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Optimizing Gel Formulations Using Carbopol 940 and Sodium Alginate Containing *Andrographis paniculata* Extract for Burn-Wound Healing

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Abstract

Background: *Sambiloto leaves (Andrographis paniculata (Burm.f.) Nees) contain andrographolide (diterpene lactone), flavonoids, quinic acid, steroids, saponins, alkaloids, and tannins, which act as anti-inflammatory, antioxidant, antibacterial, and wound healing.* ***Objective:*** *This study optimizes the gelling ingredient in Sambiloto extract gel preparations (Andrographis paniculata (Burm.f.) Ness) as a wound healer in male Wistar rats. The gelling agent is an important component that can affect active substance release.* ***Methods:*** *Formula optimization was developed using the Regular Two-Level 2^2 Factorial Design method in Design-Expert 12 software. This study used 0.5%-1% carbopol 940 and 1%-5% sodium alginate. Carbopol 940 and Sodium Alginate have different characteristics, so they need to be optimized to produce a gel with good characteristics.* ***Results:*** *Physical property evaluation using factorial design revealed the optimal formula at 0.5% carbopol and 5% sodium alginate, with average pH, viscosity, and adhesion values of 5.17 ± 0.04; 2934.452±286.871 cPs; and 194.236±3.684 s. Centrifugation and cycling tests indicated no organoleptic changes, phase separation, or significant changes in pH. ANOVA analysis showed that the gel with 10% Sambiloto leaf extract had similar burn healing activity to the positive control, with a recovery rate of 99.72 ± 0.47% in 20 days. Scabs formed on the 8th day and peeled off on the 12th day.* ***Conclusion:*** *Sambiloto extract gel in the optimum formula has the potential to be developed as a burn wound-healing drug.*

Keywords: *Andrographis paniculata (Burm.f.) Nees, burn wound-healing, gel, optimization*

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INTRODUCTION

Skin is the human body's main protector from foreign objects considered dangerous. One of the triggers for diseases that can appear on the skin is the presence of open wounds. An open wound is damage to a unit or tissue component where specifically there is a damaged or missing tissue substance (Wijaya & Putri, 2013). Open wounds can be caused by burns by exposure to heat sources such as air, fire, chemicals, electricity, and radiation. Burns damage the skin and affect all body systems (Jeschke *et al*., 2020; Tiwari, 2012).

The depth of tissue damage due to burns depends on the degree of heat from the source of the burn, the cause of the burn and the duration of contact with the body (Noer, 2006). According to the American Burn Association, second-degree burns (partial thickness burns) are burns whose depth of tissue can reach the dermis, usually found to be painful, pale when pressed, and marked by bullae filled with exudate fluid that come out of the blood vessels due to increased permeability of the walls (Shetty et al., 2021).

Sambiloto (*Andrographis paniculata* (Burm.f.) Nees) is one of the Indonesian plants that have the potential to heal burns due to the content of secondary metabolites such as andrographolide (diterpene lactone), flavonoids, quinic acid, steroids, saponins, alkaloids and tannins (Mussard *et al*., 2020). Sambiloto has many pharmacological activities, such as antibacterial, antioxidant, antiviral, antifungal, and anti-inflammatory properties, and it enhances the immune system (Rajanna *et al*., 2021). Antibacterial, anti-inflammatory and antioxidant activities are closely related to the healing process of burns because they can accelerate epithelization and repair and strengthen skin cells. Based on research by Al-Bayaty *et al*. (2011), histologically, burn wounds treated with 10% bitter extract showed good healing. Research from Selvaraj *et al*. (2022) proves that bitter extract has an anti-inflammatory valuable mechanism in wound healing. The recent research from Ariawan *et al*. (2023) demonstrated that Andrographis paniculata extract affected wound healing, such as the rate of re-epithelialization, collagen density, angiogenesis, and wound length.

Burns can be treated with topical, oral, and other treatments. However, topical treatment is more comfortable for burn sufferers. A gel preparation is one of the preparations that are suitable for treating burns. Gel preparations have a cooling effect because they contain a lot of water, so substances penetrate tissues better and accelerate wound healing (Rinaldi *et al*., 2019). An essential component in the manufacture of gel is the gelling agent.

