Jurnal Sains Farmasi & Klinis
p-ISSN: 2407-7062 | e-ISSN: 2442-5435
homepage: http://jsfk.ffarmasi.unand.ac.id
DOI : 10.25077/jsfk.11.3.197-207.2024

ORIGINAL ARTICLE J Sains Farm Klin 11(3):197–207 2024 | DOI: 10.25077/jsfk.11.3.197-207.2024

Formulation of Serum Sunflower (*Helianthus annuus* L.) Seed Oil as Antiacne

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ABSTRACT: Acne is caused by a microbial imbalance due to the overgrowth of *Propionibacterium acnes* in the skin microbiome. Sunflower seed oil (*Helianthus annuus* L.), which contains linoleic acid and terpenoids, has an antibacterial activity against *Propionibacterium acnes* This study aimed to increase the availability of anti-acne compounds from natural resources by formulating an anti-acne serum containing sunflower seed oil. The research methods included antibacterial activity testing of sunflower seed oil, serum formulation, product evaluation, and serum effectiveness testing. The results of the antibacterial activity test of sunflower seed oil against *Propionibacterium acnes* ATCC 6919 showed a minimum inhibitory concentration (MIC) of 100 mg/mL and a minimum bactericidal concentration (MBC) of 200 mg/mL. The serum evaluation and stability testing during the freeze-thaw cycling test depicted stable formulation for all formulas based on organoleptic parameters, homogeneity, pH, viscosity, and spreadability. The effectiveness test results of the serum preparation showed that formula 3, containing 200 mg/L of sunflower seed oil, was the best formula with an inhibition zone diameter of 12.76 ± 0.03 mm. In conclusion, sunflower seed oil demonstrates the ability to prevent and treat acne-prone skin, making it a suitable alternative anti-acne compound.

Keywords: **oil; seed; *Helianthus annuus* L.; serum; *Propionibacterium acnes*.**

Introduction

Acne is one of the skin problems caused by the bacterium *Propionibacterium acnes*. This bacterium produces lipase that breaks down free fatty acids from skin lipids, leading to the formation of acne [1]. Although not life-threatening, if not addressed promptly, acne can become progressive and worsen if left untreated, potentially lasting for years and causing scars like pockmarks on the face, accompanied by pain and even bleeding. Acne is also known to have serious psychosocial effects such as decreased self-esteem, depression, frustration, and withdrawal from social interactions [2].

The management of acne is carried out based on its severity. Therapy for mild cases involves topical treatments such as retinoic acid or benzoyl peroxide. In contrast, for moderate to severe cases, topical retinoid isotretinoin can be used with oral therapy with doxycycline, clindamycin, or other antibiotics [3]. In recent years, the market has transitioned from synthetic to natural materials . This transition is connected to the current environmental landscape, where consumers increasingly recognize the significance of choosing products that do not harm the environment or health. Natural cosmetics provide several health advantages and have a reduced impact on the environment [4]. Based on a review from the Centre for the Promotion of Imports from Developing Countries (CBI), one of the natural ingredients predicted to become a trend in the coming years is sunflower seed oil due to its high linoleic acid content, which is 51.5-73.5% of its total fatty acids. This value is higher than the linoleic acid content in argan oil, which is 28-36% [5-7]. Sunflower seed oil (*Helianthus annuus* L.) has been reported to suppress acne, causing microorganisms due to its content of linoleic acid and terpenoids. Terpenoids destroy the cell membranes of acne-causing bacteria, while linoleic acid is an anti-inflammatory agent that reduces inflammation caused by acne. Sunflower seed oil has antibacterial properties with a Minimum Inhibitory Concentration (MIC) of 1.5% against *Propionibacterium acnes* [8].

Based on previous research, sunflower seed oil in cream formulation tends to have high viscosity, resulting in low spreadability [8]. The spreadability of a formulation is related to

Article history
Received: 26 Dec 2024
Accepted: 19 Mar 2025
Published: 10 Apr 2025
Access this article

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its contact with the skin surface, so formulations with low spreadability are often considered to have lower effectiveness [6]. In this study, sunflower seed oil will be formulated as serum preparation. The serum formulation was chosen because its viscosity is low, providing a more comfortable effect and easy spreading on the skin surface. Additionally, its higher active ingredient content than other formulations like creams makes the serum faster and more effective in addressing skin problems [9].

Methods

The research included of confirmation the bacterium Propionibacterium acnes ATCC 6919, tested the anti-acne activity of sunflower seed oil, formulationed sunflower seed oil serum effectiveness, and tested as anti acne. This study has been conducted in accordance with biosafety guidelines for microbial studies in accordance with ISO 15189 standards. Since there are no humans or animal subject involved, negating the need the ethical approval.

Materials

The sample used in this study is sunflower seed oil from Textron Plimon with a linoleic acid content of 57.7% based on the Certificate of Analysis. The other material used are glycerin , ceteareth-33, PEG-100 stearate , stearyl alcohol , dimethicone , phenyl trimethicone , butylated hydroxytoluene, phenoxyethanol, DMDM hydantoin , DMSO, 1 % sulfuric acid (H_2SO_4), 1% barium chloride ($BaCl_2$) , and hydrogen peroxide (H_2O_2) . The bacterium utilized in this research is Propionibacterium acnes ATCC 6919. Bacteria media used are Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB).

