

# Formulation and Activity Test of Grapefruit Peel Extract Anti-Acne Gel Preparation (*Citrus maxima* Merr.) Against *Staphylococcus aureus* and *Propionibacterium acne* Bacteria

Saparuddin Latu<sup>1\*</sup>, Jangga<sup>1</sup>, Abdul Wahid Suleman<sup>1</sup>, Hardyanti Eka Putri<sup>2</sup>, Sriyana Herman<sup>3</sup>, Rifa'atul Mahmudah<sup>2</sup>, Rusli<sup>4</sup>, Nurhikmah<sup>5</sup>

<sup>1</sup>Faculty of Pharmacy, University of Megarezky Makassar, Indonesia

<sup>2</sup>Magister of Pharmacy, Postgraduate Program, University of Megarezky Makassar, Indonesia

<sup>3</sup>Magister of Reproductive Health, Postgraduate Program, University of Megarezky Makassar, Indonesia

<sup>4</sup>Department of Physiotherapy, Faculty of Health and Sport Sciences, State University of Makassar, Indonesia

<sup>5</sup>Department of physical education health and recreation, State University Muhammdiyah, Luwuk Banggai, Indonesia

\*Corresponding author

**Dr. H. Saparuddin Latu, S.Si., M.H., M.Kes., Apt**

Master of Pharmacy Program, Postgraduate Program, University of Megarezky Makassar, Indonesia.

Email: [saparuddinlatu@unimerz.ac.id](mailto:saparuddinlatu@unimerz.ac.id)

DOI: <https://doi.org/10.36685/phi.v11i3.1015>

Copyright: © 2025 the Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium provided the original work is properly cited.

## Abstract

**Background:** Grapefruit (*Citrus maxima* Merr.) peel is known to contain bioactive compounds such as alkaloids, tannins, and flavonoids, which exhibit potential antibacterial properties, particularly against acne-causing bacteria.

**Objective:** This study aimed to formulate an anti-acne gel incorporating grapefruit peel extract, evaluate its antibacterial activity against *Staphylococcus aureus* (SA) and *Propionibacterium acnes* (PA), and determine the optimal extract concentration for inhibiting bacterial growth.

**Methods:** A laboratory-based experimental study was conducted using the well diffusion (pitting) method to assess antibacterial activity. Gel formulations containing grapefruit peel extract at concentrations of 5%, 10%, and 15% were tested against SA and PA.

**Results:** The formulated gels demonstrated acceptable physical stability. The inhibition zones against SA were as follows: 5% (F1) = 10.2 mm, 10% (F2) = 11.2 mm, and 15% (F3) = 11.1 mm. Against PA, the inhibition zones were: F1 = 11.7 mm, F2 = 13.3 mm, and F3 = 11.7 mm. The 10% formulation (F2) exhibited the highest antibacterial activity against both bacterial strains, falling within the "strong" inhibition category.

**Conclusion:** Grapefruit peel extract can be successfully formulated into an anti-acne gel and demonstrates effective antibacterial activity against *Staphylococcus aureus* and *Propionibacterium acnes*. Among the tested concentrations, 10% was identified as the most effective for bacterial inhibition.

**Keywords:** grapefruit peel; *Citrus maxima*; extract; antibacterial activity; anti-acne gel

## Article History:

Received 7 December 2024

Revised 24 February 2025

Accepted 9 December 2024

## Background

Indonesia, in a tropical region, experiences a hot climate characterized by abundant sunlight throughout the year. This climatic condition can stimulate the skin to produce excess oil, particularly on the face, leading to a higher prevalence of skin issues among the population. Acne is a common skin condition, particularly among adolescents, and it can negatively affect self-confidence (Nasution et al., 2022). This skin condition is caused by chronic inflammation of the

polysebaceous follicles, with nearly everyone experiencing acne at some point in their lives. The highest prevalence of acne occurs among females aged 14-17 years (83-85%) and males aged 16-19 years (95-100%). This indicates a significant age-related trend in the incidence of acne.

According to surveys conducted in Southeast Asia, the prevalence of acne ranges from 40% to 80%. In Indonesia, records from the Indonesian Aesthetic Dermatology Research indicate that the number of cases rose from 60% in 2006 to 80% in 2007, and by 2009, it reached 90%. It is reported that 80% of teenagers have experienced this condition, which is characterized by clinical features such as blackheads, papules, pustules, nodules, and scarring, all of which can interfere with appearance. Several factors influence the development of acne, including changes in keratinization patterns, increased sebum production, the formation of free fatty acid fractions, elevated bacterial counts, increased androgen hormones, and psychological factors. Additionally, factors such as age, race, diet, and environmental conditions can trigger the onset of this skin disorder (Sifatullah & Zulkarnain, 2021).

Given the high prevalence of acne, the demand for effective skincare solutions has significantly influenced the growth of the cosmetics industry. According to the Ministry of Industry, the national cosmetics industry recorded a 20% growth increase or four times the national economic growth in 2017. According to Airlangga, the Ministry has placed the cosmetics industry as a mainstay sector as stipulated in the National Industrial Development Master Plan (RIPIN) 2015-2035. The domestic cosmetics industry increased by 153 companies in 2017, so currently, the number reaches more than 760 companies. Airlangga revealed that Indonesia is one of the large cosmetics markets, so this business will be prospective and promising for manufacturers who want to develop it in the country. Based on the growth of the cosmetics industry, there are many choices in beauty products (Saniati et al., 2020).

As the cosmetics industry in Indonesia continues to grow, there is an increasing demand for innovative skincare products. This presents an opportunity for the development of natural acne treatments, such as those using Grapefruit Peel Extract (GPE), which may address both the rise in acne cases and the growing consumer interest in natural skincare solutions.

