

Formulation of Lip Balm from Nanoparticles of Beetroot Extract (*Beta vulgaris* L) and Testing of Sunscreen Activity

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Abstract: Background: As a tropical country, Indonesia faces the risk of skin damage due to year-round sun exposure, including on the lips, which are susceptible to UV effects such as dryness, cracking, and hyperpigmentation. This study utilizes the betacyanin content in beetroot (*Beta vulgaris* L.) a phenolic compound with photoprotective properties that can counteract UV free radicals. This study applies nanotechnology through the formulation of beetroot extract nanoparticles to optimize sunscreen activity. **Aim:** This study aims to evaluate and compare the sunscreen activity between lip balm formulations containing beetroot extract nanoparticles and beetroot extract lip balm formulations, as well as to determine the extent of the effectiveness of nanoparticle-based lip balm in providing protection against UV rays. **Materials and Methods:** This study extracted beetroot (*Beta vulgaris* L.) using the maceration method with 96% ethanol, yielding a concentrated extract (yield 34%) with a dark red color. **Results:** Phytochemical analysis revealed the presence of flavonoids, phenolics (20 mg GAE/g), tannins, and alkaloids in beetroot. The nanoparticle extract was successfully formulated with a size of 1.05 nm (PDI 0.403) and a zeta potential of -21.0 mV. Among the seven lip balm formulations, F6 (30% nano extract) demonstrated the best performance with an SPF of 25.25 (ultra category), %Te of 0.26%, and %Tp of 0.61%, as well as optimal physical stability (homogeneous, melting point 70°C, pH 4.5–6.5). The nano extract formulas (F4–F6) significantly outperformed conventional extracts (F1–F3) in sunscreen activity, demonstrating the effectiveness of nanotechnology in enhancing UV protection. **Conclusion:** These findings demonstrate that beetroot nanoparticle extracts have potential as an effective and safe natural sunscreen lip balm for lip protection.

Keywords: Lip balm, nanoparticles, beetroot, sunscreen, SPF

INTRODUCTION

Excessive sun exposure can cause skin damage, premature aging, and skin cancer (Sholehah et al., 2022a). Lips are highly susceptible to UV rays because they have a thin stratum corneum (3–4 layers), lack hair follicles or sweat glands and are prone to dryness, cracking, and hyperpigmentation (Sholehah et al., 2022b). External protection such as lip balm with sunscreen is necessary to prevent the harmful effects of UV rays (Sabzevari et al., 2021). However, chemical sunscreens (zinc oxide/titanium dioxide) can trigger allergic reactions, so safer natural alternatives are needed (Warnida & Nurhasnawati, 2017). Therefore, innovation in lip balm formulations using natural plant extracts is needed to minimize the side effects of chemical sunscreens. One type of natural plant that can be used is beetroot (*Beta vulgaris* L.). Beetroot contains photoprotective compounds such as betacyanin, which can absorb UV rays through its chromophore groups and reduce free radicals (de Castro et al., 2022).

Beetroot has previously been formulated as a gel formulation with sunscreen activity. According to research by Auliani et al., (2020), testing the SPF value of the beetroot extract-based gel showed that the highest SPF value was 6.7, produced from a formulation with a 25% extract concentration. These results indicate that the SPF value produced is still classified as minimal protection and needs to be improved. In an effort to optimize sunscreen activity, this study will apply nanotechnology in the form of nanoparticle extraction. Based on research by Wilapangga et al., (2023) the application of nanoparticles in turmeric extract successfully increased the SPF value from 16.77 to 19.88. This study will develop a nanoparticle formulation of beetroot extract using the ionic gelation method, evaluate the sunscreen activity of the resulting lip balm, compare its effectiveness with conventional beetroot extract-based lip balm, and analyse the evaluation results of the nanoparticle lip balm formulation.

METHODS

Extraction

Beetroot powder was macerated using 96% ethanol with a ratio of powder to solvent of 1:3 for 24 hours, followed by three rounds of remaceration. Extraction was carried out in an inert container, namely a glass jar. The maceration filtrate was then filtered and evaporated using a rotary evaporator at a temperature of 40°C.

