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# Optimization of Antibacterial Gel Preparation Formula of Aloe Vera Leaf Flesh Fraction (Aloe Vera L.) Against Staphylococcus Epidermidis Using Simplex Lattice Design Method

Dewi Anggraheni Setyaningrum[1*], Ilham Kuncahyo[2], Ismi Rahmawati[3]
Department of Pharmacy, Faculty of Pharmacy, Setiabudi University
**Corresponding Author:** Dewi Anggraheni Setyaningrum
pinanggraheni@gmail.com

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A B S T R A C T

Acne is experienced by 90% of teenagers due to infection with the bacteria *Staphylococcus epidermidis*. Bacterial infections are treated with antibiotics, but excessive use causes resistance, so medicinal plants are used that do not have side effects. (*Aloe vera* L.) have antibacterial activity because contain anthraquinone compounds. The aim of research is update the optimization of aloe vera leaf gel to increase the effectiveness of use. The research method uses *Simplex Laticce Design* with HPMC, Karbopol and TEA. The optimum formula concentration of 1.3379% HPMC, 2.540% Carbopol and 0.481% TEA produced predicted and experimental values for adhesion power (7.977 and 7.5 seconds), spreadability (7 and 6.87 cm), pH (6.128 and 6 ), viscosity (246.775 and 265.3 dPa.S) and inhibition zone (23.772 and 22.66mm). The results of the Paired Sample Test for verification data show a p value of 0.430 > 0.05, is not significant difference between the predicted and the experimental.

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## INTRODUCTION

Acne occurs due to bacterial infection, one of which is Staphylococcus epidermidis with its ability to change sebaceous triglycerides into fatty acids that cause superficial infections in the sebaceous unit in the skin and can block the glands, causing inflammation to the skin tissue. Chronic inflammation that occurs is a special characteristic of acne with the formation of seborrhea, inflammatory lesions, blackheads, and nodules that appear on the face, neck, chest and back 23. Acne occurs in 80% of pubertal adolescents and has psychosocial effects (Saragih *et al*,. 2016). In cases where *S. epidermidis* resistance to penicillin and methicillin antibiotics has increased by more than 80% (Otto, M. 2012). Clindamycin has the potential as an antibacterial both bacteriostatic and bactericidal against *S. epidermidis* (Ardhany *et al*,. 2023). However, long-term use of antibiotics causes resistance to bacteria, so that the development of natural antibacterial products that have lower side effects is carried out [14]. Aloe vera (*Aloe vera* L.) is one of the medicinal plants that has antibacterial activity due to its active compound content of anthraquinone, flavonoids and saponins ((Bashir *et al*., 2011). Ethanol extract of 96% aloe vera leaves with a concentration of 2% produced an inhibition zone of 14.75 mm against *S.epidermidis* (Yasir *et al*,. 2022).

In the treatment, a good delivery system is needed, namely fast penetration of active substances so that the right gel preparation is made for acne treatment needs because its water content is high so that it can hydrate the skin to be permeable and its absorption is good (Musyirna *et al*,. 2023). Ethanol extract of 70% aloe vera leaves with a concentration of 4% still has antibacterial activity against S. epidermidis after being formulated into a gel preparation with a methylcellulose gelling agent producing an inhibition zone of 14 mm (Ministry of Health RI, 2017).

In the manufacture of gel preparations, optimization was carried out with three variables of gel preparation bases, namely HPMC, Carbopol and Triethanolamine using Simplex Lattice Design. So far, no research has been carried out related to the optimization of antibacterial gel preparations against S. epidermidis from the most active fraction of aloe vera leaves (Aloe vera L.). Based on the above information, research was carried out as an effort to renew with a combination of gelling agents HPMC, carbopol and TEA as alkalizing agents.

## THEORETICAL REVIEW

### *Definition of Acne*

Acne is a disease caused by bacterial infection and is commonly found in adolescence. This disease is found in almost all (90%) at the age of 15-19 years during puberty, adults and can also be in the elderly. Acne is an inflammation accompanied by blockage of the oil and hair gland ducts (pilosebaceous ducts). If the pilosebaceous duct is blocked, then the skin oil (sebum) cannot come out and collects in the duct, causing swelling and causing blackheads. Blackheads are the beginning of acne, either open comedones (blackheads) or closed comedones (whiteheads) (Hafsari *et al.*, 2015). Some factors that cause acne are infections from the activity of S. epidermidis bacteria. These bacteria cause inflammation caused by oil that collects on the skin and results in infection. Infection is a local or systemic reaction of the body due to the invasion of bacteria or toxins that enter the body. Infection is a complex disease and is often suffered by most people (Silvia, 2015).

### *Antibacterial Activity*

Aloe vera has antibacterial activity against Gram-positive and Gram-negative bacteria. The active compounds in aloe vera have strong antibacterial activity derived from anthraquinone compounds. Anthraquinone isolated from aloe vera exudate shows broad antibacterial activity. The highest anthraquinone content is found in the latex layer or brownish-yellow exudate found between the skin and flesh of aloe vera. So to obtain antibacterial content from anthraquinone, the extract processed is not only from aloe vera gel but also the latex or exudate part of the aloe vera (Hamman, 2008).

