



# Nanogel Formulation of Butterfly Pea Flower Extract (*Clitoria ternatea* L.) Against Propionibacterium acnes Bacteria Inhibitory Test

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**Abstract:** The chemical components of butterfly pea flowers (*Clitoria ternatea* L.) have a strong bacterial inhibition zone. The aim of this research is to make nanogels using the active ingredient ethanol extract of butterfly pea flowers and demonstrate the antibacterial activity of nanogels against Propionibacterium acnes bacteria. Because nanogels are more stable, translucent, and have small particle sizes making them efficient in releasing active chemicals and increasing bioavailability, nanogels are used in tropical medicine. Maceration is the extraction technique used. Nanogel of butterfly pea flower ethanol extract (BPFEE) was added to the nanogel formulation at concentrations of F1 (5%), F2 (10%), and F3 (15%). Transmission testing is part of the ideal formula; Other tests for characterization include phytochemical, organoleptic, particle size, pH, adhesion, spreadability and viscosity tests. Next, antibacterial activity was tested against Propionibacterium acnes using the well method. The research results showed that the % transmittance was 84-88%, the organoleptic results were in the form of a gel, color and distinctive aroma, particle size 114.9 - 283.9 mm, pH 5.6 - 6, adhesive power 1.36 - 2.1 seconds, spreadability 4.8-5.6 cm, viscosity 4071 - 3043.75 mPa.S. Telang flower ethanol extract nanogel can inhibit the growth of P. acnes bacteria, namely 8.2-11.7 mm.

**Keywords:** Antibacterial activity; Butterfly pea flower; Nanogel

## Introduction

Telang flowers are increasingly popular in Indonesia as flowers that provide many health benefits. Fresh or dried butterfly pea flowers can be made into butterfly pea flower drinks or in other forms of snacks. Butterfly pea flowers are also widely available in the neighborhood; In fact, most people grow it for family needs in their yard. The efficacy of butterfly pea flower extract (*Clitoria ternatea* L.) has been proven in a number of studies. These benefits include antibacterial which can treat acne, antioxidant, anti-diabetic, anti-obesity, anti-inflammatory and anti-cancer (Yulandasari et al., 2023).

According to Habibi et al. (2019), the production of excess oil gland secretions which causes irritation and skin infections is called acne. Even though acne is not a big problem, if it is not treated, acne can cause discomfort on facial skin and get worse. Because oil and dust clog facial pores, acne causes pain by inflaming the layers of the skin (Kindangen et al., 2018; Jawa La & Sawiji, 2024). This inflammation is generally triggered by several types of bacteria such as Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes (Wardani, 2020; Amalia & Sari, 2017; Hakim et al., 2019; Bia & Minarsih, 2025).

Gram-positive, pleomorphic, anaerobic Propionibacterium acnes is a bacterium that can infect

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the digestive system and skin. The typical microbiota found in the sebaceous glands of human skin is *Propionibacterium acne*. By producing lipase, which breaks down free fatty acids from skin lipids, these bacteria cause acne. Sebum thickens due to *Propionibacterium acne* which converts unsaturated fatty acids into saturated fatty acids. An effective preparation for topical therapy to treat *Propionibacterium acne* bacteria on the skin which can cause acne is an acne medication preparation in nanogel form (Meilina et al., 2018).

Hybrid nanogels have been created in recent decades in response to the increasing need for new materials. Currently, the use of multifunctional hybrid nanogels in nanomedicine is a major focus of research efforts. This research is important because nanogels are more stable, transparent, and contain small particles that effectively release active substances to increase bioavailability, they are used in cosmetic products.

## Method

### *Preparation of Test Materials*

Telang flower samples were collected from Besusu Tengah Village, East Palu Regency, Central Sulawesi for use in this research. Butterfly pea flowers are harvested, sorted, then rinsed with running water to remove any remaining dirt or dust. Then chopped to make the drying process easier, then air-dried for 5 x 24 hours. Furthermore, after drying, it is separated from dirt, and powdered so that the surface area becomes wider and contact with the solvent increases.

