JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA), Vol. 9, No.1, 2024
Chemistry Education Study Program, Universitas Sebelas Maret
https://jurnal.uns.ac.id/jkpk
pp. 85-101
ISSN 2503-4146
ISSN 2503-4154 (online)

FORMULATION, CHARACTERIZATION, AND ANTIBACTERIAL TESTING OF CREAM SUPPLEMENTED WITH ALOE VERA (*Aloe vera L.*) GEL AND NUTGRASS (*Cyperus rotundus L.*) RHIZOME ETHANOL EXTRACT

Sri Mulyani*, Salsabila Adzani Rahmadina, Elfi Susanti VH, Sri Retno Dwi Ariani, Suryadi Budi Utomo, Muhammad Hizbul Wathon

Chemistry Education Study Program, Universitas Sebelas Maret,Surakarta, Central Java, Indonesia

ARTICLE INFO

Keywords:
Cream;
aloe vera;
Nutgrass rhizome;
antibacterial;
organoleptic

Article History:
Received: 2024-02-01
Accepted: 2024-04-21
Published: 2024-04-29
**Corresponding Author*
Email:
srimulyaniuns@staff.uns.ac.id
doi:10.20961/jkpk.v9i1.84205

ABSTRACT

Various brands and forms offer a wide array of cream-based cosmetic products. However, only some of these products meet safety specifications. *Aloe vera* and nutgrass, two plants rich in bioactive compounds, possess anti-inflammatory, soothing, and antibacterial properties, making them ideal for health-safe cosmetic formulations. This research was conducted to develop antibacterial cream formulations incorporating these natural additives. The study focused on quality, organoleptic testing, and the formulated cream's antibacterial assessment. The research methodology encompassed several key processes: preparation of *Aloe vera* gel and nutgrass rhizome ethanol extract, formulation of the cream, quality testing by SNI 16-6069-1999 standards, organoleptic testing as per SNI 01-2346-2006, and antibacterial activity testing using *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 bacterial strains. Results indicated that the cream formula enriched with *Aloe vera* gel and nutgrass rhizome ethanol extract meets the standards for skin-lightening creams and exhibits significant antibacterial capabilities. Furthermore, it was observed that the antibacterial effectiveness of the cream increases with the concentration of the nutgrass rhizome ethanol extract. These findings highlight the potential for integrating natural ingredients into modern cosmetic formulations, providing therapeutic and aesthetic benefits. This approach not only ensures compliance with safety standards but also leverages the natural properties of *Aloe vera* and nutgrass to enhance the product's functionality in skin care applications.
.

How to cite: S. Mulyani, S. A. Rahmadina, E. Susanti VH, S. R. Dwi Ariani, S. B. Utomo, and M. H. Wathon, "Formulation, characterization, and antibacterial testing of cream supplemented with Aloe vera (Aloe vera L.) gel and Nutgrass (Cyperus rotundus L.) rhizome ethanol extract," *Jurnal Kimia dan Pendidikan Kimia (JKPK)*, vol. 9, no. 1, pp. 85-101, 2024. [Online]. Available: http://dx.doi.org/10.20961/jkpk.v9i1.84205

INTRODUCTION

Cream is a semi-solid formulation widely used in the pharmaceutical and cosmetic industries, comprising active ingredients either dissolved or dispersed in a suitable base [1]. The benefits of the cream include convenience, a cooling sensation upon application, ease of removal, non-greasiness, suitability for hairy areas, and a comfortable, non-irritating experience [2]. These advantages have made creams preferred for protecting and maintaining skin freshness, particularly when enhanced with natural antibacterial agents.

Developing an antibacterial cream is vital, as *S. aureus*, a bacterium commonly found on the skin, can cause infections under conditions of excessive growth or weakened immune response. Commercially available antibacterial creams are often expensive, which underscores the need for formulations incorporating natural antibacterial agents. *Aloe vera* is renowned for its effective antibacterial properties [3], and nutgrass rhizome is recognized as a potential antimicrobial agent [4]. Integrating these natural ingredients into cream formulations has significant potential to enhance skin protection against bacteria and maintain skin freshness effectively.

Amidst the proliferation of cosmetic products, ensuring the safety and efficacy of these products remains a critical concern. Unfortunately, not all cosmetic products meet the required safety and efficiency standards, with many still containing harmful chemicals such as parabens [5]. Parabens, commonly used as preservatives, have raised health concerns related to long-term exposure, including skin irritation and potential links to breast cancer [6]. Moreover, synthetic additives like butylated hydroxytoluene (BHT), known for their antibacterial properties, can lead to neurotoxicity when used in high doses [7]. The primary challenge in cosmetic safety is the effective regulation and monitoring of these products across different countries, which have varying regulations regarding chemical usage in cosmetics. This disparity often results in the availability of products containing hazardous chemicals like parabens on the market.

Natural product trends have paved the way for safer and health-conscious cosmetic alternatives [8]. Incorporating safe and natural additives in cream formulations is crucial for enhancing the overall product value, providing increased safety, and offering potential antibacterial properties. Plants offer many compounds with potential medicinal properties, including antibacterial effects. Recent studies have explored using natural ingredients such as turmeric rhizome extract, green tea leaf extract, and jackfruit leaf extract in cream formulations as alternative antibacterial agents [9]. *Aloe vera* and nutgrass exhibit unique characteristics that distinguish them from other natural ingredients. *Aloe vera* is renowned for its exceptional skin healing properties and ability to reduce inflammation [10], while nutgrass shows promise due to its anti-inflammatory and antimicrobial properties [11]. Further research into *Aloe vera* and nutgrass could provide valuable insights into their effective use in cosmetic cream formulations as safe and effective natural alternatives.

