarta, Indonesia.

Received: November 19, 2025
Revised: February 06, 2026
Accepted: April 25, 2026
Published: April 30, 2026

Corresponding Author:
Novena Yety Lindawati
novena_yl@stikesnas.ac.id

DOI: 10.29303/jppipa.v12i4.13517

Abstract: The ethanol extract of purple roselle flowers (*Hibiscus sabdariffa* L.) is known to exhibit very strong antioxidant activity and contains flavonoids, anthocyanins, and tannins that contribute to free radical scavenging through hydrogen donation mechanisms. This study aimed to formulate an antioxidant essence based on purified purple roselle extract. The extract was obtained by maceration using 70% ethanol and purified through liquid–liquid extraction using an aqueous–ethyl acetate solvent system. The purified extract was formulated into essence preparations at concentrations of 1% (FI), 3% (FII), and 5% (FIII), with the base formula (FIV) as a negative control and quercetin (FV) as a positive control. Evaluations included organoleptic properties, homogeneity, viscosity, pH, and freeze–thaw stability, while antioxidant activity was assessed using the DPPH method. All formulations met the required physical quality standards, and Formulas I–IV remained stable during testing. Formula V exhibited a color change from yellow to reddish-brown, indicating the temperature sensitivity of quercetin. The IC_{50} values of FI, FII, and FIII were 57.96 ppm (strong), 47.01 ppm (very strong), and 33.63 ppm (very strong), respectively, while FV showed an IC_{50} of 29.86 ppm. Formula III demonstrated the best performance with very strong antioxidant activity and good physical stability.

Keywords: Anthocyanins; Antioxidant activity; DPPH method; Essence formulation; IC_{50}; Purified extract of purple roselle; Stability

Introduction

Ultraviolet radiation is part of the electromagnetic spectrum originating from sunlight and is classified into UV-A (320–400 nm), UV-B (290–320 nm), and UV-C (<290 nm). Exposure to UV-A and UV-B is known to induce oxidative stress through increased formation of reactive oxygen species (ROS), which contribute to skin damage such as erythema, hyperpigmentation, premature aging, and carcinogenesis (Asanah et al., 2023; Dhar et al., 2021). Meanwhile, UV-C radiation does not have a direct impact on the skin as it is mostly absorbed by the ozone layer (Oktavia & Sutoyo, 2021).

Free radicals are molecules with unpaired electrons that are highly reactive and capable of damaging cellular lipids, proteins, and DNA. Antioxidants play an essential role in neutralizing free radicals through electron or hydrogen donation mechanisms. However, the long-term use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has been associated with potential toxic effects, including carcinogenicity and hepatotoxicity at high doses. Therefore, the exploration of natural antioxidants has become a safer and more sustainable approach (Abdelfattah et al., 2024; Fardani et al., 2023; Forman & Zhang, 2021; Sies & Jones, 2020).

How to Cite:
Lindawati, N. Y., Utami, N., Cahyadi, D. N., & Murtisiwi, L. (2026). Optimization of Essence Formulation Containing Purified Extract of Purple Rosella (Hibiscus sabdariffa L.) and Evaluation of Its Antioxidant Activity Using DPPH Method. *Jurnal Penelitian Pendidikan IPA*, *12*(4), 889–901. https://doi.org/10.29303/jppipa.v12i4.13517

Purple roselle (*Hibiscus sabdariffa* L.) is a natural source of antioxidants rich in flavonoids, particularly anthocyanins, as well as tannins, which act as free radical scavengers (Liu et al., 2021). The antioxidant activity of roselle has been reported to be very strong, with an IC_{50} value of approximately 38.29 ppm based on the DPPH method, indicating its high potential as an active ingredient in topical formulations. Anthocyanins, as the major compounds, function through hydrogen donation and stabilization of free radicals via resonance of their aromatic structure (Shikov et al., 2022). However, these compounds exhibit relatively low stability when exposed to environmental factors such as temperature, pH, and light, which may reduce their effectiveness.

In addition to active compounds, plant extracts also contain non-therapeutic components (ballast substances) such as carbohydrates, proteins, and pectin, which may affect the purity and biological activity of the extract (Lestari, 2022; Singh et al., 2023). Therefore, purification is an important strategy to enhance the concentration of bioactive compounds and optimize antioxidant activity (Aldila et al., 2024; Zou et al., 2025).

In addition to the active compounds, the delivery system plays a crucial role in determining the effectiveness of topical formulations. Essence is a skincare preparation with low viscosity that enables faster and deeper penetration of active ingredients into the epidermal layer compared to more viscous formulations such as creams. This system is considered capable of enhancing the bioavailability of active compounds while improving user comfort. The use of humectants in the formulation also contributes to maintaining skin hydration and facilitating the penetration of active compounds without significant degradation (Dzulhija et al., 2024; Husna et al., 2022; Mulangsri & Zulfa, 2020).

The novelty of this study lies in the integration of purified purple roselle extract with an essence-based delivery system characterized by low viscosity to enhance both stability and antioxidant activity. Previous studies have generally focused on crude extracts or conventional dosage forms without combining purification strategies with advanced delivery systems. Therefore, this research is important to provide a scientific basis for the development of more effective, stable, and safer natural-based cosmetic products.

Based on the above considerations, this study aims to optimize the formulation and evaluate the antioxidant activity of essence preparations containing various concentrations of purified purple roselle (*Hibiscus sabdariffa* L.) extract, as well as to assess their physical quality, including organoleptic properties, homogeneity, viscosity, pH, and stability using the freeze–thaw method.

Method

Materials

In this study, the materials used included purple roselle flowers, 70% ethanol, $FeCl_3$, DPPH (Sigma), quercetin (Sigma), and phytochemical screening reagents.

Sample Preparation

Purple roselle flowers (*Hibiscus sabdariffa* L.) were obtained and identified by the Functional Implementation Unit of the Traditional Health Center, Tawangmangu, RSUP dr. Sardjito. The dried roselle flower simplicia was then ground into powder and sieved using a 40-mesh sieve (Khalid et al., 2023).

Preparation of Purple Roselle Flower Ethanol Extract

A total of 1000 g of simplicia powder was macerated using 5000 mL of 70% ethanol (ratio 1:5 w/v) for 3 days with stirring every 24 hours, followed by filtration using flannel cloth. The residue was remacerated using the same solvent. All filtrates were combined and evaporated using a rotary evaporator at 40°C with a speed of 100 rpm until a thick extract was obtained. The extract was stored in a closed container lined with aluminum foil at 4°C (Fardani et al., 2023). The yield obtained represents the percentage of extract weight relative to the weight of the simplicia powder used. The extraction yield can be calculated using the following formula:

$$\text{Yeild}(\%) = \frac{\text{Extract weight (g)}}{\text{Simplisia's weight (g)}} \times 100\% \quad (1)$$

The general requirement for raw material extract yield is >10% (Mulangsri & Zulfa, 2020).

