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Formulation, Physical Quality, and Microbial Contamination Tests of Anti-Acne Cream from Longan (*Euphoria longan* [Lour. Steud.]) Seed Extract

St. Ratnah*, Alfrida M. Salasa, Dwi S. F. Ramadhan

Department of Pharmacy, Makassar Health Polytechnic of the Ministry of Health, Makassar, South Sulawesi, Indonesia.

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ABSTRACT

Acne is commonly associated with *Propionibacterium acnes*, a bacterium that can be treated by giving out anti-acne medications. These medications' anti-acne effects are derived from natural plant chemicals, like those in the seeds of longan (*Euphoria longan* [Lour. Steud.]) fruits. Longan seed extract needs to be made into a cream dosage form before it can be utilized as an anti-acne treatment. Therefore, the present study was aimed at formulating longan seed extract cream with an anti-acne activity that meets the stability requirements of physical quality and microbial contamination tests. The longan seeds were extracted. Cream preparations with vanishing cream bases were formulated and evaluated for their anti-acne activity, physical stability, and microbial contamination. The antibacterial effect was tested, while minimum inhibitory and minimum bactericidal concentration analyses were done using the broth dilution method. The results showed that the extract at a concentration of 1% had bacteriostatic effect, while extracts at concentrations of 2, 4, and 8% showed bactericidal activity. All cream formulas showed anti-acne activity and met the requirements for microbial contamination testing. In terms of physical stability, all formulations met the standards. However, formulas I, II, and III did not meet the requirements of the pH test, while formulas V and VI did not meet the requirements of the spreadability test. The findings of this study reveal that the extract of longan seed provided inhibitory activity against *Propionibacterium acnes*, and the formula IV qualified for physical quality and microbial contamination tests, making it an effective anti-acne cream.

Keywords: *Euphoria Longan*, Anti-acne cream, Formulation

Introduction

Skin is the outermost layer of the body and serves as a barrier against physical injury, infection, and damage brought on by environmental exposure such as ultraviolet (UV) rays and harmful chemicals. Facial skin is the part that is often treated the most because it is frequently contaminated by bacteria that give acne its disturbing appearance. As a result, people are constantly looking for solutions to these problems. Acne is a common concern that is characterized by inflammation and blockage of the oil glands (*pilosebaceous unit*). This is due to the fact that clogged sebaceous glands generate an accumulation of sebum and the development of blackheads by preventing internal sebum from reaching the skin's surface. Blackheads are the beginning of acne formation.[1] The anaerobic bacterium, *Propionibacterium acnes* is thought to play an important role in the pathophysiology of common skin diseases, such as acne vulgaris. Increased activity of androgens during puberty in humans triggers the growth of sebaceous glands and increased production of sebum. Sebum consists of glycerides, which can be converted to free fatty acids and glycerol by the lipase enzyme that is produced by *Propionibacterium acness*. The free fatty acids can irritate the sebaceous follicle walls, causing increased inflammation and cell remodeling that may lead to acne.[2]

*Corresponding author. E mail: ratnah.mansjur@poltekkes-mks.ac.id
Tel: 62 821-9131-5962

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Acne can be treated using traditional medicines, such as longan fruit seeds. The ethanol extract from longan fruit seeds contains polyphenols and flavonoids, with a total polyphenol content of 17.24% gallic acid. The extract demonstrated active antioxidant properties, as indicated by an IC_{50} value of 57.24 ppm (active antioxidant power is defined as 50–100 ppm).[3] Furthermore, this extract showed antibacterial activity against *Propionibacterium acnes*, the bacterium commonly associated with acne, with an optimal concentration of 8% w/v.[4] Longan (*Euphoria longan* [Lour. Steud.]) seed extract can be formulated into a pharmaceutical dosage form to make it simple to apply to facial skin. One of the pharmaceutical dosage forms that are widely used as an anti-acne medicine formula is cream preparations. Cream is a semi-solid dosage form that typically contains one or more active ingredients, dissolved or dispersed in a suitable base. Its preparations have the advantage of being easy to use, comfortable to apply, non-sticky, and simple to wash with water. The cream has traditionally been employed in preparations that are semi-solid but have a somewhat liquid viscosity, such as water-in-oil (w/o) or oil-in-water (o/w) emulsions.[5] Until now, it has never been reported that longan seed extract is made into cream preparations and tested for its inhibitory activity against *Propionibacterium acness*.

Therefore, the aims of this study were to formulate a cream using the active substance in longan seed extract, test the stability of the cream and investigate its inhibitory activity against the acne-causing bacteria, *Propionibacterium acness*.

Materials and Methods

Plant sample collection

Longan fruits were collected from the Herb Garden, Sunu Street, Lembo Village, Tallo District, Makassar City, South Sulawesi, Indonesia. The collection of samples took place in April and May 2022. The seeds were obtained by separating them from longan fruit flesh. Longan seeds were identified and confirmed by Sesilia Rante Pa Jadi (LAB/2022/V/15) at

the Natural Product Chemistry Laboratory, Department of Pharmacy, Makassar Health Polytechnic of the Ministry of Health, Indonesia.

