

Formulation of lotion ethanolic extract of rosella flower petals (*Hibiscus sabdariffa L.*) and antioxidant activity



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ABSTRACT

Rosella flowers (*Hibiscus sabdariffa L.*) are plants that are useful as antioxidants. The content that acts as an antioxidant is a flavonoid compound. This study aimed to determine the effect of giving calliandra honey as a multiple active ingredient and single active ingredient (rosella petal extract, calliandra honey) and ethyl vitamin C as an active ingredient. The lotion of ethanolic extract of rosella flower petals was made with three active ingredients: ethanolic extract of roselle flower petals with a concentration of 2.5%, calliandra honey 2.5%, and ethyl vitamin C 2%. Then, calliandra honey and ethanol extract of roselle flowers are combined with a concentration of extract 2.5% and calliandra honey (1.5%, 2%, and 2.5%). The physical and stability tests are the organoleptic, homogeneity, viscosity, dispersibility, pH, lotion stability, and the value of lotion's antioxidant activity. The activity test was carried out using the 2,2-Diphenyl-1-Picrylhydrazil (DPPH) method, and the IC₅₀ of inhibition was calculated. The results of the study showed that all lotion formulations had antioxidant activity. The highest antioxidant activity was obtained in formula three, which had a value of 95.2 ppm (2.5: 2.5 %). Based on the Kruskal Wallis test, the asymp value was obtained. Sig. 0.000, which indicates that there is a significant difference in antioxidant activity. This is because the percent inhibition of each sample has a large enough difference.

Keywords: Antioxidant, Calliandra honey, Lotion, Rosella flower petals

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INTRODUCTION

The Rosella plant (*Hibiscus sabdariffa L.*) is a tropical plant that has been present in Indonesia since the 1970s. This plant has attracted much special attention because it has the potential to be a natural dye and pharmaceutical ingredient (Chang et al., 2014). The high value of rosella plant benefits is due to the potential content of natural phytochemical compounds in all parts of the plant, namely the rosella leaves, stems, and flowers. These potential phytochemical components include phenols, alkaloids, tannins, flavonoids, saponins, organic acids, anthocyanins, and polysaccharides (Hakim et al., 2022)

Using rosella flower petals as an antioxidant benefits the skin's collagen formation. The research of Nurnasari & Khuluq (2017) that the ethanol extract of rosella flower petals with a concentration of 2.5% can produce antioxidants of 15.626% to 17.773%. The inhibition rate has no antioxidant activity so that the research will use the roselle petal plant as an active ingredient, carried out in combination to increase antioxidant activity.

Honey is an ingredient that is known to have many uses and benefits. Honey is a sweet liquid bees produce from flower extract or other parts of plants or insect excretions (Fadhilah & Rizkika, 2015). Honey, as a natural ingredient, can be used as a moisturizer because it has humectant, emollient, and antioxidant properties (Megantara et al., 2017). In research, Ustadi et al. (2017) said that The honey used calliandra honey because it had natural flavonoids of 156.27 mg QE/100 g and a phenolic level of 557.93 mg GAE/100 g. Calliandra honey had a higher antioxidant potential than rubber honey and raw honey, as seen from this figure. Phenolic compounds are antioxidant compounds in honey that have a major role in reducing free radicals (Cianciosi et al., 2018). Combining these two

ingredients can be used as a natural substance in a lotion preparation to determine antioxidant activity using the DPPH method.

The lotion is a cosmetic preparation in the form of an emulsion that contains more water than oil. Some of the properties possessed by this preparation are as a source of moisturizing on the skin, as a softener, and are easy to apply (Irmayanti et al., 2021). Lotions are the choice for cosmetic preparations because they have several advantages, namely being able to retain skin moisture, prevent water loss, retain active substances, quickly use them evenly on the skin surface, and can also be used as solvents, fragrances, and preservatives (Chayati & Miladiyah, 2016). The physical properties of the rosella petal ethanol extract lotion as a moisturizer will be influenced by the type of emulsifier used; the emulsifier is easily oxidized. However, anionic emulsifiers are still frequently used for antioxidant products such as three vitamin C creams. However, they are emulsifiers used on the basis that does not affect the effectiveness of the active ingredient or antioxidants. Some emulsifiers used in oil in water include sodium lauryl sulfate, triethanolamine stearate, self-emulsifying glyceryl monostearate, and so on (Chayati & Miladiyah, 2016).

Based on the explanation above, the researchers are interested in researching the ethanol extract of rosella flowers as an antioxidant in lotion products.