Carbopol is a gel base that has a clear appearance, good spreading power on the skin, and a cooling effect; it does not clog skin pores and is easily washed off with water (Niyaz *et al*., 2011). The concentration of carbopol 940 as a gelling agent ranges from 0.5 – 2.0% (Rowe *et al*., 2009). Sodium alginate is produced from brown algae with a mucilage content of up to 40%. Gel containing sodium alginate shows excellent distribution; besides that, sodium alginate has slippery properties, is not sticky, does not feel when used and shows emollient properties (moisturizes the skin) (Agoes, 2012). The concentration of sodium alginate as a gelling agent ranges from 1 – 5% (Rowe *et al*., 2009). Singh *et al*. (2013) made diclofenac gel using gelling agents such as Carbopol 940, HPMC, gelatin, sodium alginate and Na CMC. The results of this study prove that the use of sodium alginate can increase the spreadability of the gel compared to other gelling agents, while the use of carbopol 940 can increase the release of the active substance compared to other gelling agents. Liu *et al*. (2008) also proved that the use of carbopol 940 was able to increase drug penetration and preparation stability. The combination of Carbopol 940 and sodium alginate in this study is expected to improve the characteristics of the gel.

Based on the description above, it is necessary to conduct research in the form of "Optimization of Gelling Agent in Sambiloto Extract Gel (Andrographis paniculata (Burm.f.) Ness) as Wound Healer in Wistar Strain Male Rats". The ethanol extract of Sambiloto leaves (Andrographis paniculata (Burm.f.) Ness) was prepared using the maceration method with a 96% ethanol solvent. Formula optimization was developed using the Regular Two-Level Factorial Design method in Design-Expert 12 software. This study used two factors, namely factor A as carbopol-940 and factor B as sodium alginate. Sambiloto extract gel (Andrographis paniculata (Burm.f.) Ness) was evaluated to obtain the optimum formula and then tested for burn healing activity for 20 days.

MATERIALS AND METHODS

Materials

The materials used in this study were Sambiloto leaves (*Andrographis paniculate* (Burm.f.) Ness from Lampung, Indonesia), Filter Paper (Whatman®, Indonesia), Distilled Water (Bratachem®, Indonesia), 96% ethanol (Bratachem®, Indonesia), Sodium Alginate

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(Techno Phramchem®, Indonesia), Carbopol 940 (Bratachem®, Indonesia), Propylene glycol (Bratachem®, Indonesia), Methyl Paraben (Bratachem®, Indonesia), Propyl Paraben (Bratachem®, Indonesia), Triethanolamine (Bratachem®, Indonesia), Alcohol 70% (Bratachem®, Indonesia), Lidocaine 2%®, Veet ®, Binagel®.

Tools

The tools used in this study included analytical balances 0.001 g and 0.0001 g (Ohaus®), oven (IMU55L®), pH meter (Lutron® pH Electrode PE-03), rotary evaporator (Biobase®), blender (Philips®), UV-Vis (Biobase®), magnetic stirrer (IKA C-MAG HS4®), spin bar, viscometer (Grace Instrument M3400®), micropipette (Dragon Lab®), glassware (Pyrex®), iron plate, stopwatch, mouse cage, drinking bottle, refrigerator (LG®).

Method

Preparation of sambiloto extract

The sambiloto plant used was identified at the Laboratorium Karakterisasi Kebun Raya Eka Karya Bali, BRIN with ID Number 53688. The simplicia powder of Sambiloto leaves was macerated using 96% ethanol with a ratio of 1:10 for 72 hours while stirring once every 6 hours in a dark place. The resulting filtrate was concentrated in a rotary evaporator at 50°C until an ethanol extract of Sambiloto leaves was obtained. The resulting extract was stored in a refrigerator at 4°C (Fardiyah *et al*., 2020). The percent yield of the extract is calculated using the equation:

$$\% \text{ Extract yield} = \frac{\text{Extract weigth}}{\text{Simplicia weight}} \times 100$$

Phytochemical screening

The phytochemical screening method is qualitative by looking at the occurring colour reactions. The test parameters that were carried out included the identification of flavonoids, saponins, tannins, alkaloids, steroids, and triterpenoids. Alkaloid testing was carried out using Dragendorf and Wagner reagents. If the reaction is positive for alkaloids, a red precipitate is formed using the Dragendorf reagent, and a brown precipitate is formed using Wagner's reagent. Flavonoid testing was done by adding magnesium and hydrochloric acid to the extract. Yellow, red, or orange solutions indicate the presence of flavonoids. Saponin testing is done by observing the foam formed from an extract and water mixture. The formation of persistent foam for 10 minutes indicates the presence of saponin. Tannin testing is carried out with FeCl3 reagent. The blackish-green colour indicates the presence of tannin. Testing for steroids and triterpenoids was performed using glacial acetic acid and concentrated sulfuric acid reagents. A solution that is blue or green indicates the presence of steroids, while red or purple suggests the presence of triterpenoids (Depkes RI, 2017).

