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Formulation and Antibacterial Activity Test Of Green Tea Leave (*Camellia sinensis* L.) Ethanol Extract Serum with Variation Concentrations of Xanthan Gum Against *Propionibacterium acnes* ATCC 11827

Formulasi dan Uji Aktivitas Antibakteri Sediaan Serum Ekstrak Etanol Daun Teh Hijau (*Camellia sinensis* L.) dengan Variasi Konsentrasi Xanthan Gum Terhadap *Propionibacterium acnes* ATCC 11827

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Abstract

Green tea leaves contain secondary metabolites of the flavonoid group (catechins), alkaloids, saponins, steroids, and tannins which have an antibacterial activity that causes acne. Anti-acne from green tea leaf ethanol extract was developed in serum preparations to facilitate topical use. This study aims to determine the effect of varying concentrations of xanthan gum on physical quality, stability, and antibacterial activity and determine which formula has good physical quality and stability and can inhibit *P. acnes* bacteria effectively.

Green tea leaves were extracted by the maceration method using 96% ethanol formulated into three formulas with 8% green tea leaf extract concentration and variation of xanthan gum concentration, respectively 1; 1,2; and 1.4%. The serum preparations were tested for organoleptic, homogeneity, *p*H, viscosity, dispersibility, adhesion, and stability, and then, the antibacterial activity test was continued using the hole method. The data were then subjected to a statistical analysis test using the SPSS program.

The results showed that serum preparations of green tea leaf extract concentration of 8% with 1% and 1.2% variations of xanthan gum concentrations had good physical quality and stability. The inhibition power of serum preparations against *P. acnes* bacteria in F1 is 15.42 mm, F2 is 15.31 mm, F3 is 13.97 mm, and K(-) is 2.57 mm. The results also showed that formula 1 with a 1% concentration of xanthan gum in the serum had the best physical quality and stability and effectively inhibited *P. acnes* bacteria.

keywords: *Propionibacterium acnes, Camellia sinensis* L., serum, xanthan gum

Abstrak

Daun teh hijau mengandung metabolit sekunder golongan flavonoid (katekin), alkaloid, saponin, steroid dan tanin yang mempunyai aktivitas sebagai antibakteri penyebab jerawat. Antijerawat dari ekstrak etanol daun teh hijau dikembangkan dalam sediaan serum untuk memudahkan dalam penggunaan secara topikal. Penelitian ini bertujuan untuk mengetahui pengaruh variasi konsentrasi xanthan gum terhadap mutu fisik, stabilitas, dan aktivitas antibakteri, juga untuk mengetahui formula yang memiliki mutu fisik dan stabilitas yang baik, serta mampu menghambat bakteri *P. acnes* secara efektif.

Daun teh hijau diekstraksi dengan metode maserasi menggunakan etanol 96% diformulasikan ke dalam tiga formula dengan konsentrasi ekstrak daun teh hijau 8% dan variasi konsentrasi xanthan gum berturut-turut 1; 1,2; dan 1,4%. Sediaan serum diuji organoleptik, homogenitas, *p*H, viskositas, daya sebar, daya lekat, stabilitas dan dilanjutkan uji aktivitas antibakteri dengan metode sumuran. Hasil data yang diperoleh dilanjutkan dengan uji analisis statistik menggunakan program SPSS.

Hasil penelitian menunjukkan bahwa sediaan serum ekstrak daun teh hijau konsentrasi 8% dengan variasi konsentrasi xanthan gum 1% dan 1,2% mempunyai mutu fisik dan stabilitas yang baik. Daya hambat sediaan serum terhadap bakteri *P. acnes* pada F1 sebesar 15,42 mm, F2 sebesar 15,31 mm, F3 sebesar 13,97 mm dan K(-) sebesar 2,57 mm. Hasil penelitian juga menunjukkan formula 1 dengan konsentrasi xanthan gum 1% pada sediaan serum memiliki mutu fisik dan stabilitas paling baik serta efektif menghambat bakteri *P. acnes.*

kata kunci: *Propionibacterium acnes, Camellia sinensis* L., serum, xanthan gum

Introduction

Acne is the most common skin problem experienced by people. Acne commonly attacks 85% of the world's population aged 11-30 years [1]. Bacteria are one of the factors that cause acne. 78.8% of acne lesions are caused by Propionibacterium acnes bacteria (2). Propionibacterium acnes is a Gram-positive bacterium that plays a role in causing acne infection by producing metabolites that can react with sebum to increase inflammation. These bacteria are the most common microorganisms in acne lesions [3].

