



MOISTURIZING EFFECTIVENESS OF ORGANIC LIP CREAM FORMULATION
WITH BEETROOT EXTRACT (*Beta vulgaris* L) AND CHIA SEED OIL (*Salvia
hispanica*)

By

Dela Amalia Putri¹, Chaidir², Muhammad Hanafi³

¹Master of Pharmacy. Natural Cosmetics, Universitas Pancasila, Jakarta, 12640, Indonesia

^{2,3}Faculty of Pharmacy. Universitas Pancasila, Jakarta, 12640, Indonesia

E-mail: putridela@gmail.com

Abstract

Objective: The purpose of this study was to evaluate the quality parameters, safety and analyze the moisturizing effectiveness activity of Organic Lip Cream with beetroots extract (*Beta vulgaris* L) and Chia Seed Oil (*Salvia hispanica*). **Methods:** The beetroot extraction was optimized using 3 solvents and 2 types of cultivation (Conventional and Organic) and was determined its total Betalain assay (Betaine and Vulgaxhantin) was. The quality parameter of lip cream was evaluated as physical appearance, texture, viscosity, adhesion test, pH, homogeneity, hedonic test, acute dermal irritation test, and stability. Testing the moisturizing effectiveness by measuring lip moisture content immediately after use, 4 hours after use, and after 3 days of routine use. **Results:** Organic beetroots contain a total of Betalain pigment concentration significantly higher than beetroots from conventional cultivation. The highest total betalain assay in the extract was performed by 96% ethanol solvent with the addition of 1-2 % citric acid (pH 5) compared to 70% ethanol solvent and water solvent. Organic lip cream with beetroots extract and chia seed oil is significantly effective to increase lip moisture immediately after use, 4 hours after use and after 3 days of routine use compare to baseline (before use). Organic lip cream with beetroot extract and chia seed oil was formulated with result meet specifications of good lip cream, are not irritative, has effectively increased lip moisture, acceptable by hedonic test (appearance of the preparation, sensation on the lips, adhesion and smearing power) and stable for 4 weeks at 40°C. **Conclusion:** Organic lip cream with beetroots extract and chia seed oil are meets specifications of quality control, safety, and efficacy (moisturizing effectiveness).

Keywords: Chia Seed Oil, Beets, Betalain, Organic, Moisturizer.

INTRODUCTION

Lip cream is one of the cosmetic preparations favored by women [1]. The lip cream is applied to the lips to give shape and color and protect the lips from the environment, however, the number of harmful chemical dyes such as tar-based coal (coal tar) such as tartrazine can cause allergies, nausea, dermatitis, and cause dry lips because it is often consumed by users [2, 3]. The use of lip cream with dye synthesis such as eosin has been considered a risk factor for developing systemic lupus erythematosus (SLE) [4]. To prevent this

from happening, it is necessary to develop dyes and moisturizers from organic ingredients that are safe to apply to the lips, such as the pigment betalain from beetroots extract (*Beta vulgaris* L.) and the fatty oil from chia seed (*Salvia hispanica*) [5].

By using organic beets as natural dyes and oil derived from organic chia seeds as a natural moisturizer, it aims that it can reduce problems on the lips and provide safer results compared to using synthetic or conventional cultivation ingredients. Organic beetroot extract obtained from organic beets can be

extracted by maceration method with ethanol, ethanol: citric acid, and polar water solvent to extract betalain pigment content.

The organic lip cream formulation with beetroots extract will be tested for physical quality, effectiveness, safety, acceptability, and stability. Physical quality tests include organoleptic, viscosity, pH, and homogeneity. The effectiveness test includes adhesion, spreadability, and moisture test. The safety test includes the hedonic acceptability test, the irritation test, and the stability test which includes organoleptic stability, pH, and color homogeneity.

MATERIAL AND METHODS

Material

Organic beetroots used are from Parung Farm, Bogor, Indonesia. Beetroots used are *Beta vulgaris* L has been determined by the Indonesian Institute of Sciences, Research Center for Plant Conservation and Botanical Gardens, Bogor, Indonesia). Organic chia seeds oil (*Salvia hispanica*), ethanol, citric acid, water, beetroot thick extract, caprylic triglyceride, octyl dodecanol, beeswax, carnauba wax, mica powder, glycerin, ascorbic acid, potassium sorbate potassium hydroxide, KI solution, phenolphthalein (pp), chloroform, Wijs reagent, toluene and sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$).

