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Evaluation Anti-Acne Gel Combination of Ethanol Extract of Cayenne Pepper (*Capsicum frutescens* L.) and Soursop (*Annona muricata* L.) Leaves

(Evaluasi Gel Anti Jerawat Kombinasi Ekstrak Etanol Daun Cabai Rawit (*Capsicum frutescens* L.) dan Daun Sirsak (*Annona muricata* L.))

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Abstract: Acne is common near adulthood, where the causes are *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Staphylococcus aureus*. One alternative acne treatment is cayenne pepper leaves (*Capsicum frutescens* L.) and soursop leaves (*Annona muricata* L.). This study aimed to determine the best formulation, and antibacterial activity of gel preparations of a combination of ethanol extract of cayenne pepper leaves (EECPL) and ethanol extract of soursop leaves (EESL) as an inhibitor of acne-causing bacteria. The combination gel preparations of EECPL and EESL with formulations namely F1 (1% and 1%), F2 (3% and 3%), and F3 (5% and 5%) were analyzed for physical properties, antibacterial activity and stability of gel preparations with one-way ANOVA $p<0.05$. Gel preparations are stable in organoleptic, homogeneity, adhesion, spreadability, and viscosity, while pH changes after physical stability testing using the cycling test method. *S. aureus* was best inhibited by gel preparation F2 with an average inhibition zone diameter of 10.83 mm, *S. epidermidis*, and *P. acnes* were best inhibited by gel preparation F3 with an average inhibition zone diameter of 9.56 mm and 10.4 mm. Therefore, combination of EECPL and EESL in gel preparation can be used as an anti-acne gel with moderate and strong inhibition.

Keywords: Antibacterial, cayenne pepper leaf, gel, soursop leaf

Abstrak: Jerawat biasa terjadi mendekati usia dewasa. Jerawat disebabkan oleh *Staphylococcus epidermidis*, *Propionibacterium acnes* dan *Staphylococcus aureus*. Salah satu alternatif pengobatan jerawat yaitu menggunakan daun cabai rawit (*Capsicum frutescens* L.) dan daun sirsak (*Annona muricata* L.). Tujuan penelitian ini bertujuan mengetahui formulasi terbaik dan aktivitas antibakteri sediaan gel kombinasi ekstrak etanol daun cabai rawit dan esktrak etanol daun sirsak sebagai penghambat bakteri penyebab jerawat. Sediaan gel kombinasi ekstrak etanol daun cabai rawit dan ekstrak etanol daun sirsak dengan formulasi yaitu F1 (1% dan 1%), F2 (3% dan 3%), dan F3 (5% dan 5%) dianalisis sifat fisik, aktivitas antibakteri dan stabilitas sediaan gel dengan one-way ANOVA $p<0,05$. Hasil sediaan gel stabil pada organoleptis, homogenitas, daya lekat, daya sebar dan viskositas, sedangkan pada pH terjadi perubahan setelah uji stabilitas fisik menggunakan metode *cycling test*. *S. aureus* paling baik dihambat oleh sediaan gel F2 dengan diameter zona hambat rata-rata sebesar 10,83 mm, *S. epidermidis* dan *P. acnes* dihambat paling baik oleh sediaan gel F3 dengan rata-rata diameter zona hambat pada 9,56 mm dan 10,4 mm. Kesimpulan kombinasi EECPL dan EESL dalam sediaan gel dapat digunakan sebagai gel antijerawat dengan daya hambat sedang dan kuat.

Kata kunci: Antibakteri, daun cabai rawit, daun sirsak, gel

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INTRODUCTION

ACNE vulgaris is one of the most common skin diseases that occur when approaching adulthood. The cause of acne is a bacterial infection, and bacteria will secrete the lipase enzyme so that free fatty acids are split from skin lipids which will then cause inflammation in the tissues[1]. Places that are usually overgrown with acne are the back, chest, neck, and face[2]. Some bacteria that cause acne is *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Staphylococcus aureus*[3,4]. Acne can generally be treated using comedolytic compounds and antibiotics, such as sulfur, retinoids, and benzoyl peroxide, while antibiotics, for example, tetracycline, erythromycin, and clindamycin. Side effects of prolonged use of comedolytic compounds will irritate the skin as well as trigger allergic reactions, and the use of antibiotics has side effects of resistance[5].