Phytochemical Screening

- Flavonoid: 2 ml of beetroot extract with 0.1 grams of magnesium and 5 drops of concentrated HCl. The formation of a red or orange color indicates a positive result for the presence of flavonoids (Septiani, 2021).
- Phenol: 2 drops of FeCl₃ into 2 ml of extract. A color change to green, purple, red, or dark blue-black indicates the presence of phenolic compounds (Septiani, 2021).
- Saponin: Add hot water to the extract, shake vigorously, then add 2N HCl. The formation of stable foam 1–2 cm high for 10 minutes indicates a positive result for saponins (Wahid & Safwan, 2020).
- Tannin: Add 5 drops of FeCl₃ to the extract, then shake gently. A color change to dark green indicates the presence of tannins (Septiani, 2021).
- Steroid/triterpenoid: Add glacial acetic acid and H₂SO₄ to the extract, where a blue or green color indicates steroids, while red or purple indicates triterpenoids (Septiani, 2021).
- Alkaloid: Add concentrated HCl and Dragendorff/Mayer reagent to the extract, where an orange precipitate (Dragendorff) or white precipitate (Mayer) indicates a positive result for alkaloids (Septiani, 2021).

Total Phenolic Content

- Preparation of gallic acid stock solution and gallic acid standard curve

A 1000 ppm gallic acid stock solution was prepared by dissolving 1 gram of gallic acid in p.a. ethanol using a 1000 mL volumetric flask. Next, this stock solution was diluted to prepare a series of standard solutions with concentrations of 20, 40, 60, 80, and 100 ppm using a 10 mL volumetric flask. From each standard solution, 1 mL was taken and placed into separate reaction tubes, then 0.5 mL of Folin-Ciocalteu reagent and 4 mL of 7% sodium carbonate solution were added. The mixture was shaken until homogeneous and left to stand for 30 minutes before measuring the absorbance using a UV-Vis spectrophotometer at a wavelength of 752 nm. This process aims to obtain a calibration curve linking gallic acid concentration with absorbance values, enabling quantitative analysis of phenolic compounds in the tested sample (Damayanti & Wijayanti, 2020).

- Determination of total phenolic content in beetroot extract

The sample extract solution was prepared by dissolving 0.02 grams of extract in p.a ethanol using a 10 mL measuring flask. One milliliter of the sample solution was pipetted into a test tube, then 0.5 mL of Folin-Ciocalteu reagent and 4 mL of 7% Na₂CO₃ were added. The mixture was homogenized, allowed to stand for 30 minutes, and its absorbance was measured at λ 752 nm using a UV-Vis spectrophotometer. The total phenolic compound content was calculated using the linear regression equation ($y = bx + a$) from the gallic acid standard curve, where y is the sample absorbance and x is the equivalent concentration of gallic acid (ppm). The calculation results were converted to milligrams of gallic acid per gram of extract (mg GAE/g) using the formula: (Widiawati & Qodri, 2023):

$$\text{Total Phenolic Content} = \frac{C \times V \times Fp}{g}$$

Description

C: Concentration of sample from standard curve (mg/ml)

V: Volume of extract

Fp: Dilution factor

g: Weight of sample

Nanoparticle Formulation

The preparation of beetroot extract nanoparticles involved preparing a 2% acetic acid solution by dissolving 2 mL of glacial acetic acid in 100 mL of distilled water (Utami et al., 2022). Next, a 0.1% chitosan solution was prepared by dissolving 1 mg/mL chitosan in a 2% acetic acid solution and homogenized using a magnetic stirrer, while a 0.4 mg/mL NaTPP solution was prepared by dissolving NaTPP in distilled water (Maharini et al., 2017). The nanoparticles were then formed by mixing 40 mL of beetroot extract and 10 mL of chitosan solution (ratio 4:1), stirring at 400 rpm for 1 hour, slowly adding the NaTPP solution, and stirring for 2 hours followed by sonication for 30 minutes until a colloidal dispersion formed (Maharini et al., 2017).