Aloe vera is well-known for its skin health benefits. The gel from *Aloe vera* can penetrate skin tissues, maintain moisture, and prevent excessive fluid loss [10]. Its antibacterial and antifungal properties are linked to twelve anthraquinone compounds [11]. Additionally, *Aloe vera* gel contains various other compounds such as quinones, saponins, aminoglycosides, lupeol, salicylic acid, tannins, urea nitrogen, cinnamic acid, phenols, sulfur, flavonoids, and essential oils, all contributing to its antimicrobial activity. Recent developments in combining *Aloe vera* with nutgrass (*Cyperus rotundus*) are particularly compelling for further investigation. Nutgrass also has antimicrobial and anti-inflammatory properties that could benefit skin health. However, the combined effects of these two plants on skin health have yet to be thoroughly explored [12].

Nutgrass, a perennial weed thriving in tropical and subtropical regions, possesses creeping rhizomes that form nutrient-rich tubers [13]. These tubers contain various chemical components such as essential oils, alkaloids, flavonoids, polyphenols, resins, starch, tannins, triterpenes, d-glucose, d-fructose, and non-reducing sugars [14]. Alkaloids in nutgrass have shown various effects, including nervous system stimulation, blood pressure elevation, and antibacterial properties. Flavonoids in nutgrass have demonstrated antibacterial activity against *E. coli* and *S. aureus* [15]. Including nutgrass in cosmetic products marks an exciting innovation, offering potential as an effective natural antibacterial agent capable of combating bacteria such as *E. coli* and *S. aureus* [13].

The current market experiences a need for more cosmetic products, especially antibacterial creams, that utilize natural and skin-safe ingredients. This research aims to formulate a cream containing a combination of *Aloe vera* gel and nutgrass rhizome ethanol extract as natural antibacterial agents. This formulation provides a safe and effective alternative to conventional antibacterial creams, hoping to eliminate parabens and BHT while meeting the market demand for more natural and safe cosmetic products. This research overviews the study and its potential implications for the cosmetic industry and consumers seeking safer, natural, and effective skincare products.

METHODS

1. Equipment and Materials:

This research utilized essential laboratory equipment, including trays for holding ingredients during the blending and grinding process; burners and heaters for heating mixtures and reducing volume as necessary; thermometers for monitoring temperature during the heating process; pH meters for checking and controlling the pH of the cream mixture; refrigerators for storing ingredients at low temperatures; and calipers for precisely measuring the dimensions of materials, such as the length and diameter of nutgrass tubers. Specialized equipment like a rotary evaporator was also employed to concentrate natural materials such as aloe vera gel and nutgrass rhizome extract by gradually removing their solvents, aiding in obtaining purer and more concentrated extracts.

The primary materials used in this research included aloe vera sourced from Gondang Winangun, Jogonalan Subdistrict, and nutgrass tubers from Ampel, Boyolali Regency. Additional materials encompassed various chemicals, water, filter paper, and specific bacterial strains used for antibacterial testing.

2. Research Procedure:

a. Preparation of Aloe vera Gel and Ethanol Extract of Nutgrass Rhizomes:

The preparation of aloe vera gel involved washing the midrib, cutting, and draining to eliminate latex. After another wash and cut, the pulp was blended and filtered to produce the gel. Filtration utilized filter paper with a specific mesh size to achieve the desired consistency. The gel was then preserved, sterilized, and securely stored in a refrigerator. To extract nutgrass rhizomes, approximately 500 grams of nutgrass powder was immersed in ethanol for three days with intermittent stirring, allowing active ingredients to dissolve into the solvent. After this period, the mixture was filtered, and the soaking and filtration process was repeated. The combined liquid extracts from these sessions were then concentrated using a rotary evaporator, resulting in the ethanol extract.

b. Formulation and Cream Preparation

Cream formulations F0, F1, F2, and F3 were prepared with different compositions, as detailed in Table 1. The process began by mixing the water phase ingredients at a temperature of 70°C, while the oil phase ingredients were mixed separately at the same temperature. This temperature was maintained using an accurate temperature control device to ensure uniform heating. A temperature of 70°C is optimal because it lowers the viscosity of water and oil phase ingredients, facilitating even mixing without degrading or damaging them, thus allowing the cream-making process to proceed smoothly.

Table 1. Cream Formulations supplemented a Combination of Aloe Vera Gel and Nutgrass Rhizome Extract

Composition	F0	F1	F2	F3
Aloe vera gel	5 %	5 %	5 %	5 %
Nutgrass rhizome extract	-	25	30 %	35 %
emulsifier	3 %	3 %	3 %	3 %
Emollient	8 %	8 %	8 %	8 %
pH stabilizer	1 %	1 %	1 %	1 %
Humectant	12 %	12 %	12 %	12 %
Distilled water	ad 100	ad 100	ad 100	ad 100

(Note: "ad sufficient," meaning "sufficient quantity" or "to make up to" 100%. The percentages listed represent the amounts of the ingredients used in each cream formulation.)

3. Organoleptic (Descriptive and Hedonic) Testing

Organoleptic testing was conducted by 30 untrained panelists who assessed the cream's formula based on color, odor, texture/consistency, and ease of application. Panelists, selected randomly from the general population, were instructed to evaluate the samples objectively without bias, following clear guidelines on the assessment parameters. A descriptive and hedonic quality scale from 1 to 9 was utilized, with the average quality scores for each panelist calculated according to SNI 01-2346-2006 standards.

