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Exploring Rosella (*Hibiscus sabdariffa* L.) Calyx Extracts as Natural Dye and Antioxidant in Lip Cream Product: Formulation and Evaluation

Donna Maretta Ariestanti[1], Abdul Mun'im[1*], Joshita Djajadisastra[1], Riska Amalia Putri Hutami[1], Redhalfi Fadhila[1], Richard Johari James[2,3]

[1]Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia
[2]Integrative Pharmacogenomics Institute, Universiti Teknologi MARA, Selangor, Malaysia
[3]Faculty of Pharmacy, Universiti Teknologi MARA, Selangor, Malaysia

ABSTRACT

Rosella, also known as *Hibiscus sabdariffa* L., is a plant that contains anthocyanin compounds in its calyx, which are known for their antioxidant properties. These compounds give the plant a captivating red color and can be used as natural dyes. This study aimed to exploit anthocyanins in rosella calyx as natural dyes and antioxidants in lip cream product. The formulations employed extracts from rosella calyx, a viscous extract from maceration in 96% ethanol (F1) and a commercially available powdered rosella extract (F2). Evaluation on the formulations demonstrated that both F1 and F2 effectively maintained the color integrity of the lip cream. Moreover, an irritation test among 10 respondents showed no adverse reactions. Additionally, a hedonic test of 30 respondents revealed uniformity in homogeneity, odor, and spreadability on both formulations. However, a noticeable contrast emerged in color intensity, with F2 exhibiting greater intensity and preference over F1. The rosella calyx extract also demonstrated its antioxidant potential with an IC_{50} value of 130.11 ± 2.08 µg/ml. The study highlights the utility of rosella calyx extracts as a dual-functioning component in lip cosmetics. The findings indicate the variations in formulation efficacy and color intensity, emphasizing the potential of rosella-derived extracts in developing cosmetics with enhanced aesthetic appeal and functional antioxidant attributes.

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**corresponding author*
Email: munim@farmasi.ui.ac.id

INTRODUCTION

Lip color is an essential cosmetic product that aims to enhance the aesthetic appeal of facial makeup. Lip color formulations are available in diverse forms like liquids, stick, and creams, which aim to impart a distinct and vibrant layer upon application (Sharma et al., 2018; Meliana et al., 2022). Different variants of lip makeup, including lipstick, lip cream, lip gloss, and lip sealers, have evolved into symbolic representations of feminine beauty and sensuality in modern society (Draelos, 2010). However, concerns have been raised regarding the use of synthetic dyes, as specific variants, such as rhodamine B, are associated with potential health risks (Hakami et al., 2021; Juliano et al., 2017). Moreover, environmental pollutants can affect the natural red color of our lips, which comes from the blood vessels underneath the skin, leading to the fading or changing of the lip color (Kadu et al., 2017; Khan & Alam, 2019). To mitigate this issue, antioxidants are added to lip color products to protect the lips from discoloration or any possible changes (Sango & Binder, 2016; Heusèle et al., 2022).

Amidst concerns about synthetic ingredients and their associated side effects, there is an escalating demand among cosmetic users for products derived from natural sources (Mahesh et al., 2019). Using natural ingredients, classified as "food grade" by the FDA, offers a safer alternative compared to synthetic chemicals, which may contain toxic contaminants, even in trace amounts (Chattopadhyay et al., 2008; Palou et al., 2016). One such ingredient is rosella (*Hibiscus sabdariffa* L.), which contains anthocyanin compounds within its flowers (Shruthi et al., 2016). Anthocyanin, a plant pigment attributing a red color, also acts as an antioxidant, protecting against cell damage due to excessive UV light exposure (Tsai et al., 2002). The safety profile of rosella is also supported by its extensive usage in a wide range of food and beverage items.

Previous research has explored rosella as a coloring agent in lipstick formulations at varying concentrations. Notably, a 10% concentration exhibited optimal color intensity with a single application (Safitri, 2010).

Building upon this groundwork, this study aims to formulate lip cream product utilizing rosella calyx extract as a natural dye and antioxidant at different concentrations and material compositions. This research highlights the potential of rosella calyx extract in cosmetics, providing safer alternatives in lip color products and addressing concerns regarding synthetic ingredients. The study also includes antioxidant activity evaluations of the extract using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method.