Preparation of Purified Purple Roselle Flower Extract

A total of 200 g of ethanol extract was suspended in warm water until homogeneous, then allowed to cool to room temperature. The suspension was transferred into a separatory funnel, followed by the addition of ethyl acetate with a water:ethyl acetate ratio of 1:1. The mixture was gently shaken until two phases were formed. The ethyl acetate phase was separated, while the aqueous phase was re-extracted with ethyl acetate until the ethyl acetate phase became clear. All ethyl acetate fractions were collected and evaporated using a rotary evaporator at 50°C to obtain the purified extract (Lestari, 2022).

Phytochemical Screening

Flavonoids

A total of 40 mg of extract was added to 100 mL of hot water, heated for 5 minutes, and filtered. Then, 5 mL of filtrate was mixed with magnesium powder in a

sufficient amount (tip of a spatula) and 1 mL of concentrated HCl, followed by vigorous shaking. A positive result is indicated by the formation of red, yellow, or orange coloration (Mulangsri & Zulfa, 2020).

Anthocyanins

Heat the sample with 2 M HCl at 100°C for 2 minutes and observe the color. If the red color of the sample remains unchanged (constant), this indicates the presence of anthocyanins (Mulangsri & Zulfa, 2020).

Alkaloids

Weigh 2.0 grams of the purified thick extract and add 5.0 mL of 2N HCl. Divide the mixture into two portions: to the first portion, add three drops of Dragendorff's reagent; a positive result is indicated by the formation of an orange precipitate. To the second portion, add three drops of Mayer's reagent; a positive result is shown by the formation of a yellow precipitate (Mulangsri & Zulfa, 2020).

Tannins

Weigh 40.0 mg of the purified extract and dissolve it in 4.0 mL of water. Take 2.0 mL of the solution and add two drops of 1% $FeCl_3$ reagent. A positive tannin result is indicated by a blue-black or greenish-black coloration (Mulangsri & Zulfa, 2020).

Saponins

Weigh 40.0 mg of the purified extract, add 10.0 mL of distilled water, heat until boiling for 10 minutes, and filter. Transfer the filtrate into a test tube, shake vigorously for 10 seconds, and add 2N HCl. A positive result is shown by foam formation between 1 and 10 cm in height that remains stable for 10 minutes (Mulangsri & Zulfa, 2020).

Table 1. Formulation design for the preparation of purple roselle flower extract essence (Mulangsri & Zulfa, 2020)

Ingredients	F I	F II	F III	F IV	F V	Function
Quercetin	-	-	-	-	0.05	Active ingredient
Purified purple roselle flower extract	1	3	5	-	-	Active ingredient
PEG-40 hydrogenated castor oil	0.20	0.20	0.20	0.20	0.20	Surfactant
Butylene glycol	5	5	5	5	5	Humectant
Sodium polyacrylate	0.20	0.20	0.20	0.20	0.20	Thickening agent
Methylparaben	0.30	0.30	0.30	0.30	0.30	Preservative
Aquadest ad	100	100	100	100	100	Solvent

Essence Formulation Design of Purple Roselle Flowers
Preparation of Essence Formulation

Dissolve sodium polyacrylate in a mortar with distilled water and add butylene glycol, then triturate until a homogeneous mixture is formed (mixture I). Dissolve methylparaben in a small amount of hot water (mixture II). Dissolve PEG-40 hydrogenated castor oil and the purified purple roselle extract in a small amount of distilled water (mixture III). Gradually add mixture II into mixture I and stir until a homogeneous mass is obtained, then add mixture III and continue stirring until homogeneous (Mulangsri & Zulfa, 2020).

Physical Quality Evaluation
Organoleptic Test

This test is carried out through visual observation, assessing parameters such as appearance, texture, color, and odor of the essence formulation (Sari et al., 2024).

Homogeneity Test

Place a drop of the essence on an object glass and cover it with a cover slip. Observe visually. A good essence should show no visible lumps (Sari et al., 2024).

Viscosity Test

Place the sample into a container, insert the spindle into the sample, and turn on the viscometer. The viscosity value is obtained from the instrument display. A good viscosity range is 2.3–11.5 poise (Adinda et al., 2023).

pH Test

Prepare the sample in a beaker glass and insert the pH meter into the sample. Wait until the pH reading becomes constant. The acceptable average pH range for facial cosmetic preparations is 4.5–6.5 (Sari et al., 2024).

Stability Test of the Essence

In this test, the freeze–thaw method was used, following the procedure in which the formulation was stored at 4°C for 24 hours and then heated to 40°C for 24 hours (one cycle). The test was carried out for six cycles, followed by observations of changes in pH and viscosity (Sari et al., 2024).

Antioxidant Activity Test
Preparation of the DPPH Stock Solution

Accurately weigh approximately 10.0 mg of DPPH and add ethanol p.a to a final volume of 100.0 mL to obtain a concentration of 100 ppm. Prepare a 40 ppm DPPH stock solution by pipetting 40.0 mL of the 100 ppm solution into a 100.0 mL volumetric flask, then add ethanol p.a to the mark and shake until homogeneous (Ningrum et al., 2024).

Determination of Maximum Wavelength (λmax).

2.0 mL of the 40 ppm DPPH solution and add 2.0 mL of ethanol p.a into a test tube. Incubate the mixture in a closed tube covered with aluminum foil for 30 minutes at room temperature in the dark. Measure the absorbance at the predetermined λmax (Ningrum et al., 2024; Mulangsri & Zulfa, 2020).

Measurement of DPPH Absorbance

Prepare graded concentrations of 10, 20, 30, 40, and 50 ppm for each essence formulation. Create a 1000 ppm stock solution by weighing 100.0 mg of the purified purple roselle essence and dissolving it in a 100.0 mL volumetric flask with ethanol p.a to the mark. Prepare a 100 ppm intermediate solution by pipetting 10.0 mL of the 1000 ppm stock into a 100.0 mL volumetric flask and adding ethanol p.a to the mark. Serial solutions of 10, 20, 30, 40, and 50 ppm are then prepared by pipetting 1, 2, 3, 4, and 5 mL of the 100 ppm solution into separate 10 mL volumetric flasks, filling each with ethanol p.a to the mark, and shaking until homogeneous. Perform all preparations in triplicate (Ningrum et al., 2024; Parapat et al., 2025).

Determination of IC_{50}

2.0 mL of each test solution and add 2.0 mL of 40 ppm DPPH solution. Vortex for 30 seconds and incubate for 30 minutes in a closed tube wrapped with aluminum foil, at room temperature and in the dark. Measure absorbance at λmax against an ethanol blank. Calculate the percentage of radical scavenging activity, and repeat the test three times (Ningrum et al., 2024; Mulangsri & Zulfa, 2020).