With a population of around 250 million, Indonesia is a promising market for cosmetics companies. The majority of the cosmetics industry targets women as its main consumers and recently started to innovate with products for men. According to data from the Association of Indonesian Cosmetic Companies (Perkosmi) in the Ministry of Industry of the Republic of Indonesia, in 2012 sales of imported cosmetics reached Rp 2.44 trillion, up 30% from Rp 1.87 trillion in 2011. In 2013, sales of imported cosmetic products are projected to rise another 30 percent to IDR 3.17 trillion. The increase was supported by an increase in sales volume and a decrease in import duty rates in line with free trade agreements (Putri, 2018).

As cosmetic products have now become part of people's daily needs, both for men and women, they serve various purposes. Cosmetics are products that are used on the outside of the body for cleansing, beautifying, changing appearance, and repairing or maintaining the body in good condition. External appearance plays an important role in social interaction, and in various cultures, there is still a general perspective on appearance, namely that someone who has white skin is considered a sign of beauty in both men and women (Septianingrum et al., 2023). Based on its use, cosmetics are divided into two groups: makeup cosmetics (make-up), which are needed to beautify the appearance of the skin, and skincare cosmetics, which are prioritized to maintain skin cleanliness and health, sometimes even to eliminate abnormalities in the skin (Briliani et al., 2016). There are so many pharmaceutical preparations that can be made as acne medications derived from active plant compounds, but acne treatment using gels is reportedly better than cream preparations because gel preparations are easier to remove from the skin surface due to polar solvents and gels do not contain oils that can aggravate acne (Febriyani et al., 2018).

Acne, clinically known as *Acne Vulgaris*, is a prevalent skin condition characterized by the presence of comedones, papules, and pustules, primarily caused by the proliferation of *Propionibacterium acne* (PA) and *Staphylococcus aureus* (SA). Recent studies have highlighted the antibacterial properties of grapefruit peel (*Citrus maxima* Merr.), which contains various bioactive compounds that may inhibit the growth of these acne-causing bacteria.

Several studies (Aliyah et al., 2022; Pariury et al., 2021; Saputra et al., 2017) have demonstrated that grapefruit peel contains antibacterial compounds like flavonoids, alkaloids, and tannins, which have shown inhibitory effects against acne-causing bacteria such as PA and SA. For instance (Pariury et al., 2021) found that a 75% ethanol extract of grapefruit peel can inhibit PA, with an inhibition zone diameter of 4.99 mm, indicating weak antibacterial activity. However, an 80% concentration of grapefruit peel ethanol extract showed moderate antibacterial effects against PA, with an inhibition zone diameter of 5.29 mm. Additionally (Saputra et al., 2017) reported that grapefruit peel possesses antibacterial activity against *E. coli* and SA, with the degree of inhibition being directly proportional to the concentration of essential oils, particularly flavonoids, used in the extracts.

Furthermore, the nutritional components of grapefruit peel have been extensively studied. Aliyah et al., (2022) identified several key compounds, including alkaloids, flavonoids, terpenoids, lycopene, and vitamin C, with phenolic compounds such as pectin and tannin constituting approximately 23% of grapefruit peel. These compounds exhibit

antibacterial properties by disrupting bacterial metabolism, thereby inhibiting growth and survival. Also emphasized the richness of grapefruit in essential nutrients, noting that the antimicrobial potential extends to its leaves, which contain similar metabolites to those found in sweet orange (*Citrus sinensis*) and lemon (*Citrus limon*) leaves (Ben Hsouna et al., 2023).

Despite the promising antibacterial properties of grapefruit peel, its potential as an acne treatment remains underexplored in the scientific community. This research aims to fill that gap by testing the antibacterial effects of GPE against SA and PA, both of which are associated with acne development.

## Methods

### Design, Place, and Time

This research design is a type of pre-clinical trial, this research is to formulate and test the antibacterial activity of Anti-Acne Gel preparations of Ethanol Extract of Grapefruit Peel (*Citrus maxima* Merr.) against the growth of SA and PA bacteria. This research was conducted in the Laboratory, namely the Pharmaceutical Preparation Technology Laboratory, Phytochemistry Laboratory and Pharmaceutical Microbiology at Megarezky University Makassar in April 2023.

### Material and Tools

In this study, a variety of tools and materials were utilized to achieve the desired objectives. The tools employed included an analytical balance (Osuka®), autoclave (Gea®), beaker (Iwaki®), beaker glass, blender (Panasonic®), 101arbop burner, coarse cloth, drop pipette, 101arbopol101r flask (Pyrex®), filter paper, fine cloth, gauze wire, gel container, glass funnel, glass jar, incubator (B-One®), laboratory equipment, lemon perfume, measuring cup (Pyrex®), mortar, oven (B-One®), Ph meter (Mediatech®), petri dish (Normax®), porcelain cup (Holdenwanger®), propylene glycol, refrigerator (Polytron®), rotary evaporator (Eyela®), round ose needle, scissors, spirit lamp, spray bottle, sterile cotton, stirring rod, triangle, transparent glass, tweezers, and vernier caliper (Insize®). Additionally, the materials used in this study comprised alcohol, 101arbopol, DMDM hydantoin, glycerin, GPE (*Citrus maxim* Merr.), physiological sodium chloride, culture of SA and PA, Wardah Acnederm®, and triethanolamine. The careful selection and application of these tools and materials were crucial in ensuring the success of the experiments and obtaining accurate results.

