

# Formulation and Evaluation of Anti-Acne Activity of Gel Pineapple Peel (*Ananas comosus L.*) Extract on the Growth of *Propionibacterium acnes*

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**ABSTRACT:** Pineapple peel contains many compounds, namely flavonoids, tannins, saponins, phenolics, steroids, alkaloids and bromelin enzymes. Acne is a chronic inflammatory disease of the pilosebaceous glands. One of the bacteria that causes acne is *Propionibacterium acne*. Preparations that can be made for anti-acne are gel preparations. This study aims to determine whether the ethanol extract of pineapple peel (*Ananas comosus L.*) can be made into an anti-acne gel preparation and to find out at what concentration the ethanol extract of pineapple peel (*Ananas comosus L.*) in an anti-acne gel preparation can provide inhibitory activity on the development of *Propionibacterium acne* bacteria. This research method is true experimental by making anti-acne gel preparations of pineapple peel extract (*Ananas Comosus L.*) with various concentrations of 2%, 3%, 5% and testing the activity of the preparation against *Propionibacterium acne* using the agar well method. The results of the gel preparation formula showed that there were significant differences before and after the cycling test in both organoleptic, homogeneity, pH, viscosity, spreadability and adhesion tests. The activity test results obtained were that the largest inhibition zone was the 5% concentration, namely 12.7 mm, which was in the strong category, while the 3% concentration, which was 11.03 mm, and the 2% concentration, which was 8.8 mm, was included in the moderate category.

**KEYWORDS:** Anti-acne; Gel; Pineapple Skin; *Propionibacterium acne*.

## 1. INTRODUCTION

Acne is one of the many skin problems faced by most people, both men and women. Acne is not a serious problem, but if left untreated it can continue to grow and cause pain in the facial skin caused by inflammation of the lining. The bacteria that cause inflammation are *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* (Ofirnia et al., 2018).

Anti-acne drugs that are widely used in the market contain synthetic antibiotics such as erythromycin and clindamycin, but a large number have side effects such as irritation, develop resistance with long-term use, and damage organs, damage and even immune hypersensitivity (Ofirnia et al., 2018).

One of the bacteria that causes acne on the skin is the *Propionibacterium acnes* bacteria. *P. acnes*, is a Gram-positive bacterium which morphologically and structurally belongs to the Corynebacterium group of bacteria, but is not toxic. These bacteria form the normal flora of the skin. *P. acnes* is a bacterium that plays an important role in the pathogenesis of acne vulgaris by producing lipases that break down free fatty acids from skin lipids. These fatty acids can cause inflammation. As for the immune system, it destroys tissue and encourages the development of acne vulgaris. *P. acnes* is a slow growing bacterium. This bacterium is a typical Gram-positive anaerobe that is tolerant of air (Halimatus Z et al., 2018).

Several drug dosage forms intended for use on the skin to treat acne, including ointments, creams, lotions, topical solutions, and tinctures, are the most commonly used dermatological dosage forms, both for their ability to act as skin protectants, emollients, moisturizers, and so on. – other, or because of the special action of existing medicinal ingredients. Over-the-counter preparations containing a mixture of medicinal ingredients often cause skin infections, itching, burns, insect bites and bites, daphnia, corns, thickened and hardened warts, dandruff, acne, chronic skin conditions and eczema (Ansel, 1989).

The gel formulation is easy to use, spreads well on the skin, and is clearly visible by its clear color, which is why many patients prefer gel cosmetics over other formulations. The active ingredient in a gel formulation is placed in a base or carrier and the medication touches the skin surface. The gel form has several advantages, including not sticky. The gel has a thixotropic and pseudoplastic flow. This means that the gel is solid when stored and dissolves quickly when shaken. The concentration of the gelling agent needed is small to obtain a good gel mass for gel formation. Viscosity did not change significantly at storage temperature. The carriers used in topical formulations have a significant impact on drug absorption and can have beneficial effects when properly selected (Lieberman, 1989).