Preparation of longan seed extract

Longan fruits were properly washed before the seeds were removed, cleaned of any impurities, and dried. Then, they were coarsely ground and dried in an oven at a temperature of 60°C.[6] The extractor lid was filled with the plant material to be extracted. The distillation flask was then filled with the solvent and placed on top of a heating source. Above the flask, the extractor and condenser were correctly assembled. By heating the solvent in the distillation flask, the solvent vapor condensed and dripped into the extraction vessel. This process allowed the active substances to be extracted from the plant material and returned to the distillation flask. The extract was then evaporated using a rotary evaporator and dried in a water bath. The weight of the dried extract was measured, and the yield was calculated by comparing it to the initial amount of plant material used for extraction.[7]

Determination of the antibacterial activity of longan seed extract against Propionibacterium acnes by disc diffusion method

The antibacterial activity of longan seed extract was determined by the disc diffusion method, as previously described.[8] Sterile Mueller-Hinton agar (MHA) was placed into 5 sterile Petri dishes and allowed to solidify. The suspension of *Propionibacterium acnes* was prepared in accordance with the standard McFarland 0.5 turbidity scales. Prior to this, the bacteria were cultured by inoculating them onto a slanted nutrient agar medium and incubating them at a temperature of 37°C for 24 hours. Paper discs were also made and saturated with longan seed extract at concentrations of 1, 2, 4, and 8% that were diluted with dimethyl sulfoxide (DMSO), with DMSO serving as the negative control (-). Using a sterile swab, the bacterial suspension was spread on the MHA media surface, allowed to sit for about 15 minutes, and then aseptically placed on top of the paper disc. The Petri dishes were then incubated for 24 hours at a temperature of 37°C. The clear region surrounding the paper disc provided as an indicator of the inhibition zone's diameter, which was observed and measured.

Determination of the minimum inhibitory concentration and minimum bactericidal concentration.

In determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), a serial dilution of longan seed extract consisting of 0.156, 0.375, 0.625, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 5, and 10 concentrations were prepared using DMSO. Additionally, 5 mL of nutrient broth (NB) medium was prepared in a sterile test tube for each dilution. An aliquot of 1 mL of each dilution of longan seed extract was placed aseptically into each sterile test tube. Then, 0.5 mL of *Propionibacterium acnes* suspension was inoculated into each test tube. Each tube was replicated three times. A negative control was also prepared by adding the solvent of the extract and *Propionibacterium acnes* suspension to the NB medium. All test tubes, including the negative control, were incubated at 37°C for 24 hours. By comparing the test tube with the control tube, the turbidity observed was used to detect the presence or absence of bacterial growth. Test tubes that did not show cloudiness (negative for bacterial growth) were incubated for an additional 24 hours.

Formulation of an anti-acne cream

The steps involved in the formulation of anti-acne cream made of longan seed extract are shown in Table 1. Stearic acid and glycerin were first melted (oil phase), then sodium tetraborate and nipagin were dissolved in hot water to create the water phase. After adding the water phase and stirring till cream was formed, the oil phase was added to the hot mortar. The crushed triethanolamine was added and mixed until a homogenous mixture was obtained. Finally, the extract was added to the mortar along with the cream base, which was then blended until homogeneous.

Physical quality stability test of longan seed extract cream

To determine the physical stability of the cream, an evaluation of the physical quality of anti-acne cream compositions using longan seed extract was done. The accelerated stability approach was used to perform this process both before and after testing, and the results were compared. With extract stored at a constant temperature of 25°C acting as the control group, the test examined the effects of temperature stress caused by freeze-thaw cycles. The cream preparation was treated to two temperature settings for the freeze-thaw cycle: 4°C for the initial 48 hours and 40°C for the subsequent 48 hours. A storage phase at 4°C and a thawing period at 40°C make up the freeze-thaw cycle. Organoleptic testing was carried out on the prepared cream to determine its physical appearance. This test was carried out by observing the texture, smell, and color of the cream prepared (Anief, 1998). After preparing cream, its homogeneity was assessed by applying approximately 0.5 grams of cream at the top, middle, and bottom of a glass surface. This test was aimed at visually evaluating the physical uniformity of the cream shape throughout its entirety. Cream preparation is considered homogeneous when there are no lumps or coarse grains in any one portion of each part. There were no particles that agglomerated or mixed. Using a pH meter, pH was measured. To ensure accuracy, the pH meter was calibrated using buffer solutions of pH 4 and pH 7. The evaluation was carried out by dipping the pH meter electrode into a prepared solution. The solution was prepared by diluting 1 gram of cream with 10 mL of distilled water. Cream viscosity was determined using a Brookfield viscometer, and each formulation was tested in triplicate. A 30-gram container containing a sample of cream preparation was filled with the spindle attached and the rotor activated. The viscosity measurements were recorded immediately after the viscometer needle displayed a stable reading after five rotations.

Table 1: Anti-acne cream formula from longan seed extract.

No	Material Name	Utility	Cream Formula in %					
			F1	F2	F3	F4	F5	F6
1.	Longan Fruit Seed Extract	Active ingredients	1.75	2	2.5	3	6	9
2.	Stearic acid	Emulsifier and shaper of consistency	14.2	14.2	14.2	14.2	14.2	14.2
3.	Glycerin	Humectants	10	10	10	10	10	10
4.	Na. tetraborate	Preservative and pH control	0.25	0.25	0.25	0.25	0.25	0.25
5.	Triethanolamine	Stabilizer and emulsifier	1	1	1	1	1	1
6.	Nipagin	Preservative	0.15	0.15	0.15	0.15	0.15	0.15
7.	Aquadest	Solvent	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

The prepared cream, weighing approximately 1 gram, was placed on a round glass surface with a diameter of 15 cm. Another glass was then placed on top of the cream, and left undisturbed for 1 minute. The diameter of the cream spread was measured to assess its initial spreadability, and 125 grams of weight were placed on top of the glass assembly. The entire setup was allowed to stand for 1 minute and measured again to evaluate its resistance to deformation under increased weight.

