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Na-CMC and glycerine optimization in Binahong leaf extract (*Anredera cordifolia*) liposome gel and its burn wound healing activity

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ABSTRACT

Burns represent a critical global health issue, contributing to considerable morbidity and mortality rates, particularly within the Southeast Asian region. The administration of appropriate burn therapy is essential to prevent infections and promote effective wound healing. The binahong leaf (*Anredera cordifolia*) represents a highly promising natural substance for burn therapy, attributed to its ursolic acid content, which is acknowledged for its wound healing properties. However, the limitation caused by its limited solubility and bioavailibility requires the use of nanoparticle technology, such as liposomes, to enhance its efficacy. The aim of this research was to determine an optimal formulation of gel containing liposome-encapsulated binahong leaf extract, with the goal of promoting burn wound healing and examining its *in vivo* wound healing activity. Histological analysis was employed to provide additional insights into the activity of the gel. The formulation was assessed through a factorial design, exploring various amounts of Na-CMC utilized as a gelling agent alongside glycerine employed as a humectant. The results were subsequently analyzed utilizing Design Expert v13 software. The gel's viscosity, spreadability, and uniformity were assessed. Na-CMC increased viscosity while reducing spreadability, whereas glycerine had the opposite effect. The optimal formulation contained 2.78–4 g Na-CMC and 5–10 g glycerine. Statistical validation confirmed the model's accuracy. In vivo studies demonstrated that liposomal binahong gel significantly accelerated burn wound healing compared to controls. The results suggest that 10% binahong liposomal gel is a promising alternative for burn treatment.

Keywords: burn treatment, binahong leaf extract, liposome gel, Na-CMC, glycerine, wound healing

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INTRODUCTION

The skin is the largest organ of human body with its role as a barrier with immunological, sensory, and protective capabilities. Exposure to external environments could cause damage and injury (Wang et al., 2018). If left untreated, burn could lead to infection and sepsis (D'Abbondanza & Shahrokhi, 2021). When an individual sustains a burn, the risk of infection and complications on the skin increases (Priamsari & Yuniawati, 2019).

Burns are a global health concern that require attention due to their significant morbidity and mortality rates worldwide. In 2019, around 46% of burn cases globally originated from Asia, with Southeast Asia being one of the most burdened regions (Collier et al., 2022). According to the Riset Kesehatan Dasar report (Riskesdas, 2018) report, the incidence of burns in Indonesia ranked second among injury occurrences, with a total of 1.3%. Given the frequency of these events, proper treatment is necessary, as poorly managed burns can result in a prolonged inflammatory phase, slowing down the wound healing process (Holzer-Geissler et al., 2022).

Currently, various topical formulations are available for burn treatment, one of which is silver sulfadiazine (SSD). However, silver sulfadiazine is known to cause side effects such as nephrotoxicity and leukopenia (Sukrama et al., 2017). Therefore, formulations derived from natural ingredients can serve as an alternative, as they are known to have fewer side effects compared to modern drugs. One promising natural ingredient for burn healing is the binahong leaf (*Anredera cordifolia*), which contains ursolic acid, a compound known to enhance the wound healing process (Samirana et al., 2016; Yuliani et al., 2016).

Ursolic acid, the main active component of binahong extract has poor water solubility, thus nanoparticle technology, such as liposomes, can be used to improve its solubility (Dwiastuti & Ardiyati, 2020). The extract is encapsulated in liposomes, and the binahong leaf extract liposomes are formulated into a gel preparation. Gel is a semi-solid formulation with excellent drug release properties. It is chosen because it is comfortable to use, non-sticky, easy to wash off with water, has good adhesion, is easily absorbed, and allows for prolonged drug contact duration.

Na-CMC works as a thickening agent in gel formulations, while glycerine is used to retain moisture. These two ingredients are key to determining the gel's texture and behavior. High levels of Na-CMC can result in a thicker, denser gel, which might make it harder for the gel to spread evenly (Handayani et al., 2019). On the other hand, glycerine helps keep the gel stable by drawing in moisture and slowing down water evaporation. Since glycerine is a liquid, it can make the gel less viscous, which helps improve its spreadability (Sayuti, 2015). However, when Na-CMC and glycerine are combined, they can interact in a way that increases the gel's viscosity, which in turn reduces how well it spreads (Suradnyana et al., 2020). In order to achieve a gel with the desired physical properties it's important to optimize the amounts of the excipients. In this study, we focused on optimizing the formulation of a binahong leaf extract liposome gel for burn treatment, as burn wounds are distinct from other wound types due to their depth, extent of tissue damage, and high risk of infection. They require specialized treatment strategies, such as those targeting inflammation, promoting angiogenesis, and stimulating collagen synthesis. These properties align well with the bioactive compounds found in binahong, such as flavonoids and saponins, which are known for their wound-healing and antimicrobial activities. In this research we also utilizing a factorial design to examine how different amounts of Na-CMC and glycerine, as well as their interaction, affected the properties of the gel. The data was analyzed using the Design Expert v.13 (*free-trial)* software. Afterwards, the gel was tested for its burn wound healing activity and histological investigation was carried out to further investigate the wound healing effect of the gel.

