

Formulation and Evaluation of Spirulina-Based Gel with Varying Carbopol Concentrations for Anti-Acne Activity against *Staphylococcus epidermidis*

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Abstract

Spirulina platensis is a blue-green microalga known for its antibacterial properties, offering potential as a natural alternative in acne treatment. Acne vulgaris, often caused by *Staphylococcus epidermidis*, requires effective topical solutions. Gels are favored for their non-greasy texture, ease of application, and good skin absorption. This study aimed to formulate and evaluate anti-acne gels containing 25% *Spirulina* extract with varying Carbopol concentrations (0.5%, 1%, 1.5%). Each formulation was assessed for physical properties, stability over 21 days, and antibacterial activity against *S. epidermidis*. All gel formulations met quality standards for pH, homogeneity, viscosity, spreadability, and adhesiveness. The gel with 0.5% Carbopol (F1) showed the best spreadability, ideal viscosity, and good adhesiveness, along with the highest antibacterial activity, exhibiting an inhibition zone of 16.5 mm—comparable to tetracycline. In conclusion, *Spirulina*-based gel with 0.5% Carbopol offers an effective, stable, and natural anti-acne option. These findings highlight the potential of *Spirulina* as a bioactive agent in topical formulations and encourage further research for clinical applications in acne management.

Keywords: antibacterial gel; gel formulation; natural antimicrobia; *Spirulina platensis*; *Staphylococcus epidermidis*.

INTRODUCTION

Skin, the body's largest organ, is the first line of defense against environmental threats, including microbial pathogens. However, it is not immune to infections, as seen in cases of acne vulgaris a chronic inflammatory disorder affecting the pilosebaceous unit (Novaryatiin et al., 2024). Acne is commonly triggered by the proliferation of bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* in combination with excessive sebum production and clogged follicles (Du et al. 2021). This condition is particularly prevalent among adolescents and young adults, negatively affecting self-esteem and quality of life (Sidharta, Malaha, and Mursyanti 2021). Therefore, developing safe and effective topical treatments is critical in acne management. *Spirulina*, a blue-green microalga from the genus *Arthrospira*, has garnered attention for its rich biochemical composition, including essential fatty acids, phycocyanin, chlorophyll, proteins, vitamins, and minerals (Anvara & Nowruzib, 2021). Several studies highlight its therapeutic potential, particularly its antibacterial and anti-inflammatory effects (Kumar et al., 2022). Notably, its effectiveness against *Staphylococcus epidermidis* a common acne-associated bacterium has

been demonstrated through various antimicrobial assays (Sidharta et al., 2021). To enhance its application, spirulina can be formulated into a topical gel, a preparation type known for its ease of application, quick absorption, and aesthetically pleasing texture (Severn & Horswill, 2023). Gels also offer the advantage of faster drug release compared to creams and ointments, which contribute to the rapid drying of acne lesions (Sugiyarto, 2024).

Despite the known antimicrobial potential of spirulina, limited formulations have been developed to harness its benefits in a stable and effective gel form for acne treatment. The main research problem is optimizing spirulina-based gel formulations that maintain antibacterial activity, physical stability, and patient acceptability. The general solution is to investigate the formulation of spirulina extract into a topical gel using suitable gelling agents, then evaluate its antibacterial effectiveness and physical stability through standardized methods.

The use of gel formulations in dermatological therapy is well-supported due to their favorable characteristics such as pseudoplastic flow, high water content, and ease of spreadability (Nofita, Sofyan, and Saputra Yasir, 2024). Among various gelling agents, carbopol

(Carbomer) is widely preferred for its ability to produce transparent gels and stabilize active ingredients. It is typically used at concentrations between 0.5–1% and requires the addition of triethanolamine (TEA) to neutralize its acidity and form a stable gel matrix (Sharon, Yuliet, and Sulistiana, 2023). Such formulations are non-greasy and quickly absorbed, ideal for acne-prone skin. To assess antimicrobial performance, the disc diffusion method is commonly employed. This method allows for qualitative measurement of antibacterial activity by observing inhibition zones around paper discs impregnated with the test compound (Hidayati, Amanda, and Setiawansyah, 2024). *Spirulina*'s extract has demonstrated inhibitory activity against several gram-positive bacteria, including *Staphylococcus epidermidis* and *S. aureus*, due to its bioactive compounds like phycocyanin and phenolic acids (Mulyani, Kusumawardani, and Pangesti, 2022). Recent studies also reveal spirulina's efficacy as a topical agent, showing significant antibacterial activity and reduction in lesion size in in vivo and in vitro models (Yasir et al., 2023).