4. Antibacterial Testing

The antibacterial properties of the cream formula were evaluated using the good diffusion method, which is simple, reproducible, and allows for accurate visual observations of inhibition zones. This method measures the diameter of zones where active cream components inhibit microbial growth. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were chosen as test organisms due to their relevance to human skin infections. The antibacterial testing commenced with subculturing the bacteria on Nutrient Agar (NA) medium, incubated at 37°C for 18-24 hours. A homogeneous suspension of the test bacteria was then prepared in a 0.9% NaCl solution, adjusted to the turbidity of a McFarland 0.5 standard. The Mueller-Hinton Agar (MHA) medium surface was inoculated with this suspension, and wells were created for sample placement. After placing the samples, including controls, in the wells, the media was incubated at 37°C for 18-24 hours. The experiment was performed in triplicate, and the inhibition zones were measured by three individuals using calipers.

5. Data Analysis and Findings

Data were statistically analyzed using SPSS 25.0 software. A one-way ANOVA was conducted to explore the impact of varying concentrations of nutgrass rhizome extract on the antibacterial activity of the cream formula. The Kruskal-Wallis and Mann-Whitney tests were employed where data showed non-normal distribution or heterogeneity. Descriptive analysis of both antibacterial activity data and organoleptic test results provided a comprehensive overview of the cream formula's characteristics, focusing on attributes such as color, odor, and texture.

RESULTS AND DISCUSSION

1. Aloe Vera Gel Production

The production of *Aloe vera* gel begins with the careful selection of mature *Aloe vera* leaves, which are subsequently washed thoroughly to remove any impurities or contaminants. The leaves are filleted to extract the rich, creamy gel, which typically varies in color from off-white to a pale-yellow shade, as visualized in Figure 1a. *Aloe vera* gel is renowned for its myriad skincare benefits, including intense moisturization, soothing irritation, and reducing inflammation. Additionally, its gelatinous consistency enhances the texture of cream formulations, promoting easy spreadability and absorption upon application. The gel is blended during processing to achieve a smooth and uniform consistency, ensuring even distribution when incorporated into various formulations. Preservatives may be added to the gel to enhance shelf life and prevent microbial growth. The quality of the *Aloe vera* gel is crucial, as it directly impacts the effectiveness and sensory attributes of the final cream product.

2. Nutgrass Rhizome Extract Production

The Nutgrass rhizome extract, depicted as a concentrated brown liquid in Figure 1b, is produced through a meticulous extraction process. The bulbs of the Nutgrass plant are harvested, cleaned, and subsequently dried to eliminate any moisture that could interfere with the extraction process. Once sufficiently dried, the bulbs are ground into a fine powder to facilitate the efficient extraction of bioactive compounds. The maceration method employs an ethanol solvent to extract these compounds into a liquid form. The solvent is carefully evaporated after extraction, resulting in a potent, concentrated extract. This extract is rich in bioactive compounds, such as flavonoids and alkaloids. It is known for its antioxidant, anti-inflammatory, and antibacterial properties, making it a valuable ingredient for skincare formulations.

In the context of cream formulations, Nutgrass rhizome extract not only contributes to the potential therapeutic properties of the product but also influences other attributes such as color, fragrance, and consistency. Integrating this extract into cream formulations must be executed judiciously to ensure that the beneficial properties are harnessed while maintaining the cream's overall aesthetic and sensory quality.

(a) **(b)**

Figure 1. (a) Aloe Vera Gel, **(b)** Extract of Nutgrass Rhizome

3. pH Test

According to the Indonesian National Standard (SNI) 16-6069-1999, the pH requirement for skin-lightening cream formulations should range from 3.5 to 8.5. The pH of skincare products is critical as it directly affects skin compatibility and the product's efficacy. The pH scale measures how acidic or basic a substance is, with values ranging from 0 (most acidic) to 14 (most basic) and a pH of 7 being neutral. The natural pH of human skin typically ranges from 4.5 to 5.5, slightly acidic, which helps maintain the acid mantle that protects against microbes and retains moisture.

Adding Nutgrass rhizome extract to cream formulations can influence the pH due to its bioactive compounds' acidic or alkaline properties. Therefore, adjusting the extract concentration to achieve a pH that supports healthy skin conditions is crucial. Excessively acidic or alkaline products can irritate the skin or disrupt its natural protective barrier.

Table 2. The results of the pH measurements for the cream formulas contain a combination of aloe vera gel and nutgrass bulb extract.

Formula	**pH**			**Average ± RSD**	**Description**
	Test 1	**Test 2**	**Test 3**		
F0	7,4	7,3	7,3	7,3 ± 0,06	MR
F1	7,1	7,1	7,2	7,1 ± 0,06	MR
F2	6,8	6,8	6,9	6,8 ± 0,06	MR
F3	6,7	6,6	6,6	6,6 ± 0,06	MR

Description: MR (Meets Requirements); DTMR (Does Not Meet Requirements)

Cream formulations (F0 to F3) were tested for their pH values; the results are presented in Table 2. Each formulation remained within the acceptable range set by

SNI 16-6069-1999. An inverse relationship was observed between the concentration of Nutgrass bulb extract and pH levels; as the concentration increased, the pH value decreased towards the acidic side of the scale. This reduction in pH is likely due to the presence of organic acids in the Nutgrass bulb extract. According to the Bronsted-Lowry theory, an acid is a substance that donates a proton (H+), while a base accepts a proton. In our formulations, the Nutgrass bulb extract acts as an acid, donating protons and increasing the solution's acidity.

The decrease in pH with higher concentrations of Nutgrass bulb extract is aligned with findings from [16-17], which noted the presence of bioactive compounds such as alkaloids and flavonoids in the extract, contributing to the pH shift. Although a slightly acidic pH benefits skin health, overly acidic conditions may irritate or harm the skin's natural barrier. The interaction of Nutgrass bulb extract with other ingredients in the cream formulations requires further exploration to fully understand its impact and potential as a significant skincare ingredient.

4. Spreading Test

The spreading ability of cream on the skin is evaluated using the "Spreading Test," which measures the cream's capacity to distribute uniformly over the skin's surface. This property is essential to ensure active ingredients are applied evenly, optimizing their effectiveness.

