OPTIMIZATION AND FORMULATION OF SKIN LOTION CONTAINING BUTTERFLY PEA (*Clitoria ternatea*) FLOWER EXTRACT AND STUDY ON ITS ANTIOXIDANT ACTIVITY

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Abstract. Butterfly pea flower is rich in phenolic and flavonoid antioxidants that protect the skin from free radicals, which can lead to oxidative damage. This study aimed to optimize a lotion formulation containing butterfly pea flower extract (BPFE), using triethanolamine (TEA) and stearic acid as emulsifiers, and to evaluate its antioxidant activity. Eight lotion formulations were prepared using the Simplex Lattice Design method (Design Expert v13.0), focusing on optimizing the stearic acid to TEA ratio. Antioxidant activity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) at a wavelength of 516 nm. Key parameters such as spreadability, adhesion, pH, and viscosity were assessed. The maceration method yielded BPFE with a 49.66% extraction efficiency. The optimized formulation demonstrated a spreadability of 6.26±0.38 cm, adhesion of 3.2±0.2 s, a pH of 7.19±0.005, and a viscosity of 120 dPas. Both BPFE and the optimized lotion formulation exhibited strong antioxidant activity, with IC50 values of 59.87±0.802 µg/ml and 84.52±1.418 µg/ml, respectively. The optimized BPFE lotion formulation presents promising potential for development as a cosmeceutical product.

Keywords: *Clitoria ternatea*, antioxidant, simplex lattice design, lotion, optimization

1. Introduction

Air pollution is a significant contributor to the generation of free radical compounds, which pose a threat to human health, particularly the skin [1]. As the outermost layer of the body, the skin is directly exposed to these harmful compounds. Free radicals, specifically reactive oxygen species (ROS), are highly reactive molecules with unpaired electrons. The formation of these free radicals is a natural consequence of oxidation, a process occurring in all living organisms. Elevated levels of ROS can induce oxidative stress, which, if left unchecked, can lead to various skin conditions, including hyperpigmentation, premature aging, dryness, dullness, scaling, and potentially, skin cancer. To mitigate the damaging effects of free radicals and ultraviolet (UV) radiation on the skin, the application of antioxidants is essential [2].

Several plants in Indonesia are known to contain potent antioxidants capable of neutralizing free radicals, including *Clitoria ternatea* (butterfly pea flower), which is rich in phenolic compounds and flavonoids. Flavonoids, in particular, exhibit antioxidant properties through mechanisms involving oxidation [3]. The ethanolic extract of *Clitoria ternatea* has demonstrated a notable antioxidant capacity, with an IC50 value of 41.36±1.19 µg/mL [4].

Lotion formulations serve as a barrier to prevent the penetration of free radicals into the skin's epidermis. The selection of an effective and aesthetically pleasing lotion enhances its appeal and ensures it meets the protective needs of the skin [5]. Emulsifiers, which facilitate the blending of the water and oil phases in lotions, play a critical role in determining the quality and comfort of the product. The appropriate concentration of emulsifying agents is crucial in emulsion formation, necessitating optimization to ensure the lotion is both effective and pleasant to use [6].

At the appropriate concentration, the combination of triethanolamine (TEA) and stearic acid can yield a lotion with desirable physical properties and ease of application [7]. When TEA reacts with stearic acid, it forms an anionic soap with a pH of approximately 8, which functions as an emulsifier. As an anionic emulsifier, TEA stearate facilitates the formation of an oil-in-water (O/W) emulsion by encapsulating oil droplets and uniformly dispersing them within the water phase [8]. Oil-in-water emulsions are particularly favored in topical formulations due to their ease of

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Table 1. Formula of BPFE lotion

