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Antioxidant activity of formulation of Roselle calyx herbal syrup as a functional beverage

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ABSTRACT

Roselle (*Hibiscus sabdariffa*) flowers are rich in anthocyanins and organic acids, which contribute to their characteristic red-colored calyx and sour flavor, along with notable biological activities. This study aimed to develop and optimize a functional roselle syrup formulation by incorporating selected spices—cinnamon, cloves, and cardamom—and using xanthan gum and Tween 60 as stabilizing agents. Six formulations (F1–F6) were prepared and evaluated based on their physicochemical characteristics, antioxidant activity, and stability. Among the formulations, F3 (containing 1% xanthan gum and 0.1% Tween 60) showed the most favorable results, with a pH of 2.73, viscosity of 403.3 cP, total anthocyanin content of 67.85 mg/L, and DPPH free radical inhibition of 50.43% and 64.05% at concentrations of 1% v/v and 2% v/v, respectively. Upon reconstitution at a 1:5 ratio, the syrup retained appealing organoleptic qualities, including a bright red color, sweet-sour taste, and herbal aroma. During a 42-day storage study, the syrup maintained stable viscosity and antioxidant activity for up to 21 days, with pH remaining within the acceptable range (2–4) despite a slight increase. However, anthocyanin content showed significant degradation after day 7. These findings highlight the need for further formulation improvement to enhance anthocyanin and antioxidant stability in roselle syrup products.

Keywords: antioxidant, functional beverages, foods, *Hibiscus sabdariffa*, roselle, syrups

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INTRODUCTION

Functional beverages are a subset of functional foods, a term that is more of a marketing concept without legal definitions in many countries. Academically, functional beverages are commonly defined as drinks that contain biologically active ingredients offering health benefits that go beyond basic nutritional value (Cong et al., 2020). Functional beverages offer specific physiological effects on the body, including reducing blood pressure, enhancing immune function, and helping to prevent chronic illnesses. These beverages must also provide sensory satisfaction, such as enjoyable taste and texture, to appeal to consumers. Functional beverages have become the most popular category of functional foods due to their functionality, nutritional and bioactive compound content, and convenience in terms of consumption, flexibility in form, size, storage, and flavor (Maleš et al., 2022). Generally, functional beverages are categorized into fruit-based drinks (juice, squash, nectar, syrup, etc.), stimulant beverages (including tea, coffee, and sports drinks), and those derived from milk, plants, or combinations of various ingredients mixed with water to deliver energy and nutritional benefits (Aadil et al., 2019).

Syrup is a concentrated or saturated aqueous solution, thick in consistency, containing sucrose or sugar substitutes with or without flavoring agents and medicinal substances in purified water. Simple syrup typically contains 65–66.7% w/w sucrose (Shaikh et al., 2024). In the food industry, syrup is a type of soft drink made as a thick sugar solution with or without food additives, in accordance with applicable regulations, and with a variety of flavors depending on the main ingredients used.The advantages of this form of formulation include its ease of serving and relatively long shelf life (Sari et al., 2016).

Medicinal plants are a source of bioactive compounds that can be utilized in the production of functional beverages due to their chemical composition, aroma, taste, and health benefits. Roselle (*Hibiscus sabdariffa*) is one such plant that meets these characteristics (Maleš et al., 2022). The calyces of roselle have been scientifically proven to exhibit antioxidant, antidiabetic, antihypertensive, anticancer, immunomodulatory, hepatoprotective, and other biological activities (Izquierdo-Vega et al., 2020). These biological activities are attributed to its diverse chemical content, particularly phenolic acids, flavonoids, anthocyanins, and organic acids (Hapsari & Setyaningsih, 2021; Izquierdo-Vega et al., 2020). In addition to their biological activities, anthocyanins and organic acids also contribute to the red color and sour taste of roselle calyces (Izquierdo-Vega et al., 2020; Kartini et al., 2023; Millinia et al., 2024). While the sour taste of roselle-based beverages can provide a refreshing flavor sensation, excessively high acidity may be intolerable for certain consumer groups. Therefore, combining roselle with other medicinal and aromatic herbal plants is essential to improve its taste and aroma. Several studies have been conducted to develop roselle into functional foods and beverages, including herbal tea, jam, syrup, jelly candy, and carbonated juice (Ananingsih et al., 2023 ; Ashaye & Adeleke, 2009; Darnal et al., 2021; Halim et al., 2022; Nguyen & Chuyen, 2020).

However, research on formulating roselle into syrup combined with other herbs remain very limited. Therefore, this study formulated roselle syrup combined with cinnamon bark, clove buds, and cardamom fruits. Formulation optimization was carried out using xanthan gum and Tween 60 at various concentrations. Tween 60, a nonionic surfactant, is commonly used to improve the solubility and dispersion of hydrophobic compounds in aqueous systems, and has been applied in various liquid formulations to enhance stability and bioavailability (Hussein & El-Naggar, 2021; Ziani et al., 2012). Tween 60 is especially useful in functional formulations due to its ability to maintain dispersion and emulsification, which supports the stability of active compounds in aqueous environments. Meanwhile, xanthan gum is widely utilized as a natural thickening and stabilizing agent in both food and pharmaceutical syrups, owing to its high viscosity even at low concentrations and its effectiveness in preventing phase separation (Garcıa-Ochoa et al., 2000; Yang et al., 2025). Kim and Yoo (2006) showed that xanthan gum helped maintain syrup consistency and color stability during heating and storage (Kim & Yoo, 2006). These properties make xanthan gum ideal for ensuring the homogeneity and shelf stability of herbal syrup formulations. Therefore, these two excipients were selected to optimize the physical and