Several studies have explored the bioactivity of spirulina, focusing on its antioxidant, anti-inflammatory, and antimicrobial effects. For instance, Wils et al. 2021 demonstrated spirulina's protective properties against oxidative stress, while Pez Jaeschke et al. 2021 confirmed its antimicrobial action in topical applications. Additionally, gel formulations containing herbal extracts have been widely developed for skin-related diseases (Zhang et al., 2025); however, their long-term stability and antimicrobial consistency remain inconsistent. Most spirulina formulations focus on oral or dietary supplements rather than topical delivery systems tailored for acne management. Moreover, while carbopol-based gels are established in cosmetic and pharmaceutical fields, the interaction between spirulina's bioactive compounds and carbopol matrices under varying storage conditions remains underexplored. Accelerated stability studies are essential to predict the shelf life of such products. However, limited research exists on the stability of spirulina-containing gels, especially under elevated temperature conditions simulating tropical climates (I Ragusa et al., 2021). This reveals a crucial research gap in developing and evaluating a spirulina-based gel with proven antibacterial activity and physical stability suitable for practical use in acne therapy.

This study aims to formulate and evaluate a spirulina-based gel with antibacterial activity against *Staphylococcus epidermidis*, focusing on the physical characteristics and antimicrobial efficacy of the formulation. The novelty lies in combining spirulina extract with carbopol gel base to produce a stable, anti-acne topical preparation, validated through disc diffusion assay and accelerated stability testing. The scope of the study encompasses the extraction of spirulina, formulation of topical gel using carbopol and TEA,

evaluation of physical properties (viscosity, pH, spreadability), and assessment of antibacterial activity and stability under various storage conditions.

MATERIALS AND METHODS

This study aimed to develop and evaluate a *Spirulina*-based anti-acne gel by extracting proteins from *Spirulina platensis*, formulating a topical preparation, and testing its physical properties and antibacterial activity against *Staphylococcus epidermidis*. The research design involved systematic extraction, purification, gel formulation, microbial testing, and analysis to assess the product's effectiveness and stability. The materials, instruments, and procedures used in the study are described below.

Materials

The main active ingredient in this study was *Spirulina platensis* powder. For protein extraction, the powder was mixed with distilled water in a 1:25 ratio and kept at 20–25°C for 24 hours, shielded from light using aluminum foil. Ammonium sulfate ((NH₄)₂SO₄) was used for protein precipitation, and 0.005 M sodium phosphate buffer (pH 7.0) was used to redissolve the precipitate. The protein content was confirmed by biuret and ninhydrin tests, indicated by violet and blue-purple coloration, respectively (Prete et al., 2024). For the gel formulation, three different concentrations of Carbopol (0.5%, 1%, and 1.5%) were used, while the concentration of *Spirulina* was kept constant at 25%. Other components included triethanolamine (TEA), propylene glycol (PG), methylparaben, glycerin, and distilled water. Antibacterial testing was conducted using *Staphylococcus epidermidis*, cultured on Mannitol Salt Agar (MSA) and tested on Mueller-Hinton Agar (MHA). Tetracycline was a positive control, and a blank gel was a negative control.

Instrumentation

Several instruments were employed throughout the study. A sonicator operating at 40 kHz and a centrifuge (1000 rpm and 4000 rpm settings) were used for protein extraction and purification. A Brookfield viscometer with spindle number 7 measured gel viscosity. A vernier caliper was used to measure inhibition zones. An autoclave sterilized non-heat-sensitive equipment at 121°C for 15 minutes, while alcohol and flame were used for sterilizing heat-sensitive tools. Additional tools included analytical balances, incubators, glassware, and Petri dishes.

Procedure

1) Protein Extraction and Identification

Spirulina platensis powder was macerated in distilled water for 24 hours at room temperature (20–25°C), shielded from light. The solution was sonicated at 40

kHz for 45 minutes and centrifuged at 1000 rpm for 20 minutes. The resulting supernatant, containing water-soluble proteins, was purified by adding 100 mg of ammonium sulfate and stirring for 120 minutes. It was then centrifuged at 4000 rpm for 15 minutes. The blue precipitate was dissolved in 0.005 M sodium phosphate buffer (pH 7.0). Protein presence was confirmed through biuret and ninhydrin tests (Li et al., 2025).

2) Gel Formulation

Three formulations were prepared by varying the Carbopol concentration (FI = 0.5%, FII = 1%, FIII = 1.5%), while maintaining the *Spirulina* content at 25%. The gel was formulated by dissolving Carbopol in warm distilled water, followed by the sequential addition of TEA, PG, methylparaben, *Spirulina*, and glycerin. The final mixture was adjusted to 100% with distilled water and stirred until homogeneous. (Table 1) presents the composition of each formulation.