MATERIALS AND METHOD

Equipment

The equipment used in this study consisted of an analytical balance (Pioneer), a hotplate (IKA® C-MAG HS7), a blender (Philips), a thermometer, Universal pH strips (Merck), a Rion viscometer, an oven (Memmert), a refrigerator (Samsung), the Design Expert v13 software (free-trial version), Pyrex glassware, 1.5 cm metal plates, a water bath, and millimeter block paper.

Materials

The materials used in this study included a liposome suspension of binahong leaf extract, prepared following the method described by (Dwiastuti & Ardiyati, 2020). Na-CMC (pharmaceutical grade), glycerine (pharmaceutical grade), methyl parabene, propylene glycol, plastic wrap, 24 male Wistar strain white rats (*Rattus norvegicus* L.) weighing 200-300 grams, ethyl chloride (pharmaceutical grade), and Bioplacenton gel.

Gel preparation

The binahong extract liposome suspension, previously prepared based on research done by (Dwiastuti & Ardiyati, 2020) was placed into a mortar, and Na-CMC was sprinkled evenly on the surface. The mortar was then covered with plastic wrap and left for 15 minutes to allow Na-CMC to swell, forming gel matrix. The mixture was then ground with a pestle. The gel was prepared according to the formula outlined in Table 1. Once homogeneous, glycerine, propylene glycol, and methyl paraben (previously dissolved in ethanol) were added to the mixture. The mixture was then blended for 3 minutes (Dwiastuti & Ardiyati, 2020; Mardiana et al., 2020). The formula used described at Table 1.

Table 1. Binahong extract liposome gel formula

Component	Formula				Function
	1	**A**	**B**	**AB**	
Binahong leaf extract liposome suspension (mL)	100	100	100	100	Active agent
Na-CMC (g)	**2**	**4**	**2**	**4**	Gelling agent
Glycerine (g)	**5**	**5**	**10**	**10**	Humectant
Methyl Parabene (g)	0.16	0.16	0.16	0.16	Preservative
Propylene glycol (g)	10	10	10	10	Permeation enhancer

Gel physical properties test

Organoleptic Observation

Organoleptic observations were made by examining the color, odor, and texture of each gel formula (Rahayu et al., 2016) by placing each 200 miligrams of the gel formula on a glass.

Homogeneity Observation

Homogeneity was observed visually. About 0.2 grams of the gel was applied to a transparent glass slide and covered with another transparent slide. The gel was considered homogeneous if no coarse particles were visible (Rahayu et al., 2016).

pH Observation

The pH observation was performed using universal pH indicators. The pH indicator paper was dipped into the gel formulation up to the marked level. The pH value of the formulation was determined by comparing the color of the universal pH indicator paper with a standard pH color chart. A good gel had a pH equivalent to that of the skin, which ranged from 4.5 to 6.5 (Alfiany et al., 2021).

Viscosity Test

The viscosity test was conducted to measure the thickness of the formulation using a viscometer. The spindle was immersed in the gel about 0.5 cm from the base until a constant reading was shown, which was then recorded. Each formulation was tested five times. A good gel had a viscosity between 20-40 dPas (Rahayu et al., 2016).

Spreadability Test

The spreadability test was done by gradually increasing the applied weight from 50 grams to 200 grams. The spreadability range of 5–7 cm refers to the final spread diameter measured after the maximum weight of 200 grams is applied. This range is used as a benchmark to evaluate whether the gel meets the criteria for good spreadability, as defined by (Fahrezi et al., 2021).