functional characteristics of the roselle syrup formulation in this study. The optimal formulation was then tested for stability over 42 days, with evaluation parameters including organoleptic properties, viscosity, pH, total anthocyanin content, and antioxidant activity. To the best of our knowledge, studies on the formulation of roselle syrup combined with cinnamon bark, clove buds, and cardamom fruits, particularly optimized using xanthan gum and Tween 60 to improve its stability and antioxidant properties, have not been reported. Therefore, this study provides novel insights into the development of roselle-based functional beverages with enhanced sensory qualities and shelf stability through appropriate excipient optimization.

MATERIALS AND METHOD

Materials

The plant materials used in this study included the red and purple variants of roselle calyces, clove buds, cardamom fruits, and cinnamon bark. The authenticity of the plant materials was verified by the Center for Information and Development of Traditional Medicine, University of Surabaya. The food-grade chemicals used comprised sucrose (PT. Sweet Indolampung, Indonesia), xanthan gum, Tween 60, and sodium benzoate (C_6H_5COONa) (Qingdao Shengda Commercial & Trade Co., Ltd., Qingdao, China). Other pro-analytical grade chemicals were sourced from Merck KGaA (Darmstadt, Germany), including potassium chloride (KCl), sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), disodium phosphate (Na_2HPO_4), hydrochloric acid (HCl), and ethanol. Additionally, ascorbic acid ($C_6H_8O_6$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from HiMedia Laboratories, LLC (Pennsylvania, USA).

Methods

Formulation of roselle syrup

In this study, roselle syrup was prepared using a combination of direct heating and natural ingredient extraction methods. The syrup was prepared in six different variations (Table 1), differing in the concentrations of xanthan gum and Tween 60. The selected concentration of Tween 60 (0.1–0.2% w/v) reflects its function as a nonionic emulsifier used in the final syrup formulation to ensure uniform dispersion and enhance physical stability without compromising sensory quality (Hussein & El-Naggar, 2021; Ziani et al., 2012). Xanthan gum (0.2–1.0% w/v) was included for its proven role as a thickening and stabilizing agent at low concentrations, improving viscosity and stability while preserving pourability and palatability (Garcıa-Ochoa et al., 2000; Kim & Yoo, 2006; Yang et al., 2025). These ranges facilitated assessment of their effects on the syrup's physicochemical and sensory characteristics.

Table 1. Roselle syrup composition

Component*	F1	F2	F3	F4	F5	F6	Function
Red and purple roselle, 1:1 (% w/v)	8.4	8.4	8.4	8.4	8.4	8.4	Main herb
Cardamom (% w/v)	2.1	2.1	2.1	2.1	2.1	2.1	Complementary herb
Clove (% w/v)	2.1	2.1	2.1	2.1	2.1	2.1	Complementary herb
Cinnamon (% w/v)	8.4	8.4	8.4	8.4	8.4	8.4	Complementary herb
Sucrose (% w/v)	70	70	70	70	70	70	Sweetener
Tween 60 (% w/v)	0.1	0.1	0.1	0.2	0.2	0.2	Emulsifier, stabilizer
Xanthan gum (% w/v)	0.2	0.4	1	0.2	0.4	1	Thickener, stabilizer
Sodium benzoate (% w/v)	0.2	0.2	0.2	0.2	0.2	0.2	Preservative
Water up to (% v/v)	100	100	100	100	100	100	Solvent

* Each syrup formulation at the laboratory scale was prepared in a quantity of 1000 mL

The preparation of the syrup began by weighing 21 g each of cardamom, cloves, and 84 g of cinnamon bark. These ingredients were then heated on a hot plate magnetic stirrer (Cimarec from Thermo Fisher Scientific, Waltham, MA, USA) with 1600 mL of water at 100 °C for 15 minutes. After

heating was stopped, 84 g of purple and red roselle calyces (1:1) were added to the mixture and left to stand for 1 hour. The mixture was then filtered, and a portion of the filtrate was used to dissolve 700 g of sucrose, xanthan gum (weight as per formulation), and 2 g of sodium benzoate separately. The xanthan gum solution was subsequently mixed with Tween 60 (weight as per formulation). Tween 60 was added after filtration to facilitate uniform dispersion and improve the physical stability of the syrup, acting as a food-grade emulsifier commonly used in liquid formulations. The three solutions were then combined until homogeneous and reheated using a hot plate at 80–90°C until the final syrup volume reached 1000 mL. The syrup was then allowed to cool to room temperature before being packaged in 100 mL bottles. The schematic process for syrup preparation is shown in Figure 1.

Figure 1. Flowchart of the roselle syrup preparation process

Evaluation of physicochemical characteristics and antioxidant activity of roselle syrup

The physicochemical characteristics of roselle syrup were evaluated through organoleptic observations, viscosity determination, pH measurement, and total anthocyanin content analysis. Meanwhile, the antioxidant activity of the syrup was determined using the DPPH free radical scavenging assay.