Activity testing of longan seed extract anti-acne cream on the growth of Propionibacterium acnes

The sterile MHA medium was prepared and poured into a sterile Petri dish in aseptic conditions, with a volume of 15 mL, and allowed to solidify. Paper discs were soaked in cream and base preparations. A sterile swab was dipped into the bacterial suspension and then used to inoculate the surface of the sterile MHA medium. The inoculated medium was allowed to sit for approximately 15 minutes, allowing the bacteria to settle and adhere to the surface. Using sterile tweezers, a paper disc that had been soaked in the desired preparation was carefully placed on the surface of the MHA medium, ensuring proper adherence without shifting. The petri dish was then incubated at a temperature of 35-37ºC for 24 hours. The inhibition zone was observed in the form of a clear zone around the paper disc, and then the diameter was measured using a vernier caliper.

Microorganism contamination test

For the standard plate count, dilutions of 10^{-1} to 10^{-6} of extract were made, with 1 mL of each pipetted and placed into sterile Petri dishes (made in duplicate). Furthermore, 15-20 ml of PCA were added, homogenized, and solidified. The solution was incubated at 35-37ºC for 24-48 hours. Blanks of diluents and media were prepared to determine the sterility. For the total mold and yeast assay, a sterile test tube was filled with 9 ml of diluents, and 1 ml of the diluted sample from the 10^{-1} dilution was added. The process was repeated sequentially until a 10^{-6} dilution was achieved. To ensure proper mixing for homogeneity, 0.5 mL of each diluted sample was taken, placed on a Petri dish, and then covered with potato dextrose agar (PDA). Furthermore, the sterility of every Petri dish was tested by incubating it at 20 -25ºC for 5-7 days with blanks of the solvent and media.

Contamination of pathogenic bacteria test

To test for *Staphylococcus aureus* contamination, a total of 1 ml of 10^{-1} test sample was placed into 9 ml of TSB media, then incubated at 30-35^{0}C for 18-24 hours (replicated twice). After incubation, a small amount of TSB medium from each sample was taken using an inoculating loop and transferred onto mannitol salt agar (MSA). The inoculated MSA plates were then incubated at 30-35°C for 18-72 hours. *Staphylococcus aureus* was present when convex colonies, which were often white or yellow and encircled by a yellow zone, were present. To test for *Pseudomonas aeruginosa* contamination, a total of 1 ml of 10^{-1} test sample was placed into 9 ml of TSB medium, then incubated at 30-35^{0}C for 18-24 hours (replicated twice). After incubation, a loopful of TSB medium from each sample (enrichment) was taken and streaked evenly onto CETA (cetrimide agar) in a Petri dish. All inoculated Petri dishes were then incubated at 30-35°C for 18-72 hours. Specific colonies that grew were observed with the characteristic greenish colonies.

Statistical analysis

The antibacterial potency of longan seed extract was analyzed using Kruskall-Walli test to assess any difference between the various treatments. The Mann-Whitney test was then conducted with a significance level of $p<0.05$ and a confidence level of 95% to determine specific variations between treatments. The paired sample T-test was used to examine any variations between the pH, viscosity, and spreadability of the cream before and after formulation. Also, one-way analysis of variance (ANOVA) was used to evaluate the antibacterial potency of the anti-acne formula containing longan seed extract. This was followed by the LSD post hoc test to examine specific variations among the different treatments with a significance level of $p<0.05$.

Results and Discussion

Natural ingredients offer numerous health benefits and can be utilized for the development of traditional medicinal preparations. However, before incorporating them into products, it is crucial to conduct supporting studies to ensure their efficacy and safety. The findings from the preliminary study can then be applied to create products that contribute positively to society. One potential source of natural ingredients is often-underutilized parts of plants, known as organic waste. These parts, such as seeds, contain various phytochemical compounds, including tannins, flavonoids, and alkaloids, which have benefits to the human body.[9] Based on previous studies, longan seed extract had a strong antioxidant activity (IC_{50}) of 57.24 ppm and contained 17.24% total phenol, which is the gallic acid equivalent.[3]

Yield of longan seed extract

During the extraction process, 717.84 g of simplicia were used, yielding 74.56 g of extract and a 10.39% yield (Table 2). The result was lower than previous studies conducted using the same simplicia, which yielded 23.55%.[9] One contributing factor to this decrease in yield was the storage of plant materials, which could negatively impact its stability and introduce contamination.[10]

Table 2: Yield of longan seed extract

Simplicia weight (g)	Extract weight (g)	Yield %
717.84	74.56	10.39

Table 3: Antibacterial potency of longan seed extract on the growth of *Propionibacterium acnes*

Replication	Inhibition zone diameter(mm)				
	1%	2%	4%	8%	Control (-)
1	13.0	15.3	16.0	17.0	0.0
2	13.0	15.0	16.3	17.0	0.0
3	11.3	14.3	15.0	16.7	0.0
4	12.7	15.0	16.3	17.7	0.0
5	12.3	14.3	15.3	16.3	0.0
Average	12.5	14.8	15.8	16.9	0.0

