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Anti-microbial Activity of Sodium Decanoyl/Dodecanoyl Lactylate Against Malassezia furfur and Cutibacterium Acnes

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1. Introduction

Surfactants play a crucial role in cosmetic formulations due to their ability to interact with both oil and water [1]. This dual compatibility makes them widely utilized for their cleansing, foaming, and emulsifying properties. Recently, growing public awareness of safety, environmental, and sustainability issues has led to an increasing demand for natural ingredients that retain effective efficacy in cosmetics. This trend has prompted the introduction of various natural raw materials for emulsification, cleansing, and other functions, promoting the development of greener consumer products. Consequently, "edible cosmetics" have gained significant popularity in the market.

Malassezia furfur and Cutibacterium acnes are two microorganisms commonly associated with dermatological conditions. Malassezia furfur is a yeast linked to dandruff and seborrheic dermatitis, while Cutibacterium acnes is a bacterium associated with acne vulgaris [2, 3]. Both conditions affect a significant portion of the population, underscoring the need for effective and gentle treatment. Currently, many anti-dandruff and anti-acne products rely on synthetic and petrochemical-derived ingredients, raising concerns regarding skin sensitivity and environmental impact.

Sodium Decanoyl/Dodecanoyl Lactylate is traditionally utilized in the food industry for its emulsifying and solubilizing properties [4, 5]. It serves as an emulsifier in bakery products, stabilizing emulsions in doughs and batters, thereby enhancing texture and shelf life. However, its potential applications in cosmetic formulations remain largely unexplored. Additionally, the combined anti-microbial efficacy against Malassezia furfur and Cutibacterium acnes has not been thoroughly investigated.

This paper focuses on the anti-microbial activity and emulsification properties of a mixture of Sodium Decanoyl/Dodecanoyl Lactylate in cosmetic applications. Our research reveals a remarkably low minimum inhibitory concentration (MIC) against fungi, a significant finding given that many natural ingredients typically have limited antifungal effectiveness. Notably, this compound demonstrated exceptional performance in reducing Malassezia furfur, making it a

promising and cost-effective natural active ingredient for anti-dandruff formulations. Additionally, a low MIC was also observed against *Cutibacterium acnes*, indicating that this compound may serve as a valuable ingredient for anti-acne formulations.

Traditional anti-dandruff and anti-acne active ingredients are often synthetic, water-insoluble, and can have toxicity issues. In our experiments, we identified a crucial process of adjusting the pH to a suitable level, which resulted in a transparent solution when dissolved in water. In addition to its excellent antimicrobial properties, Sodium Decanoyl/Dodecanoyl Lactylate exhibited outstanding foaming and foam-stabilizing capabilitie. Given its significant antimicrobial activity against *Malassezia furfur* and *Cutibacterium acnes*, this compound presents itself as a mild, naturally derived, and water-soluble active ingredient suitable for modern cosmetic formulations.

2. Materials and Methods

2.1 Materials

Sodium Decanoyl/Dodecanoyl Lactylate samples (Batch numbers: 25394, 24599, and 25752, Hydrior AG, Switzerland; hereafter referred to as Sample A) were prepared for this study. All culture media were obtained from Qingdao Hope Biotechnology, China, and included the following: Nutrient Broth (NB), Nutrient Agar (NA), Cation-Adjusted Mueller–Hinton Broth (CAMHB), Tryptic Soy Broth (TSB), Reinforced Clostridium Medium (RCM), YM Broth, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), Modified Dixon (mDixon), and Tryptic Soy Agar (TSA). The microbial strains used in the study included *Escherichia coli* (Migula) Castellani and Chalmers (ATCC 8739; *E. coli*), *Staphylococcus aureus* (ATCC 23235; *S. aureus*), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC 15442; *Pa*), *Cutibacterium acnes* Scholz and Kilian (ATCC 6919; *C. acnes*), *Candida albicans* (Robin) Berkhout (ATCC 10231; *C. albicans*), *Aspergillus brasiliensis* Varga et al. (ATCC 16404; *A. brasiliensis*), and *Malassezia furfur* (Robin) Baillon (ATCC 14521; *M. furfur*). All microbial strains were purchased from the American Type Culture Collection (ATCC) and stored as freeze-dried cultures. Jojoba oil, refined olive oil, mineral oil, and caprylic/capric triglyceride were sourced from Ceratec Sarl, the Natura-tec division, France. Equipment used included Sepiplus 400 from Seppic, a homogenizer and blender from IKA, and a Ross-Miles Foam Tester from the Beijing Glass Group Company.