Many plants have the potential to have pharmacological activity and can be used in medication (Fawwaz et al., 2023). Pineapple (*Ananas comosus L.*) is a fruit that is commonly consumed as a health food because it contains many nutritional values such as calcium, potassium, vitamin C, carbohydrates, crude fiber, water and various minerals. Pineapple skin contains nutritious compounds, but in general pineapple is only used for the fruit and the skin is often wasted (Harahap et al., 2019).

The chemical compounds contained in pineapple skin are flavonoids, tannins, saponins, phenolics, steroids, alkaloids and bromelain enzymes (Rini et al., 2017). One of the compounds that act as an antibacterial is a flavonoid compound which works by inhibiting nucleic acid synthesis, inhibiting the function of cell membranes and inhibiting energy metabolism. In addition, there is also a bromelain enzyme compound which works to catalyze the hydrolysis reaction of protein (Yudha et al., 2018).

In Dedhi Setiawan's research, entitled Formulation of Serum Gel Anti-Acne Extract Ethanol Peel of Bauh Pineapple (*Ananas comosus L.*) and Activity Test Against *Staphylococcus aureus* ATCC 25923, it was explained that the serum formulation made had antibacterial activity inhibition against *Staphylococcus aureus* ATCC 25923 which causes pimples of 8.6 mm in formula 1 with 2% extract concentration, 13 mm in formula 2 with 3% extract concentration and 21.3 mm in formula 3 with 5% extract concentration. The greater the concentration of the extract used, the greater the inhibition produced, the greatest inhibition of the growth of *Staphylococcus aureus* ATCC 25923 was in formula 3 of 21.3 mm in the category of very strong inhibition.

Here, we explained that pineapple peel has potential as an antibacterial seen from the content of chemical compounds present in the pineapple skin, namely flavonoid compounds and bromelain enzymes against gram-positive bacteria, such as *Staphylococcus aureus* bacteria

## 2. EXPERIMENTAL SECTION

### 2.1. Research Design

This research is a true experimental study by making anti-acne gel preparations of pineapple peel extract (*Ananas Comosus L.*) on the activity of bacteria that cause acne infection *Propionibacterium acnes* bacteria using the agar well method. The research design based on the formula which can be seen in the **Table 1**.

### 2.2. Tools and Materials

Glassware, blender, maceration vessel, water bath, analytical balance, petri dish, erlenmeyer, test tube, round loop, straight loop, autoclave, oven, incubator, viscometer, spirit lamp, reservoir, beaker and tweezers. The materials used in this study were 96% ethanol, pineapple peel (*Ananas comosus*), HPMC, triethanolamine, propylene glycol, methyl paraben, distilled water, sodium agar, 0.9% NaCl, Azarin gel and using *Propionibacterium acnes* bacteria.

### 2.3. Formulation

**Table 1.** Anti-Acne Gel Formulation of Pineapple Skin Extract (*Ananas comosus L.*)

Bahan	Kegunaan	Konsentrasi				
		F1	F2	F3	F4	F5
Ekstrak kulit nanas ( <i>Ananas comocus L.</i> )	Senyawa aktif	2%	3%	5%	-	+
Hpmc	Gelling agent	2	2	2	2	+
Triethanolamin	Alkalizing agent	0,5	0,5	0,5	0,5	+
Propilen glikol	Humektan	7,5	7,5	7,5	7,5	+
Metil paraben	Pengawet	0,2	0,2	0,2	0,2	+
Aquadest	Pelarut	Ad 100	Ad 100	Ad 100	Ad 100	+

## 2.4. Methods

### 2.4.1. Sample Processing

The sample used is pineapple skin (*Ananas comosus L.*) which is cleaned and washed with clean running water. Then cut into small pieces and dried for 3 days. Then mashed with a blender and then weighed.

