

Antibacterial Activity Test Of Facial Serum Formulation Of Pegagan Leaf Extract (*Centella asiatica* L.) And Pandan Leaf Extract (*Pandanus amaryllifolius* R.) Against *Cutibacterium acnes* Bacteria

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This study aimed to evaluate the physical quality and antibacterial activity of facial serum formulations combining pegagan and pandan leaf extracts. Extraction was performed using maceration with 96% ethanol, yielding 11.75% for pegagan and 14.10% for pandan. The Minimum Inhibitory Concentration (MIC) of pegagan extract against *Cutibacterium acnes* was 12.5%. Serum formulations consisted of F1 (8% pegagan), F2 (4%:4% combination), and F3 (8% pandan). Physical evaluation showed that all formulations were homogeneous with a slightly viscous liquid consistency. Viscosities were F1 (573.33 cP), F2 (612.00 cP), and F3 (436.00 cP), all fulfilling the requirements with pseudoplastic flow behavior. Spreadability values for F1 (6.00 cm) and F2 (5.46 cm) met the good category criteria, while F3 (7.39 cm) exceeded the standard. Preparations remained stable through 6 freeze-thaw cycles without organoleptic changes, and pH values remained consistent within the range of 5.496–5.847. Well diffusion tests showed that facial serum formulations F1, F2, and F3 did not produce any inhibition zone diameter (0 mm) because the extract concentration in the preparations was below the Minimum Inhibitory Concentration (MIC). The combination facial serum demonstrated good physical stability but had not yet provided inhibitory activity against *Cutibacterium acnes* bacteria at these concentration levels.



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The skin is the body's outermost organ, serving as the primary barrier against pathogens, chemicals, and environmental changes. Facial skin consists of the epidermis, dermis, and subcutis layers, which house pilosebaceous units responsible for

sebum production. While sebum helps maintain moisture, elasticity, and microbial defense, excessive sebum production in the T-zone (forehead, nose, and chin) can obstruct the pilosebaceous ducts, leading to the development of acne (*acne vulgaris*)[1]. Chronic

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inflammation of hair follicles and sebaceous glands triggers the onset of acne (acne vulgaris), which is commonly characterized by the presence of comedones, papules, and pustules [2]. The bacterium primary responsible for the development of acne (acne vulgaris) is *Cutibacterium acnes* [3].

The topical use of clindamycin antibiotics is effective in treating acne caused by bacterial infections. However, long-term use carries the risk of skin irritation, dryness, and bacterial resistance in *Cutibacterium acnes*. Therefore, the use of natural ingredients is highly necessary due to their antibacterial activity, high biocompatibility, low risk of resistance, and minimal side effects [4]. Natural-based facial serums are considered more effective than creams due to their lightweight texture and rapid, uniform distribution of active ingredients. A study by Nabila and Rosmiati (2024) demonstrated that the penetration capability of serums is significantly superior, reaching $12,789.08 \mu\text{g}/\text{cm}^2$ compared to creams, which only achieved $4,325.78 \mu\text{g}/\text{cm}^2$. Through their optimal active delivery efficiency, serum formulations have become the primary choice in current natural cosmetic formulation trends [5]. In line with growing public awareness regarding product safety, the use of natural ingredients in serum formulations continues to expand due to their minimal side effects [6].

Pegagan leaves (*Centella asiatica* L.) are known to contain active compounds such as triterpenoids, saponins, flavonoids, alkaloids, and tannins, which play roles as anti-inflammatory, antibacterial, and antioxidant agents [7]. Pegagan leaf extract (*Centella asiatica* L.) has been proven to exhibit antibacterial effectiveness against *Cutibacterium acnes*, as previously demonstrated [8]. The pegagan leaf extract serum formulation at a concentration of 5% exhibited an average zone of inhibition of 7.68 mm against *Cutibacterium acnes*. While the inhibitory activity at this concentration falls into the moderate category, the study did not determine the Minimum Inhibitory Concentration (MIC), which is essential for identifying the lowest concentration effective in inhibiting bacterial growth

[9]. Additionally, pandan leaves (*Pandanus amaryllifolius* R.) are also known to possess antibacterial activity derived from their flavonoid, alkaloid, and tannin content, as demonstrated in a previous study [10], fragrant pandan leaf extract obtained using 96% ethanol solvent exhibited antibacterial activity against *Cutibacterium acnes* with an MIC₅₀ value of 3.565 mg/mL. Based on the potential of both plants, the combination of pegagan leaf extract and pandan leaf extract is expected to exert a synergistic effect in inhibiting the growth of acne-causing bacteria.