Treatment of acne with natural ingredients provides fewer side effects than chemical drugs, the plants to be used are also readily available around us, and the price is relatively lower. Green tea (*Camellia sinensis* L.) is a natural ingredient with anti-acne activity [4]. Green tea leaves (*Camellia sinensis* L.) contain secondary metabolites from the class of flavonoids (catechins), alkaloids, saponins, steroids, and tannins [5]. The main acne-causing antibacterial compounds in green tea leaves are catechins with a mechanism n that inhibits fatty acid synthesis in bacteria and the production of toxin metabolites in bacteria [6]. Anti-acne from green tea leaf extract was developed in serum preparations.

The serum is a formulation with very concentrated active ingredients and a low viscosity. It can form a thin layer of active ingredients on the skin's surface so that it is quickly absorbed and penetrates the deeper layers of the skin. The advantage of serum is that it contains natural active substances that are good for the skin compared to other preparations, easily penetrates the skin layers, gives a soft and moist feeling after use, works locally, and is easily applied to the skin surface [7]. One component that makes up serum preparations is the gelling agent (thickener). Differences in the concentration of the gelling agent in serum preparations will affect the physical quality of the preparations. The most commonly used gelling agent is xanthan gum, which produces a fairly stable viscosity against pH, temperature, and salt effects. According to previous studies, it was stated that variations in the concentration of xanthan gum 1; 1,2; and 1.4% in tomato extract serum preparations could affect organoleptic and pH during the storage period as indicated by changes in texture and changes in the pH value of the preparation [8].

Green tea leaf extract serum formulation has never been tested for *Propionibacterium acnes* antibacterial before, so it is hoped that this research can increase knowledge in the utilization of green tea leaves and be beneficial for all people. The formulation in this study was also carried out by varying the concentration of xanthan gum as a gelling agent to obtain serum preparations that meet the physical requirements of good physical quality. This study aims to determine the effect of varying concentrations of xanthan gum on physical quality, stability, and antibacterial activity and determine which formula has good physical quality and stability and can inhibit *P. acnes* bacteria effectively.

Research Method

Tools and Materials

The tools used in this study were gloves, masks, knives, sieve mesh no 40, analytical balance, blender, flannel cloth, filter paper, vacuum evaporator set, water bath, rotary evaporator, Erlenmeyer tube, maceration bottle, measuring cup, beaker glass, stirring rod, Moisture balance, Sterling Bidwell, separating funnel, porcelain exchanger, oven, refrigerator, Bunsen, Ose needle, cotton swab, sterile cotton swab, tweezers, permanent marker, petri dish, test tube, test tube rack, object glass, incubator,

dropping pipettes, spirit lamps, laminar air flow, paper discs, mortar, stampers, glassware (measuring flasks, measuring cups, watch glasses, beakers, etc.), spatulas, pipettes, porcelain cups, pH meters, Brookfield viscometers, serum preparation containers.

The materials used in this study were green tea leaves (*Camellia sinensis* L.), extracts of green tea leaves which were filtered with 96% ethanol using the maceration method were used as active ingredients, xanthan gum, glycerin, DMDM hydantoin, sodium metabisulfite, distilled water, 96% ethanol, toluene, methylated spirit, Propionibacterium acnes bacterial isolate, Mg powder, concentrated HCL, amyl alcohol, H2SO4, FeCl3, Dragendor reagent ff, Mayer reagent, Nutrient Agar (NA) Media, Mueller Hinton Agar (MHA) Media, blood plasma, 0.9% NaCl.

Procedure

Collection and determination of plants. Green tea leaf plants (*Camellia sinensis* L.) were obtained from Tawangmangu, Karanganyar, Central Java. Furthermore, the plants were determined at the UPT Laboratory of the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT), Tawangmangu, Central Java.

Production of green tea leaf powder. Green tea leaves are washed in running water and dried in the sun with a black cloth. Dry green tea leaves are sorted dry beforehand to separate foreign matter from entering. Then the green tea leaves are powdered using a blender. After that, it was sieved with a 40-mesh sieve. The sieving process is useful for obtaining powders of the same size so that the active substances can be released evenly.