Aloe vera leaves have pharmacological activities as antibacterials, making them one of the many types of plants that are good for your health. Aloe vera leaves have the potential to be antibacterial because they contain active molecules called anthraquinone compounds, which have antibacterial and antifungal properties (D. N. Sari, 2017). According to research by Bashir et al. (2011), aloe vera's anthraquinone, flavonoid, and saponin chemical composition contributes to its antibacterial effectiveness against a variety of acne infections. The findings of his study, which show that 75.3% of the isolated bacteria, specifically S. epidermidis, are effectively inhibited from growing, provide evidence of this. Nevertheless, the results of a more successful inhibition test were obtained in a study by Yasir et al., 2021; specifically, from a concentration of 2% 96% aloe vera ethanol extract creating an average inhibition of 14.75 mm against the development of S. epidermidis bacteria.

### *Definition of Gel*

Gel is a semi-solid preparation, penetrates the liquid and moisturizes the stratum corneum through a suspension of large organic molecules or small inorganic particles. Both the drug's and the stratum corneum's permeability may vary as a result of this. The gel's high water content can reduce the possibility of further irritation brought on by lipid buildup in the pores. This is because the bacteria that cause acne feed on lipids. As a result, this substance can be used as a component in products that treat acne (Roudhaini, 2013). Gel dosage forms contain hydrophilic base chemicals with a soft consistency that gives the skin a refreshing and cooling sensation. The benefit of gel preparations is that, once dry, they leave behind a thin, elastic layer that is sticky and does not clog pores, making them easy to wash off with water. The drawback is that, because active ingredients in gel preparations must be soluble in water, so solubility enhancers like surfactants are required to keep the gel clear and transparent throughout temperature fluctuations. However, surfactant content can also irritate skin(Voigt, 1994).

***Optimization of Simplex Lattice Design***

An approach or experimental plan called optimization aims to make the gathering and analysis of mathematical data easier. Design Expert, a program used for process optimization in the primary response generated by several factors, is one tool that may be used to find the best formula. The idea is to maximize the response. Mixture design, which finds the best formula, is one of the many design alternatives offered by Design Expert, each with its own function. Typically, the application of Simplex Lattice Design yields the optimal formula (SLD). The SLD method is an optimization method to determine the physical properties of two or more mixtures. With this method, it is expected that the trial and error coefficient in formula design can be reduced and with this method a researcher can also predict the properties of the mixture and all comparisons (Bolton, 1997).

## METHODOLOGY

***Material***

The materials used were 96% ethanol, carbopol 940, HPMC, triethanolamine, propylene glycol, methyl paraben, n-hexane, ethyl acetate, water, ether, concentrated sulfuric acid, acetic acid, NaOH, $H2SO4$, the test media used in this study were Nutrient Agar (NA) medium, Muller Hilton Agar (MHA) and Nutrient Broth (NB).

***Instrument***

The tools used are digital scales (OHAMS), inkas, platinum ose, petri dishes (Normax), Erlenmeyer flasks (Pyrex), test tubes (Pyrex), measuring cups (Iwaki), dropper pipettes, analytical balance (OHAUS), tweezers, incubators, flannel cloth, cotton (Selection), glass funnels, borer props, micropipettes (dragon), sterile cotton buds, autoclaves, water baths, filter paper, binder ovens (Binder ED 53), spirit lamps, blenders (cosmos), maceration bottles, rotary evaporators (IKA RV 10), gel containers, pH meters (starter 3100), viscometers.

***Extraction***

The aloe vera leaf samples obtained were washed clean and then separated between the skin or outermost layer and the leaf flesh. The aloe vera leaf flesh that was still slimy brownish (exudate) was weighed as much as 1500 grams, then mashed and soaked using 7500 mL of 96% ethanol solvent (ratio 1:5) with a closed container and protected from light for 24 hours [18]. Then the maceration results were separated from the solvent using a rotary vacuum evaporator to obtain a thick extract where the extract could no longer be poured. The temperature used was below the boiling point of 96% ethanol, which was 78.4 °C due to a decrease in vapor pressure. After being concentrated, the resulting extract could still be poured so it was concentrated again with the help of a water bath. The temperature used in the water bath was 40 °C below the boiling point of 96% ethanol until a thick extract was obtained (Sari *et al.*, 2018).

***Extract Identification***

a. *Determination of water content*

Weigh approximately 10 grams of sample, put it into a container that has been tared, dry at a temperature of 105°C for 5 hours, then weighed. Then repeated drying is carried out at intervals of 1 hour until the difference in weighing is no more than 12.5% (Sari *et al*., 2018).

b. *Determination of total ash content*

Weigh 2-3 grams of extract, put it into a silica crucible, ignite until the charcoal is gone and tared, ignite slowly, cool and weigh. If the charcoal cannot be removed, add hot water, stir and filter with ash-free filter paper. Ignite the filter paper with the remaining filter in the same container. Place the filtrate in a crucible, evaporate until it reaches a temperature of 800±50°C. The total ash content is calculated relative to the weight of the test material and expressed in % w/w. According to the Indonesian Herbal Pharmacopoeia, a good total ash content is less than 4.9%(Sari *et al*., 2018).

c. *Determination of acid-insoluble ash content*

The ash obtained in the determination of total ash content was heated in 25 mL of dilute hydrochloric acid for 5 minutes. Collect the acid-insoluble part, filter it with ash-free filter paper, wash it using hot water and ignite it in a crucible until the weight remains at a temperature of 800±50°C. The acid-insoluble ash content is calculated relative to the weight of the test material and expressed in % w/w. According to the Indonesian Herbal Pharmacopoeia, a good acid-insoluble ash content is less than 0.5%(Sari *et al*., 2018).