### *Preparation of Ethanol Extract of Butterfly Flower*

Simplicia powder is extracted by maceration with 96% ethanol 3 X 24 hours. One gram of simplicia powder can weigh 800 grams. Rotary ovens and evaporators are used to concentrate the extracted material (Dewanti et al., 2023; Crendhuty et al., 2020; Pertiwi et al., 2022; Pradana et al., 2023).

### *Making Nanogel Formulation*

The manufacturing process by preparing all the ingredients used. Weighing and measuring the components according to the formulation strategy. The process of making nano butterfly pea flower extract first combines Tween 80 and PEG 400, homogenizes the mixture with a distiller for five minutes, adds butterfly pea flower extract gradually, then distills for thirty minutes at 60°C and 1000 rpm (Putri et al., 2022). After adding water little by little to the mixture, sonicate for 30 minutes at 37°C (1 cycle) and stir for 30 minutes at 60°C at 1000 rpm. To make the base gel, first dissolve carbophol 940 in hot water. Then add TEA (time 1), propylene glycol, and methyl paraben, then stir well

(time 2). After mass 1 and mass 2 are mixed, stir until smooth and a gel base is formed. Nanogel BPFEE was added to the gel base at concentrations of 5%, 10%, and 15% after being formed and blended. The three nanogel formulations were then stored overnight at room temperature, between 10°C and 15°C (Jain et al., 2024).

### *Phytochemical Screening Test*

#### *Flavonoid Test*

The Nanogel BPFEE was weighed as much as 0.5 grams, put into a test tube, then 10 mL of distilled water was added and heated over a water bath then filtered, then dissolved in 1 mL of ethanol (96%) with the addition of magnesium P powder, after which it was dissolved in 10 mL of concentrated hydrochloric acid P, if the Nanogel BPFEE color occurs, it indicates the presence of flavonoids and if the yellow color occurs, it indicates the presence of flavones, chalcone and aurone (Dewi, 2020).

#### *Alkaloid Test*

The alkaloid test was carried out with Dragendorff reagent. A positive test for alkaloids is indicated by the presence of an orange-to-yellow precipitate (Ma'rif et al., 2023).

#### *Saponin Test*

The Saponin test was carried out with hot distilled water and 2N HCl. Stable foam indicates a positive saponin test, and the foam does not disappear when added with HCl 2 N (Larasati & Putri, 2023).

#### *Tannin Test*

0.5 grams of Nanogel BPFEE into a test tube, then add 5 mL of distilled water and boil for 5 minutes. Then filtered, the filtrate was added with 5 drops of 1% FeCl<sub>3</sub> (w/v). The dark blue or greenish black color that forms indicates the presence of tannin compounds (Dewi, 2020).

### *Nanogel Formula Optimization*

The nanogel formula optimization was carried out by testing the % transmittance. After adding the nanoparticle mixture solution into the cuvette, a UV-Vis spectrophotometer was used to measure the percentage transmittance at a sample wavelength of 650 nm (Jain et al., 2024).

### *Nanogel Characterization*

The shape, color, and odor of the nanogel preparation made were seen directly during organoleptic testing. The PSA (Particle Size Analyzer) instrument was used to measure the nanogel. Three replicates of 10 milliliter samples were made for each recipe. Someone put the nanogel into a cuvette. The PSA was then turned on. A pH meter was used to check the

pH. A sample weighed 0.5 grams and dissolved in 5 milliliters of distilled water was placed in a container. The pH meter was then dipped into the sample container, and the rest was moved until it was at a constant position. The pH value of the preparation is indicated by the numbers on the pH meter. Using a spreader, measure the diameter of the sample by 0.5 grams, place it between two glass plates, put a weight on it, and let it sit for a while. Then use a vernier caliper to determine its spreading power. Using an adhesion instrument, test the adhesion by applying a sample weighing 0.5 grams to a glass object, waiting five minutes, and calculating how long it takes to separate the two glass objects. Viscosity examination using a Brookfield viscometer. The nanogel formula is taken as much as 50 grams, put into a beaker, then the spindle and speed are adjusted, wait until the viscosity value is read (Vinay, 2019; Kurniasari & Atun, 2017).