Preparation of green tea leaf extract. The extract was prepared by the maceration method, 800 grams of green tea leaf powder dissolved in 8 liters of 96% ethanol (1: 10). The extraction process is left to stand for 24 hours with occasional shaking. After 24 hours, the filtrate was filtered, then concentrated using a rotary evaporator at 60°C at a speed of 50 rpm, then thickened again by evaporating it in a water bath at 40-65°C to obtain a thick green tea leaf extract.

Identification of chemical constituents of green tea leaf extract. The green tea leaf extract to be identified was weighed as much as 1 g, added 50 ml of distilled water, and heated for 10 minutes, then cooled, filtered, and the filtrate was taken to add the test reagent.

Identification of flavonoids. The filtrate was put into a test tube of as much as 1 ml, then magnesium powder was added to taste, 1 ml of hydrochloric acid, and 2 ml of amyl alcohol, shaken vigorously, then allowed to separate. Positive results of flavonoids are indicated by the formation of red/yellow/orange color on the amyl alcohol layer [9].

Identification of alkaloids. The Mayer, Bouchardat, and Dragendorff methods carried out the identification of alkaloids. Weigh 500 mg of the extract, add 1 ml of 2 N HCl and 9 ml of distilled water, heat for 2 minutes, cool, and then filter. The filtrate was divided into 3 test tubes, then each tube was added with 2 drops of Mayer's, Bouchardat's, and Dragendorff's reagents. Positive alkaloid results were indicated by the formation of a white precipitate in Mayer's reagent, the formation of a reddish-brown color in Bouchardat's reagent, and the formation of an orange color in Dragendorff's reagent [10].

Identification of saponins. 1 ml of filtrate was put into a test tube then, 10 ml of hot water was cooled and shaken vigorously for 10 seconds. Let sit for 30 seconds, then add concentrated hydrochloric acid. Positive for containing saponins if the foam is formed as high as 1-10 cm for not less than 10 minutes, and on adding one drop of HCL 2 N, the foam does not disappear [11].

Steroid identification. Green tea leaf extract as much as 1 ml added 2 ml of 70% ethanol then stirred, added 2 ml of chloroform, added 2 ml of concentrated H2SO4 by dripping slowly from the side of the test tube wall. A positive steroid result is indicated by the formation of a red ring [12].

Identification of tannins. 1 ml of filtrate was put into a test tube, then three drops of 1% FeCl3 reagent were added. Tannin-positive results are indicated by the formation of black-green color [13].

Identification of *P. acnes* bacteria

Identification of *P. acnes* on *Blood Agar Plate* (BAP). *P. acnes* bacterial suspension was inoculated by streaking the bacterial culture on BAP media and incubated at 37°C for 24 hours.

Gram stain. Identification of Gram stain was carried out by dripping one drop of sterile distilled water in the middle of the object glass aseptically to obtain the smear preparation, then inoculating the bacteria using an Ose needle on top of the object glass after that is fixing the object glass over a Bunsen flame quickly and carefully until the preparation is dry and there is no aqua dest left. The preparation was dripped with Gram A paint (Crystal Violet dye) and left for 2 minutes, then washed with running distilled water and dried. After that, it was dripped with Gram B paint (Lugol Iodine dye) and left for 1 minute and then washed with running distilled water and dried, then dripped with Gram C paint (alcohol) until the color thinned, finally covered with Gram D paint (Safranin dye) as the opposite paint, left for 30 seconds and washed using distilled water. The microscope is dripped with immersion oil and then observed under 100x magnification. *Propionibacterium acnes* positive results will show a purple color and form pairs of rods or short chains in bacterial cells [14].

Catalase test. The catalase test was carried out by taking 1 Ose of *Propionibacterium acnes* bacteria, placing it on an object glass, and dripping it with 1-2 drops of H2O2 solution. The results of a positive reaction for *Propionibacterium acnes* indicate the presence of air bubbles [15]

Coagulase test. The test was carried out using 200 µl of blood plasma and adding 3-4 Ose of *P. acnes* bacteria, homogenizing slowly, incubating for 24 hours at 37°C, observe for sediment at the bottom of the tube. Positive reaction results are indicated by the presence of lumps or deposits at the bottom of the tube [16].