d. *Ethanol-free test*

Identification of the ethanol-free test on the extract is to put 2 drops of extract into a test tube, then add 2 drops of H2SO4, then add 2 drops of acetic acid, observe the change in odor, namely if it smells of ether then it is still not free from ethanol, but if the odor is typical of aloe vera extract then the extract does not contain ethanol (Sari *et al*., 2018).

e. *Fractionation*

extract 50 gr is put into a separating funnel and 250 mL of water is added. Next, 250 mL of n-hexane solvent is added which is then shaken in a separating funnel and left for 30-60 minutes then the bottom layer (water) and the top layer (n-hexane layer) will be formed. The phase taken is the n-hexane layer formed in the top layer. After that, the n-hexane phase that is taken is concentrated again in a water bath at a temperature of 50oC until a thick extract is obtained. Then the water phase is partitioned by adding 250 mL of ethyl acetate which is then shaken in a separating funnel and left for 30-60 minutes then the bottom layer (water) and the top layer (ethyl acetate) are formed. The phase taken is the ethyl acetate layer formed in the bottom layer and water in the remainder of the fractionation. After that, the upper phase (ethyl acetate) and lower phase (water) were each concentrated again in a water bath at a temperature of 50oC until a thick extract was obtained (Department of Health, 2008).

***Phytochemical Screening***

a. *Flavonoid test*

0.1 gram of extract was put into a test tube, then 0.5 mg of magnesium powder was added and 3 drops of concentrated HCl were added. Yellow, green, black, orange and orange colors indicate positive flavonoids (Kursia *et al*., 2016).

b. *Saponin test*

0.1 g of extract is put into a test tube, then 10 mL of warm or hot water is added and shaken for 30 minutes. After that, the foam that appears is observed and how many cm of foam is formed is measured. Leave it for 5 minutes and if the foam does not disappear, 2 N HCl is added. If there is still constant foam, it shows a positive result (Kursia *et al*., 2016).

c. *Tannin test*

0.1 g of extract was put into a test tube, plus 3 drops of FeCl3. Blue color indicates the presence of tannin (Kursia *et al*., 2016).

d. *Phenol test*

1% FeCl3 reagent is added to the extract until a color change occurs, then the color is compared with the pure extract, the color will appear blacker if positive (Ikalinus *et al*,. 2015).

e. *Anthraquinone test*

1 mL of sample is then added with 1 mL of ethanol. The mixture is filtered using filter paper. Then 1 mL of ammonia solution is added, then shaken. A positive anthraquinone test result is indicated by a red color change. A red NaOH layer indicates the presence of anthraquinone glycosides (Dasopang, 2017).

***Identification of Bacteria***

a. *Observation of microscopic objects*

Take distilled water and drop it on the object glass, add 1 loop of sample culture, then fix it over the fire. Drop crystal violet staining and leave for 1 minute, wash with running water, then drop lugol leave for one minute and wash again with running water. Next, drop 96% alcohol leave for 10-20 seconds, wash with running water and add safranin leave for 20-30 seconds then wash again with running water. The next step is to dry using absorbent paper and add immersion oil and observe under a microscope. If the staining results are red bacteria, then the bacteria are gram-negative bacteria, while if the bacteria are purple, then the bacteria are gram-positive (Lenni Fitri, 2011).

b. *Catalase Test*

The catalase test is carried out by dripping H2O2 solution onto a glass object, then taking a smear of bacteria using an ose needle and smearing it on the solution on the glass object, observing the reaction that is formed if bubbles form indicating a positive reaction (Lenni Fitri, 2011).

c. *Coagulase Test*

Coagulase test is done by taking the test bacterial isolate using an ose needle then inserting it into Nutrient Broth media ±2 mL then incubating at 37℃ for 24 hours. Next, add 1 mL of rabbit citrate plasma coagulase then incubate for the first 4 hours at 37℃ to observe the presence or absence of fine sand-like clots. Positive results are indicated by the formation of clots (Hayati *et al*., 2019).

***Antibacterial Activity Test of Extracts and Fractions***

All tools and materials to be used were sterilized first. Testing the antibacterial activity of 96% ethanol extract, n-hexane fraction, ethyl acetate and water with 0.3% clindamycin positive control and 10% dimethyl sulfoxide (DMSO) negative control was carried out using the disc diffusion method on Nutrient Agar (NA) media using paper discs. Antibacterial activity tests were carried out in triplicate. The NA media was planted with S.epidermidis test bacteria by scratching the test bacteria that had been suspended in a test tube using sterile cotton swabs evenly over the entire surface of the media. Furthermore, it was incubated in an incubator at a temperature of 37℃ for 24 to 48 hours. The petri dish that had been filled with media and planted with bacteria was then divided into 6 parts. The disc paper was dripped with samples, namely 96% ethanol extract of aloe vera leaves 4%, n-hexane fraction 4%, ethyl acetate 4%, water 4%, positive control clindamycin 0.3%, and negative control DMSO 10% then placed on NA media that had been inoculated with test bacteria. Incubation was carried out at a temperature of 37˚C for 24 hours. Observation of the results of the antibacterial activity test was carried out on the formation of an inhibition zone around the disc paper (Cinthya, Y., & Silalahi, E.2020).

***Formulation Design with Simplex Lattice Design***

Optimization for the selection of the optimum formula was carried out using Design Expert 12 software with parameters of pH response, viscosity, spreadability, adhesion and antibacterial activity. All gel formulas obtained were formulated based on the sequence of runs 1-17 then tested for the physical properties of the preparation and its antibacterial activity, the data obtained were processed using Design Expert software with the SLD method using 3 factors. The factors studied were HPMC as factor A, carbopol as factor B and TEA as factor C. Each factor has a range of levels used, including for HPMC is 1-2%, for carbopol is 2-3% and for TEA 0.4-1.4% (Tsabitah *et al*., 2020).

