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Optimization of HPMC and Carbopol 940 as the Gelling Agents for the Physical Properties and Stability of Anti-Acne Gel from *Binahong* Leaf (*Anredera cordifolia* (Ten.) Steenis) Extract

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ABSTRACT

Binahong (*Anredera cordifolia* (*Ten.*) Steenis) is recognized as a widespread plant species in Indonesia and known to possess strong antibacterial properties, particularly against *Cutibacterium acnes*. Given the promising antibacterial activity of *Binahong,* there is growing interest in developing pharmaceutical formulations utilizing its therapeutic potential. This study aims to determine the effect and optimum proportion of hydroxypropyl methylcellulose (HPMC) and Carbopol 940 as the gelling agents for the physical properties of *Binahong* anti-acne gel and to confirm its topical safety on rabbits' skin. The simplex lattice design (SLD) method to optimize the gel base was performed on the Design-Expert® software version 13.0.0. The independent variables in SLD were the amounts of HPMC and Carbopol 940, while the responses included the pH, viscosity, spreadability, and adhesion. The optimal formula proposed by SLD was a combination of 1.96% HPMC and 0.53% Carbopol 940 that showed no significant difference when compared to the test results. The optimal formula had 5.7 pH, which is within the range of suitable pH to maintain the natural barrier function of the skin, 16,463 cP of viscosity, 4.63 cm of spreadability, and 12 seconds of adhesion, all of which are in accordance with SNI 16-4399-1996. The optimum formulation proved to be physically stable after three freeze-thaw cycles and showed no irritation upon application.

Keywords: *Binahong* leaves, gel, optimization, HPMC, carbopol 940

INTRODUCTION

Acne vulgaris is a common skin inflammatory disorder affecting the pilosebaceous glands and clogged pores (Sutaria et al., 2023). This condition can be exacerbated by multiplying bacterial activity, such as from *Cutibacterium acnes* (*C. acnes*). This organism releases a large number of polypeptides, such as extracellular enzymes that include protease, hyaluronidase, neuraminidase, and other enzymatic components that penetrate the sebum and lead to the formation of microcomedones and acnes (Platsidaki & Dessinioti, 2019). This condition is more prevalent in regions with tropical climates characterized by high temperatures and humidity, such as Indonesia. Although acne is not fatal, it can last for years and cause severe psychosocial effects, such as low self-esteem, depression, anxiety, and social withdrawal (Hazarika & Archana, 2016). Herbal plants and traditional remedies have been widely used to treat acne, especially in Indonesia that has rich biodiversity in plants with beneficial active substances, one of which is *Binahong* or heartleaf (*Anredera cordifolia* (Ten.) Steenis). *Binahong* is one of the widely grown plants in Indonesia that contains several phytochemical components, such as saponins, flavonoids, alkaloids, polyphenols, and aurons. Research findings have revealed many biological activities of *Binahong*, such as analgesic, antibacterial, wound healing, anti-inflammatory, and antioxidant properties (Alba et al., 2020).

In this study, the highlighted function of *Binahong* is its property as an antiacne. The ethanol extract of *Binahong* leaves is known to exhibit antibacterial properties against *C. acnes* with a minimum inhibitory concentration of 1.86% for gel formulation (Dewi, 2023). Another study shows that 5% *Binahong* leaf ethanol extract has a minimum inhibitory zone (MIC) of 14.66±0,57 mm and minimum bactericidal zone (MBC) of 11.66±0.57 mm against *P. acnes* (Sasebohe et al., 2023). It is also reported that the emulgel of *Binahong* extract is more effective than 1.2% clindamycin phosphate as a positive control. The minimum bactericidal concentration (MBC) of the leaf ethanol extract in a scratch test is 500 ppm (Yani et al., 2016). The topical use of *Binahong* ethanol extract is less comfortable due to its thick consistency and poor water solubility (Dwiastuti & Ardiyati, 2020). Therefore, it needs to be formulated with the appropriate choice of dosage form and formulation. These factors will influence the amount and rate of active substance to be ideally absorbed. Studies show that ointments and gels made from *Binahong* extract demonstrate antibacterial properties and are efficient for wound healing (Alba et al., 2020).