Therefore, this study was conducted to evaluate the antibacterial activity of a facial serum combining pegagan and pandan extracts against *Cutibacterium acnes*, as well as to determine the MIC of pegagan extract. The 8% single concentration and 4%:4% combination were selected based on preliminary formulation feasibility to maintain the serum's physical stability, clarity, and viscosity while delivering the active phytoconstituents. The findings of this study are expected to provide scientific evidence regarding the effectiveness of the combination serum formulation as a natural-based anti-acne product.

Results and Discussion

Extraction and Ethanol Free Test

Maceration of 500 g of crude drug (*simplicia*) using 96% ethanol yielded an extract recovery of 11.75% (58.7661 g) for Pegagan leaves (*Centella asiatica* L.) and 14.10% (70.5044 g) for Pandan leaves (*Pandanus amaryllifolius* R.). Both results met the minimum standard of the Indonesian Herbal Pharmacopoeia [11], which requires not less than 7.3% and 10%, respectively. Qualitative testing of solvent residue using $K_2Cr_2O_7$ and concentrated H_2SO_4 yielded negative results (the solution remained orange), indicating that the ethanol had evaporated completely and was safe for biological testing.

Determination of Minimum Inhibitory Concentration (MIC) Value

The results of the macrodilution test in Mueller Hinton Broth (MHB) medium are shown in **Table 2**. The results indicated that the Pegagan leaf extract at concentrations ranging from 0.39% to 6.25% remained turbid due to the growth of *Cutibacterium acnes*. The turbidity disappeared, and the solution appeared consistently clear at concentrations of 12.5%

and 25%. Thus, the MIC value of Pegagan leaf extract against *Cutibacterium acnes* was determined to be 12.5% (equivalent to 125 mg/mL).

Physical Quality Evaluation of Facial Gel Serum Formulations

The facial gel serum formulations were divided into three test groups, namely F1 (8% Pegagan), F2 (4%:4% combination), and F3 (8% Pandan).