### Population and Sample

Grapefruit peel samples were collected in Kandeapi, Segeri District, Pangkep Regency, South Sulawesi. The grapefruit is washed under running water and then peeled to separate from the skin. In the next stage, a chopping process is carried out to speed up the drying process. The grapefruit peel is washed in running water and drained. The drying process is carried out by aerating at room temperature, which is not protected from direct sunlight, and typically takes between 8 to 12 hours to ensure the grapefruit peel samples are adequately dried. The dried simplisia was blended until a coarse powder was obtained.

### Preparation of Extract

GPE is done by maceration method. Put 500 grams of coarse powder into a jar, then soak in 96% ethanol solvent. Close the jar and stir occasionally, let stand for 3 days, change the solvent every 24 hours to produce a clear filtrate, then the filtrate is concentrated with a rotary evaporator until the extract is thick.

### Formulation of Gel GPE

**Table 1.** Formulation of Gel GPE

Ingredient	Function	Amount (%)				
		F1	F2	F3	K-	K+
GPE	Active Substance	5	10	15	-	
Carbopol	Base	0.5	0.5	0.5	0.5	
Triethanolamine	Alkalizing Agent	0.4	0.4	0.4	0.4	
Propylene Glycol	Humectant	10	10	10	10	Wardah
Glycerin	Emollient	2	2	2	2	Acnederm®
DMDM Hydantoin	Preservative	0.5	0.5	0.5	0.5	
Lemon Fragrance	Fragrance	q.s	q.s	q.s	q.s	
Aquadest	Solvent	ad 100	ad 100	ad 100	ad 100	

Notes: 5% of gel GPE (F1); 10% of gel GPE (F2); 15% of gel GPE (F3); Positive control (K+); Negative control (K-).

The tools and materials needed for the preparation have been prepared. In the formulation, a ratio of three concentrations using GPE (*Citrus maxima* Merr.) is employed, with the dry extract being weighed at concentrations of 5%,

10%, and 15%. The ingredients are weighed according to the specified formula. For gel preparation, Carbopol is dissolved in hot water at 80°C and stirred until homogeneous. Triethanolamine, propylene glycol, glycerin, and DMDM hydantoin are added into a beaker and stirred until homogeneous. This mixture is then gradually added into a mortar containing Carbopol and ground until homogeneous. Five milligrams of GPE are added. Then, 100 ml of distilled water and an appropriate amount of lemon perfume are added, stirred until homogeneous, and finally, the mixture is transferred into the prepared container.

### **Stability Test of GPE Gel**

#### **Organoleptic**

Organoleptic tests are carried out to see whether the preparation has been physically finished by observation using the senses including color, shape, and smell to see whether there are changes or not after manufacture (Febrina Karim, 2021). Organoleptic testing is done directly to see the shape, color, and smell of the gel. Gels are usually clear with a semi-solid consistency (Suhery et al., 2023).

#### **Homogeneity**

The homogeneity test is carried out to determine whether the active ingredients are evenly distributed to see if the base and active ingredients are well mixed. This examination is carried out by applying the preparation to the objective glass, and then closing the objective glass above it, then pressing until the entire surface is evenly covered (Febrina Karim, 2021). Testing can be seen based on the absence of coarse grains, or materials that do not mix well, and form lumps (Suhery et al., 2023).

#### **pH**

The pH test is carried out to see that the pH stability is still within the limits of the pH requirements for topical preparations of 4.5-6.5 and for a good facial skin pH of 4-8 so that no irritation occurs after application, which is done by measuring the preparation using a pH meter (Febrina Karim, 2021). Testing the pH value is a characteristic that must be considered in the formulation of topical preparations. The pH test is intended to determine whether the pH value of a preparation is acceptable to the skin. The recommended pH value for topical preparations is between 4.5 and 6.5. Conditions that are too acidic will cause the skin to become irritated while conditions that are too alkaline can make the skin scaly. The pH value according to the standard (SNI No.06-2588) is 4.5-6.5 (Suhery et al., 2023).

#### **Viscosity**

The viscosity test was carried out to see the viscosity of the preparation using a viscometer (Febrina Karim, 2021). The viscosity test was carried out using a Brookfield viscometer. When the gel preparation is put into a 50 ml beaker with as much as 1 g gel, dissolved with 20 ml of distilled water, the viscosity is measured using a rotary 4 at a speed of 60 rpm. The measurement is carried out with a Brookfield viscometer, the spindle is dipped into the gel preparation until the marked line on the spindle, then the device is turned on (Aliyah et al., 2022). The viscosity of a good gel preparation <4,000 cps (Nurdianti et al., 2018).

#### **Spreadability**

The gel is placed on a glass with a scale. Then the top is given the same glass and given a load of 50 grams, and a time of 1-2 minutes, then the spread diameter is measured when the preparation stops spreading (Febrina Karim, 2021). The spreadability of a good gel is 5-7 cm (Suhery et al., 2023).

#### **Adhesion**

Tests of adhesion are carried out on gel preparations, to determine the ability of adhesion to the hair and it is expected to be able to provide a moisturizing effect on the hair. Put enough gel on the glass object, put another glass object on top of the gel, press it with a load of 20 grams, let it stand for 5 minutes, then lift the load and record the time until the two glass objects are released, the adhesion requirement is not less than 4 seconds (Suhery et al., 2023).

#### **Cycling test**

In the cycling test method, gel samples are stored at a cold temperature of 4 °C and a hot temperature of 40 °C within 24 hours for one cycle. The test was carried out for 6 cycles and then observed for phase separation or not (Febrina Karim, 2021).