*Antibacterial Activity Test Using Diffusion Well Method*

The Muller Hinton Agar (MHA) diffusion well technique was used to test the bacterial inhibition zone. 7.2 grams of distilled water was added, homogenized, and heated until clear. Three petri dishes were filled with MHA media, then allowed to solidify. Each colony of test bacteria (pathogenic bacteria) was spread into 0.1 mL (10 µL) of MHA (Muller Hinton Agar) media after being developed according to McFarland's criteria using a micropipette (Pratiwi, 2017; Kementerian Kesehatan RI, 2021). Holes (wells) were made in three different Muller Hinton Agar (MHA) media using a sterile perforator. A total of five holes were made in each medium: one for 1% glidamycin gel as a positive control, one for distilled water as a negative control, one for F1, F2, and F3 as the fifth and final hole. After that, all finished samples were concentrated for 24 hours at 37°C with the dish inverted. There will be an inhibition zone after the incubation period. The diameter of the inhibition zone was calculated using the shear period. (Maqbool et al., 2020).

**Table 3.** Organoleptic Observation Results

Formula	Organoleptic Observation			
	Day-0	Day-7	Day-14	Day-21
F1	Gel, green in color, has a distinctive aroma of telang flowers	Green gel, green in color, has a distinctive aroma of telang flowers	Green in color, butterfly pea flower extract has a pronounced aroma to its gel.	Gel, green in color, distinctive aroma of butterfly pea flower extract.
F2	Green gel, green in color, has a distinctive aroma of telang flowers	Green gel, green in color, has a distinctive aroma of telang flowers	Green gel, green in color, has a distinctive aroma of telang flowers	Green gel, green in color, has a distinctive aroma of telang flowers
F3	Green gel, green in color, has a distinctive aroma of telang flowers	Green gel, green in color, has a distinctive aroma of telang flowers	Green gel, green in color, has a distinctive aroma of telang flowers	Green gel, green in color, has a distinctive aroma of telang flowers

*Data Analysis*

The data obtained in the form of a spread power test were analyzed descriptively while the data from the inhibition power test against Propionibacterium acnes bacteria were analyzed statistically using Kruskal Wallis for differences between treatments. Data processing using software programs.

**Result and Discussion**

*Results*

*Phytochemical Screening Test*

Phytochemical testing on Nanogel BPFEE was carried out to confirm the secondary metabolite compounds contained in the nanogel (Sudjarwo, 2017). The results of the phytochemical test can be seen in Table 1 below.

**Table 1.** Phytochemical Test Result for Nanogel BPFEE

Bioactive Compound	Result
Flavonoid	+
Alkaloid	+
Saponin	+
Tannin	+

Note: (+): Contains tested compound (-): Does not contain the compounds tested

Phytochemical screening tests show that the nanogel BPFEE has secondary metabolites, namely, alkaloids, flavonoids, saponins and tannins (Cahyaningsih et al., 2019; Achrifia et al., 2024). These secondary metabolite compounds show that nanogel BPFEE have pharmacological effects and have the ability to act as medicinal ingredients.

**Table 2.** % Transmittance Results of the Preparation

Formula	Absorbance (nm)	Transmittance (%)
F1	0.055	88
F2	0.065	86
F3	0.073	84

Based on the optimization of the nanogel BPFEE which can be seen in Table 2, in the transmittance test, the results obtained were in formula 1 with a transmittance of 88%, in formula 2 it was 86%, in formula 3 it was 84%. This shows that the transmittance results approaching 100% or the highest indicate that the final formula is good, stable and indicates the clearest formula.

Organoleptic testing of nanogel BPFEE was carried out for 21 days of storage with testing on days 1, 7, 14, and 21. The results of observations on the shape, color and aroma of the nanogel can be seen in Table 3, in organoleptic testing there was no difference in each formulation. This is in accordance with the literature stating that nanogel is stable during storage.