Formulation of gel containing sambiloto extract

The gel formula design was determined using Design-Expert series 12 software. Formula optimization was developed using the Regular Two-Level Factorial Design method in Design-Expert 12 software. The formulas used in this study were four formulas using two factors, namely carbopol-940 and sodium alginate, at two successive levels of 0.5-1% and 1-5% (Table 1).

Table 1. Formula of gel containing sambiloto extract

Material	Concentration (%)			
	F1	F2	F3	F4
Extract	10	10	10	10
Carbopol-940	**1**	**0.5**	**1**	**0.5**
Sodium Alginate	**1**	**1**	**5**	**5**
Triethanolamine	1	1	1	1
Propylene glycol	15	15	15	15
Methyl Paraben	0.18	0.18	0.18	0.18
Propyl Paraben	0.02	0.02	0.02	0.02
Distilled Water	Ad 100 mL	Ad 100 mL	Ad 100 mL	Ad 100 mL

The process begins with weighing the ingredients and continues with the preparation of the gel base. Sodium alginate developed with aquadest. Carbopol-940 was dispersed in distilled water and added with triethanolamine until a gel base was formed. The dispersed sodium alginate and carbopol mass are put into a mortar and ground until homogeneous. Methyl paraben and propyl paraben were dissolved in propylene glycol, added to the gel mass, and stirred until homogeneous. The ethanol extract of Sambiloto was added little by little to the gel mass, and 100 mL of distilled water was added and then stored in a tightly closed container (Mardiyanto *et al*., 2022).

Evaluation of gel containing sambiloto extract

Organoleptic

Organoleptic tests were carried out by directly observing the shape of the gel prepared, its color, and its smell (Titaley, 2014).

Homogeneity

Preparations were taken at three different sampling points and smeared on transparent glass. The test preparation is declared homogeneous if there are no coarse grains (Nikam, 2017).

pH

The pH test is carried out by turning on the pH meter and then dipping the pH meter electrode into the

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gel formula, which has been dissolved with distilled water. Topical preparations must match the skin's pH, namely 4.5-6.5 (Tranggono, 2007).

Viscosity

Viscosity was done using a viscometer. The speed is set at 60 rpm, and then the pointer on the tool is observed for the number on the viscosity scale. The gel has viscosity values ranging from 2000-4000 cps (Garg *et al*., 2002).

Spreadability

The gel preparation was placed between two glass plates with weights on glass plates with a mass of 125 g. The diameter formed after 1 minute was measured. The specification for the diameter of the spread is 5-7 cm (Garg *et al*., 2002).

Adhesion

The gel was placed between two glass objects and then pressed with a 1 Kg load for 5 minutes. The load is lifted from the object glass, and then the object glass is mounted on the test equipment with a load of 80 g. Good adhesion requires more than 4 seconds (Mukhlishah *et al*., 2016).

Determine the optimum formula

Optimum formula selection was made using Design Expert 12 software based on desirability values close to 1 with response parameters pH, viscosity, spreadability, and adhesion. The closer the desirability value is to 1, the more perfect the desired formula is in the program (Raissi & Farsani, 2009).

Stability test

The physical stability test of the preparations was carried out using the centrifugation method and the cycling test. The centrifugation test was carried out at 3800 rpm for 5 hours, while the cycling test was carried out at 4°C and 40°C for 24 hours for six cycles. Parameters observed were organoleptic changes in the preparation, pH, and phase separation in gel preparations (Apriani *et al*., 2018; Mardiyanto *et al*., 2022).

Burn wound-healing activity test

Testing on animals has received ethical approval from Ahmad Dahlan University, number 02210066. The test animals were male white rats of the Wistar strain, aged 2-3 months, weighing 180-250g, and acclimatized for one week. The test animals were anesthetized first using lidocaine at a dose of 0.4 mL/KgBW subcutaneously. The rat's back was shaved about 3 cm below the ear using Veet® and a razor, marked with a rectangular shape with a size of 3 x 2 cm. An iron plate with a diameter of 1 cm was previously heated in boiling water for 5 minutes to achieve sterile conditions and then induced on the rats' backs for 10 seconds (Akhoondinasab *et al*., 2014).

The gel was administered by applying it to the wound on the rat twice a day, i.e., in the morning and evening, for 20 days after the burn induction. Wound healing parameters were determined from the area of wound healing, wound healing time, and the time the scab fell off.