There are side effects to the use of comedolytic compounds and antibiotics, and it is necessary to have alternative acne treatment using natural plants that have relatively smaller side effects. One alternative treatment for acne is to use a garden such as cayenne pepper leaves (*Capsicum frutescens* L.) which contain flavonoids compounds that are inhibitors of *Staphylococcus aureus* and soursop leaves (*Annona muricata* L.) which contain flavonoids which are inhibitors of *Staphylococcus epidermidis* and *Propionibacterium acnes*[6,7].

Applying acne treatment using plants will be easier and more convenient if it is made into a cosmetic preparation, one of which is a gel preparation. Gel preparations are often used to treat acne because gels are solvent-based preparations with a high level of polarity so that they are easily washed or cleaned from the skin's surface, then can reduce the severity of acne[8]. The study aimed to determine the best formulation and antibacterial activity of a combination gel preparation of ethanol extract of cayenne pepper leaves (*Capsicum frutescens* L.) (EECPL) and ethanol extract of soursop leaves (*Annona muricata* L.) (EESL) against acne-causing bacteria such as *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Staphylococcus aureus*.

MATERIALS AND METHODS

MATERIALS. Cayenne pepper leaves (*Capsicum frutescens* L.) (Banjarnegara, Indonesia), clindamycin gel 1.2%, soursop leaves (*Annona muricata* L.) (Banyumas, Indonesia), Hydroxypropyl methylcellulose (HPMC), propyleneglycol, glycerin, alcohol 96%, TEA, aquadest, Mueller Hinton agar media, *Staphylococcus epidermidis* test bacteria from Yogyakarta Health and Calibration Laboratory, *Propionibacterium acnes* and *Staphylococcus aureus* from Biology Laboratory, Faculty of Medicine, University of Sultan Agung, Semarang.

Tools. Sieve with mesh no. 200, analytical balance sheet Labtronics GH-223 (PT. Yahong Trading Indonesia, Jakarta Barat, Indonesia), blender Miyako BL102GS (PT. Kencana Gemilang, Jakarta Barat, Indonesia), filter paper, object glass, funnel, ose needle, stirring rod, erlenmeyer Pyrex (PT. Iwaki Glass Indonesia, Sumedang, Indonesia), test tube, mixer, spatial, tweezer, bunsen fire, gel pot, measuring cup, rotary evaporator Biobase RE100-Pro, waterbath Memmert WNB 14 (PT. Sumber Aneka Karya Abadi, Jakarta, Indonesia), petri dish, pH meter Hanna 98107 (Jakarta, Indonesia), weighers, test tube rack, crossbar, autoclave GEA 35 liter (Global Sinar Medika, Jakarta, Indonesia), incubator, hot plate, and micropipette, viscometer rotary NDJ-1 (Jakarta, Indonesia).

METHODS. Plant Determination. Determination was carried out to ensure that the plants used were true *Capsicum frutescens* L. and *Annona muricata* L. Determination were conducted at the Environmental Laboratory, Faculty of Biology, Jenderal Soedirman University with the results in decision letter number 089/HP.LL/VIII/2021.

Extraction of Cayenne Pepper Leaves and Soursop Leaves. Raw materials of cayenne pepper leaves (*Capsicum frutescens* L.) were wet, chopped, washed and then drained. After that, for seven days, the leaves were dried in the direct sunlight. Dry cayenne pepper leaves simplisia is reduced in size using a blender. Extraction was carried out using 800 grams of dried cayenne pepper leaves simplisia, then macerated with 96% ethanol solvent as much as 8 liters for three days with stirring once a day. The obtained macerate was evaporated with a rotary evaporator and water bath. The exact process was also carried out on soursop leaves (*Annona muricata* L.).

Phytochemical Screening. Identifying secondary metabolites from EECPL and EESL include flavonoids, alkaloids, tannins, terpenoids, saponins, and steroids. The test solution was prepared by dissolving 200 mg of viscous extracts (EECPL and EESL) with 25 mL of 70% ethanol.