2.2 Methods

2.2.1 pH adjustment of sample solution

The critical step in achieving transparency for Sample A in water is to first adjust the pH. The samples were dissolved in Milli-Q water at a 1:10 (w/w) ratio and stirred until fully dissolved. They were then hydrolyzed using a 30% NaOH solution, and the mixtures were vortexed until the aqueous solution became clear. The pH of the sample solutions was adjusted to 5.0, 5.5, 6.0, 6.5, and 7.0 using a 30% citric acid solution. The sample solutions were stored in a refrigerator at 4°C.

2.2.2 Antimicrobial activity

To assess the inhibitory efficacy of Sample A against various bacteria or fungi, the broth microdilution method was employed. First, a bacterial inoculum was prepared by making a direct saline suspension of isolated colonies selected from a pre-incubated non-selective agar plate. This inoculum was adjusted to a concentration of 108 CFU/ml by measuring the optical density at 600 nm using a spectrophotometer, targeting an absorbance range of 0.1 to 0.2. For spore suspension, a 0.05% polysorbate 80 solution in saline was used, and the concentration was adjusted to 10^6 spores/ml, measured at 530 nm with an absorbance range of 0.09 to 0.13. All working cultures used for testing were maintained at no more than five passages from the ATCC reference culture. The inoculum of each strain was diluted to intermediate concentration (2X), 1×10^6 CFU/ml for bacteria, 1×10^3 to 5×10^3 CFU/ml for yeast and 0.8×10^4 to 10×10^4 for spore suspension. The average bacterial titer of each strain was determined using plate counting and a hemocytometer. The sample solutions at different pH values were also diluted to an intermediate concentration (2X) using suitable broth. In a 96-well microplate, 200 μ L of the sample solution was dispensed into each well of the columns. The inoculum suspension was vortexed uniformly and added to each well with 100 μ L. Before incubation, the absorbance of the inoculated plates was measured at 600 nm for all cultures except *A. brasiliensis*, which was measured at 530 nm, and the absorbance values were recorded. The inoculated 96-well plates were then incubated under optimal temperature, time, and atmospheric conditions (as shown in Table 1). After incubation, the absorbance values were measured again. A net increase in absorbance after incubation indicated bacterial growth.

2.2.3 Emulsification Efficacy

The performance of emulsification was tested by preparing emulsions containing 5% oil (Refined Olive Oil, Mineral Oil, and Caprylic/Capric Triglyceride) along with different percentages of emulsifier and water (as shown in Table 2).

2.2.4 Foaming Properties

To determine the foamability of Sample A solution and the stability of the foam produced, the standard Ross-Miles method (ISO 696-1975) was employed. Briefly, 2.5 g of facial cleanser with or without Sample A was dissolved in hard water (150 mg/kg) to prepare a 1 L solution, which was maintained at 40°C. A 200 mL aliquot of the Sample solution was poured from a height of 50 cm into a measuring cylinder containing 50 mL of the same solution. The volume of foam produced was measured immediately after pouring and again at 5 minutes. Hard water without Sample A served as the control.