Table 2. Treatment group of burn wound-healing activity test

Group	Note
Negative Control	Gel base
Positive Control	*Binagel*
Sample	Optimum formula for sambiloto extract gel

Observations on the healing of burns were carried out from days 0, 4, 8, 12, 16, and 20. Measurements of the wound area were carried out at intervals of 4 days. Binagel® was chosen as the positive control because it is a marketed burn preparation containing plant extracts.

Data analysis

Data analysis on the evaluation of gel preparations was carried out by calculating the factorial design using the Design Expert 12 Program. Data analysis was carried out by calculating the coefficient values of each factor, namely carbopol-940 and sodium alginate, to obtain an equation for the relationship between factors and responses. Data analysis on burn wound healing used the SPSS® application with a data normality test performed with Shapiro-Wilk to see the normality of data distribution. It continued with One-Way ANOVA, and then a Post Hoc Duncan Test was conducted if the p-value <0.05.

RESULTS AND DISCUSSION

The sambiloto extract produced in this study is dark green, has a distinctive smell, and is thick, with a yield percentage of 20.20%. The results of the phytochemical screening showed that Sambiloto extract contains flavonoids, saponins, tannins, steroids, triterpenoids, and alkaloids (Table 3). The results of this study are in line with the research by Hita *et al*. (2022), where the 96% ethanol extract of Sambiloto leaves was proven positive for containing flavonoids, alkaloids, tannins, triterpenoids, and saponins.

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Table 4. Results of evaluation of gel containing sambiloto extract

Parameter	Formula			
	F1	F2	F3	F4
Organoleptic	Dark green, distinctive smell and thick	Dark green, distinctive smell and thick	Dark green, distinctive smell and thick	Dark green, distinctive smell and thick
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	5.25±0.03	5.56±0.01	5.38±0.03	5.17±0.04
Viscosity (cPs)	2391.66± 135.94	2079.66± 93.11	3732.33± 173.07	2822.66± 370.93
Spreadability (cm)	5.93±0.12	6.53±0.50	5.63±0.40	5.73±0.47
Adhesion (s)	90.67±2.08	39±1.00	195±5.00	162.67±2.52

Table 5. Results of model analysis

Parameter	Parameter				
	R^2	Adjusted R^2	Predicted R^2	Adequateprecision	p-value
pH	0.9651	0.9521	0.9216	19.6780	<0.0001
Viscosity	0.9226	0.8936	0.8259	12.9745	<0.0001
Spreadability	0.7287	0.6270	0.3896	6.4286	0.0018
Adhesion	0.9984	0.9977	0.9963	89.2441	<0.0001

Table 3. Result of phytochemical screening

Secondary Metabolites	Result
Flavonoid	+
Saponin	+
Tanin	+
Alkaloid	+
Steroid & Triterpenoid	+

Note : + indicates metabolites are present

Sambiloto extract is used as an active ingredient in making gel. In the manufacture of gels, the gelling agent is an essential factor in determining the results of the characterization of the resulting preparations. In this study, variations in the concentration of the gelling agent, namely carbopol-940 and sodium alginate, were carried out to produce four formulas. The gel preparations made in this study can be seen in Figure 1. The results of the evaluation of the preparations from the four formulas can be seen in Table 4.

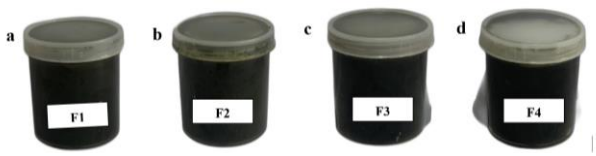

Figure 1. Gel containing sambiloto extract

Based on Table 4, it is known that the four formulas have met the evaluation requirements for gel preparations, namely pH ranging between 4.5-6.5, viscosity with a value range of 2000-4000 cPs, spreadability ranging from 5-7 cm and adhesion is in the range of 2-3000 seconds (Garg *et al*., 2002). Measured parameters such as pH, viscosity, spreadability, and adhesion were continued for the optimization process by factorial design analysis using the Design-Expert 12® program. Data analysis was carried out broadly divided into two, namely model analysis and response analysis.

The model analysis determines which parameters can be used to determine the optimum formula. A model can be good if the p-value is less than 0.05 with an R2 value greater than 0.7, the difference between adjusted R2 and predicted R2 is not more than 0.2, and the adequate precision value is more than 4 (Apriani *et al*., 2023). The model analysis results of measured parameters such as pH, viscosity, spreadability, and adhesion can be seen in Table 5.

