

## Development of standardized kaffir lime (*Citrus hystrix*) fruit peel extract as a gel for antioxidant and anti-acne

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### ABSTRACT

Topical acne treatment using antibiotics causes an increase in the resistance of acne-causing bacteria. Using natural ingredients is an effort to avoid resistance, such as kaffir lime fruit peel which contains antibacterial substances, namely alkaloids, flavonoids, and tannins. This study aims to determine the effect of increasing the concentration of condensed extract of kaffir lime fruit peel in gel dosage form on physical quality (pH value, viscosity, dispersion) and effectiveness as an antioxidant and anti-acne. The condensed extract was obtained by maceration with 95% ethanol and then standardization of specific and non-specific extracts was carried out. The dosage form chosen is hydrophilic gel. The concentrations of the condensed kaffir lime fruit peel extract used in the gel are F1 (10%), F2 (15%), and F3 (20%). The gel preparation was tested for physical quality and effectiveness, consisting of antioxidant activity ( $IC_{50}$ ) using the DPPH method and antibacterial activity against *Cutibacterium acnes* using the diffusion method. Experimental data between batches and between formulas were analyzed using the One-Way ANOVA statistical method. If there is a significant difference in statistical analysis between formulas, then the test is continued using the Tukey post-hoc test method. The experimental results showed that increasing the concentration of kaffir lime fruit peel extract (*Citrus hystrix*) caused a decrease in pH and viscosity values as well as an increase in the ability to spread the gel preparation. Increasing the extract concentration also causes an increase in the anti-acne effect with the largest inhibition zone ( $18.27 \pm 0.306$  mm), and effectiveness as an antioxidant with the smallest  $IC_{50}$  value ( $15.51 \pm 0.15$  mg/mL) in formula 3. It was concluded that the best antioxidant and anti-acne gel is the F3 formula with an extract concentration of 20%.

**Keywords:** anti-acne, antioxidant, *Citrus hystrix*, kaffir lime fruit peel extract, gel

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## INTRODUCTION

Acne is a facial condition with clogged skin pores due to excess oil (sebum) production (Krysandrika, 2023). Acne treatment can be oral or topical. The advantages of using oral and topical antibiotics are more effective in dealing with acne. At the same time, the drawback is easy to develop bacterial resistance to antibiotics, such as irritation (Dessinioti et al., 2022; Kayiran et al., 2020) which states that an increase in the prevalence of resistant *Cutibacterium acnes* was found in clindamycin antibiotics from 45% to 91% and tetracycline antibiotics from 5% to 26.4%.

Based on this, it is necessary to develop anti-acne preparations made from natural ingredients that are expected to be effective and more friendly to facial skin, with the risk of antibiotic resistance being avoided. Many natural ingredients have compounds that have medicinal properties so they have the potential to become an alternative in treatment. kaffir lime (*Citrus hystrix*) is a natural ingredient that is widely used by the community and kaffir lime contains alkaloids, flavonoids (naringenin and hesperidin, and tannins as antibacterial) (Rusmiat et al., 2023). Meanwhile, the most common flavones in kaffir lime fruit peel are naringenin and hesperidin (Guan et al., 2020). The potential effect of kaffir lime fruit peel which has been widely studied, namely as an antioxidant using the DPPH method at a concentration of 100 mg/mL, gives a result of 92.78% of inhibition higher than the percentage of inhibition for other extracts (Ramli et al., 2020). In another study, the IC<sub>50</sub> value of kaffir lime leaves was 279.03 mg/mL tested using the DPPH method (Othman et al., 2023).

This research was conducted to increase the effectiveness of using kaffir lime peel in the form of a condensed extract as an anti-acne by developing a formulation in hydrophilic gel preparations. The gel form has a better penetration speed than the cream or ointment form, considering that the gel base has a high-water content, which causes an increase in the hydration, making it easier for the gel to penetrate, easy to wash off, and provides a cooling feeling. Application on the skin can reduce the risk of inflammation due to oil production in the skin pores, making it suitable for use as an anti-acne preparation. In addition, the gel form spreads quickly on the skin, the appearance of the color is clear, and it causes a cold sensation so that the acceptability by consumers is greater (Safitri et al., 2020). This study aimed to determine the effect of increasing the concentration of kaffir lime fruit peel extract gel preparations on physical quality and effectiveness and the correlation between increased antioxidant activity and its anti-acne (antibacterial) properties. The utilization of kaffir lime fruit peel (*Citrus hystrix*), a native plant of Indonesia, as a cosmetic product is expected to contribute to the discovery of complementary and alternative therapies for anti-acne.

