

The Antioxidant Activity Test of Rosella Flower (*Hibiscus sabdariffa* Linn.) with DPPH (1,1 - Dhenyl-2-picrylhydrazyl) and its Application for Lipstick Provisions

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Abstract: Rosella (*Hibiscus sabdariffa* Linn) grows a lot in the Java region and has never been analyzed and applied optimally in the health sector. The purpose of this study was to determine the antioxidant activity and application of anthocyanin dyes in lipstick preparations. This research using maceration and reflux extraction methods with ethanol fraction was carried out by Uv-Visible Spectrophotometry through DPPH reagents to obtain IC50 inhibitory concentration. The results of the test data were analyzed descriptively, and the extract was applied in the preparation of the lipstick preparations that were tested including organoleptic (physical, aroma, texture, and color), homogeneity test of lipstick preparations, test smear, melt temperature test, pH test, color intensity test, and irritation test. The results of the research showed that the yield of rosella extract with maceration method was 88.72 g or 88.72% w/w, and for reflux method was 48.33 g or 48.24% w/w, the results of antioxidant test with IC50 value of extract rosella maceration is 11,940 µg / mL while rosella reflux extract is 25,942 µg / mL, this means that both extracts are very active as antioxidants (very high category), and the results of homogeneous lipstick formulation test, pH 4, melting point 50-70^oC, strength test 600-900 grams, and non-irritating.

Keywords: antioxidant, DPPH, rosella, lipstick

1. Introduction

Lipstick is used to beautify lips with attractive colors, protect lips from drying out, and can accentuating the good side and disguising bad lip shape. [1]

Based on the results of routine supervision of the POM Agency Throughout Indonesia there are cosmetics in circulation from October 2014 to September 2015, found 30 types of cosmetics containing hazardous ingredients consisting of 13 types of cosmetics in foreign production and 17 types of cosmetics in domestic production. The hazardous substances identified are contained in the cosmetics, namely K3 red and K10 red (rhodamine B) dyes, retinoic acid, mercury, and hydroquinone. Based on the Head of POM RI Regulation No. 2 of 2014 concerning the Second Amendment to the Regulation of the Head of POM No. Hk.03.1.23.08.11.07517 of 2011 concerning Technical Requirements for Cosmetic Materials, these materials are included in the list of hazardous substances that are prohibited for use in the manufacture of cosmetics [2]. Based on this, it is necessary to have a lipstick product that is safe and has benefits that are suitable for its use. As products derived from natural ingredients that are safer for the skin. One type of natural colorant from plants that are used as a substitute for synthetic dyes is the Rosella flower (*Hibiscus sabdariffa* Linn.). Roselle flower produces anthocyanin pigment as a natural coloring agent and is one of the plants that can be used as a preservative because it contains antioxidants and antibacterial.

Free radicals that are produced continuously during normal metabolic processes, are considered to be the cause of

damage to the functioning of the body's cells which ultimately triggers the onset of degenerative diseases [3]. Free radicals in the body are very reactive and will interact destructively through oxidation reactions with parts of the body and certain cells composed of fat, protein, carbohydrates, DNA, and RNA that trigger various diseases such as coronary heart disease, premature aging, and cancer. Therefore antioxidants are needed to overcome free radicals [4]. This study aims to determine the antioxidant activity and application of anthocyanin dyes in lipstick preparations from rosella flowers (*Hibiscus sabdariffa* Linn).

2. Research Methodology

- Tools: maceration tools, round bottom flask, condenser, heater, stirring rod, mortar, stamper, lipstick mold, glassware, freezer, blender.
- Ingredients: roselle flower, citric acid, aquadest, Cera alba, lanolin, cetyl alcohol, paraffin wax, methylparaben, propylparaben, castor oil, methanol, DPPH.

3. Research Procedure

Extraction Process with Maceration

Weigh Rosella flower powder by 100 grams, then put the simplicia powder into the vessel then add 1000 ml of solvent where the solvent contains 70% ethanol mixture: 1% citric acid (9: 1), then the vessel is tightly closed and left for 5 days protected from the light, stirring occasionally. The extract is filtered using a flannel cloth and liquid extract will be obtained then the liquid is evaporated by direct evaporation until thick, and ethanol-free test.

Reflux Extraction Procedure

By using the same solvent as maceration, the reflux process is carried out at temperatures ranging from 70-80°C for approximately 3 hours. The extract obtained is then evaporated until thick.

Anthocyanin Qualitative Test

The extract was heated to 10 mL 2M HCl then heated at 100°C for 5 minutes. The characteristic of anthocyanin is the red color will not fade. Extract added 2M NaOH solution drop by drop until the result is the red color changes to blue-green and fades slowly.