## Antibacterial Activity

Petri dishes were divided into 5 sections using a permanent marker. Add Mueller Hinton Agar (MHA) media into each Petri dish up to 5 ml using a syringe and let it solidify, then insert 5 plugs and arrange them so that there is a good area to observe the inhibition zone that occurs. Then add 10 ml of MHA media into the vial, then add 0.1 ml of bacterial suspension in the vial and homogenize it. The MHA media is first poured into a Petri dish and allowed to solidify to create a stable surface. Once solidified, the test bacterial suspension is spread evenly over the surface. Afterward, the inhibition zone is created by removing a portion of the solidified media to form a well, which is then used to test the gel preparation. Then incubated in an incubator at 37° C for 1 x 24 hours then observed the inhibition zone that occurred around the well, and measured the inhibition zone vertically, horizontally, and diagonally using a caliper (Febrina Karim, 2021).

## Results

**Table 2.** Organoleptic Observations of GPE

Formula	Observation	Before Cycling Test	After Cycling Test
F1	Shape	Gel	Gel
	Color	Pale Yellow	Pale Yellow
	Odor	Lemon	Lemon
F2	Shape	Gel	Gel
	Color	Dark Yellow	Dark Yellow
	Odor	Lemon	Lemon
F3	Shape	Gel	Gel
	Color	Orange	Orange
	Odor	Lemon	Lemon
K (-)	Shape	Gel	Gel
	Color	Clear	Clear
	Odor	Characteristic Carbopol	Characteristic Carbopol

Based on Table 2, it is shown that the gel preparations of grapefruit peel extract (*Citrus maxima* Merr) at concentrations of 5%, 10%, and 15% have remained stable in terms of organoleptic properties, with no changes in shape, color, or odor after the cycling test.

The research results showed that all gel formulations (F1, F2, F3) and the negative control (K-) were homogeneous both before and after the cycling test. This indicates that the components in the preparations were evenly mixed, with no coarse lumps or inconsistencies in color observed. Table 1 summarizes the pH values of the gel preparations before and after the cycling test.

**Table 3.** pH values

Sample	pH Value	
	Before Cycling Test	After Cycling Test
F1	4.57	5.12
F2	4.65	5.09
F3	4.65	4.89
K-	4.95	5.51

Table 3 presents the pH values of the gel preparations remained within the acceptable range for skin compatibility (4-8), indicating their suitability for facial application. Notably, a slight increase in pH values was observed following the cycling test, which may be attributed to factors such as gas absorption and temperature fluctuations during storage. This maintenance of an appropriate pH level is crucial for ensuring user comfort and minimizing the risk of skin irritation.

**Table 4.** Viscosity

Sample	Viscosity	
	Value Before Cycling Test (mPa·s)	Value After Cycling Test (mPa·s)
F1	3690	3360
F2	3590	3309
F3	3980	3380
K-	3990	3590

Table 4 presents the viscosity values of the gel preparations before and after the cycling test. All formulations exhibited a decrease in viscosity after the cycling test, which is likely due to the effects of temperature changes during storage. Despite the reduction, the viscosity values remained within the normal range for gel preparations, indicating that the formulations retained their desired consistency and stability.

**Table 5. Spreadability**

Sample	Spreadability	
	Before Cycling Test (cm)	After Cycling Test (cm)
F1	6.0	6.2
F2	6.3	6.4
F3	6.1	6.1
K-	5.9	6.1

Table 5 illustrates the spreadability of the gel preparations before and after the cycling test. The results indicate an increase in spreadability for F1 and F2 after the cycling test, which can be attributed to the decrease in viscosity. This enhanced spreadability is beneficial for application on the skin, ensuring that the gel can be easily distributed and absorbed.

**Table 6. Adhesion**

Sample	Adhesion Time	
	Before Cycling Test (seconds)	After Cycling Test (seconds)
F1	12.52	11.73
F2	11.32	11.10
F3	11.25	10.75
K-	13.96	12.45

Table 6 presents the adhesion times of the gel preparations before and after the cycling test. The results show a slight decrease in adhesion times for all formulations after the cycling test, which may be related to the changes in viscosity. Adequate adhesion is crucial for ensuring that the active ingredients remain in contact with the skin for optimal therapeutic effects. All formulations maintained adhesion times above the acceptable threshold of 4 seconds, indicating their effectiveness for topical application.

The cycling test demonstrated that all gel formulations successfully maintained their physical integrity throughout the duration of the test. Observations revealed no significant changes in appearance, color, or consistency of the gels, indicating that the formulations remained stable under the tested conditions.

Furthermore, the organoleptic properties, including odor and texture, were consistent before and after the cycling test. This stability suggests that the formulations are not only effective but also suitable for prolonged storage, ensuring their quality and usability for consumers. These findings underscore the reliability of the gel formulations in maintaining their intended characteristics despite exposure to varying temperature conditions.

**Table 7. Antibacterial Activity**

F1	<b>10.2 ± 0.21*</b>	<b>11.7 ± 0.65*</b>	<b>Strong</b>
F2	11.2 ± 0.33*	13.3 ± 2.66*	Strong
F3	11.1 ± 0.51*	11.7 ± 1.14*	Strong
K-	0	0	Strong
K+	12.2 ± 0*	12.5 ± 0.55*	Strong

Notes: Significantly different ( $p < 0.05$ ) from K- (\*)

Table 7 presents the inhibition zone diameters for both SA and PA across different concentrations of GPE. The results indicate that the formulations exhibit varying degrees of antibacterial activity, with F2 showing the highest inhibition zones for both bacteria.

## Discussion

This study is based on previous research by Pariury et al., (2021), which found that GPE (*Citrus maxima* Merr.) possesses significant antibacterial properties against acne-causing bacteria due to its antioxidant content. To validate these findings and ensure that the research results can be widely utilized, the extract was formulated into a pharmaceutical preparation in this case, a gel which is considered to have various advantages, including ease of removal from the skin surface and the absence of oil that could exacerbate acne.