The R2 value is used to see how much the data population influences the factors used. For example, the value of R2 at a pH of 0.9651 means that 96.51% of the data population is influenced by carbopol-940, sodium alginate, and the interaction between the two. The difference value of adjusted R2 and predicted R2 describes the difference between the system's predicted value and the (actual) measured value. The smaller than 0.2, the closer the similarity of the resulting values is. The adequate precision value indicates the robustness of the model, while the p-value indicates the significance of the effect. Based on Table 5, the pH, viscosity, and adhesion parameters show good model results. In contrast, the spreadability parameter shows poor model analysis results where the difference between adjusted

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R2 and predicted R2 is more than 0.2. Based on these results, the parameters that can be continued to determine the optimum formula are pH, viscosity, and adhesion.

The following analysis performed was response analysis. Response analysis was carried out to see the effect of the carbopol-940, sodium alginate, and the interaction of the two factors on the parameters of pH, viscosity, and adhesion. The response analysis results can be seen in Table 6 and Figure 2.

Table 6. Results of response analysis

Parameter		Intercept	A (Carbopol 940)	B (Sodium Alginate)	AB (Interaction)
pH	Coefficient	5.346	-0.024	-0.064	0.129
	p-value		0.0393*	0.0002*	<0.0001*
	% contribution		2.635	18.582	73.297
	Response Equation	y = 5.346 – 0.024 A – 0.064 B + 0.129 AB			
Viscosity	Coefficient	2756.580	305.417	520.917	149.417
	p-value		0.0014*	<0.0001*	0.0470*
	% contribution		22.241	64.699	5.323
	Response Equation	y = 2756.58 + 305.417 A + 520.917 B + 149.417 AB			
Adhesion	Coefficient	121.833	4.833	57.000	-27.000
	p-value		0.0006*	<0.0001*	<0.0001*
	% contribution		0.628	87.351	11.857
	Response Equation	121.833 + 4.833 A + 57.000 B – 27.000 AB			