Test solution for flavonoid was added with magnesium and dripped with 2N hydrochloric acid. Heat the mixture on a hot plate for 30 minutes and then filter. The resulting filtrate was dripped with amyl alcohol, and vigorous shaking was done. A yellow to red colored solution indicates the presence of flavonoid[9].

The test solution for testing tannin was added with 10% $FeCl_3$ solution drops. The presence of tan-

nins is indicated if the color of the solution becomes bluish-black[9].

The test solution for testing alkaloid was dripped with chloroform and then with Wagner, Mayer, and Dragendorf reagents. The presence of alkaloids is indicated if the color of the solution becomes brownish-white[9].

The test solution for testing terpenoid was added with glacial acetic acid and H_2SO_4. The presence of terpenoids is indicated by the solution turning yellow[9].

The test solution for testing steroid was added with glacial acetic acid and H_2SO_4. The presence of steroids is indicated by the solution turning a yellow color[9].

The test solution for testing saponin was added with 5 mL of distilled water and shaken within 30 seconds. The presence of saponins is characterized by 1-3 cm high foam that is stable for 30 seconds[9].

Gel Preparations. The composition of the gel formulation is shown in Table 1. A total of 1.5 grams of HPMC was put into a cup, and enough warm water was added until it expanded. After HPMC expanded, it was stirred until it was completely dispersed. After that, 12 mL of propylene glycol, 20 mL of glycerin, and 2 mL of TEA were added and stirred until homogeneous using a stirring rod. Next, EECPL and EESL were added, and then 100 mL of distilled water was added and stirred until homogeneous. Then the three gel formulas were stored at room temperature.

Table 1. Formulation of gel preparations.

Component	Concentration (%)		
	F1	F2	F3
EECPL	1	3	5
EESL	1	3	5
HPMC	1.5	1.5	1.5
Glycerin	20	20	20
Propyleneglycol	12	12	12
TEA	2	2	2
Aquadest ad	100 mL	100 mL	100 mL

Test of Physical Properties in Gel Preparations. The resulting gel preparation was analyzed using the cycling test method for its physical properties in organoleptic tests, homogeneity, pH, adhesion, spreadability, viscosity, and stability.

Organoleptic Test. The organoleptic test is the gel's color, shape, and smell.

Homogeneity Test. A total of 0.1 gram of gel is applied to clear glass, then observe the results. The gel has good homogeneity if it looks homogeneous and has no coarse grains[10].

pH test. One gram of gel was dissolved using 10 mL of distilled water and stirred until evenly distributed. Furthermore, the solution was tested with a pH meter. The pH requirements of a good gel if it meets the requirements of the skin pH criteria, namely 4-8[11].

Spread Power Test. The gel preparation was placed in a petri dish. The diameter of the gel was measured starting from no load; then weights were placed starting from 50 grams, 100 grams, 150 grams, and 200 grams. Spreadability is good if it meets the requirements of the spreadability test ranging from 5-7 cm[12].

Adhesion Test. The gel preparation is placed in the center between 2 glass objects, placed on the test device, given a load weighing 80 grams, and recorded how long the glass object is detached from the test device. The requirement for adhesion is met if it is at least 4 seconds[13].

Viscosity Test. A total of 100 mL of gel preparation was tested for viscosity using a viscometer. Viscosity is qualified if the viscosity value is 2000-4000 cps[14].

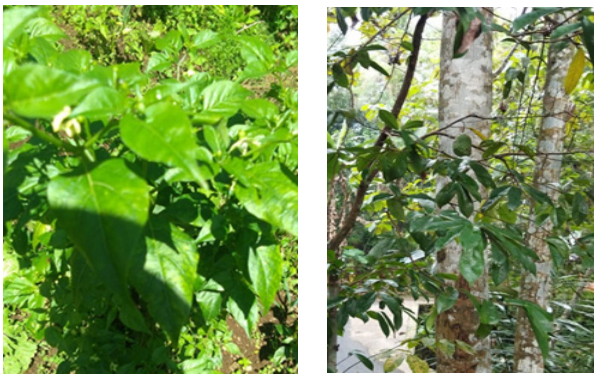
Stability Test. The cycling test method is used to test the stability of gel preparations. The gel preparation was stored at ± 4 °C (stored in a refrigerator) for 24 hours, after which the gel preparation was transferred to a temperature of ± 40 °C (stored in an oven) for 24 hours (one cycle). Tests were carried out from the 1st cycle to the 6th cycle[15].

