JFSP Vol.9, No.2, May-August 2023, Page: 140-147 pISSN: 2549-9068, eISSN: 2579-4558
Jurnal Farmasi Sains dan Praktis
(JFSP)
http://journal.unimma.ac.id/index.php/pharmacy

OPTIMIZATION AND PHYSICAL STABILITY OF KEMBANG BULAN (*Tithonia diversifolia* [A.Gray] EXTRACT CREAM FORMULA

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https://doi.org/10.31603/pharmacy.v9i2.8404

Article info:
Submitted : 26-12-2022
Revised : 31-05-2022
Accepted : 06-06-2023

Publisher:
Universitas Muhammadiyah Magelang

ABSTRACT

Based on recent studies, the ethanolic extract of Kembang Bulan leaves (*Tithonia diversifolia*[A.Gray]) has an anti-keloid effect. This study aims to determine the optimum formula for cream preparations with *paraffinum liquidum*, cetyl alcohol, and stearyl alcohol. Inappropriate comparison of additional ingredients results in the physical properties of the preparation becoming unstable during storage. The optimum formula was obtained from the optimization using Simplex Lattice Design (SLD) by software Design Expert version 10.0. The parameters measured were viscosity, pH, spreadability, adhesive ability, and accelerated study (centrifugation). The optimum cream formula consists of 19.11% b / b; Cetyl alcohol with a level of 2.01% b / b; stearyl alcohol with a level of 1.49% b / b with a viscosity of 134.35 dPa.s, a spread of 5.78 cm, pH 6.243, and adhesive ability of 13.61 seconds. The cream base produced is milky white, has a viscosity of 143.99 dPa.s, a spread of 5.7 cm, pH 6.305, and adhesion to 14.57 seconds. There is a significant difference in viscosity and pH of the experimental and software prediction. However, there is no significant difference in adhesive ability and spreadability response. The cream base and ethanolic extract of Kembang Bulan leaves have a significant difference in physical stability except for its spreadability. During the 4 weeks of storage, both of the creams are relatively stable. The cream is stable after centrifugation.

Keywords: Cream; Extract; Kembang bulan; Simplex Lattice Design

1. INTRODUCTION

Injuries to the skin are common. Scars that arise after the wound has healed, however, are often aesthetically and functionally disturbing. The scars that arise and exceed the boundaries of the wound and do not decrease over time are called keloids (Ogawa, 2017). Some classify keloids as benign tumors, hence many drugs with cytotoxic function are currently being developed as anti-keloids. Previous studies had shown that one of the benefits of the ethanolic extract of the Kembang Bulan is that it is cytotoxic to skin cancer melanoma cells, therefore it can be used as an anti-keloid, although it still requires data on toxicity and clinical studies. Most of the terpenoid compounds contained are sesquiterpene lactones, one of which is the Tagitinin compound which shows various pharmacological activities so that it is now a research center (Ajao and Moteetee, 2017).

The Tagitinin C compound obtained from the isolation of Kembang Bulan leaves is a sesquiterpene lactone that has anti-fibroblast activity, which can inhibit the accumulation of collagen. This collagen buildup can form keloids (Wahyuningsih et al., 2015). The development of non-invasive therapies continues to be carried out to provide alternative therapies that are comfortable to use for patients. An alternative that can be developed is in the form of a cream. A cream is a semisolid preparation where the active substance is dispersed in a suitable vehicle (Anonim, 2020). Cream has the advantage that is local and does not go through first-pass metabolism (Sidgwick et al., 2015). Therefore, it is more targeted for localized keloids. These

dosage forms are generally easy to clean and easy to spread (Allen and McPherson, 2021). This can increase patient compliance with using creams (Anwar, 2012; Kusuma et al., 2021).