Burn wound healing assay

We submitted an ethical clearance application to the Health Research Ethics Committee of Universitas Respati Yogyakarta (UNRIYO), Yogyakarta (084.3/FIKES/PL/VII/2024). The effectiveness of binahong leaf extract was tested *in vivo* using healthy male Wistar strain white rats (*Rattus norvegicus* L.) weighing 200-300 grams, aged 6-8 weeks, and without physical defects such as blindness, unsteady walking, or wounds. Male rats were used because their biological condition is more stable than females, which undergo estrus cycles. Experimental animals without defects were selected to standardize the condition of the test subjects, minimizing errors from the experimental animals and ensuring they are in good health.

The rats were anesthetized with ethyl chloride to reduce the pain caused by burn induction. Back area were shaved to an area of 2 cm × 2 cm. Burns were created by gently press a 2 cm diameter metal plate, pre-heated in boiling water at 99°C for 3 minutes, for 5 seconds (Wahyuningsih et al., 2021). The resulting burns were shallow second-degree burns, characterized by redness and edema on the skin (Priamsari & Yuniawati, 2019).

The experimental animals in this study were randomly divided into six groups, with a specific number of animals in each group. The number of experimental animals per group was determined using Federer's formula (Federer, 1669). Based on the results of Federer's formula, 4 experimental animals were used in each group. A total of 24 animals were used in the study, which involved 6 groups of experimental animals with different treatments as could be seen on Table 2.

Table 2. Test animal groups

Group Name	Description
Positive Control	Rats treated using bioplasenton gel (Neomycin sulfate 0.5% and placenta ex bovine 10%)
Gel Basis Control	Rats treated by gel base without binahong leaf extract liposome
Negative Control	Rats with no treatment
Treatment 1	Rats treated with a gel containing 10% liposomal binahong leaf extract
Treatment 2	Rats treated with a gel containing 30% liposomal binahong leaf extract
Treatment 3	Rats treated with a gel containing 50% liposomal binahong leaf extract

The rats were then treated according to their respective groups for 21 days. The treatment (approximately 200 mg of gel extract) was administered twice daily with a 12-hour interval; (Betriksia et al., 2018; Saputri & Darmawan, 2017). Wound closure was observed by measuring the surface area of the burn, conducted daily before the second treatment in the afternoon. Burn wound healing was indicated by the narrowing and closure of the wound (Wahyuningsih et al., 2021).

Histological analysis

In order to prepare for H&E staining of the experimental animal skin at day 21, the experimental animals skin area is carefully removed with appropriate margins. Following a 24-hour fixation in 10% formalin liquid, the tissue is dehydrated using graded ethanol, cleared with xylene, and embedded in paraffin. Using a microtome, thin pieces of 4–6 µm are sliced and then positioned on glass slides covered with adhesive. Drying of the slides is done at 37°C for 1-2 hours or at 60°C for 20-30 minutes. Prior to staining, the sections undergo deparaffinization by submerging the slides in xylene and then rehydration using a succession of decreasing ethanol solutions. The nuclear staining is performed with hematoxylin, followed by differentiation in acidic alcohol and counterstaining with eosin for the cytoplasm and extracellular components. Once the slides have been dehydrated and cleared, they are affixed with coverslips. An analysis of the produced slides is conducted using a light microscope, and photographs are taken. To measure the epidermis/dermis thickness, we utilized a single field of view to obtain the measurements. Using ImageJ software, the boundaries of the epidermis (from the outermost layer to the basal membrane) and the dermis (from the basal membrane to the subcutaneous layer) were identified within this field of view. A perpendicular line was drawn from the top to the bottom of the respective layer, and the thickness was measured after calibrating the scale based on the provided magnification.

Data Analysis

The physical properties of the gel, including viscosity and spreadability result, were analyzed using a two-way ANOVA with a 95% confidence level through the Design Expert v13 software (free trial version). This analysis determined the effects of Na-CMC, glycerine, and their interaction on the physical characteristics of the gel. A contour plot was then created to determine the optimal area of Na-CMC and glycerine through an overlay plot.

The validation of the optimal composition area was performed by selecting one point randomly from the optimal area, after which the binahong leaf extract liposome gel was re-prepared. The difference between the validation data and the theoretical data was assessed using a One Sample T-Test. The factorial design equation was considered valid if the p-value was greater than 0.05 (Suradnyana et al., 2020).