Organoleptic observations

The concentrated roselle syrup and its reconstituted form with water (1:5) were analyzed organoleptically for color, taste, and aroma.

pH measurement

The pH analysis was conducted to determine the acidity level of the syrup using a pH meter (Mettler Toledo FP-20; Ohio, US). Prior to measurement, the pH meter was calibrated with buffer solutions of

pH 4.0 and 7.0. Ten milliliters of syrup were placed into a 50 mL beaker, and the electrode was immersed in the syrup. The pH value of the formulation was recorded at room temperature.

Viscosity determination

Viscosity analysis of the concentrated roselle syrup was conducted using a VT-04 viscometer (Rion Co., Ltd., China). Prior to each day's measurements, the viscometer was calibrated at 25 ± 1 °C using a certified silicone oil standard (350 cP at 25 °C), and the instrument constant Kv was recalculated according to the manufacturer's protocol. The analysis was then performed at a spindle speed of 60 rpm with spindle number 3. The spindle was inserted into the sample, allowed to rotate until torque stabilized within the recommended 10–90% range, and the displayed viscosity value was recorded.

Determination of total anthocyanin content (TAC)

Total anthocyanin content (TAC) was determined using the pH differential method as outlined by Lee et al. (2005), with some modifications (Lee et al., 2005). Initially, 300 µL of syrup was mixed with 2700 µL of a pH 1.0 buffer solution, which was prepared by dissolving 1.86 g of potassium chloride (KCl) in distilled water, adjusting the pH to 1.0 using approximately 6.3 mL of HCl, and diluting the solution to 1 L. Another 300 µL aliquot of the same syrup sample was combined with 2700 µL of a pH 4.5 buffer solution, prepared by dissolving 54.43 g of sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) in distilled water, adjusting the pH to 4.5 with about 20 mL of HCl, and diluting to 1 L. These two buffered solutions were used to assess anthocyanin content based on their absorbance, measured with a UV-Vis spectrophotometer (Shimadzu UV 1900; Shimadzu, Kyoto, Japan) at 520 nm and 700 nm. The final absorbance (A) was calculated using the following equation, $A = [(A_{520} - A_{700})\, pH_{1.0} - (A_{520} - A_{700})\, pH_{4.5}]$. TAC was then expressed as cyanidin-3-glucoside equivalents using the appropriate calculation formula (1).

$$\text{TAC (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad \text{.....(1)}$$

where, A = final absorbance, MW = molecular weight of cyanidin-3-glucoside, which is 449.2 g/mol, DF = dilution factor, ε = molar extinction coefficient of cyanidin-3-glucoside (26,900 L x mol^{-1} x cm^{-1}), l = path length of the cuvette (1 cm), and 10^3 = conversion factor from grams to miligrams.

Antioxidant activity assay

The antioxidant activity of the syrup was assessed using the DPPH free radical scavenging assay (Kartini et al., 2019), with ascorbic acid as the reference compound. A 100 µL aliquot of the sample or reference solution was transferred into a well of a 96-well clear polystyrene microplate, followed by the addition of 50 µL of DPPH solution (0.026% in ethanol). The mixture was then thoroughly homogenized and incubated in the dark for 10 minutes. After the incubation period, the absorbance was measured using a microplate reader (UVM 340 Biochrom; Biochrom Ltd., Cambridge, UK) at a wavelength of 517 nm. For the blank, 100 µL of the appropriate sample solution was mixed with 50 µL of ethanol. The absorbance of the DPPH solution mixed with water (50 and 100 µL) was also measured to determine the control absorbance. The radical scavenging activity was then calculated as percentage of inhibition using the Equation (2).

$$\text{Inhibition (\%)} = \frac{(A_c - A_s)}{A_c} \times 100\% \quad \text{..... (2)}$$

where, A_c = control absorbance and A_s = sample absorbance.

Stability test

After evaluating the syrup formulations, the formulation with the best characteristics was selected for stability testing. The test was conducted by placing the formulation in a dark box equipped with a thermohygrometer and storing it at room temperature (27 ± 2 °C). The syrup was stored for 6 weeks (42 days), with samples taken at six time points: day 0, 7, 14, 21, 28, and 42 (referred to as t0, t7, t14, t21, t28, and t42). This short-term stability evaluation was based on previously established protocols for liquid herbal formulations under ambient conditions (OECD, 2004). The time intervals selected were intended to monitor early-phase physical and chemical changes, as short-term stability testing typically spans 4 to 6 weeks. Storage in a dark environment was applied to minimize photodegradation, especially of light-sensitive anthocyanins. The selected time intervals were intended to monitor changes in physical and chemical characteristics throughout the storage period. The testing parameters included organoleptic evaluation, pH value, viscosity, TAC, and DPPH free radical scavenging activity.

Data Analysis

Statistical analysis was performed using two methods: descriptive and inferential statistics. Descriptive statistics were used to compare the obtained data with acceptance criteria, while inferential statistics were applied to compare data between formulations and across sampling times. Inferential analysis ($\alpha = 0.05$) was performed using one-way ANOVA or the Kruskal-Wallis test, followed by post hoc tests such as LSD or Mann-Whitney U, depending on the data distribution and variance. The analyses were performed using SPSS version 26 (SPSS Inc.; Illinois, US). Unless otherwise stated, all experiments were performed with three replicates.