## MATERIALS AND METHOD

### Materials

The kaffir lime fruit peel was used as an active ingredient obtained from the local market around Surabaya. Gel base excipients consisting of carbopol 941 (Corel Pharma, Meshana, Gujarat- India), TEA (Petronas Chemicals, Labuan, Malaysia), Tween 80 (PT Brataco, Surabaya-Indonesia) were selected. Glycerin (PT Suma Asih, Indonesia) and IPM (Oleon Port Klang, Selangor, Malaysia.) as a solubilizer and penetrant enhancer was added to increase the penetration speed of the gel, and distilled water was used as a solvent. All excipients used were of analytical grade. Other ingredients with a pro analytical grade are DPPH reagent (Sigma Aldrich, Germany) as a reagent used in the antioxidant activity test, Ethanol 96% as a solvent (Merck, Darmstadt, Germany), Tryptic Soy Agar as a growth medium for *Propionibacterium acnes* (Merck, Darmstadt, Germany), bacteria *Cutibacterium acnes* obtained from the Microbiology Laboratory of the Faculty of Pharmacy – WMCSU.

### Methods

#### Preparation and Standardization of dried simplicia powder of kaffir lime fruit peel

The kaffir lime fruit used is immature, characterized by green skin with a rough texture. The peel of the kaffir lime fruit dried in an oven at a temperature of around 50°C. The dried powder simplicial of kaffir lime peel is standardized to be specific and non-specific. The Specific standardization includes: water content, total ash content, acid soluble ash content. Water soluble ash content. Specific

standardization includes: organoleptic, pH, solubility in water, solubility in ethanol (Departemen Kesehatan RI, 2000; Darsono et al., 2020).

#### **Preparation and standardization of kaffir lime fruit peel condensed extract (*Citrus hystrix*)**

Preparation of condensed extract of kaffir lime fruit peel by weighing 500 grams, then put into a macerator and 96% ethanol solvent (1:2) at room temperature 25°C for 24 hours. Yield presentation was calculated against the volume of the liquid extract before being evaporated. The condensed extract of kaffir lime peel is standardized to be specific and non-specific (Departemen Kesehatan RI, 2000; Darsono et al., 2022b).

#### **Determination of the profile of active compounds: naringenin and hesperidin in the condensed extract of kaffir lime fruit peel (*Citrus hystrix*)**

Analysis of active compounds of naringenin and hesperidin refer to Dianingati et al. (2017) using the TLC method modification. As a standard solution, naringenin and hesperidin were used with a concentration of 0.5% w/v. Each 8 µl of extract and standard solution was individually spotted onto silica gel 60 GF<sub>254</sub> TLC plates (250 µm thickness). The plates were eluted in ethyl acetate: methanol: formic acid (95:5:0,5 %v/v). After drying, the TLC plates were examined under UV light at 366 and 254 nm (Camag UV cabinet, USA). The R<sub>f</sub> values of the interesting spots were calculated.

#### **Preparation of antioxidant and anti-acne gel containing condensed extract of the kaffir lime fruit Peel (*Citrus hystrix*)**

The gel formula based on antioxidant and anti-acne containing a condensed extract of kaffir lime fruit peel in this study refers to the research of Forestryana & Rahman, 2020 and Wulaningsih, 2010, which has been modified as shown in Table 1. Modification of the formula was carried out at the concentration of condensed extract kaffir lime fruit peel starting with a concentration of 10% (F1), 15% (F2), 20% (F3) and gel bases (FB), and adding isopropyl myristate (IPM) as a penetrant enhancer to increase penetration ability can reach the intended target site and glycerin for moisturizing.

**Table 1. Reference formula and modified formula for antioxidant and anti-acne gel containing condensed extract of kaffir lime fruit peel (*Citrus hystrix*)**

Composition Reference formula	Composition Formula	Modified	Modified Formula (%)			
			F1	F2	F3	FB
	<b>Kaffir lime fruit peel condensed extract</b>	<b>10</b>	<b>15</b>	<b>20</b>	<b>-</b>	
Carbopol 940	Carbopol 940	0.5	0.5	0.5	0.5	
TEA	TEA	0.5	0.5	0.5	0.5	
Tween 80	Tween 80	0.1	0.1	0.1	0.1	
	<b>IPM</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>	
	<b>Glycerin</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	
Aquadest	Aquadest (until)	100	100	100	100	

#### **Physical quality test of antioxidant and anti-acne gel containing of kaffir lime fruit peel extract (*Citrus hystrix*)**

##### *Appearance*

The appearance of the prepared formulations including constitution, color, smell, and homogeneity was observed by visual observation.

##### *pH test*

Thoroughly weigh 100 grams of antioxidant and anti-acne gel in a glass beaker and determine the pH using a Methron 744 pH meter. The pH of antioxidant and anti-acne gel tests were replicated three

times for each batch. The general requirement for the pH of the gel preparation is the skin pH, which is 4.5-6.5. The expected specifications are pH  $5.0 \pm 0.5$ .

#### *Viscosity test*

The viscosity test refers to the study of [Flieger et al., 2021](#) with the following modifications. A viscosity test was conducted using a Brookfield viscometer LVDV-I+ (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) with spindle S63 and speed 0.6 rpm for 250 mL of antioxidant and anti-acne gel. The general requirements for the viscosity of a gel preparation containing the gelling agent carbopol 940 NF Polymer will provide a thickness between 40,000-60,000 cPs at a pH value of 7.5-7.7 ([Power and Ecu, 2013](#)). The expected viscosity specification is 40,000 s / d > 60,000 cPs ([Safitri et al., 2020](#)).