Nanoparticle Characterization Testing

- Determination of particle size using PSA

Particle size was determined using a Particle Size Analyzer (PSA) with a sample of 0.25 mg of beetroot extract nanoparticles dissolved in 2.5 mL of aqua pro injection. Nanoparticles were considered acceptable if their particle size was within the range of 1–100 nm (Abdassah et al., 2009).

2. Measurement of zeta potential

The zeta potential was measured using a 1 mL sample of beetroot extract nanoparticles and placed in a zeta potential cuvette. Nanoparticles are considered suitable if the zeta potential value is less than -30 mV and greater than +30 mV, indicating higher stability (Zulfa & Puspitasari, 2019).

Lip Balm Formulation

The procedure for making lip balm begins by melting the Vaseline album base and cera alba at a temperature between 60 and 65°C (mass A). Then, phenoxyethanol, BHT, and glycerine are mixed in (mass B). Mass A is then mixed into mass B while continuing to stir. Once the temperature reaches a point that is not too hot, beetroot extract nanoparticles or red beetroot extract are added while continuing to stir. The mixture is then poured into a mold that has been previously coated with glycerine. The mixture is left to solidify at room temperature (Sholehah et al., 2022b).

Table 1. Lip Balm Formulation							
Material	Formulation Without Nanoparticles (%)			Formulation With Nanoparticles (%)			Base
	1	2	3	4	5	6	7
Beetroot extract	20	25	30	-	-	-	-
Beetroot extract nanoparticles	-	-	-	20	25	30	-
Glycerin	8	8	8	8	8	8	8
Cera alba	20	20	20	20	20	20	20
Phenoxyethanol	0,5	0,5	0,5	0,5	0,5	0,5	0,5
BHT	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Vaseline album	Ad 100%						

Sunscreen activity test on lip balm preparations

a. SPF Test

SPF testing was performed by dissolving 0.1 g of lip balm preparation (F1-F7) in p.a ethanol to 10 mL (concentration 10,000 ppm). A total of 200 µL of sample solution from each formula was taken for testing. The solution's absorbance was measured using UV-Vis spectrophotometry in the 290-320 nm range with three replicates. The SPF value can be calculated using the following equation.

$$SPF = CF \times \sum_{290}^{320} EE \times I(\lambda) \times Abs(\lambda)$$

Description

CF: Correction Factor (10)

EE: Erythral Effect Spectrum

I: Intensity Spectrum of the Sun

Abs: Absorbance of the sample

Table 2. EE×I value at wavelength 290-320 nm

Wavelength (nm)	EE×I
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180

b. Erythema Transmission Value (%Te)

The erythema transmission (Te) value of lip balm preparations was tested by preparing sample solutions of each formula (F1 to F7) at a concentration of 10,000 ppm. Samples were prepared by weighing exactly 0.1 grams of the preparation, then dissolving it in p.a. ethanol up to the mark in a 10 mL measuring flask. A total of 200 µL of the sample solution from each formula was taken for testing, including the extract formulas (20%, 25%, 30%), the nanoparticle extract formulas (20%, 25%, 30%), and the base as a control. Absorbance measurements were performed using a UV-Vis spectrophotometer in the wavelength range of 292.5–317.5 nm. Erythema transmission (Te) was calculated using the formula:

$$Te = T \times Fe$$

The amount of erythema flux transmitted by sunscreen (Ee) is calculated using the formula:

$$Ee = \sum Te$$

The erythema transmission percentage is calculated using the following formula:

$$\%Te = \frac{Ee}{\sum Fe} = \frac{\sum (T \times Fe)}{\sum Fe}$$

Description:

Te: Percentage value of Erythema Transmission

Fe: Erythema flux value at wavelength (292.5-317.5 nm)

Ee: $\sum (T \cdot Fe)$ = Amount of erythema flux transmitted by sunscreen

Table 3. Erythmia flux value (Yetti, 2023)