Preparation of green tea leaf ethanol extract serum

Xanthan gum is dissolved with 10 mL of glycerin, let stand for ± 10 minutes, then add ± 50 mL of distilled water, gradually stirring until a serum base is formed (mixture 1). Sodium metabisulfite and DMDM hydantoin are dissolved in ± 2 mL of distilled water, then stirred until dissolved (mixture 2). Green tea leaf extract was dissolved in distilled water and stirred until homogeneous (mixture 3). Mixture 2 was added to Mixture 1 little by little while stirring until homogeneous, then added to Mixture 3 and stirred again until homogeneous. The mixed containers 2 and 3 are rinsed with a little distilled water, then the rinse results are added to all the mixed ingredients. Serum

added with 100 ml of distilled water ad, stir until homogeneous. The finished serum is put into the preparation container.

Table 1. Green tea leaf ethanol extract serum preparation formula

Material	Function	Concentration (%)				
		F1	F2	F3	K (-)	K (+)
Green tea leaf ethanol extract	Antibacterial active substance	8	8	8	-	Clindamycin
Xanthan gum	*Gelling agent*	1	1,2	1,4	1,2	
Glycerin	Humectants	10	10	10	10	
DMDM hydantoin	Preservative	0,3	0,3	0,3	0,3	
Sodium metabisulfite	Antioxidant	0,1	0,1	0,1	0,1	
Aquades	Solvent	Ad 100	Ad 100	Ad 100	Ad 100	

Explanation:
F1 : Anti-acne serum containing 1% concentration of xanthan gum
F2 : Anti-acne serum containing 1.2% concentration of xanthan gum
F3 : Anti-acne serum containing 1.4% concentration of xanthan gum
Control (-) : Serum base without extracts
Control (+) : Clindamycin antibiotics

Physical quality and stability testing

The physical quality test of serum preparations included organoleptic, homogeneity, pH, viscosity, spreadability, and adhesion. Organoleptic testing was carried out by observing the preparation's shape, color, and smell. Homogeneity testing is done by observing whether the ingredients are evenly mixed and there are no material granules. The pH measurement was carried out using a pH meter which was previously calibrated with a standard buffer solution of pH four and pH 7. Viscosity was measured using a Brookfield viscometer. The spreadability test was carried out by measuring the diameter of the spread of the serum preparation with the addition of 50 g, 100 g, and 150 g loads on a round glass. The adhesion test was carried out by recording the time the two glass objects separated. Then testing the stability of the preparation was carried out using the cycling test method, namely by storing the preparation at ±4°C for 24 hours, then transferring it to an oven at ±40°C for 24 hours for six cycles, then the physical condition of the preparation after the experiment was compared with the physical condition of the preparation before the experiment [18].

Testing the antibacterial activity of serum preparations

The antibacterial activity test was carried out using the well-diffusion method, namely by taking a bacterial suspension that had been equated with Mc. Farland 0.5 immediately before use with a sterile cotton swab and then rubbed onto the surface of the MHA media, then flattened and allowed to stand for 15 minutes, after which five wells with a diameter of 8 mm were made aseptically using a *boorprop* tool. Serum preparations of ethanol extract of green tea leaves formula 1, formula 2, formula 3, negative control, and positive control clindamycin were put into the well using a micropipette as much as 50 µl and incubated for 24 hours at 37°C. A circular clear zone around the well area indicated antibacterial activity. The diameter of the inhibition zone was measured with a vernier caliper. Then the results were averaged to obtain the inhibition zone from the serum preparation of green tea leaf ethanol extract [19].

Result and Discussion

Collection and determination of plants. The results of the determinations that have been made show that the plant used for the research was *Camellia sinensis* (L.)

Kuntze. The determination aims to ensure that the plants used are truly green tea plants.

Green tea leaf powder. The weight of the powder obtained was 1000 grams from 1200 grams of dry leaves. The yield obtained from the weight of the powder to the weight of dry leaves was 83.33%.

Green tea leaf extract. The resulting green tea leaf extract is 237 grams with an extract yield percentage of 29.625%. This result meets the requirements: the green tea leaf extract yield is not less than 7.8% [20].

Chemical content of green tea leaf extract. The chemical content of green tea leaf extract was identified by test tube through qualitative observation of the color change and precipitate reaction. The results of determining the chemical content of green tea leaf extract can be seen in Table 2.