#### *Spreadability test*

The spreadability test aims to determine the ability of the preparation to spread on the skin's surface. The spreadability test refers to the research of [Shriwas et al. 2019](#) with the following modifications. The spreadability of the anti-acne gel was measured by spreading 0.5 g of the gel on a 2 cm diameter circle marked on a glass plate, then a second glass plate was used and pressed using two types of weight (50 grams and 100 grams). The diameter of the circle after spreading the gel was determined. The general requirements for dispersion for gel are 5-7 cm. The specifications expected in this study are  $5.0 \pm 0.5$  cm, with criteria easy to spread.

### **Antioxidant activity of antioxidant and anti-acne gel containing condensed extract of kaffir lime fruit peel (*Citrus hystrix*) using the DPPH method with a spectrophotometer microplate reader**

#### *Preparation of 0.5 mM of DPPH solution*

The DPPH 0.5 mM solution (concentration 200 ppm) was pipetted as much as 5 mL, put into a measuring flask, and diluted with methanol ad 25 mL as a blank solution (40  $\mu\text{g/mL}$ ). The DPPH solution with a concentration of 40  $\mu\text{g/mL}$  was homogenized and the absorption was measured at a wavelength of 400-800 nm ([Molyneux, 2004](#)).

#### *Preparation of test solution*

The gel preparation of each formula (F1, F2, and F3) was weighed as much as 1 gram and dissolved in 10 mL methanol pro analysis and then homogenized using vortex. The preparation of the control solution consisted of 100  $\mu\text{L}$  of DPPH solution mixed with 100  $\mu\text{L}$  of methanol pa. The preparation of the sample solution consisted of a test solution mixed with a 0.5 mM DPPH solution at a ratio of 1:1. The preparation of a blank sample solution consisted of 100  $\mu\text{L}$  of the test solution and 100  $\mu\text{L}$  of methanol pro analysis. The preparation of the control solution, sample solution, and blank solution were pipetted into a 96-well plate well. After incubation for 45 min at room temperature in the dark, the decrease in absorbance of each solution was read at 517 nm using a microplate reader (Multiscan GO *Microplate Reader* (Thermoscientific, Finlandia) against a blank (methanol). A solution of l-ascorbic acid (100  $\mu\text{L}$ ), at concentrations between 5 and 50  $\mu\text{g/mL}$  in methanol was used as a positive control. All experiments were determined in triplicate. Furthermore, the absorbance value obtained is used to determine the  $\text{IC}_{50}$  value. Based on the data obtained, the correlation between extract concentration and increase in the  $\text{IC}_{50}$  value was determined with the parameter in the form of calculated R values at  $\alpha = 0.05$  and  $\text{df} = 1$  ([Darsono et al., 2020](#)).

### **Antibacterial activity of anti-acne gel containing condensed extract of kaffir lime fruit peel (*Citrus hystrix*) by well diffusion method**

#### *Preparation of Bacteria Suspension*

*Cutibacterium acnes* in TSA slanted taken one ose, suspended into the TSB, and then measured for turbidity with standard Mc Farland I ( $1.5 \times 10^8$  CFU/mL).

### *Preparation of test solution*

Test solutions 10%, 15%, and 20% w/v were made by weighing 0.1 g; 0.15 g; and 0.2 g of kaffir lime fruit peel extract (*Citrus hystrix*), then each was dissolved in 1 mL of 1% DMSO.

### *Diffusion method antibacterial activity test*

The bacterial suspension was first equalized to Mc Farland I ( $1.5 \times 10^8$  CFU/ml), then 0.1 mL was inoculated into 10 mL TSA and poured into sterile Petri dishes. Then pre-curing for 1.5-2 hours at 37°C, then perforating with a 6 mm diameter perforator. Kaffir lime peel extract (*Citrus hystrix*) with a concentration of 10%, 15%, and 20% was put into the well as much as 20  $\mu$ L. Added positive controls using the antibiotic clindamycin 2  $\mu$ G/20 $\mu$ L into the wellbore, and negative control was introduced using 1% DMSO 20  $\mu$ L. The media was incubated for 24 hours at 37°C (Binder, Germany). After incubation, a clear area around the hole was observed, indicating microbial growth inhibition. The antimicrobial activities were measured as the diameter (mm) of the inhibition zone ( $n = 3$ ). Gel preparations have antibacterial activity if the DHP value is more significant than 6.0 mm (Soegianto et al., 2016).