**Table 4.** Results of Nanogel Particle Size

Formula	Nanogel Particle Size(nm)			Average ± SD
	I	II	III	
F1	112.6	113.3	118.7	114.9 ± 3.3
F2	119.0	203.9	241.7	188.2 ± 62.8
F3	235.9	279.6	336.2	283.9 ± 50.3

In testing the particle size of BPFEE nanogel, it was carried out to determine the distribution of nanogel sizes from the preparation, using PSA (Particle Size Analyzer). The results obtained can be seen in table 5, namely in formula 1 the value of 114.9 nm was obtained. Formula 2 is 188.2 nm, in formula 3 is 283.9 nm. So, it can be said that the size of the nanogel has a good particle size and distribution. Based on the results of statistical data processing for the particle size test, the nanogel can be distributed normally and homogeneously, then a parametric test of One-Way ANOVA analysis was carried out where the results obtained (sig. 012) were not significantly different (> 0.05).

**Table 5.** pH Test Results

Formula	pH Nanogel				Average ± SD
	Day-0	Day-7	Day-14	Day-21	
F1	5.9	5.7	5.5	5.4	5.6 ± 0.22
F2	6.1	5.8	5.6	5.5	5.8 ± 0.26
F3	6.2	6.0	5.9	5.7	6.0 ± 0.21

The pH test on the nanogel BPFEE aims to determine the safety of the preparation when applied to the skin so that it does not cause skin irritation. Based on the observation results in table 4.5, pH testing for 21 days of storage, the results of pH measurements with an average of 5.6 in F1 were obtained, 5.8 in F2, and 6.0 in F3. not less than 4.5 and not more than 6.5. Based on the SPSS results for the pH test, the nanogel can be distributed normally and homogeneously, then a parametric One-Way ANOVA analysis test was carried

out where the results obtained (sig.193) were not significantly different (> 0.05).

**Table 6.** Results of Adhesion Test

Formula	Adhesive Force Observation Data (sec)		Average ± SD
	0 g	20 g	
F1	1.5	1.22	1.36 ± 0.20
F2	1.7	2.16	1.9 ± 0.33
F3	2.1	2.19	2.1 ± 0.06

The adhesion test of nanogel BPFEE was carried out by testing at a load of 0 gr and 20 gr for 5 minutes as shown in Table 6, the results obtained were on average, namely in formula 1 the results were 1.36 seconds, in formula 2 the results were 1.9 seconds, and in formula 3 the results were 2.1 seconds. This states that topical preparations that adhere longer will increase the potential for drug absorption on the skin longer. Topical preparations that adhere longer will increase the potential for drug absorption on the skin better. Based on the SPSS results for the bacterial adhesion test, nanogels cannot be distributed homogeneously but are normally distributed, then a non-parametric Kruskal Wallis test was carried out where the results obtained (sig. 021) were not significantly different (> 0.05).

**Table 7.** Results of the Spread Power Test

Formula	Spread Power Test (cm <sup>2</sup> )						Average ± SD
	0 g	5 g	10 g	15 g	20 g	25 g	
F1	4.3	4.8	5.3	5.8	6.3	6.8	5.6 ± 94
F2	3.7	4.2	4.7	5.2	5.7	6.2	5.0 ± 94
F3	3.7	4.1	4.5	5.1	5.5	5.9	4.8 ± 85

The spreadability test of nanogel BPFEE was carried out by testing at loads of 0 gr, 5 gr, 10 gr, 15 gr, 20 gr, and 25 gr can be seen in table 4.6, in the spreadability test the average value was obtained, namely in formula 1 the value was 5.6 cm, in formula 2 the value was 5.0 cm, in formula 3 the value was 4.8 cm, based on Table 7, the results showed an increase in spreadability with the addition of each load used. This states that the higher the concentration of the formulation, the smaller the spreadability of the preparation. Based on the SPSS results for the particle size test, the nanogel can be distributed normally and homogeneously, then the parametric test of One-Way ANOVA analysis was carried out where the results obtained (sig.343) were not significantly different (> 0.05).