Table 4: Mann Whitney analysis of antibacterial potential of longan seed extract on the growth of *Propionibacterium acnes*

Extract Concentration (%)	N	Zone of inhibition of the growth of test bacteria				
		Means	std. dev	Median	Min	Max
1	5	12.4600	0.70922	12.7000	11.30	13.00
2	5	14.7800	0.45497	15.0000	14.30	15.30
4	5	15.7800	0.59749	16.0000	15.00	16.30
8	5	16.9400	0.51284	17.0000	16.30	17.70

Table 5: Antibacterial potency of longan seed extract on the growth of *Propionibacterium acnes*

Replication	Concentration (mm)				
	1%	2%	4%	8%	Control (-)
1	0.0	7.0	8.0	9.0	0.0
2	0.0	8.0	10.0	11.0	0.0
3	0.0	7.0	7.0	8.0	0.0
4	0.0	8.0	9.0	10.0	0.0
5	0.0	8.0	8.0	9.0	0.0
Average	0.0	7.6	8.4	9.4	0.0

Antibacterial activity of longan seed extract against Propionibacterium acnes

The diameter of the inhibition zone, which is represented by the clear region surrounding the paper disc, was used to estimate the antibacterial activity of longan seed extract. The widest one was observed at an 8% concentration and results were consistent with preliminary research.[4] The normality test results indicated that antibacterial activity followed a normal distribution ($p>0.05$), while homogeneity showed the acquired data was not homogeneous ($p<0.05$). The Kruskal-Walli test revealed significant differences between the treatments ($p<0.05$). The Mann-Whitney test was used to determine the specific variations. The results obtained showed significant differences among all treatments ($p<0.05$) as presented in Table 3. These results showed that all concentrations exhibited a bacteriostatic effect by inhibiting bacterial growth, with its optimal concentration (8%). Further testing was conducted to determine whether the extract solely possessed bacteriostatic or also bactericidal properties, with an extended incubation of 24 hours. The results indicated that the 1% concentration did not exhibit any inhibition zones around the paper disc, implying it had a bacteriostatic effect, while the others demonstrated bactericidal activity (Table 4). After conducting the Mann-Whitney statistical test, it was observed that the 2% concentration did not significantly differ from the 4% concentration but was significantly different from the 8%. As demonstrated in Table 5, the 4% concentration did not differ significantly from the 2 and 8% concentrations. Following a 48-hour incubation, the optimal concentration was determined to be 4% (Table 6). Bacteriostatic agents inhibit bacterial growth, and once the antimicrobial compound is no longer present, bacterial multiplication reoccurs.[11] According to a study,[12] the diameter of the inhibition zone was influenced by the thickness of the growth medium and the duration of incubation, leading to variations observed in the five replicates used.

Table 6: Mann Whitney analysis antibacterial potential of longan seed extract on the growth of *Propionibacterium acnes*

Extract Concentration (%)	n	Zone of inhibition of the growth of test bacteria				
		Means	std. dev	Median	Min	Max
1	5	0	0	0	0	0
2	5	7.6000	0.54772	8.0000[a]	7.00	8.00
4	5	8.4000	1.14018	8.0000[ab]	7.00	10.00
8	5	7.9843	1.14018	9.0000[b]	8.00	1..00

[b]: No difference between groups (based on the Mann-Whitney test with $p<0.05$).

Table 7: Minimum inhibitory concentration (MIC) of longan seed extract on the growth of *Propionibacterium acnes*

Concentration (%)	Test result		
	1	2	3
10	-	-	-
5	-	-	-
2.5	-	-	-
2.25	-	-	-
2	-	-	-
1.75	-	-	-
1.5	-	+	+
1.25	+	+	+
0.625	+	+	+
0.3125	+	+	+
0.156	+	+	+
MIC value = 1.75 %			

The minimum inhibitory concentration and minimum bactericidal concentration of longan seed extract on the growth of Propionibacterium acnes

The MIC and MBC tests were conducted to determine the minimum concentration of extract capable of inhibiting and killing test bacteria, respectively.[13] The broth dilution test was employed for MIC and MBC assessments. The results indicated that the smallest concentration of longan seed extract effective against *Propionibacterium acnes* growth was determined to be 1.75%. The presence or absence of bacterial growth was observed by observing turbidity (indicating bacterial growth) or lack of cloudiness (indicating no bacterial growth). The Test solution with the lowest concentration that remained clear without bacterial growth was designated as MIC (Table 7). Following the determination of the test solution's MIC, NB media were grown without the addition of the test bacteria and incubated for 24 to 28 hours. Liquid media which remained clear after incubation were designated as MBC,[14] and the value obtained in this test was 2.5% (Table 8). The MIC and MBC values were higher and observed to be 2 and 3%, respectively.[15] These variations could be attributed to differences in the levels of secondary metabolites present in each simplicia. Factors such as varying growth locations, harvesting periods of plant products, and storage time of the simplicia may contribute to these differences.[10]

Table 8: MKC test results of longan seed extract on the growth of *Propionibacterium acnes*

Concentration (%)	Test result		
	1	2	3
10	-	-	-
5	-	-	-
2.5	-	-	-
2.25	+	+	-
2	+	+	-
1.75	-	+	+
MKC Value = 2.5 %			

Table 9: Organoleptic test observation results.