Table 3. The results of the spreading test for cream formulas supplemented with a combination of aloe vera gel and the Nutgrass bulb extract.

Formula	Initial Spread Cream Diameter (cm)	Final Cream Spread Diameter (cm)	Average ± RSD (cm)	Description
F0	5,4	5,5	5,45 ± 0,07	MR
F1	5,0	5,2	5,10 ± 0,14	MR
F2	4,9	5,0	4,95 ± 0,07	DTMR
F3	4,8	4,9	4,85 ± 0,07	DTMR

Description: MR (Meets Requirements); DTMR (Does Not Meet Requirements)

The test has shown that higher concentrations of Nutgrass bulb extract increase the cream's viscosity, reducing its ability to spread easily. Although Nutgrass bulb extract enhances the cream with beneficial properties, its higher concentrations may render the product less user-friendly due to decreased spreadability. This reduction could impact consumer satisfaction and the product's overall efficacy. The molecular interactions between the Nutgrass bulb extract and the cream's base contribute to this decreased spreadability. As extract concentration increases, it may enhance intermolecular forces, thereby increasing resistance to flow (or spread). According to the Kinetic Molecular Theory, the behavior of matter can be explained by the motion of its molecules. In this scenario, a higher concentration of Nutgrass bulb extract likely causes increased molecular interactions, restricting their free movement and thus reducing spreadability.

The Spreading Test assesses a cream's ability to cover the skin adequately, which is critical for uniform application and optimal absorption of ingredients. For optimal spreading, a diameter range of 5 cm to 7 cm is recommended [18]. In our study, creams that combined aloe vera gel and Nutgrass bulb extract demonstrated varying spreading capabilities. The results, as detailed in Table 3, include initial and final spread diameters, their averages, and the Relative Standard Deviation (RSD). Data analysis indicates that the Nutgrass bulb extract concentration directly influences spreading ability. Formulas F2 and F3 did not meet the criteria set by Garg et al. [9], indicating reduced spreadability with increased concentrations of Nutgrass extract. This reduction is likely due to higher extract amounts creating a denser cream, which impedes effective spreading. Similar observations were made by [9], who noted that higher concentrations of Nutgrass or thickening agents can increase the viscosity of creams, affecting their spreadability.

In conclusion, the concentration of Nutgrass bulb extract is pivotal for determining cream spreadability. Excessive amounts of the extract result in denser creams that spread poorly. Therefore, further adjustments to the cream formulation are necessary to balance the benefits of Nutgrass bulb extract with the product's efficiency and user-friendliness on the skin.

5. Homogeneity Test

Homogeneity testing, pivotal in cream development, ensures uniform ingredient distribution in cosmetic products. This consistency prevents clumps and ensures that each application delivers a steady concentration of active components. Our results (Table 4) confirmed a consistent, well-blended ingredient mix for creams with aloe vera gel and Nutgrass bulb extract. This data denotes an even spread of aloe vera, Nutgrass bulb extract, and other elements, culminating in a smooth product.

Thorough mixing and stabilization during the formulation process will result in homogeneous creams. The absence of agglomerated particles suggests that the active ingredients, such as Aloe vera gel and Nutgrass extract, are uniformly dispersed throughout the cream base. A homogeneous cream guarantees a consistent therapeutic effect for users with each application. From a manufacturing standpoint, it confirms that the formulation process effectively blends and stabilizes the ingredients, ensuring consistent quality. Creams are complex colloidal systems. Achieving homogeneity involves overcoming potential ingredient segregation due to differences in particle sizes, densities, and physicochemical properties. Cams can exhibit phase separation, sedimentation, or creaming if incorrectly formulated. The Theory of Sedimentation, governed by Stoke's Law, explains how particles can settle over time if not uniformly distributed. The achieved homogeneity in these formulations implies that these unwanted phenomena are effectively countered.

During testing, creams undergo visual and potential microscopic inspection to identify non-uniformities. A clump-free product assures quality, implying that users consistently reap the intended benefits. Homogeneity is paramount for consistent performance, pleasant texture, user trust, and safety. Uneven distributions can cause skin irritation or diminished efficacy. Aligning with standards like SNI 16-6069-1999 ensures product safety and quality. Meeting these benchmarks signifies a well-produced cream that promises uniform benefits. In summary, homogeneity is essential to cream development. Our aloe vera and Nutgrass creams, being consistent and up to standard, guarantee user satisfaction and confidence in their safety and effectiveness.

Table 4. The results of the Homogeneity Test for the cream formulas containing a combination of aloe vera gel and the Nutgrass bulb extract.

Formula	Observation Results	Homogeneity Test	Description
F0	No agglomerated particles	homogeneous	Meets Requirements
F1	No agglomerated particles	homogeneous	Meets Requirements
F2	No agglomerated particles	homogeneous	Meets Requirements
F3	No agglomerated particles	homogeneous	Meets Requirements

The process of homogeneity testing is pivotal in the development of cosmetic products. This testing ensures that the ingredients are uniformly mixed, preventing clumping and ensuring that each use delivers consistent active components. In our study involving creams formulated with aloe vera gel and Nutgrass bulb extract, the data from Table 4 verified a consistent and well-blended mix of ingredients, resulting in a smooth final product. Effective mixing and stabilization during the formulation process contribute to the homogeneity of the creams. The absence of agglomerated particles indicates that the active ingredients, such as *Aloe vera* gel and Nutgrass extract, are evenly dispersed throughout the cream base, thus guaranteeing consistent therapeutic effects with each application.

From a manufacturing perspective, achieving homogeneity in creams—a complex colloidal system—requires overcoming the potential segregation of ingredients due to differences in particle sizes, densities, and physicochemical properties. Creams can exhibit phase separation, sedimentation, or creaming if incorrectly formulated. Governed by Stoke's Law, the Theory of Sedimentation describes how particles can settle over time if not uniformly distributed [2]. The homogeneity of these formulations suggests that these undesirable phenomena are effectively countered.