## **RESULT AND DISCUSSION**

### **Results of standardization of dried powder of simplicia of kaffir lime fruit peel (*Citrus hystrix*)**

Standardization carried out includes non-specific and specific. The results of the non-specific standardization of dried simplicia powder of kaffir lime fruit peel (*Citrus Hystrix.*) can be seen in Table 2. Non-specific standardization of Simplicia powder of kaffir lime fruit peel (*Citrus hystrix.*) with parameters of water content aims to provide a minimum limit or range regarding the amount of water content in a material. If the water content value is relatively high, microbes and bacteria will easily contaminate the material. Enzymatic reactions quickly occur, which causes the active ingredients to be hydrolyzed. The results obtained for determining the water content are  $8.89 \pm 0.89\%$ , where these results meet the general requirements for water content that have been set, which is less than 10% (Departemen Kesehatan RI, 2000). The total ash content was determined the mineral content in the simplicial powder of kaffir lime fruit peel (*Citrus hystrix*). The test results were obtained at  $5.52 \pm 0.23\%$ , which means that the mineral content in the simplicial powder of kaffir lime peel (*Citrus hystrix*) met the requirements which are less than 7%.

### *Result of standardization of condensed extract of kaffir lime peel extract (*Citrus hystrix*)*

The results of making a condensed extract of kaffir lime fruit peel (*Citrus hystrix*) obtained an average yield calculated based on the weight of the dry simplicia powder, which was  $17.59 \pm 1.70\%$ . The standardization includes non-specific and specific. The results of standardization of the condensed extract of kaffir lime fruit peel extract can be seen in Table 2. The results of an organoleptic test of the condensed extract of kaffir lime fruit peel (*Citrus hystrix*) had a thick greenish-yellow color and a characteristic odor of kaffir lime fruit. The pH value of the condensed extract of kaffir lime fruit peel was  $5.59 \pm 0.02$ , which indicated that the viscous extract was acidic. Carried out the determination of water-soluble extract content and ethanol soluble extract content to determine the content of compounds in certain solvents according to their polarity level, kaffir lime peel has a higher solubility in ethanol than water solvents. The results of the determination of the water-soluble extract content of the kaffir lime peel (*Citrus hystrix*) were  $58.11 \pm 0.78\%$  and the results of the determination of the ethanol-soluble extract content of  $65.93 \pm 0.35\%$ . The full results of the standardization of condensed extracts can be seen in Table 2.

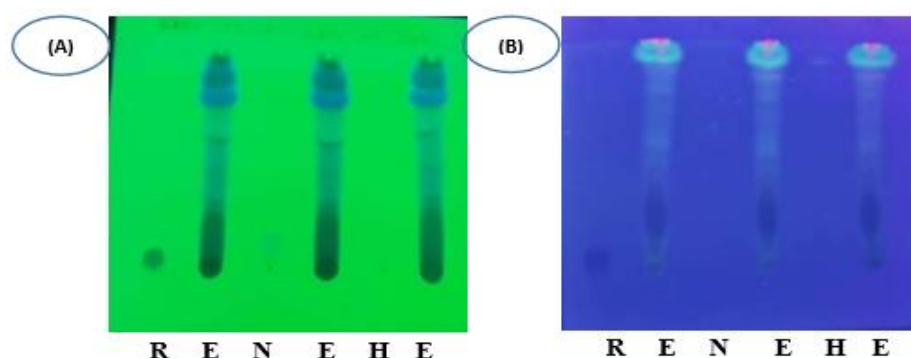
The active compound profile of condensed extract of kaffir lime fruit peel: hesperidin, or naringenin, was determined by thin layer chromatography with UV light 254 and 366 nm VIS. The results of the active substance profiles in the extract with the mobile phase of ethyl acetate: methanol: formic acid (95: 5: 0.5) %v/v and the stationary phase of silica gel 60 GF<sub>254</sub> showed that in the extract containing active compounds, namely hesperidin, naringenin, and routine.



The stain detected under 366 nm has a value of Rf 0.70 for a fluorescent blue colour is hesperidin, for a bluish-green fluorescent stain with a value of Rf 0.14 is naringenin. It is mostly for a green stain with an Rf of 0.07. The theoretical Rf values for the active substances rutin, naringenin, and hesperidin were 0.167, 0.88, and 0.07, respectively. The complete result of the TLC profile can be seen in Figure 1. The difference in Rf value is due to several factors, including extraction and solvent used during the TLC analysis process. Still, in general, it can say that the extract contains active substances that have anti-acne properties.