***Gel Preparation Preparation***

The aquadest was heated to a temperature of 70℃ then the carbopol was dispersed in the aquadest using a stirrer at a speed of 70 rpm until homogeneous. After being homogeneous and the foam was gone, TEA was added to form a gel. Furthermore, HPMC was dispersed with aquadest until it expanded, then added to the carbopol that had previously been added with TEA, stirred until homogeneous. Then methyl paraben was dissolved in hot water, after dissolving it was added to the gel mass. The most active fraction of the aloe vera leaf flesh extract (Aloe vera L.) and propylene glycol were added to the gel mass and stirred with a stirrer until homogeneous while adding the remaining aquadest (Tambunan, 2018).

***Physical Quality Test of Preparation***

a. *pH Test*

The pH test is carried out by attaching a pH stick to the gel preparation to be tested, then observing the color change on the pH stick, adjusting the color to the specified pH indicator paper. The pH value of a good topical preparation is a pH value that is close to the skin pH which ranges from 4.5-7 (Tambunan, 2018).

b. *Adhesion test*

The adhesion test is carried out by placing 0.25 grams of gel on two predetermined object glasses, then pressing with a load of 1 kg for 5 minutes. After that, the object glass is installed on the test tool and then an 80 gram load is added to the test tool, then the release time from the object glass is recorded. The higher the adhesion of the gel indicates the stronger the bond between the gel and the skin, allowing for higher drug absorption by the skin. Good adhesion of the preparation is not less than 4 seconds (Tambunan, 2018).

c. *Spreadability test*

The spreadability test was carried out by weighing the gel preparation as much as 0.5 grams, after which the gel was placed right under a round glass with a diameter scale underneath, then covered with another glass that had been weighed and left for one minute, after which the spread diameter was measured. After 1 minute, a load of 50 grams was added and left for 1 minute, then the spread diameter was measured. The same thing was done every 1 minute with the addition of a load of 50 grams until a sufficient diameter was obtained to see the effect of the load on the spread diameter of the gel preparation. The results of a good spreadability of the gel preparation are 5-7 cm (Tambunan, 2018.

d. *Viscosity test*

Viscosity determination is carried out using a VT 04 series viscometer. The gel is inserted into the tube on the viscotester, then rotor number 2 is installed until the spindle is completely submerged in the gel. The device is turned on and the rotor number 2 pointer is observed on the viscosity scale until it stops stable. The number indicated by the pointer is in dPa.S units (1 dPa.S = 1 poise). A good gel viscosity value is in the range of 150-300 dPa.S, because with this viscosity the gel is able to spread well when applied (Tambunan, 2018).

e. *Antibacterial Activity Test of Gel Preparation*

Antibacterial activity test was conducted using the disc diffusion method. This antibacterial test was conducted in a *laminar air flow* (LAF) room. The first disc diffusion test was conducted by pouring sterilized NA media into a petri dish. The NA media that had cooled and solidified was then planted with bacteria. The NA media was planted with S.epidermidis test bacteria by scratching the test bacteria that had been suspended in a test tube using sterile cotton buds evenly over the entire surface of the media. Furthermore, it was incubated in an incubator at a temperature of 37°C for 24 to 48 hours. In two petri dishes that had been filled with media, 6 wells were then made in each part of each dish. Furthermore, bacteria were planted by scratching using sterile cotton buds, then 17 gel formulas of the most active fraction of aloe vera leaf flesh extract were each taken using a micropipette and inserted into the well. After that, it was incubated for 1 x 24 hours at a temperature of 37 °C. Then the inhibition zone was observed and measured using a vernier caliper (Cinthya, Y., & Silalahi, E. 2020).

f. *Formula Optimization Preparation*

Determination of the optimum formula of aloe vera leaf flesh gel preparation (Aloe vera L.) was determined using *Design Expert* software with the *Simplex Lattice Design* method. The data to be used in determining the optimum formula is to look at the critical parameters including spreadability, viscosity adhesion, pH and antibacterial activity of 17 formulas. The expected final result by referring to the critical parameters of the optimum formula of the most active fraction gel preparation of aloe vera leaf flesh with good physical properties of the gel and antibacterial activity equivalent to the most active fraction, namely having a large spreadability and adhesion, a viscosity value of 150-300 dPas, a pH value in the range of 4.5-7 and having antibacterial activity with a strong category in the inhibition zone of 10-20 mm.

***Data Analysis***

Data obtained from the pH test, adhesive power, spread power, viscosity and antibacterial of the most active fraction gel preparation of aloe vera leaves were analyzed using Design Expert software with the SLD method to obtain an optimum formula, the optimum formula obtained was verified by comparing the physical properties of the gel preparation made with the predicted physical properties of the gel using a paired sample test with a 95% confidence level. The data obtained is said to have no significant difference if the significance value is ($p>0.05$).