The study commenced with the collection of grapefruit in the morning, when the plants are most effective in photosynthesis. To prevent microbial growth, the grapefruit peels were dried. Prior to testing, extraction of the grapefruit peel was performed using the maceration method, where 500 grams of grapefruit peel were dissolved in 96% ethanol.

The maceration method was selected due to its simplicity and minimal impact on the chemical components of the extract, as it does not involve high temperatures that could damage heat-sensitive compounds. According to Pariury et al., (2021), ethanol is a universal and polar solvent that is readily available. The choice of 96% ethanol was based on its selectivity, non-toxicity, good absorption, and high filtration capacity, which allows for effective extraction of nonpolar, semipolar, and polar compounds. High concentrations of ethanol facilitate better penetration into the sample cell walls, resulting in a more concentrated extract.

The maceration extraction process involved soaking the mashed grapefruit peel, ground into a coarse powder, in 96% ethanol for three 24-hour periods, with occasional stirring. The solvent was changed every 24 hours, and the mixture was subsequently filtered using gauze. The resulting filtrate was concentrated using a rotary evaporator until a thick extract was formed. Furthermore, the filtrate was transferred to an open container to allow the remaining solvent to evaporate, resulting in a more concentrated extract. In this study, a thick extract weighing 56.08 grams was obtained, yielding 11.216%. According to Subaryanti et al., (2022), the sample yield significantly influences the amount of extract obtained during the extraction process. Higher yields typically indicate the presence of more active compounds; thus, a yield above 10% is considered favorable.

The formulation of anti-acne gel preparations from GPE was created with concentrations of 5%, 10%, and 15%, which served as active substances. In addition to GPE, several other ingredients were incorporated, including carbopol, triethanolamine, propylene glycol, glycerin, DMDM hydantoin, lemon fragrance, and distilled water. Carbopol was utilized as a gelling agent due to its effectiveness in formulating semi-solid preparations and increasing viscosity, thereby enhancing adhesion. The selection of carbopol is attributed to its ability to create a clear gel that is easily dispersed in water and provides sufficient viscosity at low concentrations (0.05-2%). Triethanolamine was included as a neutralizing agent to balance the acidity of the carbopol, ensuring that the gel preparation remains clear. The optimal concentration for triethanolamine is between 0.4% and 0.5%. Additionally, humectants play a crucial role in preventing water loss from the gel, thereby increasing its stability. Propylene glycol was chosen for its humectant properties, maintaining moisture levels in the range of 5%-30%. Glycerin acts as an emollient, contributing to the stability of propylene glycol (Sumule, 2020). DMDM hydantoin was selected as a preservative.

The gel preparation of GPE was formulated into three different concentrations along with a negative control, followed by a physical stability test known as the cycling test. This cycling test was designed to assess the stability of the preparations under varying storage temperatures and specific time intervals, thereby accelerating the changes that typically occur under normal conditions. Conducted over six cycles, the test involved storing the gel preparation at a cold temperature of 4°C for 24 hours, after which it was transferred to a warmer environment at 40°C; this entire process constituted one cycle (Suryani et al., 2017). The necessity of physical stability tests arises from the need to ensure that the quality, safety, and efficacy of the gel meet the expected specifications and remain stable during storage. These stability assessments encompassed a series of tests, including organoleptic evaluations, homogeneity assessments, pH measurements, viscosity evaluations, spreadability tests, and adhesion tests on the preparations.

Organoleptic observation refers to the direct assessment of a gel preparation using the senses of sight, smell, and touch to evaluate its shape, color, and aroma. These observations were conducted both before and after the cycling test. As illustrated in Table 2, the formulations F1, F2, F3, and K- exhibited a consistent gel shape before and after the cycling test, indicating no change in shape during storage. In terms of color, F1 presented a pale-yellow hue, F2 a dark yellow, F3 an orange, and K- a clear appearance. Notably, all formulations maintained their color stability throughout the cycling test, suggesting they are stable during storage. Regarding aroma, F1, F2, and F3 retained a lemon scent before and after the cycling test, indicating physical stability during storage. Conversely, K- exhibited a distinctive carbopol smell, also indicating physical stability. The results indicate that higher concentrations of extract lead to a more concentrated preparation. The negative control (K-) appears clear and white, as it contains no extract. The gel preparations, derived from various concentrations, maintain a gel shape and a characteristic lemon scent, attributed to the addition of lemon fragrance. The stability of the gel is influenced by the use of carbopol as a base, which serves not only as a carrier for active substances but also as a stabilizer. Additionally, triethanolamine contributes to the formation of a well-structured organoleptic gel by enhancing the consistency of carbopol, resulting in a thicker and clearer base (Derbisbekova et al., 2017; Nurman et al., 2019).

Following the organoleptic observation, the homogeneity test was conducted to assess whether the components of the preparation were evenly and thoroughly mixed. The homogeneity of the preparation can be affected by the manufacturing process, including stirring and the temperature during mixing. The formulations F1, F2, F3, and K- were homogeneous both before and after the cycling test, as evidenced by the absence of coarse lumps and uniform color distribution, indicating stability in terms of homogeneity. The homogeneity of the preparation is evaluated based on color uniformity, the absence of insoluble particles, and the lack of lumps on the glass surface (Suryani et al., 2017).