Formulas	Form		Color		Smell	
	Before Cycling test	After Cycling test	Before Cycling test	After Cycling test	Before Cycling test	After Cycling test
Base	Semi-solid	Semi-solid	White	White	Typical smell	Typical smell
FI	Semi-solid	Semi-solid	Beige	Beige	Typical Longan smell	Typical Longan smell
FII	Semi-solid	Semi-solid	Beige	Beige	Typical Longan smell	Typical Longan smell
FIII	Semi-solid	Semi-solid	Beige	Beige	Typical Longan smell	Typical Longan smell
FIV	Semi-solid	Semi-solid	Beige	Beige	Typical Longan smell	Typical Longan smell
FV	Semi-solid	Semi-solid	Chocolate	Chocolate	Typical Longan smell	Typical Longan smell
FVI	Semi-solid	Semi-solid	Chocolate	Chocolate	Typical Longan smell	Typical Longan smell

Table 10: Homogeneity test observation results

Formula	Homogeneity	
	Before cycling test	After cycling test
Base	Homogeneous	Homogeneous
FI	Homogeneous	Homogeneous
FII	Homogeneous	Homogeneous
FIII	Homogeneous	Homogeneous
FIV	Homogeneous	Homogeneous
FV	Homogeneous	Homogeneous
FVI	Homogeneous	Homogeneous

Physical quality stability of longan seed extract cream

The results from the disc diffusion method and the broth dilution test confirmed that longan seed extract act as an antibacterial agent against *Propionibacterium acnes*. This was attributed to chemical extracts such as tannins, flavonoids, and alkaloids.[9] However, to enhance utility and ease of application, the extract was formulated into an O/W (oil-in-water) type cream, also known as vanishing cream. Creams are semi-solid emulsions that exhibit various degrees of opacity and translucency, depending on the type of emulsion.[16] To qualify the requirements and ensured the desired quality of anti-acne cream, physical quality stability test was conducted. This test included evaluations of organoleptic properties, homogeneity, viscosity, pH, and spreadability. Extract concentrations used were 1.75, 2, 2.5, 3, 6, and 9%. Tests were performed both before and after storage using a climatic chamber.

The organoleptic test includes evaluating the color, aroma, and texture of the preparation. Formulas I to IV of longan seed anti-acne cream had a milky color due to the extract addition. However, formulas V and VI turned brown as the concentration of extract increased. All formulas exhibited a semi-solid texture and emitted a distinctive longan aroma. Importantly, test results remained unchanged even after storage, indicating the stability of formulations (Table 9). Homogeneity is a crucial aspect of cream preparations as it ensures smooth and uniform application on the skin without any coarse particle feel.[17] The results obtained from all formulas were homogeneous due to the lack of small granules and coarse particles. Importantly, the homogeneity of formulations remained consistent even after undergoing freeze-thaw cycles as shown in Table 10.

When making cream preparations, pH levels must be carefully taken into account because too much acidity may irritate the skin while too much alkalinity may leave the skin feeling slippery, dry, and scaly.[18] The baseline pH of the preparations was 5.52 before storage, and it increased to 6.73 after. When the extract was added, pH rose above its initial value; higher extract concentrations led to a more acidic pH. The presence of acidic polyphenolic chemicals in the extract may be responsible for this. Longan seed extract also contained polyphenolic compounds such as flavonoids and tannins,[9] as well as a total phenol content of 17.24%.[3] The pH range of the formulas before storage was 4.48 to 6.28, and it is consistent with the results from previous studies.[19] Afterward, the pH increased from 5.68 to 7.88 due to the reactions between several compounds in the formula. The pH values of all formulae prior to storage met the standards for skin pH, which normally range from 4.5 to 8.0.[17] These also met the particular pH criteria for facial skin, which are ideally between 4.5 and 6.5.[18] After storage, the pH of base formulas I to III exceeded the desired range for facial skin. Each formula maintained the pH levels within the parameters. For all formulae and bases, statistical analysis using the paired samples T-test indicated significant differences in pH levels before and after storage (Table 11).

A viscosity test was performed to evaluate the thickness of a preparation. The value of the base used before storage was measured at 5.555 cps, which exhibited a decrease of 3.943 cps afterward. The addition of extract resulted in a viscosity increase, where the greater its concentrations, the higher the viscosity because less amount of water was added. The viscosity test results before storage ranged from 6.639 to 16.663 cps. After storage, it decreased from 4.545 to 14.841 cps. This can be attributed to increased particle diameter, resulting in reduced surface area and subsequent decrease in viscosity. These results are consistent with a previous study.[20] Statistical analysis using the paired samples T-test revealed significant differences in viscosity before and after storage for the base and all formulas containing various extract concentrations. The viscosity of the base and all formulas before and after storage met the requirements,[17] which specified a range of 2,000 to 50,000 cps (Table 12).