The viscosity test of the ethanol extract nanogel BPFEE was carried out using a viscometer with a speed of 60 rpm and a spindle rotor of 4, the results obtained were average, namely in formula 1 with a result of 4071 mPa.S, in formula 2 with a result of 3733.75 mPa.S, and in formula 3 with a result of 3043.75 mPa.S. The three

unstable formulas experienced a decrease in viscosity after storage on the 21st day. The decrease in nanogel viscosity can be caused by external factors such as temperature and storage method (Ayu Martini et al., 2020). Based on the SPSS results for the viscosity test, the nanogel can be distributed normally and homogeneously, then the One Way ANOVA parametric test was carried out where the results obtained were significantly different (<0.05), then a further test was carried out using LSD where F1 and F2 were not significantly different, F1 and F3 were significantly different (<0.05), F2 and F1 were not significantly different (>0.05), F2 and F3 were significantly different (<0.05), F3 and F1 were significantly different (<0.05), F3 and F2 were significantly different (<0.05).

**Table 8.** Viscosity Test Results

Formula	Nanogel viscosity (mPa.S)				Average ± SD
	Day-0	Day-7	Day-14	Day-21	
F1	3965	3996	4122	4201	4071 ± 110.09
F2	3607	3674	3756	3898	3733.75 ± 125.31
F3	2873	2978	3115	3209	3043.75 ± 148.17

**Table 9.** Results of Nanogel Antibacterial Activity Test

Formula	Antibacterial inhibition diameter (mm)			Average
	I	II	III	
K-	0	0	0	0 ± 0
K+	25.4	26	25.8	25.7 ± 0.31
F1	8	8.5	8	8.2 ± 0.29
F2	10.2	10	10.3	10.2 ± 0.15
F3	11.5	12	11.5	11.7 ± 0.29

Note: K (-) = Aquadest; K (+) = Gel Clyndamicin; F1 = Formula nanogel BPFEE 5 %; F2 = Formula nanogel BPFEE 10%; F3= Formula nanogel BPFEE 15%

The antibacterial activity test aims to see whether the nanogel BPFEE has inhibitory power against propanibactetium acnes bacteria using the well method, in Table 9, the results can be seen that the higher the concentration of the nanogel preparation formula, the higher the diameter of the bacterial growth inhibition zone which is indicated by a larger diameter of the inhibition zone with an average value of 3 replications, namely, the negative control did not get an inhibition zone, in F1 with an extract concentration of 5% with an inhibition zone of 8.2 mm, in F2 with an extract concentration of 10% with an inhibition zone of 10.2 mm, in F3 with an extract concentration of 15% with an inhibition zone of 11.7 mm (Magani et al., 2020; Amalia et al., 2024).

*Discussion*

Butterfly pea flowers were extracted using a maceration process using 96% ethanol filter fluid. A rotary vacuum evaporator was used to break down the material, and the result was a thick extract of 66.4 grams

with an extract yield percentage of 8.14%. This figure is in accordance with the Indonesian herbal pharmacopoeia which states that the yield value is not less than 5.6% in ethanol solvent (Hamka et al., 2022). The comparison of the amount of extract produced during extraction with the initial weight multiplied by 100% is called the yield (Hamka et al., 2022). Therefore in this study a new formulation was made that has better bioavailability than the extract, namely nanogel preparations. Good stability, good drug transport at the intracellular level, reduces skin irritation, protects against degradation, and cures acne (Ariyani & Wulandari, 2020).

Propionibacterium acnes is an anaerobic gram-positive bacteria that can cause inflammation of the skin. This bacteria is the main organism that plays a role in the formation of acne (Meilina & Hasanah, 2018).