Cams undergo visual and potentially microscopic inspections during quality control to identify non-uniformities. A clump-free product assures quality and implies that users can consistently benefit from the intended effects. Homogeneity is crucial for consistent performance, appealing texture, user trust, and safety. Uneven distributions can lead to skin irritation or reduced efficacy. Adhering to standards such as SNI 16-6069-1999 is paramount for ensuring product safety and quality. Meeting these benchmarks underscores a well-produced cream that offers uniform benefits. In conclusion, maintaining homogeneity is vital to cream development, ensuring that our aloe vera and Nutgrass creams are consistent, safe, and effective, guaranteeing user satisfaction and confidence in their therapeutic benefits.

6. Emulsion Type Test

The emulsion type testing results, detailed in Table 5, confirm that the cream formulations are characterized as water-in-oil (W/O) emulsions. This classification is evidenced by observing a blue color with the methylene blue dye, which dissolves in the dispersing phase (water phase), indicating the presence of a W/O emulsion. In this type of emulsion, "M" denotes the dispersed or internal phase (oil phase), and "A" represents the continuous or external phase (water phase). Emulsions in skincare are composed of a water phase and an oil phase, where the nature of the emulsion affects the product's properties, such as its feel on the skin and its moisturizing capabilities.

Table 5. The results of the emulsion type testing for the cream formulas were supplemented with a combination of Aloe vera gel and the Nutgrass bulb extract.

Formula	Observation	Emulsion Type
F0	methylene blue is soluble in water	M/A
F1	methylene blue is soluble in water	M/A
F2	methylene blue is soluble in water	M/A
F3	methylene blue is soluble in water	M/A

Water-in-oil emulsions encapsulate water droplets within an external oil phase. These emulsions are known for their moisturizing properties because the oil phase creates a barrier on the skin that helps prevent water loss. The oil phase seals in moisture and provides a protective layer that enhances the product's hydrating effectiveness, making it particularly beneficial for dry or sensitive skin types. The emulsification process involves dispersing one liquid phase in another immiscible liquid, which is stabilized by emulsifying agents or surfactants. The type of emulsion formed is influenced by the Hydrophilic-Lipophilic Balance (HLB) value of the surfactant; surfactants with low HLB values are more conducive to forming W/O emulsions, as they are more oil-soluble. This is further supported by the Bancroft rule, which states that the phase in which the emulsifying agent is more soluble will dominate as the continuous phase. Therefore, the surfactants used in these formulations likely have lower HLB values, favoring the formation of W/O emulsions.

In such emulsions, the continuous water phase, containing active components like *Aloe vera* gel and Nutgrass bulb extract, is interspersed with tiny oil droplets. This arrangement contributes to a smooth, non-greasy feel while enhancing skin moisturization. The dominant water phase attracts and retains moisture on the skin, forming a protective layer that alleviates dryness. Stability in W/O emulsions is crucial for maintaining product quality and performance over time. The consistent formation of water-in-oil emulsions, facilitated by *Aloe vera* gel and Nutgrass extract, underlines the product's reliability and potential benefits. Although these creams show promising attributes for skincare due to their moisturizing properties and stability, further research is essential to optimize and understand these benefits fully, enhancing their efficacy in real-world applications.

7. Organoleptic Testing for Color, Odor, Texture, and Ease of Application Attributes

Organoleptic testing employs the hedonic quality scale in the sensory evaluation of a cream product, focusing on subjective preferences and perceptions of its attributes—color, aroma, texture, and ease of application. Results from this evaluation are detailed in Table 6, which summarizes assessments made by 30 untrained panelists on cream formulations F0, F1, F2, and F3.

The **color** attribute significantly influences consumer attraction and initial perception, as visually appealing colors are often associated with higher quality. According to the results, creams with brighter and more vibrant colors scored higher, confirming existing research that suggests consumers are drawn to aesthetically pleasing products [18]. Conversely, creams with duller colors tended to receive lower scores, potentially due to negative associations with product effectiveness or appeal.

Aroma plays a critical role in the acceptability of skincare products. In this study, the distinct nutgrass aroma was a focal point. Formulations with a pronounced nutgrass scent were preferred, suggesting that a stronger aroma correlates with perceived natural content and effectiveness. Conversely, creams with a faint or absent nutgrass aroma were rated lower, possibly indicating to consumers a lack of potency or authenticity [19].

Texture and consistency are key factors that affect user experience and perception of a cream's functionality. Creams with thicker consistency were favored, often perceived as more luxurious and effective. This preference for a richer texture aligns with consumer expectations of a cream's nourishing properties [20]. In contrast, less viscous creams were scored lower, as they might be perceived as less effective in delivering skincare benefits.

Ease of application is another vital attribute in assessing the cream's practicality. Formulations that spread easily and left no residue were highly rated, underscoring consumer preference for creams that integrate seamlessly into their skincare routines without adding unwanted greasiness or stickiness [21]. Products that were difficult to apply or left a sticky feeling were not favored, as they detract from the user experience and could discourage regular use.

Through the hedonic quality scale, researchers and formulators can garner insights into consumer preferences and perceptions, which are instrumental in refining cream formulations. This approach ensures that the final products meet and exceed consumer expectations, enhancing satisfaction and competitiveness in the skincare market. Sensory evaluation techniques like the hedonic quality scale are crucial for advancing product development and ensuring high customer satisfaction in the dynamic cosmetic industry.

Table 6. The hedonic score for Color, Odor, Texture, and Ease of Application Attributes of Cream Formulas F0, F1, F2, and F3.