Wavelength (nm)	Erythmia flux (Fe)
292,5	0,1105
297,5	0,6720
302,5	1,0000
307,5	0,2008
312,5	0,1364
317,5	0,1125
Total	2,2322

c. Determination of Pigmentation Transmission Value

Sample solutions of lip balm (F1-F7) at 10,000 ppm were prepared by dissolving 0.1 g of the preparation in 10 mL of ethanol p.a. A total of 200 µL of each solution was tested on a UV-Vis spectrophotometer (λ 222.5-372.5 nm) to evaluate the extract formula (20-30%), extract nanoparticles (20-30%), and base. The Tp value was calculated based on absorbance, weight factor, and measurement interval, where a lower Tp indicates better UV protection against skin pigmentation. The test results determined the effectiveness of the formula in preventing hyperpigmentation. Pigmentation transmission (Tp) was calculated using the formula:

$$Tp = T \times Fp$$

The amount of pigmentation flux transmitted by sunscreen (Ep) is calculated using the formula:

$$Ep = \sum Tp$$

The pigmentation transmission percentage is calculated using the following formula:

$$\%Te = \frac{Ep}{\sum Fp} = \frac{\sum (T \times Fp)}{\sum Fp}$$

Description:

Te: Percentage value of pigmentation Transmission

Fe: Erythema flux value at wavelength (292.5-317.5 nm)

Ee: $\sum (T \cdot Fe)$ = Amount of pigmentation flux transmitted by sunscreen

Table 4. Pigmentation flux value (Yetti, 2023)

Wavelength (nm)	Pigmentation Flux
322,5	0,1079
327,5	0,1020
332,5	0,0936
337,5	0,0798
342,5	0,0669
347,5	0,0570
352,5	0,0448
357,5	0,0456
362,5	0,0356
367,5	0,0310
372,5	0,0260
Total	0,6942

Lip balm testing

- Organoleptic Test: Evaluation using the five senses (sight, smell) to assess the shape, aroma, and color of lip balm (Arisanty et al., 2021)
- Homogeneity Test: 1 gram of sample is placed between two glass slides and observed for coarse particles/lumps. Standard: no lumps or coarse texture (Suleman et al., 2022).
- Melting Point Test: The sample is heated in an oven at 50°C for 5 minutes. The ideal melting point according to SNI 16-5769-1998 is 50–70°C (Saputri et al., 2023)
- pH Test: A 1% solution of the sample (0.5 g in 50 mL of distilled water) is measured with a pH meter. The pH value is taken from the average of 3 repetitions (Setiawan, 2022).
- Adhesion Test: A 0.25-gram sample is pressed with a 1-kg load for 5 minutes. Standard: release time >1 second (Ambari et al., 2020).
- Spreadability Test: A 0.5-gram sample was pressed with a graduated load (50–200 grams), then the spread diameter was measured. Standard: 5–7 cm (Sholehah et al., 2022b).

RESULT AND DISCUSSION

Extraction

Beetroot (*Beta vulgaris*) extraction was carried out using the maceration method using 96% ethanol as the main solvent. The selection of 96% ethanol was based on its semi-polar properties which are able to dissolve polar compounds such as phenolics. The extraction results showed a yield of 34% with a thick extract weight reaching 262 grams, which is in line with the findings of previous studies that obtained similar yields. This high yield value reflects the efficiency of the extraction process and the abundant content of bioactive compounds in beetroot. The resulting extract has physical characteristics in the form of a deep red color and a distinctive aroma.

Phytochemical Screening

Phytochemical screening is done to identify secondary metabolite compounds. This method involves adding reagents to the extract and observing color changes or precipitates that indicate the presence of certain compounds.

Table 5. Phytochemical Screening Results.

Compound Group	Reagent	Results
Flavanoids	Concentrated HCl	Orange (+)
Phenol	FeCl ₃	Greenish (+)
Saponin	Hot water and HCl 2 N	(-)
Tannin	FeCl ₃	Blackish Green (+)
Steroids/tripenoids	acetic acid & H ₂ SO ₄	(-)

Alkaloids	Reagen Mayer	White Sediment (+)
	Reagen Dragendorff	Brown Sediment (+)

Total Phenolic Content

Determination of total phenolic content was carried out using a gallic acid calibration curve which showed a linear relationship ($y = 0.0031x + 0.088$; $r = 0.9891$), according to the Lambert-Beer law. Based on this equation, the absorbance of the sample was converted to total phenolic content in mg GAE / g. The results of the analysis showed that the ethanol extract of 2000 ppm red beet tubers contained 20 mg GAE / g total phenolics ((Andriani & Murtisiwi, 2018).