### 2.4.2. Gel Preparation

Preparation of anti-acne gel from pineapple peel extract is carried out by dispersing the carbopol with a portion of distilled water which has been heated to a temperature of 80 - 90 °C, allowed to swell and stirred until homogeneous. Triethanolamine was added, stirred until homogeneous and a gel mass was formed. Then added pineapple peel extract according to the concentration of the formulation and methyl paraben which had been dissolved with propylene glycol, crushed homogeneously and dissolved in the remaining distilled water, stirred until homogeneous.

### 2.4.3. Gel Stability Test

- a. Uji organoleptis

Observations of the preparations included the aroma, color and texture of each anti-acne gel serum formulation that were observed.

#### b. Homogeneity Test

The preparations were tested using two glass slides, where the sample was placed on one of the slides and placed evenly. A good preparation must be homogeneous and free from clumping particles.

#### c. pH Test

The pH test was carried out using a universal pH paper dipped in a diluted gel sample. The color changes that occur are matched with universal pH standards. Gel preparations generally have a pH value between 4-6.

#### d. Spreadability Test

This test is carried out by weighing as much as 0.5 gram of gel then placed in a round glass, another glass is placed on it and left for 1 minute. After that, 150 g of load was added, allowed to stand for 1 minute and the constant diameter was measured.

#### e. Stickiness Test

A sample of 0.25 g was placed between 2 glass objects on the stickiness test apparatus, then pressed by a 1 kg load for 5 minutes, the load was lifted and the time for releasing the gel was recorded.

#### f. Viscosity Test

The viscosity test of the preparation was carried out using a viscometer by immersing the spindle in the viscometer in the preparation container and at a speed of 60 rpm. The viscosity of the preparation is seen on the scale in the tool after stability is achieved.

#### g. Cycling Test Examination Test

The gel preparations were stored at 4°C for 24 hours, then removed and placed at 40°C, this was done for 24 hours. This treatment is one cycle. The experiment was repeated for 6 cycles and observed and measured changes in each cycle.

### 2.4.4. Base media preparation (NA)

2.1 gram of NA medium was weighed, then dissolved with 100 mL of distilled water in an Erlenmeyer flask, and heated until the material dissolved completely until it became clear. Then sterilized by autoclave at 121°C for 15-20 minutes. Media that has been sterilized is put into a petri dish and allowed to solidify at room temperature.

### 2.4.5. Preparation of the test bacterial suspension

Take 1 ose of the results of rejuvenation of the pure culture of *Propionibacterium acnes* bacteria, put it in a test tube that already contains 3 mL of 0.9% physiological fluid, then homogenize it and put a mark on each test tube.

### 2.4.6. Antibacterial activity testing

The antibacterial activity test was carried out by pouring 5 mL of NA into each of 5 petri dishes as a base layer, then making wells with a diameter of 7 mm using a buffer and then allowing the NA media to solidify. After solidification, *Propionibacterium acnes* bacteria which had been inoculated in 0.9% NaCl were taken and mixed with the remaining NA medium, 10 mL each per petri dish. Anti-acne gel preparation Pineapple peel extract with various gel formulas F1 (2% extract), F2 (3% extract) and F3 (5% extract), F4 (negative control) and F5 (positive control) were added to the wells that had been made. Then incubated in an incubator at 37 °C for 24 hours. Measurements were made on the clear zone formed around the wells which indicated the zone of inhibition of bacterial growth activity.

## 3. RESULTS

### 3.1. Pineapple skin extraction results

**Table 2.** Yield of sample extraction of Pineapple Skin Extract (*Ananas comosus L.*)

Sampel	Jenis pelarut	Berat Sampel Kering (g)	Berat Ekstrak Kental (g)	Rendaman (%)
Ekstrak Kulit Nanas ( <i>Ananas comosus L.</i> )	Etanol 96%	500	20,49	4,89%

### 3.2. Phytochemical screening results

**Table 2.** Phytochemical screening of Pineapple Skin Extract (*Ananas comosus L.*)

Kandungan kimia	Pereaksi	Hasil pengamatan	Hasil pustaka	Keterangan
Flavonoid	HCL pekat dan mg	Terbentuk warna kuning	Terbentuk warna kuning	+
Saponin	HCL	Terbentuk busa	Terbentuk busa	+
Tanin	FeCl <sub>3</sub>	Terbentuk hijau kehitaman	Terbentuk hijau kehitaman	+
Alkaloid	Fragen mayer	Terbentuk endapan putih	Terbentuk endapan putih	+