**RESULTS**

***Extract Identification***

The weight of the obtained simplicia was 1500 gr, after extraction, the thick extract yield was 285 gr with a yield of 19.0%. The yield value in this study was not far from the yield value obtained in the study conducted by (Yasir & Angin2022). which obtained a yield of 96% ethanol extract of aloe vera leaves of 19.63% using the same solvent used in this study, namely 96% ethanol. The total ash content of the aloe vera leaf extract in this study was 6.48% ± 1.05. The total ash content value shows that the inorganic compound content obtained from 3 grams of aloe vera leaf extract has a total ash content of 6.48% ± 1.05. The results of the total ash content value do not meet the requirements for the total ash content value, which does not exceed 4.9% (Ministry of Health RI, 2017). The results of 10 grams of aloe vera leaf extract used in determining the water content obtained a value of 11.377% ± 0.46, this value has met the parameters of the water content value in the Indonesian Herbal Pharmacopoeia, which is not more than 12.5% [5]. The results of the acid-insoluble ash content of the aloe vera leaf extract in this study were 1.716% ± 0.49. The acid-insoluble ash content value shows that the results of the replication of the determination of the total ash content of the aloe vera leaf extract have an organic compound content of 1.716% ± 0.49. The acid-insoluble ash content indicates the presence of mineral or metal contamination that is not acid-soluble in a product. The resulting value does not meet the parameter criteria in the Indonesian Herbal Pharmacopoeia, which is not more than 0.5%(Ministry of Health RI, 2017). The results of the ethanol-free test of the aloe vera leaf extract in this study were negative, which means that the aloe vera leaf extract is truly pure, not contaminated by ethanol and has antibacterial activity that is pure from its constituent compounds, not from the effects of ethanol. A negative result is indicated by the distinctive odor of ether which is not detected in the extract after being dripped with H2SO4 and acetic acid (Sari *et al*., 2018).

***Phytochemical Screening***

Table 1. *Results of phytochemical screening of aloe vera leaf extracts and fractions (Aloe vera L.)*

| Compound | Extract | n-hexane | Ethyl acetate | Water | Reaction | Library |
|---|---|---|---|---|---|---|
| Anthraquinon | + | - | + | + | Red | The red NaOH layer indicates the presence of anthraquinone glycosides |
| Taninn | + | - | - | + | Blue | The blue color indicates the presence of tannins |
| Flavonoids | + | + | + | + | Orange | Yellow, green, black, orange and orange colors indicate positive flavonoids |
| Saponin | + | + | + | + | Foam | If there is still constant foam, it indicates a positive result |
| Phenol | + | - | - | + | Blackish | The color change appears blacker if positive |

***Identification of Bacteria***

The results of the catalase test are in accordance with the research conducted by [11], which stated that the catalase test on S. epidermidis contained bubbles, which means the catalase test results were positive. S. epidermidis is a bacteria that has the catalase enzyme, where the catalase enzyme can catalyze the decomposition of hydrogen peroxide into H2O and O2. The catalase test on coccus-shaped bacteria is used to distinguish between *Staphylococcus* and *Streptococcus* (Rosmalawati & Widiatmoko, 2022). The results of the coagulase test are in accordance with the research conducted by [11], which stated that the coagulase test on *S. epidermidis* bacteria did not contain clots, which means the coagulase test results were negative. *S. epidermidis* does not produce the coagulase enzyme that can clot plasma. The coagulase test is used to distinguish the bacterial genera S. *aureus* and *S. epidermidis* (Purbowati , 2017).

***Results of Antibacterial Activity Test of Extracts and Fractions***

Table 2. *Results of antibacterial activity tests of aloe vera leaf extracts and fractions (Aloe vera L.)*

| Sampel | Replication | | | Average inhibition diameter | Normality | Homogeneity | *Anova* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | | | | |
| Control + | 32 | 35 | 33 | 33,3 ± 1,52 | 0,637 | 0,802 | 0,000 |
| Extract | 18 | 16 | 17 | 17,0 ± 1,0 | | | |
| Water fraction | 20 | 21 | 20 | 20,3 ± 0,57 | | | |
| Ethyl acetate fraction | 14 | 15 | 14 | 14,3 ± 0,57 | | | |
| n-hexane fraction | 0 | 0 | 0 | 0 | | | |
| Control – | 0 | 0 | 0 | 0 | | | |

Description: Normality Test p value > 0.05 (data is normally distributed)
Homogeneity Test p value > 0.05 (data is homogeneous)
One Way ANOVA Test p value < 0.05 (there is a significant difference in data)

The results of the antibacterial activity test of the extract and fraction of aloe vera leaves (*Aloe vera* L.) in table 2. show that the highest antibacterial activity is produced by the water fraction, which is 20.3 ± 0.57 mm. The results of data analysis from the data in table 2. show that the distribution of the data is stated as normal and homogeneous with a p value (Asymp. Sig.) > 0.05. The results of data analysis using the One Way ANOVA test obtained a p result (Asymp. Sig.) < 0.05 which means that the data has a significant difference in antibacterial activity.

***Results of Physical Quality Test of Preparations***

The results of physical quality testing of gel preparations include the results of pH, adhesive power, spread power, viscosity and antibacterial activity tests.