MATERIALS AND METHODS

Materials

Rosella calyx (*Hibiscus sabdariffa L.*), rosella calyx extract powder (Changsha Nutramax, China), castor oil (TCO Group, Thailand), olive oil (Fabri, Italia), carnauba wax, candelilla wax, beeswax, vaseline album, frambozen essence, glycerin (Brataco, Indonesia), dimethicone (KCC Corporation, South Korea), butyl hydroxy toluene, propyl paraben (Stan Chem, United Kingdom), DPPH (Sigma Aldrich, Singapore), ethanol, vitamin C, (Brataco, Indonesia), methanol (Petronas, Malaysia), KCl, HCl, $CH_3CO_2Na.3H_2O$, NaCl (Merck, United States of America).

Extraction of Rosella Calyx

Calyx of rosella simplicia was obtained from Bumi Herbal Dago, Dago Pakar, Bandung, and was determined by the School of Life Sciences and Technology, Bandung Institute of Technology. The extraction process was conducted following the procedure outlined in Suzery et al., (2010), with some modifications. The simplicia was then ground and macerated with 96% ethanol in a 1:2 (simplicia:solvent) ratio for 24 hours under light-protected conditions. Later, the macerate was filtered, and the filtrate was concentrated using a rotating vacuum evaporator (Buchi, Switzerland) to get viscous extract at 40°C. The powder extract purchased from China was used as a comparison.

Anthocyanin Quantification from Rosella Calyx Extract

The total anthocyanin content of rosella calyx extract was measured using UV-Vis Spectrophotometer (Shimadzu UV-1601, Japan). Extract was diluted using a solution of pH 1.0 (KCl 25 mM) and pH 4.5 ($CH_3COO.Na$ 400 mM) with 1:4 ratio (simplicia:ethanol). The absorbance of each dilution samples was measured at wavelengths of 520 and 700 nm (Mun'im et al., 2008).

The absorbance value was calculated according to the following equation:

$$A = (A_{520\ nm} - A_{700\ nm})\text{pH 1.0} - (A_{520\ nm} - A_{700\ nm})\text{pH 4.5} \quad (1)$$

From the absorbance obtained, formula below is used to determine the anthocyanin content:

$$\text{Anthocyanin content (mg/g)} = \frac{A \times MW \times DF \times \left(\frac{V}{Wt}\right) \times 10^3}{\varepsilon \times l} \quad (2)$$

Note:

A	: Total absorbance of anthocyanin
ε	: Molar absorptivity of cyanidin-3-glucoside (26900 L/mol.cm)
l	: Cuvete length (1 cm)
MW	: Molecular weight of cyanidin-3-glucoside (449,2 g/mol)
DF	: Dilution factor
V	: Dilution volume (L)
Wt	: Initial weight of sample (g)

Antioxidant Activity of Rosella Calyx against DPPH (2,2-Diphenyl-1-picrylhidrazyl) radical

The DPPH free radical scavenging method assessed the antioxidant activity of rosella calyx ethanolic extract based on Adhitia et al., (2017). Samples were generated at concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml, 125 µg/ml, and 150 µg/ml. As much as 2 mL of each concentration was pipetted to reaction tube, added with 1 mL of methanol, and 1 mL DPPH (100 µg/mL). The solution was then incubated for 30 minutes in the dark at 37°C, and the reduction of absorbance was measured using UV-Vis Spectrophotometer at 517 nm.

The scavenging DPPH activity was calculated according to the following equation:

$$\text{Inhibition (\%)} = \left[\frac{Control\ absorbance - sample\ absorbance}{Control\ absorbance}\right] \times 100\% \quad (3)$$

The IC_{50} value states the concentration of the sample when it can scavenge 50% of DPPH radical. Vitamin C was used as positive control.

Formulation of Rosella Calyx Extract-Based Lip Cream Preparations

Formula 1 and 2 were prepared as lip creams following meticulous procedural steps. All ingredients are measured precisely according to the amounts specified in the formula (Table 1).

Initially, a mixture of vaseline album and candelilla wax was heated until the wax phase melted (Beaker A). Subsequently, propyl paraben was dissolved in glycerin in a separate beaker and mixed with rosella calyx extracts until completely dissolved (Beaker B). In a separate container (Beaker C), castor oil, olive oil, and BHT were homogeneously mixed and then incorporated into the extract mixture in Beaker B. Afterwards, the melted wax phase was continuously stirring (Beaker A) into the extract mixture (Beaker B). After mixing, the preparation was left to solidify.

Table 1. Formulation of rosella calyx extract-based lip cream

Materials	F1 (w/w%)	F2 (w/w%)	Function
Rosella extract	12	-	Natural pigment
Rosella extract powder	-	8	Natural pigment
Dimethicon	18	18	Emollient
Vaseline album	20	20	Stiffening agent
Candelilla wax	4	4	Stiffening agent
Castor oil	20	20	Emollient
Olive oil	20	20	Emollient
Glycerine	5	9	Solvent
Propil paraben	0.6	0.6	Preservative
BHT	0.1	0.1	Antioxidant
Frambozen essence	0.3	0.3	Fragrance
Total	100	100	

Post-solidification, a homogenizer formed cream by agitating the preparation while adding frambozen essence until uniformity was achieved. Finally, the final mixture was transferred into a suitable container for storage and application.