3. Results

3.1. Figures, Tables and Schemes

Table 1. Inoculation Conditions for the microbial culture

Microbial strain	Culture media	Incubation temperature	Incubation time	Incubation atmosphere
Bacteria				
E. coli	CAMHB	37°C	18-24 hours	Aerobic
S. aureus	CAMHB	37°C	18-24 hours	Aerobic
Pa	CAMHB	37°C	18-24 hours	Aerobic
C. acnes	RCM	37°C	72 hours	Anaerobic
Fungi				
C. albicans	YM broth	35°C	24 hours	Aerobic
A. brasiliensis	PDB	35°C	48-50 hours	Aerobic
M. furfur	mDixon	30°C	24 hours	Aerobic

Table 2. Composition of Emulsions in the Emulsification Efficacy Test

Ingredients	Percentage (%)				
Sample A	1	2	3	4	5
Oil	5	5	5	5	5
Water	94	93	92	91	90
Total	100	100	100	100	100

Table 3. Effect of pH adjustment on the transparency of 1% Sample A aqueous solution

Sample A to 30% NaOH (w/w)	0.00	0.25	0.50	0.75
Appearance Photo	Non-transparent	Non-transparent	Clear	Clear

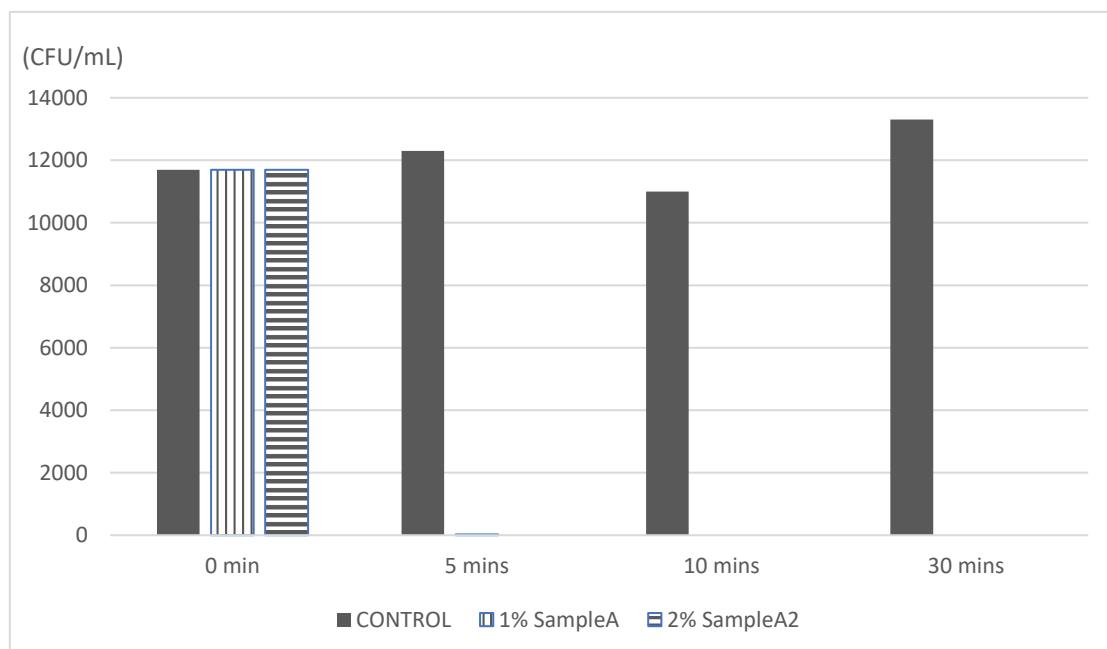
Table 4. MIC Value of Samples Against Various Microorganisms

pH value	Bacteria				Fungi	
	E. coli	Pa	S. aureus	C. acnes	C. albicans	A. brasiliensis
	GNB	GNB	GPB	GPB	GPB	GPB
5.00	0.500	0.125	0.016	0.026	0.031	0.052
5.50	2.000	0.125	0.016	0.026	0.063	0.031
6.00	≥ 2	0.125	0.016	0.026	0.063	0.016
6.50	≥ 2	0.250	0.016	0.026	0.063	0.016
7.00	≥ 2	0.250	0.031	0.026	0.063	0.016

(GNB: Gram-negative bacteria; GPB: Gram-positive bacteria)