Table 2. Results of identification of chemical content of green tea leaf extract

Chemical content		Reaction results	References	Interpretation of results
Flavonoids		A red color forms on the amyl alcohol layer	The formation of a red/yellow/orange color on the amyl alcohol layer [9].	(+)
Alkaloids	*Mayer*	No white precipitate formed	The formation of a white precipitate [17]	(-)
	Bouchardat	A reddish-brown precipitate formed	The formation of a reddish-brown precipitate [17]	(+)
	Dragendorff	Orange color formed	The formation of the orange color [17]	(+)
Saponins		2 cm high stable foam was formed	Formed foam as high as 1-10 cm for not less than 10 minutes [17]	(+)
Steroids/triterpenoids		A red ring is formed	Formation of a red color ring [12]	(+)
Tannins		The blackish-green color formed	A blackish-green color is formed [13]	(+)

Explanation:
(+) : there are compounds
(-) : no compounds

The results of identifying the chemical content in Table 2 show the same results as previous studies: green tea leaf extract contains flavonoids, alkaloids, saponins, steroids, and tannins [5].

Results of identification of *Propionibacterium acnes* bacteria

Identification of *P. acnes* on media *Blood Agar Plate* (BAP). The formation of small white bacterial colonies indicates a positive result for P. acnes. The colony's surface is convex and has a dense consistency on BAP media [21].

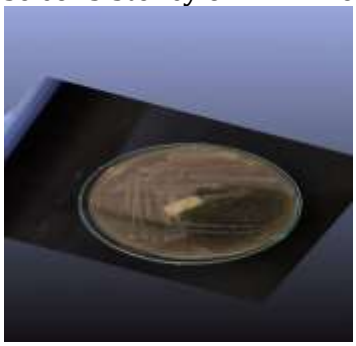

Image 1. BAP media test results

Gram stain. The observations of bacteria in the form of rod cells, small, scattered, and purple in color, show that the bacteria identified by Gram staining were indeed *Propionibacterium acnes* bacteria.

Image 2. Gram stain results

Catalase test. The test results showed the presence of gas bubbles (O2) on the surface of the glass object. This is because the *P. acnes* bacteria produce catalase enzymes which can decompose hydrogen peroxide (H2O2) into water (H2O) and gas bubbles (O2).

Image 3. Catalase test results

Coagulase test. The coagulase test results for *P. acnes* bacteria gave positive results, which were indicated by the formation of clots in the plasma, and when turned over, the plasma did not spill and remained attached to the test tube wall. Plasma clots are formed because the *P. acnes* bacteria can produce coagulase, which converts fibrinogen into plasma. Coagulase is a protein from *P. acnes* that can coagulate plasma when added with citrate or oxalate.

Image 4. Coagulase test results

Results of physical quality test of serum preparations

Organoleptic testing. The results of organoleptic testing of serum preparations can be seen in Table 3.

Table 3. Organoleptic test results for green tea leaf extract serum preparations

Formula	Phase	Color	Scent
F1	Thick like a gel	Green	Typical extract
F2	Thick like a gel	Green	Typical extract
F3	Thick like a gel	Green	Typical extract
K (-)	Thick like a gel	Bubbly clear	No odor

The results of organoleptic observations of serum preparations showed that there were no differences in the dosage form. Differences are shown in the color and scent of the preparations between F1, F2, F3, and K (-). Formula 1, formula 2, and

formula three were added with green tea leaf extract at a concentration of 8%, resulting in a greenish color and atypical green tea extract in the preparation. At the same time, the negative control gave the original color of the formula a transparent color and no odor because it was only a base without a mixture of extracts.

Homogeneity testing. The results of testing the homogeneity of serum preparations can be seen in Table 4.

Table 4. Results of homogeneity testing of green tea leaf extract serum preparation

Formula	Homogeneity
F1	Homogenous
F2	Homogenous
F3	Homogenous
K (-)	Homogenous

The test results showed that all the formulations were evenly mixed, marked by the absence of agglomerated particles from the active substance or the ingredients contained therein.

pH testing. The pH test is carried out to determine the level of acidity or alkalinity of the green tea extract serum prepared for its use on facial skin, so it must be ensured that it is safe when used. A pH that is too alkaline will cause the skin to become scaly, while a pH that is too acidic will cause skin irritation. Preparations meet the requirements if they are in the pH range of facial skin, namely pH 4.5-6.5 [22]. The results of the pH test can be seen in Table 5.

Table 5. Results of testing the pH of green tea leaf extract serum preparations

Formula	pH±SD
F1	5,03±0,01
F2	5,08±0,01
F3	5,31±0,01
K (-)	7,38±0,09

The pH test results showed that the serum formula containing green tea leaf extract had a value according to the requirements, which ranged from pH 5.03 to 5.31. In contrast, the negative control without extract did not meet the criteria because it had a pH value of 7.38. The concentration of xanthan gum can affect the pH of the preparation. The higher the concentration of xanthan gum, the higher the pH of the preparation. This can be due to the nature of xanthan gum, which has a neutral pH (pH=6.95) [23].