Confirmation Test of *Propionibacterium acnes* ATCC 6919

The confirmation test of bacteria was carried out by observing the morphology of bacterial colonies, microscopic observation, and biochemical reaction tests on the test bacteria *Propionibacterium acnes* ATCC 6919. The test bacteria are first rejuvenated by isolating *Propionibacterium acnes* from a pure culture using a sterile loop. Then, the bacteria arenoculated into MHA media and incubated for 1 x 24 hours at room temperature 37°C in an incubator.

Macroscopic Observation of Colony Morphology

The morphology of bacterial colonies was observed macroscopically and microscopically. Macroscopic observation is performed by inoculating the test bacteria *Propionibacterium acnes* ATCC 6919 from the rejuvenated bacterial colony using a sterile loop into MHA media, then incubated for 1 x 24 hours at room temperature 37°C in an incubator.

Microscopic Observation of Colony Morphology

Microscopic morphological observation was conducted using the Gram staining method. The rejuvenated bacteria were taken in a single loop and placed on a glass slide. The bacteria were then stained with crystal violet for 5 minutes. Next, the dye was discarded, and Lugol's iodine solution was applied and allowed to sit for 45-60 seconds. After that, the test bacteria were rinsed with 96% alcohol until no dye remained. The bacteria were stained with safranin for 2 minutes, rinsed with distilled water, and then dried before being observed under a microscope to examine the shape and color of the test bacteria cells. Gram-positive bacteria will show purple colonies, while gram-negative bacteria will show red colonies [9,10].

Biochemical Reaction Test of Propionibacterium acnes ATCC 6919

The catalase test is performed by dropping hydrogen peroxide (H_2O_2) reagent onto a sterile glass slide and then applying the culture onto the slide using an inoculating loop. Next, the suspension is mixed slowly using the loop. P. acnes will show a positive result, as indicated by the formation of air bubbles after adding H_2O_2. Meanwhile, the indole test is performed by taking a portion of the colony from MHA using a sterile loop and inoculating it into the indole medium by stirring, then incubating it at 37°C for 24 hours. After that, 1-2 drops of Kovac's reagent were added. *Propionibacterium acnes* will produce a positive result marked by forming a red ring on the surface of the medium [11].

Testing the Antibacterial Activity of Sunflower Seed Oil

The determination of the antibacterial properties of sunflower seed oil is carried out through several stages as follows

Preparation of Sunflower Seed Oil Samples

Weigh 8 g of sunflower seed oil and then add 2 g of DMSO solution to obtain a stock solution of 800 mg/mL or 80%. Dilution will later be performed stepwise in sterile test tubes.

Preparation of Test Media

There are two test media used in this study, namely MHA media and MHB media, which are prepared as follows:

MHA Test Media

MHA media is prepared by dissolving 19.5 g of MHA media into 250 mL of sterile distilled water in a 1000 mL Erlenmeyer flask, heating it using a hotplate stirrer until dissolved, covering it with aluminum foil, and sterilizing it in an autoclave at 121°C for 15 minutes [12].

MHB Test Media

MHB media is prepared by dissolving 3.25 grams of MHB media into 250 mL of sterile distilled water in a 1000 mL Erlenmeyer flask. Afterward, the prepared MHB media in the Erlenmeyer flask is covered with aluminum foil and sterilized in an autoclave at 121°C for 15 minutes [13].

Preparation of standard 0.5 McFarland

The McFarland turbidity standard is prepared by adding 0.05 mL of 1% $BaCl_2$ to

9.95 mL of 1% H_2SO_4. The standardization is carried out using a UV-Vis Spectrophotometer with a standard absorbance value of 0.08 – 0.10 at 600-625 nm wavelength [14].

Determination of MBC

Eight test tubes were prepared and labeled 1-8. 1 mL of MHB medium was added to tubes 1-8, then 1 mL of sunflower seed oil solution with a concentration of 800 mg/mL was added to tube 2, resulting in a 400 mg/mL test concentration. From these tubes, 1 mL was taken and added to tube 3, and so on until tube 7. In the well of tube 7, 1 mL of the test solution was discarded, resulting in concentration variations of 400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and

12.5 mg/mL. Then, 1 mL of the test bacterial suspension with turbidity equivalent to 1 x 10^6 CFU/mL was inoculated into each tube, except for tube 1, which served as the negative control. Thus, the following test concentrations were obtained: 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL. The test media were then incubated at 37°C for 18 hours. The Minimum Bactericidal Concentration (MBC) value is located in the tube with the lowest concentration that does not show any cell sedimentation before the next lowest concentration that shows sedimentation. The MIC results that did not show any sediment were subcultured with 20 µL on the surface of 20 mL MHA media. The test media were incubated at 37°C for 18 hours. The MBC value is at the lowest concentration, showing a colony growth of 0.1% from the final inoculation (1 x 104 CFU/mL) or no colony growth [15].

Sunflower Seed Oil Serum Formulation

The sunflower seed oil serum was prepared using stearic alcohol as a serum base. The serum formula conducted in this research is shown in Table 1.