RESULT AND DISCUSSION

Organoleptic properties of roselle syrup

In general, roselle syrup from the six formulations (F1–F6) exhibited similar organoleptic characteristics. They were thick, pourable liquids with a reddish-purple or dark red color, a very sweet and sour taste, and a distinct herbal aroma from roselle, cinnamon, cardamom, and cloves. After being reconstituted with water at a 1:5 ratio, the syrups became thin liquids with a bright red color, a sweet and sour taste, and a similar herbal aroma to the original formulation. The differences among F1–F6 were in the concentrations of xanthan gum and Tween 60. The lack of variation in organoleptic properties might be due to xanthan gum's neutral taste, which does not affect the flavor even at higher concentrations (Ramadhan et al., 2015; Rowe et al., 2009). Meanwhile, Tween 60 is slightly yellow with a weak characteristic odor and a mildly bitter taste (Heng et al., 2014). Because it was used in very small amounts (0.1 and 0.2 %v/v), its addition did not impact the organoleptic properties of the syrup. An example of the visual characteristics of the roselle syrup before and after reconstitution is shown in Figure 2.

Figure 2. Roselle syrup before (A) and after reconstitution with water at a 1:5 ratio (B)

The red color of the roselle syrup is due to the anthocyanin content in roselle calyx, water-soluble pigments such as delphinidin-3-O-sambubioside, cyanidin-3-O-sambubioside, cyanidin-3-glucoside,

and delphinidin pentoside-glycoside (Hapsari & Setyaningsih, 2021; Izquierdo-Vega et al., 2020; Wu et al., 2018). Before reconstitution, the roselle syrup tastes extremely sweet due to its high sucrose content (70%), intended to serve as a preservative. Sucrose is a widely applied additive, functioning as both a sweetener and a preservative. Sugar, a common ingredient in food and confectionery, plays a vital role in the formulation of syrups by contributing to viscosity, sweetness, and stability. Additionally, it has therapeutic applications, such as in sugar pastes, where it promotes wound healing (Rowe et al., 2009). In this study, the inclusion of sucrose enhanced the texture and stability of the roselle syrup, contributing to its overall sensory and physical properties.

However, after reconstitution with water at an appropriate ratio, the syrup produces a sweetness level acceptable to the palate. Roselle contains various organic acids, such as citric acid, hydroxycitric acid, hibiscus acid, ascorbic acid, malic acid, and tartaric acid, which contribute to the syrup's sour taste (Izquierdo-Vega et al., 2020). The distinctive herbal taste and aroma of the formulation result not only from roselle as the primary ingredient but also from the inclusion of cardamom fruits, clove flowers, and cinnamon bark. These ingredients are abundant in aromatic compounds, including 1,8-cineole, α-pinene, linalyl acetate, limonene, myrcene, eugenol, chavicol, cinnamaldehyde, linalool, terpineol, and others (Ashokkumar et al. 2020; Błaszczyk et al., 2021; Haro-González et al., 2021).

pH Value, viscosity, and TAC of the roselle syrup

The pH measurement was conducted to determine the acidity level of the syrup. Table 2 indicates that the pH values of the six roselle syrup formulations ranged from 2.53 to 2.73, with no significant differences among the formulations. Although not statistically different, there was a slight tendency for the pH to increase as the concentration of xanthan gum was elevated. Previous studies have shown that roselle calyx extract can retain its red color in media with a pH range of 2.6–4; however, at pH 5–7, the color shifts to bright red or reddish-brown (Kartini et al., 2023).

Anthocyanins are flavonoid compounds whose stability is influenced by various variables such as light, temperature, pH, enzymes, oxygen, and oxidizing agents. Anthocyanin color is pH-dependent due to the ionic characteristics of their molecular structure. In acidic environments, anthocyanins typically appear red, transition to purple at neutral pH, and shift to blue under alkaline conditions. Anthocyanins are more stable in low-pH solutions because the formation of flavylium cations enhances their water solubility (Khoo et al., 2017).

From Table 2, a pattern emerges showing an increase in viscosity from F1 to F3, ranging from 166.7 to 403.3 cP. A similar trend is observed from F4 to F6, with viscosities ranging from 136.7 to 340.0 cP. Among the six syrup formulations, F3 exhibits the highest viscosity. This can be attributed to the concentration of xanthan gum, which is at its highest in both F3 and F6. However, F6 contains a greater amount of Tween 60 compared to F3. These data suggest that as xanthan gum concentration increases, the viscosity of the syrup also increases. Conversely, a higher concentration of Tween 60 tends to reduce the viscosity. This reduction may occur because Tween 60, a low-viscosity nonionic surfactant, can interfere with the three-dimensional network formed by xanthan gum, disrupting hydrogen bonding and polymer entanglements that contribute to thickening. Surfactants are known to act as plasticizers in hydrocolloid systems, which can reduce intermolecular interactions and lower viscosity (Espert et al., 2019). Xanthan gum serves as a thickening agent, where it forms complex polysaccharide polymer chain bonds between xanthan molecules. Therefore, as the concentration of xanthan gum increases, the viscosity of the rosella syrup also increases. Xanthan gum is an odorless and tasteless compound, so its presence does not affect the taste of the syrup (Rowe et al., 2009).

In general, the TAC in F1, F2, and F3 is higher compared to F4, F5, and F6, with the highest content found in F3, which is 67.85 mg/L. The stability of anthocyanin color can improve with the increase in xanthan gum concentration. This is attributed to xanthan gum, a polysaccharide capable of forming hydrophobic interactions—such as covalent and van der Waals bonds—with anthocyanins. These interactions help shield the chromophore groups from nucleophilic attacks by water, thereby inhibiting the conversion of anthocyanins into their colorless hemiketal form. Additionally, anthocyanins that are

strongly bound to polysaccharides can enhance their ability to withstand external environmental factors (Zhao et al., 2020).