Antibacterial Activity Test. The disc diffusion method carried out antibacterial activity testing against *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Staphylococcus aureus*. Mueller Hinton Agar media was prepared and then planted with bacterial test culture, which was put into an incubator at 37 ºC and incubated for 24 hours. They dipped a 0.55 cm diameter paper disc into the EECPL and EESL combination gel. Then the disc paper was transferred to the medium and culture in a petri dish, then incubated at 37 ºC for 24 hours. Measure the clear zone around the disc paper using a calliper[16].

Data Analysis. The data analysis used was one-way ANOVA on SPSS version 25, which has a 95% confidence level, aims to see differences between groups and is continued by using the LSD test on data that provides significant differences. Data analysis was carried out on the physical properties of the gel before and after the cycling test, which included pH, spreadability, viscosity, adhesion, and pH tests. In the antibacterial activity results, the inhibition value was compared with the inhibition diameter category.

RESULTS AND DISCUSSION

Determination Plants. Based on the decision letter number 089/HP.LL/VIII/2021 from the Environmental Laboratory, Faculty of Biology, Jenderal



(A) (B)

Figure 1. (A) Cayenne pepper leaves (B) Soursop leaves.

Soedirman University, shows that the cayenne pepper plants used are true *Capsicum frutescens* (L.) and the soursop plants used are true *Annona muricata* (L.). The cayenne pepper and soursop plants used are shown in Figure 1.

Extraction Results of Cayenne Pepper Leaves and Soursop Leaves. The extraction process uses 96% ethanol solvent with maceration within three days and stirring daily. The extraction by maceration method aims to prevent damage to the active substances[17]. Ethanol 96% was chosen because it is a universal solvent, is polar, selective, and non-toxic, and can extract all compounds present in cayenne pepper and soursop leaves[30]. The extraction yield of cayenne pepper leaves obtained a product of 6.05% is different from other studies with a yield of 7.79%, and this is due to different soaking times where this study was conducted for three days while the study was for five days[18]. The extraction yield of soursop leaves obtained a yield of 4.41%, and the extraction yield is not much different from other studies with a yield of 4.63%[19].

Phytochemical Screening Results. EECPL and EESL were subjected to phytochemical screening to determine the content of secondary metabolites using colour reagents. The colour reagent method is used because it is easier and faster to show results. Phytochemical screening of EECPL showed the presence of tannins, flavonoids, saponins, terpenoids, and steroids. This differs from other studies, which state that EECPL contains saponins, flavonoids, alkaloids, phenols, and tannins[20]. The results of EESL phytochemical screening showed the presence of compounds such as flavonoids, alkaloids, tannins, terpenoids, and saponins. This is similar to the effects of other studies, which state that EESL has secondary metabolite compounds in saponins, triterpenoids, tannins, alkaloids, and flavonoids[21]. The results of phytochemical screening can be observed in section Table 2.

Formulation Results, Physical Properties Test and Gel Preparation Stability Test. This study uses

Table 2. EECPL and EESL phytochemical screening results.

Metabolite coumpounds	Sample	
	EECPL	EESL
Alkaloid	-	+
Flavonoid	+	+
Tanin	+	+
Saponin	+	+
Terpenoid	+	+
Steroid	+	-

Description: (+) Contains secondary metabolites
(-) Does not contain secondary metabolites.

HPMC as a gel base. HPMC is a semi-synthetic polymer that can make gels clear, neutral, easily soluble in water and has good resistance to microorganisms[22]. Each gel preparation formulation was made with three replicates. The gel preparation was then analyzed for physical properties, including organoleptic test, homogeneity test, pH test, adhesion test, viscosity test, and spreadability test, and continued with a stability test using the cycling test method. The physical properties and stability of the preparation can be observed in Table 3 and Table 4.