Formula	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)
BPFE	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Stearic acid	2	3	2.5	3	4	4	2	3.5
Triethanolamine	3	2	2.5	2	1	1	3	1.5
Cetyl alcohol	2	2	2	2	2	2	2	2
Glycerin	5	5	5	5	5	5	5	5
Lanolin	8	8	8	8	8	8	8	8
Propyl parabens	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Methyl parabens	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lavender oil	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop
Water	up to 50	up to 50	up to 50	up to 50	up to 50	up to 50	up to 50	up to 50

application and removal from the skin. The concentration of anionic emulsifiers, such as TEA stearate, can be tailored to meet the specific requirements of topical lotion formulations. When TEA and stearic acid are combined as emulsifiers, they undergo a saponification reaction, resulting in the formation of triethanolamine stearate salts, commonly referred to as amine soap. This process occurs through the reaction of fatty acids (stearic acid) with bases (TEA), undergoing hydrolysis to release hydrogen ions from the stearic acid and hydroxyl groups from the TEA, ultimately producing TEA stearate and water [9,10].

The objective of this study was to optimize the formulation of a lotion containing butterfly pea flower extract (BPFE), using TEA and stearic acid as emulsifiers. In addition, the study aimed to evaluate the antioxidant activity of the formulated lotion.

2. Methods

2.1 Materials

Butterfly pea flowers (*Clitoria ternatea*) were sourced from the Laweyan region in Surakarta, Central Java, Indonesia. Triethanolamine (TEA), stearic acid, cetyl alcohol, lanolin, glycerin, propylparaben, and methylparaben were obtained from Brataco. Ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), and vitamin E were purchased from Sigma-Aldric.

2.2 BPFE Preparation

The identification of butterfly pea flowers was confirmed at the UPT-Laboratory of Setia Budi University. The petals and calyx of the flowers were separated, thoroughly washed, and air-dried before being dehydrated in a drying cabinet at 40 °C for 24 hours. The extraction process was carried out using the maceration method. A total of 145 g of dried flower powder was placed in a sealed glass container with 1,450 mL of 70% ethanol as the solvent. The mixture was allowed to be macerated for three days with periodic stirring. After filtration, the solvent was evaporated at 50 °C using a rotary evaporator. The extract was further concentrated in a water bath at 50 °C until a thick BPFE (butterfly pea flower extract) was obtained. Re-maceration was conducted using the same conditions with 70% ethanol as the solvent [11].

2.3 BPFE Lotion Formulation

The optimal lotion formulation was determined using Design Expert software (version 13). Upper and lower limit values were input into the software, resulting in the generation of eight different formulas (Table 1). Response variables, including adhesion, spreadability, viscosity, and pH, were assessed, and the physical evaluation results were input into the software to identify the optimum BPFE lotion formula.

The preparation of the lotion involved the following steps: for the oil phase, stearic acid, cetyl alcohol, lanolin, and propylparaben were melted together. Simultaneously, the aqueous phase was prepared by dissolving glycerin, TEA, and methylparaben in water. Both the oil and aqueous phases were heated separately over a water bath at 75 °C and stirred until homogeneous. The lotion was created by gradually adding the aqueous phase into the oil phase while stirring continuously with a hot mortar until uniform consistency was achieved. After cooling, the BPFE and lavender essential oil were incorporated into the mixture, which was stirred until homogeneous and subsequently filled into containers [12].

2.4 Physical Evaluation of BPFE Lotion

2.4.1 pH

The pH of the BPFE lotion was determined using a calibrated pH meter (ST3100-B, OHAUS). The