The evaluation of cream spreadability refers to its ability to cover the skin upon application evenly. A higher spreadability depicts a wider contact area between the cream and the skin surface, facilitating proper distribution of the active substances. Typically, a good cream should have a spreadability within the range of 5 to 7 cm in diameter.[21] The initial spreadability measurement of the base cream before and after storage was found to be 5.07 cm and 6.13 cm, respectively. These results showed that the cream met the criterion. When the extract was added to the formula before storage, the spreadability was within the

range of 3.40 cm to 6.40 cm. It increased from 4.30 cm to 6.83 cm after storage. It was observed that the concentration of extract inversely affected this factor. Higher extract concentrations resulted in increased viscosity, leading to reduced spreadability. The results were in accordance with the previous study.[22] Statistical analysis using the paired sample T-test revealed significant differences in spreadability between the base cream and all formulas before and after storage. However, both formulas V and VI, before and after storage, did not qualify for the desired threshold of 5 cm (Table 13).

Activity of longan seed extract anti-acne cream on the growth of Propionibacterium acnes

Propionibacterium acnes have the capacity to form biofilms by adhering to surfaces or substrates. These biofilms play a role in disease development.[23] To prevent disease progression, one approach is to inhibit its formation.[24] Longan seed extract exhibits antibacterial properties against *Propionibacterium acnes.*[15] It contained phytochemical compounds, such as flavonoids, tannins, and alkaloids.[9] These plant-derived secondary metabolites can impede bacterial attachment to surfaces, thereby preventing the formation of mature biofilms and inhibiting bacterial growth.[25] Anti-acne cream was made using the antibacterial capabilities of longan seed extract against *Propionibacterium acnes*. To test if the chemicals included in the extract kept their efficacy in the manufactured product, the antibacterial activity of an anti-acne cream was reassessed. The results showed that the anti-acne cream of longan seed extract exhibited similar antibacterial activity in extract form as depicted in Table 14. This demonstrated that the cream base was not preventing the phytochemical components in the extract from diffusing. An increase in extract concentration resulted in a wider diameter of the inhibitory zone. Statistical analysis using LSD revealed no significant difference in antibacterial activity between formulas II and III.

Microorganism contamination test outcome

Microorganism contamination test was essential to conduct during the preparation of cream formulations, as contamination can potentially occur at any stage of the process. Contaminated preparations can lead to health issues; therefore, thorough testing was required. Common tests include total plate count, total mold and yeast, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.[2617] The standard plate count (SPC) test was conducted to quantify the total count of viable bacteria, regardless of the species present. Each individual cell had the ability to form a visible colony when placed in an appropriate growth medium. In this research, plate count agar (PCA) was utilized as the medium. Cream samples were appropriately diluted and then inoculated onto PCA plates using the pouring method. The plates were then incubated at 30 to 35°C for 3 to 5 days. A minimum of two Petri dishes were tested for each dilution. The plate with the highest number of colonies with less than 250, from a dilution level was selected for analysis.[5]

Table 11: Observation results of pH test

Formula	pH		Standard	Significant
	Before Cycling Test	After Cycling Test		
Base	5.52 ± 0.021	6.73 ± 0.035	4.5-8.0	0.000
FI	6.28 ± 0.021	7.88 ± 0.070		0.000
FII	5.97 ± 0.015	7.84 ± 0.012		0.000
FIII	5.43 ± 0.015	7.31 ± 0.015		0.000
FIV	4.99 ± 0.040	6.20 ± 0.016		0.000
FV	4.73 ± 0.035	5.93 ± 0.025		0.000
FVI	4.48 ± 0.021	5.68 ± 0.015		0.000

Table 12: Viscosity test results

Formula	Viscosity (cps)		Standard	Significant
	Before Cycling Test	After Cycling Test		
Base	5.555 ± 4.509	3.943 ± 4.509	2000-50000	0.000
FI	6.639 ± 3.512	4.545 ± 3.000		0.000
FII	6.843 ± 2.517	5.028 ± 3.215		0.000
FIII	7.171 ± 3.512	5.399 ± 4.583		0.000
FIV	8.037 ± 3.606	6.265 ± 3.512		0.000
FV	8.088 ± 2.517	6.426 ± 3.000		0.000
FVI	16.663 ± 3.055	14.841 ± 3.512		0.000

Table 13: Spread power test results

Formula	Spreadability (cm)		Standard	Significant
	Before Cycling Test	After Cycling Test		
Base	5.07 ± 0.208	6.13 ± 0.153	5-7	0.001
FI	6.40 ± 0.100	6.83 ± 0.153		0.006
FII	6.27 ± 0.252	6.70 ± 0.100		0.039
FIII	5.87 ± 0.153	6.43 ± 0.208		0.003
FIV	5.40 ± 0.361	6.13 ± 0.153		0.032
FV	4.23 ± 0.252	4.67 ± 0.306		0.006
FVI	3.40 ± 0.361	4.30 ± 0.300		0.004

Table 14: Activity test results of longan seed extract anti-acne cream on the growth of *Propionibacterium acnes.*

Replication	Formula Resistance (mm)						
	F1	F2	F3	F4	F5	F6	Base
1	11.0	15.0	16.0	17.5	19.5	22.5	0.0
2	13.0	14.0	15.0	19.5	19.5	22.0	0.0
3	10.0	13.0	14.0	17.5	19.5	20.0	0.0
4	11.0	12.0	13.0	19.0	21.0	23.5	0.0
5	12.0	13.0	14.0	16.5	23.0	22.5	0.0
Average	11.4	13.4	14.4	18.0	20.5	22.1	0.0

Table 15: LSD analysis of the activity of anti-acne cream from longan seed extract on the growth of *Propionibacterium acnes*

Formula	n	Zone of inhibition of the growth of test bacteria				
		Means	Std. dev	Median	Min	Max
Base	5	0	0	0	0	0
F1	5	11.40	1.14	11.00	10.00	13.00
FII	5	13.40	1.14	13.00[a]	12.00	15.00
FIII	5	14.40	1.14	14.00[a]	13.00	16.00
FIV	5	18.00	1.23	17.50	16.50	19.50
FV	5	20.50	1.54	19.50	19.50	23.00
FVI	5	22.10	1.29	22.50	20.00	23.50

[a]: No difference between groups (based on the LSD test with $p<0.05$).