The formulation of GPE gel necessitates a thorough evaluation of its physicochemical properties to ensure compatibility with skin health. In addition to homogeneity, the pH test was conducted to determine whether the GPE gel

preparation has a pH compatible with skin pH, which typically ranges from 4 to 8. A pH outside this range may lead to skin dryness or irritation. The pH measurements (Table 3) indicated that before the cycling test, F1 had a pH of 4.57, F2 had 4.65, F3 had 4.65, and K- had 4.95. After the cycling test, the pH values increased: F1 to 5.12, F2 to 5.09, F3 to 4.89, and K- to 5.51. This aligns with research, which noted pH changes in gel preparations. The increase in pH post-cycling test may be attributed to factors such as gas absorption and storage temperature. The cycling test involved six cycles, alternating between cold (4°C) and hot (40°C) temperatures, which contributed to the observed pH changes. Users are advised to monitor the storage temperature of the preparation, ideally keeping it at room temperature. Nevertheless, the gel preparation meets the pH requirements for facial skin, providing comfort during use. A pH that is too acidic can irritate the skin, while an excessively alkaline pH may lead to dryness and peeling.

The viscosity test was conducted to determine the viscosity value of the grapefruit peel extract gel preparation. The results (Table 4) indicated that before the cycling test, F1 had a viscosity of 3690 mPa·s, F2 had 3590 mPa·s, F3 had 3980 mPa·s, and K- had 3990 mPa·s. After the cycling test, the viscosity values decreased: F1 to 3360 mPa·s, F2 to 3309 mPa·s, F3 to 3380 mPa·s, and K- to 3590 mPa·s. The decrease in viscosity across all preparations can be attributed to temperature fluctuations during the storage period, yet all values remained within the normal range for gel preparations. This finding is consistent with research conducted by (Febrina Karim, 2021), which reported a decrease in viscosity in all preparations post-cycling test, influenced by storage temperature. The viscosity of a preparation can be affected by several factors, including the mixing process, ingredient selection, and particle size. The observed decrease in viscosity is particularly notable as the final cycle of the cycling test was conducted at a high temperature, which increases the distance between particles and reduces the intermolecular forces, thereby lowering viscosity. The viscosity of a good gel preparation <4,000 cps (Nurdianti et al., 2018; Shiehzadeh et al., 2023).

The spreadability test (Table 5) was conducted to evaluate how well the grapefruit peel extract gel preparation can spread on the skin's surface. Before the cycling test, the spreadability measurements were as follows: F1 with a spreadability of 6, F2 with 6.3, F3 with 6.1, and K- with 5.9. After the cycling test, the gel preparations exhibited an increase in spreadability: F1 at 6.2, F2 at 6.4, F3 at 6.1, and K- at 6.1. This increase can be attributed to the decrease in viscosity of the gel preparations, which is consistent with findings by (Febrina Karim, 2021). The temperature effects during the storage period, which involved six cycles at 4°C and 40°C, contributed to this increase in spreadability. The enhanced spreadability post-cycling test correlates with the viscosity changes, as higher temperatures tend to dilute the gel, reducing particle interaction and allowing for easier application. An ideal gel preparation should exhibit a spreadability of 5-7 cm (Suhery et al., 2023).

The adhesion test (Table 6) was performed to determine the duration the gel preparation remains adhered to the skin. Longer adhesion times are indicative of better formulations, as they allow for more active substances to diffuse into the skin, optimizing therapeutic effects. The adhesion test results prior to the cycling test showed F1 with an adhesion time of 12.52 seconds, F2 at 11.32 seconds, F3 at 11.25 seconds, and K- at 13.96 seconds. Post-cycling test, the adhesion times decreased: F1 to 11.73 seconds, F2 to 11.10 seconds, F3 to 10.75 seconds, and K- to 12.45 seconds. This reduction in adhesion can be attributed to the decrease in viscosity during storage, although all values remained within acceptable limits. The adhesion of a preparation is directly proportional to its viscosity; thus, higher viscosity correlates with longer adhesion times. However, excessive adhesion can block skin pores, while insufficient adhesion may hinder therapeutic efficacy. The requirement for adhesion is a minimum of 4 seconds (Suhery et al., 2023). In conclusion, the grapefruit peel extract gel preparation demonstrates favorable physicochemical properties, including pH compatibility with skin, acceptable viscosity, enhanced spreadability, and adequate adhesion. These characteristics suggest that the gel is suitable for facial application, providing comfort and efficacy while minimizing the risk of skin irritation or dryness.

The primary objective of the antibacterial activity test of GPE gel is to evaluate their effectiveness against specific bacterial strains, SA and PA. This assessment aims to determine the potential of GPE gel as a viable antibacterial agent, particularly in the context of skin-related applications, where these bacteria are commonly implicated. By investigating the antibacterial properties of the gel at varying concentrations, the study seeks to establish a correlation between concentration levels and antibacterial efficacy, thereby contributing to the development of effective natural antibacterial formulations.

*Staphylococcus aureus* (SA), a gram-positive bacterium, is commonly found as part of the normal flora on human skin (Febrina Karim, 2021). The antibacterial activity was assessed through varying concentrations of the gel preparations, specifically F1 (5%), F2 (10%), and F3 (15%). The results, as presented in Table 7, specifically the F2 gel preparation exhibited the best inhibition zone, closely approaching that of the positive control and significantly different ( $p < 0.05$ ) to the negative control. This suggests that the antibacterial activity of the GPE gel preparations is independent of the extract concentration, with F2 showing the highest efficacy, followed by F3 and F1.