The serum is made by grouping the ingredients into two phases, namely, the aqueous phase and the oil phase. When both phases have reached 70°C, mix the two, then stired using a mechanical stirrer. After mixing, added sunflower seed and stired again until homogeneous [16,17]. The concentration of sunflower seed oil used for Formulas 1, 2, and 3 depends on the antibacterial activity test values. ceteareth-33 as the emulsifier was differentiated in the three formulas at 1%, 1.25%, and 1.5%, respectively. The variation in the amount of ceteareth-33 is based on the amount of sunflower seed oil used. The more significant the amount of oil used, the greater the ceteareth-33 added to the formulation; this is to ensure that the emulsifier can bind the oil effectively. However, this results in an increase

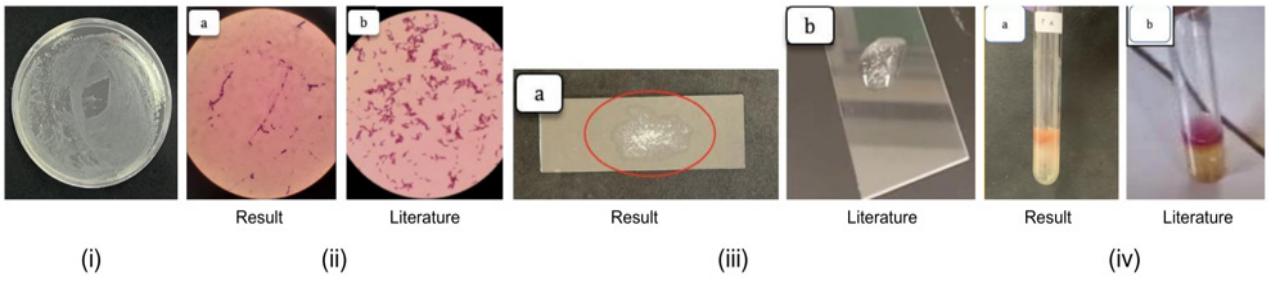

Figure 1. Confirmation Test of P.acnes ATCC 6919, (i) Morphology of *Propionibacterium acnes* ATCC 6919 Colonies, (ii) Gram staining of Propionibacterium acnes ATCC 6919 Colonies, (iii) Catalase test of *Propionibacterium acnes* ATCC 6919, (iv) Indole test of *Propionibacterium acnes* ATCC 6919.

in viscosity. Therefore, the effect of adding ceteareth-33 and the increase in viscosity in the serum base used is observed, with an evaluation of the serum, particularly its antibacterial ability.

Evaluation and Stability Test of Sunflower Seed Oil Serum

The evaluation of the formulation used to test the stability of the new formulation is the freeze-thaw cycling test, which is a stability test by placing the test formulation in two alternating temperature conditions, namely 4°C ± 2°C and 40°C

± 2°C, each for 24 hours in one cycle. This test is conducted for six cycles with seven sampling points in the cycle (T), named C0, C1, C2, C3, C4, C5, and C6. The serum evaluation test is conducted by testing several parameters, such as organoleptic tests to observe the physical stability of the serum formulation, homogeneity test, spreadability test, pH test, and viscosity test [16].

Organoleptic Test

The organoleptic test is conducted visually without the use of auxiliary tools. This observation includes the smell, color, and shape [17].

Homogeneity Test

The serum homogeneity test is conducted by applying the serum on two glass slides. The serum sample is placed on one of the glass slides and then evenly spread. Homogeneity testing was conducted to evaluate the uniformity of the serum formulation by examining the consistency of particle distribution [8].

Spreadability Test

The spreadability test is conducted using a watch glass or a microscope slide. 0.5 g of serum is applied to the glass slide, and its diameter is measured. Next, a load weighing approximately 50 g is added, and its diameter is measured [18]. A good spreadability has a spread range of 5-7 cm [19,20].

pH Test

The pH testing is conducted using a Mettler Toledo SevenCompact pH meter, calibrated using pH 4.01 and 7.01 buffer solutions. The pH meter electrode is rinsed with distilled water and dried. The electrode is then dipped into the serum formula and left for 1 minute until a constant pH value is obtained [8]. The safe pH range for application on the skin is pH 4.5 – 7 [21].

Viscosity Test

The viscosity test is conducted using a Lamy Rheology B-One Plus Brookfield viscometer, calibrated using viscosity standard fluid. The result is recorded to calculate the total calibration tolerance. Place the viscometer spindle at the appropriate depth in the serum preparation. The spindle used is spindle No. 3, set at a speed of 50 rpm, and then the viscosity measurement results are recorded. Based on SNI 16-4399-1996, the ideal serum has viscosity criteria of 200-50000 mPa.s (2-500 dPas) [22,23].

Effectiveness Testing of Sunflower Seed Oil Serum

The antibacterial activity test was conducted to evaluate the anti-acne properties of the sunflower seed oil serum. This test was performed using the agar diffusion method with serum concentrations tested according to the concentrations obtained from the Minimum Inhibitory Concentration (MIC) method and 1% clindamycin antibiotic as a comparison. A total of 20 µL of bacterial suspension at 0.5 McFarland was placed into a sterile petri dish, followed by the addition of 20 mL of MHA media at 40-45°C. The media was allowed to solidify and then perforated using an 8 mm-sized perforator. 100 µL from each test concentration was introduced into the wells created in the test media. All test media, the positive control (MHA media inoculated with the test bacteria), and the negative control (MHA media only) were incubated at 37°C for 18 hours. The resulting inhibition zone diameter was measured using calipers.