Analysis

The IC_{50} value represents the concentration of the essence formulation required to achieve 50% radical scavenging activity compared to the control, determined through the linear regression equation between concentration and percentage radical scavenging activity (Mulangsri & Zulfa, 2020).

$$\% \text{ Scavenging} = \frac{\text{DPPH absorbance} - \text{Absorbance of the test sample}}{\text{DPPH absorbance}} \times 100\% \quad (2)$$

Based on the percentage of radical scavenging at each concentration, a regression curve was constructed using the linear equation $y = bx + a$. The extract concentration (ppm) was plotted on the horizontal axis (x-axis), while the percentage of radical scavenging was plotted on the vertical axis (y-axis) (Mulangsri & Zulfa, 2020).

Data Analysis

The data obtained from the physical quality evaluation, stability testing of the essence, and antioxidant activity assay were statistically analyzed using SPSS version 24. Normality was assessed using the Shapiro–Wilk test to determine whether the data were normally distributed. If the data met the normality assumption, further analysis was performed using ANOVA (Mulangsri & Zulfa, 2020).

Result and Discussion

Plant Determination

Purple roselle flowers (*Hibiscus sabdariffa* L.) were obtained from the Roselle Flower Coffee & Tea Plantation, Borobudur, Krajan I, RT 2/RW 1, Kiyudan, Majaksingi, Borobudur District, Magelang Regency, Central Java 56553, Indonesia. The fresh purple roselle flowers that had been collected were then subjected to a determination process. Plant determination was carried out to confirm the taxonomic identity of the sample, ensuring that all extraction, formulation, and testing procedures were conducted using the correct plant material (Abdelfattah et al., 2024). The results of the plant identification confirmed that the sample used was indeed *Hibiscus sabdariffa* L., belonging to the Malvaceae family. This was verified by the issuance of the determination registration number TL.02.04/D.XI.6/12452.435/2025.

Table 2. Extraction yield

Weight of Simplisia (gram)	Weight of Extract (gram)	Extract Yield (%)
2000	301.6	15.08

The extract yield of 15.08% indicates an optimal result and meets the general standard of >10% (Dzulhija et al., 2024). The use of 70% ethanol as the extraction solvent for purple roselle flowers was based on its polarity, which enables effective dissolution of secondary metabolites, particularly polar compounds such as flavonoids and anthocyanins. Seventy percent ethanol is considered the most effective solvent because the balance between ethanol and water enhances the diffusion of active constituents from the plant matrix while maintaining the stability of thermolabile compounds. Previous studies have also reported that 70% ethanol is superior in extracting total phenolics and anthocyanins compared to solvents of either higher or lower ethanol concentrations, thereby contributing to

stronger antioxidant activity (Adinda et al., 2023; Darmayanti et al., 2024; Husna et al., 2022; Marbun, et al., 2024). Thus, the use of 70% ethanol not only preserves anthocyanin stability during extraction but also maximizes the flavonoid content obtained, making it highly relevant to the objectives of this study.

Extract Purification

Table 3. Purification results of the Purple Roselle (*Hibiscus sabdariffa* L.) extract

Weight of Extract (gram)	Weight of Purified Extract (gram)	% Yield of Purified Extract (%)
40	7.16	17.9%

Based on the results of the purification process, a high extract yield was obtained. This indicates that the purification using ethyl acetate through a liquid–liquid extraction method successfully enhanced the purity while preserving a significant number of bioactive compounds (Mulangsri & Zulfa, 2020). The effectiveness of ethyl acetate as a purification solvent is attributed to its intermediate polarity, which is ideal for extracting semi-polar compounds such as flavonoids. In contrast, highly non-polar or highly polar constituents remain in the previous extract or aqueous phase, allowing the ethyl acetate fraction to achieve a higher purity of active compounds. Another advantage of using ethyl acetate is its ease of evaporation during rotary evaporation due to its relatively low boiling point (approximately 77°C), which prevents degradation of thermolabile compounds. Its low density and viscosity also facilitate rapid phase separation with minimal risk of emulsion formation. Additionally, ethyl acetate is relatively safe, exhibits low toxicity, and is more environmentally friendly compared to halogenated solvents (Aldila et al., 2024; Ananda et al., 2025; Shikov et al., 2022; Wulandari & Yuniarti, 2023)

Phytochemical Screening

Table 4. Phytochemical screening results of the purified Purple Roselle (*Hibiscus sabdariffa* L.) extract

Screening	Result	Description
Flavonoid	Yellow-colored solution	Positive
Anthocyanin	Reddish-brown solution	Positive
Alkaloid	Dragendorff reagent: No precipitate, solution turns reddish-brown; Mayer reagent: No precipitate, solution turns yellow	Negative
Tannin	Dark green solution	Positive
Saponin	No foam formation	Negative

The phytochemical screening of the purified purple roselle (*Hibiscus sabdariffa* L.) extract showed the presence of flavonoids, anthocyanins, and tannins, while alkaloids and saponins were not detected. These results are consistent with previous studies reporting the predominance of polyphenolic secondary metabolites in purple roselle flowers (Ananda et al., 2025; Fardani et al., 2023; Wulandari & Yuniarti, 2023). The reaction schemes for the phytochemical screening are presented as follows.

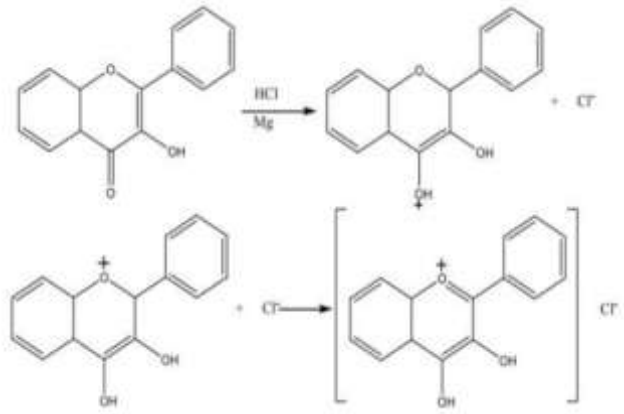

Figure 1. Flavonoid screening reaction equation (HARIS) (Rasmi et al., 2023)

Figure 1 illustrates the reduction process of flavonoid compounds present in the extract, in which the reaction with Mg^{2+} and concentrated HCl forms a yellow-colored $[Mg(OAr)_6]^{4-}$ complex (Oktapiya et al., 2022).

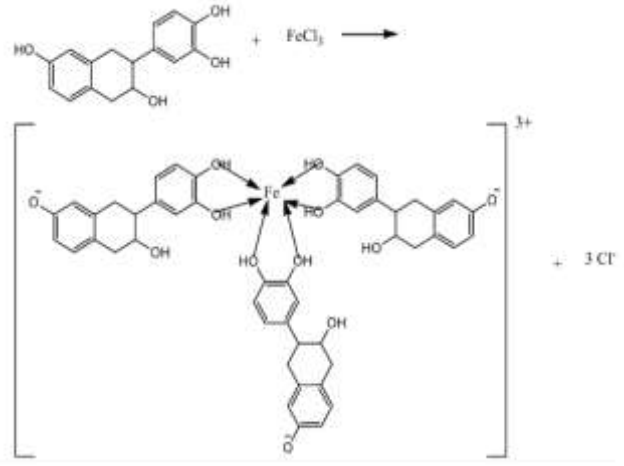

Figure 2. Tannin screening reaction equation (Darmayanti et al., 2024)

Figure 2 describes the reaction occurring during the tannin content test in the purified *Hibiscus sabdariffa* extract, in which the addition of $FeCl_3$ solution results in the formation of a colored complex dark green coloration, indicating the formation of a complex between tannins and Fe^{3+}) (Oktavia & Sutoyo, 2021).