The results obtained from the SPC test for anti-acne cream containing longan seed extract, base, and all formulas met the required standards (27), which is $\leq 10^7$ colonies/g (Table 15).
The total mold and yeast (TMY) test involved inoculating the diluted cream sample into Sabouraud dextrose agar (SDA) medium using the pour plate method. Replicates of the test were conducted, with a minimum of two Petri dishes used for each dilution. The plates were then incubated at a temperature within the range of 20 to 25°C for 5 to 7 days. Each colony, whether small, large, or wide, was counted as one. Total mold and yeast value was determined based on the dilution with the highest number of colonies, provided that the count was less than 50 colonies.[5] The recommended limit of TMY for cream preparations is $\leq 10^4$ colonies/g.[26] The total mold and yeast test results for the cream base and all formulas passed the required standards (Table 16).

Contamination of pathogenic bacteria testing outcome
Cream preparations containing natural ingredients should ideally not contain *Staphylococcus aureus* and *Pseudomonas aeruginosa.*[26] These two bacteria, when present in contaminated cream, could multiply and have a risk of causing diseases. To identify pathogenic bacteria, a common method involved enriching the sample using appropriate media and then inoculating it onto selective media. These selective media contain specific ingredients that could be metabolized by certain bacteria. The resulting metabolites could then be used as a basis for identification. The presence of *Staphylococcus aureus* contamination was determined by following a specific testing procedure. First, the sample was inoculated into tryptic soy broth (TSB) enrichment media and then incubated at a temperature of 30 to 35ºC for 18 to 24 hours. Subsequently, the sample was transferred to selective MSA media where it was cultured once again for 18 to 72 hours at 30 to 35 °C. The positive result was indicated by the presence of white or yellow colonies surrounded by a yellow zone.[5] The analysis of anti-acne cream preparations containing longan seed extract revealed that it was free from *Staphylococcus aureus* contamination, as shown in Table 17. Testing for *Pseudomonas aeruginosa* contamination involved using TSB enrichment media, with the same incubation temperature and duration as the *Staphylococcus aureus*. For its selective growth, CETA was used as the specific medium, and it was incubated at a temperature of 30 to 35ºC for 18 to 72 hours. The presence of *Pseudomonas aeruginosa* contamination was indicated by the colonies that exhibited green fluorescence.[5] As indicated in Table 18, every anti-acne cream formula and base tested negative during the experiment.

Conclusion

The effectiveness of longan seed extract in inhibiting the growth of *Propionibacterium acnes* was evaluated at a concentration of 1% and showed a bacteriostatic effect, while concentrations of 2, 4, and 8% demonstrated bactericidal effects. Among the tested formulas, formula IV was found to qualify the stability requirements for physical quality, and microbial contamination tests, and shows antibacterial activity against *Propionibacterium acnes*, making it an effective anti-acne cream.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 16: Standard plate count of anti-acne cream from longan seed extract

Formulas	Replication	Dilution		Result (colony/g)	Requirements (BPOM, 2019)
		10^{-1}	10^{-2}		
Base	1	0	0	$<1 \times 10^1$	$\leq 10^7$ colonies/g
	2	0	0		
FI	1	1	0	1×10^1	
	2	0	0		
FII	1	4	0	2×10^1	
	2	0	0		
FIII	1	0	3	2×10^2	
	2	0	0		
FIV	1	0	0	$<1 \times 10^1$	
	2	0	0		
FV	1	0	0	$<1 \times 10^1$	
	2	0	0		
FVI	1	0	0	$<1 \times 10^1$	
	2	0	0		

Table 17: Total mold and yeast test results of anti-acne cream from longan seed extract

Formulas	Replication	Dilution		Result (colony/g)	Requirements (BPOM. 2019)
		10^{-1}	10^{-2}		
Base	1	0	0	$<1 \times 10^1$	$\leq 10^4$ colonies/g
	2	0	0		
FI	1	0	0	$<1 \times 10^1$	
	2	0	0		
FII	1	0	0	$<1 \times 10^1$	
	2	0	0		
FIII	1	0	0	$<1 \times 10^1$	
	2	0	0		
FIV	1	0	0	$<1 \times 10^1$	
	2	0	0		
FV	1	0	0	$<1 \times 10^1$	
	2	0	0		
FVI	1	0	0	$<1 \times 10^1$	
	2	0	0		

Table 18: Test results of pathogenic microbial contamination of anti-acne cream from longan seed extract

Microbial contamination	Formulas							Requirements (BPOM, 2019)
	Base	FI	FII	FIII	FIV	FV	FVI	
Staphylococcus aureus	negative	negative	negative	negative	negative	negative	negative	negative
Pseudomonas aeruginosa	negative	negative	negative	negative	negative	negative	negative	negative