Viscosity testing. Viscosity is a test parameter to determine the flowability/thickness of a preparation. Viscosity measurement of serum preparations was measured with a Brookfield viscometer using spindle no. 2 with a speed of 30 rpm. The viscosity requirements for good serum preparations are 230-1150 cPs [24]. The results of the viscosity test can be seen in Table 6.

Table 6. The results of testing the viscosity of green tea leaf extract serum preparations

Formula	Viscosity (cPs)±SD
F1	697,3±62,35
F2	1121±4,00
F3	1526,33±5,77
K (-)	1288±21,38

The viscosity of F1 and F2 serum preparations met the requirements, while F3 and K(-) exceeded the maximum viscosity limit of serum preparations. The results showed that variations of xanthan gum and extracts affected the viscosity of the preparations. The higher the concentration of xanthan gum, the higher the viscosity of the preparation. This can be seen from the viscosity values of the formulas F1, F2, and

F3 with variations of xanthan gum, respectively, namely 1%, 1.2%, and 1.4%, directly proportional to the increase in the viscosity value. This increase in viscosity is due to the nature of xanthan gum, which is perfectly dispersed in water so that when the concentration of xanthan gum is increased, the emulsion will be thicker. This increase was also due to the formation of more complex polysaccharide polymer chain bonds between xanthan gum molecules [25].

Spreadability test. A spreadability test was carried out to determine the ability of the serum preparation to spread and contact the skin. Preparations meet the requirements if they are f 5-7 cm [26]. The results of the spreading power test can be seen in Table 7.

Table 7. Results of testing the spreadability of green tea leaf extract serum preparation

Formula	Spreadability (cm)±SD
F1	6,07±0,52
F2	5,96±0,48
F3	4,73±0,18
K (-)	6,01±0,69

The test results for all formulas obtained spreadability values between 4.73-6.07 cm, which means that formulas F1, F2, and K(-) met the criteria for good coverage, but formula three did not meet the requirements for serum coverage. The tendency of decreasing spreadability of serum preparations along with increasing concentration of xanthan gum in the formulation. This is related to the consistency and viscosity of the resulting serum preparation. Increasing the concentration of xanthan gum will cause the serum's consistency to be thicker and the viscosity of the serum to be greater so that the spreading power of the serum is getting smaller [27]. The greater the spreading power produced, the ability of the active substance to spread and contact with the skin is wider [27].

Adhesion test. The adhesion test was carried out to describe the serum preparation sticking to the skin. Preparations are stated to have good adhesion to the skin if they have an adhesion of > 1 second. The results of the adhesiveness test can be seen in Table 8.

Table 8. The results of testing the adhesion of green tea leaf extract serum preparations

Formula	Adhesiveness (second)±SD
F1	1,45±0,04
F2	1,50±0,03
F3	1,52±0,02
K (-)	1,52±0,04

The results of the adhesion test for all serum formulas had an adhesion of >1 second, this indicated that the green tea leaf extract serum had good adhesion to the skin. Serum adhesion increases with increasing concentration of xanthan gum.

Results of testing the stability of serum preparations

Organoleptic stability testing. Organoleptic stability testing was carried out to see whether there was a change in the preparation's shape, color, and smell due to changes in temperature and time. The results of the organoleptic stability test for green tea leaf extract serum preparations can be seen in Table 9.

Table 9. Results of organoleptic stability testing of green tea leaf extract serum preparations

Organoleptic						
Formula	Before *cycling test*			After *cycling test*		
	Phase	Color	Odor	Phase	Color	Odor
F1	Thick, like a gel	Green	Typical extract	Gel	Green	Typical extract
F2	Thick, like a gel	Green	Typical extract	Gel	Green	Typical extract
F3	Thick, like a gel	Green	Typical extract	Gel	Green	Typical extract
K (-)	Thick, like a gel	Bubbly clear	No odor	Gel	Bubbly clear	No odor

The results of the stability test for green tea extract serum preparations for all formulas were seen visually, and they did not show a change in color and odor before and after stability. Still, there was a change in the thickness of the preparation, which became slightly runny after the stability test was carried out. This change in the consistency of the preparation could be due to the storage temperature. So it can be concluded that the serum preparations at F1, F2, F3, and K(-) are organoleptically stable in color and odor. Still, the difference in storage temperature affects the form of the thickness of the preparation. Stable organoleptic will provide comfort for long-term use.