Table 5. Absorbance measurements of Sample A against Malassezia furfur Across various pH levels and concentrations

pH	Concentration of Sample A (ppm)					Growth	Sterile
	10000	5000	2500	1250	625	control	control
pH=5.0	0.074	0.119	0.149	0.184	0.2	0.554	0.14
pH=5.5	0.073	0.134	0.147	0.296	0.208	0.289	0.098
pH=6.0	0.075	0.141	0.175	0.179	0.435	0.288	0.093
pH=6.5	0.075	0.13	0.177	0.243	0.231	0.395	0.093
pH=7.0	0.081	0.14	0.186	0.304	0.251	0.383	0.1

**Figure 1. Reduction of Malassezia furfur at different time intervals following 30 mins treatment**

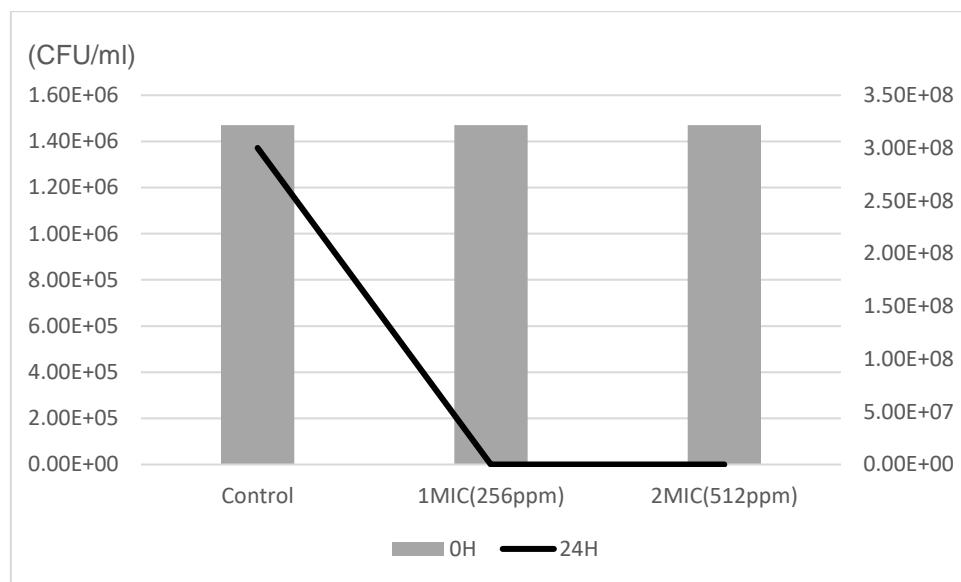


Figure 2. Reduction of *Cutibacterium acnes* after 24 hours treatment

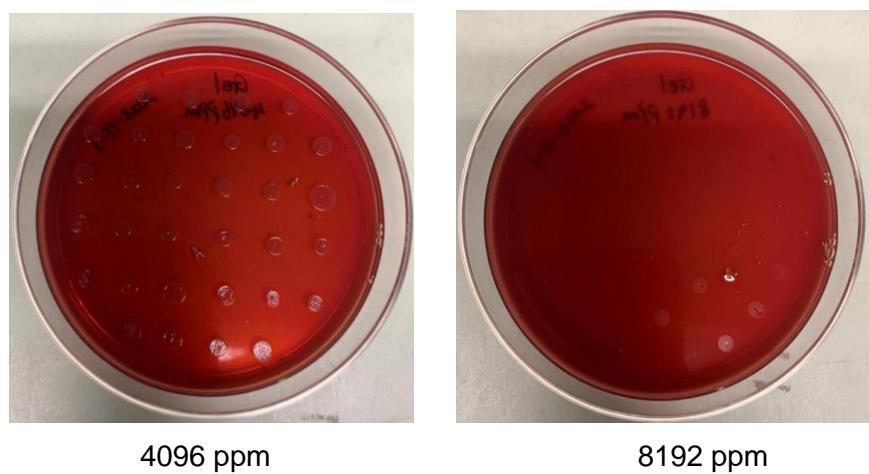


Figure 3. Reduction of *Cutibacterium acnes* after 24 hours treatment

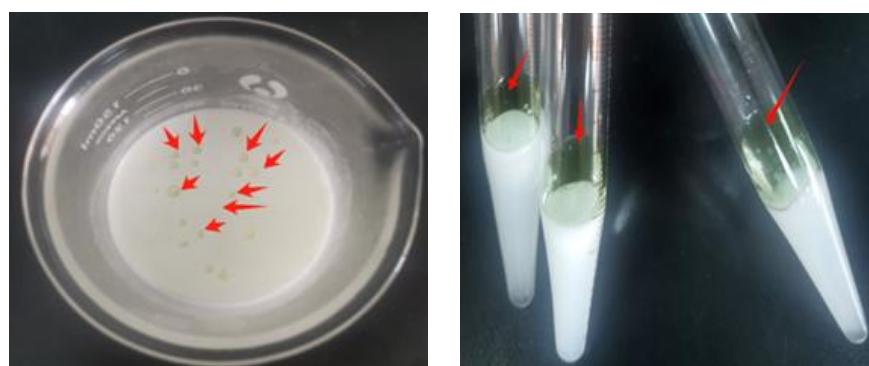


Figure 4. a: Emulsification after 1hour

b: centrifugation

foam(cm) time(min)	Sample A 1%	Control 0%
0	44	41
5	31	26

Table 6. Result of Ross-Miles method

4. Discussion

4.1 pH adjustment

Transparency is often associated with purity and quality. Clear cosmetic formulations enhance the visual appeal of a product, making it more attractive to consumers. Therefore, the ability of a surfactant to remain transparent in cosmetic formulations is beneficial, particularly for cleansers and other cosmetic products.

The primary goal of this study was to explore methods to maintain transparency of Sample A at a pH suitable for cosmetic formulation. The optimal pH range for cosmetics, which is ideal for our skin, is between 4 and 7. Initially, the Sample A aqueous solution was found to be non-transparent within this pH range. The pH was then adjusted to alkaline using a 30% Sodium Hydroxide (NaOH) solution (as illustrated in Table 3). When the Sample A to NaOH ratio increased to 1:0.5 (w/w) or 1:0.75 (w/w), the resulting solutions became clear; however, their pH values exceeded the cosmetic