The surface area of the burn was measured by capturing images, which were then analyzed using the Image J program to obtain the measurement of the wound area in mm², with data collected from day 1 to 21 (Hapsari et al., 2017). The burn wound healing percentage was then calculated using equation (1) bellow.

$$L\% \frac{L1-Ln}{L1} \text{X } 100\ \% \qquad (1)$$

L% = percentage of wound healing
L1 = initial burn area on day 0 (D0)
Ln = burn area on day n

RESULT AND DISCUSSION

Organoleptic observations were conducted to evaluate the conformity of the formulation's appearance, color, and odor based on desired criteria (Rahayu et al., 2016). The results indicated that formulas 1 and B had a thinner gel consistency, with formula B being more liquid than formula 1. Both formulas appeared as cloudy brownish-green, had a characteristic binahong leaf extract odor, and contained small bubbles. Formulas A and AB had a thicker gel consistency, with formula AB being thicker than formula A. These two formulas also appeared as cloudy brownish-green, had the characteristic odor of binahong leaf extract, and contained fewer small bubbles.

Illahi's research (2018) showed that gels without liposomes resulted in a clear gel, while gels containing liposomes as the active ingredient produced a bone-white colored gel. Therefore, the cloudy brownish-green color of the four gel formulas in this study may be attributed to the use of liposome suspensions, which have a concentrated brownish-green color.

Homogeneity observation was carried out to evaluate and ensure that the gel formulation was thoroughly mixed. This observation involved placing 0.2 grams of each gel formula on a transparent glass slide and covering it with another transparent glass slide as can be seen at Figure 1. A gel was considered homogeneous if no coarse particles were present (Rahayu et al., 2016). The observation results indicated that all four formulas were free of large particles and were perfectly mixed.

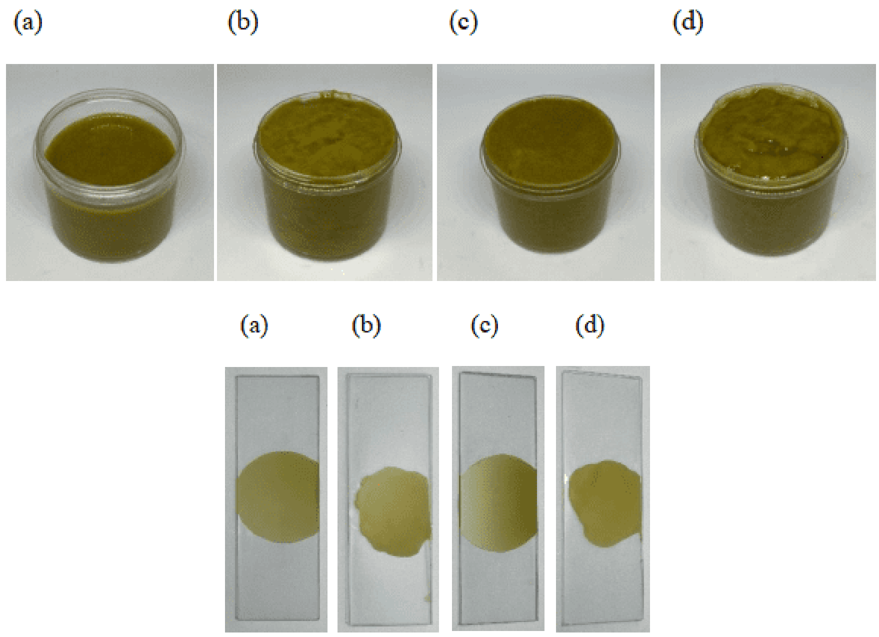

Figure 1. Physical appearance (upper) and homogeneity observation (lower) of the binahong extract liposome gel with different formulas: a (F1); b (FA); c (FB); and d (FAB)

The pH observation was conducted to evaluate the acidity or alkalinity of a formulation to ensure its compatibility with the skin. A good gel formulation should have a pH close to that of the skin, which ranges from 4.5 to 6.5. A formulation with high acidity may cause skin irritation, while one that high in alkalinity may lead to dry skin (Sayuti, 2015). The pH observation of the binahong leaf extract liposome gel was carried out using a universal pH indicator. The pH value of all four gel formulas was 5, indicating that the formulations are safe for use on the skin.