Phytochemical tests carried out on the ethanol extract nanogel of butterfly pea flowers obtained the results in table 1, namely positive for alkaloids, saponins, tannins and flavonoids. This is in accordance with previous studies which stated that the alkaloid test using dragendroff reagent with the results of the formation of a red-orange color is indicated as positive for containing alkaloids, in the flavonoid test using Concentrated HCl reagent, Mg metal and 96% ethanol if an orange color is formed it means positive for containing flavonoids, in the tannin test using FeCl3 reagent the results obtained were blackish green indicated as positive for containing tannins, in the saponin test the results obtained were the formation of foam which was indicated as positive for containing saponin compounds with the addition of 2N HCl reagent (Tandi et al., 2020).

Based on the optimization of formulation nanogel BPFEE which can be seen in table 2 in the transmittance test, the results were obtained in formula 1 with a transmittance of 88%, in formula 2 it was 86%, in formula 3 it was 84%. This shows that the transmittance results approaching 100% or the highest indicate that the final formula is good, stable and indicates the clearest formula (Juliantoni et al., 2020). Based on the characterization of the ethanol extract nanogel formulation of butterfly pea flowers, characterization tests were carried out including organoleptic tests, transmittance tests, nanoparticle size tests, pH tests, viscosity tests, adhesive power tests, and absorption power tests, and inhibition tests (Windy et al., 2022). Organoleptic testing of the ethanol extract nanogel of butterfly pea flowers was carried out for 21 days of storage with testing on days 1, 7, 14, and 21. The results of observations on the shape, color and aroma of the nanogel can be seen in Table 3, in organoleptic testing there was no difference in each formulation. This is consistent with research showing that nanogels

maintain their stability over time (Ariyani & Wulandari, 2020).

In testing the particle size of the ethanol extract nanogel of butterfly pea flower, it was carried out to determine the distribution of the nanogel size of the preparation, using PSA (Particle Size Analyzer). The results obtained can be seen in table 4, namely in formula 1 the value of 114.9 nm was obtained. Formula 2 is 188.2 nm, in formula 3 is 283.9 nm. This is in accordance with the literature which states that nanogels have particle sizes between 1 - 1000 nm (Li et al., 2022). So it can be concluded that the size of the nanogel has a good particle size and distribution. Nanogel can be dispersed well and uniformly based on the results of the particle size test from SPSS. Then the parametric test of One-Way ANOVA analysis was carried out and the results (sig. 012) were not statistically different ( $> 0.05$ ).

The purpose of the pH test on the ethanol extract nanogel of butterfly pea flower is to ensure whether the product is safe for topical use and does not cause skin irritation. Based on the observation results in table 5, pH testing for 21 days of storage, the average pH measurement values for F1, F2, and F3 were 5.8 and 6.0, respectively. not lower or higher than 4.5 or 6.5. This is in accordance with research that shows the ideal pH range for the skin is between 4.5 and 6.5; it should not be too acidic or too alkaline. Ariyani et al. (2020) stated that pH levels that are too alkaline can cause dry and sensitive skin, while pH levels that are too acidic can worsen skin irritation. Based on the findings of the pH test from SPSS, nanogel can be applied thoroughly and uniformly. Then the One-Way ANOVA parametric analysis test was carried out and the results (sig.193) were not statistically different ( $>0.05$ ).

The nanogel BPFEE was tested for its spreadability at loads of 0 gr, 5 gr, 10 gr, 15 gr, 20 gr, and 25 gr. Table 6 shows the average values obtained from the spreadability test. Specifically, formula 1 produces a value of 5.6 cm, formula 2 produces a value of 5.0 cm, and formula 3 produces a value of 4.8 cm. This is in accordance with research showing that the spreadability of the preparation decreases with the concentration of the formulation (Ariyani & Wulandari, 2020). Based on the SPSS results for the particle size test, the nanogel can be distributed normally and homogeneously, then a parametric test of One-Way ANOVA analysis was carried out where the results obtained (sig.343) were not significantly different ( $> 0.05$ ).