Table 2. Physical characterization of BPFE lotion

Formula	TEA	Stearic Acid	Spreadability (cm)	Adhesion (sec)	pH	Viscosity (dpas)
1	3	2	6.95 ± 0.08	1.4 ± 0.11	8.36 ± 0.011	90 ± 0.0
2	2	3	6.49 ± 0.13	1.9 ± 0.05	8.0 ± 0.017	110 ± 0.0
3	2,5	2.5	6.68 ± 0.07	1.7 ± 0.15	8.3 ± 0.0	100 ± 0.0
4	2	3	6.50 ± 0.06	1.9 ± 0.15	8.08 ± 0.03	110 ± 0.0
5	1	4	6.32 ± 0.06	3.3 ± 0.30	7.19 ± 0.011	120 ± 0.0
6	1	4	6.1 ± 0.02	3.9 ± 0.2	6.99 ± 0.005	130 ± 0.0
7	3	2	7.1 ± 0.04	1.1 ± 0.20	8.46 ± 0.0	90 ± 0.0
8	1,5	3.5	6.43 ± 0.04	2.5 ± 0.25	7.76 ± 0.028	120 ± 0.0

lotion was applied to the electrode, and the pH was recorded once the meter displayed a stable and consistent reading [13] .

2.4.2 Spreadability

The spreadability of the lotion was assessed by placing a sample in the center of a round glass plate, which was then covered with another round glass. A 100 g weight was placed on top, and the setup was left undisturbed for 1 minute. The diameter of the spread lotion was measured. This process was repeated with incremental weights of 100 g, up to a maximum of 400 g, with the diameter recorded at each interval [14,15].

2.4.3 Adhesion Test

A 0.5 g sample of the lotion was placed between two glass slides, which were pressed together until fused. A 100 g load was applied for 5 minutes. The adhesion test device was then used to measure the time taken for the two glass slides to slip apart, recording the duration of adhesion [16].

2.4.4 Viscosity Test

The viscosity of the lotion was measured using a viscometer (RION VT-06). Rotor No. 2 was immersed in the lotion, and the start button was pressed. The viscosity value was recorded once the reading stabilized [17].

2.5 Antioxidant assay of BPFE lotion using DPPH method

The antioxidant activity of the BPFE lotion was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. First, 0.7 mL of 0.4 mM DPPH solution was added to a 5 mL volumetric flask and diluted with ethanol up to the 5 mL mark. The mixture was shaken until homogeneous and left to stand for 30 minutes in the dark. The absorbance of the solution was measured at a wavelength of 516 nm using a UV-visible spectrophotometer (UV SHIMADZU 1280), which served as the control [18].

The antioxidant activity of BPFE was assessed as follows: 10 mg of BPFE was dissolved in 10 mL of ethanol, and a series of concentrations were prepared from this stock solution. Each concentration was mixed with 0.7 mL of DPPH stock solution and ethanol was added to a final volume of 5 mL. The solutions were incubated in the dark for 30 minutes before measuring absorbance at 516 nm. Vitamin E was used as the positive control and underwent the same treatment [19].

The antioxidant activity of the BPFE lotion was measured similarly. A 10 mg sample of BPFE lotion was dissolved in 10 mL of ethanol and centrifuged for 10 minutes. The supernatant was collected and prepared in a series of concentrations. Each solution was transferred to a 5 mL volumetric flask, followed by the addition of 0.7 mL of DPPH solution and ethanol up to 5 mL. After standing for 30 minutes in the dark, the absorbance of each solution was measured at 516 nm [20.

3. Result and Discussion

The identification confirmed that the butterfly pea flowers selected for the study belonged to *Clitoria ternatea* L., a species in the Fabaceae family. The extraction process yielded a thick extract of 72.02 g, with an extraction efficiency of 49.66%.

3.1 Physical Evaluation of BPFE Lotion

The physical properties of the eight BPFE lotion formulations varied according to the proportions of TEA and stearic acid used. The physical evaluation results for the eight formulations are presented in Table 2.