Characteristic Evaluation

Organoleptic

The organoleptic evaluation includes the examination of texture color, scent, and uniformity. To assess homogeneity, a specified quantity of lip cream was applied to a glass object and then cut lengthwise. The product must be homogeneous, devoid of any noticeable coarse granules to meet the criteria (Warnida et al., 2020).

Determination of pH

One gram of the lip cream was melted with 100 mL of hot water, and its pH value was measured using a pH-meter type 510 (Eutech Instrument, Singapore) (Lestari et al*.,* 2018).

Spreadability test

This test conducted through visual examination, applying the product five times on the skin at the back of the hand. A product possesses good spreading power if the color adheres substantially and evenly to the skin after multiple applications at a specific pressure. Conversely, if the color adheres minimally and unevenly, the product is considered as poor spreadability (Lestari et al*.,* 2018).

Physical stability test of products

Texture, consistency and aroma of the lip cream were observed at room temperature (±25ºC) on days 1, 5, 10, 15, 20, 25, 30, 35, 40, and 45 (Mishra et al., 2010; Vishwakarma et al., 2011). Observation of changes in color of lip cream was carried out by placing the product under sunlight at 09.00-14.00 during storage on days 0, 5, 10, 15, 20, 25, 30, and 45. The color changes were then compared using a pantone card.

Irritation Test

The patch test technique was employed to conduct an irritation test on the inner forearm of 10 participants. All subjects participating in this study have given informed consent. The product was applied to a cleaned adhesion site (2.5 x 2.5 cm), covered with a silver patch for 24 hours, and the condition of the application site was subsequently observed. Inclusion criteria for the irritation test specified women aged 18-30 years, physically healthy, without a history of allergic disease, and gave consent to participate. Conversely, exclusion criteria encompassed women over 30 years old with physical health issues. The collected data were then processed to calculate the skin irritation index (Primary Irritation Index) using a designated formula (Nawanopparatsakul et al., 2005):

$$\text{PII} = \frac{\sum eritema\ score + \sum edema\ score}{total\ participants} \times \text{variable factors} \qquad (4)$$

note:

Variable factors: number of skin types used x observation time

Preference/Likeability/Hedonic Test

Preference test was conducted on 30 respondents, including those 10 participating in the irritation test.

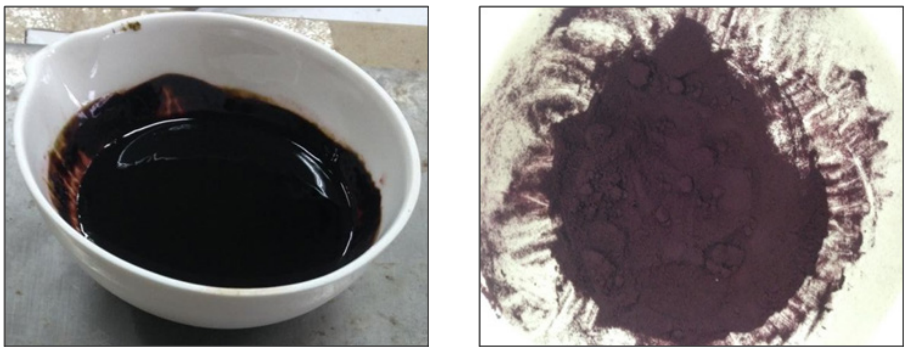

Figure 1. Rosella calyx extracts used in formulations. (A) Viscous rosella extract; (B) Rosella extract powder

Figure 2. Physical appearance of formula 1 and formula 2 preparations

Each respondent was asked to apply a formulation with various rosella calyx extract concentrations on the back of their hand. Subsequently, respondents selected their preferred lip cream product . They rated their preferences on a scale from the x-axis displays respondent preference, ranging from 1 (dislike very much), 2 (dislike moderately), 3 (dislike slightly), 4 (neither like nor dislike), 5 (like slightly), 6 (like moderately) to 7 (like very much). Observation parameters for the preference test included ease of application, aroma, homogeneity, and color intensity of the lip cream when applied to the back of the hand. The percentage of preference for each formulation was then calculated.