Table 1. Anti-Acne Gel Formulations with *Spirulina*.

Material	Formula			
	FI %	FII %	FIII %	Control - %
Serbuk <i>Spirulina platensis</i>	25	25	25	-
Carbopol	0,5	1	1,5	0,5
TEA	1	1	1	1
Propilen glikol	10,0	10,0	10,0	10,0
Metil paraben	0,18	0,18	0,18	0,18
Gliserin	15	15	15	15
Destiled water	ad 100	ad 100	ad 100	ad 100

Notes: F1: Carbopol 0.5% combination; F2: Carbopol 1% combination; F3: Carbopol 1.5% combination; Control -: Negative control; Control +: Tetracycline disk

3) Physical Evaluation

The gels were tested for organoleptic properties (color, smell, consistency), pH, homogeneity, viscosity, spreadability, and adhesiveness. Viscosity was measured with a Brookfield viscometer over 5 seconds. Spreadability was evaluated by placing 1 g of gel between two glass plates under weights ranging from 5 to 250 g and measuring the spread diameter. Adhesiveness was measured by determining the time it took for two adhered plates to separate under a 1 kg load dropped from an 80 g weight.

4) Stability Testing

Stability was assessed using a six-cycle freeze-thaw test, alternating storage at 4°C and 40°C for 24 hours each. After each cycle, the gel's appearance, pH, and viscosity were evaluated to determine stability (Hoskin et al., 2023).

5) Antibacterial Testing

The agar disk diffusion method was used to evaluate antibacterial activity. *Staphylococcus epidermidis* suspensions were adjusted to 1.5×10^8 CFU/mL using a 0.5 McFarland standard and spread onto MHA plates. Paper soaked in gel samples was placed on the agar, along with tetracycline and blank gel disks as controls. Plates were incubated at 37°C for 24 hours. Inhibition zones were measured in millimeters using a vernier caliper. Identification of *S. epidermidis* was confirmed by colony morphology on MSA, Gram staining, catalase, and coagulase tests.

6) Data Collection and Analysis

All experiments were performed in triplicate. Quantitative data such as inhibition zone diameters, viscosity, spreadability, and adhesiveness were recorded and averaged. Data were analyzed descriptively and compared across formulations. Results were interpreted to assess formulation effectiveness and consistency over time.

RESULTS AND DISCUSSION

The preliminary tests conducted on *Spirulina* involved evaluating its powdered and extracted forms at concentrations of 20%, 25%, and 30%. The *Spirulina* powder, being dry, required no additional testing, whereas the extract was characterized by a precipitate resulting from the extraction process. Both forms were utilized for antibacterial testing against *Staphylococcus epidermidis* (Iyer, Raut, and Dasgupta, 2021; Saputri et al., 2024). To determine the presence of protein, the Biuret and Ninhydrin tests were employed. Positive results were observed both the *Spirulina* powder and extract. The Biuret test produced a color shift from green to light purple, and the Ninhydrin assay turned from green to dark purple, confirming the presence of proteins (Meray, Utami, and Nurazizah, 2024).

The antibacterial activity of *Spirulina* powder and extract was assessed using Mueller Hinton Agar (MHA) media and the disc diffusion method. After 16–18 hours of incubation, inhibition zones were measured, with

results indicating effective antibacterial activity. The zones ranged from 10.9 mm to 16.5 mm, with tetracycline as a positive control and 0.005 M sodium phosphate buffer (pH 7.0) as a negative control (D'Angelo Costa & Maia Campos, 2024). The results of the antibacterial activity of Spirulina powder and extract are shown in (Table 2).

Table 2. Antibacterial activity results of Spirulina powder and extract.

Formula	Inhibition Zone (mm)		Range
	Spirulina Extract	Spirulina Powder	
Control +	20.8±0.13	21.1±0.21	0.3%
Control -	0	0	0%
20%	10.9±0.13	10.9±0.13	0%
25%	12.3±0.06	10.9±0.13	0.5%
30%	16.5±0.11	15.1±0.19	1.4%

Information:

Control + : Tetracycline antibiotic discs

Control - : Sodium phosphate buffer 0.005 M (pH range-7.0).

Evaluation of the Spirulina gel formulations involved testing their physical quality. Organoleptic analysis revealed that all three formulas were dark green, aromatic, and semi-solid on day one but faded to light green by day 21 due to the thermal instability of phycocyanin (Irene Ragusa et al., 2021). The organoleptic results of the Spirulina gel formulation can be seen in (Table 3).