The adhesion test of the nanogel BPFEE was carried out by testing at a load of 0 gr and 20 gr for 5 minutes as seen in table 6, the results obtained were on average, namely in formula 1 the results were 1.36 seconds, in formula 2 the results were 1.9 seconds, and in formula 3 the results were 2.1 seconds. This is in accordance with research showing that topical preparations that last

longer on the skin have a higher chance of allowing the drug to be absorbed there. Topical preparations that last longer have a higher chance of increasing drug absorption in the skin. For semi-solid preparations, the adhesion is more than one second (Ariyani & Wulandari, 2020). Nanogels cannot be spread homogeneously; instead, they are distributed regularly, according to SPSS findings for bacterial adhesion tests. Furthermore, the Kruskal Wallis non-parametric test was carried out and the results (sig. 021) were not statistically different ( $> 0.05$ ).

A viscometer with a speed of 60 rpm and a spindle rotator 4 was used to measure the viscosity of the ethanol extract of butterfly pea flower nanogel, as can be seen in table 8, the results obtained were on average, namely in formula 1 with a result of 4071 mPa.S, in formula 2 with a result of 3733.75 mPa.S, and in formula 3 with a result of 3043.75 mPa.S. On the 21st day, the viscosity of the third unstable formula decreased after storage. The viscosity of the nanogel may decrease due to environmental variables such as storage conditions and temperature (Ariyani & Wulandari, 2020). Based on the SPSS results for the viscosity test, the nanogel can be distributed normally and homogeneously, then the One Way ANOVA parametric test was carried out where the results obtained were significantly different ( $<0.05$ ), then a further test was carried out using LSD where F1 and F2 were not significantly different, F1 and F3 were significantly different ( $<0.05$ ), F2 and F1 were not significantly different ( $>0.05$ ), F2 and F3 were significantly different ( $<0.05$ ), F3 and F1 were significantly different ( $<0.05$ ), F3 and F2 were significantly different ( $<0.05$ ).

The purpose of the antibacterial activity test was to determine whether the ethanol extract of butterfly pea flowers in nanogel form can inhibit the growth of bacteria. *Propanibacterium acnes* using the well technique. Table 8 shows that the diameter of the bacterial growth inhibition zone increases with increasing concentration of the nanogel preparation formula. This can be seen from the increasing diameter of the inhibition zone if the three average values are calculated, namely at an extract concentration of 5% in F1, the inhibition zone measures 8.2 mm; in F2 with an extract concentration of 10%, the inhibition zone is measured at 10.2 mm; and in F3 with an extract concentration of 15%, the inhibition zone is measured at 11.7 mm. The negative control did not obtain an inhibition zone. The resulting inhibition zone has the following diameter: light, namely between 0 and 5 mm, moderate, namely between 5 and 10 mm, strong, namely between 10 and 20 mm, and very strong, namely above 20 mm (Emelda et al., 2021). Previous studies have shown that positive control clindamycin produces an inhibition zone with a diameter of 21.83 mm against

*Propionibacterium acnes* bacteria. The average diameter of the inhibition zone in the positive control of 25.7 mm is included in the very strong category (Chandra et al., 2021). Nanogel cannot be distributed normally and homogeneously based on SPSS results for bacterial inhibition tests. Therefore, a non-parametric Kruskal Wallis test will be carried out, the results of which are significantly different (0.05). Furthermore, further testing will be carried out using Mann-Whitney where K- does not have an inhibition zone and F1, F2, and F3 have an inhibition zone but do not have a K+ inhibition zone.