Table 2. pH values, viscosity, and *total anthocyanin content* (TAC) of roselle syrup

Formulation	pH	Viscosity (cP)	TAC (mg/L)
F1	2.53 ± 0.13[a]	166.7 ± 15.3[a]	59.80 ± 14.42[a, b]
F2	2.63 ± 0.03[a]	230.0 ± 43.6[a, b, c]	64.59 ± 9.06[a, b]
F3	2.73 ± 0.02[a]	403.3 ± 90.2[d]	67.85 ± 4.45[b]
F4	2.68 ± 0.05[a]	136.7 ± 87.4[a, e]	45.83 ± 2.34[a, c]
F5	2.60 ± 0.01[a]	223.3 ± 35.1[a, c]	24.68 ± 5.37[d]
F6	2.71 ± 0.18[a]	340.0 ± 36.1[c, d]	21.96 ± 2.77[d]

Notes:
Values within the same column that are followed by different letters indicate a statistically significant difference ($P < 0.05$)

Antioxidant activity

In the food industry, antioxidants are recognized for their ability to prolong product shelf life by preventing the formation of free radicals. In this study, however, antioxidant activity was evaluated as a functional characteristic derived from the intrinsic properties of the ingredients used in the syrup formulation. Roselle is particularly rich in antioxidant compounds, especially anthocyanins and phenolic acids (Wu et al., 2018). Beyond their antioxidant function, anthocyanins also serve as natural colorants and offer various health-promoting effects, including antimicrobial activity and protection against chronic diseases (Khoo et al., 2017). These multifunctional properties contribute to the functional value of the syrup formulation, rather than serving as added preservatives or excipients.

The comparison of DPPH free radical inhibition activity among the six roselle syrup formulations is presented in Table 3. At a sample concentration of 1% v/v, there was no significant difference in the inhibition percentages among the six formulations, which ranged from 50.11% to 51.95%. However, at a sample concentration of 2% v/v, the inhibition values varied (52.28%–64.05%), with the highest value observed in F3. Xanthan gum is a polysaccharide composed of heteropolysaccharide units, which contain numerous hydroxyl groups, allowing it to exhibit antioxidative effects. Therefore, xanthan gum is also recognized as a potential source of antioxidants (Ramadhan et al., 2015). Although xanthan gum concentration influences viscosity and may aid in the retention of antioxidant compounds, the antioxidant activity of the syrup did not increase linearly with xanthan gum content. The interplay between xanthan gum and Tween 60 concentrations likely contributed to the variations observed in DPPH scavenging activity among the formulations.

Table 3. Antioxidant activity of roselle syrup based on DPPH radical scavenging assay

Formulation	Inhibition against DPPH (%)	
	1% v/v	2% v/v
F1	50.11 ± 1.27[a]	52.28 ± 0.93[a]
F2	50.45 ± 0.95[a]	60.37 ± 1.34[b, c]
F3	50.43 ± 1.90[a]	64.05 ± 2.19[b]
F4	51.44 ± 2.12[a]	58.83 ± 2.76[c, d]
F5	51.25 ± 1.36[a]	62.09 ± 1.34[b, c]
F6	51.95 ± 0.38[a]	55.47 ± 0.89[a, d]

Notes:
Values within the same column that are followed by different letters indicate a statistically significant difference ($P < 0.05$).

Figure 3 shows the results of method validation on antioxidant activity assay using ascorbic acid. A linear regression equation of concentration *vs* inhibition, y = 1.6958x + 32.732, was obtained. From this equation, the IC_{50} value of ascorbic acid was determined to be 10.1828 µg/mL. These findings are in agreement with previous research (Kartini et al., 2023), indicating that the antioxidant activity testing method used in this study can be applied to samples with valid results.

Anthocyanins are known to exhibit absorbance in the visible range, particularly around 500–550 nm, which may potentially overlap with the DPPH absorbance measured at 517 nm. To minimize spectral interference, each sample was measured against its respective blank (sample plus ethanol, without DPPH), and the resulting absorbance (A_{bl}) was subtracted from the sample reading (A_s). This approach allowed correction for background absorbance due to color compounds such as anthocyanins. Although this method reduces the likelihood of interference, the use of additional antioxidant assays operating at different wavelengths, such as ABTS or FRAP, is recommended for more comprehensive validation of antioxidant activity.

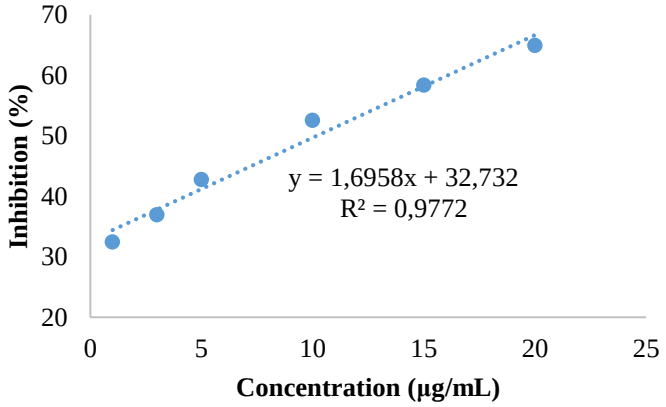

Figure 3. The relationship between ascorbic acid concentration and DPPH inhibition

Stability of roselle syrup

The evaluation of physicochemical characteristics and DPPH free radical scavenging activity indicated that syrup F3 had the best characteristics. Therefore, stability testing was conducted on F3 by storing the formulation at room temperature away from light for 42 days. The testing parameters included organoleptic properties (color, taste, odor), viscosity, pH, TAC, and DPPH free radical scavenging activity. Organoleptic observations were performed on two forms of the formulation: the concentrated syrup and the syrup reconstituted with water in a 1:5 ratio.