Following the assessment of SA, the antibacterial activity of the GPE gel was further tested against PA, a primary etiological agent of acne inflammation (Febrina Karim, 2021). The results of this testing are summarized in Table 7. Similar

to the findings with SA, the F2 gel preparation again demonstrated the most significant inhibition zone, indicating a consistent pattern of antibacterial activity across different bacterial strains. The significant differences in antibacterial activity among the gel preparations confirm the observation that F2 exhibited the highest activity, followed by F3 and F1. This trend proves that the statement that aligns with the general principle that higher concentrations of antibacterial agents typically yield greater activity is not always true, this is thought to be because the various compound components contained in the extract can work synergistically or antagonistically.

These findings are also not in line with the research conducted by (Zeniusa et al., 2019), which highlighted an inverse relationship between extract concentration and the resulting inhibition zone. In Zeniusa's study, lower concentrations of the extract were found to exhibit effective antibacterial activity, potentially due to the diffusion characteristics of the extracts in the media. The diffusion process can be influenced by solubility factors; as the concentration of the extract increases, its solubility may decrease, thereby hindering the diffusion of active substances into the medium. This phenomenon could explain the reduced efficacy of higher concentration formulas in inhibiting the growth of both SA and PA. Based on these results, GPE gel preparations in both F1, F2, and F3 are in a strong category as well as the positive control while the negative control has no antibacterial activity. This is also in line with the results of research conducted by Zeniusa et al., (2019) where there is an inverse comparison between the concentration of the extract and the inhibition zone produced, in research it is precisely the extract with a low concentration that has effective antibacterial activity. This can occur due to the lack of diffusion of extracts in the media. The extract diffusion process can be influenced by solubility factors, where the higher the extract, the lower the solubility so that the diffusion process of the active substance will be reduced or slower as a result it can reduce the ability of the extract into the media so that it can reduce the ability of formulas with high concentrations in inhibiting the growth of SA and PA (Zeniusa et al., 2019).

Moreover, according to Zeniusa et al., (2019), several factors can influence the diameter of the inhibition zone, including the turbidity of the bacterial suspension. A less turbid suspension typically results in a larger inhibition zone, while a more turbid suspension may yield a smaller zone. Additionally, the thickness of the agar medium plays a crucial role; the effective thickness for optimal diffusion is approximately 4 mm. If the agar thickness is less than this, diffusion occurs more rapidly, whereas greater thickness can impede diffusion. In this study, the specific thickness of the Muller Hinton Agar (MHA) used was not measured, which limits the ability to draw precise conclusions regarding its impact on the results. The diameter of the inhibition zone is influenced by various factors, including diffusion speed, the nature of the agar medium, the inoculum size, bacterial growth rate, chemical concentration, and incubation conditions. Furthermore, it is essential to note that the diameter of the inhibition zone does not always increase proportionally with the concentration of the antibacterial agent. Variations in diffusion rates of different antibacterial compounds and their respective concentrations can lead to differing inhibition zone diameters.

In conclusion, the GPE gel preparations from *Citrus maxima* Merr. demonstrate significant antibacterial activity against both *Staphylococcus aureus* and *Propionibacterium acnes*, with optimal efficacy observed at specific concentrations. These findings contribute to the understanding of the antibacterial potential of natural extracts and their application in developing effective antibacterial formulations.

## Conclusion

In conclusion, the study demonstrates that Grapefruit Peel Extract (GPE) can be effectively formulated into a stable anti-acne gel preparation. This GPE anti-acne gel exhibits significant antibacterial activity against both *Staphylococcus aureus* (SA) and *Propionibacterium acnes* (PA) bacteria. The optimal concentration for antibacterial efficacy was identified in formulation F2, which contains a concentration of 10%. It is recommended that future research extend the duration of cycle testing to allow for a more comprehensive evaluation of the stability of the GPE gel preparation over an extended period. This will provide valuable insights into the long-term viability and effectiveness of the formulation in practical applications.

### Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

### Funding

This research received no grant from any funding agency.

### Author contributions

SL and JG designed the research. AW and HE performed the research, analyzed the data, and wrote the manuscript. MS and SH contributed to statistical analysis, RM, RL, and NH contributed to research administration. All authors read and approved the final manuscript.

## Author's Biographies

*Saparuddin Latu* is a lecturer in Faculty of Pharmacy, University of Megarezky, Makassar, Indonesia

*Jangga* is a lecturer in Faculty of Pharmacy, University of Megarezky, Makassar, Indonesia

*Abdul Wahid Suleman* is a lecturer in the Magister of Pharmacy, Postgraduate Program, University of Megarezky Makassar, Indonesia

*Hardyanti Eka Putri* is a lecturer in the Magister of Pharmacy, Postgraduate Program, University of Megarezky Makassar, Indonesia

*Sriyana Herman* is a lecturer in the Department of Reproductive Health, Postgraduate Programme, Universitas Megarezky, Makassar, Indonesia

*Rifa'atul Mahmudah* is a lecturer in the Magister of Pharmacy, Postgraduate Program, University of Megarezky Makassar, Indonesia

*Rusli* is a lecturer in the Department of Physiotherapy, Faculty of Health and Sport Science, Universitas Negeri Makassar, Makassar, Indonesia

*Nurhikmah* is a lecturer in the Department of Physical Education Health and Recreation, State University Muhammadiyah, Luwuk Banggai, Indonesia