Generally, a cream consists of 2 phases, namely the oil phase and the water phase. A cream in which its oil phase is dispersed in the water phase is called m/a cream. These creams do not feel greasy when used (Kusuma et al., 2021). In addition, when applied to the skin, the water phase will evaporate and the concentration of the drug in the remaining water will increase. This will accelerate the absorption of the drug (Shovyana and Zulkarnain, 2015)

The cream consists of several components: active substances, emulsifiers, thickeners, emollients, solvents, preservatives, and other additives. The use of thickeners in the making of cream can increase the viscosity and stability of the product (Sugihartini et al., 2021). The higher the viscosity, the more difficult it is for the cream to spread (Elcistia and Zulkarnain, 2019).

Cetyl alcohol and stearyl alcohol are thickening components in creams. The thickener in the cream affects the viscosity of the cream. Apart from being a thickener, cetyl alcohol is an emollient that functions as a protective layer that provides moisture. The use of cetyl alcohol mixed with other alcohol oils, such as stearyl alcohol, will increase the ability of cetyl alcohol to improve stability and other properties (Rowe et al., 2011).

To achieve physical stability in anti-keloid creams that use keloid extracts, it is necessary to optimize cetyl alcohol as a thickener and emollient, stearyl alcohol as a thickener, and emollient and liquid paraffin as an emollient. This anti-keloid of Kembang Bulan extract m/a cream is expected to be an alternative therapy for keloid treatment.

2. METHODS

Instruments: water bath(memmert®), analytical balance (Adventurer™, Ohaus), viscosimeter (*Brookfield* DV-I Prime Jepang), ultra turrax T25 (JANKE & KUNKEL IKA®-Labortechnik),, stopwatch (Alba *Digital Stopwatch*), adhesion test kit (Pharm.Tech.Lab.), UV-Vis spectrophotometry (Hitachi, UV-3200, Japan), pH meter (Hanna Ltd).

Ingredients: Kembang Bulan extract, cetyl alcohol, stearyl alcohol, stearic acid, triethanolamine, methyl paraben, propyl paraben, glycerin, aquadest, paraffin liquidum, citric acid, propylene glycol, tween 80, span 80, carbopol (all pharmaceutical grades). The formula is shown in **Table 1**.

Table 1. Cream formula o/w Kembang Bulan extract

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Stearic Acid	8	8	8	8	8	8	8	8	8	8	8	8	8
TEA	2	2	2	2	2	2	2	2	2	2	2	2	2
Stearyl alcohol	0.6	1.3	0.9	1.6	0.9	1.9	2.6	1.6	0.6	0.6	0.6	2.6	0.6
Cetyl Alcohol	4	2.7	3.3	3	2.3	2.3	2	2	4	3	2	2	2
Paraffin Liquidum	18	18.7	18.3	18	19.3	18.3	18	19	18	19	20	18	20
Span 80	3	3	3	3	3	3	3	3	3	3	3	3	3
Tween 80	7	7	7	7	7	7	7	7	7	7	7	7	7
Propylene Glycol	2	2	2	2	2	2	2	2	2	2	2	2	2
Carbopol	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Ethanolic Extract	1	1	1	1	1	1	1	1	1	1	1	1	1
Citric Acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glycerin	15	15	15	15	15	15	15	15	15	15	15	15	15
Propyl Paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Aquadest (ad)	100	100	100	100	100	100	100	100	100	100	100	100	100

2.1. Optimization of a Cream Formula

Optimization of the cream formula was carried out using the simplex lattice design method with the help of software design expert v.10 on liquid paraffin, cetyl alcohol, and stearyl alcohol. The formula is shown in **Table 1**.

2.2. Preparation of Curcuma Mangga Extract

A total of 4 kilograms of Curcuma mangga rhizome powder was extracted using the maceration method, dissolved in 8 L of 70% ethanol, and soaked for 24 hours. After that, the macerate was separated with a vacuum Buchner, evaporated using a rotatory evaporator, and evaporated again in a water bath until a thick extract of temu mango rhizome was obtained (Arizona & Zulkarnain, 2018).

2.3. Making of the Cream Formula

Kembang Bulan extract is dissolved in tween 80. The oil phase and the water phase were mixed and heated at 75° - 85 °C in a water bath respectively. The water phase was added to the oil phase while stirred using ultra turrax T25. Then, the Kembang Bulan extract, which had been dissolved, was added in tween 80 and stirred until homogeneous. Cream preparations that had been formed were put into a closed container.