Table 3. Organoleptic results of Spirulina gel formulations.

Time	Formula	Organoleptic Test	Time	Organoleptic Test
Day 1	FI	Dark green, Spirulina odor, gel	Day 21	Light green, strong Spirulina odor, gel
Day 1	FII	Dark green, Spirulina odor, gel	Day 21	Light green, strong Spirulina odor, gel
Day 1	FIII	Dark green, Spirulina odor, gel	Day 21	Light green, strong Spirulina odor, gel
Day 1	Control -	Dark green, Spirulina odor, gel	Day 21	Light green, strong Spirulina odor, gel

Information:

FI: Formula I with Carbopol concentration of 0.5%

FII: Formula II with Carbopol concentration of 1%

FIII: Formula III with Carbopol concentration of 1.5%

Control -: Negative control with Carbopol concentration of 0.5%

The pH values remained within the skin-friendly range of 4 to 7 throughout the 21-day observation. The slightly acidic pH was attributed to Spirulina's protein and essential amino acid content (Wiratantri, Peranginangin, and Sulaiman, 2024). The pH value of the Spirulina gel formulation can be seen in (

Table 4).

Table 4. pH values of Spirulina gel formulations.

Time	Formula	pH	Time	pH
Day 1	FI	5.58±0.33	Day 21	5.19±0.03
Day 1	FII	4.81±0.22	Day 21	4.27±0.01
Day 1	FIII	5.00±0.10	Day 21	4.07±0.01
Day 1	K-	6.01±0.15	Day 21	5.14±0.01

Homogeneity testing confirmed that all formulations maintained uniform dispersion without separation during the study period, indicating a stable gel matrix (Setyawaty, Gustin, and Setiyabudi, 2021). The results of the homogeneity of the Spirulina gel formulation can be seen in (Table 5).

Table 5. Homogeneity of Spirulina gel formulations.

Time	Formula	Homogeneity	Time	Homogeneity
Day 1	FI	Homogeneous	Day 21	Homogeneous
Day 1	FII	Homogeneous	Day 21	Homogeneous
Day 1	FIII	Homogeneous	Day 21	Homogeneous
Day 1	Control -	Homogeneous	Day 21	Homogeneous

Viscosity increased proportionally with higher Carbopol concentrations and Spirulina addition, likely due to the molecular interactions between the polymer matrix and Spirulina compounds (Chwil et al., 2024). The results of the viscosity of the Spirulina gel formulation can be seen in (Table 6).

Table 6. Viscosity of Spirulina gel formulations.

Time	Formula	Viscosity (cP)	Time	Viscosity (cP)
Day 1	FI	46.31±1.23	Day 21	35.95±0.76
Day 1	FII	58.78±3.64	Day 21	48.26±2.22
Day 1	FIII	65.42±0.62	Day 21	57.07±8.49
Day 1	Control -	41.86±0.78	Day 21	39.77±0.33

Spreadability values fell within an ideal range for topical gels, and a slight decrease in spreadability with increasing Carbopol concentration was noted (Safitri, Nawangsari, and Febrina, 2021) The spreadability of the Spirulina gel formulation can be seen in (Table 7).

Table 7. Spreadability of Spirulina gel formulations.

Time	Formula	Spreadability (cm)	Time	Spreadability (cm)
Day 1	FI	5.28±0.09	Day 21	5.24±0.08
Day 1	FII	5.46±0.06	Day 21	5.30±0.09
Day 1	FIII	5.68±0.10	Day 21	5.52±0.27
Day 1	Control -	5.22±0.03	Day 21	5.22±0.04

Adhesion testing showed good retention time across all formulas, with FIII exhibiting the highest adhesion time. All formulations exceeded the minimum threshold of 4 seconds (Ikeda, Sydney, and Sydney, 2022). The

adhesion results of the Spirulina gel formulation can be seen in Table 8.

Table 8. Adhesion of Spirulina gel formulations.

Time	Formula	Adhesion Time (s)	Time	Adhesion Time (s)
Day 1	FI	5.92±0.29	Day 21	4.92±0.34
Day 1	FII	5.50±0.81	Day 21	6.41±0.45
Day 1	FIII	6.78±0.17	Day 21	5.58±0.67
Day 1	Control -	5.50±0.55	Day 21	5.15±0.58

Bacterial identification confirmed the presence of *S. epidermidis*. Macroscopic observations on mannitol salt agar (MSA) indicated no mannitol fermentation, as seen in the red-black colony color, verifying species identity. The results of macroscopic observations can be seen in (Figure 1).