Organoleptic testing uses direct observation of colour, odour, and shape for all gel preparation formulations and their replications. The results showed that the EECPL gel preparation combined with EESL was blackish-green, had a distinctive odour, and had a gel form. Then the stability test was carried out, and the results of the gel preparation did not change, where the gel preparation remained blackish-green in colour, with a distinctive odour of extract and semi-solid form (gel) from cycles 1-6, so it can be said that all gel preparation formulations have stable organoleptic properties.

The requirement for a gel with good homogeneity is that the gel looks homogeneous and there are no coarse grains[10]. The purpose of the homogeneity test is to determine whether the gel components can mix well. The results of testing the homogeneity of each gel preparation formulation with three repetitions showed that the three formulations were homogeneous, marked in the gel preparation without coarse particles. The homogeneity of the preparation after the stability test is carried out, the results of the gel preparation remain homogeneous, and there are no coarse grains.

The pH test is used to determine the gel preparation's acidity and basicity to match the skin's pH condition. The pH test results of each gel preparation formulation with three replicates showed that the three formulations were at good pH criteria for the skin, namely 4-8[11]. Then the stability test was carried out, and the results of the pH value in cycle 1 to cycle 3 were still within the normal skin pH range of 4-8[11].

Table 3. Test results of physical properties of gel preparations.

Physical Properties	Average physical properties test results		
	F1	F2	F3
Organoleptic			
a. Color	Slightly blackish green	Slightly blackish green	Slightly blackish green
b. Smell	Characteristic smell	Characteristic smell	Characteristic smell
c. Shape	Gel (semi-solid)	Gel (semi-solid)	Gel (semi-solid)
Homogeneity	Homogeneous	Homogeneous	Homogeneous
pH (4-8)	7.6	7.4	7.7
Dispersion power (5-7 cm)	5.2	5.1	5.0
Adhesion (>4 seconds)	4.85	5.62	6.30
Viscosity (2000-4000 cp)	2543	3034	3113

Table 4. The average result of the gel preparation stability test.

Physical Properties	The average result of the gel stability test					
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Organoleptic						
a. Color	Slightly blackish green	Slightly blackish green	Slightly blackish green	Slightly blackish green	Slightly blackish green	Slightly blackish green
b. Smell	Characteristic smell	Characteristic smell	Characteristic smell	Characteristic smell	Characteristic smell	Characteristic smell
c. Shape	Gel (semi-solid)	Gel (semi-solid)	Gel (semi-solid)	Gel (semi-solid)	Gel (semi-solid)	Gel (semi-solid)
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH (4-8)						
a. F1	7.5	7.7	7.7	8.5	8.6	8.7
b. F2	7.5	7.8	7.8	8.2	8.2	8.1
c. F3	7.5	7.7	7.7	7.9	7.9	7.8
Dispersion power (5-7 cm)	5.1	5.2	5.1	5.3	5.4	5.5
a. F1	5.5	6.0	5.7	5.3	5.4	5.7
b. F2	5.3	5.3	5.8	6.3	6.4	6.6
c. F3						
Adhesion (>4 seconds)	6.03	5.76	5.23	4.23	4.14	4.29
a. F1	6.51	5.46	5.51	4.31	4.21	4.16
b. F2	7.55	6.09	5.78	4.39	4.23	4.23
c. F3						
Viscosity (2000-4000 cps)						
a. F1	2603	2538	2394	2204	2122	2052
b. F2	2174	2198	2495	2192	2261	2220
c. F3	3069	2757	2535	2237	2556	2475

Furthermore, from the 4th to the 6th cycle, the pH value of the gel preparation turned more alkaline, which was thought to be caused by the influence of temperature[23]. If the pH of the gel preparation is <4, it will cause skin irritation, while if the pH of the gel preparation is >8, it will cause the skin to dry[24].

Spreadability testing is used to determine the level of spread of the gel on the skin. In testing the spreadability of each gel preparation formulation with three replications, the results showed that the three formulations had good spreadability of 5-7 cm[12]. The gel preparations produced during the cycling test experienced an increase and decrease in the spreadability value due to the influence of temperature during storage. Changes in temperature can cause changes in the viscosity of the gel, which will change its spreadability[25]. The increase and decrease in the spreadability value of the results before and after the stability test still meet the requirements of the gel preparation spreadability test, which is between 5-7 cm[12].