Gels have more water content which enable them to provide better drug delivery than ointments and protect the skin from excessive dehydration while providing moisture. Compared to other topical dosage forms, gel preparations have more advantages because of their ease of application and equal distribution. They also deliver a cooling sensation and leave no scars on the skin. They are non-toxic and non-irritating, making them excellent for repeated use (Safitri et al., 2020). The physical properties, structure, and stability of a gel are determined by the gelling agent(s) used, including HPMC and Carbopol 940 used in this study. Carbopol is a synthetic acrylic polymer with high molecular weight and acts as a gelling agent in the concentration range of 0.5–2% (Rowe et al., 2020). It has an extensive viscosity range, which spans from 40,000 to 60,000 cP. Therefore, a low concentration of Carbopol is adequate to form a viscous gel. Meanwhile, higher concentrations of Carbopol 940 result in more acidic gels due to their acidic characteristics (Safitri et al., 2020). Hydroxypropyl methylcellulose (HPMC) is a semisynthetic cellulose derivative that serves as a suspending agent. When compared with other gelling agents, HPMC produces clearer and neutral gel with a cooling effect and stable viscosity without clogging the pores (Noval et al., 2019; Rowe et al., 2020). Furthermore, HPMC is an excellent hydrogel-producing material as it expands well in water. This compound also shows a strong antimicrobial resistance and provides good release of active substance (Jaber et al., 2023). HPMC undergoes a thermo-reversible transformation upon heating and cooling. Its solubility in water increases at low temperatures and decreases at high temperatures. At low temperatures, water molecules dissolve HPMC molecules in water to produce HPMC solutions. On the other hand, increasing water temperatures lead to the formation of HPMC networks (Yang et al., 2022). A combination of HPMC and CMC-Na shows good stability and physical characteristics, but it foams up when added to *Binahong* extract (Rahmani & Zulkarnain, 2023; Dewi, 2023). Considering the physicochemical properties of the extract, HPMC and Carbopol 940 need to be combined to compensate for the shortcomings of each of these materials and create a gel with optimal physical properties and stability. Further research shows that a concentration of HPMC higher than that of Carbopol 940 results in a more desirable and firm gel, and vice versa (Firmansyah et al., 2022).

Different ratios of gelling agents can affect the physical properties and stability of gel preparations. Therefore, it is essential to optimize these components to obtain the ideal composition range for a good and stable gel. One of the approaches to predict the ideal formulations is to conduct trial and error. However, this method is both labor-intensive and costly, requiring substantial efforts to establish and supervise. To address this issue, it is imperative to ascertain the most desirable compositions which will enable the development of pharmaceuticals in a more effective manner (Afrin & Gupta, 2023). In light of this, computer programs are now more desirable to be a guide in creating and improving formulations more rapidly (Jameel et al, 2019). Therefore, to obtain the optimal composition of the gelling agents, the simplex lattice design (SLD) method is used. This approach forms an equation that expresses the relationship between the variation in the composition of the gelling agents and the physical properties of the gel using multiple samples. The SLD method equation reveals the effect of the concentration of each component on the measured reaction of physical characteristics. It also helps find the ideal physical properties by considering the combination of the gelling agent concentrations (Bolton & Bon, 2010; Rahmani & Zulkarnain, 2023).

This study aims to optimize the ratio of HPMC and Carbopol 940 to make a physically stable gel from *Binahong* leaf extract, one of the many Indonesian herbal plants that has good anti-acne properties. Ernawati (2018) reports that the *Binahong* gel can be safely administered topically to a rabbit's skin in previous in-vivo research. However, there is still a lack of evidence of the irritation reaction upon the application of *Binahong* gel onto the surface of the skin. This study provides a new perspective on the optimal gel formulation of *Binahong* leaf extract using HPMC and Carbopol 940 as the gelling agents. This study also aims to confirm the safety of *Binahong* gel as a potential anti-acne skincare product.

MATERIALS AND METHODS

The instruments used in this study were glassware (Iwaki, Pyrex), analytical balance, drop pipette, spatula, glass stirring rod, oven, evaporating dish, Büchner funnel, filter paper, gel pot, hotplate, overhead stirrer (IKA RW 20), pH meter (HANNA), viscometer (Lamy Rheology B-ONE PLUS), spreadability test kit, adhesion test kit, stopwatch (Alba), climatic chamber, and refrigerator (LG). The ingredients used in this study consisted of fresh *Binahong* leaves (Kelompok Tani Surya Hijau), 70% ethanol (pharmaceutical grade), HPMC (pharmaceutical grade), Carbopol 940 (pharmaceutical grade), propylene glycol (pharmaceutical grade), triethanolamine (TEA) (pharmaceutical grade), nipagin (pharmaceutical grade), nipasol (pharmaceutical grade), and aquadest (pharmaceutical grade).