**Table 2. Standardization of dried powder simplicia and condensed extract of kaffir lime fruit peel (*Citrus hystrix*)**

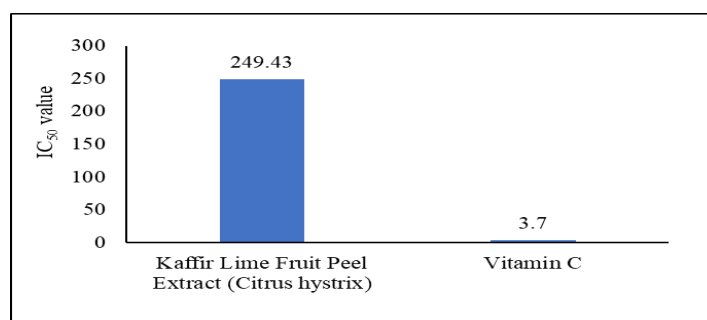
Test Type	Observation result	
	dried powder simplicia	condensed extract
<b>Specific Standardization</b>		
Form	Powder	Condensed
Colour	Yellow green	Yellow green
Smell	Kaffir lime	Kaffir Lime
pH (1% solution)	-	5.59 ± 0.02
Water Soluble Extract Level ( $\bar{x} \pm SD$ ) (%)	27.82 ± 3.77	58.11 ± 0.78
Ethanol Soluble Extract Level ( $\bar{x} \pm SD$ ) (%)	20.81 ± 0.58	65.93 ± 0.35
<b>Non Specific Standardization</b>		
Water content ( $\bar{x} \pm SD$ ) (%)	8.89 ± 0.89	17.82 ± 0.86
Total Ash Content ( $\bar{x} \pm SD$ ) (%)	5.52 ± 0.23	2.17 ± 0.22
Acid Insoluble Ash Content ( $\bar{x} \pm SD$ ) (%)	0.61 ± 0.001	0.04 ± 0.001
Water Soluble Ash Content ( $\bar{x} \pm SD$ ) (%)	5.17 ± 0.13	2.17 ± 0.42



**Figure 1. The comparative stain profile of hesperidin, naringenin, rutin, and condensed extract of kaffir lime peel (*Citrus hystrix*) by Thin Layer Chromatography (TLC) under UV light 254 nm (A), and UV light 366 nm (B) with R = rutin, N = naringenin, H = hesperidin, E = condensed extract of kaffir lime fruit peel**

#### **Results of antioxidant activity test of condensed extract of kaffir lime fruit peel (*Citrus hystrix*)**

The results of testing the antioxidant activity of the condensed extract of the kaffir lime fruit peel (*Citrus hystrix*) can be seen in Figure 2. Based on the results of the antioxidant power test of the condensed extract of kaffir lime fruit peel, it is known that it has antioxidant power according to the expected specifications. The lower the IC<sub>50</sub> value of a compound, the greater the antioxidant activity. The test results found that the IC<sub>50</sub> value of the condensed extract of kaffir lime fruit peel was 2.49 mg/mL (249.43 ± 0.51 µg/mL) and vitamin C was 3.70 ± 0.01 µg/mL.



**Figure 2. Antioxidant activity of kaffir lime fruit peel extract (*Citrus hystrix*) and vitamin c**

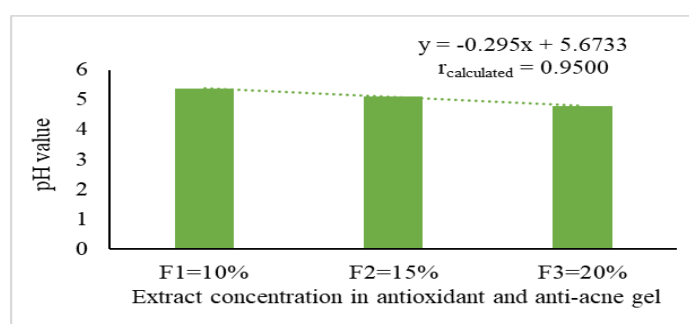
### Physical quality test results of anti-acne gel containing condensed extract of Kaffir Lime Fruit Peel (*Citrus hystrix*)

#### Organoleptic test results

Kaffir lime fruit peel extract has an organoleptic appearance such as yellow-green color, opaque gel form, and a characteristic kaffir lime odor. The blank formula has a white appearance, an opaque gel form, and no odor.

#### pH test results

The purpose of the pH observation was to determine the pH value of the anti-acne gel preparation of kaffir lime peel extract, following the pH of the skin to prevent skin irritation. The pH test results obtained can be seen in [Figure 3](#). Formulas F1, F2, and F3 gave pH values of  $5.37 \pm 0.06$ ,  $5.10 \pm 0.01$ , and  $4.78 \pm 0.00$ , respectively, which met the dosage specifications of  $5.00 \pm 0.05$ . The pH value of the blank formula (FB) is  $6.97 \pm 0.02$ . Based on the results of statistical analysis using the one-way ANOVA method, the calculated Fvalue ( $0.000 < F_{table}$  ( $\alpha = 0.05$ )) was obtained with a significance value (sig.) or p-value obtained of  $0.000 < 0.05$  which shows a significant difference between the formulas. The blank formula with the formula with condensed extract of kaffir lime peel has a different pH due to the influence of the acidic pH of the condensed extract, which is around  $5.59 \pm 0.02$ . The formula with a condensed extract of kaffir lime peel has a different pH value for each formula, and this is because the effect of the concentration of the extract used is increasing, increasing the acidity of the preparation.