Up to day 21, the syrup formulation showed no significant organoleptic changes. The syrup remained reddish-purple in color, with a sweet and sour taste, a distinctive herbal aroma, and very minimal sediment after reconstitution. However, from day 28 to day 42, there was a noticeable decline in the sourness and the characteristic herbal aroma. During the 42-day storage period, a slight increase in pH was observed, from 2.73 to 2.95 (Figure 4). This change may be attributed to minor degradation of organic acids that could have occurred under storage conditions. In parallel, the viscosity of the syrup also exhibited gradual changes. These variations in viscosity might result from the structural rearrangement or partial degradation of xanthan gum, the primary gelling agent used in the formulation. Although pH and viscosity changes may occur independently, acidic conditions can influence the conformation and solubility of hydrocolloids such as xanthan gum. A rise in pH may reduce the tight coiling of xanthan chains, leading to altered viscosity. Therefore, the observed shifts in pH during storage may have contributed, at least in part, to the corresponding changes in viscosity. Despite this significant difference, the pH remained within the range of 2–4, allowing the syrup to retain its red color effectively (Kartini et al., 2023).

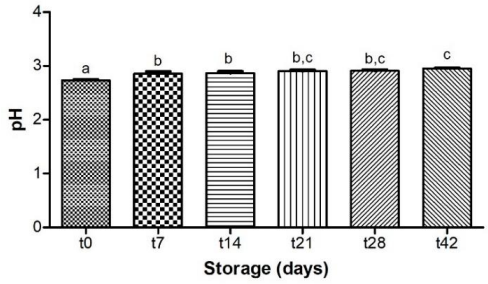

Figure 4. Changes in the pH of roselle syrup during 42 days of storage
Note: Different letters (a, b, c, d) within each series denote statistically significant differences between means at P < 0.05

Conversely, the syrup experienced a decrease in viscosity from 403.3 cP at t_0 to 286.7 cP at t42 (Figure 5). However, this decrease was not significant. These results are consistent with previous studies, which have shown that adding xanthan gum to fruit syrups can improve their quality and stability, particularly in terms of viscosity during heating and storage (Pongsawatmanit et al., 2011).

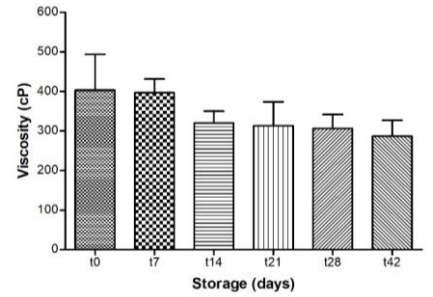

Figure 5. Changes in the viscosity of roselle syrup during 42 days of storage

The TAC of the syrup did not show significant changes up to day 7, remaining within the range of 67.85–60.17 mg/L. However, after that, it continued to decrease significantly, reaching 26.05 mg/L by day 42 (Figure 6). The exact factors causing this decline are not yet fully understood. Further development of the syrup formulation is needed to maintain TAC stability, such as by adding external antioxidants or conducting additional research focusing on product packaging.

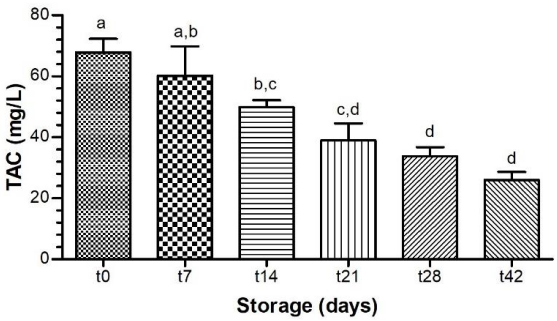

Figure 6. Changes in *total anthocyanin content (*TAC) of roselle syrup during 42 days of storage
Note: Different letters (a, b, c, d) within each series denote statistically significant differences between means at P < 0.05

The DPPH scavenging activity of the syrup at a 1% v/v concentration did not change until day 21, with inhibition ranging from 50.43% to 45.25%. However, after that, the activity decreased to 43.34% by day 42. Meanwhile, the sample at a 2% v/v concentration did not show a significant decrease in activity until day 7, but its activity also decreased by day 42 (Figure 7). This decline in activity corresponds with the reduction in TAC, which also decreased after day 7. This phenomenon further supports previous research data suggesting that anthocyanins are among the compounds responsible for the antioxidant activity in roselle flowers.