Different from previous research conducted by Ariyani et al. (2020). The findings of the antibacterial activity test on *Propionibacterium acnes* bacteria with a bacterial inhibition value of 8.2 mm for the ethanol extract nanogel formulation of butterfly pea flowers (*Clitoria ternatea* L.), better than the sunflower seed oil extract nanogel preparation (*Helianthus annuus* L.) on *Staphylococcus aureus* bacteria, which is 1.88 mm. This states that the ethanol extract nanogel preparation of butterfly pea flowers (*Clitoria ternatea* L.) has a greater inhibitory power than the sunflower seed oil extract nanogel preparation (*Helianthus annuus* L.). This can be caused by the antibacterial activity of the nanogel preparation being influenced by several things including the concentration, size and shape of the nanogel, and the microorganisms tested (Farhan, 2023). Because various butterfly pea flower food ingredients contain several bioactive chemicals that can inhibit the development and activity of microorganisms in bacteria, butterfly pea flowers have the potential to be natural antibacterials. This is because the product provides a good inhibition zone for bacteria. The active ingredient of butterfly pea flower, namely tannin, has the ability to activate enzymes, transport proteins in cell membranes, and microbial adhesins. Inhibiting growth and causing bacterial cell death, this process covers the bacterial cell membrane and disrupts the genetic function of the cell. In addition, the small size of the nanogel increases its tendency to interact with the bacterial cell wall and penetrate deeper, thereby damaging the membrane. Different from previous research conducted by Purwandari et al. (2020).

The results of the antibacterial activity test on *Propionibacterium acnes* bacteria with a bacterial inhibition value of 8.2 mm for the nanogel BPFEE, better than the nanogel preparation of cinnamon extract (*Cinamomum burmannii*) on *Staphylococcus aureus* bacteria, which is 6.7-7.1 mm. This states that the nanogel BPFEE has a greater inhibitory power than the nanogel preparation of cinnamon extract (*Cinamomum burmannii*). This can be caused by the antibacterial activity of the nanogel preparation being influenced by several things including the

concentration, size and shape of the nanogel, and the microorganisms tested. Because various butterfly pea flower foods contain several bioactive chemicals that can inhibit the development and activity of microorganisms in bacteria, butterfly pea flowers have the potential to be natural antibacterials. This is because the product provides a good inhibition zone for bacteria. The active ingredient of butterfly pea flowers, namely tannin, has the ability to activate enzymes, transport proteins in cell membranes, and microbial adhesion. This process weakens the bacterial cell membrane and disrupts the cell's genetic activity, inhibiting growth and causing bacterial cell death. In addition, the small size of the nanogels increases their tendency to interact with the bacterial cell wall and penetrate deeper, damaging the membrane.

Different from previous research conducted by Ariyani et al. (2020). The results of the antibacterial activity test of the nanogel BPFEE against *Propionibacterium acnes* bacteria, with a bacterial inhibition value of 8.2 - 11.7 mm, better than the olive oil extract nanogel preparation on *Staphylococcus aureus* bacteria, which is 2.5 - 7.5 mm. This states that the nanogel BPFEE has a greater inhibitory power than the olive oil extract nanogel preparation. This is due to the fact that the concentration, size, and shape of the nanogel, along with the microorganisms studied, can all affect the antibacterial efficacy of the preparation. Because various butterfly pea flower food ingredients contain several bioactive chemicals that can inhibit the development and activity of microorganisms in bacteria, butterfly pea flowers have the potential to be natural antibacterials (Nabila et al., 2022). This is because the product provides a good inhibition zone for bacteria. The active ingredient of butterfly pea flower, namely tannin, has the ability to activate enzymes, transport proteins in cell membranes, and microbial adhesins. This process weakens the bacterial cell membrane and disrupts the genetic activity of the cell, thereby inhibiting growth and causing bacterial cell death. In addition, the small size of the nanogel increases its tendency to interact with the bacterial cell wall and penetrate deeper, thereby damaging the membrane.

## Conclusion

Based on the results of the research that has been done, it can be concluded that BPFEE nanogel can be made in the form of nanogel preparations with 3 variations of stable extract concentrations and has a high inhibition zone for antibacterial activity against *Propionibacterium acnes* bacteria.

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### Author Contributions

Research design and manuscript preparation, data collection, analysis, and interpretation, N.P.D., R., M., and I.K.U.; reviewing, editing, and validating the manuscript, Y.R., N.R., N., and N.K.M. All authors have read and approved the final version of the manuscript.

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### Conflicts of Interest

The authors declare no conflict of interest.

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