**Figure 3. The relationship between pH values and gel formula containing condensed extracts of kaffir lime fruit peel (*Citrus hystrix*) at various concentrations**

#### Homogeneity test results

A homogeneity test was conducted to determine the level of admixture of additives and active ingredients, in this case, the condensed extract of kaffir lime fruit peel. Homogeneous antioxidant and

anti-acne gel preparations can be seen whether there are lumps or coarse particles. The observations showed that the anti-acne gel preparations of condensed extract of kaffir lime fruit peel in each formula, namely F1, F2, F3, and FB, had a good level of homogeneity. Each formula provides an even texture, and no coarse particulates are visible, so the gel preparation can be said to be homogeny.

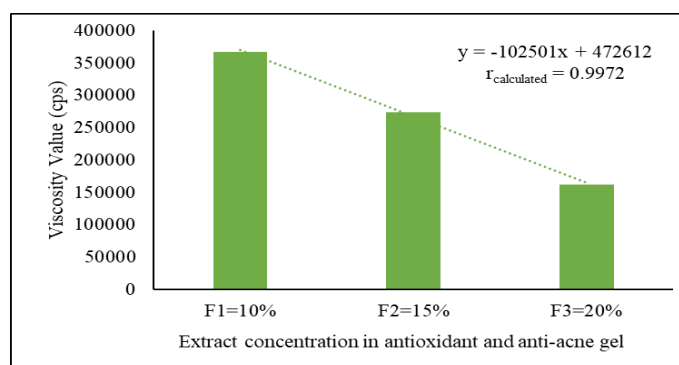
#### *Viscosity test results*

The viscosity test results obtained can be seen in [Figure 4](#). Viscosity testing determines the viscosity level of the anti-acne gel preparation of kaffir lime peel extract, which affects the dispersion parameters when applied to the skin surface. Viscosity with a low value will affect a large spread area. If the viscosity value is high, it will reduce the spread area, considering that increasingly viscous preparations will make it difficult to apply on skin reinforcement because the dispersion power is not good. The viscosity value of the anti-acne gel preparation of kaffir lime fruit peel extract is 40,000 to > 60,000 cPs ([Power and Ecu, 2013](#); [Safitri et al., 2020](#)). Viscosity observations of each formula were F1, F2, F3 and Fb, respectively:  $367,000 \pm 21,000$ ,  $273,833 \pm 1,833$ ,  $161,999 \pm 2,334$  and  $36,367 \pm 5,067$ . Based on the results of statistical analysis, they are using the one-way ANOVA method, and the calculated  $F_{\text{value}} (0.000) < F_{\text{table}} (\alpha = 0.05)$  shows that the data between formulas is significantly different. The ANOVA analysis shows that the significance value (sig.) or p-value obtained is 0.000, and this indicates that there are significant differences between the three tested formulas (sig. < 0.05 means that there is a significant difference between the tested groups). The formula with a condensed extract of kaffir lime peel has a different pH value for each formula. The increasing concentration of kaffir lime peel extract causes the viscosity value of the gel preparation to be smaller where the consistency of the preparation becomes thinner. Considering that the optimum pH of carbopol® as a gelling agent is in the range of pH values that are not too acidic, which is around neutral at pH 7.5 - 7.7 because carbopol® is stable at that pH and carbopol® is incompatible with strong acids ([Shah et al., 2021](#)). In its neutral form, carbopol is soluble in water, alcohol, and glycerin and will form a clear and stable gel. In an acidic solution (pH 3.5-4.0), carbopol® dispersion shows a low to medium viscosity, and at a pH of 5.0-10.0 and temperatures above 75°C will show optimal viscosity. In addition, the content of active substances that were successfully extracted in the kaffir lime peel extract also influenced the viscosity of the preparation, wherein the presence of the kaffir lime peel extract. The gel preparations formed a thicker mass with a consistency that could still pour because of the presence of astringent and ballast substances.