Table 3. *Results of physical quality tests of gel preparations*

| **Run** | **Fomula** | | | **Adhesion Spreadability** | **Spreading power** | **pH** | **Viscosity** | **Antibacterial activity** |
|---|---|---|---|---|---|---|---|---|
| | **HPMC** | **Carbopol** | **TEA** | | | | | |
| 1 | 1,4 | 2,3 | 0,7 | 10,5 | 5,3 | 8 | 352 | 20,4 |
| 2 | 1,2 | 2,1 | 1,1 | 11,5 | 4,2 | 8 | 444,1 | 19,5 |
| 3 | 1,4 | 2,3 | 0,7 | 10 | 5,5 | 7 | 349 | 21 |
| 4 | 1,5 | 2 | 0,9 | 9,8 | 5,9 | 7 | 322 | 22 |
| 5 | 1,4 | 2,6 | 0,4 | 7,4 | 7,5 | 5 | 214,6 | 23,7 |
| 6 | 1,4 | 2,3 | 0,7 | 10 | 5,4 | 7 | 362 | 20 |
| 7 | 1,4 | 2,3 | 0,7 | 10,5 | 5,5 | 8 | 345 | 21,9 |
| 8 | 2 | 2 | 0,4 | 5,2 | 8,4 | 5 | 298,4 | 20,8 |
| 9 | 1 | 3 | 0,4 | 7,7 | 7,5 | 6 | 235,7 | 23 |
| 10 | 1 | 2 | 1,4 | 12,7 | 3,8 | 8 | 465,2 | 18,3 |
| 11 | 1,2 | 2,6 | 0,6 | 8,5 | 6,7 | 6 | 269,9 | 22.5 |
| 12 | 1,7 | 2 | 0,7 | 9,1 | 6,4 | 6 | 298 | 22,1 |
| 13 | 1 | 1,5 | 0,9 | 11 | 5,1 | 7 | 388 | 20,7 |
| 14 | 1 | 2,5 | 0,9 | 11,1 | 5,3 | 7 | 365 | 20 |
| 15 | 1,5 | 2 | 0,9 | 9 | 5,9 | 6 | 332 | 21,6 |
| 16 | 1,7 | 2,3 | 0,4 | 5,9 | 8 | 5 | 209,6 | 25,6 |
| 17 | 1,5 | 2,5 | 0,4 | 6,6 | 7,7 | 6 | 212,3 | 25,6 |

a. *The results of the adhesion test of the preparation*

The results of the adhesion test of the gel preparation from 17 formulas showed that formula 10 had the highest adhesion with a value of 12.7 seconds with variations in the gel preparation formula of 1% HPMC, 2% Carbopol and 1.4% TEA. The differences in the data obtained were significantly different in the significant model parameters and the lack of fit was not significant, meaning that the replication formula on the same component had a different response. The equation formula obtained from the Simplex Lattice Design application is as follows,Y = +5,1831 (A) + 7,6924 (B) + 12,6844 (C) + 0,81669 (AB) + 1,6907 (AC) + 3,3463 (BC)

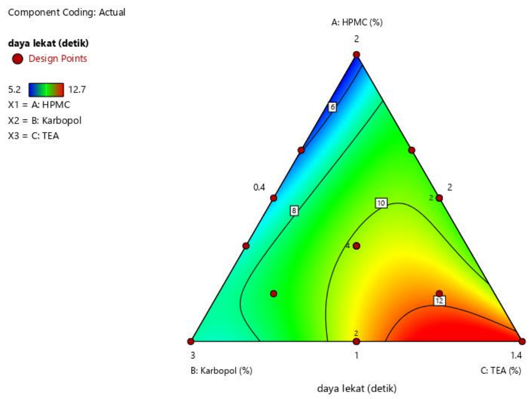


**Figure 1. Counter plot of adhesion values**

Based on the Counter plot and the equation above, it can be seen that there is an interaction between formula ingredients in influencing the increase in the value of the adhesive power of the preparation. However, the one that has the most influence on the increase in the value of the adhesive power of the preparation and the one that has the most dominant influence is TEA as an alkalizing agent as evidenced by the highest value of (+ 12.6844) and the best interaction to increase the adhesive power is Carbopol 940 and TEA with a positive value (+ 3.3463). The results of this study are the same as the results of the study conducted (Ikalinus *et al*,. 2015), which showed that the results of the adhesive power equation from Carbopol 940 had a greater influence on increasing the adhesive power of the preparation than HPMC.

b. *Spreadability test results of the preparation*

The spreadability test results of the gel preparation from 17 formulas showed that formula 11 had the highest spreadability with a value of 6.7 cm with variations in the gel preparation formula of HPMC 1.2%, Carbopol 2.6% and TEA 0.6%. The difference in the data obtained was significantly different in the significant model parameters and the lack of fit was not significant, meaning that the replication formula on the same component had a different response. The equation formula obtained from the Simplex Lattice Design application is as follows, Y = +8,3975 (A) + 7,4991 (B) + 3,7973 (C) – 0,9350 (AB) – 0,8197 (AC) – 1,8101 (BC)

Based on the Counter plot and the equation of spreading power above, it can be concluded that the three variables get positive results which means HPMC 1000DB, Carbopol 940 and TEA can cause an increase in the value of the gel spreading power and the one with the highest value is HPMC as evidenced by a positive value (+8.3975) which means that HPMC has the most dominant influence in increasing the value of the preparation spreading power. The interaction between the three materials has no effect in increasing the preparation spreading power but the one that has the least effect is the interaction between Carbopol 940 and TEA which is evidenced by a negative result (-1.8101).