Result and Discussion

Confirmation Test of *Propionibacterium acnes* ATCC 6919

The results from the confirmation test of *Propionibacterium acnes* ATCC 6919 were observed through macroscopic, microscopic, and biochemical testing. Figure 1(i) illustrates the round, thin, and white colonies of *Propionibacterium acnes* observed under macroscopic examination. The microscopic analysis of *Propionibacterium acnes* ATCC 6919 morphology using Gram staining was conducted to determine whether the bacteria are Gram-negative or Gram-positive. Figure 1(ii) illustrates the rod-shaped (bacillus) cells with a purple color of Gram-stained *Propionibacterium acnes* ATCC 6919 under 100x magnification. This observation confirms that *Propionibacterium acnes* ATCC 6919 is a Gram-positive bacterium characterized by a thick peptidoglycan layer. This result aligns with the previous research, which categorized *Propionibacterium acnes* as a Gram-positive bacterium with a thick peptidoglycan

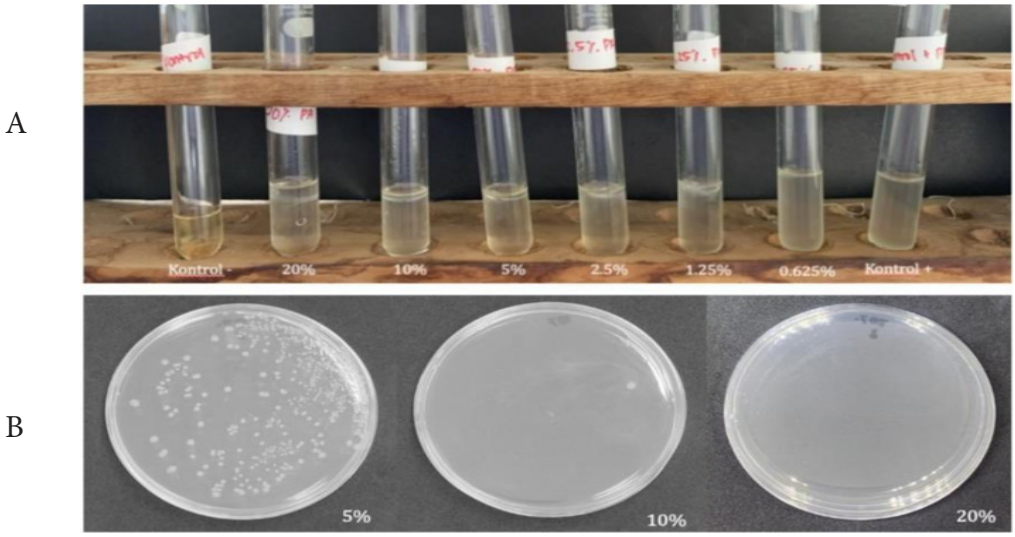

Figure 2. Antibacterial activity test of sunflower seed oil, (A) Results of the microdilution test of sunflower seed oil on *Propionibacterium acnes* ATCC 6919, (B) Results of the minimum bactericidal concentration (MBC) test of sunflower seed oil against *Propionibacterium acnes* ATCC 6919 (n=3).

structure [24].

Biochemical tests of *Propionibacterium acnes* ATCC 6919 included catalase and indole reaction tests. The catalase test was conducted by exposing the test bacteria to hydrogen peroxide (H_2O_2) reagent. The catalase enzyme in bacteria facilitates the decomposition of H_2O_2 into H_2O and O_2, resulting in the formation of gas bubbles, which signify a positive reaction [25]. The test results revealed the formation of gas bubbles, as shown in Figure 1(iii), confirming that *Propionibacterium acnes* ATCC 6919 possesses the catalase enzyme.

The indole test was performed to detect the presence of the tryptophanase enzyme in the bacteria by using Kovac's reagent to evaluate the organism's ability to break down indole from the amino acid tryptophan, which signifies the presence of the tryptophanase enzyme, as indicated by the appearance of a red colored ring layer [26]. The indole test results were positive, demonstrated by the formation of a red-colored ring layer on the surface of the test medium. These findings indicate the presence of tryptophanase enzyme in *Propionibacterium acnes* ATCC 6919 [27,28]. Based on the research results, as shown in Figure 1d, it can be concluded that the test bacterium used in this study showed a positive result for *P. acnes*.

Antibacterial Activity Test of Sunflower Seed Oil

The antibacterial activity test aims to assess the highest sensitivity of a compound in inhibiting specific bacterial growth. This study chose the liquid dilution method due to the nonpolar characteristics of sunflower seed oil compounds. The liquid dilution method is suitable for determining a compound's minimum

Figure 3. Organoleptic parameter of all serum formulation, (A) Organoleptic of base formulation, (B) Organoleptic of formula 1 (sunflower seed oil 10%), (C) Organoleptic of formula 2 (sunflower seed oil 15%), (D) Organoleptic of formula 3 (sunflower seed oil 20%).