Calculation	Cream Formulas			
	F0	**F1**	**F2**	**F3**
	Hedonic score for Color Attribute			
Average (x)	8,133	5,933	4,300	3,767
Standard deviation (s)	0,340	1,031	1,716	1,892
Interval (P min)	8,012	5,565	3,686	3,090
Interval (P max)	8,255	6,302	4,914	4,444
P	8,012 ≤μ≥ 8,255	5,565 ≤μ≥ 6,302	3,686 ≤μ≥ 4,914	3,090 ≤μ≥ 4,444
The Hedonic Quality Scale	8	6	4 - 5	3 – 4
	Hedonic score for Odor Attribute			
Average (x)	5,167	6,300	6,933	7,567
Standard deviation (s)	1,551	0,971	0,854	1,086
Interval (P min)	4,612	5,952	6,628	7,178
Interval (P max)	5,722	6,648	7,239	7,955
P	4,612 ≤μ≥ 5,722	5,952 ≤μ≥ 6,648	6,628 ≤μ≥ 7,239	7,178 ≤μ≥ 7,955
The Hedonic Quality Scale	5 - 6	6 - 7	7	7- 8
	Hedonic score for Texture Attribute			
Average (x)	7,200	7,067	7,133	7,067
Standard deviation (s)	0,833	0,573	0,670	1,031
Interval (P min)	6,902	6,861	6,894	6,698
Interval (P max)	7,498	7,272	7,373	7,435
P	6,902 ≤μ≥ 7,498	6,861 ≤μ≥ 7,272	6,894 ≤μ≥ 7,373	6,698 ≤μ≥ 7,435
The Hedonic Quality Scale	7	7	7	7
	The hedonic score for Ease of Application Attribute			
Average (x)	7,867	7,400	6,967	6,633
Standard deviation (s)	0,427	0,663	0,752	0,706
Interval (P min)	7,714	7,163	6,698	6,381
Interval (P max)	8,019	7,637	7,236	6,886
P	7,714 ≤μ≥ 8,019	7,163 ≤μ≥ 7,637	6,698 ≤μ≥ 7,236	6,381 ≤μ≥ 6,886
The Hedonic Quality Scale	8	7 - 8	7	6 – 7

Attributes Scale:

Score	Color Attribute	Odor Attribute	Texture Attribute	Ease of Application Attribute
9	Bright color, very appealing	Very strong nutgrass aroma	Very thick	Very easy to apply
8	Bright color, appealing	Strong nutgrass aroma	Thick	Easy to apply
7	Quite a bright color, quite appealing	Quite strong nutgrass aroma	Quite thick	Quite easy to apply
6	Less bright color, appealing	Less strong nutgrass aroma	Less thick	Quite easy to apply, slightly sticky
5	Moderately bright & appealing color	Starting to have a nutgrass aroma	Slightly thick	Quite easy to apply, quite sticky
4	Less dark brown color, appealing	No nutgrass aroma, not rancid	Less runny	Quite difficult to apply, slightly sticky
3	It is quite a dark brown color, appealing	No nutgrass aroma, somewhat rancid	Quite runny	Quite difficult to apply, quite sticky
2	Dark brown color, unappealing	No nutgrass aroma, rancid	Runny	Difficult to apply, sticky
1	Dark brown color, very unappealing	There is no nutgrass aroma, and very rancid	Very runny	Very difficult to apply, very sticky

8. Antibacterial Test

The antibacterial activity test assesses the efficacy of chemical compounds, specifically aloe vera gel and Nutgrass bulb extract, against targeted bacteria [19]. Aloe vera gel comprises bioactive components such as saponins, sterols, acemannan, and anthraquinones, while Nutgrass bulb extract contains alkaloids, tannins, Aperol, and flavonoids. The effectiveness of these antibacterial agents is evaluated by measuring the diameter of the inhibition zone using the disc diffusion method [20]. A broader inhibition zone signifies more potent antibacterial activity of the cream that combines aloe vera gel and Nutgrass bulb extract. Distilled water is the negative control in this setup and is used to dilute the formulation [21].

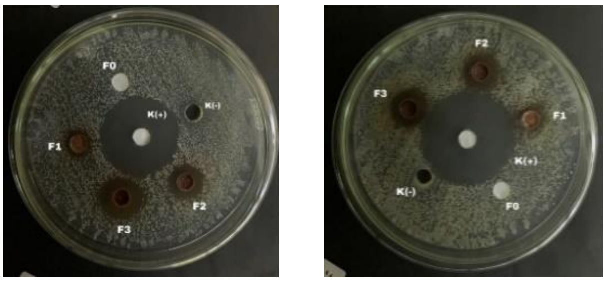
The disc diffusion method is prevalent for determining the antimicrobial activity of various substances. In this procedure, paper discs impregnated with cream formulations are placed on agar plates previously inoculated with a specific bacterial strain. The active compounds in the cream diffuse into the agar, inhibiting bacterial growth around the disc and creating a clear inhibition zone (Fig. 2). The diameter of this zone is measured, with a larger zone indicating stronger antibacterial effects.

The synergy between aloe vera gel and Nutgrass bulb extract in the cream is believed to enhance antibacterial activity, leveraging different bioactive compounds with potential antibacterial properties. Saponins disrupt bacterial cell membranes, while alkaloids and flavonoids inhibit bacterial growth through their known antibacterial actions.

Assessing antibacterial activity is crucial for determining the potential therapeutic applications of the cream. The cream could be used topically for various skin conditions driven by bacterial infections if the significant antibacterial activity is demonstrated. However, further studies and clinical trials must confirm its efficacy and safety in practical applications.