### 3.3. Evaluation results of gel preparations

#### a. Organoleptic Test

**Table 3.** Organoleptic properties of gel

Formula	Bentuk		Warna		Bau	
	Sebelum cycling test	Sesudah cycling test	Sebelum cycling test	Sesudah cycling test	Sebelum cycling test	Sesudah cycling test
F1	Semi padat	Semi padat	Coklat muda	Coklat muda	Khas ekstrak	Khas ekstrak
F2	Semi padat	Semi padat	Coklat agak tua	Coklat agak tua	Khas ekstrak	Khas ekstrak
F3	Semi padat	Semi padat	Coklat tua	Coklat tua	Khas ekstrak	Khas ekstrak
F4 (-)	Semi padat	Semi padat	Bening	Bening	Khas	Khas

#### b. pH Test

**Table 4.** Acidity properties of gel

Formula	Uji Ph		Syarat	signifikansi
	Sebelum Cycling Test	Setelah Cycling Test		
F1	4,26	4,91	4,5 – 6,5	Sig: 0,068 > 0,05
F2	4,22	5,09		
F3	4,21	5,65		
K (-)	5,78	6,09		

#### c. Homogeneity Test

**Table 5.** Homogeneity properties of gel

Formula	Homogenitas		Syarat
	Sebelum cycling test	Setelah cycling test	
F1	Homogen	Homogen	Homogen
F2	Homogen	Homogen	
F3	Homogen	Homogen	
F4 (-)	Homogen	Homogen	

## d. Viscosity Test

Table 6. Viscosity properties of gel

Formula	Uji Viskositas		Syarat	Signifikan
	Sebelum Cycling Test	Setelah Cycling Test		
F1	2680	2470	2000-4000	Sig: 0,083 > 0,05
F2	2740	2610		
F3	3330	3010		
F4 (-)	2390	2380		

## e. Spreadability test

Table 7. Spreadability properties of gel

Formula	Uji Daya Sebar		Syarat	Signifikan
	Sebelum Cycling Test	Setelah Cycling Test		
F1	5,6	5,7	5-7 cm	Sig: 0,092 > 0,05
F2	5,4	5,5		
F3	5	5,2		
F4 (-)	5,3	5,3		

## f. Stickiness test

Table 8. Stickiness properties of gel

Formula	Uji Daya Lekat		Syarat	Signifikan
	Sebelum Cycling Test	Setelah Cycling Test		
F1	61 detik	61 detik	> 1 detik	Sig: 0,180 > 0,05
F2	64 detik	50 detik		
F3	64 detik	64 detik		
F4 (-)	61 detik	60 detik		

## g. Antibacterial Inhibitory activity test

Table 9. Antibacterial properties of gel

Formula	Replikasi			Diameter rata-rata (mm)	Kategori	Signifikansi
	I	II	III			
F1	9,65	8,25	8,5	8,8	Sedang	Sig: 0,017 > 0,05
F2	14,9	9,35	9,75	11,03	Sedang	
F3	15,25	11,05	11,8	12,7	Kuat	
F4 (-)	0	0	0	0	Lemah	
F5 (+)	8,5	9,05	8,3	8,61	Sedang	

## 4. DISCUSSION

The sample used in this study was pineapple skin (*Ananas Comosus L.*) originating from the Makassar eggplant market, South Sulawesi. The chemical compounds contained in pineapple skin are flavonoids, tannins, saponins, phenolics, steroids, alkaloids and bromelin enzymes which can be used as antibacterial. This research was initiated by

carrying out the maceration process on samples of pineapple (*Ananas Comosus L.*) using 96% ethanol solvent. The extraction process uses the maceration method because using the maceration method provides a number of advantages, including guarantees that the extracted active ingredients will not be damaged (Engineering et al, 2019). The choice of 96% ethanol solvent as the solvent is because it is universal, can attract polar and non-polar compounds and can provide protection against contamination from microbes during the extract manufacturing process. Furthermore, the maceration results were evaporated using a rotary evaporator. The evaporator in the study was used to evaporate the extract solvent contained in the maceration results to produce a thick extract (Reo et al, 2017). The yield of the extract soaking obtained was 4.89%.