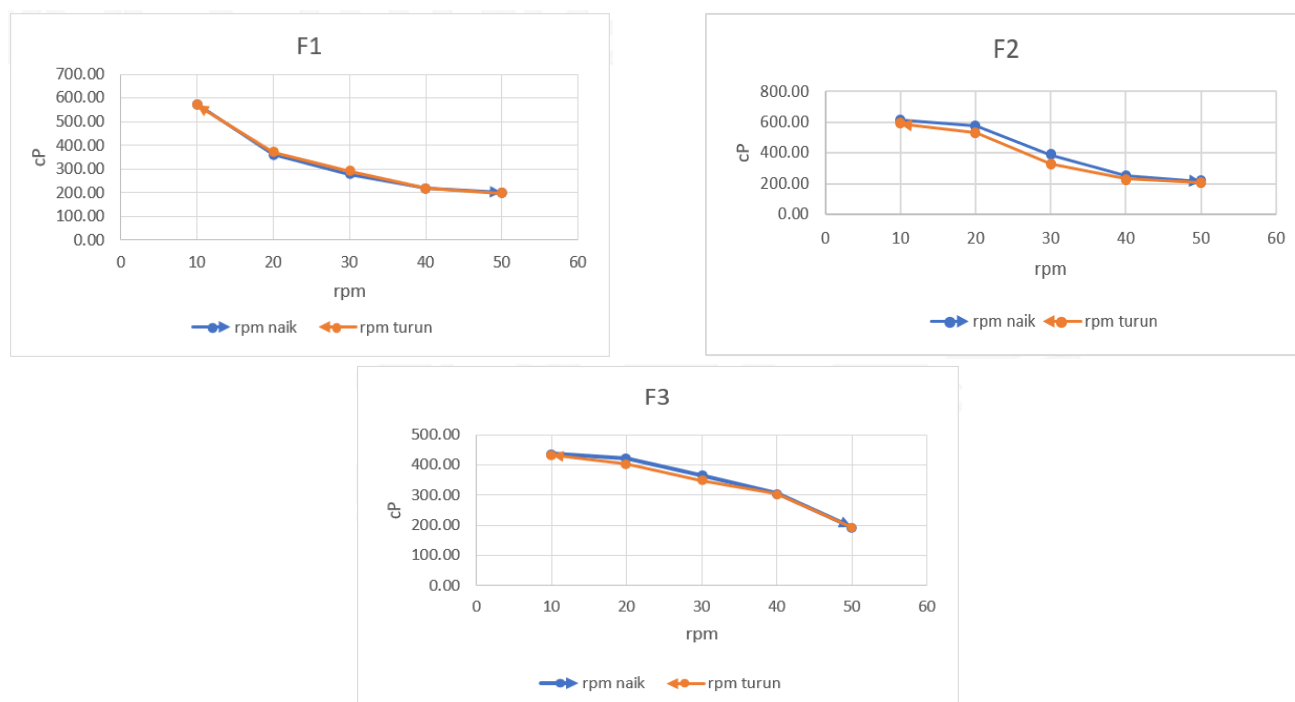


Figure 1. Flow behavior graph

Organoleptic and Homogeneity

All three formulations possessed a physical form of a slightly viscous liquid that was completely homogeneous without any visible coarse particles. The variation in active ingredients influenced the color and aroma: F1 exhibited a dark brownish-green color with a strong herbal Pegagan aroma; F2 displayed a dark green color with a combined Pegagan–Pandan aroma; whereas F3 was green-brownish in color with a dominant aromatic Pandan scent.

Viscosity and Flow Behavior

Viscosity measurements (spindle 2, 10 rpm) showed mean values for F1, F2, and F3 of 573.33 ± 10.07 cP, 612.00 ± 4.00 cP, and 436.00 ± 18.33 cP,

respectively. All formulations met the ideal standard for facial serums (230–1150 cP) [9]. One-Way ANOVA results confirmed a significant difference in viscosity values among the formulations ($p < 0.05$), which was influenced by variations in the active ingredients on the density of the carbomer gel base.

The flow behavior characteristics of the formulations are shown in **Figure 1.**, which illustrates the relationship between rotational speed (rpm) and viscosity. The graph profile indicates that all serum formulations follow a non-Newtonian pseudoplastic (*shear-thinning*) flow behavior, in which the viscosity of the preparation consistently decreases with an increase in spindle rotational speed (from 10 rpm to 50 rpm)[12].

Spreadability

The spreadability diameters of the preparations were 6.00 ± 0.32 cm for F1, 5.46 ± 0.08 cm for F2, and 7.39 ± 0.19 cm for F3. Formulations F1 and the F2 combination met the ideal topical range (5–7 cm), whereas F3 exceeded the standard due to its lower viscosity. *Post-Hoc* Tukey analysis confirmed that the difference in spreadability for F3 was highly significant compared to the other formulations ($p < 0.05$).

Physical Stability Test (Freeze-Thaw)

Testing under extreme conditions for 6 cycles demonstrated excellent physical stability of the preparations. Based on organoleptic evaluation, all formulations (F1, F2, and F3) maintained their original texture, color, and aroma consistency without showing any phase separation. The stability of the preparations was also supported by consistent pH values within the skin-safe range (4.5 to 6.5), with mean changes from the initial cycle to the final cycle as follows: F1 (5.847 to 5.756), F2 (5.568 to 5.496), and F3 (5.743 to 5.670). Statistical analysis using a *Paired Sample T-Test* yielded $p > 0.05$ for all three formulations, confirming that the changes in pH values during testing were not statistically significant.