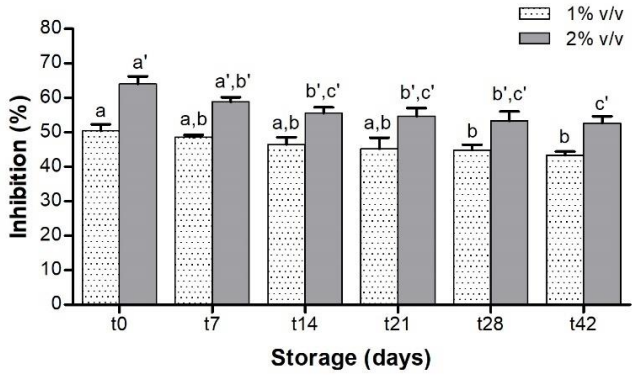

Figure 7. Changes in DPPH scavenging activity of roselle syrup during 42 days of storage at 1% v/v and 2% v/v

Note: Values with different letters (a, b, etc.) within the same series are significantly different ($P < 0.05$)

CONCLUSION

The formulated roselle syrup combined with clove, cinnamon bark, and cardamom fruit using 1% xanthan gum and 0.1% Tween 60 resulted in a stable, pourable, dark red syrup with a sweet-sour herbal taste. It exhibited good antioxidant activity, inhibiting DPPH free radicals by 50.43% at 1% v/v and 64.05% at 2% v/v concentrations. During storage, the syrup maintained its organoleptic properties and viscosity up to day 21, while pH remained within the expected range (2–4) until day 42. However, total anthocyanin content decreased significantly after day 7, although antioxidant activity remained relatively stable up to day 21. Overall, the formulation was successful, but further development is needed to improve anthocyanin stability during storage.

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REFERENCES

Aadil, R. M., Roobab, U., Sahar, A., ur Rahman, U., & Khalil, A. A. (2019). Functionality of bioactive nutrients in beverages *Nutrients in beverages*, 237-276, Elsevier.

Ananingsih, V. K., Hartajanie, L., Connie, F., Soedarini, B., & Pratiwi, A. R. (2023). Formulation of Low-Calorie Roselle Syrup Using Sorbitol and Carboxymethyl Cellulose. *ICFSE Full Paper ID 124 Soegijapranata Catholic University Repository*. http://repository.unika.ac.id/id/eprint/31614

Ashaye, O., & Adeleke, T. (2009). Quality attributes of stored Roselle jam. *International Food Research Journal*, *16*(3), 363-371

Ashokkumar, K., Murugan, M., Dhanya, M., & Warkentin, T. D. (2020). Botany, traditional uses, phytochemistry and biological activities of cardamom [*Elettaria cardamomum* (L.) Maton]–A

critical review. *Journal of Ethnopharmacology, 246*, 112244. https://doi.org/10.1016/j.jep.2019.112244

Błaszczyk, N., Rosiak, A., & Kałużna-Czaplińska, J. (2021). The potential role of cinnamon in human health. *Forests, 12*(5), 648. https://doi.org/10.3390/f12050648

Cong, L., Bremer, P., & Mirosa, M. (2020). Functional beverages in selected countries of asia pacific region: a review. *Beverages, 6*(2), 21. https://doi.org/10.3390/beverages6020021

Darnal, A., Kunathigan, V., Tipvarakarnkonn, T., & Panprivech, S. (2021, 17-18 June). *Determination of sensorial characteristics of carbonated roselle juices using rapid sensory profiling techniques.* Paper presented at the The 23rd Food Innovation Asia Conference 2021 (FIAC 2021).

Espert, M., Salvador, A., & Sanz, T. (2019). Rheological and microstructural behaviour of xanthan gum and xanthan gum-Tween 80 emulsions during in vitro digestion. *Food Hydrocolloids, 95*, 454-461. https://doi.org/10.1016/j.foodhyd.2019.05.004

Garcıa-Ochoa, F., Santos, V., Casas, J., & Gómez, E. (2000). Xanthan gum: production, recovery, and properties. *Biotechnology Advances, 18*(7), 549-579. https://doi.org/10.1016/S0734-9750(00)00050-1

Halim, Y., Evelyne, C., Rosa, D., & Ramli, S. (2022). Development of roselle (*Hibiscus sabdariffa* L.) calyx jelly candy. *Caraka Tani: Journal of Sustainable Agriculture, 37*(2), 357-372. http://dx.doi.org/10.20961/carakatani.v37i2.61537

Hapsari, B. W., & Setyaningsih, W. (2021). Methodologies in the analysis of phenolic compounds in Roselle (*Hibiscus sabdariffa* L.): composition, biological activity, and beneficial effects on human health. *Horticulturae, 7*(2), 35. https://doi.org/10.3390/horticulturae7020035

Haro-González, J. N., Castillo-Herrera, G. A., Martínez-Velázquez, M., & Espinosa-Andrews, H. (2021). Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. *Molecules, 26*(21), 6387. https://doi.org/10.3390/molecules26216387

Heng, K., Kei, T., Singh, K., Hairui, L., Ai-Ling, P., & Lifeng, K. (2014). *Handbook of cosmeceutical excipients and their safeties*: Elsevier.