Figure 3. Essence formulation

The negative results for alkaloid and saponin tests can be explained by the use of ethyl acetate as the solvent. Ethyl acetate is a medium-polarity solvent that selectively extracts polyphenolic compounds such as flavonoids, anthocyanins, and tannins, but is less effective in dissolving alkaloids and saponins, which tend to be more soluble in polar or specific semi-polar solvents (Mulangsri & Zulfa, 2020). Thus, the use of ethyl acetate in the fractionation process effectively enriches phenolic compounds as the primary bioactive constituents responsible for antioxidant activity, while reducing ballast compounds or non-essential secondary metabolites. This enhances the purity and pharmacological potential of the purified purple roselle extract (Sari, 2020).

Essence Preparation Formulation

Visual observations of the essence formulations showed differences in color appearance across all formulas. The negative control appeared white because it contained only the base ingredients without any active compound. The positive control exhibited a characteristic yellow color, corresponding to the description of pure quercetin, which is a yellow powder that dissolves in the formulation base. Meanwhile, Formulas FI–FIII containing purified purple roselle extract showed a brown coloration. The intensity of this color increased with higher concentrations of the purified extract, indicating that the greater the amount of extract incorporated into the formulation, the darker the resulting preparation.

Evaluation of the Physical Properties of the Preparation
Organoleptic Test

Table 5. Organoleptic test results of the essence preparation

Formula	Shape	Texture	Colour	Odour
I (1%)	Liquid	Non-sticky	Brown+	Odorless
II (3%)	Liquid	Non-sticky	Brown++	Odorless
III (5%)	Liquid	Non-sticky	Brown+++	Odorless
IV	Liquid	Non-sticky	White	Odorless
V	Liquid	Non-sticky	Yellow	Odorless

Description:
+, ++, +++ = Indicates the intensity of color depth

Organoleptic testing is an evaluation method of a preparation based on human sensory perception, including observations of shape, texture, color, and aroma, which aims to assess product acceptability as well as the consistency of its visual quality (Khalid et al., 2023; Suiyarti et al., 2025). Based on the results presented in Table 4, all essence formulations containing the purified purple roselle extract exhibited a liquid form with a non-sticky texture and odorless aroma, indicating that the addition of the purified extract did not affect the aroma of the essence preparation. A distinct difference was observed in the color parameter, in which formulations I–III showed a gradation of color intensity (+ to +++), corresponding to the increasing concentration of the purified extract. In contrast, formulation IV appeared white and formulation V exhibited a yellow color, reflecting differences in the composition of the ingredients used in the formulation and the bioactive content. Thus, the organoleptic evaluation demonstrates that the essence formulations containing purified purple roselle extract possess acceptable organoleptic characteristics.

Homogeneity Test

Table 6. Results of homogeneity test of the essence preparation

Formula	Result
I (1%)	Homogeneous
II (3%)	Homogeneous
III (5%)	Homogeneous
IV (Negative Control)	Homogeneous
V (Positive Control)	Homogeneous

Homogeneity test is an important parameter in the quality evaluation of a preparation, aimed at ensuring that both active and auxiliary ingredients are uniformly

dispersed without the presence of lumps, sediment, or phase separation, thereby guaranteeing the quality, stability, and dose consistency of the product. Based on the results presented in Table 6, all essence formulations containing the purified roselle extract (I–V) exhibited homogeneous characteristics, indicating that the mixing process was conducted properly and produced a uniform dosage system. The homogeneous condition also reflects good compatibility between the purified extract or quercetin and the base ingredients, ensuring that the bioactive compounds are evenly dispersed throughout the essence preparation.

Viscosity Test

Table 7. Viscosity Test Result

Formula	Result (poise) ± SD
I (1%)	5.87 ± 0.06
II (3%)	5.36 ± 0.06
III (5%)	5.42 ± 0.01
IV (Negative Control)	6.37 ± 0.03
V (Positive Control)	7.53 ± 0.01

Viscosity test result reflects the ease of use and the comfort of product application on the skin (Asanah et al., 2023). Based on Table 5, the purified purple roselle extract essence exhibited lower viscosity values compared with the negative and positive controls. The decrease observed in Formulas I–II is attributed to the interaction between the bioactive compounds of purple roselle (such as anthocyanins and polyphenols) and the solvent matrix or base ingredients, which subsequently influences the system's thickness. Nevertheless, the resulting viscosity remained within the acceptable range for a well-formulated essence (2.3–11.5 poise), indicating a sufficiently fluid consistency that facilitates ease of use and skin penetration without any signs of phase separation (Singh et al., 2023).

pH Test

pH test is one of the critical parameters in the quality evaluation of topical formulations, as pH influences the stability of active ingredients, product safety, and user comfort on the skin. The normal pH of human skin generally ranges from 4.5 to 6.5; therefore, formulations with a pH close to this range are more acceptable and less likely to cause irritation (Rahmadhena et al., 2024). Based on Table 8, the essence formulations containing purified purple roselle extract exhibited pH values ranging from 4.5 to 4.7 (Formulas I–III), while the negative control showed a pH of 5.0 and the positive control 5.7. The lower pH in the roselle-containing formulas is presumed to be influenced by the presence of anthocyanins and natural organic acids in the purified extract, which contribute to acidity and consequently decrease the pH of the preparation. Nevertheless, all formulations remain within the physiological pH range of the skin, indicating that they are safe for use and unlikely to disrupt the skin's protective barrier. Thus, the incorporation of roselle extract affects the reduction in pH, yet the final values remain compliant with the safety standards for topical formulations (Asanah et al., 2023).

Table 8. pH test result

Formula	Result
I (1%)	4.7
II (3%)	4.5
III (5%)	4.5
IV (Negative Control)	5.0
V (Positive Control)	5.7

Physical Stability Test of Essence Preparation

Based on the evaluation of physical properties, including organoleptic assessment, homogeneity, pH, and viscosity, all essence formulations containing purified purple roselle extract demonstrated characteristics that meet the required quality standards. Therefore, the analysis was continued with a stability test using the freeze–thaw method, which aims to assess the physical and chemical resilience of the formulation against extreme temperature variations and repeated cooling–heating cycles. This procedure ensures the consistency of quality, safety, and efficacy of the product during storage and distribution.