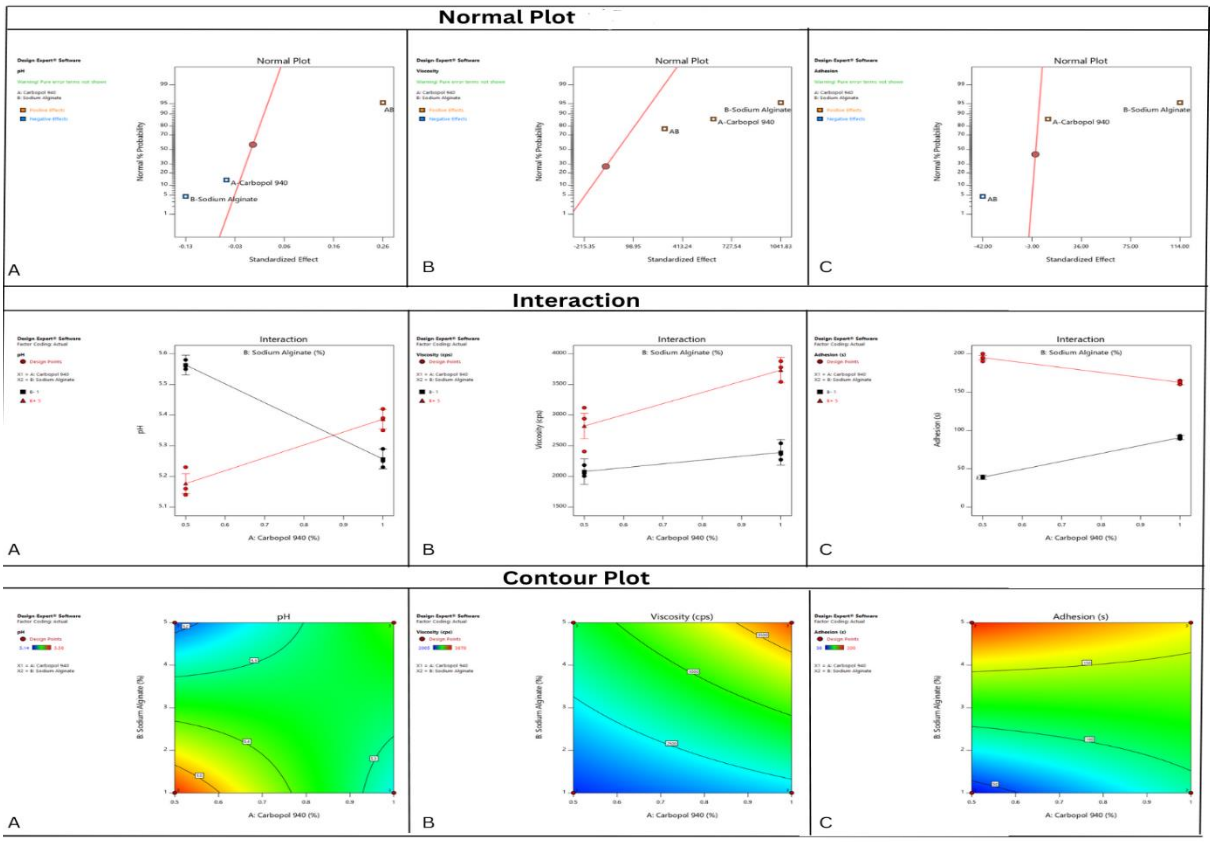

Figure 3. Graph of normal plot, interaction and contour plot of the parameters pH (A), Viscosity (B) and adhesion (C)

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Based on Table 6, the concentrations of carbopol-940 and sodium alginate and their interactions significantly affect pH, viscosity, and adhesion parameters with a p-value <0.05. These results can also be supported by Figure 3, which is related to the normal plot graph where the factor points are outside the straight line, which indicates that the factor has an influence, both positive and negative. This positive and negative influence can be observed from where the point is in the negative or positive area. Besides that, it can also be seen from the notation of the coefficients whether there is a negative sign or not. The concentration of each carbopol-940 and sodium alginate has a negative effect on the pH value, where the higher the concentration used, the lower the pH value of the resulting gel preparation, while the interaction of the two factors has a negative effect on adhesion. The relationship between the interactions of carbopol-940 and sodium alginate can also be illustrated by the interaction graph in Figure 3. The pH parameter has a line crossing on the interaction graph, indicating a strong interaction between carbopol-940 and sodium alginate. These results can also be supported by the percent contribution from AB to a large pH of 73.297%. There is no line crossing when observed from the interaction graph on the parameters of viscosity and adhesion. However, the angle of inclination between the two lines is different, so interaction can occur even though it is small and is also supported by the percentage contribution of AB to the viscosity and adhesion of 5.323% and 11.857%.

The interaction between carbopol-940 and sodium alginate strongly influences the pH. The higher the interaction of the two factors, the higher the pH of the preparation. The increased concentration of carbopol-940 will lead to the concentration use of triethanolamine as a base agent to help develop carbopol 940. In addition, sodium alginate has a pH range of 6-8. This statement is the reason why the pH value of the gel preparation will increase when carbopol-940 and sodium alginate are used in high concentrations. The viscosity factor strongly influences the viscosity factor, where the percent contribution reaches 64,699%. Sodium alginate has more effect on viscosity than carbopol-940 because the concentration of sodium alginate used is more remarkable than carbopol-940.

In addition, carbopol-940 has a ,significant ability to absorb water so that the amount of free water contained in the gel also increases, causing the texture of the gel to get softer (Nurman *et al*., 2019). The viscosity of sodium alginate is affected by temperature, solution level, and degree of polymerization. The main property of sodium alginate is its ability to form gels in the presence of divalent cations (Cardenas *et al*., 2003). If a lot of Ca2+ ions are released into the gel-making process, more cross-links that can be formed between alginate molecules are obtained from the increasing concentration of CaCO3 (Remaggi *et al*., 2022). Sodium alginate also influences the adhesion parameter, namely 87.351%. This result is related to the high viscosity of sodium alginate. The higher the viscosity, the higher the adhesive power of the preparation will be. Gel adhesion can affect the therapeutic effect. The longer the gel is attached to the skin, the more active substances will be absorbed by the skin so that the gel can provide a more prolonged therapeutic effect, and its use becomes more effective (Loyald *et al*., 2014).

After analyzing the response, then proceed with determining the optimum formula. The optimum formula was determined using the Design Expert tool version 12. The optimum formula suggested by the system was a formula with a carbopol-940 concentration of 0.5% and a sodium alginate concentration of 5% with a desirability value of 0.997%. The results of confirmation of the characteristics of the optimum formula can be seen in Table 7. The optimum formula shows values between 95% PI low and 95% PI high. The optimum formula was further tested: stability test and burn healing activity test. The results of the stability test can be seen in Table 8.

