

Effect Of Cetyl Alcohol Variation On Formulation and Stability Of Belimbing Wuluh (*Averrhoa bilimbi* L.) Leaf Extract Cream

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Abstract

Belimbing wuluh (*Averrhoa bilimbi* L.) extract has antioxidant activity that can help prevent skin wrinkles and makes it potential to be formulated into a cream. Cetyl alcohol is a common thickener and emulsifier in a cream formulation. This study investigated the effect of cetyl alcohol concentration variation on physical properties and stability of belimbing wuluh (*Averrhoa bilimbi* L.) cream preparation. The extract was formulated into three creams with different cetyl alcohol concentrations: FI (3% cetyl alcohol), FII (4% cetyl alcohol), and FIII (5% cetyl alcohol). The creams were evaluated for their physical characteristics, including organoleptic properties, homogeneity, cream type, adhesion, spreadability, pH, and viscosity. Stability was tested using a freeze and thaw cycling test over six cycles. All data were analyzed statistically. Statistical analysis showed significant differences between the formulations in adhesion and pH. Furthermore, significant differences were found between FIII and the other formulations in spreadability and viscosity. The stability test revealed that FI and FII were unstable, as indicated by changes in their adhesion, spreadability, and viscosity. In contrast, FIII demonstrated good stability across all evaluations. In conclusion, the concentration of cetyl alcohol significantly impacted the physical properties and stability of the belimbing wuluh extract cream.

Keywords: *Averrhoa bilimbi* L., cetyl alcohol, physical evaluation, cycling test, leaf.

1. INTRODUCTION

Skin aging is a natural part of growing older and this process can be accelerated by external factors like air pollution and ultraviolet (UV) light. UV-B light, in particular, penetrates the skin's dermis layer, which can lead to wrinkles and an increased risk of skin cancer (Kirana *et al.*, 2023). The acceleration of wrinkle formation is initiated by the production of free radicals known as Reactive Oxygen Species (ROS). ROS are a byproduct of aerobic metabolism, which is a cellular response to radiation, such as UV light (Jia *et al.*, 2014). The continuous exposure to UV light leads to an overproduction of ROS, which in turn degrades the skin's elasticity. This occurs by triggering the breakdown of the extracellular matrix by the enzyme elastase (Natanael *et al.*, 2021). This degradation can be effectively mitigated with the use of antioxidants.

Belimbing wuluh (*Averrhoa bilimbi* L.) is a herbal plant that contains antioxidant-rich secondary metabolites, including flavonoids, alkaloids, phenols, coumarins, and tannins (Setyawan *et al.*, 2021; Valsan & Raphael, 2016). Its ethanol extract has been shown to have potent antioxidant and anti-inflammatory properties (Hasim *et al.*, 2019). Similarly, Kusuma *et*

al. (2023) confirmed the strong antioxidant activity of belimbing wuluh ethanolic extract using the DPPH method. Since creams are a common topical vehicle for antioxidants, a study by Ariem *et al.* (2020) formulated a cream with 7% belimbing wuluh extract. While it showed strong antioxidant activity, its consistency was too liquid.

A cream's liquid texture is directly related to its low viscosity, which negatively impacts its spreadability and adhesion. Although a cream should have a thick consistency, it must still be easy to apply. Cetyl alcohol is an excipient used in topical form as an emulsifier and to elevate the viscosity of the preparation (Rowe *et al.*, 2009). Below 2% concentration of the cetyl alcohol used in the formulation will produce low viscosity cream. Otherwise, when the concentration over than 10%, it produce a cream with high viscosity form (Manna & Thalib, 2023). Given this factor, this study was conducted to investigated the effect of cetyl alcohol concentration variation on physical properties and stability of belimbing wuluh (*Averrhoa bilimbi* L.) extract cream preparation.

2. METHOD

Tools and Materials

Tools used in the study were maceration container, stirring rod, glass funnel, filter paper, stand, clamp, glass bottle, porcelain evaporating dish, petri dish, ointment pot, waterbath, oven (Mettler), container, handmixer (Ocooker), volumetric flask (Iwaki CTE33 Asahi Glass), beaker glass 100 ml (Duran 100ml), double ended spatula, spatel, digital balance (Acis AD-600i), refrigerator (Sharp), vernier calipers, glass plate, viskometer Brookfield (ND J-9S Digital Viscometer), and pH meter (Hanna instrument).