Table 1. Final Formulation of Anti-Acne Serum Containing Sunflower Seed Oil.

Compound	Function	Formula Concentration (%) w/w			
		Base	F1	F2	F3
Oil Phase					
Sunflower seed oil	Active ingredients	-	10	15	20
Glyceryl stearate	Emulsifier	1	1	1	1
Ceteareth-33	Emulsifier	1	1	1.25	1.5
Stearyl alcohol	Base	0.75	0.75	0.75	0.75
Phenyl trimethicone	Emollient	1	1	1	1
Dimethicone	Emollient	2	2	2	2
Polyacrylamide, C13-14 Isoparaffin, Laureth-7	Viscosity control	0.5	0.5	0.5	0.5
Bamboo aloe	Fragrance	0.5	0.5	0.5	0.5
BHT stabilizer	Stabilizer	0.15	0.15	0.15	0.15
Water Phase					
Glycerin	Humectant	3	3	3	3
Triethanolamine 1.5%	pH control	1	1	1	1
Phenoxyethanol	Preservative	1	1	1	1
Aqua	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

Description:F1 = Formula 1 F2 = Formula 2 F3 = Formula 3

inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). For this test, a series of concentrations of the test compound was prepared in a liquid MHB medium containing the test bacteria, and turbidity was observed. The MIC was identified as the lowest concentration that showed no visible bacterial growth, which was determined through turbidimetry by assessing whether bacterial cell sedimentation was present at each test concentration.

The microdilution test of sunflower seed oil against *Propionibacterium acnes* ATCC 6919 revealed that the 100 mg/mL concentration did not show bacterial cell sedimentation, as illustrated in Figure 2(A). This concentration was then subcultured by spreading it on solid media (MHA) at the lowest observed concentration (100 mg/mL) and two other concentrations surrounding it: 50 mg/mL and 200 mg/mL. The results indicated that at 50 mg/mL, *P. acnes* colonies were still visible. Only one colony was observed at 100 mg/mL, while no bacterial colonies were present at 200 mg/mL, as shown in Figure 2(B). Based on these observations, sunflower seed oil's minimum inhibitory concentration (MIC) against *Propionibacterium acnes* is 100 mg/mL, while the minimum bactericidal concentration (MBC) is 200 mg/mL. These concentrations will be incorporated into the formulation of an anti-acne serum based on sunflower seed oil.

Formulation of Sunflower Seed Oil Serum

The anti-acne serum was prepared using different concentrations of sunflower seed oil based on previous testing results, which fell within the range of the oil's minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The concentrations used in the formulation were 100 mg/mL, 150 mg/mL, and 200 mg/mL, equivalent to 10%, 15%, and 20%, respectively. Each serum formulation, as shown in Table 1, will undergo evaluation for stability using the freeze-thaw cycling test method. Figure 3 illustrates the organoleptic properties of serum preparations in a thick white liquid.

Evaluation and Stability Testing of Sunflower Seed Oil Serum

The stability evaluation of the anti-acne serum from sunflower seed oil was conducted using the freeze-thaw

Table 2. Antibacterial Effectiveness of Sunflower Seed Oil Serum Against *Propionibacterium acnes*.

Test Sample	Inhibition Zone Diameter (mm)			Average Inhibition Zone (mm)	Antibacterial Activity
	1	2	3		
Clindamycin 1%	16.70	16.67	16.67	16.68 ± 0.02	Strong
Base	0	0	0	0	None
F1	10.70	10.60	10.87	10.73 ± 0.14	Strong
F2	11.83	11.67	11.53	11.67 ± 0.15	Strong
F3	12.78	12.73	12.77	12.76 ± 0.03	Strong
Clindamycin 1%	16.70	16.69	16.68	16.69 ± 0.01	Strong
Base 1	0	0	0	0	None
Base 2	0	0	0	0	None
Base 3	0	0	0	0	None

Description:
Base : Serum base formulation
F1 : Anti-acne serum formulation containing 100 mg/mL sunflower seed oil
F2 : Anti-acne serum formulation containing 150 mg/mL sunflower seed oil
F3 : Anti-acne serum formulation containing 200 mg/mL sunflower seed oil
Base 1 : Anti-acne serum base formulation containing 1% (10 mg/mL) of the emulsifier ceteareth-33
Base 2 : Anti-acne serum base formulation containing 1.25% (12.5 mg/mL) of the emulsifier ceteareth-33
Base 3 : Anti-acne serum base formulation containing 1.5% (15 mg/mL) of the emulsifier ceteareth-33
Perforator size : 8 mm

cycling test method. This method exposed the serum to alternating temperatures of 4°C ± 2°C and 40°C ± 2°C for 24 hours each over a series of six cycles. Following these six freeze-thaw cycles, the serum's physical and chemical characteristics were analyzed, including organoleptic properties, homogeneity, pH, viscosity, and spreadability.