References

1. Tranggono, Iswari R, Latifah F. Buku Pegangan Ilmu Pengetahuan Kosmetik. PT. Gramedia Pustaka; 2007.
2. Sukandar EY, Retnosari A, Sigit JI, Adnyana IK, Setiadi, AP AA. ISO Farmakoterapi 2. Penerbit ISFI; 2011.
3. Salasa, A.M., Ratnah S. Determination of Total Phenolic Content (TPC) and Antioxidant Avtivity of Longan (Euphoria longan) Seed and Peel Extract. In: Interproprofessional Proceedings Collaboration on Urban Health Vol. 2. No.1. Politeknik Kesehatan Makassar; 2019.
4. Sapan, M.V., Salasa, A.M. Ratnah S. Uji Aktivitas Antibakteri Biji Buah Kelengkeng (Euphoria longan Stend) Terhadap Pertumbuhan Propionibacterium acnes.; 2018.
5. Dirjen Kefarmasian dan Alat Kesehatan. Farmakope Indonesia. VI. Kementerian Kesehatan RI; 2020.
6. Litbang BB. Pedoman Umum Panen Dan Pascapanen Tanaman Obat. Kemenkes RI; 2011.

7. Egbuna C, Ifemeje JC, Udedi SC, Kumar S. Phytochemistry, Volume 1: Fundamentals, Modern Techniques, and Applications. Vol 1. Apple Academic Press Inc.; 2019.
8. Lay BW. Analisis Mikroba Di Laboratorium. PT. Raja Grapindo Persada, Jakarta; 1994.
9. Ratnah S, Salasa AM. Quality Standardization of Longan Seed Extract (*Euphoria longan* Stend). Int J Curr Pharm Res. 2022;14(2):31-35. doi:10.22159/ijcpr.2022v14i2.1948
10. Ditjen POM. Parameter Standar Umum Ekstrak Tumbuhan Obat. 1st ed. Depkes RI; 2000.
11. Karoll KC, Morse SA, Mietzner T, Miller S. Jawetz, Melnick Dan Adelberg's Mikrobiologi Kedokteran. 27th ed. Buku Kedokteran EGC; 2017.
12. Davis WW, Stout TR. Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error. Appl Microbiol. 1971;22(4):659-665. doi:10.1128/aem.22.4.659-665.1971
13. Cappucino JG, Sherman N. Manual Laboratorium Mikrobiologi. 8th ed. Buku Kedokteran EGC; 2014.
14. Pratiwi ST. Mikrobiologi Farmasi. Erlangga; 2008.
15. Ratnah S, Salasa AM. Efektivitas Ekstrak Biji Buah Kelengkeng (Euphoria longan Stend) Terhadap Pertumbuhan Staphylococcus aureus dan Propionibacterium acnes.Media Farm. 2020;16(1).
16. Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. Third. Varghese Publishing House; 1987.
17. SNI. Sediaan Tabir Surya. Vol 16.; 1996.
18. Tranggono RI, Latifah F. Buku Pegangan Ilmu Kosmetik. (Djayadisastra J, ed.). PT. Gramedia Pustaka Utama; 2007.
19. Noviardi H, Ratnasari D, Fermadianto M. Formulasi Sediaan Krim Tabir Surya dari Ekstrak Etanol Buah Bisbul (Diospyros blancoi). J Ilmu Kefarmasian Indones. 2019;17(2):262. doi:10.35814/jifi.v17i2.771
20. Purwaningsih, Septiarini AD, Wardani TS. Analisis Nilai SPF (Sun Protection Factor) Sediaan Krim Tabir Surya Ekstrak Etanol Bunga Pepaya Jantan (*Carica papaya* L.) Dengan Metode Spektrofotometri UV-Vis. J Farm Politek Indonusa Surakarta. 2021;5(2):26-32.
21. Garg A, Aggarwal D, Garg S, Singla AK. Spreading of semi-solid formulations: An update. Pharm Technol North Am. 2002;26(9):84-105.
22. Arbie S, Sugihartini N, Wahyuningsih I. Formulasi Krim M/A Dengan Variasi Konsentrasi Ekstrak Buah Pepaya (Carica papaya L.) Menggunakan Emulgator Asam Stearat Dan Trietanolamin. Media Farm. 2021;16(1):97. doi:10.32382/mf.v16i1.1420
23. Sriamornsak P, Polat A, Krongrawa W, Krüger-genge A, Storsberg J, Seidler T. Formulation of Microemulsions Containing Rambutan Peel Extract and Their Antibacterial Activities.Trop J Nat Prod Res. 2023;7(April):2730-2736.
24. de Canha MN, Komarnytsky S, Langhansova L, Lall N. Exploring the Anti-Acne Potential of Impepho [Helichrysum odoratissimum(L.) Sweet] to Combat Cutibacterium acnes Virulence. Front Pharmacol. 2020;10(January):1-21. doi:10.3389/fphar.2019.01559
25. Dwiani S, Choirunnisa AR, Haniffadli A, et al. Reduction of Microbial Contamination in Kaolin from Belitung Island, Indonesia. Trop J Nat Prod Res. 2023;7(1):2104-2106. doi:10.26538/tjnpr/v7i1.3
26. Peraturan BPOM RI No. 32. Persyaratan Keamanan dan mutu Obat Tradisional. Bpom RI. Published online 2019.