An adhesive test determines how long the gel preparation sticks to the skin. In testing the adhesion of each gel preparation formulation with three repetitions, the results showed that the three formulations met the requirements of adhesion that less than 4 seconds[13]. The gel preparations produced during the cycling test experienced an increase and decrease in adhesion due to the influence of temperature during storage. However, the test results before and after the stability test met the requirements of less than 4

seconds[13]. The longer the gel preparation, the better because the active substances contained in the gel will be absorbed completely.

Viscosity testing is used to determine the viscosity of the gel. The results of testing the viscosity of each gel preparation formulation with three replications showed that the three formulations had good viscosity with values ranging from 2000-4000 cps[14]. The viscosity value of the gel preparation during the cycling test increased and decreased in viscosity value due to the influence of temperature in storage. However, the viscosity test results before and after the stability test met the requirements of 2000-4000 cps[14]. The viscosity value influences the dispersion value. The higher the viscosity value, the lower the dispersion value and vice versa[26].

Bacterial Inhibition Results. Inhibition testing uses the agar diffusion method with the disc method. The gel preparation is absorbed on a paper disc which is then attached to an agar medium that has been homogenized with bacteria, then incubated until there is an inhibition zone in the form of a clear zone in the area around the disc[16]. The results of the inhibition test can be observed in Table 5. Inhibition testing was carried out on the test bacteria *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Staphylococcus aureus* with 5 different samples, in the form of positive control (+) using clindamycin 1.2% gel, negative control (-) using distilled water, gel preparations with varying concentrations of extract combinations, namely F1 (1% and 1%), F2 (3% and 3%), and F3 (5% and 5%) with each sample performed 3 times replication. The average diameter of the inhibition zone in the positive control (+) showed inhibition of *S. epidermidis* by 40.6 mm and *S. aureus* by 26.5 mm, indicating inhibition which is included in the very strong category, while in *P. acnes* by 15.5 mm, indicating a strong inhibition category. The average diameter of the negative control inhibition zone was zero (no clear zone formed) on *S. aureus*, *S. epidermidis*, and *P. acnes* showed weak inhibition.

The combined gel preparation of EESL and EECPL has antibacterial activity on *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Staphylococcus aureus* where the average diameter of the inhibition zone of the F1 gel preparation formulation on *S. aureus* is 9.83 mm, *S. epidermidis* 8 mm, and *P. acnes* is 8.1 mm showing moderate inhibition. The average diameter of the inhibition zone from gel preparation formulation F2 on *S. aureus* of 10.83 mm showed strong inhibition, while on *S. epidermidis* of 8.9 mm and *P. acnes* of 8.9 mm showed moderate inhibition. The average diameter of the inhibition zone from the F3 gel preparation formulation on *S. aureus* is 9.4 mm,

Table 5. The result of the inhibitory power of the gel preparation.

Sample	Average of the inhibitory zones (mm)		
	S. aureus	*S. epidermidis*	*P. acnes*
Control positive (+)	26.5 [a)]	40.66 [a)]	15.5 [b)]
Control negative (-)	0 [d)]	0 [d)]	0 [d)]
F1	9.83 [c)]	8 [c)]	8.1 [c)]
F2	10.83 [b)]	8.9 [c)]	8.9 [c)]
F3	9.4 [c)]	9.56 [c)]	10.4 [b)]

Description: Inhibitory power criteria (diameter) [35].
[a)] Very powerful (≥ 21 mm)
[b)] Powerful (10-20 mm)
[c)] Medium (5-10 mm)
[d)] Weak (≤ 5 mm)

and *S. epidermidis* is 9.56 mm, indicating moderate inhibition, while the *P. acnes* is 10.4 mm, indicating strong inhibition. The results show that any increase in the concentration of gel extracts is not linear to the increase in inhibition[27].