2.4. Physical Properties Test of m/a Kembang Bulan Extract Cream

2.4.1. Cream pH Test

The pH of the cream was tested with Hanna's semisolid pH meter. It was first calibrated with a standard buffer solution of pH 4.01, pH 7.01, and pH 10 until the tool read the pH. Then, the electrode was washed with distilled water, making sure it was dry before being used for reading. The electrode was then dipped into the container containing the sample. The number shown by the digital pH meter was the pH of the preparation. The pH measurements were carried out with three repetitions for each test. The test was carried out every week for four weeks at room temperature. As required by SNI (Sugihartini et al., 2021) that cream sunscreen preparations have a pH range of 4,5-8. pH values that are too acidic will cause irritation and pH values that are too alkaline will cause scaly and itchy skin (Arizona & Zulkarnain,, 2018).

2.4.2. Cream Viscosity Test

The viscosity was tested with a Brookfield viscometer, by putting the preparation into an adequate container, then inserting the spindle and reading the viscosity for 15 seconds at 100 rpm. The spindle used was adjusted to the hardness of the preparation. The test was carried out every week for four weeks at room temperature (Alam et al., 2020).

2.4.3. Cream Adhesion Test

The test was carried out by placing 100 mg of cream on an object glass and then covering it with another glass object. After that, it was given a load weighing 1 kg for 5 minutes. Then the separation time of the two glass objects was measured when given a towing load of 20 grams (Marchaban et al., 2015; Arifin et al., 2020; Harliatika & Noval, 2021).

2.4.4. Cream Spreadability Test

Approximately 0.5 grams of cream was spread in the middle of a round glass that had been prepared as part of the spreadability test kit. Then, the other glass was weighed first and the glass was placed on top of the preparation and left for 1 minute. Next, the diameter of the cream that spread was calculated (by calculating the average diameter of several sides). Then, additional weight was added until the final load was 300 grams per minute. Each time the load added was 50 grams and was left for 1 minute, and the diameter of the distribution was recorded. The test was carried out every week for four weeks at room temperature (Marchaban et al., 2015). Good gel spreadability values range from 5-7 cm (Rahmawati et al., 2018).

2.4.5. Cream Emulsion Separation Test

The cream was put into two test tubes scaled to a certain scale. The scale test tubes containing cream preparations were tested using centrifugation at speeds of 1500, 3000, and 4500 rpm for 30 minutes. Observations were made for each 5-minute increase (Arizona & Zulkarnain,, 2018); (Nurfita et al., 2021).

3. RESULTS AND DISCUSSION

3.1. Optimum Formula Determination

The optimization of the Kembang Bulan m/a cream formula was carried out with variations in the concentration of the ingredients. The evaluation of pH, viscosity, adhesion, and spreadability was carried out with the "Targets" that were within a certain range. Software Design Expert provides 3 formula solutions according to the desired optimization target. The solution chosen was the formula with the highest desirability value, which was the formula with a desirability value of 0.960. The location of the selected optimum formula was indicated by a white dot on the contour plot desirability as illustrated in **Figure 1**. The optimum formula chosen had a composition of liquid paraffin with a concentration of 19.11% w/w; Cetyl alcohol with a content of 2.01% w/w; and stearyl alcohol with a concentration of 1.49% w/w. The selected formula was predicted to provide a pH of 6.058; viscosity of 146.00 dPa.s; adhesion of 13.945 seconds; and spreadability of 17.2145 cm2. The plot overlay is shown in **Figure 1** and **Figure 2**.