RESEARCH METHOD

Materials

The tools used in this research include a scale (Ohaus PA214), Brookfield viscometer (Fungilab Viscolead), UV-Vis spectrophotometer (BEL engineering M51), Vacuum rotary evaporator (RE-2000E), hot plate stirrer (Thermo et al.), pH meter (Ohaus). The materials used in this study were roselle petal extract, calliandra honey (Different standard), ethyl vitamin C (Different standard), and stearic acid. (pharmaceutical), cetyl alcohol (pharmaceutical), glycerin (pharmaceutical), ethanol 70% (technical), methylparaben (pharmaceutical), Triethanolamine (pharmaceutical).

Methods

This research was conducted at the Laboratory of Pharmaceutical Technology, Faculty of Health Science, University of Darussalam Gontor, from January to April 2021. This research was conducted by testing the physical quality and stability of the lotion. The parameters to be analyzed in the study include an organoleptic test, homogeneity test, pH test, spreadability test, viscosity test, and antioxidant test.

1. The Process of Making Rosella Flower Petal Extract Lotion

The manufacturing process begins with weighing the ingredients used in making rosella petal extract lotions. The materials used consist of an oil phase and a water phase. Materials included in the oil phase are made by successively melting stearic acid and cetyl alcohol at a temperature of 70°C. Materials, including the water phase, are made by dissolving methylparaben in hot water at 70°C and adding glycerin, then triethanolamine, maintained at 70°C. After the dissolved phase, the oil phase is put into the mortar and mixed with the water phase while stirring using a magnetic stirrer for 3 minutes at a constant speed until it is homogeneous and a lotion base is formed. After that, the extract of rosella petals and calliandra honey is added and stirred until homogeneous (Rahmawanty et al., 2020).

2. Antioxidant Testing

Stock solution (roselle petal extract lotion) has been made into five series of dilutions; each is taken 4 ml and then mixed with 1 ml DPPH solution of 0.4 mM. The mixture was incubated during the previously obtained operating time and read on its absorbance at the maximum wavelength of DPPH. Blank absorbance can be obtained by measuring the absorbance of 1 ml of 0.4 mM DPPH solution and 4 ml of methanol with various concentrations and measuring its absorbance at a wavelength of 517nm in 6 minutes.

Data Analysis

To ensure that rosella extract, calliandra honey, and ethyl vitamin C have substantial antioxidant activity differences, an analysis was performed using SPSS 16.0. The test is carried out first, namely the Shapiro-Wilk normality test. The results of the analysis obtained a p-value of 0.0000. After it was found that it was not normally distributed, the Kruskal-Wallis analysis test was carried out, and then the Mann-Whitney test was continued.

Based on the Kruskal Wallis test, the asymp value was obtained. Sig. 0.000, which indicates that there is a significant difference in antioxidant activity. This is because the % inhibition of each sample has a large enough difference.

RESULT AND DISCUSSION

Table 1. Formulations of the lotion combination of rosella flower petal extract with calliandra honey.

Ingredient	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	Function
Rosella flower petal extract	2.5	2.5	2.5	-	-	2.5	Active substance
Calliandra honey	1.5	2	2.5	2.5	-	-	Active substance
Ethyl vitamin c	-	-	-	-	2	-	Active substance
Stearic acid	4	4	4	4	4	4	Emulgator
Triethanolamine	8	8	8	8	8	8	Emulgator
Cetyl alcohol	2	2	2	2	2	2	Emollient
Glycerin	10	10	10	10	10	10	Humectant
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	preservative
Aquadest	Add 100	Add 100	Add 100	Add 100	Add 100	Add 100	Solvent

Table 1. show the components used in the manufacture of the lotion were the aqueous phase and the oil phase. Adding an emulsifier can reduce the interfacial tension of the preparations in the oil and water phases. This emulsifier will surround the dispersed droplets in a strong layer to avoid collation and separation between the dispersed phases. Stearic acid and triethanolamine are used because they are not toxic or irritating, and their functions are closely related to the formulation because they can form stable anionic emulsions (Slamet & Waznah, 2019). So that the lotion preparation remains stable. The water phases used in this study were glycerin, triethanolamine, methylparaben, and distilled water. Methylparaben is intended to inhibit the growth of bacteria or fungi to be used as a preservative. (Rowe et al., 2009). The oil phases used in this study were cetyl alcohol and stearic acid. The cetyl alcohol in the formulation increases stability and softens the skin (Slamet & Waznah, 2019). Excess cetyl alcohol can combine the liquid solution of the semi-extender to form a continuous viscoelastic phase that gives it semi-solid properties.