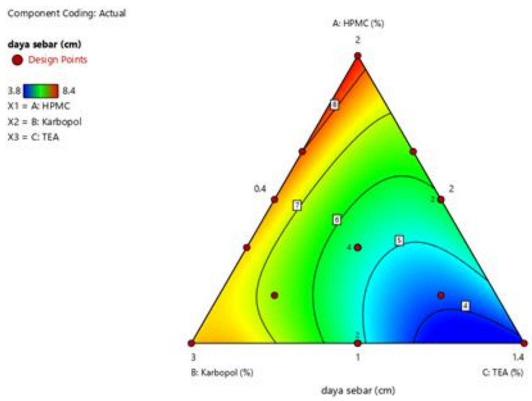


**Figure 2. Counter plot of spreading power values**

The results of this study are the same as the results of the study conducted (Ikalinus *et al*,. 2015), which showed that the results of the HPMC spreadability equation had a greater effect on increasing the spreadability of the preparation than Carbopol. The study conducted Nursiah *et al*,. (2011) showed that the HPMC gelling agent has hydrophilic properties and has advantages in producing good spreadability, a cooling effect, does not clog pores, is easy to wash with water and is able to release active substances maximally. Based on the data above, it can be seen that the addition of HPMC 1000DB can increase the spreadability.

c. *Results of pH test of the preparation*

The results of the pH test of the gel preparation from 17 formulas showed that there were only 13 formulas that met the criteria for a good pH of the gel preparation used on the skin with a pH range of 4.5-7. The differences in the data obtained were significantly different in the significant model parameters and the lack of fit was not significant, meaning that the replication formula on the same component had a different response. The equation formula obtained from the Simplex Lattice Design application is as follows,Y = +5,0203 (A) + 5,7493 (B) + 8,0952 (C) – 0,3471 (AB) – 0,0617 (AC) + 0,1423 (BC)

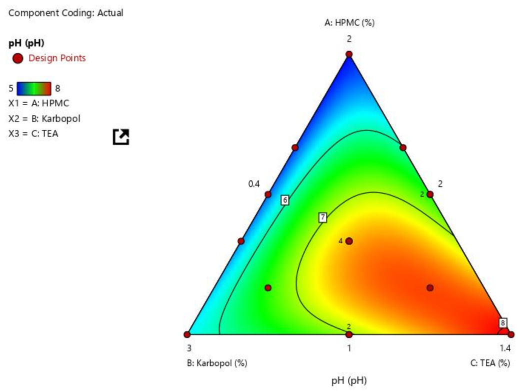


**Figure 3. Counter plot of pH values**

Based on the Counter plot and the equation of spreading power above, it can be concluded that the three variables get positive results which means that HPMC 1000DB, Carbopol 940 and TEA can cause an increase in the value of the gel spreading power and the one with the highest value is TEA as evidenced by a positive value (+8.0952) which means that TEA has the most dominant influence in increasing the value of the preparation spreading power. This shows that the greater the proportion of TEA is able to increase the pH of the preparation. The interaction between Carbopol 940 and TEA has a positive result (+0.1423) which shows that the interaction of both is able to increase the pH value of the preparation. The results of this study are the same as the results of the study conducted (Ikalinus *et al*,. 2015), which showed that the results of the pH equation from TEA most influenced the increase in the pH of the preparation.

d. *Viscosity test results of the preparation*

The viscosity test results of the gel preparation from 17 formulas showed that formula 8 had the highest viscosity with a value of 298.4 dPa.S with variations in the gel preparation formula of HPMC 2%, Carbopol 2% and TEA 0.4%. The difference in the data obtained was significantly different in the significant model parameters and the lack of fit was not significant, meaning that the replication formula on the same component had a different response. The equation formula obtained from the *Simplex Lattice Design* application is as follows, Y= +298,008 (A) + 235,366 (B) + 465,103 (C) – 234,875 (AB) – 219,307 (AC) + 104,441 (BC) + 1562,22 (ABC)

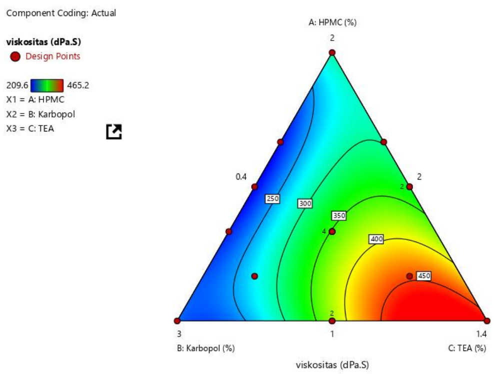


**Figure 4.** *Counter plot of viscosity values*

Based on the Counter plot and the equation above, it can be concluded that the interaction between Carbopol 940 and TEA gives a positive value (+104.441) which means that Carbopol 940 and TEA can increase the viscosity of the gel preparation, the interaction between HPMC 1000DB and TEA gives a negative value (-219.307) which means that it will decrease the viscosity of the gel preparation and of the three materials, the most dominant one that increases viscosity is TEA which is proven by a positive value (+465.103). This shows that the greater the proportion of Carbopol 940 and TEA, the greater the viscosity. Conversely, if the greater the proportion of HPMC 1000DB and TEA used, the lower the viscosity response. Based on the data above, it can be seen that the addition of Carbopol 940 can increase the viscosity of the gel preparation. The results of this study are the same as the results of the study conducted (Ikalinus *et al*,. 2015), which showed that the viscosity equation results of Carbopol 940 had a greater influence on increasing the viscosity of the preparation than HPMC.