On the other hand, in different studies, each extract obtained weak antibacterial activity without being combined and made into a peel-off gel mask preparation. The study states that EECPL in the preparation of peel-off gel masks at a concentration of 5% can inhibit *Staphylococcus aureus* with an average inhibition zone diameter of 4.11 mm which shows weak inhibition, then at a concentration of 10% the average inhibition zone diameter is 5.48 mm and at a concentration of 15% the average inhibition zone diameter is 7.11 mm which shows a moderate inhibition category[28]. The greatest inhibition occurred in *Staphylococcus aureus*, namely at a concentration of 15% rather than concentrations of 5% and 10%[28]. Where the greater the concentration of EECPL, the greater the inhibitory activity.

Soursop leaves in peel-off gel mask preparations have no antibacterial activity at concentrations of 5%, 10%, and 15%[29]. On the other hand, the antibacterial activity of EESL can inhibit *Staphylococcus epidermidis* bacteria with a strong category, namely at a concentration of 0.5% has an average inhibition zone diameter of 11.2 mm, at a concentration of 1% has an average inhibition zone diameter of 15.2 mm, at a concentration of 3% has an average inhibition zone diameter of 11.7 mm, and at a concentration of 5% has an average inhibition zone diameter of 16.7 mm[31]. The concentration of soursop leaves extract shows that the higher the concentration, the smaller the inhibition against *Staphylococcus epidermidis*.

The zone of inhibition around the disk can occur due to the active flavonoids in EECPL and EESL[32,33]. Flavonoid compounds in EECPL have antibacterial activity against *Staphylococcus aureus*, and flavonoid compounds in EESL have antibacterial activity against

Staphylococcus epidermidis and *Propionibacterium acnes*[6][7]. Flavonoid compounds work with three mechanisms. The first is inhibiting nucleic acid synthesis, using the β ring contained in flavonoids that inhibit DNA and RNA synthesis in intercalation or nucleic acid bases that accumulate with hydrogen bonds[34]. The second mechanism, flavonoids, are compounds that can inhibit bacteria in their cell membrane function, disrupting its integrity due to complex bonds with soluble proteins outside the cell. The third mechanism is to inhibit energy metabolism carried out when bacteria undergo the respiration process. As a result, the activity of metabolite absorption and biosynthesis of bacterial macromolecules does not run perfectly because the energy is inhibited[34].

Data Analysis. The results of the gel's spreadability, stickiness, viscosity, and pH were analyzed using one-way ANOVA to see whether there were differences between test groups (Table 6). Based on the results of the one-way ANOVA test, there is a significant difference, indicating that the variation in concentration of the combination of EESL and EECPL affects the physical properties of the preparation, namely on the spreadability, pH, and adhesion of F1, F2, and F3 (sig. value <0.05), while the viscosity values of F1, F2, and F3 show no difference in each cycle. The test was continued with the LSD test, and results showed that the spreadability, pH, and stickiness of the F1, F2, and F3 gel preparations before and after the stability test had no significant difference.

CONCLUSION

Table 6. Data analysis results (*One-Way ANOVA*).

Formulas	Sig. value			
	Spread power	Adhesion	Viscosity	pH
F1	0.000[a)]	0.000[a)]	0.083[b)]	0.000[a)]
F2	0.000[a)]	0.000[a)]	0.651[b)]	0.000[a)]
F3	0.000[a)]	0.000[a)]	0.266[b)]	0.016[a)]

Description:
[a)] Sig. value <0.05: have a difference in values in each cycle
[b)] Sig. value >0.05: has no difference in values in each cycle

All gel preparations obtained are good and stable and have antibacterial activity on *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* so that they can be used as anti-acne gels. *Staphylococcus aureus* was inhibited best by gel preparation F2 with EESL and EECPL concentrations of 3% and 3%, respectively, with an average inhibition zone diameter of 10.83 mm. Furthermore, *Staphylococcus epidermidis*, and *Propionibacterium acnes* were best inhibited by gel preparation F3 with the respective concentrations of EESL and EECPL, namely 5% and 5%, namely with an average diameter of the inhibition zone on *Staphylococcus epidermidis* of 9.56 mm and on *Propionibacterium acnes* of 10.4 mm.

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