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Table 3. Organoleptic and homogeneity of BPFE lotions

Formula	Texture	Color	Smell	Homogeneous
1	Soft	Green- Bluish	Lavender	Homogeneous
2	Soft	Green- Bluish	Lavender	Homogeneous
3	Soft	Green- Bluish	Lavender	Homogeneous
4	Soft	Green- Bluish	Lavender	Homogeneous
5	Soft	Blue	Lavender	Homogeneous
6	Soft	Blue	Lavender	Homogeneous
7	Soft	Green- Bluish	Lavender	Homogeneous
8	Soft	Blue	Lavender	Homogeneous

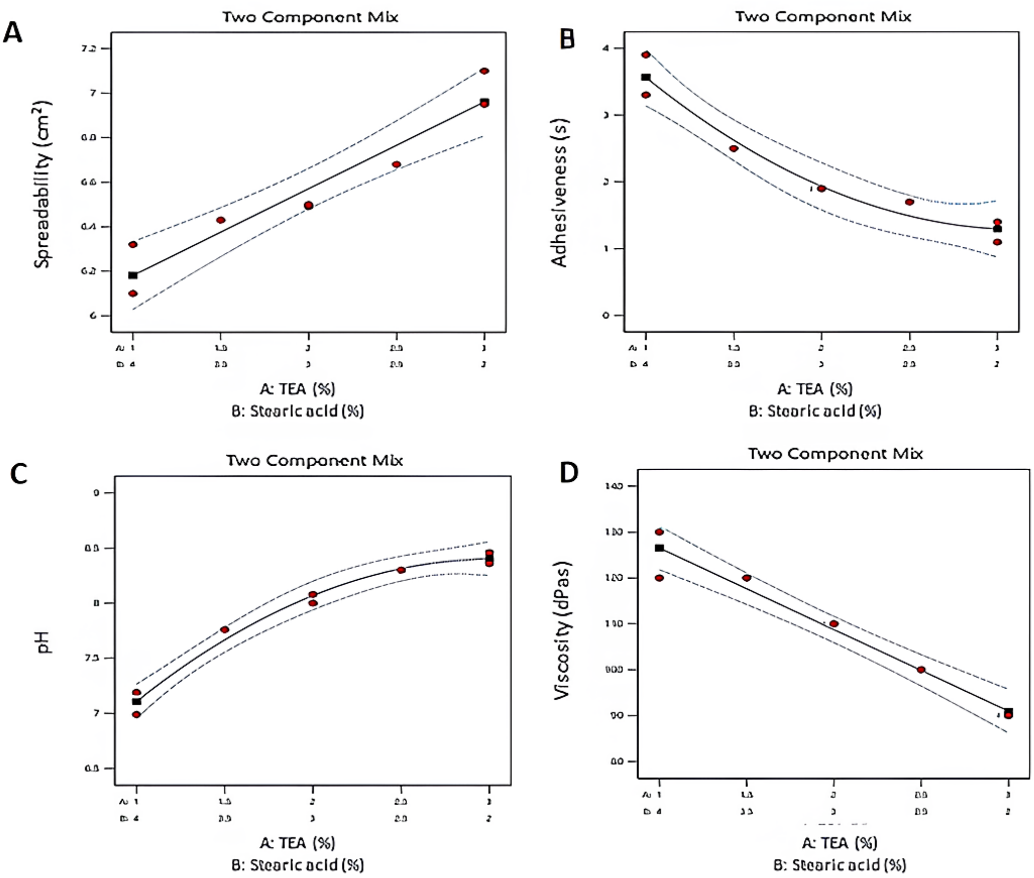

Fig. 1. The response surfaces of the BPFE lotions characteristics estimated by Design-Expert software, as a function of the percentage of TEA and stearic acid

The spreadability of the lotions ranged between 6.1 and 7.1 cm, which meets the standard requirements for topical applications [21]. A longer adhesion time in the BPFE lotions is advantageous, as it would enhance the antioxidant effect, providing greater protection against free radicals. The pH values of all eight formulations were within the acceptable range for topical lotions (4.5–8.0), making them suitable for external use. The pH levels varied based on the concentration of TEA, with higher TEA levels correlating with an increased pH. It is important to note that excessively acidic lotions may irritate the skin, while overly alkaline lotions can lead to dryness and flakiness [22]. The viscosity of each lotion formulation was also influenced by the composition of the emulsifier. The use of stearic acid as an emulsifying agent contributed to the thickness of the lotions, with higher TEA content further increasing viscosity [23].