Figure 1. Macroscopic identification of *S. epidermidis*.

Further validation was performed through Gram staining, where microscopic observation confirmed Gram-positive cocci in clusters, consistent with the morphology of *S. epidermidis*. The results of microscopic observations can be seen in Figure 2.

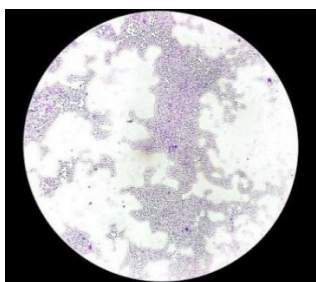


Figure 2. Microscopic identification.

The catalase and coagulase tests supported these results. Positive catalase activity was seen through bubble formation, and clumping in the coagulase test confirmed enzyme presence. The results of the catalase and coagulase tests can be seen in Figure 3.

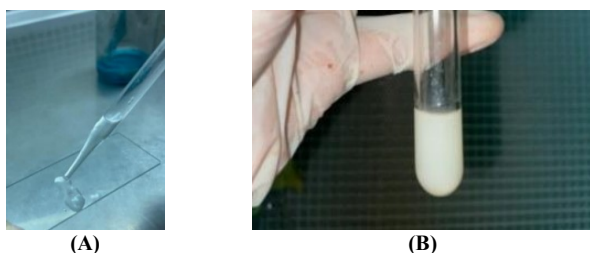


Figure 3. (A) Catalase test and (B) Coagulase test

Additional bacterial analysis through the IMViC test revealed negative results for indole, methyl red, and citrate, but a positive result for Voges-Proskauer, providing biochemical evidence to support the bacterial classification. The IMViC *S. epidermidis* test results can be seen in Table 9.

Table 9. IMViC test results of *S. epidermidis*.

Test	Result
Indole	Negative
Methyl Red	Negative
Voges-Proskauer	Positive
Citrate	Negative

Moreover, the Triple Sugar Iron Agar (TSIA) test showed a red slant and yellow butt without gas or H_2S production, further confirming the identity of *S. epidermidis*. The results of the TSIA *S. epidermidis* test can be seen in Table 10.

Table 10. TSIA test results of *S. epidermidis*.

cha	Interpretation
Red slant / Yellow butt	Glucose fermentation only
Gas	Negative
H_2S	Negative

Collectively, these results demonstrate that Spirulina-based gel formulations are effective against *S. epidermidis*, with both powdered and extracted forms showing antibacterial activity. The presence of proteins, and favorable physical characteristics such as appropriate pH, viscosity, spreadability, and adhesion, further supports the formulation's potential for topical application. This aligns with previous findings on Spirulina's antimicrobial and biochemical properties, reinforcing its value in pharmaceutical and cosmetic products. However, a key limitation observed was the instability of phycocyanin, which affects product aesthetics over time. Furthermore, the current study only assessed in vitro activity, necessitating future in vivo studies to confirm efficacy. Future work should aim to improve pigment stability, assess longer-term physical properties under varying storage conditions, and investigate synergistic combinations of Spirulina with other natural actives for enhanced antimicrobial activity.

CONCLUSION

This study successfully formulated *Spirulina*-enriched anti-acne gels using varying concentrations of Carbopol (0.5%, 1%, and 1.5%) and evaluated their physical properties and antibacterial activity against *Staphylococcus epidermidis*. All formulations met acceptable standards for organoleptic characteristics, pH, viscosity, spreadability, and homogeneity over 21 days.

The first formulation (FI, 0.5% Carbopol) demonstrated the best balance of physical characteristics—high spreadability, stable pH, and good adhesiveness—while also exhibiting the highest antibacterial activity, with inhibition zones reaching up to 16.5 mm, indicating *Spirulina*'s significant antimicrobial potential. Stability testing confirmed that all formulations maintained structural integrity under accelerated conditions. The findings suggest *Spirulina platensis* is a promising natural agent for topical antibacterial applications, particularly in managing acne caused by *S. epidermidis*. The gel formulation not only preserves the bioactivity of *Spirulina* but also ensures acceptable physicochemical properties essential for consumer use. This supports the viability of *Spirulina* as an eco-friendly alternative to synthetic agents in dermatological preparations. Further research should investigate the clinical efficacy of *Spirulina* gel formulations in vivo, including human trials for acne treatment. Optimization of formulation for enhanced stability, particularly under tropical climate conditions, is recommended. Additionally, future studies could explore the synergistic potential of *Spirulina* with other natural antimicrobials and assess long-term storage behavior and consumer acceptability.

Competing Interests: The authors declare that there are no competing interests.

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