Identification of *Binahong* leaves

Five kilograms of fresh *Binahong* leaves were obtained from Kelompok Tani Surya Hijau plantation in Suryodiningratan, Mantrijeron, Yogyakarta. The identification of the leaves was carried out to match the morphological characteristics by referring to the 2017 Indonesian Herbal Pharmacopoeia or *Farmakope Herbal Indonesia (2017)*.

Preparation of *Binahong* leaf ethanol extract

The extract was prepared by weighing 2.5 kg of *Binahong* leaves and washing them to remove dirt and impurities. Subsequently, the leaves were oven-dried for 24 hours at 50°C. The dried leaves were then mashed and stored in a plastic container filled with a silica gel. For each maceration process, 500 g of the leaves was macerated using 5 L of 70% ethanol (1:10 ratio) in a glass container, which was then covered with an aluminum foil. The container was placed in a closed cabinet, left for three days with occasional stirring, and then filtered with filter paper to produce a filtrate and residue. The residue was then remacerated twice every two days with 2.5 L of 70% ethanol while being stirred. The macerate was filtered by using a Büchner funnel, and the filtrate was concentrated in a rotary evaporator at a temperature of 50^0C. The rotary evaporator uses a vacuum to lower the boiling point of ethanol, thus resulting in an extract that is completely free of ethanol. The extraction process was repeated for another batch.

Characterization of *Binahong* leaf ethanol extract

The ethanol extract of *Binahong* leaves was then characterized through several tests, including an organoleptic test (odor, color and texture), determination of the yield weight, and pH test.

Binahong extract gel formulation

A 2^3 factorial design was used to create eight formulas of the gel. The formulation process began by dispersing the gelling agents. Carbopol 940 was dispersed slowly with aquadest and heated to 70°C while being allowed to expand. Then, TEA was added to neutralize the pH and to form a clear gel mass. In another beaker glass, HPMC was dispersed slowly with propylene glycol over hot distilled water at 70-80°C while being homogenized until a gel mass was formed. Both of the gel masses that had been formed were then mixed in a beaker glass, added with nipagin as well as nipasol, and stirred until homogenous. Lastly, the *Binahong* extract was added along with the remaining aquadest until the mixture reached 50 mL, and then it was gradually homogenized into a gel without heating. The gel was then left for 24 hours (Table I).

Evaluation of the physical properties of the gel

Organoleptic test

The organoleptic test was carried out visually by observing the changes in the color, smell, odor, and homogeneity of the gel.

pH test

The pH value was measured by using a pH universal indicator for all the formulas made. By smearing the gel onto a pH paper and waiting until it changed color, a pH value was obtained and recorded.

Viscosity test

The viscosity of the sample was measured in a Lamy Viscometer B-One Plus with RV 7 system at a weight of 50 g and a speed of 100 rpm for 60 seconds.

Table I. Gel Formulations of *Binahong* Leaf Extract

Ingredients	Function	Formula (%)							
		F1	F2	F3	F4	F5	F6	F7	F8
Binahong extract	API	1.86	1.86	1.86	1.86	1.86	1.86	1.86	1.86
HPMC	Gelling agent	2	1.75	1.625	1.5	1.875	1.75	2	1.5
Carbopol 940	Gelling agent	0.5	0.75	0.875	1	0.625	0.75	0.5	1
Propylene glycol	Humectant	5	5	5	5	5	5	5	5
TEA	Emulsifier	1	1	1	1	1	1	1	1
Nipagin	Preservative	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Nipasol	Preservative	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009
Aquadest	Solvent	ad 50	ad 50	ad 50	ad 50	ad 50	ad 50	ad 50	ad 50

Spreadability test

A total of 0.5 g of the gel was weighed and placed in the middle of a round glass scale. Another round glass was placed on top of the gel and left for 1 minute, and the spread was recorded. Then, a 50-gram weight was placed on top of the round glass and left for 1 minute. The spread was measured on the four sides by using a ruler. Another 50-gram weight was added to the load until the spread was constant. The spreadability was determined as the width of the area covered by the sample. The test was replicated three times, and the average spreadability was calculated (Marchaban et al., 2017).