Inclusion criteria for the preference test included women aged 18-30 years, physically healthy, possessing knowledge and experience in organoleptic assessment, and gave consent to participate. Meanwhile, exclusion criteria for the preference test encompassed women over 30 years old in a state of physical illness.

RESULTS AND DISCUSSION

Rosella Calyx Extraction

Rosella calyxes were macerated using 96% ethanol through 6 re-maceration process and evaporated, resulting in an extract yielding 12.387%. Physical appearance of viscous extract can be seen in Figure 1A. Anthocyanins are a group of glycoside flavonoids, making polar solvents such as ethanol suitable for extracting anthocyanins from plants. Ethanol can also extract other components, such as other flavonoids, phenols, and organic acids, interacting with anthocyanins, reducing degradation and improving anthocyanin stability (Escobar et al., 2022). Additionally, ethanol has minimal toxic effects compared to methanol, making it widely used as a solvent for extraction (Oancea et al., 2012). The obtained extract has a pH of 3.8, resulting in a deep red color. The rosella extract powder used for comparison has a pH of 3.4 with a reddish-purple color (Figure 1 B). The slight difference is due to the pH-dependent nature of anthocyanins, as their molecular structure is ionic. Generally, at pH 2-4, anthocyanins are relatively stable, displaying a reddish color primarily in the form of flavylium cation (Turturică et al., 2015).

The maximum wavelength (λ max) of anthocyanins in the sample is 520.50 nm, consistent with Harborne, (1973) states that the wavelength of anthocyanins ranges from 475 to 550 nm and approximately 275 nm. At a wavelength of approximately 500 nm, there is specific evidence of anthocyanins, while around 200 nm indicates the presence of flavonoid compounds in general, as anthocyanins belong to the flavonoid group.

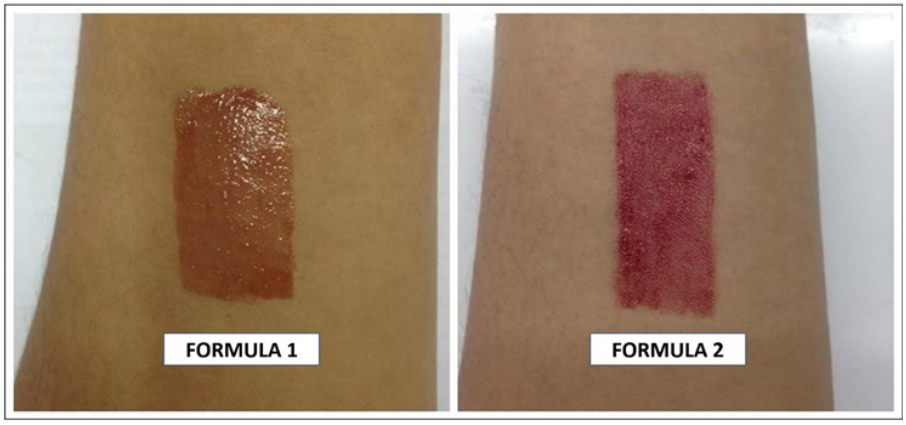

Figure 3. Texture of formula 1 and formula 2 upon spread ability test

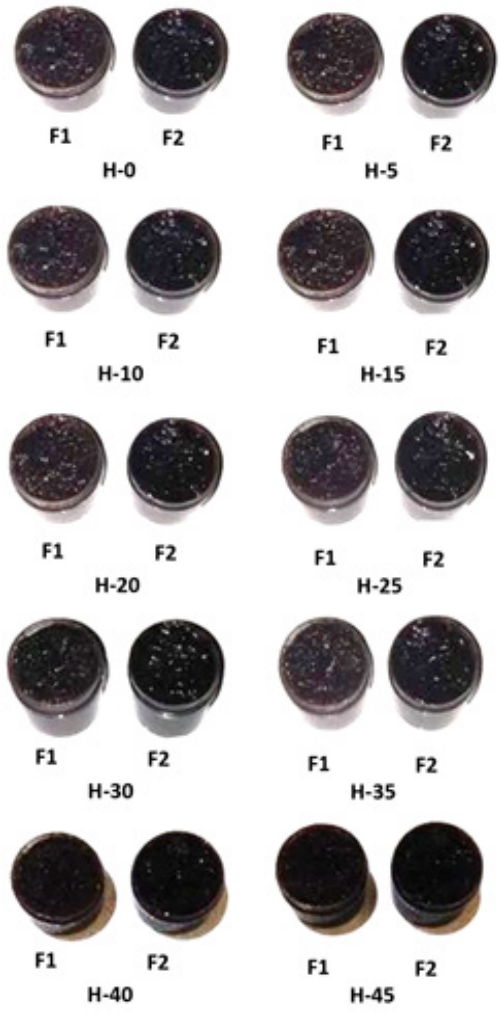

Figure 4. Physical stability test results for formula 1 and formula 2 at 25ºC for 45 days