Method

Extraction of Conventional Organic Beets and Beets with 3 solvents:

- 1) Maceration extraction with an ethanolic solvent
25 g of beetroots extracted by maceration method using 70% ethanol for 40 minutes. Filtered macerate and analyzed the total betalain assay.
- 2) Maceration extraction with ethanol + citric acid solvent
25 g of beetroots extracted with solvent (96% ethanol + citric acid) pH 5 for 40 minutes, filtered macerate, and analyzed the total betalain assay.

3) Extraction with water solvent

25 g of beetroots washed and chopped, add water and blended, let stand for 40 minutes, filtered macerate, and analyzed the total betalain assay.

Analysis of Betalain assay from 2 Types of Beets and 3 Solvents

The test parameters carried out on the results of extracting beetroot from 3 types of solvents were betalain assay using UV-Visible Spectrophotometry (UV-1240 Shimadzu, Japan) at three wavelengths are 476, 538, and 600 nm. The absorbance results obtained were then calculated by the formula

$$C \text{ betaine} = \frac{x}{1120} \times \text{dissolving factor}$$

$$C \text{ vulgaxantin} - 1 = \frac{y}{750} \times \text{dissolving factor}$$

The pigment content of betacyanin is an amount of C betaine + C vulgaxanthin-1 in mg / 100g units of betaine vulgaxanthin-I shown in fig.1

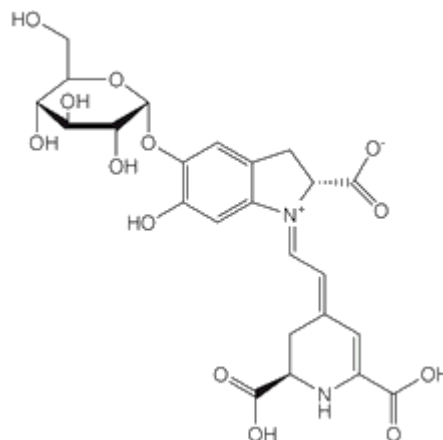


Fig. 1 Betacyanin (betalain) structure (Amdree, 2015)

The results of the total betalain content were compared, and the most effective solvent was determined to extract the betalain pigment.

Lip cream formulation

Each ingredient was weighed carefully according to the desired weight. Base lip cream (triglyceride, octyl dodecanol, chia seed oil, beeswax, carnauba wax, glycerin) was heated on a hotplate at a temperature of 75-85°C until



it melts completely. This process results in phase A.

The color mixture was made by weighing the thick extract of beets, then mixed with mica powder, potassium sorbate, ascorbic acid, and other ingredients, crushed in a mortar until completely homogeneous and no color in powder form or until all dissolved homogeneously. This process produces phase B.

Phase B is mixed into phase A and stirred until homogeneous. After everything is mixed homogeneously, cool to 45 ° C, then the mixture is poured into the lip cream container mold then attach the applicator brush and close it.

Lip cream Evaluation

1) Physical appearance

The examination was observed the physical appearance of the lip cream.

2) Texture

Testing was observed by applying lip cream to the skin, then seeing whether the texture is rough or smooth.

3) Viscosity Test of the lip cream

Viscosity test was observed using Viscometer Brookfield using the appropriate spindle (spindle 3, speed rpm 3).

4) Adhesion test of the lip cream

Testing the adhesion of lip cream preparations was carried out by 0.03 g of lip cream is applied to the glass object, then another glass object is placed on top and pressed with a load of 1000 g for 1 minute. The glass object is attached to the test apparatus and removed with a load of 80 g. The timing is stopped until the two glass objects are separated.

5) Determination of the pH of the preparation

The sample was made in a concentration of 1%, then weighed 1 g of the preparation and melted in a glass beaker with 100 ml of distilled water over a water bath.

6) Homogeneity examination

Each lip cream preparation was checked for homogeneity by applying a certain amount of preparation on a transparent glass.

Acute Dermal Irritation Testing

The animal test used was an albino rabbit (*Oryctolagus cuniculus*) following PerKaBPOM No.7 / 2014 Guidelines for In vivo Non-Clinical Toxicity Test for testing acute dermal irritation which obtained ethical clearance from the Health Research Ethics Committee of the Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia, dated 30 Dec 2020.

The test preparation is exposed to a skin area of ± 6 (2 x 3) cm² with the location of exposure, then the exposure location is covered with gauze and plastered with a non-irritant plaster. Clinical Observation and Assessment of Skin Reactions were performed. All test animals were observed for the presence or absence of erythema and edema, response assessment carried out at 1, 24, 48, and 72 hours after opening the patch (for non-corrosive / irritant test preparations).