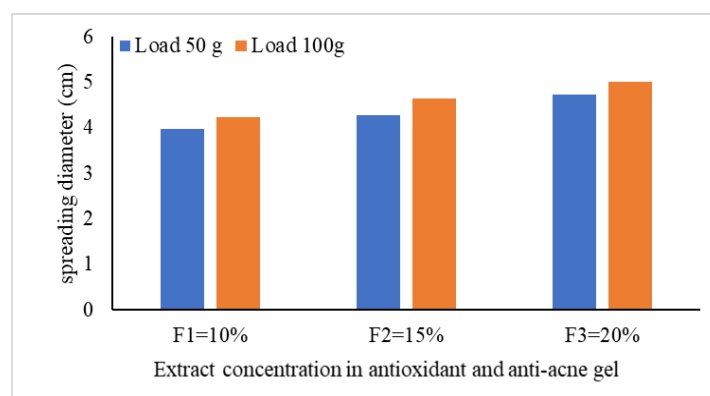
#### *Spreadability test results*

The spreadability test was carried out on each formula with the aim of knowing the ease with which the preparation spread when used. Determination of spreadability using two types of weights, namely 50 and 100 grams, aims to determine the amount of hand pressure when applied to the surface of facial skin, which provides good spreadability. The results of the dispersion test obtained can be seen in [Figure 5](#). The results of observations of dispersion with a load of 50 grams from each formula F1, F2, F3, and FB are as follows:  $3.98 \pm 0.00$ ,  $4.28 \pm 0.11$ ,  $4.73 \pm 0.12$ ,  $5.53 \pm 0.20$ . As for the load of 100 grams, the results were as follows:  $4.23 \pm 0.00$  (F1),  $4.65 \pm 0.20$  (F2),  $5.01 \pm 0.18$  (F3) and  $6.09 \pm 0.02$  (FB). Based on the results of statistical analysis using the one-way ANOVA method, the calculated  $F_{\text{value}} (0.000) > F_{\text{table}} (\alpha = 0.05)$  shows that the data between formulas is significantly different. The ANOVA analysis shows that the significance value (sig.) or p-value obtained is 0.000, and this indicates that there are significant differences between the three tested formulas (sig. < 0.05 means that there is a significant difference between the tested groups). The ability of the preparation dispersion to the viscosity value has an inversely proportional value. Each formula has a high viscosity, but low spreadability but is still within the required range. Conversely, if the formula has a low viscosity and high dispersion value. The higher the concentration of the kaffir lime peel extract used in the formula, the lower the viscosity value, so it can say that the preparation is getting dilute, which impacts the higher dispersing ability ([Chellathurai et al., 2023](#)).





**Figure 4. Relationship between viscosity value and gel formula containing condensed extracts of kaffir lime fruit peel (*Citrus hystrix*) at various concentrations**



**Figure 5. Relationship between spreadability and gel formula containing condensed extracts of kaffir lime fruit peel (*Citrus hystrix*) at various concentrations**

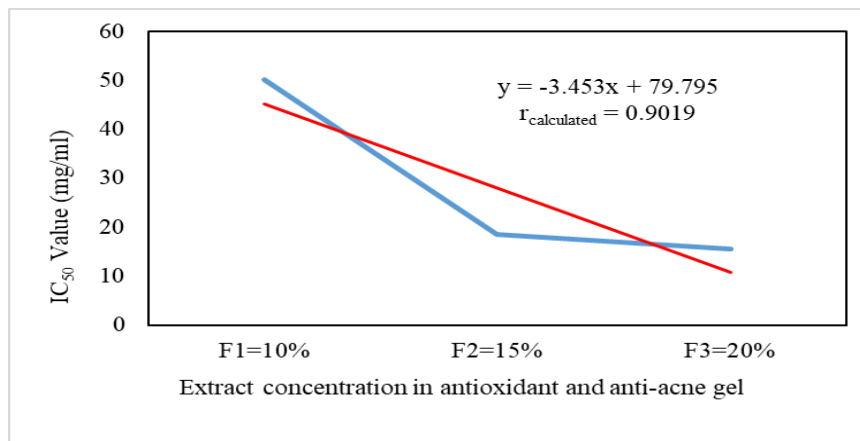
### Test results of the effectiveness of antioxidant and anti-acne gel containing condensed extract of kaffir lime fruit peel (*Citrus hystrix*)

#### Antioxidant test results

The results of the antioxidant test preparations obtained can be seen in Figure 6. From the previous research data, the  $IC_{50}$  value of the condensed extract of kaffir lime fruit peel was found to be 2.49 mg/mL and the Vitamin C as a comparison was found to be 37  $\mu$ g/mL. In this research, three gel preparation formulations were made with 3 different concentration of condensed extract of kaffir lime fruit peel (F1, F2, and F3) and the antioxidant activity was tested for each formulation. It was found that the  $IC_{50}$  value for F1 was  $50.04 \pm 11.28$  mg/mL, for F2 was  $18.45 \pm 2.89$  mg/mL, and for F3 was  $15.51 \pm 0.15$  mg/mL. The difference in antioxidant power that occurs is due to an increase in the concentration of kaffir lime peel extract from a concentration of 10% (F1), 15% (F2), and 20% (F3), increasing the number of nutritious active compounds contained in the extract so that the impact on increasing their antioxidant power.

The antioxidant activity of kaffir lime peel is due to the content of flavonoid compounds, namely naringenin and hesperidin, which have antioxidant activity. Judging from the basic structure of flavonoids, the presence of hydroxyl group (-OH) attached to the aromatic ring can break down free radicals, or also called ROS (Reactive Oxygen Species) because they have high reactivity as hydrogen donors which will stabilize free radicals. Increasing the concentration in formula 3 causes the amount of flavonoids contained in it to be greater. Therefore, the  $IC_{50}$  value in formula 3 shows the lowest

value, or it could be said that the antioxidant activity of formula 3 is the highest compared to the other two formulas. Furthermore, statistical tests were conducted to determine whether there was a significant difference between the formula and the antioxidant activity obtained. The calculation result shows a p-value of 0.02, which is smaller than 0.05 and indicates a significant difference between the formula and the antioxidant activity.