Anthocyanin Quantification from Rosella Calyx Extract

Ethanolic extract of rosella calyx contains anthocyanin with a concentration of 418.90 mg/L, equivalent to 6.76%. The powdered extract of rosella calyx used as a reference has a concentration of 10%. The powder was sourced from China, and the difference in concentration may be attributed to the variation in geographical locations where the plants grow (Zargoosh et al., 2019). In this study, the anthocyanin content is higher than in previous research conducted by Yang et al. (2012). It was observed that the duration of the extraction process and the quantity of plant material and solvent used would influence the extracted anthocyanin.

Antioxidant Activity of Rosella Calyx against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical

Based on the results of the antioxidant activity test of the rosella calyx extract against DPPH,an IC_{50} value of 130.11 ± 2.08 ppm was obtained. In comparison, vitamin C as a positive control yielded an IC_{50} value of 2.25 ± 0.05 ppm. From the DPPH assay, the rosella extract is classified as a medium-strength antioxidant (IC_{50} ranging from 100-500 µg/mL). In contrast, vitamin C exhibits a powerful antioxidant capacity (IC_{50} lower than 50 µg/mL) (Molyneux, 2004). Anthocyanins, known for their high antioxidant capacity, perform primarily as scavengers of free radicals by capturing H atoms in DPPH (2,2-diphenyl-1-picrylhydrazyl) to produce a stable DPPH molecule. Notably, the degree of hydroxylation at the B-ring structure's 3′ and 4' positions is a crucial determinant of their radical scavenging activity (Wu et al., 2018).

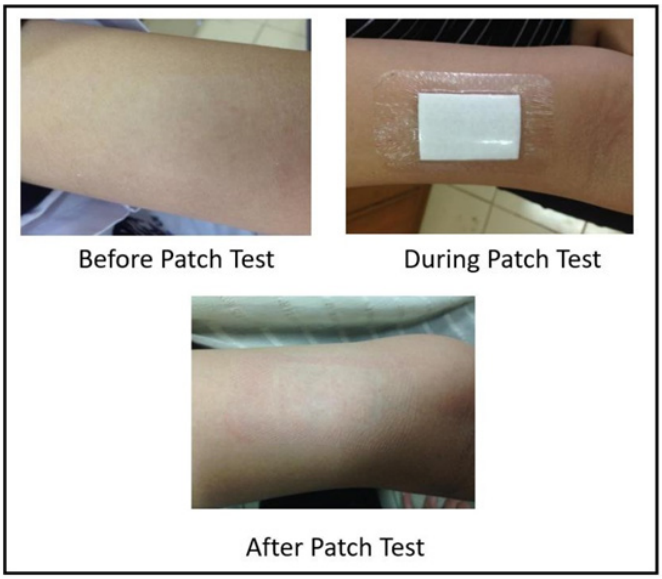

Figure 5. Skin condition before, during and after the irritation test

Characteristics Evaluation of Rosella Calyx Extract Lip Cream Products

Organoleptic

The visual assessment of the product, encompassing characteristics such as texture, color, and aroma, reveals distinct attributes in formula 1 and 2. The appropriate amount for the viscous and powdered extracts used in the formulas has been determined based on the level of anthocyanin concentrations in both extracts. According to the concentration of anthocyanins in each extract, 12% of viscous extract will have an equal anthocyanin level with 8% of powdered extract. Both formulations exhibit a cream-like texture and possess a noticeable aroma associated with rosella. Nevertheless, while formula 1 showcases a dark red coloration, formula 2 tends toward a darker shade of purple. Since both formulas contain similar levels of anthocyanins, it is reasonable to conclude that color differences are unlikely to be caused by differences in anthocyanin concentrations. The difference in color between the two products can be attributed to the pH level, which significantly influences the color expression of anthocyanins in both the rosella extract and powder.

These anthocyanins are the primary constituents responsible for the colorful appearance of rosella (Alshoosh et al., 1997). They are known to be sensitive to pH levels due to structural changes within the molecules at different pH levels (Turturică et al., 2015). Given that the pH values in the two formulas are noticeably different, it can be inferred that the resulting color disparities suggest a direct correlation between pH differences and the observed color differences.