standard. Consequently, the pH values of these solutions were adjusted to 5.0, 5.5, 6.0, 6.5, and 7.0 using a 30% citric acid solution, and the resulting aqueous solutions remained clear. This finding highlights the potential of Sample A for formulating transparent cosmetic products, including anti-dandruff shampoos and anti-acne cleansing gels.

4.2 Antimicrobial activity

4.2.1 MIC Value of Samples Against Various Microorganism

Minimum Inhibitory Concentrations (MIC) refer to the minimal dosage of an active ingredient required to inhibit the growth of a specific microorganism. The MIC values of Sample A against various microorganisms, as illustrated in Table 4, reveal several significant observations. Notably, the MIC values for samples against fungi were not higher than those for bacteria, which contrasts with typical findings for common antimicrobial agents. Specifically, *C. albicans* maintained a consistently low MIC value across different pH levels, comparable to the effectiveness observed in *S. aureus*. This suggests that both types of microorganisms can be effectively targeted by Sample A. In contrast, the MIC value for *A. brasiliensis* decreased from 0.052 at pH 5.00, to 0.031 at pH 6.00 and further to 0.016 at pH 7.00. This decreasing trend indicates a potent antimicrobial effect of Sample A against this fungus, which is remarkably different from the general understanding that fungi often exhibit higher resistance to antimicrobial agents. Additionally, the MIC values for bacteria, particularly *E. coli*, showed higher values at certain pH levels compared to fungi. Typically, antimicrobial agents exhibit lower inhibitory effects against fungi due to their more complex cell wall structure, making them more resistant to certain agents. These observations highlight the complexity of microbial

resistance, therefore further investigation into the mechanisms of the antimicrobial effect would be beneficial.