Organoleptic Test

Table 9. Results of organoleptic evaluation before and after cycling test

Formula	Before the Cycling Test				After the Cycling Test			
	Shape	Texture	Colour	Odour	Shape	Texture	Colour	Odour
I (1 %)	Liquid	Non-sticky	Brown+	Odourless	Liquid	Non-sticky	Brown+	Odourless
II (3 %)	Liquid	Non-sticky	Brown++	Odourless	Liquid	Non-sticky	Brown++	Odourless
III (5 %)	Liquid	Non-sticky	Brown+++	Odourless	Liquid	Non-sticky	Brown+++	Odourless
IV	Liquid	Non-sticky	White	Odourless	Liquid	Non-sticky	White	Odourless
V	Liquid	Non-sticky	Yellow	Odourless	Liquid	Non-sticky	Reddish brown	Odourless

Based on the organoleptic evaluation after the cycling test, all essence formulations remained in liquid form with a non sticky texture and were odorless, with the main difference observed in the color parameter, where Formula V as the positive control containing quercetin showed a color change from yellow to reddish brown, while Formulas I to IV remained stable. This change is related to the chemical nature of quercetin, which is highly sensitive to environmental conditions. As a flavonol with a conjugated system, quercetin easily undergoes oxidation of its phenolic groups when exposed repeatedly to extreme temperatures during the freeze thaw test, forming reactive o quinone compounds that can further undergo dimerization and polymerization, resulting in a darker color. In contrast, the roselle extract formulations showed better color stability due to the presence of a complex phytochemical matrix. The anthocyanin compounds, mainly delphinidin 3 sambubioside and cyanidin 3 sambubioside, interact with other phenolic compounds through hydrogen bonding and π interactions, creating a copigmentation effect that stabilizes the flavylium cation structure and reduces the susceptibility of anthocyanins to oxidation. These interactions also limit oxygen exposure and slow down the oxidation process, leading to better physicochemical stability compared to pure quercetin, which does not have this type of natural molecular protection (Darmayanti et al., 2024; Michelutti et al., 2020; Ruff, 2021; Supriatno et al., 2024; Woodby et al., 2020; Zou et al., 2025).

Viscosity Test

Table 11. Viscosity test results before and after cycling test

Formula	Before the Cycling Test (poise)	After the Cycling Test (poise)	Homogeneity Test	Normality Test	P Value
I (1 %)	5.90 ± 0.06	4.40 ± 0.15			
II (3 %)	5.40 ± 0.05	4.30 ± 0.03			
III (5 %)	5.40 ± 0.01	4.30 ± 0.01	sig. <0.05	sig. >0.05	0.001
IV	6.40 ± 0.02	5.70 ± 0.08			
V	7.50 ± 0.01	5.90 ± 0.03			

Based on the viscosity test results before and after the cycling test, all formulations exhibited a decrease in viscosity, particularly Formulas I–III containing the purified purple roselle extract, whereas the control formulas (IV and V) remained relatively stable. This decrease is presumed to be associated with weakened intermolecular interactions and phase redistribution induced by repeated temperature cycling, however, it did not result in any physical quality failure of the preparation (Ningrum et al., 2024). The statistical analysis showed that the data were normally distributed (sig. > 0.05) but not homogeneous (sig. < 0.05), thus the evaluation was continued using the Welch ANOVA test. The Welch ANOVA results demonstrated a significant difference between the measurements taken before and after the cycling test ($p < 0.05$). Despite the decrease in viscosity observed across all formulas, the values remained within the acceptable range for essence formulations, namely 2.3–11.5 poise (Asanah et al., 2023). Thus, all formulas, both before and after the cycling test, remained within the acceptable viscosity range. These results indicate that the essence formulations containing purified purple roselle extract at various concentrations continue to meet the required viscosity specifications and exhibit adequate physical stability despite exposure to extreme temperature conditions.

Homogeneity Test

Based on the homogeneity test results presented in Table 8, all formulations (I–V) exhibited homogeneous characteristics both before and after the cycling test. This indicates that the active ingredients and excipients in the essence were evenly dispersed without the presence of clumps, sediment, or phase separation, thereby maintaining the physical quality of the preparation. The absence of changes in homogeneity following the cycling test also demonstrates that the formulations were able to maintain physical stability despite exposure to extreme temperature fluctuations, which typically have the potential to induce coalescence, particle aggregation, or phase separation in emulsion and dispersion systems (Dzulhija et al., 2024). Thus, all essence formulations containing purified purple roselle extract exhibited good homogeneity stability, ensuring that they remain safe to use and maintain consistent quality throughout storage.

Table 10. Results of homogeneity evaluation before and after cycling test

Formula	Before the Cycling Test	After the Cycling Test
I	Homogeneity	Homogeneity
II	Homogeneity	Homogeneity
III	Homogeneity	Homogeneity
IV	Homogeneity	Homogeneity
V	Homogeneity	Homogeneity

pH Test

Table 12. pH test results before and after cycling test

Formula	Before the Cycling Test (poise)	After the Cycling Test (poise)	Homogeneity Test	Normality Test	P Value
I (1 %)	4.70 ± 0.06	6.20 ± 0.06			
II (3 %)	4.50 ± 0	5.70 ± 0.06			
III (5 %)	4.40 ± 0.06	5.50 ± 0	sig. < 0.05	sig. > 0.05	0.001
IV	5 ± 0.06	5.20 ± 0			
V	5.60 ± 0.06	6 ± 0.06			

Based on the pH evaluation before and after the cycling test, all formulas exhibited only minor changes in pH values and remained within the physiological skin range (4.5–6.5), indicating that they are safe and appropriate for essence formulations. Formulas I–III, which contain purified purple roselle extract, showed a slight increase in pH after the treatment. This increase may be associated with partial degradation of phenolic components or shifts in ion distribution induced by repeated temperature cycling during the freeze–thaw process, which can influence the acid–base equilibrium within the system (Asanah et al., 2023; Nabilah & Mentari, 2023). Statistical analysis showed that the data were normally distributed (sig. > 0.05) but not homogeneous (sig. < 0.05), thus the Welch ANOVA was used as an alternative to conventional ANOVA. The Welch test indicated significant differences among the formulas before and after the cycling test ($p < 0.05$). However, based on the pH values of all formulas, the purified purple roselle essence preparations remained within the acceptable pH range for essence formulations and demonstrated good pH stability even after exposure to extreme temperature conditions.

Antioxidant Activity of the Essence Formulations

In the antioxidant activity assay of the essence preparations using the DPPH method, the maximum wavelength (λmax) was determined to be 516 nm, with the absorbance of the DPPH solution measured at 0.5333. The operating time applied in the measurement was 30 minutes (Dzulhija et al., 2024). The next step involved preparing a series of concentrations of the essence formulations, each of which was added with DPPH solution at the same concentration and volume. The uniformity of the added DPPH volume ensured that differences in absorbance values were solely influenced by variations in the sample concentrations, rather than by differences in the amount of DPPH radicals available for reaction. This approach guarantees that the resulting data are valid and comparable across treatments (Wołosiak et al., 2021). After the incubation process (30 minutes), the absorbance of each sample was measured at the maximum wavelength for all concentrations, allowing the calculation of the percentage of free-radical scavenging and the determination of the IC_{50} value as an indicator of antioxidant potency (Cahyono et al., 2020).