Homogeneity stability testing. Homogeneity stability testing was carried out to see whether temperature and storage time changes affected the preparation's homogeneity. The results of testing the stability of the homogeneity of the serum preparation of green tea leaf extract can be seen in Table 10.

Table 10. The results of testing the stability of the homogeneity of green tea leaf extract serum preparations

Formula	Homogeneity	
	Before *cycling test*	After *cycling test*
F1	Homogenous	Homogenous
F2	Homogenous	Homogenous
F3	Homogenous	Homogenous
K (-)	Homogenous	Homogenous

The results of testing the stability of the homogeneity of the serum preparation showed that there was no separation, the color was even, and there were no coarse lumps, which means that the serum preparation remained homogeneous during storage. The test results showed that the difference in storage temperature did not affect the homogeneity of the serum preparation.

pH stability testing. The results of pH stability testing of green tea leaf extract serum preparations can be seen in Table 11.

Table 11. Results of pH stability testing of green tea leaf extract serum preparations

Formula	pH±SD	
	Before *cycling test*	After *cycling test*
F1	5,03±0,01	5,01±0,01
F2	5,08±0,01	5,08±0,00
F3	5,31±0,01	5,30±0,00
K (-)	7,38±0.09	7,27±0,01

Statistical analysis was carried out using the *paired T-test* to determine the difference in the pH of the serum preparation before and after the stability test. The results of the analysis preparation obtained sig 0.083 (p> 0.05). There is no significant difference in the pH of the serum preparation before and after the stability test. Changes in the pH value that usually occurs can be influenced by the media decomposing at storage temperature, producing a pH with acidic or basic properties. Changes in storage

temperature can lower the pH of the preparation due to the effect of contact with the humidity of the preparation, where CO_2 gas in the air can react with water in the preparation to form acid [28].

Viscosity stability testing. The results of the viscosity stability test for green tea leaf extract serum preparations can be seen in Table 12.

Table 12. The results of testing the viscosity stability of green tea leaf extract serum preparations

Formula	Viscosity (cPs)±SD	
	Before *cycling test*	After *cycling test*
F1	697,3±62,35	589,2±67,41
F2	1121±4,00	1011,77±34,00
F3	1526,33±5,77	1313,33±23,63
K (-)	1288±21,38	1123±6,00

The results of the viscosity stability test were statistically analyzed using the *paired T-test* to determine the differences in the viscosity of the serum before and after the stability test. The results of the analysis obtained sig 0.000 ($p < 0.05$). This indicated that the viscosity of the serum preparation before and after the stability test was significantly different. A decrease in viscosity values indicates viscosity changes. This decrease can be caused by storage temperature. Preparations stored in an oven temperature of ± 40°C can cause the polymer chain to uncoil to form a ball or disentangle, decreasing the viscosity of the serum preparation. In comparison, preparations stored at a cold temperature of ± 4°C will cause the polymer chains to shorten and will join together so that, over time, the serum will shrink or entangle, resulting in a change in viscosity [29].

Spreadability stability testing. The results of testing the stability of the spreadability of green tea leaf extract serum preparations can be seen in Table 13.

Table 13. The results of testing the stability of the spreadability of green tea leaf extract serum preparations

Formula	Spreadability (cm)±SD	
	Before *cycling test*	After *cycling test*
F1	6,07±0,52	6,24±0,79
F2	5,96±0,48	5,98±0,65
F3	4,73±0,18	5,86±0,68
K (-)	6,01±0,69	6,10±0,62

The results of the spreadability stability test of the preparation were analyzed statistically using the *paired T-test* to determine differences in the dispersing ability of the serum preparation before and after the stability test. The analysis results obtained were sig 0.000 ($p < 0.05$), indicating that the serum preparation's dispersing ability was significantly different before and after the stability test. This difference can be seen from the increased spreadability value obtained. The increase in the spreading power value is affected by the viscosity of the preparation, which has decreased due to the length of storage.

Adhesion stability test. The results of testing the stability of the adhesion of green tea leaf extract serum preparations can be seen in Table 14.