## References

- Aliyah, H., Rahmatullah, S., & Rahmasari, K. S. (2022). Uji efektivitas antibakteri deo lotion ekstrak etanol kulit jeruk bali (*Citrus maxima* (Burm.) Merr) terhadap bakteri *Staphylococcus aureus* ATCC 25923 PK/5.
- Ben Hsouna, A., Sadaka, C., Generalić Mekinić, I., Garzoli, S., Švarc-Gajić, J., Rodrigues, F., & Mnif, W. (2023). The chemical variability, nutraceutical value, and food-industry and cosmetic applications of citrus plants: A critical review. *Antioxidants*, 12(2). <https://doi.org/10.3390/antiox12020481>
- Briliani, F. A., Sudarno, & Safitri, D. (2016). Analisis kecenderungan pemilihan kosmetik wanita di kalangan mahasiswa Jurusan Statistika Universitas Diponegoro menggunakan biplot komponen utama. *Jurnal Gaussian*, 5(3).
- Derbisbekova, U. B., Datkhayev, U. M., Rakhimov, K. D., Zhuravel, I. A., Makhatova, B. G., & Zabyzbekova, T. B. (2017). Development of gel composition and technology based on pyrimidine substance. *National Journal of Physiology, Pharmacy and Pharmacology*, 7(9), 901–906. <https://doi.org/10.5455/njppp.2017.7.0518412062017>
- Febrina Karim, S. (2021). Formulasi dan uji aktivitas sediaan gel anti jerawat ekstrak daun nilam (*Pogostemon cablin* Benth) sebagai antibakteri terhadap bakteri *Staphylococcus aureus*, *Staphylococcus epidermidis* dan *Propionibacterium acnes*.
- Febriyani, E., Falah, S., Andrianto, D., & Lastini, T. (2018). Identification of active compounds and anti-acne activity from extracts and fractions of surian (*Toona sinensis*) leaves planted in Sumedang, West Java, Indonesia. *Biodiversitas*, 19(4), 1406–1412. <https://doi.org/10.13057/biodiv/d190429>
- Nasution, R., Bahi, M., Marianne, & Murianna. (2022). Beberapa tumbuhan penghasil buah dalam genus *Artocarpus* di Indonesia (Vol. 1). Syiah Kuala University Press. <https://books.google.co.id/books?id=XUKoEAAAQBAJ>
- Nurdianti, L., Rosiana, D., & Aji, N. (2018). Evaluasi sediaan emulgel. *Journal of Pharmacopolium*, 1(1).
- Nurman, S., Yulia, R., Irmayanti, Noor, E., & Sunarti, T. C. (2019). The optimization of gel preparations using the active compounds of arabica coffee ground nanoparticles. *Scientia Pharmaceutica*, 87(4). <https://doi.org/10.3390/scipharm87040032>
- Pariury, J. A., Herman, J. P. C., Rebecca, T., Veronica, E., Kamasan, G., & Arijana, N. (2021). Potensi kulit jeruk bali (*Citrus maxima* Merr) sebagai antibakteri *Propionibacterium acnes* penyebab jerawat. *Hang Tuah Medical Journal*, 19(1). <http://www.journal-medical.hangtuah.ac.id>
- Putri, A. (2018). Perkembangan penggunaan produk kosmetik di Indonesia, 2016.
- Saniati, F., Wilujeng, B. Y., S.Pd., & M.Pd. (2020). Analisis produk kosmetik make up salah satu merek global terhadap keputusan pembelian.
- Saputra, K. A., Puspawati, N. M., & Suirta, I. W. (2017). Kandungan kimia minyak atsiri dari kulit buah jeruk bali (*Citrus maxima*) serta uji aktivitas antibakteri terhadap *Staphylococcus aureus* dan *Escherichia coli*.
- Septianingrum, Y., Safrina, U., Puspita, N., & Surahman, S. (2023). Gambaran tingkat pengetahuan tentang Period After Opening (PAO) dan perilaku penyimpanan kosmetika perawatan pada remaja di Kota Tangerang. *Jurnal Sains dan Kesehatan*, 5(1), 6–13. <https://doi.org/10.25026/jsk.v5i1.1478>
- Shiehzhadeh, F., Mohebi, D., Chavoshian, O., & Daneshmand, S. (2023). Formulation, characterization, and optimization of a topical gel containing tranexamic acid to prevent superficial bleeding: In vivo and in vitro evaluations. *Turkish Journal of Pharmaceutical Sciences*, 20(4), 261–269. <https://doi.org/10.4274/tjps.galenos.2022.60687>
- Sifatullah, N., & Zulkarnain. (2021). Jerawat (*Acne vulgaris*): Review penyakit infeksi pada kulit. <http://journal.uin-alauddin.ac.id/index.php/psb>
- Subaryanti, S., Meianti, D. D., & Manalu, R. T. (2022). Potensi antimikroba ekstrak etanol daun gatal (*Urticastrum decumanum* (Roxb.) Kuntze) terhadap pertumbuhan *Staphylococcus aureus* dan *Candida albicans*. *Pharmaceutical Journal of Indonesia*, 15(2).
- Suhery, W. N., Lestari, H., Fernando, A., Aryani, F., & Novita, G. (2023). Formulation and antioxidant activity test of rice bran extract cream from two varieties of white rice. *Majalah Obat Tradisional*, 28(2), 151–156. <https://doi.org/10.22146/mot.84715>
- Sumule, A. (2020). Optimasi Carbopol 940 dan gliserin dalam formula gel lendir bekicot (*Achatina fulica* Ferr) sebagai antibakteri *Staphylococcus aureus* dengan metode simplex lattice design. *Pharmaceutical Journal of Indonesia*, 17(1).
- Suryani, E. P., Putri, A., & Agustiyani, P. (2017). Formulasi dan uji stabilitas sediaan gel ekstrak terpurifikasi daun paliasa (*Kleinhovia hospita* L.). *Pharmacon: Jurnal Ilmiah Farmasi-Unsrat*, 6(3).
- Zeniusa, P., Ramadhian, M. R., Nasution, S. H., & Karima, N. (2019). Uji daya hambat ekstrak etanol teh hijau terhadap *Escherichia coli* secara in vitro. *Majority*, 8(2).