Organoleptic Test

Organoleptic tests were carried out to see the physical appearance of the lotion, including color, aroma, and texture. Organoleptic testing is commonly also called sensory testing or sense testing. The testing method uses the human senses as the main tool (Gusnadi et al., 2018). The Organoleptic test was carried out for 12 days with two observations. The results of the organoleptic observations of lotion on day 0, F1, F2, F3 of the roselle petal extract with a combination of calliandra honey (1.5%, 2% and 2.5%) have a slightly greenish-brown color, F4 with the unique active ingredient calliandra honey (2.5%) has a white color, F5 with the unique active ingredient ethyl vitamin C (2%) had a yellowish color.

In comparison, F6, with the unique active ingredient rose petal extract (2.5%), had a slightly greenish-brown color. When formulating the combination with calliandra honey, adding various concentrations did not affect the resulting color. It has been released 33 in a study conducted by (Nurnasari & Khuluq, 2017) using the active ingredient of roselle petal ethanol extract as a lotion with the same color.

Homogeneity test

This testing aims to determine if any coarse particles are in the preparation and if the active ingredient and excipient are homogeneously mixed (Arthania et al., 2021). The homogeneity test of the preparation was carried out by applying it on a watch glass or other suitable transparent material that must show homogeneity; a small sample of hand body lotion is taken and placed between two glass slides, and then it is observed for any coarse particles. The preparation is homogeneous if no coarse particles or lumps are mixed uniformly if an evenly distributed color is seen (Mardikasari et al., 2017). The homogeneity test was conducted to determine whether the particles separated in the lotion preparation or the dispersed phase in the dispersion phase. The lotions that have been developed are very important for homogeneity testing because their use, when applied to the surface of the skin, can affect the effect of substance therapy on blemished skin (Ansel, 1989). The observations on the lotion homogeneity test showed that the six formulas were homogeneous preparations. This is also due to the perfect mix of all the ingredients, plus it shows that adding extracts and other active ingredients did not affect the homogeneity of the lotion. Homogeneity can be determined by visually observing the uniformity of the color in the preparation if the color of the lotion is uniform. Then, the lotion is homogeneous.

pH test

The pH test Performed with a pH meter aims to determine the level of acidity in a cosmetic product, which is a very important factor. Because cosmetic products used for external use will be in direct contact with the skin. The skin is a very sensitive organ with a pH of 4 – 8, as indicated by SNI-16-4399-1996, so the resulting lotion products are safe for use by the skin. All formulas have increased; this was the effect of adding active ingredients, each with an acid number. Changes in the pH value during storage indicated a reaction or damage to the constituent components of the preparation such that it may be decreased or increased the pH value of the preparation, where changes in the pH value from the effect that preparation had given when applied. Tests of the degree of acidity in the lotion preparations before and after storage at four °C and 40°C with six cycles for 12 days showed a change to determine whether the increase in viscosity was significant or not; the data were then statistically analyzed using SPSS 16.0 software with a significant level of 5%. Data were tested for normality using Shapiro-Wilk with a significance value of $P > 0.05$ (Appendix 4). This shows that the data is normally distributed. 36 Because the data is normally distributed, the analysis can continue with a parametric test, One Way ANOVA, which shows a significant value obtained (p-value < 0.05) $P = 0.047$ in cycle 6. Then, proceed with Post Hoc using Tukey. The data show that formulations 2 and 5 have a significant value < 0.05 , indicating a significant effect between the formulations and the pH of the lotion preparations. Formulation 5 has the best significance value obtained (p-value < 0.05), namely $P = 0.000$ from other formulas; although it has decreased after storage, it is still within safe limits.

Viscosity test

The purpose of the viscosity test is to determine the consistency of the viscosity of a lotion product. When the product was thick, the greater the force required to flow. Viscosity testing was performed using a Brookfield 4 shaft viscometer at a speed of 5 rpm. The spindle speed will affect the viscosity result. Because the faster the spindle turns, the lower the viscosity, and vice versa; the slower the spindle turns, the higher and thicker the viscosity (Voigt, 1989). The viscosity of the six formulations prepared is under the Indonesian National Standard 2000 – 50000 cPs. The viscosity test results of each formula show that they have increased after the stability test is carried out. Day 12 was done at a high temperature. However, the oven used by the researchers did not experience good heat, so the test preparation became thick. Storage at low temperatures will experience freezing due to decreased temperature, so the viscosity has increased. Meanwhile, F6 experienced a decrease because the oven used still had a set temperature at the time of storage. The results of the lotion viscosity test were analyzed by statistical analysis. The normality test was conducted by looking at Shapiro-Wilk because the sample was less than 50 (Supriadi et al., 2014). The normality test shows a significance value of $p > 0.05$ (appendix), meaning that the six formulas' viscosity test data are normally distributed. Because the data were normally distributed, the parametric test was continued, namely, one-way ANOVA with

a significant result of $p > 0.05$ (appendix), meaning there were no significant differences between the formulas.