Table 13. IC_{50} values of essence formulations I, II, III, IV, and V

Formula	IC_{50} (ppm)
I (1%)	57.96
II (3%)	47.01
III (5%)	33.63
IV (Negative Control)	-
V (Positive Control)	29.86

Based on the IC_{50} results presented in Table 13, Formula I (1%) exhibited an IC_{50} value of 57.96 ppm, which falls within the category of strong free-radical scavenging activity. Formula II (3%) exhibited an IC_{50} value of 47.01 ppm, while Formula III (5%) showed an IC_{50} value of 33.63 ppm; both are classified as having very strong free-radical scavenging activity. Formula IV, serving as the negative control, was not assessed for antioxidant activity because it contained only the base ingredients of the essence without any active compounds. Meanwhile, Formula V as the positive control demonstrated an IC_{50} value of 29.86 ppm, which also falls within the very strong category. These results indicate that higher concentrations of purified purple roselle extract correspond to lower IC_{50} values, thereby reflecting an increase in antioxidant activity (Rasmi Shahidi & Zhong, 2015).

Formulas I–III, which contain purified purple roselle extract, exhibited increasing antioxidant activity in line with the rising extract concentration, as reflected by the decreasing IC_{50} values. Scientifically, this phenomenon can be explained by the presence of anthocyanins primarily delphinidin-3-sambubioside and cyanidin-3-sambubioside which belong to the flavonoid group. Anthocyanins possess chemical structures that effectively neutralize free radicals through several key features: the ortho-dihydroxy groups on the B ring enable efficient hydrogen donation, the conjugated double-bond system (including the 2,3-double bond and the 4-oxo group on the C ring) facilitates electron delocalization, and the antioxidant activity is further enhanced by resonance stabilization

following electron or hydrogen donation (Har & Ismail, 2023; Inggrid et al., 2018; Lestari, 2022; Shafirany et al., 2021). The explanation of the reaction can be observed in the following chemical reaction equation.

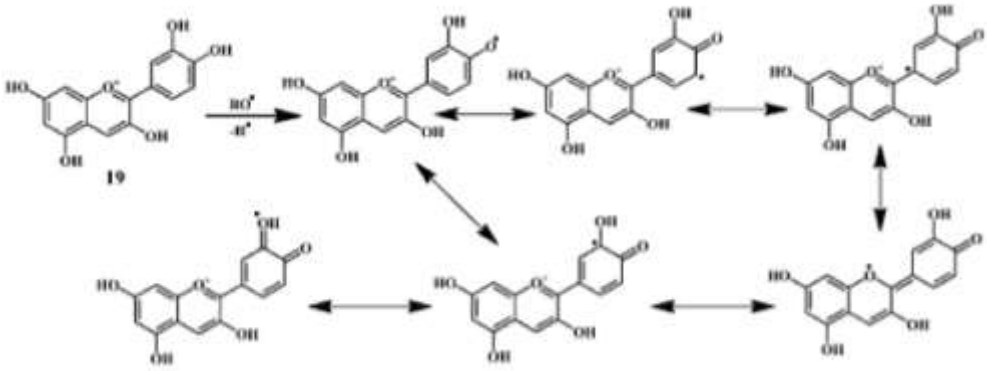

Figure 4. Mechanism of free radical scavenging by anthocyanin compounds (Lestari, 2022)

Conclusion

The purified purple roselle extract demonstrated significant antioxidant potential, with activity consistently increasing in line with the rising extract concentration. Formula I (57.96 ppm) exhibited strong antioxidant activity, while Formulas II (47.01 ppm) and III (33.63 ppm) were classified as very strong based on the standard IC_{50} classification (< 50 ppm). Formula III showed the highest antioxidant activity and fell within the same category as the quercetin control (29.86 ppm). In terms of physical stability, all formulations maintained acceptable parameters after the freeze thaw cycling test, including organoleptic properties, pH, viscosity, and homogeneity, although a color change was observed in the quercetin containing formulation due to its susceptibility to oxidative degradation. Overall, increasing the concentration of roselle extract not only enhanced antioxidant activity but also improved the physicochemical stability of the formulation, indicating its potential as a natural active ingredient in cosmetic applications. For future research, the development of nanoencapsulation systems is recommended as a strategy to enhance stability, protect bioactive compounds from oxidative degradation, and optimize bioavailability and targeted delivery. In addition, further exploration and optimization of more selective and efficient extraction methods are required to improve yield, purity, and the content of key active compounds, thereby supporting better consistency and performance of the formulation.

Acknowledgments
The authors express their sincere gratitude to the Research and Community Service Institute of Sekolah Tinggi Ilmu Kesehatan Nasional for the support and collaboration provided. Appreciation is also extended to all parties who contributed, both directly and indirectly, enabling the completion of this research and manuscript.

Author Contributions
Conceptualization, formal analysis, writing original draft preparation, N.Y.L. and D.N.C.; methodology, investigation, resources, N.Y.L., D.N.C., N.U., and L.M.; writing review and editing, D.N.C., N.U., and L.M.; visualization, D.N.C.; supervision, N.U. and L.M.; project administration, funding acquisition, N.Y.L. All authors have read and approved the published version of the manuscript.

Funding
This research was funded by the Research and Community Service Institute of Sekolah Tinggi Ilmu Kesehatan Nasional, with a total funding amount of Rp 10,000,000. The APC was funded by the same institution.

Conflicts of Interest
The authors declare no conflict of interest. The authors have no personal circumstances or interests that could be perceived as influencing the representation or interpretation of the reported research results. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

Abdelfattah, H. M. E., Hussein, H. A., Teleb, S. S., El-Demerdash, M. M., & George, N. M. (2024). Chemotaxonomy Compared to Morphological and Anatomical Taxonomy of Five Hibiscus Species. *Journal of Plant Research*, *137*(6), 967–984. https://doi.org/10.1007/s10265-024-01566-9

Adinda, A. A., Limanan, D., & Ferdinal, F. (2023). Ekstrak Bunga Rosella (Hibiscus sabdariffa): Uji Fitokimia, Total Antioksidan, dan Kadar Fenolik Total. *Jurnal Kesehatan Tambusai*, *4*(3), 3580–3586. https://doi.org/10.31004/jkt.v4i3.16215

Aldila, H., Indriawati, A., & Putro, P. A. (2024). Purification and Characterization of Monazite from Bangka Using Mechanical-Magnetic Separation Method. *Jurnal Penelitian Pendidikan IPA*, *10*(5), 2745–2751. https://doi.org/10.29303/jppipa.v10i5.6644

Ananda, K. R., Muthmainah, M., & Hikmayani, N. H. (2025). Kelakai (Stenochlaena palustris (Burm.) Bedd) Extract from Kalimantan: Determination of Total Phenol and Alkaloid Content Potential as Nutraceuticals. *Jurnal Penelitian Pendidikan IPA*, *11*(1), 551–557. https://doi.org/10.29303/jppipa.v11i1.9786