Adhesion test

The gel was placed on an object glass, and another object glass was placed on it and then pressed with a 1-kg load for 5 minutes. The object glasses attached to each other were then affixed to an adhesion test kit. An 80-gram weight was released, and the time when the load was released was recorded. The time measurement stopped until the two object glasses detached. The test was replicated three times, and the average adhesion time was calculated (Marchaban et al., 2017).

Stability test

The stability test of the gel preparation was carried out by using the freeze-thaw cycle method. The gel was placed in a tightly closed glass container and then stored at three different temperatures. First, it was put in a refrigerator for 24 hours at 4°C, then placed at a room temperature (28^{o}C) for 24 hours, and subsequently transferred into an oven for 24 hours at 40°C (1 cycle). This process was repeated for 3 cycles or 9 days, with each cycle being observed for the presence of phase separation in the gel (Agustin & Taihattu, 2020). The instability that may occur in a gel is characterized by changes in the color, odor, and texture, or by phase separation.

Irritation test

The irritation test was approved by the Ethics Committee of the Faculty of Medicine of Universitas Gadjah Mada (No. KE-FK-1892-EC-2023). The Draize method was used on three physically healthy adult albino rabbits with no diseases or allergies. The test was done in four treatments with an equal area on the rabbits' back skin: one spot with no treatment (control), one spot with 500 mg of optimum gelling agents (HPMC and Carbopol 940), and two spots with 500 mg of *Binahong* gel extract. Approximately 24 hours before the test, the rabbits' fur was removed from the dorsal area at various locations on the body. Each of the controls was applied evenly on the rabbits' skin and bandaged with sterile gauze. The skin was observed for reactions after the first hour and observed again after being left open for approximately 4, 24, 48, and 72 hours. A positive reaction was defined as erythema and edema reactions which were assessed using a skin reaction scoring method (Draize, 1959)

Data analysis

The optimization of the *Binahong* leaf gel formula was carried out on the Design-Expert® software version 13.0.0 with the simplex lattice design (SLD) method. The formula optimization was carried out by including the independent variables HPMC and Carbopol 940 in the range of 2-2.5% and 0.5-1%, respectively. The data included as the response variables consisted of the pH, viscosity, adhesiveness, and spreadability. The optimal formula obtained was in the form of a comparison of certain concentrations of both gelling agents, which could produce optimum physical properties of the *Binahong* leaf gel formula from the software prediction. Then, the optimal formula prediction was validated by testing the SLD prediction formula in the laboratory.

The results were then analyzed in the SPSS one-sample t-test with a 95% confidence level. The calculation was observed to examine whether there were significant differences in the response of the prediction and the experiment, hence to conclude the reliability of the equation.

RESULTS AND DISCUSSION

Characterization of the extract

In a maceration process, a filter fluid that is suitable for compounds with the desired activity is required. This follows the concept of "like dissolves like", in which a compound will dissolve in a solvent with the same polarity (Kemit et al., 2016). Flavonoids are polar because they bind to phenolic groups; therefore, they can be extracted by using 70% ethanol, which is a polar solvent. The resulting extract in this study was dark green in color, had a distinctive *Binahong* smell, and had a pH of 5. The yield of the ethanol extract of *Binahong* leaves was calculated based on a comparison between the weight of the final product and the weight of the simplicia powder and then expressed in percents. This obtained an exact yield of 10.95%.

Organoleptic test

A study conducted by Dewi (2023) shows that the result of a MIC test of 0.93% ethanol extract of *Binahong* leaves can inhibit the activity of *C. acnes*, but the percentage is doubled in a gel formulation because it still has to be mixed with other excipients. Therefore, in the gel formula, a 1.86% concentration of ethanol extract is used. In this study, the organoleptic observations found that all the eight runs did not show any different results in terms of the color, smell, and shape. All the formulas had a greenish color, a characteristic odor of *Binahong*, and a slightly viscous texture. Meanwhile, based on a pH test, a pH value that is too alkaline can dry the skin while a pH that is too acidic can irritate the skin. Therefore, the pH of a preparation must be adjusted to the pH of the skin in the range of 4.5-6.5 (Alberts et al., 2008). The results of the pH test with eight runs in this study show that run 3 had the lowest pH (5.33), while the remaining runs had a pH that did not exceed 6. This indicates that the pH values of all the runs are still in the range of the skin's pH value, hence being in accordance with the theory.