Spreadability test

The spreadability test determines whether the preparation's potential to spread on the skin has met the requirements, which is 5 – 7 cm (Dominicia & Handayani, 2019). 0.5 grams was weighed and placed in the center between 2 glass plates. Then, a load (50 g, 100g, 200g, and 500g) was applied and left for 1 minute, and then the spread area was measured (Megantara et al., 2017).

The dispersion value before the stability of the lotion preparation showed that F1 and F4 had decreased because the texture was thicker than the other formulations. This was evidenced by the drastic increase in viscosity of these two formulas. Because dispersibility was generally inversely proportional to viscosity, the higher the viscosity value, the lower the spreadability (Indriastuti et al., 2021). In F3, before the stabilization test, it had an average value because the viscosity value was very high compared to other formulas. After storage, it had a high spreading value because the viscosity value was close to the previous results.

The results of the lotion dispersion test were analyzed by statistical analysis. The normality test was conducted by looking at Shapiro-Wilk because the sample was less than 50 (Supriadi et al., 2014). The normality test shows a significance value of $p > 0.05$ (appendix 4), which means that the data on the dispersion power of the six formulas are normally distributed. Because the data were normally distributed, the parametric test was continued: one-way ANOVA with a significant result of $p > 0.05$ and $p > 0.22$ (appendix 4), meaning there are significant differences between formulas.

Results of antioxidant activity tests

The presence and absence of antioxidant activity in the combination of rosella flower petal lotion with calliandra honey can be seen in the IC_{50} value that describes the free radical scavenging force, which is then correlated with the concentration of the test solution, which can reduce 50% DPPH free radical test solution. The lower the IC_{50} value, the better the antioxidant activity.

Table 2. Results of antioxidant activity tests

Sample	Result of IC_{50} (ppm) \pm Std	Notes
F1	111.04 \pm 0.0138	Medium
F2	108.29 \pm 0.0154	Medium
F3	95.2 \pm 0.0064	Strong
F4	106.29 \pm 0.0138	Medium
F5	108.13 \pm 0.0048	Medium
F6	111.71 \pm 0.005	Medium

The study results in Table 2 show that Formula 3 had the strongest antioxidant activity, with a value of 95.2 ppm. Then, the addition of calliandra honey (2%) to the roselle petal extract (2.5%) had an effect on the increase in the value of antioxidant activity obtained from the roselle petal extract in formula VI as the single active ingredient with an IC_{50} value of 111.71 ppm. The lotion preparation in the IC_{50} value of formula V with the active ingredient ethyl vitamin C as a comparison had a value of 108.13 ppm. This value is small because ethyl vitamin C is susceptible to heating and quickly oxidizes. The IC_{50} values in formulas I and II are 111.04 ppm and 108.29 ppm; if the concentration of calliandra honey is small, it has a small effect on the value of the antioxidant activity. The results of statistical tests showed differences in each formula at each concentration of the active ingredient that has been designed. This research is in line with the research conducted by Ustadi et al. (2017) that calliandra honey has the highest phenolic content value of 156.27 mg QE/100 g) compared to opium honey and rubber honey. In research, Chayati and Miladiyah (2016) stated that the antioxidant activity of the DPPH method on calliandra honey was 48.02 ppm. Then, the research conducted by Nurnasari & Khuluq (2017) showed that 2.5% rosella petal extract produces antioxidant activity of 15.626 ppm to 17.773 ppm. Based on these results, 2.5% calliandra honey was added to 2.5% rosella flower extract. The effect on the increased antioxidant activity of the preparation can be seen from the value of % inhibition.

CONCLUSION

Rosella flower petal extract lotion combination of calliandra honey in formula III showed strong antioxidant activity with a value of $IC_{50} 95.2 \pm 0.0064$ ppm, indicated by its ability to dissolve the purple color of DPPH with various concentrations. It measured its absorbance at a wavelength of 517nm in 6 minutes.

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