Table 7. The result of the optimum formula confirmation analysis

Parameter	95% PI Low	95% PI High	Result
pH	4.990	5.363	5.17±0.04
Viscosity (cPs)	1613.445	4031.895	2934.452±286.871
Adhesion (sec)	178.406	211.594	194.236±3.684

Table 8. The result of optimum formula stability

Parameter	Observation result	
	Before	After
Organoleptic	Dark green, extract smell and thick	Dark green, extract smell and thick
pH	5.17±0.04	4.94±0.02
Syneresis	None	None

Based on Table 8, the optimum gel preparation formula did not experience organoleptic changes or syneresis, but there was a decrease in pH. The optimum formula in this study did not experience syneresis due to

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the ability of the gelling agent combination between carbopol-940 and sodium alginate to maintain the bond strength of the gel matrix so that the solvent contained therein was not separated and came out onto the surface of the gel. The optimum formula experienced a decrease in pH of 0.23. The results of the paired-sample test analysis show that there is a significant difference where the significance value obtained is 0.02. A reduction in pH can occur due to the influence of speed and temperature, which causes the preparation to experience cation hydrolysis from TEA as a weak base to produce H+ ions (Lewis & Zhao, 2006).

Testing the burn healing activity of Sambiloto extract gel preparation in this study was carried out experimentally on Wistar male white rats with 3 test groups: the positive, negative, and optimum formula groups. The positive group was given Binagel® gel, the negative group was given a gel base, and the optimum formula group was given the optimum sambiloto extract gel preparation. Binagel® was chosen as a positive control because the preparation contains binahong extract derived from natural ingredients.

The primary goal of wound healing involves timely wound closure, prompt pain relief, and an aesthetically acceptable scar. The results of % Recovery can be seen in Table 9 and Figure 4.

Based on the % recovery data, which can be seen in Table 9 and Figure 4, it can be seen that there was a significant increase in the positive group and the optimum formula from day 0 to 20. This result is inversely proportional to the negative group, which has a slower % recovery. The positive group had a healing percentage of up to 100% on day 20 and was not significantly different from the optimum formula based on post-hoc Duncan analysis. The percentage of wound healing on the fourth day in the positive, negative, and optimum formula groups was low. This result happened because, on day four, the wound healing process was still at the inflammatory stage. The inflammatory stage can be seen from the observation that there are signs of redness, inflammation occurs, it feels hot and painful, and it can even cause swelling in the wound. The inflammatory process allows white blood cells and platelets to limit more severe damage and accelerate wound healing (Gonzalez *et al*., 2016). Furthermore, a scab will form after the inflammatory process occurs. Observations on the formation and release of scabs can be seen in Table 10.

Table 9. Result of % recovery

Group	Average %Recovery of Each Group ± SD					
	Day					
	0	4	8	12	16	20
Negative	0.00±0.00[a]	-13.32±6.97[b]	11.53±5.42[a]	14.21±6.82[b]	33.33±9.35[b]	52.49±3.86[b]
Positive	0.00±0.00[a]	10.10±5.86[a]	6.91±2.55[a]	50.92±14.52[a]	97.37±1.21[a]	100.00±0[a]
Optimum Formula	0.00±0.00[a]	7.53±3.12[a]	7.71±2.69[a]	35.38±11.34[ab]	92.08±5.99[a]	99.72±0.48[a]

Note: Values followed by lowercase letters (a/b) in the row indicate significant differences between groups in Duncan's post hoc test

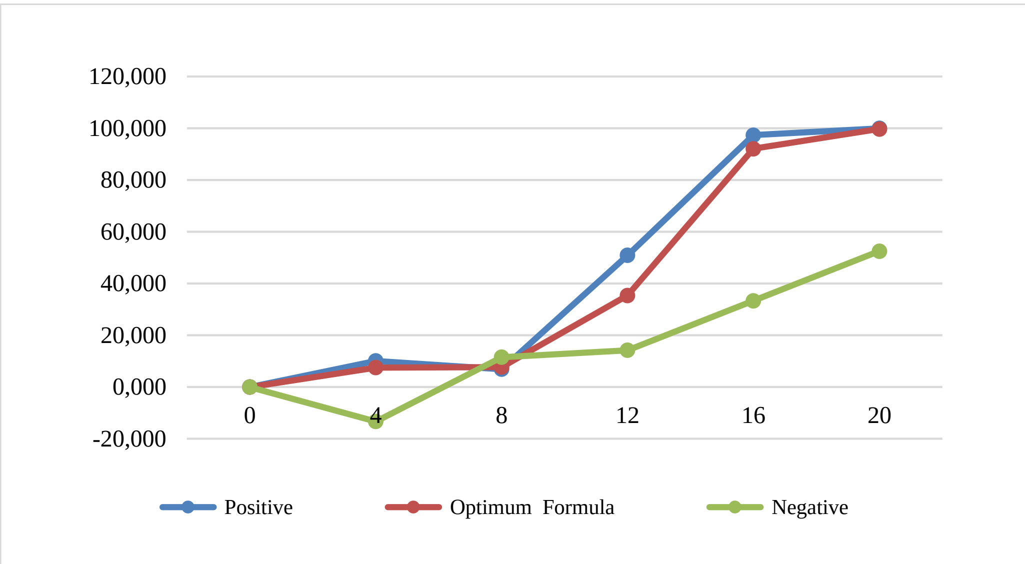

Figure 4. Graph of the percentage of burn healing for each group

Table 10. Scab observation results

Group	Average Day	
	Scab Formation	Scab Removal
Negative	12	16
Positive	8	12
Optimum Formula	8	12