The viscosity test was conducted to evaluate the thickness of the formulation. A gel with too low viscosity can reduce the contact time with the skin, affecting the activity of the active ingredient in the gel. On the other hand, a gel with too high viscosity can increase retention time, reduce spreadability, and make it difficult to dispense from its container (Haryono et al., 2021). Based on the test results of the four formulas (Table 3), formulas A and AB had good viscosity and fell within the desired range of 20-40 dPas (Rahayu et al., 2016). In contrast, formulas 1 and B did not meet the ideal viscosity range. Formula A contained 4 grams of Na-CMC as the gelling agent and 5 grams of glycerine as the humectant, while formula AB contained 4 grams of Na-CMC and 10 grams of glycerine. The addition of Na-CMC had the most dominant effect in increasing gel viscosity. The more Na-CMC used, the higher the viscosity of the gel, as more polymer chains are formed in a limited space (Anita & Lestari, 2023). Additionally, the increase in viscosity was also due to the release of Na+ ions being replaced by H+ ions, forming HCMC, which enhances viscosity through cross-linking. (Candradireja, 2014). The addition of glycerine, which has a liquid consistency, resulted in a decrease in gel viscosity (Robiatun et al., 2022). This can be seen in Table 3, where gel formulations with a high level of glycerine (10 grams) had lower viscosity compared to those with a lower glycerine level (5 grams), as observed at all four replication.

The four formulations of binahong leaf extract liposome gel were influenced by two factors: the different amounts of Na-CMC and glycerine, which were divided into low and high levels. These two factors impacted the response of the gel's physical properties. The response was analyzed using the

Design Expert v13 (free-trial version) application, employing a two-factor, two-level factorial design method. This process allowed for the identification of the optimum formulation.

The research data obtained were analyzed using the Design Expert v13 (free-trial version) application to examine the effects of Na-CMC, glycerine, and their interactions on the physical properties, specifically the viscosity and spreadability responses. The effects and contributions of Na-CMC, glycerine, and their interactions on the viscosity response can be seen in Table 4.

Table 3. Viscosity and spreadability test result of binahong leaf extract liposome gel

Formula	Viscosity (dPas)	Spreadability (cm)
1	15.00±0.71	7.71±0.07
A	32.80±0.84	5.89±0.07
B	10.80±0.84	7.71±0.10
AB	29.60±1.14	5.94±0.13

Table 4. Effect and contribution of Na-CMC, glycerin, and their interaction on the viscosity and spreadability of Binahong leaf extract liposome gel

Viscosity				
Factor	**Effect**	**Contribution%**	**p-value**	**Model p-value**
Na-CMC	18.3	95.30%	<0.0001	<0.0001 (significant)
Glycerine	-3.7	3.90%	<0.0001	
Interaksi	0.5	0.07%	0.2293	
Spreadability				
Factor	**Effect**	**Contribution%**	**p-value**	**Model p-value**
Na-CMC	-1.7975	99.06%	<0.0001	<0.0001 (significant)
Glycerine	0.0275	0.02%	0.5296	
Interaction	0.0225	0.02%	0.6062	

In the given equation, the viscosity response is denoted as Y, the quantity of Na-CMC used is denoted as X1, the quantity of glycerine used is denoted as X2, and the interaction between Na-CMC and glycerine is denoted as X1X2. An analysis of the ANOVA test findings using the free-trial edition of Design Expert v13 revealed that the viscosity response had a statistically significant model p-value of less than 0.0001 ($p\text{-value} < 0.05$). These findings suggest that the model equation is applicable for identifying the most favorable area on the overlay plot.

Spreadability response

The results of the analysis of the effects and contributions of Na-CMC, glycerine, and their interactions on the spreadability response can be seen in Table 4. Na-CMC was the most influential factor in reducing the spreadability of the gel, contributing 99.06% with an effect of -1.7975. The influence of Na-CMC on the spreadability value is related to its role as a gelling agent, where the use of a gelling agent increases the viscosity of the gel. Spreadability is inversely proportional to viscosity; as viscosity increases, spreadability decreases. Therefore, Na-CMC increases viscosity, resulting in reduced spreadability. The Na-CMC factor had a significant effect on reducing spreadability, with a p-value <0.05. However, glycerine and the interaction between Na-CMC and glycerine did not significantly affect the spreadability response (p-value >0.05). Based on the spreadability values of the four formulas, the following equation (3) was derived:

$Y = 6.88 – 0.8600X1 – 0.0375X2\ 0.0425X1X2$ ....(3)

In the given equation, the spreadability response is denoted as Y, the quantity of Na-CMC utilized is denoted as X1, the quantity of glycerine used is denoted as X2, and the interaction between Na-CMC and glycerine is denoted as X1X2. The Model ANOVA test results obtained from the free-trial edition of Design Expert v13 indicated that the spreadability response had a statistically significant model p-value of less than 0.0001 (p-value < 0.05). Hence, this equation might be employed to ascertain the most advantageous area on the overlay plot.