Materials used were belimbing wuluh (*Averrhoa bilimbi* L.) leaf simplicia, ethanol 96% solvent (technical grade), stearic acid, cetyl alcohol, triethanolamine (TEA), distilled water, glicerine, paraffin liquid, and metyl paraben. All excipient were pharmaceutical grade.

Extraction

A total of 2.3 kg of fresh belimbing wuluh leaf simplicia was collected and sun-dried for five days. The resulting dried simplicia weighed 830 g, with a moisture content of 6.78%. This met the standard requirement of less than 10% for dried simplicia (Health Ministry of the Republic of Indonesia, 2017). The dried simplicia was then pulverized into a coarse powder. This powder was extracted using the maceration method with a 1:5 simplicia powder-solvent ratio for 7 days. The resulting liquid was filtered, and the remaining solid residue was macerated a second time for four days using the same drug-to-solvent ratio. The liquids from both

maceration steps were mixed. The solvent was then evaporated using a water bath, and the extract was concentrated into a thick paste using an oven at 60°C.

Cream Formulation

Cream formulation used in this study was based on Ariem *et al.*, (2020) formulation with cetyl alcohol concentration modification.

Table 1. Belimbing wuluh leaf extract cream formulation

Composition	FI (%b/b)	FII (%b/b)	FIII (%b/b)
Belimbing wuluh leaf extract	7	7	7
Stearic acid	16	16	16
Cetyl alcohol	3	4	5
Metyl paraben	0.2	0.2	0.2
Parafin liquid	10	10	10
Glycerin	8.5	8.5	8.5
TEA	7 drops	7 drops	7 drops
Aquadest	Ad 100	Ad 100	Ad 100

The cream was prepared using a standard heating method for both the water and oil phases. The water phase, consisting of methylparaben, triethanolamine (TEA), glycerin, and distilled water, was heated to 70°C in a separate container. Concurrently, the oil phase, which included stearic acid, cetyl alcohol, and liquid paraffin, was also heated to 70°C until all components were fully melted and dissolved. The heated oil phase was then slowly added to the heated water phase while being continuously mixed with a hand mixer at a speed of 14000 rpm. Aquadest was gradually incorporated into the mixture to form a stable cream base. The belimbing wuluh leaf extract was gradually added to the cream base, and the mixture was homogenized until a uniform and consistent cream was obtained.

Cream Evaluation

Organoleptic test

The organoleptic test was performed by observing each cream formulation, including color, odor, and texture (Nurjanah *et al.*, 2019).

Cream type test

The cream type test was performed using dissolution method. A small amount of cream was dissolved in the water and its solubility was observed (Nonci *et al.*, 2016).

Homogeneity test

The homogeneity test was performed by spreading 1 gram of cream on a glass plate and observing its homogeneity. The test was done in triplicate (Suwandi *et al.*, 2023).

Adhesion test

The adhesion test was performed by placing 1 gram of cream between two transparent glass plate. A 1 kg weight was then placed on the top plate for 5 minutes. Afterward, an 80 gram weight was attached to one of the plate to pull it horizontally and the time required for two plate to separate was recorded as the adhesion time (Ilmaknun & Endriyatno, 2024).

Spreadability test

The spreadability test was performed by placing 1 gram of cream between two round transparent glass plates. A 200 gram weight was placed on the top plate for 1 minute. The resulting diameter of the cream's spread was then measured (Ain Thomas *et al.*, 2022).

pH test

The pH test was performed using a pH meter by inserting the probe into the cream and this test was conducted in triplicate (Suwandi *et al.*, 2023).

Viscosity test

The viscosity test was performed using viscometer Brookfield (Puradewa *et al.*, 2024) and its done for three replications (Nurjanah *et al.*, 2019).

Cream Stability Test

The stability test was conducted using Freeze and Thaw Cycling Test method for a total 6 cycles. The test was done by placing cream formulaation in a refrigerator at 4°C for 24 hours and then transferring it in an oven at 40°C for the next 24 hours (1 cycle). The parameters which were evaluated at the end of each cycle were organoleptic, homogeneity, pH, spreadability, adhesion, and viscosity (Mardikasari, *et al.*, 2017).