Based on the above observations, it can be seen that the positive group and the optimum formula showed faster scab formation on the 8th day and faster scab removal on the 12th day and were significantly different when compared to the negative group. The formation of a scab indicates the initial proliferation process in wound healing. The proliferative stage is marked by the formation of granulations in the wound, namely fibroblasts and inflammatory cells. This phase occurs from day 4 to day 14. The scab that forms on the wound's surface can help hemostasis and prevent wound contamination due to microorganisms. Scabs are formed due to the denaturation of the skin layer proteins in the coagulation zone (Markiewicz-Gospodarek *et al*., 2022).

On the 20th day, the percentage of wound healing in the positive group and the optimum formula reached 100 ± 0.00% and 99.72 ± 0.48%. This result can happen because the wound has entered the remodelling phase. The remodelling phase occurs after the wound has been filled with granulation tissue and the re-epithelialization process is over. This phase aims to maximize the strength and structural integrity of new tissue, filling the wound, epithelial growth, and scar tissue formation (Velnar *et al*., 2009). The negative group on the 20th day had not entered the remodelling stage because the percentage of wound healing was still minor, namely 52.49 ± 3.86%.

The optimum formula of Sambiloto extract gel is proven to have wound-healing activity because it contains flavonoids, saponins, tannins, alkaloids, steroids, and triterpenoids, which help accelerate wound healing. Phytochemical compounds such as flavonoids have various biological activities such as antibacterial, anti-inflammatory, and antioxidant effects and are believed to benefit wound healing (Acar et al., 2002). Tannin compounds support wound healing with their astringent and antimicrobial properties. Saponins function as cleansers and antiseptics to prevent the growth of microorganisms in wounds so that the wound does not experience severe infection and stimulates the formation of collagen I, a protein that plays a role in wound healing (Dong, 2020). Triterpenoids can strengthen skin cells and improve repair, stimulate blood cells and the immune system, and can be natural antibiotics (Sutardi, 2016).

Sambiloto extract has been widely studied to contain the main component, andrographolide, a triterpenoid group (Valdiani *et al*., 2012). In addition, Sambiloto extract also includes the most dominant flavonoid, quercetin (Fardiyah *et al*., 2020). Antioxidants from quercetin compounds can trigger collagen production and increase Vascular Endothelial Growth Factor (VEGF), which is anti-inflammatory and antibacterial. Based on research by Yang *et al*. (2020), quercetin compounds have an anti-inflammatory effect by inhibiting the cyclooxygenase (COX) enzyme, which induces the formation of prostaglandins as inflammatory mediators, while antibacterial activity inhibits bacterial growth through inhibition of bacterial hydrolytic enzymes. Andrographolide compounds have activity as anti-inflammatory and antioxidant (Lin *et al*., 2009). Andrographolide works as an anti-inflammatory agent by suppressing the production of the protein inducible nitric oxide synthase in RAW 264.7 cells (iNOS) (Chiou *et al*., 2000). The unsaturated α,β-lactone structure (α,β-unsaturated lactone) part of the andrographolide molecular structure can neutralize the superoxide anion so that andrographolide acts as an antioxidant (Yan *et al*., 2018).

CONCLUSION

Carbopol-940 and sodium alginate concentrations influence the evaluation of gel preparations, namely pH, viscosity, and adhesion. The higher the carbopol-940 and sodium alginate concentration will increase the viscosity and adhesiveness values, but lower the pH value. The optimum formula based on the optimization of the factorial design analysis on the evaluation response of the preparation was obtained at a concentration of 0.5% carbopol-940 and 5% sodium alginate with the average characteristics of pH, viscosity, and adhesion, respectively 5.17 ± 0.04; 2934.452±286.871 cPs; and 194.236±3.684 s. The optimum formula shows values between 95% PI low and 95% PI high. The optimum gel preparation of Sambiloto extract has fairly good physical stability. The optimum gel preparation of Sambiloto extract was also shown to have a good effect on healing burns with a %recovery of 99.72%, on the 20th day, and based on the results of ANOVA, there was no significant difference to the positive control.

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The authors declared no conflict of interest.

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