The organoleptic evaluation was performed by assessing the preparation's appearance, colour, and aroma. Figure 6 depicts the organoleptic parameters of serum after formulation; all formulas showed a thick white liquid with an aloe bamboo aroma. According to the research, it was observed that the serum base and the three anti-acne serum formulations derived from sunflower seed oil exhibited no changes in colour or aroma throughout the freeze-thaw cycling test. However, the consistency of the three anti-acne serum formulations showed changes characterized by an increase in consistency, which was first observed during the third cycle for F1 and F2 and during the second cycle for F3. These findings are consistent with the viscosity values obtained. Viscosity serves as a

Table 3. Results of the pH Values of Seed Oil Sunflower Serum during *Freeze-thaw Cycling*.

Cycling	Base	F1	F 2	F3
0	4,76 ± 0,05	5,35 ± 0,01	5,67 ± 0,02	5,70 ± 0,04
1	4,74 ± 0,03	5,19 ± 0,01	5,23 ± 0,04	5,35 ± 0,02
2	4,91 ± 0,01	5,06 ± 0,01	5,27 ± 0,01	5,45 ± 0,01
3	4,88 ± 0,02	4,87 ± 0,01	5,13 ± 0,02	5,27 ± 0,01
4	4,86 ± 0,04	5,00 ± 0,01	5,15 ± 0,01	5,22 ± 0,02
5	4,82 ± 0,08	4,78 ± 0,04	5,12 ± 0,03	5,16 ± 0,01
6	4,98 ± 0,05	4,88 ± 0,01	5,02 ± 0,02	5,15 ± 0,01

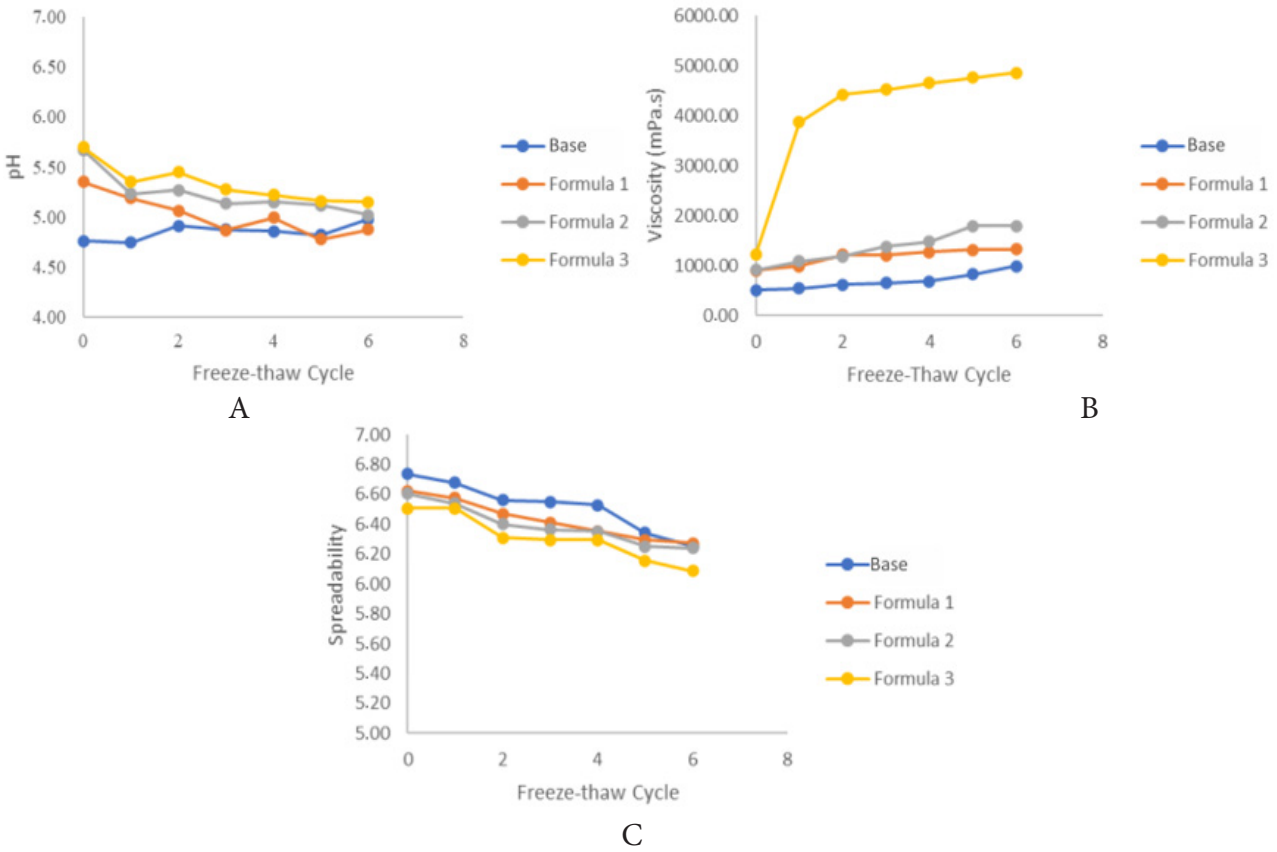

Figure 4. Freeze-thaw cycle of pharmaceutical parameters, (A) Freeze-thaw cycle of pH parameters, (B) Freeze-thaw cycle of viscosity parameters, (C) Freeze-thaw cycle of spreadability parameters.

qualitative indicator of the preparation's consistency; therefore, higher viscosity values correspond to increased formulation consistency.