Table 14. Stability of the adhesion of green tea leaf extract serum preparations

Formula	Adhesiveness (second)±SD	
	Before *cycling test*	After *cycling test*
F1	1,45±0,04	1,38±0,01
F2	1,50±0,03	1,40±0,05
F3	1,52±0,02	1,42±0,02
K (-)	1,52±0,04	1,42±0,04

The results of the adhesive stability test were followed by statistical analysis using the *paired T-test* to determine differences in the adherence ability of the serum before and after the stability test. The analysis results were sig 0.000 (p <0.05), indicating that the inherent ability of the serum before the stability test and after the stability test was significantly different. This decrease in stickiness is directly proportional to the decreased consistency of the preparation.

Antibacterial activity of serum preparations. Testing the antibacterial activity of green tea leaf extract serum against *Propionibacterium acnes* ATCC 11827 was conducted using the good diffusion method. The inhibition zone was indicated by a clear zone around the wells, indicating that the chemical compounds from green tea leaf extract in serum preparations were effective as antibacterials. The results of the antibacterial activity test of green tea leaf extract serum follow.

Table 15. Results of testing the antibacterial activity of serum preparations of ethanol extract of green tea leaves

Formula	Inhibition zone diameter (mm)			Average (mm) ± SD	Categories
	Replication 1	Replication 2	Replication 3		
F1	14,32	16,48	15,47	15,42±1,08	Strong
F2	13,78	16,90	15,25	15,31±1,56	Strong
F3	12,03	16,00	13,87	13,97±1,99	Strong
Control (+)	23,85	22,38	34,12	26,78±6,40	Very strong
Control (-)	0	4,35	3,35	2,57±02,28	Weak

The results of testing the antibacterial activity of green tea leaf extract serum preparations yielded an average diameter of inhibition in formula 1, formula 2, and formula 3, respectively 15.42; 15.31; and 13.97 mm. These results indicate that the antibacterial activity in formulas 1, 2, and 3 is included in the strong category, namely the inhibition value of more than 10 to 20 mm [30].

The results of the inhibition data obtained were then analyzed using SPSS using the *Shapiro-Wilk* test, which stated that sig > 0.05 means that the data were normally distributed, then the analysis was continued with One-way ANOVA to see whether or not there was a difference in the diameter of the resulting inhibition. The data used is not homogeneous with sig 0.016 (p <0.05). The results of the One-way ANOVA analysis obtained a sig value of 0.000 (p <0.05), which means that the diameter of the drag was significantly different. The analysis that has been carried out shows that all formulas have antibacterial activity, as indicated by the formation of the diameter of the inhibition zone. The analysis was continued with Tukey's post hoc test, which showed a significantly different negative control for all variables. The positive control also has a significant difference to all variables, and this is because the positive control has broad activity against *P. acnes* bacteria. Tukey's analysis showed that formula 1, formula 2, and formula 3 had no significant difference. The data obtained shows that the inhibition of Formula 1 is greater than Formula 2 and Formula 3. The viscosity and pH of the preparation can affect the diameter results of antibacterial inhibition. The greater the viscosity, the greater the resistance to inhibit the release of the active substance. This will result in the inhibition of *P. acnes* bacteria which has a decreasing impact. This is because the preparation inhibits the release of the active compound content from the extract to diffuse into the media so that the extract contained in the preparation is not completely released into the media (31). The pH value of the preparation also affects bacterial growth, and pH 6.5-7.5 is the optimum pH for bacterial growth (32). In this study, the pH of the preparation with the addition of the extract had an acidic pH with a

value of 5.03-5.31, which means that it is outside the optimum pH range. It causes the activity of bacterial enzymes to be disrupted and inhibits the growth of bacteria so that the inhibition of bacteria will be even greater. Based on the analysis that has been done, it can be concluded that the greater the concentration of xanthan gum, the lower the ability of the serum to inhibit *P. acnes* bacteria.

Conclusion

A higher concentration of xanthan gum affects increasing pH, viscosity, adhesion, and spreadability, and the stability of serum preparations of ethanol extract of green tea leaves (*Camellia sinensis* L.). Serum preparations of ethanol extract of green tea leaves (*Camellia sinensis* L.) with various concentrations of xanthan gum 1, 1.2, and 1.4% had antibacterial activity against *Propionibacterium acnes* with diameters of inhibition zones, respectively 15.42, 15.31, and 13.97 mm. Formula 1 is a serum preparation of ethanol extract from green tea leaves (*Camellia sinensis* L.) with various concentrations of xanthan gum, which has the best physical quality and stability and effectively inhibits *Propionibacterium acnes* bacteria.

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