The Design-Expert® software provides recommended solutions according to the desired optimization targets. The optimization was carried out by processing the data on the physical properties of *Binahong* leaf ethanol extract gel from eight run formulas that had been obtained through the experiments. The response parameters consisted of four physical properties, including the pH, viscosity, adhesion, and spreadability, because only these responses have proved to be influenced by the variations in HPMC and Carbopol 940 concentrations. For the response parameters used, the target response (goal) and appropriate degree of importance were selected to obtain the optimum formula. The chosen solution was the formula with the highest desirability value. The value of maximum desirability is one, and the closer it is to one, the better the desired value. Meanwhile, the lack-of-fit value had a p-value of >0.05, thus indicating that the value was not significant. An insignificant lack-of-fit value indicates that the desired model is suitable and has little noise.

Viscosity test

The viscosity test was performed to evaluate the thickness of the formulation. The gel preparation should have low viscosity to facilitate easy removal from the container, and it should not be too runny thus allowing the gel to be topically absorbed by the skin and to produce a therapeutic effect. Based on the results of the ANOVA test, a significance value of 0.318 was obtained (>0.05), indicating that there was no significant difference in the viscosity of the preparations during the test. Through the SLD viscosity equation listed in Table 2, it was found that Carbopol 940 (coefficient value = +18292.4) had a greater coefficient value than HPMC (coefficient value = +14852), indicating that Carbopol 940 has a more dominant role in increasing gel viscosity compared to HPMC. This is in line with the theory put forward by Firmansyah (2022) and Quinones et al. (2008) which states that the combination of Carbopol 940 > HPMC will increase the dissolution of an active substance and the viscosity of a preparation.

pH test

The analysis with the Design-Expert program for the pH data gave a probability of >F value of 0.258 (>0.05), and the results were not significant. The resulting less-than-fit value indicates that the distribution of the pH data was even, and the test data had a homogenous variant. Therefore, this model is in accordance with the existing data. Decreasing the concentration of Carbopol 940, before adding TEA, combined with increasing the concentration of HPMC will increase the pH response (Figure 1). This is based on the pH value of HPMC, which is higher than that of Carbopol 940.

Table II. SLD Equation & ANOVA of Anti-Acne Gel of *Binahong* Leaves from Design-Expert®

Parameter	Equation	p-value	R2	Significance
Viscosity	Y = 14,852A + 18,292.4B + 464.8AB + 33439.7AB(A-B)	0.318	0.713	Not significant
pH	Y = 5.91A + 5.76B	0.258	0.756	Not significant
Adhesion	Y = 8.94A + 8.14B + 10.38AB	0.046	0.709	Significant
Spreadability	Y = 4.84A + 4.16B	0.025	0.593	Significant

Y: Response; (A): HPMC; (B): Carbopol 940; (AB): Interaction between HPMC and Carbopol 940