Overlay plot

The optimization of the binahong leaf extract liposome gel formulation was carried out using a factorial design method. The objective was to observe the effects and interactions at each factor level to obtain the optimum composition of Na-CMC and glycerine that meets the requirements for good and stable gel physical properties. The response criteria used were viscosity within the range of 20-40 dPas and spreadability within the range of 5-7 cm. The contour plots of each response were combined to form an overlay plot, which was used to determine the optimal region.

The yellow region in Figure 2 corresponds to a desirable zone where the viscosity and spreadability reaction values are within the defined ranges. The gray area denotes areas where the given replies fail to satisfy the specified requirements. Using the Design Expert v13 (free-trial version) software, 100 solutions were generated based on the estimation of Na-CMC and glycerine quantities necessary to create gel formulations that fulfill the desired parameters. The overlay plot results showed that a gel with physical characteristics aligning with the necessary criteria could be obtained by varying the Na-CMC quantity from 2.78-4 grams and the glycerine amount from 5-10 grams. A model validation was performed to compare the experimental test findings of the liposome gel containing binahong leaf extract with the predicted results derived from the theoretical equation. The validation of the response equation involved analysis of the results from the viscosity and spreadability tests. The chosen amount for Na-CMC was 3.3 grammes, while for glycerine it was 8.6 grammes, as these values were within the optimum range known as the yellow zone. The aforementioned point was included into the factorial design equation, which directly represents the viscosity response equation ($Y = 2.4 + 8.4X1 – 1.04X2 + 0.1X1X2$) To predict the spreadability response, the selected point for the two factors was also input into the actual spreadability response equation ($Y = 9.565 – 0.9325X1 – 0.008X2 + 0.0045X1X2$). Given these two data points, it is expected that the gel will possess a viscosity of 24.01 dPas and a spreadability of 6.55 cm. An empirical comparison will be made between the measured viscosity and spreadability test result and the theoretical calculations utilizing a One Sample T-Test implemented in the Jamovi software.

Table 5. Comparative analysis of the model estimated outcomes of the optimal formula with actual data

Response	Theoretical estimation data	Actual data ($\bar{x} \pm SD$)	*p-value*
Viscosity (dPas)	24.01	24.20 ± 0.44	0.190
Spreadability (cm)	6.55	6.53 ± 0.03	0.317

The one sample t-test (Table 5) indicate that there is no statistically significant difference (p-value > 0.05) between the actual and theoretical estimates, therefore confirming the validity of the theoretical model equation. This result indicate that the ideal range for Na-CMC is between 2.78-4 grams, while for glycerine it is between 5-10 grams.

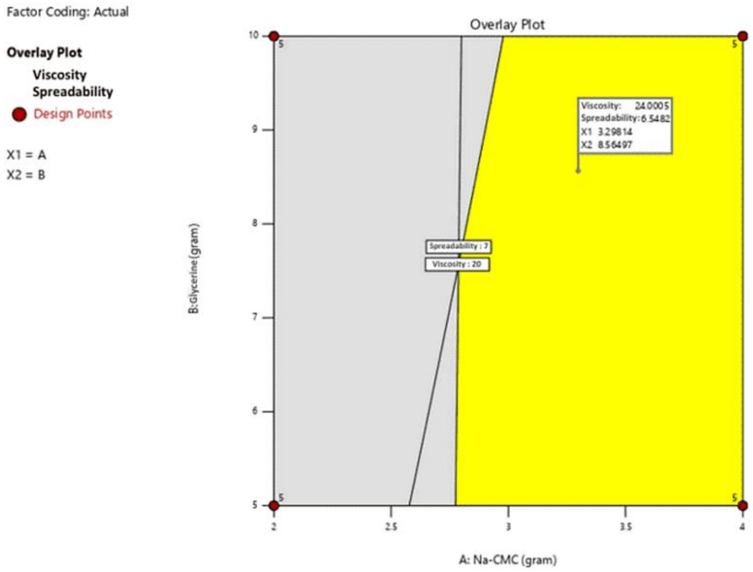

Figure 2. Overlay plot of viscosity and spreadability response

Wound healing activity

From Table 6 above and Figure 3, it could be seen that all experimental animals achieved full wound healing (as evidenced by the closure and hair covering of the wound) by day 21, as could be seen at Figure 3 with the exception of the control group (untreated) and treatment group 2. The observed results may be due to the applied gel being licked by the experimental animals, reducing the effectiveness of the treatment. The treatment groups 1, 2, and 3 exhibited the highest average wound healing % from day 1 to day 9. By day 13, it was noted that both treatment group 3 and 1 had the most rapid closure of wounds, approaching full complete wound healing. It could also be seen that the treatment group constantly giving statistically significant wound healing percentage from day 5 towards day 13, with T1 at day 5 being the exception. After the thirteenth day, it was no statistically significant difference between all treatment groups compared to untreated/negative control. We hypothesized that binahong extract liposome gel might only helps burn wound healing in the early stages of wound healing. This result somehow in accordance with a review article done by (Ikeda et al., 2008), that ursolic acid, a constituent in binahong leaf extract, are sometimes pro-inflammatory, thus prolong the inflammatory phase.