*e. Antibacterial test results of the preparation*

The antibacterial test results of the gel preparation from 17 formulas showed that formulas 16 and 17 had the highest antibacterial activity with a value of 25.6 mm with variations in the gel preparation formulas of formulas 16 and 17, respectively, namely HPMC (1.7%, 1.5%), Carbopol (2.3%, 2.5%) and TEA (0.4% and 0.4%). The difference in the data obtained was significantly different in the significant model parameters and the lack of fit was not significant, meaning that the replication formula on the same component had a different response. The equation formula obtained from the Simplex Lattice Design application is as follows, Y= +21,1758 (A) + 22,6539 (B) + 18, 2474 (C) +13,3606 (AB) + 8,33072 (AC) – 59,9956 (ABC)

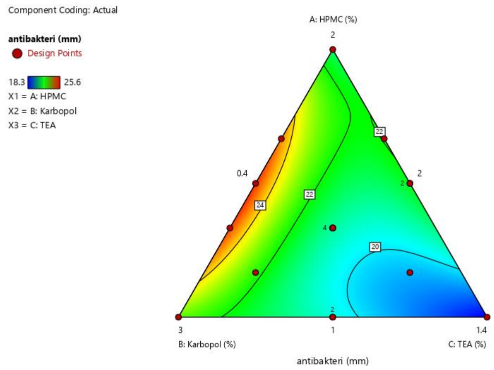


**Figure 5. Counter plot of antibacterial activity values.**

Based on the Counter plot and the equation above, it can be seen that the interaction of the three factors has a significant influence, in the interaction of HPMC 1000DB and Carbopol 940 gives a positive value (+13.3606) which means Carbopol 940 and HPMC 1000DB can increase antibacterial activity in gel preparations, and of the three ingredients of the gel preparation formula has a positive value which means it can increase the antibacterial activity of the preparation but the most dominant is Carbopol 940 which is proven by the highest value which is positive (+22.6539). This shows that the greater the proportion of HPMC 1000DB and Carbopol 940 is able to increase antibacterial activity. Conversely, if the greater the proportion of HPMC 1000DB and TEA used, the response of antibacterial activity will decrease. Based on the data above, it can be seen that the addition of Carbopol 940 can increase the antibacterial activity of the gel preparation.

### *Formula Optimization Results With Simplex Lattice Design*

After the response data is entered into the design expert application with the Simplex Lattice Design method, it will obtain the results of the solution for the variation of humectant materials from the prediction of good response values. The assessment for decision making to choose the best formula is based on critical parameters with the desired range of numbers. The results of the composition analysis and prediction of the physical properties of the optimum formula are shown in table 4.

Table 4. *Results of optimization of the dosage form with Simplex Lattice Design*

| HPMC | Carbopol | TEA | Adhesion | Spreading power | pH | Viscosity | Antibacterial activity | Desirability |
|---|---|---|---|---|---|---|---|---|
| 1,346 | 2.578 | 0.477 | 7,977 | 7 | 6,128 | 246,775 | 23,772 | 0.743 |
| 1,397 | 2,522 | 0,481 | 7,953 | 7 | 6,127 | 247,003 | 23,717 | 0,742 |
| 1,360 | 2,560 | 0,480 | 8,003 | 7 | 6,131 | 246,670 | 23,716 | 0,742 |

Based on the data prediction, it can be concluded that the formula produces a desirability value of 0.743 and Super Improsed is obtained as indicated by the yellow area that provides the optimum response, the desirability value approaching 1 indicates the desired formula and vice versa if the desirability value approaches 0 then it indicates an undesirable formula.

### *Optimization Data Verification Results*

The optimum formula predicted by the Simplex Lattice Design method was tested for its accuracy by making a gel preparation formula and conducting physical quality tests including adhesive power, spread power, pH, viscosity and antibacterial activity. The results obtained showed that the predicted and experimental data were not much different. The results of the optimum formula verification are shown in table 5.

Table 5. *Comparison of predicted values with experimental values*

| Response | Prediction value | Trial value | Conclusion |
|---|---|---|---|
| Adhesion | 7,977 | 7,5 | No significant difference |
| Spreading power | 7 | 6,87 | No significant difference |
| pH | 6,128 | 6 | No significant difference |
| Viscosity | 246,775 | 265,3 | No significant difference |
| Antibacterial activity | 23,722 | 22,66 | No significant difference |

Continued with the Paired Sample Test with the aim of comparing data before and after the application. The results of the Paired Sample Test from the optimum formula verification data show a p value (Asymp. Sig.) of 0.430> p = 0.05 which means that the data from the predicted value and the experimental value do not have a significant difference.

## CONCLUSIONS AND RECOMMENDATIONS

Based on the research results, it can be concluded that the water fraction of 96% ethanol extract of aloe vera leaves (Aloe vera L.) has the highest antibacterial activity against S. epidermidis so that a gel preparation formula is made. The optimum proportion of the aloe vera leaf water fraction gel preparation formula (Aloe vera L.) is at a concentration of HPMC 1.3379%, Carbopol 2.540% and TEA 0.491%. The optimum formula has a good effect on the physical quality of the aloe vera leaf water fraction gel preparation (Aloe vera L.) by having a pH and adhesive power that meets the criteria for gel preparations, spreadability, high viscosity and meets the criteria for gel preparations and is able to increase the antibacterial activity of the aloe vera leaf water fraction.

**FURTHER STUDY**

1. Similar research needs to be conducted with the replaced humectant, namely HPMC 1000DB replaced with CMC, to determine the physical properties of the gel preparation contained in HPMC 1000DB and CMC whether there are significant differences.
2. Testing the antibacterial activity of the gel preparation was replaced using other bacteria besides Staphylococcus epidermidis, namely Staphylococcus aureus and Propionibacterium acnes which are bacteria that cause acne.Every research is subject to limitations; thus, you can explain them here and briefly provide suggestions to further investigations.

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