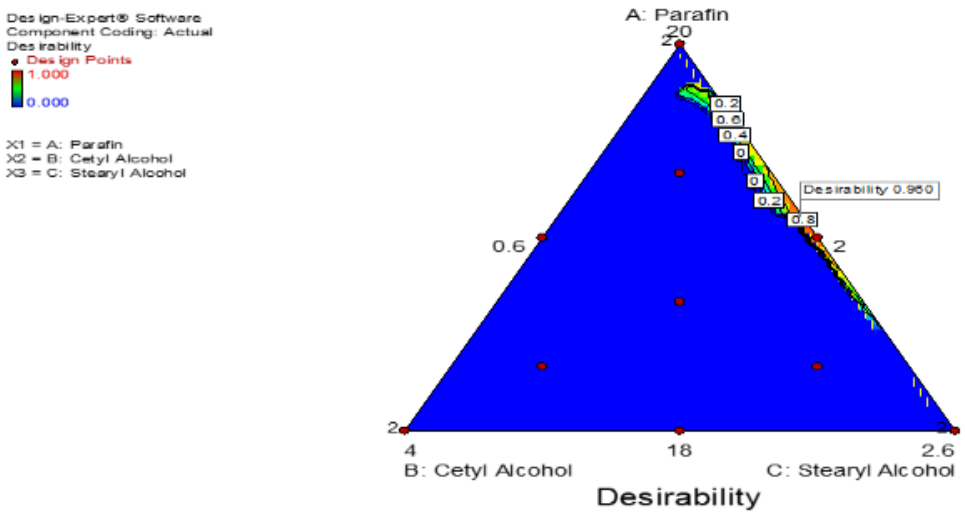

Figure 1. Desirability of cream base formula

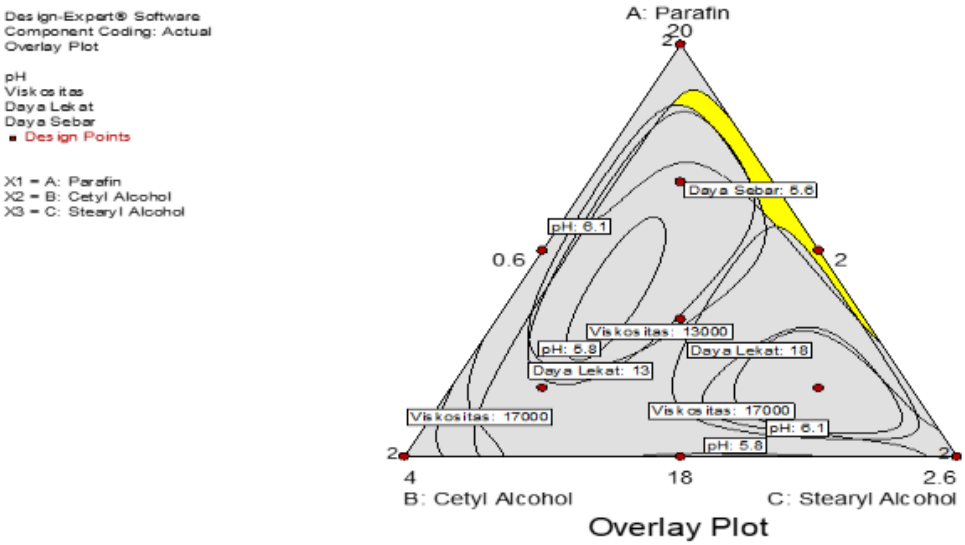

Figure 2. Overlay plot of cream base formula

The base data for m/a Kembang Bulan extract cream that was used to compare with the predictions by the software was the data from week 0. The analysis was performed with a t-test using SPSS v.25 software. The data is shown in **Table 2**.

Table 2. Optimum formula verification using one sample t-test

Response	Prediction	Test	*Sig. (2-tailed)*	Description
pH	6.058	6.305	0.001	significantly different
Viscosity (dPa.s)	146.00	143.95	0.002	significantly different
Adhesion (s)	13.95	14.58	0.095	Not significantly different
Spreadability (cm)	5.8	5.7	0.092	Not significantly different

The results of the analysis with the t-test of one sample stated that the pH and viscosity values of the cream base from the experimental results were significantly different from the predicted pH and viscosity values. The spreadability and adhesion of the predicted cream and the optimum formula were not significantly different.