Organoleptic evaluations, including assessments of smell, color, and texture, were conducted to determine the sensory characteristics of the lotions (Table 3). All formulations exhibited a soft texture with a pleasant lavender fragrance. The color varied slightly, but most were dominated by a bluish-green hue. Additionally, all lotions displayed homogeneity,

Table 4. The response equation of the physical characteristics of BPFE lotion

Response	Equation
Spreadability	Y = 6.96 (A) + 6.18 (B)
Adhesion	Y = 1.30 (A) + 3.56 (B) – 1.99(AB)
pH	Y = 8.40(A) + 7.11 (B) + 1.26 (AB)
Viscosity	Y = 90.97(A) + 126.53 (B)

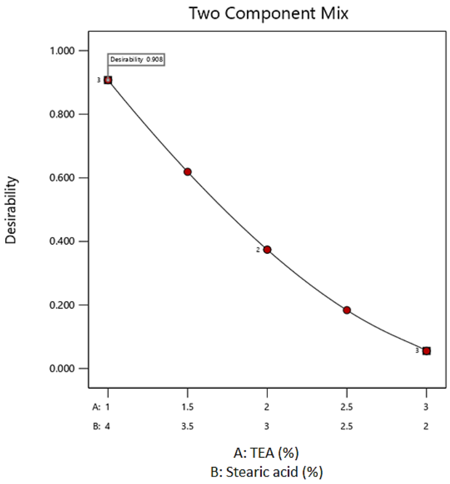

Figure 2. Graph of the optimum lotion formula

with no visible coagulation or particles, ensuring a smooth, evenly mixed appearance.

3.2 Optimization of BPFE Lotion Preparation

The formulation of BPFE lotion was optimized by evaluating four key parameters: spreadability, adhesion, pH, and viscosity. The optimization process utilized the Simplex Lattice Design model, with the relevant equations and graphical representations of the lotion's physical characteristics presented in Table 4 and Figure 1.

Spreadability, which reflects the lotion's ability to be evenly distributed on the skin, was significantly influenced by the presence of TEA and stearic acid. The p-value for spreadability was 0.0002 ($p < 0.05$), confirming the statistical significance of these factors. The equation in Table 4 indicates that both TEA and stearic acid positively contribute to spreadability, with each component having a measurable impact.

Notably, a lotion's spreadability is inversely related to its viscosity—the higher the viscosity, the less effectively it spreads. TEA, known for its softening properties, enhances the lotion's spreadability by reducing its viscosity. This effect is demonstrated in the curve shown in Figure 1, where increased levels of TEA and reduced concentrations of stearic acid lead to greater spreadability [24, 25].

The adhesion test evaluated the duration the lotion adheres to the skin post-application. ANOVA analysis produced a quadratic model with a p-value of 0.0004 ($p < 0.05$), demonstrating that both TEA and stearic acid significantly impacted adhesion. The corresponding equation, presented in Table 4, identifies TEA as Factor A with a value of +1.30, stearic acid as Factor B with a value of +3.56, and the interaction between TEA and stearic acid (Factor AB) with a value of -1.99. These results indicate that both components enhanced adhesion. Increasing the concentration of stearic acid prolonged adhesion, as stearic acid acts as a stiffening agent, thickening the lotion and increasing its viscosity, thus improving adhesion. At lower concentrations, TEA also contributed to increased adhesion by enhancing the lotion's viscosity [26].

The pH analysis aimed to assess the lotion's compatibility with the skin's pH. ANOVA results revealed a quadratic model with a p-value of 0.0001 ($p < 0.05$), confirming the significance of the findings. The Simplex Lattice Design equation, shown in Table 4, reports TEA as Factor A with a value of +8.40 and stearic acid as Factor B with a value of +7.11, while the interaction of both factors is represented by +1.26. These results suggest that increasing the concentration of free TEA or unreacted stearic acid can influence the pH of BPFE lotion [24].