The physical appearance of both products can be seen in Figure 2. For homogeneity, in the context of formulations 1 and 2, upon application to the object glass, the resulting color demonstrates a uniform composition without any noticeable coarse granules. The criteria for homogeneity in this product is the absence of visible coarse grains (Erwan et al., 2022).

pH

At room temperature, formula 1 exhibits a pH of 2.7, while formula 2 has a pH of 3.3 at week 0. Subsequently, by week 4, the pH levels are measured at 2.5 for formula 1 and 2.9 for formula 2. The decreasing pH is possibly attributed to the degradation of some compounds present in the formulation upon exposure to specific environmental factors. Exposure to light, heat, and oxygen can also result in the degradation or structural change in anthocyanin and leads to a changing in pH level (Enaru et al., 2021). Additionally, extended storage periods may weaken the hydrogen bonds that keep the cream's compounds stable, thereby altering the cream's structure and causing the pH to shift (Vargas et al., 2013). It is noteworthy that the use of preservatives in the formulation may also have contributed to the pH drop. A study by Azman et al. (2022) demonstrated that anthocyanin extract displayed increased stability in the absence of additional preservatives. This indicated that preservatives may interfere with anthocyanin self-association, resulting in their degradation. Furthermore, Supiati et al. (2022), demonstrated a similar pH decline in cream formulations containing anthocyanins, notably affected by storage time, even at colder temperatures (4°C). This decline could be attributed to reduced hydrogen bond formation over prolonged storage, ultimately impacting the cream's pH.

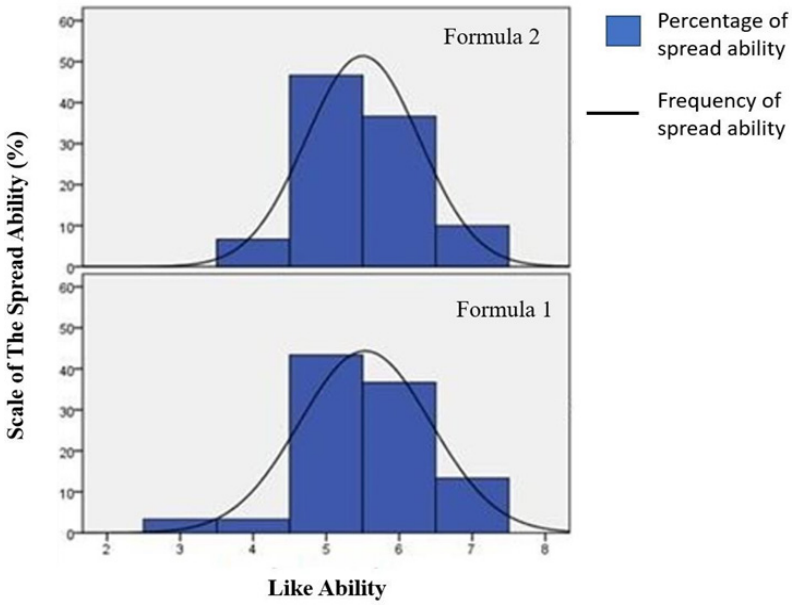

Figure 6. Graph of like ability test for the spread ability of formula 1 and formula 2

Notably, both formulations consistently maintain pH levels below the typical physiological pH range of the lips, corresponding to the skin pH ranging from 4.5 to 6.5. This deviation from the skin's pH is attributed to the acidic nature of the anthocyanin compound present in the formulations. The considerable concentration of rosella extract incorporated into the product contributes to their inherent acidity. The stability of anthocyanin compounds is known to be closely linked to an acidic environment (Wu et al., 2018). Deviation from this acidic pH may lead to alterations in the color properties of anthocyanin compounds, underscoring the necessity to maintain an acidic pH for stability.

Spread-ability

The key to superior topical properties of lip cream is the ability to deliver a concentrated, uniform, and consistent color upon application (Westfall, 2015). In the context formula 1 and 2, both exhibit coloration upon application. However, the color intensity of formula 1 appears less vibrant compared to formula 2. This difference is due to the distinct formulation methods used. Formula 2 incorporates extract powder that has a notably darker hue and a higher overall anthocyanin concentration than the viscous extract used in formula 1. The results of spread ability test can be seen in Figure 3.

On the other hand, previous research conducted by Safitri in 2010 evaluated five different lipstick formulations. Among these, the spread ability test involving five applications revealed that the formulation exhibiting robust adhesion was formulation 6, featuring a lipstick concentration of 10% rosella extract. Differences in spread ability between the previous and current studies resulted from variations in the formulations and the unique characteristics of the rosella extract used. The varying anthocyanin levels in each extract led to different color intensities, which influenced the observed differences in spread ability across the formulations.