Asanah, F. M., Suryanti, L., & Nurlaeli, L. (2023). Formulasi dan Uji Stabilitas Fisik Sediaan Essence dari Ekstrak Etanol 96% Daun Bayam Merah (Amaranthus tricolor L.) sebagai Perawatan Kulit Wajah. *Jurnal Ilmiah Farmasi Indonesia (JIFIN)*, *1*(1), 28-38. Retrieved from https://journals.stikim.ac.id/index.php/jifin/article/view/2533

Cahyono, B., Setyadewi M., C., Suzery, M., & Aminin, A. L. N. (2020). The Comparison of Spectrophotometric and TLC-Densitometric for DPPH Radical Scavenging Activity Analysis of Three Medicinal Plant Extracts. *JKPK (Jurnal Kimia dan Pendidikan Kimia)*, *5*(2), 110. https://doi.org/10.20961/jkpk.v5i2.40370

Darmayanti, D., Dewi, N. P., Mariyani, M., Tandi, J., Rachmawati, M., & Susanto, Y. (2024). Subchronic Toxicity Test of Purple Leaves Ethanol Extract (PLEE) on the Histopathological Picture of the Lymph of Wistar Rats and Antioxidant Activity. *Jurnal Penelitian Pendidikan IPA*, *10*(7), 3942–3948. https://doi.org/10.29303/jppipa.v10i7.8070

Dhar, R., Rana, M. N., Zhang, L., Li, Y., Li, N., Hu, Z., Yan, C., Wang, X., Zheng, X., Liu, H., Cui, H., Li, Z., & Tang, H. (2021). Phosphodiesterase 4B is Required for NLRP3 Inflammasome Activation by Positive Feedback with Nrf2 in the Early Phase of LPS- Induced Acute Lung Injury. *Free Radical Biology and Medicine*, *176*, 378–391. https://doi.org/10.1016/j.freeradbiomed.2021.10.007

Dzulhija, A. F., Aisiyah, S., & Harjanti, R. (2024). Formulasi dan Uji Aktivitas Antioksidan Sediaan Essence Ekstrak Etanol Bunga Telang (Clitoria ternatea L.) dengan Variasi Konsentrasi Butilen Glikol. *Jurnal Farmasi*, *13*(2), 1–21. Retrieved from https://garuda.kemdiktisaintek.go.id/documents/detail/4774473

Fardani, R., Rodiatullah, R., Halid, I., & Atfal, B. (2023). Uji Aktivitas Antioksidan Ekstrak Bunga Rosella (*Hibiscus sabdariffa*) Menggunakan Metode 1,1-Difenil-2-Pikrilhidrazyl (DPPH). *JSN: Jurnal Sains Natural*, *1*(4), 97–100. https://doi.org/10.35746/jsn.v1i4.411

Forman, H. J., & Zhang, H. (2021). Targeting Oxidative Stress in Disease: Promise and Limitations of Antioxidant Therapy. *Nature Reviews Drug Discovery*, *20*(9), 689–709. https://doi.org/10.1038/s41573-021-00233-1

Har, L. W., & Ismail, I. S. (2023). Antioxidant Activity, Total Phenolics And Total Flavonoids Of Syzygium Polyanthum (Wight) Walp Leaves. *Int. J. Med. Arom. Plants*.

Husna, N. E., Noor, E., Fahma, F., & Sunarti, T. C. (2022). Teknik Ekstraksi dan Nanoenkapsulasi Komponen Bioaktif Buah Malaka: Tinjauan Literatur. *Agrointek: Jurnal Teknologi Industri Pertanian*, *16*(2), 171–185. https://doi.org/10.21107/agrointek.v16i2.12433

Inggrid, M., Hartanto, Y., & Widjaja, J. F. (2018). Karakteristik Antioksidan pada Kelopak Bunga Rosella (Hibiscus sabdariffa Linn.). *Jurnal Rekayasa Hijau*, *2*(3). https://doi.org/10.26760/jrh.v2i3.2517

Khalid, M. M., Chadha, M. M., Nath, M. V., Mehta, D. S., Chawla, D. A., Piplani, D. S., Siddheshwar, D. S., & Deshmukh, D. R. (2023). Assessment of Organoleptic and Physicochemical Properties of Herbal Shampoos: Formulation Considerations of Fermentation Method. *Eur. Chem. Bull.*, *12*(4), 543-553. Retrieved from https://www.researchgate.net/publication/370410935

Lestari, G. A. D. (2022). Skrining Fitokimia dan Uji Aktivitas Antioksidan Ekstrak Etanol Bunga Rosella Ungu (Hibiscus sabdariffa L.). *Jambura Journal of Chemistry*, *4*(1), 17–24. https://doi.org/10.34312/jambchem.v4i1.11157

Liu, Q., Wu, H., Luo, J., Liu, J., Zhao, S., Hu, Q., & Ding, C. (2021). Effect of Dielectric Barrier Discharge Cold Plasma Treatments on Flavor Fingerprints of Brown Rice. *Food Chemistry*, *352*, 129402. https://doi.org/10.1016/j.foodchem.2021.129402

Marbun, A. P., Suryanto, D., & Elimasni, E. (2024). Phytochemical Screening, Activity Antioxidant and Activity Test of Antimicrobials Ethanol Extract of Gagatan Harimau Leaves (Paraboea leuserensis B.L Burtt). *Jurnal Penelitian Pendidikan IPA*, *10*(12), 10909–10916. https://doi.org/10.29303/jppipa.v10i12.8428

Michelutti, L., Bulfoni, M., Bolzon, V., & Nencioni, E. (2020). Preliminary Evidence of a Molecular Detection Method to Analyze Bacterial DNA as a Quality Indicator in Cosmetics. *Cosmetics*, *7*(3), 54. https://doi.org/10.3390/cosmetics7030054

Mulangsri, D. A. K., & Zulfa, E. (2020). Uji Aktivitas Antibakteri Ekstrak Terpurifikasi Daun Mangga Arumanis (Mangifera indica L.) dan Identifikasi Flavonoid dengan KLT: Antibacterial Activity of

Arumanis Mango Leaves (Mangifera indica L.) Purified Extract and Identification of Flavonoid by TLC. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *6*(1), 55–62. https://doi.org/10.22487/j24428744.2020.v6.i1.14044

Nabilah, T. U., & Mentari, I. A. (2023). Formulation and Physical Stability Test of Essence Sheet Mask Preparation from Kokang Leaf Extract (Lapisanthes Amoena). *Jurnal Mandala Pharmacon Indonesia*, *9*(1), 80–86. https://doi.org/10.35311/jmpi.v9i1.302

Ningrum, Y. D. A., Sholeh, A. B., & Ramadhini, A. (2024). Optimasi Formula dan Uji Evaluasi Fisik Sediaan Essence Kombucha dengan Variasi Konsentrasi Propylenglikol dan Gliserin. *JIFI (Jurnal Ilmiah Farmasi Imelda)*, *7*(2), 86–95. https://doi.org/10.52943/jifarmasi.v7i2.1518