Understanding the efficacy of Sample A against *Cutibacterium acnes* is crucial for developing effective acne formulations, as *C. acnes* is a primary contributor to acne development. The MIC values for *C. acnes* was 0.026 at pH level between 5.00 to 7.00, demonstrating the effectiveness of Sample A across various pH conditions. Notably, these MIC values are relatively low compared to those for some bacteria, such as *E. coli*, suggesting that Sample A is more potent against *C. acnes*. Moreover, the consistent MIC values across different pH levels indicate stability in antimicrobial effectiveness, which is advantageous for formulating products that retain efficacy on the skin, where pH can fluctuate. This versatility in pH compatibility enhances the potential for various cosmetic formulations. Given these findings, it is also essential to explore the MIC value against *M. furfur*, a common fungus associated with dandruff. Understanding the efficacy of Sample A against this organism could provide valuable insights into its broader applications in dermatological treatments.

Another observation from our study refers to the MIC values of gram-positive and gram-negative bacteria. The results indicate that the antimicrobial efficacy of Sample A is generally higher against gram-positive organisms compared to the gram-negative bacteria. This observation aligns with common microbiological knowledge, as gram-positive bacteria have a simpler cell wall structure that is more susceptible to antimicrobial agents. In contrast, gram-negative bacteria, like *E. coli*, possess a more complex outer membrane that can act as a barrier to many antimicrobial compounds, resulting in higher MIC values. The low MIC value of *C. acnes* suggests that Sample A could serve as a mild and nature active ingredient in anti-acne cosmetic products.

4.2.2 MIC Value of Samples Against *Malassezia furfur*

To further investigate the inhibitory effect of Sample A against *M. furfur*, absorbance measurements were taken across various pH levels and concentration. A test sample exhibiting strong antimicrobial activity will show no turbidity, indicating that the growth of the test microorganisms was inhibited. Samples without Sample A served as growth control, while that without *M. furfur* served as sterile control. As shown in Table 5, higher concentrations of Sample A effectively inhibit the growth of *M. furfur*, with lower absorbance values correlating to reduced microbial growth. Notably, the absorbance of Sample A at 10,000 ppm was similar to that of the sterile control, implying a minimum inhibitory concentration (MIC) at this level, which corresponds to 1%. This indicates that at this dosage, Sample A is effective in preventing microbial growth.

Additionally, while the absorbance values varied across different pH levels, suggesting that the antimicrobial activity of Sample A may not be entirely pH-independent. Higher absorbance readings at both 625 and 1250 ppm, particularly at pH 5.5 and 7.0, indicate that pH could influence the effectiveness of Sample A against *M. furfur*. However, at higher concentration including 2500, 5000 and 10000 ppm, the absorbance values were similar across various pH levels, suggesting that the antimicrobial effect becomes pH-independent at these concentrations. Further investigation is needed to fully understand the relationship between pH and efficacy.

Taken together, these results highlight the potential applications of Sample A in dermatological products, particularly for conditions such as dandruff or fungal infections. Further investigation into the mechanisms of action, stability across different formulations, and potential interactions with other ingredients in cosmetic products will ultimately enhance the development of effective antifungal solutions.

4.2.3 Time kill assay

To evaluate the efficacy of Sample A against *M. furfur* and *C. acnes*, time kill assays were performed. At 0 minutes, all samples (growth control, 256 ppm, and 512 ppm) as illustrated in Figure 1, exhibited similar microbial growth levels (1.17×10^4), indicating no immediate treatment effect against *M. furfur* (Figure 1). The 256 ppm concentration significantly reduced the microbial count to 2.5×10 , demonstrating initial antimicrobial activity, while the 512 ppm concentration completely eliminated *M. furfur* after 5 minutes. This rapid decrease in viable counts at both concentrations suggests that Sample A acts quickly against *M. furfur*, making it a potential candidate for formulations targeting fungal infections, particularly in anti-dandruff applications.