Nanoparticle Formulation

This study developed nanoparticles through ionic gelation method by utilizing electrostatic interaction between chitosan (positively charged) and sodium tripolyphosphate (NaTPP, negatively charged). The formation process involves ionic complexation, where excess chitosan binds to TPP ions through cross-linking, while polyphenol compounds from beetroot extract interact with cationic groups of chitosan (Samudra et al., 2021). The nanoparticle formulation was made by mixing 40 mL of beetroot extract and 10 mL of 0.1% chitosan solution in 2% acetic acid (ratio 4:1), producing particles measuring 30–99 nm. Visually, the formed nanoparticles have a clear appearance with a dark brown color.

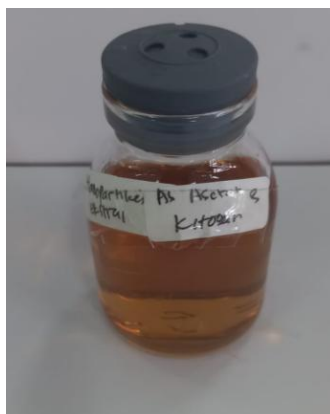


Figure 1. Beetroot Extract Nanoparticle Solution

Nanoparticle Characterization Testing

The results of the Particle Size Analyzer (PSA) test showed a particle size of 1.05 nm (volume 63%), meeting the criteria for nanoparticles (1-100 nm) (Abdassah et al., 2009). This small size increases the specific surface area, which has the potential to increase the solubility and bioavailability of active compounds. The Polydispersity Index (PDI) value of 0.403 indicates that the system is polydisperse with variations in particle size, indicating heterogeneity in the formulation. The zeta potential measurement produced a value of -21.0 mV, which is below the optimal stable threshold (± 30 mV), indicating that the colloidal system has not reached maximum stability and has the potential to aggregate (Zulfa & Puspitasari, 2019). The results of this characterization provide important information about the physicochemical properties of the developed nanoparticles, especially in terms of size, uniformity, and system stability.

Table 6. Nanoparticle Testing

Parameters	Results	Requirements
Particle size	1,05 nm with volume 63%,	1–100 nm (Abdassah et al., 2009)
Polydispersity Index (PDI)	0,403	0,3- 0,7 (Utami et al., 2022).
zeta potential	-21,0 mV	<-30 mV or >+30 mV (Zulfa & Puspitasari, 2019)

Lip Balm Formulation

Lip balm preparations were formulated using red beetroot extract which functions as a sunscreen. There were seven lip balm formulations developed, namely three formulations with red beetroot extract at concentrations of 20%, 25%, and 30% (F1 to F3); three formulations with red beetroot nanoparticle extract at the same concentration (F4 to F6); and one formulation without extract (F7).



Figure 2. Beetroot Extract Lip Balm Preparation and Beetroot Extract Nanoparticle Lip Balm

Sunscreen activity test on lip balm preparations

The lip balm formulation based on beetroot extract nanoparticles showed a significant increase in sunscreen activity compared to the formula without nanoparticles. In the non-nanoparticle formula with extract concentrations of 20%, 25%, and 30%, the resulting SPF values were 13.77; 16.38; and 17.85, respectively. The percentage of erythema transmission (%Te) was 3.81%; 2.11%; and 1.43%, respectively, and the pigmentation transmission (%Tp) was 6.24%; 4.86%; and 2.13%. After being formulated in the form of nanoparticles at the same concentration, there was an increase in the SPF value to 20.84; 24.33; and 25.25. This increase was also accompanied by a significant decrease in the %Te (0.70%; 0.34%; and 0.26%) and %Tp (1.87%; 0.99%; and 0.61%). As a comparison, the basic formula (without extract) only showed an SPF value of 8.45 with %Te and %Tp of 13.04 and 25.44, respectively. The effectiveness of sunscreen is determined by the high SPF value and the low %Te and %Tp, which reflect the protective ability against skin damage due to exposure to ultraviolet (UV) rays.