The homogeneity test was conducted to evaluate the uniformity of the tested preparation and to examine the even distribution of particles within the anti-acne serum derived from sunflower seed oil. Observations revealed that when applied to a glass slide, the three anti-acne serum formulations contained no coarse particles. Additionally, the preparations maintained a smooth texture without clumping throughout the freeze-thaw cycling test. These results suggest that the base and all three anti-acne serum formulations from sunflower seed oil have a uniform and consistent composition. Figure 3 shows that serum does not crack and is homogeneous [29].

Several evaluation aspects, including pH, viscosity, and spreadability, were observed for changes during the freeze-thaw cycle and statistically analyzed using the

Table 4. Results of the Viscosity Test of Seed Oil Sunflower Serum during *Freeze-thaw Cycling.*

Cycling	Base (mPa.s)	F1 (mPa.s)	F 2 (mPa.s)	F3 (mPa.s)
0	512 ± 26	902 ± 17	907 ± 27	1211 ± 16
1	547 ± 40	990 ± 39	1089 ± 38	3863 ± 100
2	616 ± 2	1217 ± 23	1181 ± 30	4423 ± 249
3	651 ± 24	1269 ± 42	1377 ± 38	4523 ± 199
4	679 ± 11	1311 ± 26	1472 ± 76	4647 ± 57
5	822 ± 8	1309 ± 23	1789 ± 10	4763 ± 110
6	985 ± 3	1321 ± 12	1796 ± 5	4860 ± 131

Table 5. Results of the Spreadability Test of Seed Oil Sunflower Serum during *Freeze-thaw Cycling*.

Cycling	Base (cm)	F1 (cm)	F2 (cm)	F3 (cm)
0	6,74 ± 0,12	6,62 ± 0,04	6,60 ± 0,21	6,50 ± 0,08
1	6,68 ± 0,02	6,58 ± 0,04	6,54 ± 0,09	6,50 ± 0,04
2	6,56 ± 0,06	6,47 ± 0,02	6,4 ± 0,02	6,31 ± 0,10
3	6,53 ± 0,13	6,41 ± 0,08	6,37 ± 0,08	6,30 ± 0,04
4	6,34 ± 0,08	6,35 ± 0,00	6,35 ± 0,00	6,30 ± 0,11
5	6,27 ± 0,11	6,30 ± 0,11	6,25 ± 0,12	6,16 ± 0,11
6	6,25 ± 0,12	6,27 ± 0,04	6,24 ± 0,02	6,09 ± 0,04

Statistical Package for the Social Sciences (SPSS) version 25. The pH and spreadability tests were conducted using the two-way ANOVA method because the data were normally distributed based on normality tests. Meanwhile, the viscosity test was statistically analyzed using the Friedman method because the data, based on normality test results, were not normally distributed.

Effective topical formulations should have a pH range between 4 and 6 [30]. This range is similar to the natural pH of human skin, which typically falls between 4 and 6.5 [31]. Using a facial serum with an inappropriate pH can damage the stratum corneum; a formulation with a pH that is too acidic can irritate the skin, while one that is too alkaline can cause dryness and flaking [32]. The pH test results showed that all formulations fell within the expected range, with values from 4.76 to 5.7 , that showed at table 3. During the freeze-thaw cycling test, the pH of the serum base increased to 4.98 by the final cycle, while the three anti-acne serum formulations from sunflower seed oil showed a pH decrease, ranging from 4.88 to 5.15 with significant difference ($p < 0,05$), as seen in Figure 4(A). This decline in pH is likely due to the reaction of CO_2 with the aqueous phase, which produces acid and lowers the pH of the formulation. Despite this reduction, the pH of the anti-acne serum from sunflower seed oil remains within the range specified by SNI-16-4399-1996, which is between 4 and 6.

The viscosity test was performed to observe any changes in the consistency of the formulation during the freeze-thaw cycling test. According to SNI 16-4399-1996, facial serum formulations should have a viscosity within the range of 200– 50,000 mPa.s (2–500 dPas) [22,23]. The results showed that the serum base had a viscosity value of 512 mPa.s., while the three formulations containing sunflower seed oil as the active ingredient showed viscosities ranging from 902 to 1211 mPa.s (see in table 4). Based on the graph in Figure 4(B), the freeze-thaw cycling test demonstrated a consistent increase in viscosity with each cycle, reaching a range between 985 and 4860 mPa.s with significant differences ($p < 0.05$).

The increase in viscosity is likely due to shear force or mechanical stress from the preparation and mixing process. The shear force causes the polymer structures in the formulation to loosen, temporarily lowering the viscosity during the initial mixing stages. However, as the freeze-thaw cycles continue, the polymer structure returns to its original state, which leads to an increase in viscosity and makes the formulation thicker [33]. The viscosity test revealed differences between the formulations, with higher concentrations of sunflower seed oil resulting in higher viscosities. These variations are attributed to the differences in emulsifier concentration across the formulations. The amount of emulsifier depends on the amount of sunflower seed oil used; higher oil concentrations require greater emulsifier levels to stabilize and bind the oil. Consequently, increasing emulsifier levels improves oil-binding capacity, leading to higher viscosity values [34]. Although there was a noticeable increase in viscosity across all formulations, the resulting values still fell within the acceptable range of 200–50,000 mPa.s, as specified by SNI 16-4399-1996 [22,23].