Testing the effectiveness of moisturizers by measuring moisture content

Moisturizing effectiveness test has obtained ethical clearance from the Health Research Ethics Committee of the Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia, dated 30 Dec 2020. The experimental design used was completely randomized. The number of volunteers needed is 20 people according to the data needed for statistical data processing. With this condition, each respondent gets 2 samples (2 formulas) smeared on the right and left lip:

- a) Sample 1: Lip cream Formula I (with 1% chia seed oil) on the right lip
- b) Sample 2: Lip cream Formula IV (with 4% chia seed oil) on the left lip

The inclusion criteria for volunteers were healthy women with an age range of 20-40 years and had lip moisture or water content of less than 60%. Volunteers are regular users of lip preparations (lipstick or lip cream) using a minimum of 4 times a week. Subjects must be willing to use the product at least 4 hours per



day, every day for the duration of the test of 3 days, with application/dab at least once.

The exclusion criteria for volunteers were volunteers with chronic dryness or lips (chapped lips) that would not improve with the use of lip balm products.

All volunteers have measured the initial condition of the lip skin on the test area to be applied the lip cream as a baseline. Volunteers must stop using lip preparations for at least 1 day as a baseline.

The test carried out is a moisture test using a skin analyzer. Treatment begins by applying lip cream evenly on the lips. The lip cream is applied every day for at least 4 hours with 3 applications based on the group specified above. Changes in lip skin condition were measured before use, after immediately after use, after 4 hours, and after 3 days of routine use of lip cream using a skin analyzer.

Preference Test (Hedonic Test)

The organoleptic assessment test was carried out using the Hedonic method, by analyzing according to the preference test (parameters of the color of the preparation, the sensation on the skin of the lips, the stickiness, and the smear of the dosage color) using 20 panelists who were given samples of lip cream preparations. To see the respondent's level of preference for lip cream preparations based on each parameter, a numerical scale was used.

Stability Testing

Stability testing was carried out on samples with the best formula, the product is stored at a temperature of 40 ± 2 ° C, RH $75 \pm 5\%$ for 1 month. The parameters evaluated included physical appearance, texture, pH, adhesion, spreadability, and homogeneity.

RESULT AND DISCUSSION

The results of the comparison of the assay total of betalain extracted

The results of the comparison of the assay total of betalain extracted from beets with organic cultivation (1) and beets with conventional planting (2) with 3 solvents are:

Table 1. comparison of the assay total of betalain extracted from beetroots with organic cultivation (1) and beets with conventional planting (2)

Solvent	P-Value	Tukey Method	
		Organic cultivation	Conventional cultivation
Water	0.008*	1.12805	1.08264
Alcohol	0.037*	2.84031	2.81274
Solvent 70%			
Solvent	0.000*	2.94221	2.2536
Alcohol 96%: Citric acid 1-2% pH 5			

* P <0.05, Tukey Method based on Mean grouping

Based on the results of the One-way ANOVA statistical analysis of beet extract with water solvent, the p-value <0.05 where the null hypothesis is rejected, meaning that there is a significant difference to the beet planting method planting 1 (Organic cultivation) has a higher total betalain content compared to conventional planting (planting 2), whether it is extracted with water solvent, 70% ethanol or ethanol: citric acid pH 5. The average content of total betalain pigment in organic beetroot extract is 2.8346 mg/100 g, which is higher than conventional beetroot extract, which is 2.7959 mg/100 g.

Based on the above result, it can be seen that the content of betalain, which is a secondary metabolite in organic beetroots plants, has a higher content than the beetroot from conventional cultivation, and it align with a previous study where the results showed that organic fruits tend to have higher secondary metabolite (hydrolyzable polyphenol contents)



than conventional ones, with values being 11.5% in orange peels, to 72.6% in papaya peels, higher for hydrolyzable polyphenols. This is likely to exert protection against damage of fruit and roots when grown in the absence of pesticides [14,15].