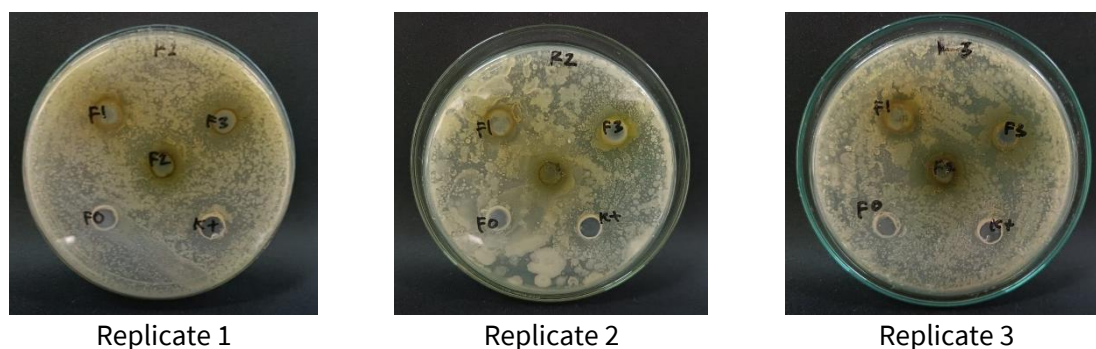


Figure 2. Antibacterial Activity Test of the Serum against *Cutibacterium acnes*

Antibacterial Activity Test

The well diffusion test revealed an inhibition zone diameter of 0 mm across all serum formulations (F1, F2, and F3) the observation of these inhibition zones can be seen in Figure 2. This absence of antibacterial activity occurred because the extract concentrations in the serum (4% and 8%) were below the Minimum Inhibitory Concentration (MIC) threshold of *Centella asiatica* extract (12.5%). Unlike [8] who obtained a 7.68 mm inhibition zone from a 5% *Centella asiatica* serum, and [10] who reported an MIC_{50} of 3.565 mg/mL for *Pandanus amaryllifolius* extract, the extracts in this study required higher concentrations to inhibit *Cutibacterium acnes*, aligning with [13] where pure extract required $\geq 30\%$ to produce inhibition (7.67 ± 0.25 mm). Furthermore, the carbomer hydrogel matrix viscosity (436.00–612.00 cP) entrapped the active phytoconstituents,

retarding their passive diffusion into the agar media, while the 4%:4% combination in F2 produced no synergistic effect to lower the required MIC threshold.

Conclusion

The 96% ethanol extracts of Pegagan leaves and Pandan leaves obtained through maceration were free from solvent residue, making them safe for use. The Minimum Inhibitory Concentration (MIC) value of the single Pegagan leaf extract against *Cutibacterium acnes* was 12.5%. All facial serum formulations (F1, F2, and F3) were proven to be homogeneous, met the ideal physical quality standards for topical preparations, and exhibited good physical and pH stability after 6 cycles of the *freeze-thaw* test. Nevertheless, neither the single nor combined facial serum formulations demonstrated antibacterial efficacy against *Cutibacterium acnes* (0 mm inhibition zone). This was attributed to the

extract concentrations in the serum formulations (4% and 8%) being below the required minimum inhibitory threshold (MIC) value of the extract.

Materials and Methods

Research Method

This laboratory experimental study evaluated the physical characteristics and antibacterial inhibitory activity of facial serum formulations containing single and combined extracts of pegagan leaves (*Centella asiatica* L.) and pandan leaves (*Pandanus amaryllifolius* R.). The study was conducted at the Pharmacy Laboratory of Ma Chung University, Malang, from January to June 2026.

Tools and Materials

The equipment used throughout this study included primary instruments such as a rotary evaporator, water bath, analytical balance, pH meter, Brookfield viscometer equipped with spindle number 2, a custom spreadability testing apparatus, glass slides, and cover slips. The microbiology and sterilization laboratory equipment utilized comprised a laminar air flow cabinet, autoclave, incubator, candle jar, sterile inoculating loop, UV-Vis spectrophotometer, vortex mixer, vernier caliper, cork borer (or sterile pipette tips), micropipettes with corresponding tips, Whatman No. 1 filter paper, and supporting laboratory glassware.