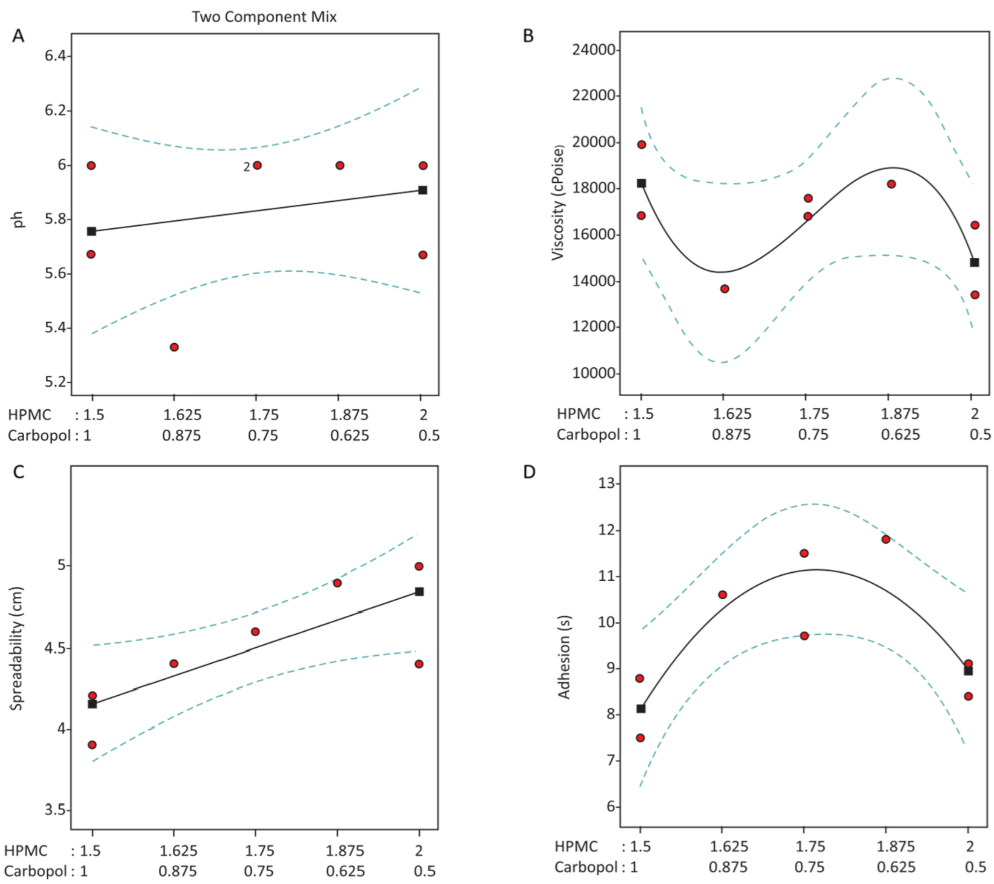

Figure 1. Two Component Mixture Graphs; (A): pH, (B): Viscosity, (C): Spreadability, (D): Adhesion

The Handbook of Pharmaceutical Excipients states that HPMC has a pH value of 8, and Carbopol 940 has a pH value of 3.5 (Rowe et al., 2020).

Adhesion test

The adhesive power of a gel preparation is directly proportional to its viscosity (Table II). Based on the SLD equation in the table, it is evident that the HPMC coefficient value (coefficient value = +8.94) was greater than that of Carbopol 940 (coefficient value = +8.14), indicating that HPMC plays a more dominant role in enhancing gel adhesion in comparison to Carbopol 940. However, because the coefficient difference between the two was not substantial, the resulting change in the adhesive power was not significant. The equation also resulted in a positive coefficient of the interaction between HPMC and Carbopol 940 (coefficient value = +10.38), indicating that the interaction of these two components has a role in increasing the viscosity of the gel. The R^2 value and *p-value* strengthen the influence of HPMC and Carbopol 940 interpretations. An R^2 value of 0.709 was obtained, indicating that the two gelling agents influence the adhesive strength because it is close to 1.

Table III. One Sample T-Test Analysis of the Optimum Formula

Parameter	Actual Data	Prediction Data	Sig. (2-tailed)	Interpretation
pH	5.7	6.0	0.423	Not significantly different
Viscosity	16463 cp	18130 cP	0.194	Not significantly different
Adhesion	12 s	10 s	0.027	Significantly different
Spreadability	4.63 cm	4.7 cm	0.749	Not significantly different

Table IV. Freeze-Thaw Test Results of the Optimum Formula

Parameter	Response		
	Cycle 1	Cycle 2	Cycle 3
Color	Dark green	Dark green	Dark green
Odor	Binahong odor	Binahong odor	Binahong odor
Homogeneity	Homogeneous	Homogeneous	Homogeneous
pH	6	6	6
Viscosity	16859	16762	16493
Adhesion	12.26	10.33	9
Spreadability	4.37	4.27	4.20

Table V. Score of Erythema and Edema After the Application of *Binahong* Gel Extract

Reaction	4h		24h		36h		72h	
	Cl	Trt	Cl	Trt	Cl	Trt	Cl	Trt
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0

Primary Irritation Index (PII) = 0/5, PII = 0 (No irritation). Cl = Control, Trt = Treatment

Spreadability test

Spreadability is inversely proportional to viscosity. A greater value of spreadability gives lower viscosity while a higher concentration of the gelling agent will increase the flow resistance of the gel. The viscosity of the gel increases as the concentration of HPMC increases in the mixture until it reaches the optimum point and then decreases again (Figure 1). The greater the spreading power, the greater the contact area between the gel and the skin, which allows for effective absorption of the active substance. Based on the ANOVA (Table II), an R^2 value of 0.593 was obtained. Because the R^2 value obtained is not close to 1, the effect of HPMC and Carbopol 940 on the adhesion strength does not seem to be very strong.