Homogeneity

In the context of formulations 1 and 2, upon application to the object glass, the resulting color demonstrates a uniform composition without any noticeable coarse granules. The criterion for homogeneity in this product is the absence of visible coarse grains (Erwan et al., 2022).

Color and physical stability

The coloration in rosella is attributed to anthocyanins, a group of compounds that are known to be pH-dependent (Sinela et al., 2017). These compounds exhibit enhanced stability under acidic conditions, with pH values ranging between 2 to 4 (Turturicăa et al., 2015). However, at higher pH levels, anthocyanins tend to degrade much more rapidly. Additionally, exposure to light, heat, and oxygen can also result in the degradation or loss of color in these compounds (Enaru et al., 2021). In their stable form, anthocyanins produce a reddish hue in the form of flavylium cation (Turturicăa et al., 2015).

The assessment of physical stability over a span of 45 days, with storage at room temperature (±25ºC), revealed no observable alterations in the shape, color, or aroma of the lip cream product. Comprehensive observations conducted on days 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 confirmed the sustained integrity of these characteristics throughout the entire observation period.

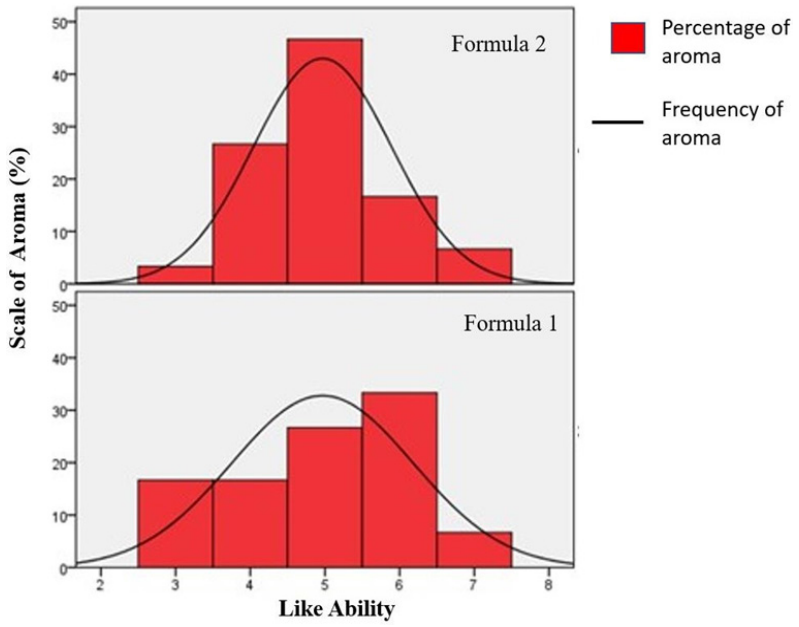

Figure 7. Graph of like ability test for aroma of formula 1 and formula 2

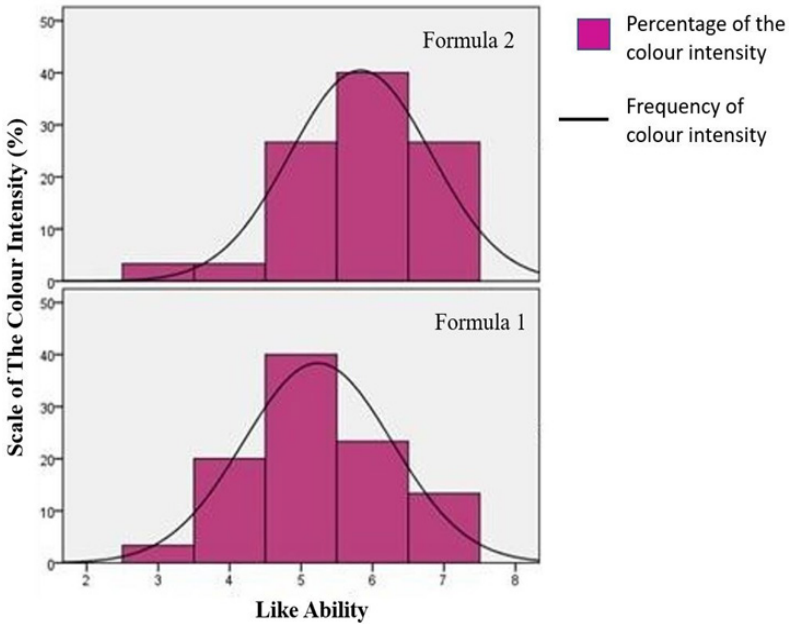

Figure 8. Graph of like ability test for homogeneity of formula 1 and formula 2

Color observations were conducted on formula 1 and 2 during its storage period, with a focus on how sunlight influenced color changes. These observations were conducted on day 1, 5, 10, 15, 20, 25, 30, 35, 40, and 45 (Figure 4). On day 25, formula 1 exhibited a slight color alteration, but it was deemed insignificant due to the product's inherent darkness, making meticulous observation challenging. The color manifested by formula 1 between days 1 and 20 closely resembled the Pantone 504 PC card. Subsequent alterations observed between days 25 and 45 aligned more closely with the Pantone 490 PC card. On the other hand, formula 2 exhibited consistent color resemblance to the Pantone 262 PC card throughout the entire observation period of days 1 to 45, displaying no visible alterations.