Antioxidant Activity Test

Roselle extract parent solution (both maceration and reflux) 1000 ppm pipette every 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml, put into a 10 ml measuring cup, then add methanol ad 10 ml shake ad homogeneous. Then it was incubated for 30 minutes and then absorbance measured at a wavelength of 450-550 nm using a UV-Vis spectrophotometer. As a blank using methanol and DPPH solution, then absorbance is measured.

Lipstick formulation

Lipstick dosage formulations are made in 3-gram weight as follows

Table 1: Roselle Flower Lipstick Formulation

Composition	Concentration (%)				
	F1	F2	F3	F4	F5
Rosella extract	0	5	10	15	20
Cera Alba	20	20	20	20	20
Lanolin	4	4	4	4	4
Setil Alcohol	5	5	5	5	5
Paraffin Wax	4	4	4	4	4
MetilParaben	0,02	0,02	0,02	0,02	0,02
Propil Paraben	0,18	0,18	0,18	0,18	0,18
Castor oil	Ad 3 gram				

Making lipstick preparations



Dissolve dyes into the oil phase. Castor oil is poured into the cup (mixture 1) and then Rosella flower extract is added. After that, melt the base (lanolin, cetyl alcohol, Cera alba, and paraffin wax) on a water bath, the base which melts completely little by little the molten base is poured into (mixture 1) while stirring continuously to avoid hardening. Then add preservatives (propylparaben) and oleum rosae (farfum), the mixture is melted briefly over the water bath until slightly melted, then poured into the mold. Then the physical properties of the lipstick preparation include: organoleptic test, pH test, homogeneity test, topical test, melting point test, lipstick strength test, irritation test.

4. Results and Discussion

Extraction process with 2 methods, namely hot and cold methods in which each method is chosen one way. The cold method uses maceration extraction, while the hot method uses reflux. The solvents used in the extraction process by these two different methods together use a 70% ethanol mixture solvent: Citric Acid 1% (9: 1), because effective

solvents for dissolving anthocyanins are polar solvents such as ethanol acidified with Acid citrate (Siahaan et al., 2014). From the extraction results with maceration, the yield was 88.72 g or 88.72% w / w, while the reflux extract obtained the extract yield

Table 2: Organoleptic Extract Test Results

Extract	Organoleptic Test	Result of Research	Picture
Maceration	Shape	Liquid	
	Color	Brown	
	Smell	Distinctive extract	
Reflux	Shape	liquid	
	Color	Reddish-brown	
	Smell	Distinctive extract	

The anthocyanin content test in both positive rosella flower extracts contained anthocyanin with marked color changes when added to HCl resulting in a deep red color that did not fade and added NaOH namely green color. This is because the addition of HCl accompanied by heating will produce acidic compounds because during heating OH-ions will be eliminated or will be released and will bind H + ions to produce a solution that is red due to acid. Likewise, the addition of 2M NaOH with a change in green color showed anthocyanin. This is because anthocyanins contained in rosella flowers are polar anthocyanin compounds when added to NaOH, the compounds will substitute OH groups in NaOH so that it becomes more alkaline making the color changes to green. The H atom of the -OH group in phenols contained in the anthocyanin structure can be substituted by active metals such as sodium and potassium, forming alkoxides and hydrogen gas so that it can be reacted with the addition of NaOH to form the Na phenoxide group and HCl to form a benzene chloride group.

Next up is the antioxidant activity test of the two extracts which results in the relationship between probit and the log concentration of each extract as follows:

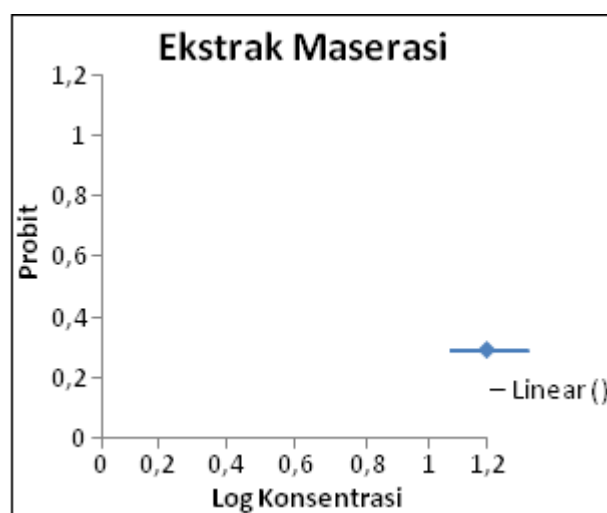


Figure 1: Diagram of the relationship between probit and log concentration from maceration extract