Optimum formula

The optimum formula was determined by analyzing the data on the response parameters obtained from the eight runs of the gel formula. The analysis revealed that the optimal proportion of HPMC and Carbopol 940 was 1.96:0.53. The outcome was a desirability value of 0.920, which was close to 1. This indicates that the use of those concentrations in the formulation greatly enhances the possibility of achieving the desired physical properties to form a good gel that meets the specified requirements (Table III).

The results of the physical property test of the optimum *Binahong* extract gel formula were compared with the predicted values obtained from the Design-Expert® by using a one sample t-test. The parametric test data showed that the three optimum formula responses had a significance value of more than 0.05; therefore, the predicted responses did not significantly differ from the values predicted by the Design-Expert®. As for the adhesion parameter, variability in the experimental conditions, such as sample measurement techniques and environmental factors, can lead to differences in the interpretation of the results. Nevertheless, this proves that the optimum formula was verified as it matched the prediction. Furthermore, the gel suits the pH of the skin, has an adhesion value of >4s, and has sufficient spreadability which provides a comfortable feeling on the skin. This is in accordance with the Indonesian National Standard (SNI) 16-4399-1996 which states that the pH of topical cosmetic preparations is in the range of 4.5–8.0 with the viscosity ranging between 2000 and 50000 cp and the spreadability being within 4-7 cm (BSN, 1996).

Stability test

The physical stability test aimed to determine the stability of the *Binahong* gel during storage. After three freeze-thaw cycles in a climatic chamber, the optimum formula did not show any changes in the shape, taste, and odor. The formula also remained homogeneous with a pH of 6, which is in the range of the pH of human skin. The spreadability, viscosity, and adhesion values of the Binahong gel was always in the required range (Table IV). The results for these tests were not significantly different and showed good physical stability in all the cycles.

Irritation test

Allergy is a condition characterized by an exaggerated immune reaction to an antigen, resulting in skin swelling and redness (James & Sunday, 2014). Erythema refers to the reddening of the skin or mucous membranes due to increased blood flow in the superficial capillaries, while edema is the accumulation of excessive serous fluid between cells in the tissue (Gatne et al., 2015). Recent animal investigations have demonstrated that *Binahong* gel extract does not induce any irritation in a slug irritation test (Yuliani et al., 2016). In this study, no positive skin reactions were seen following the application of *Binahong* gel extract. Therefore, the outcome is consistent with the findings of prior investigations. No signs of apparent skin irritation, such as edema and erythema, or inflammation were observed during the trial period in comparison to the control group. One hour after the test ingredient was removed, and continuing afterwards, it was observed that all the rabbits had a score of "0" for edema and erythema (Table V).

CONCLUSION

The organoleptic observations revealed that all the eight variations of the gel formula had similar characteristics. The selected solution from the Design Expert had the highest desirability value (0.920), which consisted of HPMC concentration at 1.96% and Carbopol 940 concentration at 0.53% resulting in desirable properties of the viscosity, pH, and adhesion, as well as adequate spreadability, thus ensuring a comfortable application on the skin. The one-sample t-test result showed that there was no significant difference in the comparison between the predicted value and the experimental value of the physical quality test of the *Binahong* gel as most of the significance values were greater than 0.05. The formulation proved to be physically stable after three freeze-thaw cycles and showed no irritation upon application. Therefore, this optimal formula shows characteristics that provide an effective gel formula for the treatment of acne using *Binahong* leaf extract.

However, this study has some limitations. The research included a limited number of samples and a narrow range of extract concentrations in the gel. It is possible that different gel base concentrations can have a better influence on the physical properties of the gel dosage form. The stability test was also conducted in a short period, which might not adequately represent a generalized conclusion of the stability of the formulation. Furthermore, it is crucial to maintain optimal experimental conditions when formulating the gel, such as sample measurement techniques and environmental factors, to ensure that all the physical properties of the gel show the same statistical interpretations. Besides, there are still a limited number of journals that study the effectiveness of *Binahong* leaf extract against *C. acnes*. It remains possible that further research will show more significant and comprehensive results for the *Binahong* leaf extract with other concentrations. Therefore, it is recommended that future research uses other variations of *Binahong* leaf extract concentrations in the gel with more sample runs and a longer duration of stability testing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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