Irritancy

Among the 10 respondents subjected to a 24-hour test, none experienced erythema or edema in the evaluation of formula 1. However, in the assessment of formula 2, only

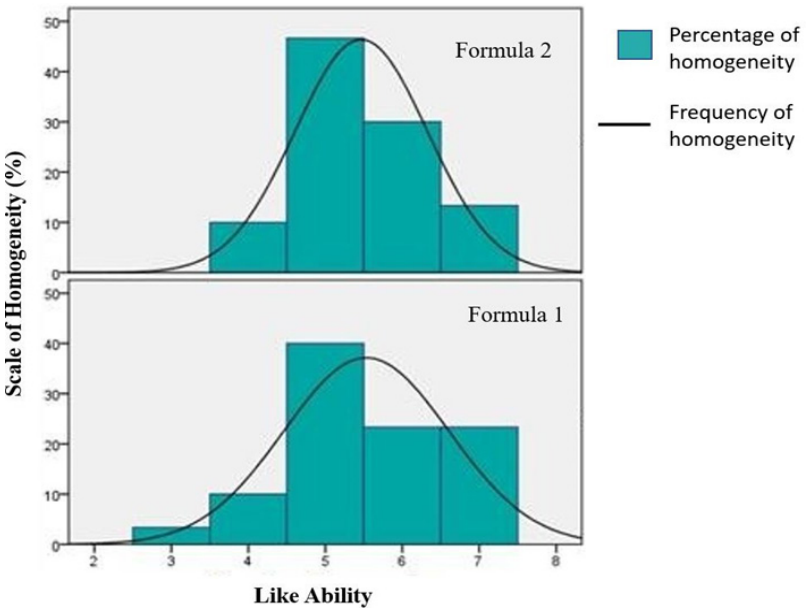

Figure 9. Graph of like ability test for the color intensity of formula 1 and formula 2

one respondent displayed mild skin redness, categorized as minimal erythema (almost imperceptible), and none exhibited edema. Both formula 1 and formula 2 obtained a primary irritation index (PII) value of 0, meeting the criteria for insignificance in irritation, as per the guidelines by Nawanopparatsakul et al. (2005). Hence, it can be concluded that neither formula 1 nor formula 2 induced irritation or were deemed to cause negligible irritation. The picture of skin condition before, during and after the irritation test (patch test) can be seen in Figure 5.

Preference/Likeability/Hedonic Test

The evaluation encompassed a preference test conducted on a cohort of 30 female respondents. To ensure the accuracy of the findings, the Mann-Whitney test method was employed in SPSS. This allowed for a thorough evaluation of various parameters, such as spread ability (Figure 6), aroma (Figure 7), homogeneity (Figure 8), and color intensity (Figure 9). In Figure 6-9, the x-axis displays respondent preference, ranging from 1 (dislike very much) to 7 (like very much), while the y-axis shows the percentage of respondents in each category. The results indicated that there were no significant differences between the two formulations in terms of spread-ability, aroma, and homogeneity. However, it was observed that formula 2 had a more appealing color intensity compared to formula 1. This can be attributed to the fact that formula 2 produces a richer color intensity, while formula 1 delivers a lighter hue.

CONCLUSION

Based on the formulation results, rosella calyx extract has proven to be an excellent component for lip cream products. Our findings also showed significant antioxidant activity of the extract, with an IC_{50} value of 130.11 ± 2.08 µg/ml, as determined by a DPPH method test. These findings emphasize the potential of rosella extract as a natural antioxidant. Moreover, a preference test involving 30 respondents indicated a noticeable difference in color intensity between formulations F1 and F2, with F2 displaying a more vibrant hue and receiving greater favorability compared to F1. While there were no noticeable differences in terms of homogeneity, spreadability, or aroma, it is evident that the unique color-enhancing properties of rosella extract make it an ideal ingredient for creating visually appealing lip cream formulations. These findings firmly establish the value of rosella extract as an effective and attractive ingredient for lip cream formulations.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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