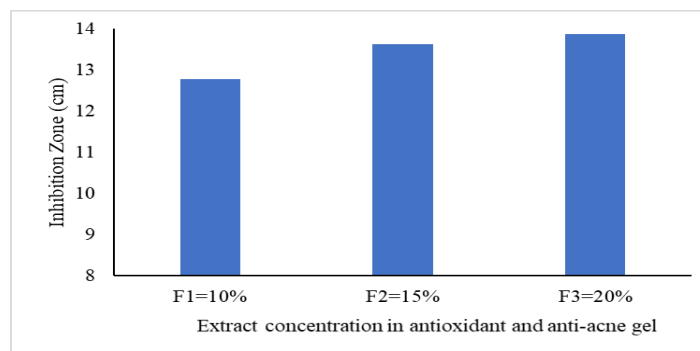
**Figure 6. Relationship between increasing concentration (%) of the condensed extract of kaffir lime fruit peel (*Citrus hystrix*) in anti-acne gel preparations on the IC<sub>50</sub> value**

#### **Results of antibacterial effectiveness test against *Cutibacterium acnes* with the well diffusion method of anti-acne gel containing condensed extract of kaffir lime fruit peel (*Citrus hystrix*)**

*The results of antibacterial activity test of kaffir lime fruit peel extract (Citrus hystix) and anti-acne gel containing kaffir lime fruit peel extract against Cutibacterium acnes*

The activity test was carried out using the diffusion method. The results of the antibacterial activity test of kaffir lime fruit peel extract (*Citrus hystix*) and kaffir lime peel extract gel preparations against *Cutibacterium acnes* can be seen in [Figures 7](#). The test results showed the presence of antibacterial activity of antioxidant and anti-acne gel containing kaffir lime fruit peel extract (*Citrus hystix*) at concentrations of 10%, 15%, and 20% with zone inhibition (ZI) areas respectively  $12.78 \pm 0.370$  mm,  $13.63 \pm 0.154$  mm,  $13.87 \pm 0.118$  mm. The results of the ZI data were analyzed using ANOVA statistics, and the significance value (sig.) or p-value was 0.000, and this indicates a significant difference between the three tested formulas (sig. < 0.05 means a significant difference between the groups tested). The test results of ZI with 1% DMSO and gel base had no activity, and this indicates 1% DMSO solution used to dissolve the kaffir lime fruit peel extract and the gel base used to make the kaffir lime peel extract gel did not affect the results of the antibacterial activity against *Cutibacterium acnes*. The difference in antimicrobial activity obtained with the ZI parameters was due to differences in the concentration of the kaffir lime fruit peel extract used. The higher the concentration, the greater the content of the active ingredients diffuse through the agar medium, thereby providing greater ZI. Besides that, it is also supported by the higher concentration of kaffir lime fruit peel extract. The more acidic the gel preparation causes the preparation to be more dilute, making it easier for the diffusion process to the agar medium containing the bacteria *Cutibacterium acnes*. The antibacterial activity of kaffir lime fruit peel extract is due to the presence of phenolic and flavonoid compounds that inhibit bacterial growth by inhibiting cell membrane function, interfering with cell membrane permeability, and inhibiting essential enzymes such as ATPase and phospholipase ([Hasan et al., 2022](#)). The antibacterial activity of gel preparations containing kaffir lime fruit peel extract was lower than that of unformulated kaffir lime fruit peel extract. The inhibition zone produced from kaffir lime fruit peel condensed extract differ from the gel form. It is due to the acidic nature of the condensed extract of

kaffir lime fruit peel, which affects the viscosity of the gel, and the ability of the gel to diffuse into media is limited. The effectiveness of carbopol as a gelling agent depends on the pH value of the gel. Based on the study's results, it was concluded that the increase in the concentration of kaffir lime peel extracts affected the physical qualities: pH, viscosity, and dispersion, as well as effectiveness as antibacterial and antioxidant. Increasing the concentration of kaffir lime peel extract led to a significant increase in effectiveness as an antioxidant and anti-acne.



**Figure 7. Antibacterial activity of Antioxidant and Anti-acne Gel containing kaffir lime fruit peel extract**

The formula was determined as the best formula that fulfilled all the specifications of the physical quality test that referred to the general requirements of the gel preparation and had the best anti-acne effectiveness and antioxidant activity. Based on the overall physical quality evaluation results, formula 3 (20% kaffir lime peel extract concentration) is a formula that better meets all test specifications.

## CONCLUSION

Increasing the concentration of kaffir lime peel extract (*Citrus hystrix*) (10%, 15%, and 20%) affects the results of physical quality (pH, viscosity, spreadability) and the effectiveness of the preparation as an anti-acne and antioxidant. Formula 3 (20% concentration of kaffir lime peel extract) is the best anti-acne gel formula that meets the specifications for physical quality and activity antibacterial against *Cutibacterium acnes*.

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