Data Analysis

All data from the physical evaluation and stability tests were analyzed statistically. A one-way ANOVA was used to analyze the physical properties data, while the stability data were analyzed using Paired Sample T-test. For the data that did not follow a normal distribution, the Kruskal-Wallis and Mann-Whitney tests were used as non-parametric alternatives. All statistical analyses were performed with a significance level of $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

Extraction Result

Maceration extraction method was chosen to prevent the degradation of thermolabile compounds found in the herbal plant (Badaring *et al.*, 2020). This method requires regular

stirring to maximize contact between the simplicia powder and the solvent (Handoyo, 2020). The belimbing wuluh simplicia extraction yielded 18.8%, which is considered a good result as it exceeds the 10% standard (Wardaningrum, 2019). This yield is higher than the 10% yield obtained in a previous study by Ariem *et al.* (2020). The difference in yield may be attributed to variations in the maceration time used in each study.

Cream Evaluation Result

Belimbing wuluh leaf cream physical evaluation result can be seen in the Table 2.

Table 2. Belimbing wuluh leaf extract cream physical evaluation result

Evaluation test	Result		
	FI	FII	FIII
Organoleptic			
<i>Color</i>	Green-brown	Green-brown	Green-brown
<i>Odor</i>	Distinctive	Distinctive	Distinctive
<i>Texture</i>	Smooth	Smooth	Smooth
Homogeneity	Homogeneous	Homogeneous	Homogeneous
Cream type	O/W	O/W	O/W
Adhesion	4.30 ± 0.45	3.62 ± 0.25	7.43 ± 0.05
Spreadability	5.42 ± 0,19	5.18 ± 0,16	4.56 ± 0.18
pH	5.42 ± 0.03	5.18 ± 0.01	5.03 ± 0.02
Viscosity	9628 ± 262.88	18698 ± 768.27	17659 ± 7053.57

The belimbing wuluh leaf extract cream was a green-brown color with a distinctive aroma and a smooth texture (Figure 1). All formulations were found to be homogeneous, exhibiting a uniform color without any visible coarse particles. The cream type was confirmed to be oil-in-water (O/W), as all formulations readily dissolved when mixed with water.



Figure 1. Belimbing wuluh leaf extract cream showed a green-brown color with a distinctive aroma and a smooth texture

The adhesion times for all formulations were within the standard range of 2 to 300 seconds. The combination of stearic acid and triethanolamine (TEA) served as an effective emulsifying system, contributing to good adhesion. Stearic acid provides a more rigid texture, while TEA helps to modify the cream's viscosity (Ilmaknun & Endriyatno, 2024). A Kruskal-Wallis test revealed a statistically significant difference in adhesion between the formulations (Asymp.Sig.=0.027<0.05). This variation is likely due to the different concentrations of cetyl alcohol, as higher concentrations of cetyl alcohol are known to increase the cream's adhesion properties (Puspitasary *et al.*, 2024).

The spreadability test was conducted to assess the cream's ability to spread evenly over skin surface, which is crucial for uniform application and a faster absorption rate of its active ingredients (Hernawan *et al.*, 2020). The test result showed that FI and FII both met the required standart range of spreadability between 5-7 cm. However, FIII failed to meet the standart. The lower spreadability of FIII can be attributed to its higher concentration of cetyl alcohol, which increased the cream's rigidity and viscosity (Manna & Thalib, 2023). A one-way ANOVA was performed to determine if there were significant differences among the formulations. The result, with an Asymp. Sig. (P-value) of 0.003, indicated a statistically significant difference ($P < 0.05$). Post-hoc analysis further revealed that the significant differences in spreadability were specifically between FI-FIII and FII-FIII.

All cream formulations had a pH within the acceptable range for topical preparations (4.5–6.5). A Kruskal-Wallis test, revealed a statistically significant difference in pH among the formulations (Asymp.Sig.=0.026<0.05). Post-hoc analysis showed significant differences between FI-FII, FI-FIII, and FII-FIII. This indicates that the variation in cetyl alcohol concentration had an effect on the pH of the formulations, likely due to its acidic properties (Dewi & Arimurni, 2021).