3.2. Kembang Bulan Cream

Optimization in each formula run without adding active substances due to the limitations of active substances. Therefore, it was necessary to do a comparison between the optimum formula cream base and the Kembang Bulan cream and to do a statistical analysis using the t-test of 2 independent samples. The results of the statistical analysis showed that there was a significant difference between the pH, viscosity, and adhesion of the optimum formula cream base and the pH, viscosity, and adhesion of the Kembang Bulan cream. While the spreading power of the optimum formula cream base with the Kembang Bulan cream was not significantly different. A comparison of the physical properties of the optimum formula cream base and Kembang Bulan cream with the t-test of 2 independent samples are listed in **Table 3**. Response optimal formula cream base and Kembang Bulan cream during storage at room temperature are listed in **Table 4**.

Table 3. Comparison of the physical properties of the optimum formula cream base and Kembang Bulan cream with the t-test of 2 independent samples

Test Parameters	Cream Base	Kembang Bulan Cream	Significance	Description
pH	6,31±0,04	6,24±0,01	0.049	significantly different
Viscosity	144,0±0,38	134,35±1,86	0.001	significantly different
Adhesion	14,58±0,09	13,61±0,28	0.001	significantly different
Spreadability	5,68±0,10	5,70±0,08	0.705	Not Significantly Different

Table 4. Optimum formula cream base response and Kembang Bulan cream during storage at room temperature

Week	pH		Viscosity		Adhesion		Spreadability	
	Cream base	Kembang Bulan Cream	Cream base	Kembang Bulan Cream	Cream base	Kembang Bulan Cream	Cream base	Kembang Bulan Cream
0	6.31±0.04	6.24±0.01	143.99±0.38	134.35±1.87	14.58±0.09	13.61±0.28	5.68±0.10	5.70±0.08
1	6.25±0.05	6.16±0.01	142.95±0.53	132.48±1.64	14.43±0.25	13.53±0.16	5.58±0.10	5.70±0.08
2	6.23±0.01	6.15±0.01	140.05±0.21	131.88±0.75	14.33±0.32	13.53±0.32	5.68±0.10	5.70±0.08
3	6.225±0.02	6.15±0.01	139.615±0.32	132.34±0.99	14.23±0.19	13.55±0.30	5.80±0.08	5.78±0.10
4	6.18±0.03	6.06±0.01	138.14±0.41	131.62±0.51	13.81±0.11	13.51±0.25	5.88±0.10	5.78±0.10

3.3. Cream stability test

The optimum physical stability of the cream base formula and the Kembang Bulan cream was carried out during 4 weeks of storage at room temperature. The test parameters include organoleptic, pH, viscosity, adhesion, and spreadability. The pH response during storage is the blue line is alkaline, and the red is the extract, as shown in **Figure 3**.

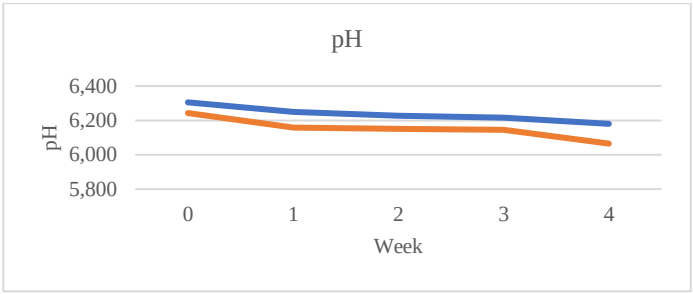

Figure 3. pH response during storage line blue is base and red is extract

Both the cream base and the Kembang Bulan cream did not change in physical form (color, smell, and homogeneity) within 4 weeks. The pH of the cream ranged from 6 to 6.3 which was close to neutral pH. The changes in pH for 4 weeks can be seen in **Table 4**. The ANOVA test conducted showed a significant change after the 2nd week on the cream base and showed a significant change from the 1st week onwards on the Kembang Bulan cream.