The reason for making gels is that gel preparations are of great interest to the drug and cosmetic industry because they have advantages over other preparations, namely good distribution on the skin, a cooling effect when applied to the skin, good drug release, and easy washing (Kaur & Guleri, 2013). Anti-acne gel consisting of HPMC, triethanolamine, propylene glycol, methyl paraben, and distilled water which is used to dissolve the ingredients and provide the desired volume. HPMC was chosen because HPMC gel base is a gelling agent that is often used in cosmetic and drug production, because it can produce a clear gel, dissolves easily in water, and has low toxicity. In addition, HPMC (Hydroxy Propyl Methyl Cellulose) produces a gel that is neutral, clear, colorless, stable at pH 3-11, has good resistance to microbial attack, and provides good film strength when it dries on the skin. Triethanolamine was chosen because it can provide an alkaline environment to HPMC so that the resulting gel becomes thick and clear. Propylene glycol is added to function as a humectant. Methyl paraben is added to function as a preservative to prevent damage and growth of microorganisms in gel preparations.

The research was continued by carrying out phytochemical screening, in the test for the content of flavonoid compounds the results were obtained by the formation of a yellow color. In the test for the content of saponin compounds, the results were obtained by the formation of foam and lasted for 5 minutes. In the test for the content of tannin compounds, the results showed the formation of a black-green color. And in the test for the content of alkaloid compounds, the results were obtained by the formation of a white precipitate at the bottom of the test tube.

The research was continued with preparation evaluation. This is done by carrying out a stability test on the preparation. The preparations were placed at 4 °C and 40 °C. This test aims to determine the stability of a preparation, at this stage an organoleptic test is carried out to see the color, odor, and dosage form. It is hoped that the preparation both before and after the cycling test will not change. The results of the stability tests of the four preparations showed no change in terms of color, aroma, and shape both before and after storage. Based on the results of the study it can be concluded that the addition of extracts can affect the organoleptic properties of the color of the preparation. The F3 preparation has a more concentrated color than the F1 and F2 preparations, this is due to the higher concentration of the extract compared to the other preparations. According to Johan & Kromo (2020), the combination of extracts can affect the organoleptic properties affecting the color of the preparation, depending on the concentration of the extract needed.

The pH test was carried out on the gel preparations. The results of the pH test showed that the four gel preparations that had been made experienced a change in pH after the cycling test was carried out. The four preparations experienced an increase in pH after the cycling test was carried out. The F1 pH test before the cycling test was 4.26 and after the cycling test was 4.91. In F2 before cycling test 4.22 and after cycling test 5.09. F3 before cycling test 4.21 and after cycling test 5.65. K(-) before the cycling test was 5.78 and after the cycling test was 6.09, where the pH results carried out on gel preparations entered into the pH requirements for topical preparations. Even though it is still within the pH requirements for topical preparations, namely 4.5-6.5. Changes in pH that occur can be caused by several factors such as storage temperature and the sensitivity of the pH meter tool used (Rasyadi, 2018). Based on the results of the SPSS output of the Tests of Normality table of the Shapiro-Wilk normality test, it is known that the Sig value before cycling is 0.003 <0.05 and the Sig value after cycling is 0.

## 5. CONCLUSION

Based on the results of the study it can be concluded that the ethanol extract of pineapple peel (*Ananas Comosus L.*) can be formulated as an anti-acne gel. Formulation with a concentration of 5% is the most effective concentration in inhibiting the growth of *Propionibacterium acne* bacteria.

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