The comparison of the extraction method with 3 solvents:

water solvent (1), Alcohol 70% solvent (2) and Alcohol: citric acid (3) solvent to Organic beetroots:

Table 2. The comparison of the extraction method with 3 solvents to Organic beetroots

Solvent	P-Value	Tukey Method
Water	0.000*	1.12805
Alcohol Solvent 70%		2.84031
Solvent Alcohol 96%: Citric acid 1-2% pH 5		2.94221

* P <0.05, Tukey Method based on Mean grouping

Based on the results of the One-way ANOVA statistical analysis, the p-value is <0.05 where the null hypothesis is rejected, meaning that there is a significant difference in the choice of solvent for beetroot extraction. After proceeding with the Tukey test, it was seen that the highest or recommended solvent for extracting organic beets was solvent 3 (Alcohol Solvent and Citric acid 1-2% pH 5) and aligns with Castellar et al. that betacyanin has a high level of stability at pH 5 [16].

Table 3. Lip cream formula with beetroot coloring and organic chia seed oil

Material	Formula (%)			
	I	II	III	IV
Organic Beetroot extract	30	30	30	30
Organic Chia seed oil	1	2	3	4
Octyl dodecanol	18	18	18	18
Beeswax	7	9	9	9
Carnauba wax	7	6	6	6
Mica Powder	15	15	15	15
Glycerin	1	1	1	1

Ascorbic acid	0.2	0.2	0.2	0.2
Potassium sorbate	0.1	0.1	0.1	0.1
Grape flavor	Qs	Qs	Qs	Qs
Caprylic triglyceride	Ad	Ad	Ad	Ad
	100	100	100	100

Based on the results of the evaluation of lip cream preparations, the organoleptic and homogeneity of the lip cream met the specifications, homogeneous, the pH of the lip cream obtained from 4.97 to 5.01 met the specifications of the lip cream, which was in the physiological pH range of the skin (4.5 - 6.5), viscosity lip cream (16 820–18 000 cP), meets the specifications, with theoretical viscosity (10 000 – 20 000 cP), lip cream adhesiveness of 11-18 seconds which indicates that the lip cream adheres well when applied to the lips but is not sticky to the surface of the lips, the resulting oil also has good lubrication, which is a lot and evenly distributed with several basting. Formulas 1,2,3 and 4 have no significant difference in the evaluation results of lip cream on all formulas. The difference in chia seed oil content in the preparation, with concentrations 1,2,3, and 4%, did not affect the quality of the resulting lip cream because the base used was the same and the pH had been adjusted.

Irritation Test for Lip cream preparations

Based on the irritation test, organic lip cream preparations with beetroot extract and chia seed oil are non-irritate (primary irritation index 0).

Moisturizing Effectiveness Test

Organic lip cream with beetroot extract and chia seed oil moisturizing effectiveness test was carried out on 20 female respondents aged 20 - 40 years. Tests were carried out on 2 formulas, Formula I with the lowest chia seed oil content (1%) and Formula IV with chia seed oil content (4%). Moisture analyzed with the SK-8 portable skin analyzer was carried out before use, immediately after use, after 4 hours of use, and after routine use for 3 days.

Statistical analysis results for Formula I (1% chia seed oil) immediately after use, after

4 hours of use, and after 3 days of routine use compared to before use (baseline):

Table 4. Statistical analysis results for Formula I (1% chia seed oil) immediately after use, after 4 hours of use, and after 3 days of routine use compared to before use (baseline)

Measurement time	P-Value	Tukey Method	
		Formula I	Baseline
Immediately after use	0.000*	55.912	38.977
After 4 hours of use	0.000*	46.878	38.977
After 3 days of routine use	0.000*	43.858	38.977

* P <0.05, Tukey Method based on Mean grouping

Based on the results of the one-way ANOVA statistical analysis, the p-value is <0.05 where the null hypothesis is rejected, meaning that there is a significant difference in the use of chia seed oil lip cream Formula I. Based on the statistical test results above, Formula I gives an increase moisture content or humidity significantly immediately after use, 4 hours after use and routine use for 3 days compared to before use.

Results of statistical analysis of Formula IV (4% chia seed oil) immediately after use, after 4 hours of use, and after 3 days of routine use compared to before use (baseline):

Table 5. Statistical analysis results for Formula IV (4% chia seed oil) immediately after use, after 4 hours of use, and after 3 days of routine use compared to before use (baseline)

Measurement time	P-Value	Tukey Method	
		Formula IV	Baseline
Immediately after use	0.000*	57.298	38.977
After 4 hours of use	0.000*	47.582	38.977
After 3 days of routine use	0.000*	43.813	38.977

*P <0.05, Tukey Method based on Mean grouping

Based on the results of the one-way ANOVA statistical analysis, the p-value is <0.05 where the null hypothesis is rejected, meaning that there is a significant difference in the use of chia seed oil lip cream Formula IV. Based on the statistical test results above, Formula IV gives an increase moisture content or humidity significantly immediately after use, 4 hours after use and routine use for 3 days compared to before use. Thus the result is aligned where 4% chia seed oils after 8 weeks of application, has significant improvements in skin hydration, lichen simplex chronicus, and prurigo nodularis were observed [8].