The materials used consisted of powdered simplicia of pegagan leaves (*Centella asiatica* L.) and pandan leaves (*Pandanus amaryllifolius* R.) obtained from UPT Laboratorium Herbal Materia Medica Batu, 96% ethanol solvent, and alcohol-free test reagents comprising 1% potassium dichromate ($K_2Cr_2O_7$) and concentrated sulfuric acid (H_2SO_4). The excipient components for the serum base included carbomer, propylene glycol, Tween 80, glycerin, methylparaben, triethanolamine (TEA), and distilled water. Meanwhile, materials for microbiological testing comprised pure cultures of *Cutibacterium acnes* strain ATCC 6919, Mueller Hinton Agar (MHA) medium, Mueller Hinton Broth (MHB) medium, 0.9% NaCl solution, 1% barium chloride ($BaCl_2$) solution and 1% H_2SO_4 for the 0.5

McFarland standard, Cindala gel 1% (positive control), and serum base without extract (negative control).

Extract Preparation and Ethanol-Free Test

A total of 500 grams of powdered simplicia from pegagan leaves and pandan leaves were individually macerated using 96% ethanol solvent (1:10) for 3 days, followed by remaceration for 2 days. The filtrate was concentrated using a rotary evaporator at 40°C to obtain a semi-solid extract, then further evaporated on a water bath until a thick extract was obtained. The ethanol-free test was performed using a 1% potassium dichromate ($K_2Cr_2O_7$) solution and concentrated H_2SO_4 , where a constant orange color indicated that the extract was alcohol-free [14].

Bacterial Preparation and Antibacterial Activity Test

The subculture of *Cutibacterium acnes* was rejuvenated on MHA slants (37°C, 48–72 hours) under anaerobic conditions using a candle jar [15]. Bacterial colonies were suspended in 0.9% NaCl solution to match the 0.5 McFarland standard (absorbance of 0.08–0.13 at 625 nm) [16], [17]. The antibacterial activity of the serum formulations was evaluated using the well diffusion method on MHA plates inoculated with the test bacteria. A volume of 100 μ L of each sample (F1, F2, F3, positive control, and negative control) was loaded into the wells, incubated for 72 hours at 37°C, and the zone of inhibition was measured using a vernier caliper [9].

Determination of the MIC Value of Pegagan Extract

The Minimum Inhibitory Concentration (MIC) value was determined using the tube macrodilution method in MHB medium with a series of pegagan extract concentrations ranging from 25% to 0.39% (Khofifah et al., 2025). Each tube was inoculated with a bacterial suspension of *Cutibacterium acnes* (1.5×10^8 CFU/mL) and incubated at 37°C for 18–24 hours. The MIC value was visually determined as the lowest concentration that appeared clear [9]. The flowchart for the MIC determination procedure is presented in **Figure 3**.