Table 6. Average burn wound healing percentage in different experimental animal groups treated with Binahong leaf extract liposome gel over 21 days

	Average wound healing percentage (%)					
Group	**Day 1**	**Day 5**	**Day 9**	**Day 13**	**Day 17**	**Day 21**
Negative control	5.71±2.48	25.82±9.98	45.99±12.37	72.17±6.35	96.91±2.38	99.64±0.40
Positive control	4.73±1.76	28.60±5.48	66.10±15.03	89.68±14.22	99.03±1.67	100
Gel Base Control	9.14±4.98	35.94±8.85	48.07±5.08	83.45±2.33*	98.76±0.85	100
T1 (10%)	8.96±7.97	44.14±15.75	72.59±16,02*	90.27±6.24*	99.93±0.12	100
T2 (30%)	14.82±4.38*	51.30±14.72	75.90±24.36	80.82±19.48	96.11±4.03	99.84±0.27
T3 (50%)	12.17±6.40	49.97±10.63*	76.65±13.88*	97.75±3.54*	99.97±0.04	100

(*) indicates statistically significant ($p<0,05$) compared to negative control treatment group

Following the completion of the experiment on day 21, a histopathological analysis was performed on the skin of rats to investigate the effect of binahong extract liposome gel. Results shows that the dermis layer in the T1 group and the positive control had the highest thickness. However, the dermis layer in the T2 and T3 treatment groups had the lowest thickness (Figure 4). The observed outcome can be attributed to the higher concentration of ursolic acid present in the binahong extract used in the treatment of both groups. By modulating the Smad2/3 pathway, ursolic acid suppresses excessive growth, migration, and collagen deposition of human dermal fibroblasts triggered by TGF-β (Zhou et al., 2023). Furthermore, the solubility and bioavaibility of ursolic acid in this research might be modulated by the incorporation of the liposome used, thus modulating the effect. Collagen, being the main component of the skin, has a significant role in dermal volume (Bravo et al., 2023). Therefore, if collagen is deposition was suppressed, the thickness of the dermis layer will also fall. Our research suggests that binahong leaves have the potential to improve the process of burn wound healing but if the concentration of the extract used is excessive, it may hinder the production of collagen. However, this finding still needs further investigation.