Hussein, J., & El-Naggar, M. E. (2021). Synthesis of an environmentally quercetin nanoemulsion to ameliorate diabetic-induced cardiotoxicity. *Biocatalysis and Agricultural Biotechnology, 33*, 101983. https://doi.org/10.1016/j.bcab.2021.101983

Izquierdo-Vega, J. A., Arteaga-Badillo, D. A., Sánchez-Gutiérrez, M., Morales-González, J. A., Vargas-Mendoza, N., Gómez-Aldapa, C. A., . . . Madrigal-Santillán, E. (2020). Organic acids from Roselle (*Hibiscus sabdariffa* L.)—A brief review of its pharmacological effects. *Biomedicines, 8*(5), 100. https://doi.org/10.3390/biomedicines8050100

Kartini, K., Avanti, C., Phechkrajang, C., & Vallisuta, O. (2019). Antioxidant Activity, HPTLC Fingerprint and Discriminant Analysis of *Plantago major* Leaves from Diverse Origins in Indonesia. *Pharmacogn. J., 11*(6s), 1483-1489. https://doi.org/10.5530/pj.2019.11.229

Kartini, K., Huda, M. B., Hayati, Z. M., Sastika, N., & Nawatila, R. (2023). Scaling up stirring-assisted extraction and transformation of roselle anthocyanins into dried powder using spray-drying and oven-drying. *Appl Food Res, 3*(2), 100357. https://doi.org/10.1016/j.afres.2023.100357

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr. Res., 61*(1), 1361779. https://doi.org/10.1080/16546628.2017.1361779

Kim, C., & Yoo, B. (2006). Rheological properties of rice starch–xanthan gum mixtures. *Journal of food engineering, 75*(1), 120-128. https://doi.org/10.1016/j.jfoodeng.2005.04.002

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J. AOAC Int., 88*(5), 1269-1278. https://doi.org/10.1093/jaoac/88.5.1269

Maleš, I., Pedisić, S., Zorić, Z., Elez-Garofulić, I., Repajić, M., You, L., . . . Dragović-Uzelac, V. (2022). The medicinal and aromatic plants as ingredients in functional beverage production. *Journal of Functional Foods, 96*, 105210. https://doi.org/10.1016/j.jff.2022.105210

Millinia, B. L., Mashithah, D., Nawatila, R., & Kartini, K. (2024). Microencapsulation of roselle (*Hibiscus sabdariffa* L.) anthocyanins: Effects of maltodextrin and trehalose matrix on selected physicochemical properties and antioxidant activities of spray-dried powder. *Future Foods, 9*, 100300. https://doi.org/10.1016/j.fufo.2024.100300

Nguyen, Q. V., & Chuyen, H. V. (2020). Processing of herbal tea from roselle (*Hibiscus sabdariffa* L.): Effects of drying temperature and brewing conditions on total soluble solid, phenolic content, antioxidant capacity and sensory quality. *Beverages, 6*(1), 2. https://doi.org/10.3390/beverages6010002

OECD. (2004). OECD guidelines for the testing of chemicals, Test No. 111: Hydrolysis as a function of pH Retrieved from https://www.oecd-ilibrary.org/docserver/9789264069701-en.pdf

Pongsawatmanit, R., Yakard, N., & Suwonsichon, T. (2011). Effect of xanthan gum on the quality of syrup thickened by modified starch during heating and storage. *Agriculture and Natural Resources, 45*(1), 128-135

Ramadhan, K., Atmaka, W., & Widowati, E. (2015). Kajian pengaruh variasi penambahan xanthan gum terhadap sifat fisik dan kimia serta organoleptik fruit leather kulit buah naga daging super merah (*Hylocereus costaricensis*). *Jurnal Teknologi Hasil Pertanian, 8*(2), 115-122. https://doi.org/10.20961/jthp.v0i0.12902

Rowe, R. C., Sheskey, P., & Quinn, M. (2009). *Handbook of pharmaceutical excipients* (Vol. 6). London: Pharmaceutical Press.

Sari, Y. W., Mustofa, A., & Kurniawati, L. (2016). Karakteristik Sirup Herbal Fungsional "Sijala"(SirihMerah-Jahe-Rosela) Sebagai Sumber Antioksidan. *JITIPARI (Jurnal Ilmiah Teknologi dan Industri Pangan UNISRI), 1*(2), 79-87. https://doi.org/10.33061/jitipari.v1i2.1520

Shaikh, P., Lokare, M., Bhoge, M., Rajmane, N., Avadhut, N., & Thavare, N. (2024). Formulation and evaluation of antidiabetic polyherbal syrup. *Journal of Pharmacognosy and Phytochemistry*, *13*(2), 10-17. https://doi.org/10.22214/ijraset.2025.67006

Wu, H.-Y., Yang, K.-M., & Chiang, P.-Y. (2018). Roselle anthocyanins: Antioxidant properties and stability to heat and pH. *Molecules, 23*(6), 1357. https://doi.org/10.3390/molecules23061357

Yang, H.-I., Min, S.-G., Yang, J.-H., Seo, H.-Y., Choi, H., Kim, H.-S., & Chung, Y.-B. (2025). Effect of hydrocolloids on the rheological properties and stability of kimchi seasoning. *International Journal of Food Properties, 28*(1), 2472966. https://doi.org/10.1080/10942912.2025.2472966

Zhao, L., Pan, F., Mehmood, A., Zhang, Y., Hao, S., Rehman, A. U., . . . Wang, Y. (2020). Protective effect and mechanism of action of xanthan gum on the color stability of black rice anthocyanins in model beverage systems. *International Journal of Biological Macromolecules, 164*, 3800-3807. https://doi.org/10.1016/j.ijbiomac.2020.09.027

Ziani, K., Fang, Y., & McClements, D. J. (2012). Encapsulation of functional lipophilic components in surfactant-based colloidal delivery systems: Vitamin E, vitamin D, and lemon oil. *Food Chemistry, 134*(2), 1106-1112. https://doi.org/10.1016/j.foodchem.2012.03.027