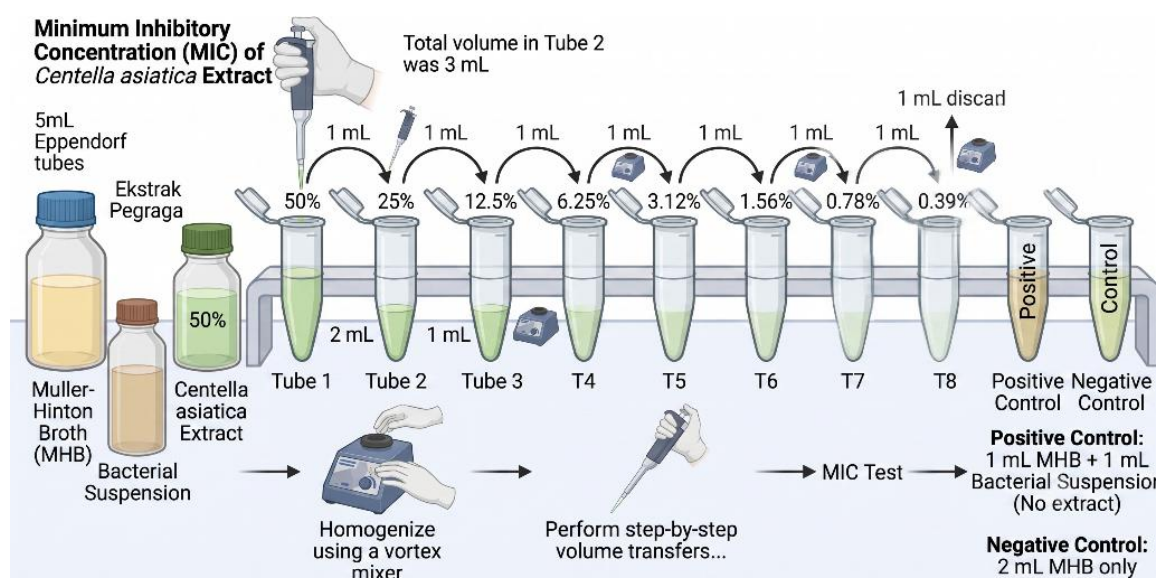


Figure 3. Illustration of MIC determination of pegagan extract (*Centella asiatica* L.)

Facial Serum Formulation and Preparation

The facial serum was prepared in three formulations: F1 (8% pegagan extract), F2 (combination of 4% pegagan + 4% pandan), and F3 (8% pandan extract) [8], [10]. The detailed composition of each formulation is shown in **Table 1**. The serum base consisted of 0.5% carbomer, 15% propylene glycol, 2%

tween 80, 1% glycerin, 0.1% methylparaben, 0.25% TEA, and distilled water *ad* 100%. Carbomer was swollen in water as the gel base, mixed with methylparaben previously dissolved in propylene glycol, and then the active ingredients (extracts) were added gradually. TEA was added to neutralize the pH until a homogeneous gel texture was formed.

Table 1. Combination Serum Formulations of Pegagan Leaf Extract (*Centella asiatica* L.) and Pandan Leaf Extract (*Pandanus amaryllifolius* R.)

Ingredients	Function	Concentration (%)		
		F1	F2	F3
Pegagan Leaf Extract	Active Ingredient [8]	8	4	-
Pandan Leaf Extract	Active Ingredient [10]	-	4	8
Carbomer	Serum base	0.5	0.5	0.5
Propylene glycol	Cosolvent	15	15	15
Tween 80	Surfactant	2	2	2
Glycerin	Humectant	1	1	1
Methylparaben	Preservative	0.1	0.1	0.1
TEA	Alkalizing agent	0.25	0.25	0.25
Aquadest	Solvent	Ad 100	Ad 100	Ad 100

Physical Quality Evaluation of Formulations

Organoleptic and Homogeneity Tests

Organoleptic evaluation was carried out by direct visual inspection, observing the consistency,

color uniformity, and odor of the prepared facial serum formulations [9]. Meanwhile, homogeneity was evaluated by spreading a small amount of serum sample evenly onto a glass slide, covering it with a cover slip, and observing it under bright light to detect

the presence of coarse particles or unblended granules [18].