The study evaluated the antibacterial efficacy of cream formulations F1, F2, F3, and F4, enriched with aloe vera gel and Nutgrass bulb extract. These components are recognized for their robust antibacterial properties contributed by saponins, sterols, acemannan, anthraquinones, alkaloids, tannins, Aperol, and flavonoids [19]-[20]—the evaluation involved measuring the diameters of inhibition zones, which reflect the antibacterial potency. Preliminary data analysis indicated a non-normal distribution ($p<0.05$), necessitating the use of nonparametric tests, specifically the Kruskal-Wallis test, which revealed significant differences across cream formulations with varying concentrations of Nutgrass bulb extract against S. aureus ATCC 25923 and E. coli ATCC 25922 bacteria. A subsequent Mann-Whitney test confirmed significant differences between each pair of formulations [22].

Research suggests that increased concentrations of the extracts intensify the presence of antibacterial compounds, enhancing their ability to penetrate bacterial cells, disrupt metabolism, and induce cell death [23]. The differential susceptibility of S. aureus (Gram-positive) and E. coli (Gram-negative) to these agents is due to their distinct cell wall structures, which may affect the penetration and effi cacy of the antibacterial agents.



Description:
F0: Cream with 5% aloe vera gel and without Nutgrass bulb Extract
F1: Cream with 5% Aloe Vera Gel and 25% Nutgrass bulb Extract
F2: Cream with 5% Aloe Vera Gel and 30% Nutgrass bulb Extract
F3: Cream with 5% Aloe Vera Gel and 35% Nutgrass bulb Extract
K (+): Gentamicin (positive control)
K (-): distilled water (negative control)

Figure 2. The result of the Antibacterial Activity Test of Cream Against (a) *S. aureus* ATCCA 25923 and (b) *E. coli* ATCC 25922 Bacteria using the Well Method

Table 7. The Result of Measurement of Inhibition Zone Diameter of Cream Supplemented with a Combination of Aloe Vera Gel and Nutgrass Rhizome Extract against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 Bacteria using the Well Method

Types of Bacteria Tests	Sample	Inhibition Zone	Category
S. aureus ATCCA 25923	F0	0,00 ± 0,00	No inhibition zone
	F1	3,42 ± 0,41	Weak
	F2	7,38 ± 0,76	Moderate
	F3	8,79 ± 0,29	Moderate
	K+ (*Gentamicin*)	23,93 ± 0,67	Strong
	K- (distilled water)	0 ± 0,00	No inhibition zone
E. coli ATCC 25922	F0	0 ± 0,00	No inhibition zone
	F1	2,47 ± 0,37	Weak
	F2	5,69 ± 0,40	Moderate
	F3	6,91 ± 0,14	Moderate
	K+ (*Gentamicin*)	26,76 ± 0,80	Strong
	K- (distilled water)	0 ± 0,00	No inhibition zone

Interestingly, cream F0, which contains 5% *Aloe vera* gel without Nutgrass extract, displayed no antibacterial effects. This outcome could be attributed to the fact that the antibacterial properties of *Aloe vera* are primarily associated with anthraquinones, which are predominantly found in the plant's latex rather than the gel [24]. While the gel offers various therapeutic benefits, its role in this study is focused more on its soothing and anti-inflammatory properties rather than direct antibacterial action. Moreover, a significant finding from the study was the synergistic effect observed when *Aloe vera* gel was combined with Nutgrass bulb extract. This combination enhances the antibacterial activity beyond a mere additive effect, suggesting a broader spectrum of therapeutic benefits.

In summary, this study highlights the substantial potential of integrating natural ingredients like *Aloe vera* gel and Nutgrass bulb extract into formulations, particularly for those seeking botanical alternatives for treating bacterial skin infections. Although the results are promising, they call for further comprehensive research and clinical trials to validate these creams' safety and efficacy fully. The findings underscore the complexity of cream formulations and emphasize the importance of understanding components' individual and combined effects in such mixtures.

CONCLUSION

Our study successfully spotlighted the enhanced antibacterial capabilities of cream formulations enriched with Aloe vera gel and Nutgrass bulb extract. When pitted against S. aureus ATCC 25923 and E. coli ATCC 25922, these formulations demonstrated marked antibacterial potency, underlining the potential of these botanical ingredients in synergistic combinations. These findings underscore the immense promise of integrating traditional botanical ingredients to address contemporary skin concerns, especially bacterial skin infections, in the broader realm of skincare and cosmetic formulations. Not only do they offer therapeutic benefits, but they also resonate with the growing consumer inclination towards natural and sustainable skincare solutions. As we progress, we should delve deeper into the mechanistic pathways these ingredients exert their effects, potentially optimizing and tailoring them for diverse skin types and conditions. Additionally, exploring these botanicals in varied cosmetic formulations could pave the way for a new era of holistic, nature-inspired skincare solutions.

ACKNOWLEDGEMEN

The authors would like to thank Sebelas Maret University, which has provided funding for this research through the 2023 Research Group grant program (Penelitian Hgr-UNS).

REFERENCES

[1] Y. Yanhendri and Y. S. W. Jenny, "Berbagai bentuk sediaan topikal dalam dermatologi," *Cerminan Dunia Kedokt.,* vol. 39, no. 6, pp. 423–430, 2012.

[2] N. F. Thamrin, "Formulasi sediaan krim dari ekstrak etanol kunyit (Curcuma domesticae. Val) dan uji efektivitas terhadap bakteri Staphylococcus *aureus*," *NASPA J.,* 2012.

[3] Badan Pengawas Obat dan Makanan (BPOM), "*Peraturan kepala badan pengawas obat dan makanan RI no. HM.03.03.1.43.12.14.7870 tentang kosmetika yang mengandung bahan berbahaya*," Jakarta, Indonesia, 2014.

[4] P. D. Darbre and P. Harvey, "Paraben esters: Review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks," *J. Appl. Toxicol.,* vol. 28, no. 5, pp. 561–578, 2008, doi: 10.1002/jat.1358.