All three formulations (FI, FII, and FIII) met the required viscosity standards. However, FIII had a lower viscosity than FII, which may have been influenced by variations in the mixing process. The speed and duration of mixing are known to decrease a cream's viscosity while simultaneously increasing its stability (Sari & Lestari, 2015). A one-way ANOVA test was conducted and revealed a statistically significant difference in viscosity among the formulations, with a P-value of 0.002 ($P < 0.05$). Post-hoc analysis showed no significant difference between FI and FII. However, significant differences were found between FI-FIII and FII-FIII. This supports the conclusion that the percentage of cetyl alcohol concentration directly influences the cream's viscosity due to cetyl alcohol's properties as a thickener (Murdiana *et al.*, 2022).

Cream Stability Test Result

After six cycles of the freeze-thaw test, all formulations (FI, FII, and FIII) remained organoleptically stable. They all retained their original green-brown color, distinctive odor, and smooth texture. The creams also maintained their homogeneity and stable oil-in-water (O/W) cream type throughout the stability test.

The adhesion test is vital for measuring how well a cream adheres to the skin, which ensures the active ingredients have enough time to be fully absorbed (Wikandita, 2022). Adhesion and spreadability have an inverse relationship; as adhesion increases, spreadability tends to decrease, and vice versa. Storage temperature is another factor that can influence adhesion (Lumentut *et al.*, 2020).

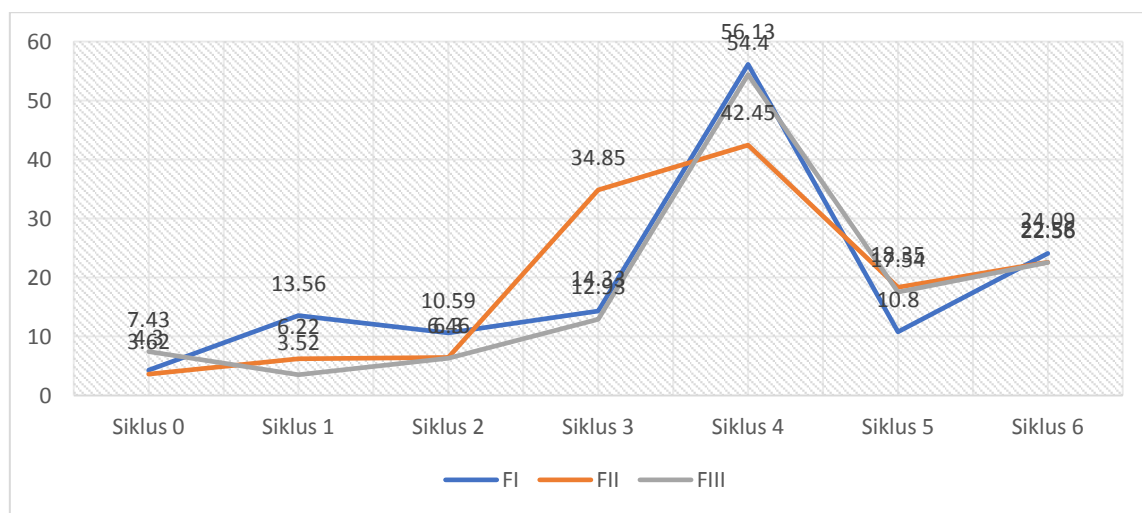


Figure 2. Adhesion stability test result

Based on the adhesion stability test result data (Figure 2) and Paired Sample T-test, FI showed significant differences (P value < 0.05) between cycles, indicating unstable adhesion properties. Otherwise, FII and FIII showed P value > 0.05 between cycles indicated no significant differences and stable adhesion properties in conclusion.

The spreadability test measures a cream's ability to spread easily on the skin without pressure (Muthoharoh & Rianti, 2020). Based on the data shown in Figure 3, the spreadability of FI and FII decreased from cycle 1 to cycle 6. This reduction may be attributed to factors such as storage duration, the properties of the cream's components, and changes in viscosity over time (Tari & Indriani, 2023). While these two formulations met the standard spreadability range at the beginning of the test, their stability was compromised. FIII, on the other hand, consistently failed to meet the required spreadability standard of 5–7 cm throughout the entire test.