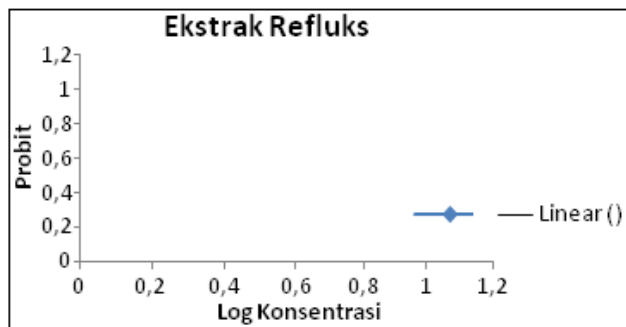


Figure 2: Diagram of the relationship between probit and log concentration from reflux extract

Furthermore, the IC₅₀ value calculated obtained the IC₅₀ value of rosella maceration extract was 11,940 µg / mL while the IC₅₀ value of rosella reflux extract was 25,942 µg / mL, this means that both extracts were very active as antioxidants because the IC₅₀ value was ≤ 100 µg /mL. Extracts that have been tested both anthocyanin and antioxidant activity then made lipstick preparations and tested physical properties which include organoleptic test, pH test, test of homogeneity, topical test, melting point test, lipstick strength test. Lipstick physical test results as follows:

1) Organoleptic Test



Figure 3: Roselle extract lipstick

The results of research on organoleptic lipstick preparations showed differences in the color of each preparation, it can be seen in the lipstick preparations from maceration extracts in formulas II-IV produce a light brown color, while in formula V produce a rather dark brown color. In the preparation of the reflux extract in the formula, II produces a light brown color, while in the formula III-V produces a dark brown color. This shows that the extract difference and extract concentration influence the organoleptic test, especially in the preparation color parameters and duration of extract storage so as to produce the extract characteristics was obtained.

2) Homogeneity Test

The homogeneity test aims to determine whether the mixing of each component in the manufacture of lipstick has been evenly mixed. The presence of coarse grains indicates the preparation of lipstick is not homogeneous because it is not dispersed between the components of lipstick. Based on homogeneity tests, each formula shows homogeneous conditions and no particles. The difference in extract and extract concentration did not affect the homogeneity test, but

according to Handayani in the process of making lipstick, the most important thing was the grinding process to produce homogeneous colors, the color mixing process must also be considered. So it can be concluded that for homogeneity testing, the preparations obtained are in accordance with the requirements.

3) pH test

The results of the test lipstick preparation of each formula with extracts both from maceration and reflux result in pH 4, while lipstick without extract produces pH 6. It can be concluded that the lipstick preparation is safe to use, the pH is close to the pH of the lips ± 4 [5], the process extraction and extract concentration difference did not affect the pH value.

4) Topical Test

In the dye release test, this research was conducted by applying lipstick to the skin of the back of the hand. Each application of each preparation of each lipstick formula is made and applied to the skin of the back of the hand, which is maxing 5 times polishing. Based on the results obtained both preparations without extracts or with extracts all produce the same results, which is more than 4x topical, where maceration and reflux extract lipsticks in formulas II-IV do not give color to the back of the hand and only formula V gives a brown color to the back of the hand.

5) Melting Point Test

The melting point test serves to determine the melting point of lipstick that will affect the storage of lipstick.

Table 3: Melting Point Test Results

Extract Refluk (°C)					
Pengamatan	F1	F2	F3	F4	F5
Start to melt	55	55	55	55	55
Whole	62,9	64,5	63,9	63,9	63,9
Ekxtract Maserasi (°C)					
Pengamatan	F1	F2	F3	F4	F5
Start to melt	55	55	55	55	55
Whole	62,9	64,5	63,9	63,9	61

Based on data from the melting point test results table, shows that each formula has a melting point that is different from both preparations that contain extracts and those without extracts, but all lipstick preparations have melting points that meet the test standards, namely the ideal lipstick melting point that is around 50-70 ° C [6].

6) Strength Test

The strength test is useful to determine the level of strength and durability of lipstick preparations to resist damage caused by various types of handling, such as production, packaging, shipping, and transportation. Based on the results of the strength test, lipstick formulation has good strength with a strong range of 600 - 900 grams, it refers to several journals with a strength test of 165-215 g [1].

7) Irritation Test

The irritation test aims to determine the reaction after the use of lipstick preparations cause irritation or not. This test was

carried out on 10 panelists by applying lipstick to the inner forearm for three consecutive days, and the results showed that all panelists gave negative results to the observed irritant reaction parameters ie the absence of red skin, itching, or the presence of swelling. From the results of the irritation test, it can be concluded that the preparation of the lipstick made is safe to use [7].

5. Conclusion

The results of the study concluded that the antioxidant test with IC50 value of rosella maceration extract was 11,940 µg / mL while the rosella reflux extract was 25,942 µg / mL, this meant that both extracts were very active as antioxidants, and the results of homogeneous lipstick formulation test, pH 4, melting point 50-70⁰C, strength test of 600-900 grams, and does not cause irritation.

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