[5] National Toxicology Program, "Bioassay of Butylated Hydroxytoluen (BHT) for Possible Carcinogenicity," *National Cancer Institute: Carcinogenesis Technical Report Series*, 1979.

[6] A. Widyasanti, A. Y. Rahayu, and S. Zein, "Pembuatan sabun cair berbasis virgin coconut oil (VCO) dengan penambahan minyak melati (Jasminum sambac) sebagai essential oil," Jurnal Teknotan, vol. 11, no. 2, pp. 1–10, 2017, doi: 10.24198/jt.vol11n2.1.

[7] I. L. Tarigan and A. Muadifah, *"Senyawa Antibakteri Bahan Alam,"* Malang, Indonesia: Media Nusa Creative (MNC Publishing), 2022.

[8] L. S. Cobra, "Aktivitas krim ekstrak rimpang kunyit (Curcuma longa Linn) terhadap bakteri Staphylococcus *aureus*," Doctoral dissertation, Stikes

Karya Putra Bangsa Tulungagung, 2018.

[9] N. Widyaningrum, M. Murrukmihadi, and S. K. Ekawati, "Pengaruh konsentrasi ekstrak etanolik daun teh hijau (Camellia sinesis L.) dalam sediaan krim terhadap sifat fisik dan aktivitas antibakteri," Sains Med. J. Kedokt. dan Kesehat., vol. 4, no. 2, pp. 147, 2012, doi: 10.30659/sainsmed.v4i2.371.

[10] N. S. Majid, P. V. Y. Yamlean, and G. Citraningtyas, "Formulasi dan uji efektivitas krim antibakteri ekstrak daun nangka (Artocarpus heterophyllus Lam.) terhadap bakteri Staphylococcus aureus," Pharmacon, vol. 8, no. 1, pp. 225, 2019, doi: 10.35799/pha.8.2019.29257.

[11] E. S. Hartanto and E. H. Lubis, "Pengolahan minuman sari lidah buaya (Aloevera linn.) (Warta IHP/J. AgroBased Industry.)," 2002.

[12] S. B. Ariyani and Hidayati, "Penambahan gel lidah buaya sebagai antibakteri pada sabun mandi cair berbahan dasar minyak kelapa additional oo aloe vera gel as antibactery in liquid bath soap based coconut oil," J. Ind. Has. Perkeb., vol. 13, no. 1, pp. 11–18, 2018, doi: 10.33104/jihp.v13i1.3675.

[13] N. Sulistyani, E. Kurniati, Yakup, and R. A. Cempaka, "Aktivitas antibakteri infusa daun lidah buaya (Aloe barbadensis miller)," *Jurnal Penelitian Saintek*, vol. 21, no. 2, pp. 121–126, 2016.

[14] P. D. A. E. Al-Snafi, "A review on Cyperus rotundus a potential medicinal plant," IOSR J. Pharm., vol. 6, no. 7, pp. 32–48, 2016, doi: 10.9790/3013-06723248.

[15] Murnah, "Pemeriksaan Kualitatif dan Kuantitatif Minyak Atsiri dan Tannin dalam Umbi Teki," Jurnal Kedokteran Diponegoro, vol. 30, pp. 3–4, 1995.

[16] I. M. Kusuma, S. Aunillah, and Y. S. Djuhariah, "Formulasi krim lulur scrub dari ekstrak etanol ubi jalar ungu (Ipomoea batatas (L.) Lam.) dan serbuk beras putih (Oryza sativa L.)," J. Farm. Udayana, vol. 10, no. 2, p. 177, 2021, doi: 10.24843/jfu.2021.v10.i02.p12.

[17] A. Wulandari, "Uji aktivitas antioksidan, antiaging, dan penghambatan enzim tirosinase dari ekstrak metanol daun kelor (Moringa oleifera) secara in vitro," Skripsi, Universitas Ahmad Dahlan, 2018.

[18] A. Garg, S. G. Aggarwal, and A. K. Sigla, "Spreading of semisolid formulation; an update," Pharm. Tecnol. North America, vol. 26, no. 9, pp. 84, 2002.

[19] R. A. Bara, G. D. Kandou, A. R. B. Ola, and J. Posangi, "Analisis senyawa antibiotik dari jamur simbion yang terdapat dalam ascidia didemnum mole di sekitar perairan bunaken-sulawesi utara," J. LPPM Bid. Sains dan Teknol., vol. 2, no. 2, pp. 28–35, 2015, doi:10.35801/jlppmsains.2.2.2015.10688

[20] G. F. Brooks, J. S. Bute, and S. A. Morse, Jawetz, Melnick, & Adelberg's Mikrobiologi Kedokteran, 23rd ed. New York: McGraw-Hill Companies Inc., 2004.

[21] M. T. Putri, "Identifikasi kandungan senyawa dan aktivitas antibakteri minyak atsiri sereh wangi (Cymbopogon nardus) terhadap bakteri Staphylococcus *aureus* dan Escherichia coli," Antimicrob. Agents Chemother., vol. 58, no. 12, pp. 7250–7257, 2014.

[22] R. Dewi and Marniza E., "International Standard of Serial Number 2622-1020

Resmila Dewi," J. Saintek Lahan Kering, vol. 2, no. 2, pp. 61–62, 2019. doi:10.32938/slk.v2i2.888

[23] R. Ningtyas, "Uji antioksidan dan antibakteri ekstrak air daun kecombrang (Etlingera elatior (Jack) R.M. Smith) sebagai pengawet alami terhadap Eschercia Coli dan Staphylococcus *aureus*," Skripsi, 2010, pp. 24–25.

[24] J. H. Hamman, "Composition and applications of Aloe vera leaf gel," Molecules, vol. 13, no. 8, pp. 1599–1616, 2008, doi: 10.3390/molecules13081599.