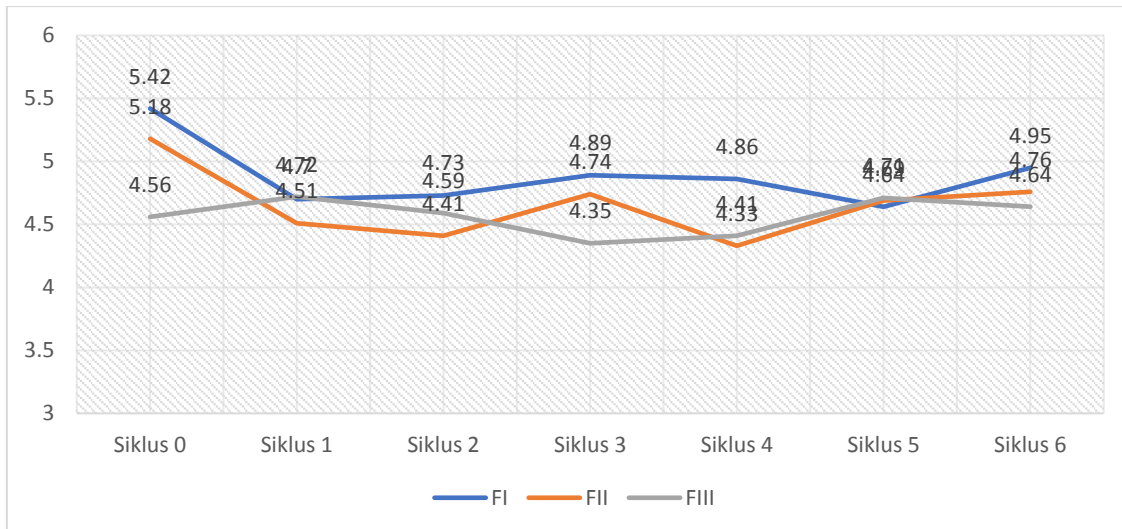


Figure 3. Spreadability stability test result

Statistical analysis of Paired Sample T-test conducted showed that FI across cycles 1, 4, and 6 showed significant differences (P value < 0.05). Similarly, FII cycles 4, 5, and 6 showed P value < 0.05 . This statistical result indicating that FI and FII spreadability were unstable. Different result showed by FIII, which showed P value > 0.05 between cycles indicating its stabil spreadability properties. However, FIII failed to meet the spreadability standart range between 5-7 cm.

According to SNI 16-4399-1996, the acceptable pH range for topical preparations is between 4.5-8. A pH below this range can cause skin irritation, while a pH above it can lead to dry skin (Tungadi *et al.*, 2024). Based on the data in Figure 4, the pH of all formulations decreased from cycle 1 to cycle 6. This change is likely due to the effects of the freeze-thaw cycles on the cream's components. Factors like storage temperature are known to influence a cream's pH over time (Lestari *et al.*, 2023). Although cetyl alcohol, which is mildly acidic, influences the overall pH, all formulations remained within the required standard pH range of 4.5-8 even after the completion of the stability test.