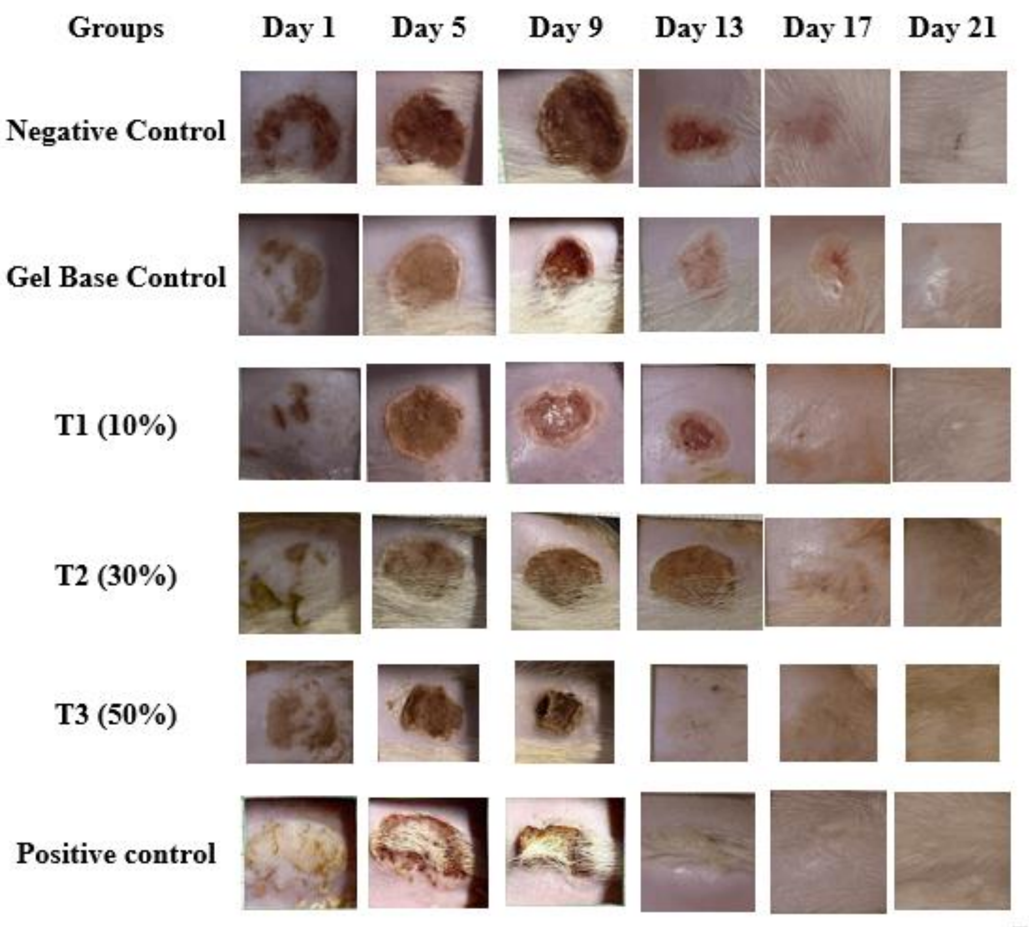

Figure 3. ***In Vivo* burn wound healing progress in experimental animal groups treated with Binahong leaf extract liposome gel on days 1, 5, 9, 13, 17, and 21**

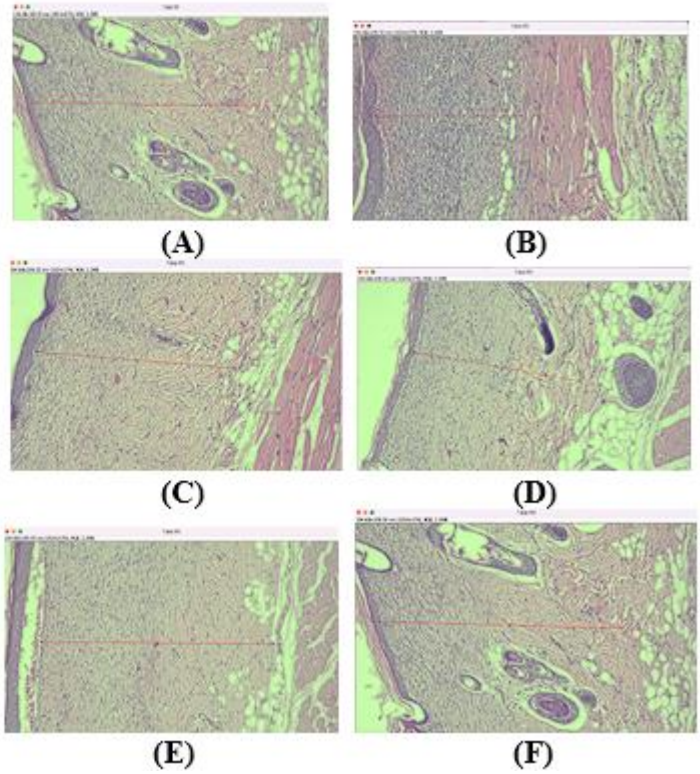

Figure 4. H&E staining histopathological results of burn wounds on day 21 showing dermis thickness; gel base control (Figure 4A) 110 µm; negative control (Figure 4B) 117 µm; T1 (10%) (Figure 4C) 135 µm; T2 (30%) (Figure 4D) 85 µm; T3 (50%) (Figure 4E) 98 µm; positive control (Figure 4F) 148 µm; measurements represent dermis layer thickness indicated by red lines

CONCLUSION

The variations in Na-CMC and glycerine amount influence the physical properties of binahong extract liposome gel, including pH, viscosity, and spreadability.The optimal formulation for the binahong leaf extract liposome gel is achieved with Na-CMC ranging from 2.78 to 4 grams and glycerine from 5 to 10 grams. The resulting gel had a pH of 5, a viscosity of 24.2 ± 0.4472 dPas, and a spreadability of 6.53 ± 0.0326 cm. However, prolonged administration of binahong leaf extract concentrations higher than 30% may impede the burn wound healing process, though further research is required.

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