The increase in effectiveness is strongly suspected to be related to the use of nanoparticle technology, which allows smaller particle sizes so that active substances can be distributed more evenly on the skin surface. This supports increased penetration, solubility, stability, and bioavailability of active compounds and allows for more efficient release. According to Susanti & Lestari, (2019), effective sunscreen must have a high SPF and low %Te and %Tp values. This finding is supported by the analysis of Spearman correlation results in this study which showed a very strong and significant negative relationship between SPF values and %Te and %Tp, each with a correlation coefficient of -0.997 ($p < 0.001$) and -0.973 ($p < 0.001$). A significance value of 0.000 ($p < 0.05$) supports the rejection of the null hypothesis (H_0), which statistically proves a real relationship between the three parameters. Clinically, this indicates that an increase in SPF value occurs with a decrease in UV light transmission, so that the nanoparticle formulation is considered more effective in providing protection against UV radiation.

Table 7. Sunscreen activity test on lip balm preparations

Formula	SPF Value average ± SD	Average of % Te ± SD	Average of % Tp ± SD
F1 (E20%)	13,77 ± 0,2315	3,81 ± 0,234	6,24 ± 0,554
F2 (E 25%)	16,38 ± 0,5417	2,11 ± 0,251	4,86 ± 0,429
F3 (E 30%)	17,85 ± 0,4407	1,43 ± 0,159	2,13 ± 0,448
F4 (NE20%)	20,84 ± 0,7767	0,7 ± 0,1	1,87 ± 0,323
F5 (N 25%)	24,01 ± 0,2371	0,34 ± 0,024	0,99 ± 0,172
F6 (NE 30%)	25,25 ± 0,1174	0,26 ± 0,006	0,61 ± 0,021
F7 (basis)	8,45 ± 0,4159	13,04 ± 0,821	25,44 ± 1,874

Lip balm testing

The evaluation results showed that all lip balm formulas, both those containing beetroot extract (F1–F3), beetroot extract nanoparticles (F4–F6), and the base formula (F7), met the criteria as good lip balm preparations. Organoleptically, F1–F3 had a brown color and a sweet aroma typical of beetroot, while F4–F6 were white with a typical vaseline aroma, reflecting the differences in the forms of active substances used. Formula F7, which did not contain active substances, only displayed the physical properties of the base material. All formulas showed a solid texture and good homogeneity, with a stable melting point at around 70°C. The pH value ranged from 5.14 to 5.95,

which is safe and suitable for use on the skin of the lips. The adhesive power of the formula was in the range of 13–17 seconds, indicating adequate adhesion, while the spread power ranged from 4.62 to 5.98 cm, reflecting ease of application and comfort when used. Thus, the addition of nanoparticles does not have a negative impact on the physical stability of the preparation, so that the entire formula still meets the requirements as a stable and suitable lip balm for use.

CONCLUSION

Beetroot (*Beta vulgaris* L.) extract nanoparticles were successfully formulated through ionic gelation method with particle size characteristics of 1.05 nm (including nano range) and PDI of 0.403 (indicating polydisperse distribution), although it has a zeta potential of -21.0 mV which is not optimal. The lip balm formula of the extract with a concentration of 20-30% showed an SPF value of 13.77-17.85 with %Te of 3.81-1.43% and %Tp of 6.24-2.13%, while the nanoparticle formula at the same concentration (20-30%) showed a significant increase with SPF of 20.84-25.25 (ultra category), %Te of 0.70-0.26%, and %Tp of 1.87-0.61%. The 30% nanoparticle formula (F6) is the most optimal with SPF 25.25, %Te 0.26, and %Tp 0.61, which is significantly better than the regular extract formula or base (SPF 8.45). This increase in sunscreen activity is due to the superiority of nanoparticles in the distribution and penetration of active substances. All formulas meet the physicochemical requirements (organoleptic, homogeneity, pH, adhesion, spreading power, melting point), proving that the nanoparticle formulation is effective as UV protection while meeting the standards of topical preparations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

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