Oktapiya, T. R., Pratama, N. P., & Purnamaningsih, N. (2022). Analisis Fitokimia dan Kromatografi Lapis Tipis Ekstrak Etanol Daun Rosella (Hibiscus sabdariffa L.). *Sasambo Journal of Pharmacy*, *3*(2), 105–110. https://doi.org/10.29303/sjp.v3i2.181

Oktavia, F. D., & Sutoyo, S. (2021). SKRIning Fitokimia, Kandungan Flavonoid Total, dan Aktivitas Antioksidan Ekstrak Etanol Tumbuhan *Selaginella doederleinii*. *Jurnal Kimia Riset*, *6*(2), 141. https://doi.org/10.20473/jkr.v6i2.30904

Parapat, A. R. B., Ridwanto, R., Yuniarti, R., & Rani, Z. (2025). Uji Aktivitas Antioksidan Ekstrak dan Fraksinasi Daun Tekelan (Chromolaena odorata (L.) R.M.King & H.Rob) Serta Uji Antibakteri Terhadap Staphylococcus aureus dan *Escherichia coli*. *Journal of Pharmaceutical and Sciences*, *8*(4), 2560–2567. https://doi.org/10.36490/journal-jps.com.v8i4.1059

Rahmadhena, M. P., Amazona, G. J., Mutmainah, N., Niladitya, R., Fitriana, F., & Wulandari, W. (2024). Effectiveness of Snakehead Fish Extract on the Healing Time of Perineal Wounds in Postpartum Women Who Experience Perineal Rupture in the Jakarta-Tangerang Region in 2024. *Jurnal Penelitian Pendidikan IPA*, *10*(11), 8552–8557. https://doi.org/10.29303/jppipa.v10i11.8726

Rasmi, D. A. C., Zulkifli, L., & Ahadia, N. (2023). Antimicrobial Activity Test of Bitter Melon (Momordica charantia L.) Plant Extract Against Staphylococcus Epidermidis, Escherichia Coli Bacteria and Candida albicans. *Jurnal Penelitian Pendidikan IPA*, *9*(2), 454–458. https://doi.org/10.29303/jppipa.v9i2.3699

Ruff, P. G. (2021). Thermal Effects of Percutaneous Application of Plasma/Radiofrequency Energy on Porcine Dermis and fibroseptal network. *Journal of Cosmetic Dermatology*, *20*(7), 2125–2131. https://doi.org/10.1111/jocd.13845

Sari, F., Hidayatul, F., Sariwati, A., Wahyuni, D., & Hesturini, R. J. (2024). Korelasi Kadar Total Flavonoid Ekstrak dan Fraksi Polar Kelopak Rosella (Hibiscus sabdariffa L.) Terhadap Aktivitas Antioksidan dengan Metode DPPH (2,2-Difenil-1-Pikrilhidrazil). *Bioscientist: Jurnal Ilmiah Biologi*, *12*(2), 1937. https://doi.org/10.33394/bioscientist.v12i2.13205

Sari, L. O. R. K. (2020). Pemanfaatan Obat Tradisional dengan Pertimbangan Manfaat dan Keamanannya. *Pharmaceutical Sciences and Research*, *3*(1), 1–7. https://doi.org/10.7454/psr.v3i1.3394

Shafirany, M. Z., Indawati, I., Sulastri, L., Sadino, A., Kusumawati, A. H., & Alkandahri, M. Y. (2021). Antioxidant Activity of Red and Purple Rosella Flower Petals Extract (Hibiscus sabdariffa L.). *Journal of Pharmaceutical Research International*, 186–192. https://doi.org/10.9734/jpri/2021/v33i46B32931

Shahidi, F., & Zhong, Y. (2015). Measurement of Antioxidant Activity. *Journal of Functional Foods*, *18*, 757–781. https://doi.org/10.1016/j.jff.2015.01.047

Shikov, A. N., Mikhailovskaya, I. Yu., Narkevich, I. A., Flisyuk, E. V., & Pozharitskaya, O. N. (2022). Methods of Extraction of Medicinal Plants. In *Evidence-Based Validation of Herbal Medicine* (pp. 771–796). Elsevier. https://doi.org/10.1016/B978-0-323-85542-6.00029-9

Sies, H., & Jones, D. P. (2020). Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. *Nature Reviews Molecular Cell Biology*, *21*(7), 363–383. https://doi.org/10.1038/s41580-020-0230-3

Singh, U., Chamolia, M., Singh, K., Lalit, M., & Maheshwari, R. K. (2023). Enormously Beneficial Chia Seeds in Modern Food Regimen. *International Journal of Environment and Health Sciences*, *4*(1). https://doi.org/10.47062/1190.0401.04

Suiyarti, W., Ervianingsih, E., Astari, C., Riska, R., & S, S. (2025). Determination of SPF (Sun Protection Factor) Value in Sunscreen Formulation of Vetiveria Zizanioides L. Essential Oil. *Jurnal Penelitian Pendidikan IPA*, *11*(4), 224–227. https://doi.org/10.29303/jppipa.v11i4.10893

Supriatno, S., Riska, R., Rahmatan, H., Nur, Y. I. M., & Ulhusna, F. A. (2024). Toxicity Test of Red-Shoot Leaves (Syzygium myrtifolium Walp.) Extract as Biolarvicide on Filariasis Vector Mortality. *Jurnal Penelitian Pendidikan IPA*, *10*(4), 1648–1654. https://doi.org/10.29303/jppipa.v10i4.5454

Wołosiak, R., Drużyńska, B., Derewiaka, D., Piecyk, M., Majewska, E., Ciecierska, M., Worobiej, E., & Pakosz, P. (2021). Verification of the Conditions for Determination of Antioxidant Activity by ABTS and DPPH Assays—A Practical Approach.

Molecules, *27*(1), 50. https://doi.org/10.3390/molecules27010050

Woodby, B., Penta, K., Pecorelli, A., Lila, M. A., & Valacchi, G. (2020). Skin Health from the Inside Out. *Annual Review of Food Science and Technology*, *11*(1), 235–254. https://doi.org/10.1146/annurev-food-032519-051722

Wulandari, P., & Yuniarti, E. (2023). Bioactivity Potential and Chemical Compounds of Cinnamomum: Literature Review. *Jurnal Penelitian Pendidikan IPA*, *9*(5), 1–7. https://doi.org/10.29303/jppipa.v9i5.3284

Zou, P., Otero, P., Mejuto, J. C., Simal-Gandara, J., Xiao, J., Cameselle, C., Chen, S., Lin, S., & Cao, H. (2025). Exploring the Mechanism of Flavonoids Modification by Dimerization Strategies and their Potential to Enhance Biological Activity. *Food Chemistry*, *467*, 142266. https://doi.org/10.1016/j.foodchem.2024.142266