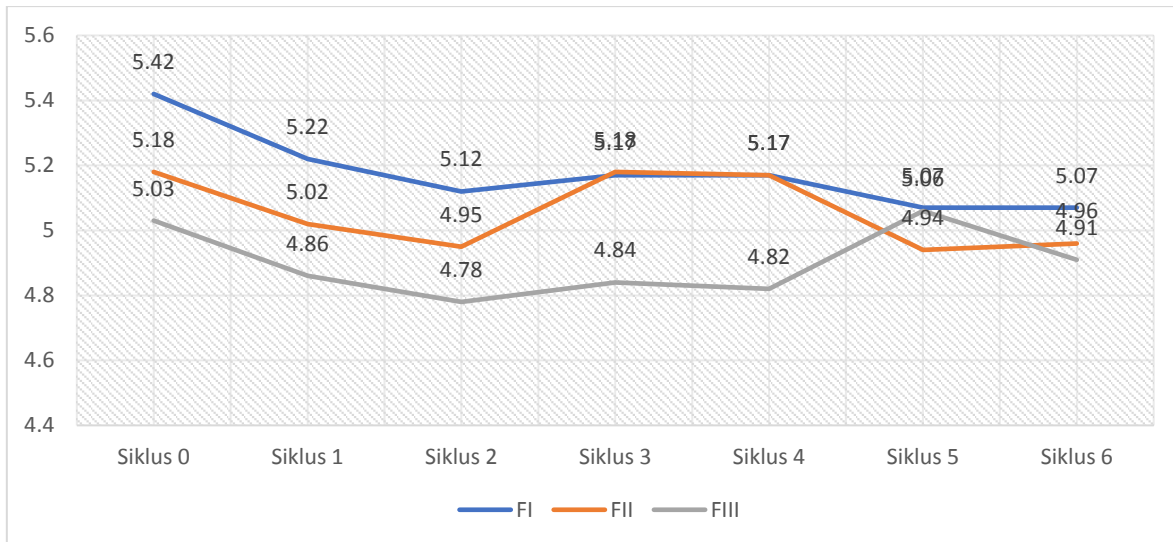


Figure 4. pH stability test result

Based on the statistical result, all formulation showed P value > 0.05 which indicating stable pH properties. Viscosity is a critical parameter that measures a cream's thickness, which directly influences the delivery of active ingredients and the product's spreadability (Ilmagnun & Endriyatno, 2024). Viscosity can be affected by various factors, including storage duration, which may cause a decrease in viscosity, and lower temperatures, which can increase it (Rabima, 2017).

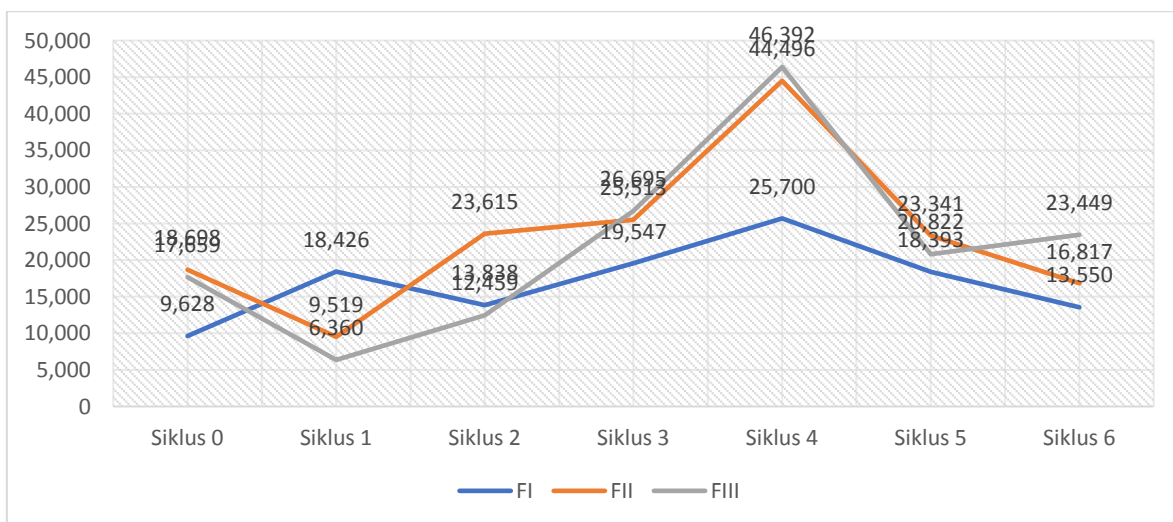


Figure 5. Viscosity stability test result

The viscosity data statistical analysis result of FI using Paired Sample T-test showed Asymp.Sig < 0.05 on all cycles. This indicated significant differences between cycles of FI's viscosity data. Different result showed by FII, which showed significant differences on cycles 1, 4, 5, and 6 that indicating unstable viscosity. Stable viscosity result was shown by FIII, with

Asymp.Sig > 0.05 on all cycles, indicating no significant differences between cycles. However, a notable increase in viscosity was observed during cycle 4, which was likely due to a lower room temperature. This environmental change can lead to a reduction in the distance between molecules and an increase in intermolecular forces, thereby raising the viscosity (Rabima, 2017). Additionally, cetyl alcohol, a waxy substance, tends to thicken at lower temperatures, which also contributes to increased viscosity (Rowe *et al.*, 2009). The impact of this temperature fluctuation was most showed in the formulation with the highest concentration of cetyl alcohol, FIII.

4. CONCLUSION

The concentration of cetyl alcohol significantly impacted the physical properties and stability of the belimbing wuluh leaf (*Averrhoa bilimbi L.*) extract cream. The variation in cetyl alcohol concentration directly influenced the cream's adhesion, spreadability, pH, and viscosity.

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