Based on the results of the statistical tests above, both Formula I and Formula IV resulted in a significant increase in moisture or moisture content. The average increase in water content after use was Formula I of 16.935% and Formula IV of 18.321%. The average increase in water content after 4 hours of use in Formula I was 7.901% and Formula IV was 8.605%. The increase in humidity after 3 days of use on average, Formula I of 4.881% and Formula IV of 4.836%, this increase is still not following the expectations of researchers, this is because the working principle of chia seed oil in increasing moisture is as an emollient, with filling in the blank cells in the skin layer to reduce evaporation, which acts on the top layer of the skin so that the increase in water content after regular use is about 4.8%.

Comparative statistical analysis between Formula I and Formula IV immediately after use, after 4 hours of use, and after 3 days of routine use as follows:

Table 6. Statistical analysis results for Formula IV (4% chia seed oil) immediately after use, after 4 hours of use, and after 3 days of routine use compared to Formula I (1% chia seed oil)

Measurement time	P-Value	Tukey Method	
		Formula I	Formula IV
Immediately after use	0.388*	55.912	57.298



After 4 hours of use	0.658 *	46.878	47.582
After 3 days of routine use	0.827 *	43.858	43.813

* P <0.05, Tukey Method based on Mean grouping

Based on the results of the one-way ANOVA statistical analysis, the p-value is > 0.05, where the null hypothesis is accepted, meaning that there is no significant difference to the use of chia seed oil lip cream Formula I and Formula IV after routine use for 3 days. Based on the results of statistical analysis comparing the effect of using Formula I and Formula IV lip cream immediately after use, after 4 hours of use, and after 3 days of routine use between Formula I and Formula IV, there was no significant difference. Formula I contains 1% chia seed oil while Formula IV contains 4% chia seed oil, with this difference in concentration does not give a different result, so 1% chia seed oil is sufficient to increase lip moisture.

Preference Test (Hedonic Test)

The preference test was carried out on Formula I and Formula IV on 20 respondents who were also respondents to the humidity effectiveness test. The parameters tested included appearance of the preparation, sensation on the lips, adhesion, and spreadability.

The statistical test results of the appearance of the preparations between Formula I and Formula IV used the Mann-Whitney test:

Table 7. Statistical analysis results of the hedonic test (appearance of the preparation, sensation on the lips, adhesion, and spreadability) between Formula I and Formula IV used the Mann-Whitney test

Parameter	P-Value*	
	Not adjusted forties	adjusted forties
the appearance of the preparation	0.860	0.0841
sensation on the lips	1.000	1.000

adhesion	0.930	0.924
spreadability	1.000	1.000

*P<0.05

Based on the results of the Mann-Whitney analysis, the comparison between Formula I and Formula IV on the appearance of the preparation, sensation on the lips, adhesion, and smearing power, all test P values are greater than 0.05 so that they receive H0 which shows no significant difference between Formula I and Formula IV.

Stability Test

Table 8. Statistical analysis results of stability test (pH and adhesion) Formula I, II, III, and IV for 4 weeks storage

Parameter	P-Value
pH	0.225*
adhesion	0.635*

* P<0.05

Formulas on the stickiness stability of lip cream Based on the results of the one-way ANOVA statistical analysis, the p-value is > 0.05, where the null hypothesis is accepted, meaning that there is no significant difference in the adhesion of the preparation to the difference in the concentration of chia seed oil. This is because the bases used are the same, and the chia oil concentration that varies from 1 to 4% does not affect pH and adhesion during storage.

CONCLUSION

Based on the results of the research that has been done it can be concluded that:

Organic beets contain significantly greater (total) betalain pigment than beets from conventional cultivation. The 96% ethanol solvent with the addition of citric acid extracted the betalain pigment from organic beets was significantly greater than other solvents. However, the resulting viscous extract has a low pH so the use of 96% ethanol with the addition of citric acid (pH 5) is not used in the



extraction of beets for lip cream formulations. Organic lip cream with organic beetroot extract and chia seed oil was formulated and has met the specifications of a good lip cream, does not irritate, can effectively moisturize lips, and is stable for 4 weeks at 40°C.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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