Viscosity and Flow Behavior Tests

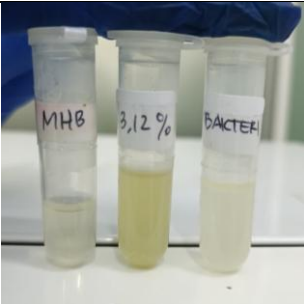
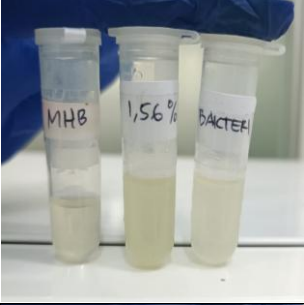
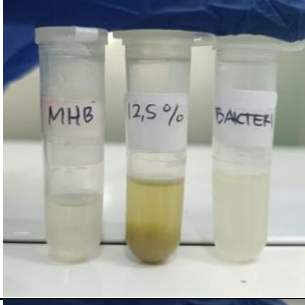
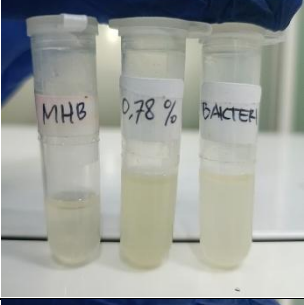
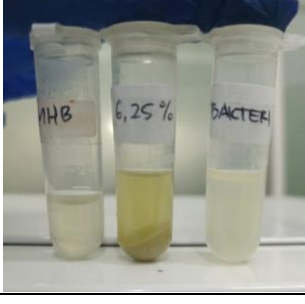
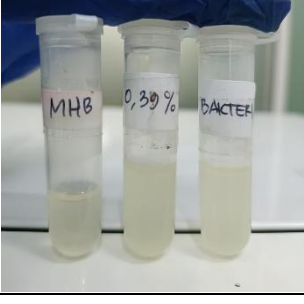
Viscosity measurement of the formulation was performed using a Brookfield viscometer equipped with spindle number 2 at multi-tier rotational speeds [8]. The test was conducted by measuring variations in ascending and descending rotational speeds ranging from 10 to 50 rpm [8]. The obtained viscosity values were then plotted into a graph showing the relationship between rpm and

viscosity to determine the flow behavior profile of the gel serum formulation [12].

Spreadability Test

The spreadability test began by weighing 0.5 grams of the serum sample, placing it precisely in the center of the glass plate of the spreadability testing apparatus, and applying an additional load of a 50 gram weight on top [19]. The gel serum formulation was allowed to stand for 1 minute until the spread area reached a constant state, after which the spread diameter was measured using a ruler from three different directions [19]

Table 2. Minimum Inhibitory Concentration (MIC) Test Results of Pegagan Leaf Extract (*Centella asiatica* L.)

Description	Observation Result	Description	Observation Result
MHB (Negative Control) and <i>Cutibacterium acnes</i> Bacteria (Positive Control)		Pegagan leaf extract concentration of 3.12% (v/v) Result: Turbid	
Pegagan leaf extract concentration of 25% (v/v) Result : Clear		Pegagan leaf extract concentration of 1.56% (v/v) Result: Turbid	
Pegagan leaf extract concentration of 12.5% (v/v) Result : Clear		Pegagan leaf extract concentration of 0.78% (v/v) Result: Turbid	
Pegagan leaf extract concentration of 6.25% (v/v) Result: Turbid		Pegagan leaf extract concentration of 0.39% (v/v) Result: Turbid	

Physical Stability Test (Freeze-Thaw)

The physical stability of the serum formulation against thermal stress was evaluated using a cycling test method for 6 cycles [20]. Each testing cycle consisted of storing the serum formulation at a cold temperature of 4°C for 24 hours, after which it was immediately transferred to an incubator at a hot temperature of 40°C for the next 24 hours [20]. Physical parameters evaluated following the completion of the 6th cycle included organoleptic stability and changes in the pH value of the formulation [20].

Antibacterial Activity Test Using Well Diffusion Method

Testing was carried out using the well diffusion method on MHA media that had been uniformly inoculated with the bacterial suspension [9]. The agar wells were filled with 100 μ L of test samples, consisting of serum formulations F1, F2, and F3, negative control (serum base without extract), and positive control (Cindala clindamycin 1% gel) [8], [10]. After incubation in a candle jar at 37°C for 72 hours, antibacterial activity was determined by measuring the diameter of the inhibition zone using a vernier caliper [9]

Data Analysis

Organoleptic and preparation homogeneity data were analyzed descriptively. Quantitative data regarding viscosity, spreadability, and pH stability were tested for normality (Shapiro-Wilk) and homogeneity (Levene) assumptions [6]. Significance testing among formula groups was evaluated using One-Way ANOVA ($\alpha = 0.05$) followed by a Tukey HSD *post-hoc* test [21],[8]. Meanwhile, the stability of pH values from the initial to the final cycle was analyzed using a Paired Sample *T-Test*. All quantitative data were processed using IBM SPSS software version 26.

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