The spreadability test was performed to determine how well and quickly the formulation spreads when applied to the skin. This property is significant for topical formulations, as it influences the rate at which the active ingredients are released and absorbed at the intended site. Typically, the spreadability of topical serums should fall within the range of 5–7 cm [20]. Figure 4(C) and table 5., showed that the formulations' spreadability declines during each freeze-thaw cycling test cycle. However, despite this decrease, the serum base and all three anti-acne serum formulations containing sunflower seed oil maintained a

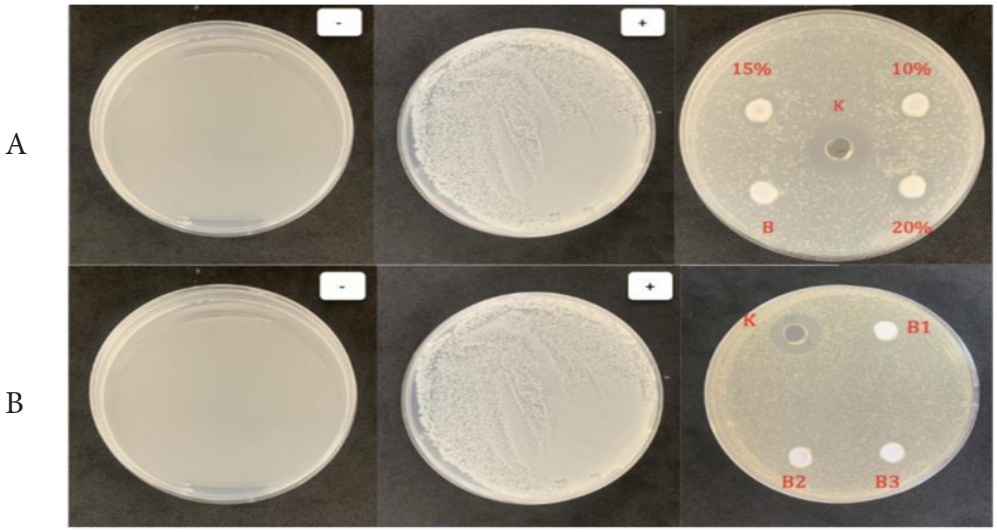

Figure 5. Effectiveness testing of anti-acne serum from sunflower seed oil against *Propionibacterium acnes* ATCC 6919, (A) Inhibition test result of serum base, (B) Inhibition test result of serum containing sunflower seed oil.

Description:

-	: MHA media control (Control -)
+	: MHA media control + Propionibacterium acnes ATCC 6919 bacteria (Control +)
K	: 1% Clindamycin as a comparator
B	: Anti-acne serum base formula
10%	: Anti-acne serum with 10% sunflower seed oil concentration
15%	: Anti-acne serum with 15% sunflower seed oil concentration
20%	: Anti-acne serum with 20% sunflower seed oil concentration
B1	: The base formula of the serum contains 1% ceteareth-33 emulsifier
B2	: The base formula of the serum contains 1.25% ceteareth-33 emulsifier
B3	: The base formula of the serum contains 1.5% ceteareth-33 emulsifier

spreadability within the acceptable range of 5–7 cm, with significant differences ($p < 0.05$). The observed reduction in spreadability is linked to the increase in viscosity within the formulations. As the viscosity of a formulation increases, its spreadability tends to decrease.

Effectiveness Test of Anti-Acne Serum from Sunflower Seed Oil

The purpose of the effectiveness test was to evaluate the antibacterial properties of sunflower seed oil after being formulated into a topical preparation. According to the results shown in Table 2, the anti-acne serum containing sunflower seed oil retained its antibacterial activity against *Propionibacterium acnes* ATCC 6919, as shown in Figure 5. The results demonstrated that all three serum formulations with sunflower seed oil exhibited strong antibacterial properties. It was noted that higher concentrations of sunflower seed oil led to larger inhibition zone diameters, indicating increased effectiveness. Among the formulations, Formula 3 showed the most excellent effectiveness, with an inhibition zone measuring 12.76 ± 0.03 mm. Furthermore, the variation in the concentration of ceteareth-33 in the base formulation did not impact the serum's antibacterial activity .

Conclusion

In conclusion, this research showed that sunflower seed oil in an anti-acne serum formulation demonstrated promising potential based on its antibacterial activity. This study confirms that sunflower seed oil in the serum formulation could be a natural and effective alternative to conventional acne treatments. Future research should focus on *in vitro* testing, *in vivo* trials, long-term stability studies, and combining sunflower seed oil with other bioactive compounds to enhance its therapeutic effects.

Conflict of Interest

All authors declare that there is no conflict of interest in this research.

Acknowledgement

This researched was funded by Universitas Padjadjaran through Grant of Accelerated Head Lecture (RPLK), 2024. (Grant number 1506/UN6.3.1/PT.00/2024).

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