In Figure 2, the data revealed that Sample A is highly effective against *C. acnes*, achieving complete microbial reduction at both concentrations (256 ppm and 512 ppm) within 24 hours. Unlike the rapid action observed against *M. furfur*, the results for *C. acnes* show that while immediate effects are not observed, long-term efficacy is significant, suggesting that Sample A may exert its antimicrobial effects over an extended duration.

Overall, the time kill assay data confirm that Sample A is a potent antimicrobial agent against both *M. furfur* and *C. acnes*, showing promise for its application in dandruff and acne treatment formulations. Further research into its safety profile will be crucial for its successful development into consumer products.

4.2.4 Evaluation of the antimicrobial activity of an anti-acne cleansing gel

To evaluate the antimicrobial activity of an anti-acne cleansing gel against *Cutibacterium acnes*, the experiment was designed using various concentrations of Sample A: 1, 2, 4, 8, 16, 32, 128, 256, 512, 1024, 2048, 4096, 8192, and 16,384 ppm. As shown in Picture 3, some white microbial growth was observed at a concentration of 4,096 ppm. However, only minimal marks were present at 8,192 ppm, indicating the absence of viable bacteria. Therefore, the minimum inhibitory concentration (MIC) of the anti-acne cleansing gel against *Cutibacterium acnes* was determined to be 8,192 ppm, or 0.8192%.

4.3 Emulsification Efficacy

To effectively evaluate the emulsification efficacy, different concentrations of the test sample were mixed with 5% of a specific oil in water. Any visible separations were noted, and the mixtures were subsequently centrifuged to assess the potential for phase separation. As shown in Figure 4, some oil spots floated on the surface of the lotion after only 1 hour of emulsification, and additional oil separated during the 30-minute centrifugation test at 3,000 RPM. These results indicate that Sample A at concentrations of 1-5% were unable to effectively emulsify 5% oil, suggesting that Sample A alone may not be sufficient and might only function as a co-emulsifier.

4.4 Foam ability and stability

To assess the foaming ability and stability of the sample, the Ross-Miles method was employed. The pH of the facial cleaning was adjusted to 5.8, with or without 1% Sample A. As shown in Table 6, Sample A produced a higher initial foam height (44 cm) compared to the control (41 cm). After 5 minutes, the foam height of Sample A decreased to 31 cm, while the control decreased to 26 cm. Although both samples experienced a reduction in foam height over time, Sample A retained more foam than the control, indicating greater foam stability. These results demonstrate that Sample A exhibits superior foaming ability and stability compared to the control, suggesting its potential for use in formulations where these characteristics are desired. However, further testing should be conducted to evaluate additional factors such as sensory properties and its compatibility with other ingredients.

5. Conclusion

This study has demonstrated that Sodium Decanoyl/Dodecanoyl Lactylate possesses significant anti-microbial activity against *Malassezia furfur* and *Cutibacterium acnes*, two key microorganisms associated with dandruff and acne. The compound showed a remarkably low minimum inhibitory concentration (MIC), highlighting its potential as an effective natural active ingredient in anti-dandruff and anti-acne formulations. Additionally, its impressive foaming and foam-stabilizing properties enhance its attractiveness for use in cosmetic products.

The findings reveal that Sodium Decanoyl/Dodecanoyl Lactylate not only serves as an effective emulsifier but also meets the growing consumer demand for naturally derived ingredients in cosmetics. By facilitating water transparency while maintaining antimicrobial efficacy, this compound offers a viable alternative to traditional synthetic ingredients, which are often associated with skin sensitivity and environmental concerns.

Incorporating Sodium Decanoyl/Dodecanoyl Lactylate into anti-dandruff shampoos and anti-acne cleansing gels could lead to the development of safer and environmentally friendly products. Further exploration of its applications in cosmetic formulations, particularly in emphasizing the importance of utilizing naturally derived ingredients that align with consumer expectations for safety and sustainability.

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