Viscosity response during storage blue line is base and red is extract as shown in **Figure 4**. The viscosity of the cream base and Kembang Bulan cream tended to decrease over time during 4 weeks of storage. The decrease in viscosity was due to the dehydration of the cream during storage which caused the cream to become brittle and reduced the resistance to rotation of the rotor resulting in a decrease in viscosity. This could be due to the depolymerization of the emulsifier in the cream (Kar et al, 2019). The cream base showed a significantly different viscosity response every week except the first week ($p>0.05$). The Kembang Bulan cream did not show a significant change even though there was a decrease.

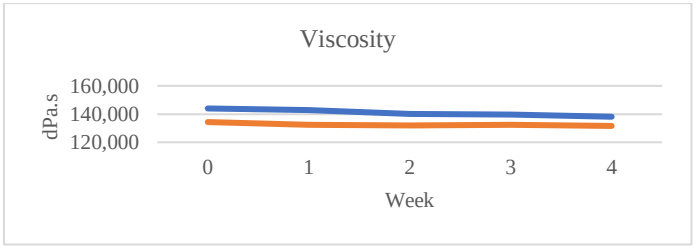

Figure 4. Viscosity response during storage line blue is base and red is extract

The stickiness of the cream base and Kembang Bulan cream tends to decrease as shown in **Figure 3**. However, the ANOVA test showed that the response was not significantly different from either the cream base or the Kembang Bulan extract cream. The decrease in adhesion was following the decrease in viscosity. Adhesion response during storage blue line is alkaline, and the red is extracted, as shown in **Figure 5**.

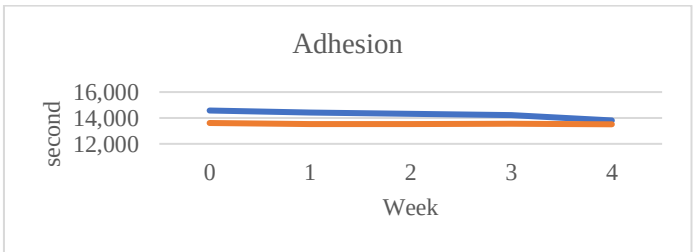

Figure 5. Adhesion response during storage line blue is base and red is extract

Scattering response during storage, the blue line is the base, and the red is the extract, as shown in **Figure 6**. The spreadability of the cream base tended to increase. The spreadability of the Kembang Bulan cream tended to fluctuate. The increased spreadability was due to the

decrease in the viscosity of the cream which then facilitated the spread of the preparation. The statistical test results showed no significant changes every week.

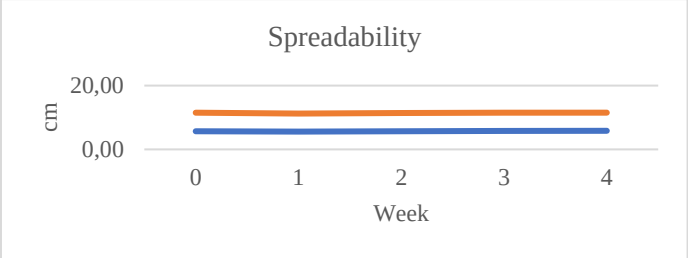

Figure 6. Spreadability response during storage line blue is base and red is extract

The stability test was accelerated by centrifugation. This test was a mechanical accelerated stability test with the addition of force (Martin & Sinko, 2011). The higher the speed used, the higher the separation that occured due to the separation of the internal phase by centrifugation which causes coalescence. The test results showed that there was no separation after treatment. This shows that the cream base and Kembang Bulan cream are stable.

4. CONCLUSION

The composition for the optimum formula is 19.11% w/w; cetyl alcohol with a content of 2.01% w/w; and stearyl alcohol with a concentration of 1.49% w/w with a desirability of 0.952. The cream obtained was stable during storage 4 weeks. There was a tendency for viscosity. The stickiness decreased during storage 4 weeks.

5. CONFLICT OF INTEREST

The author has no conflict of interest to declare in this research.

6. ACKNOWLEDGMENT

Support from the Faculty of Pharmacy UGM Yogyakarta is greatly acknowledged.

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