Viscosity data, illustrated in Figure 1c, demonstrated that higher concentrations of stearic acid resulted in increased lotion viscosity. Statistical analysis supported a linear model as the best fit for assessing viscosity, with a p-value of 0.0001 ($p < 0.05$), indicating that both TEA and stearic acid significantly affected viscosity. Figure 1 illustrates the effect of stearic acid, a saturated fatty acid, on viscosity. When combined with lower concentrations

Table 5. Physical characteristics of the optimum formula of BPFE lotion

Parameter	Predicted	Observed	P-value
Spreadability	6.187	6.26 ± 0.038	0.068
Adhesion	3.564	3.2 ± 0.20	0.088
pH	7.108	7.19 ± 0.005	0.002
Viscosity	126.528	120 ± 0	-

Table 6. Antioxidant activity of BPFE, BPFE lotion, and vitamin E

Sample	Linear Regression Equation	R^2	Average IC_{50}± SD
BPFE	y = 0.5177x + 19.004	0.9989	59.87 ± 0.812
Optimized BPFE Lotion	y = 0.5859x + 0.476	0.9996	84.52 ± 1.418
Vitamin E	y = 6.1175x – 3.651	0.9992	8.77 ± 0.16

of TEA, stearic acid increases the lotion's viscosity, producing a thicker consistency [26, 27]. This effect is further supported by the SLD equation, where TEA (Factor A) has a value of +90.97 and stearic acid (Factor B) has a value of +126.53. These values indicate that stearic acid has a greater influence on viscosity compared to TEA, and both components positively contribute to increased viscosity.

The optimization of the BPFE lotion formulation was achieved using the Simplex Lattice Design method, with TEA and stearic acid as the primary optimization factors. The optimization process resulted in a single solution with a desirability value of 0.908. The optimum formulation consisted of 1% TEA and 4% stearic acid. The optimization criteria included maximizing spreadability, adhesion, and viscosity, while maintaining the pH within an acceptable range.
Table 5 validates the predicted physical characteristics of BPFE lotion. No significant differences were observed between the predicted and actual values for spreadability and adhesion. Although there was a slight variation in pH values, it remained within the acceptable range for topical formulations. These results confirm that the Simplex Lattice Design method is an effective tool for optimizing lotion formulations with varying concentrations of triethanolamine and stearic acid.

3.3 Antioxidant activity of BPFE lotion

Antioxidant activity was assessed using the DPPH method (Table 6). Antioxidant potency is categorized into several ranges: extremely potent (≤50 ppm), potent (51–100 ppm), moderate (101–150 ppm), and weak (151–200 ppm). Based on the study's findings, both BPFE and BPFE lotion fall within the potent antioxidant category. Several factors, including the geographic location where the butterfly pea flower is cultivated, environmental conditions such as water availability, light exposure, soil type, and improper storage of the extract, can influence the yield and concentration of active metabolites. Previous research has identified flavonoids and polyphenols as key active compounds in BPFE, contributing to its strong antioxidant activity [28, 29].

4. Conclusion

The physical properties of BPFE lotion were significantly influenced by varying concentrations of TEA and stearic acid. Increasing the TEA content resulted in a decrease in viscosity and adhesion, while simultaneously raising the pH and enhancing the spreadability of the formulation. An optimal formulation was obtained by combining 1% TEA with 4% stearic acid. This formulation demonstrated potent antioxidant activity, with an IC50 value of 84.52 ± 1.41 µg/mL. However, further studies are necessary to evaluate the long-term stability of the formulation and its effects on human skin.

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The data supporting the findings of this study are available from the corresponding author, [E.W.], upon reasonable request.

E.W. conceived and supervised the project; T.W. conducted the experiments; E.W. and T.W. analyzed the data and co-authored the manuscript.

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