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Nutmeg Seed Essential Oil Hand Cream: Formulation and In Vitro Antibacterial Activity Against *Staphylococcus aureus*

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ABSTRACT

The skin of the palms is easily exposed to chemicals and microorganisms, making it prone to dryness, irritation, and infection, including Staphylococcus aureus. Excessive use of alcohol based hand sanitizers may worsen skin dryness, so an alternative hand cream with antibacterial properties is needed. This study aimed to formulate nutmeg seed essential oil into a hand cream, evaluate its physicochemical characteristics, and assess its in vitro antibacterial activity against S. aureus. This experimental study used essential oil concentrations of 5.0% (F1), 7.5% (F2), and 10.0% (F3); a formulation without oil (F0) was the negative control. Evaluations included organoleptic characteristics, homogeneity, emulsion type, pH, viscosity, spreadability, adhesiveness, freeze-thaw cycling, and hedonic tests. Antibacterial activity was evaluated by agar well diffusion (three technical replicates from one formulation batch) and analyzed using One-Way ANOVA and Tukey HSD. All formulations were homogeneous, semisolid oil-in-water (O/W) emulsions without phase separation after three freeze-thaw cycles. The pH values (6.56–7.64) did not meet the recommended skin pH range of 4.5–6.5, whereas viscosity (17,333.33–34,000.00 cPs), spreadability (6.25–6.90 cm), and adhesiveness met the reference requirements. Mean inhibition zones increased with oil concentration: 4.33 mm (F1, weak), 10.00 mm (F2, moderate), and 10.83 mm (F3, strong), versus 12.00 mm for the positive control (gentamicin 0.1% cream). ANOVA showed a significant difference among groups ($F = 35.679$; $p < 0.001$); F2 and F3 did not differ significantly from each other ($p = 0.954$) or from the positive control ($p = 0.498$ and 0.864). Nutmeg seed essential oil can be formulated into a hand cream that was physically stable under the freeze-thaw conditions tested and showed in vitro antibacterial activity; F2 (7.5%) was the most efficient concentration. These findings are preliminary: one bacterial strain and one batch were used, and irritation, moisturizing efficacy, and long-term stability were not evaluated.

Keywords: *Nutmeg essential oil; hand cream; Staphylococcus aureus; antibacterial activity*

Introduction

The skin of the palm is the part of the body most frequently exposed to air, sunlight, chemicals, and microorganisms, making it prone to dryness, irritation, and infection. Beyond its role in daily activities, palm skin also serves as a major vehicle for transmitting pathogenic bacteria, particularly *Staphylococcus aureus* (Suyasa, 2020). This gram-positive bacterium is capable of causing a wide spectrum of illnesses, ranging from superficial skin infections to life threatening systemic disease, including pneumonia, skin abscesses, and bacteremia (Y. R. Sari & Niken, 2022). Its pathogenicity is closely linked to extracellular matrix binding adhesion proteins that facilitate bacterial attachment and colonization of host tissue (Husna

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et al., 2018), and its presence on human hands has been directly confirmed in Indonesian clinical settings, including on the palms of community health center visitors (Ariyadi et al., 2023).

Given this urgency, the most practical measure for maintaining hand hygiene is the use of hand sanitizer; its main active ingredient, alcohol, functions as a disinfecting surfactant that disrupts microbial cell membranes (Rahmawati Amalia Yunia, 2020). However, frequent or prolonged use of alcohol based hand sanitizer is known to cause skin dryness and irritation, creating demand for an alternative capable of maintaining hand hygiene without compromising skin condition (Wulandari et al., 2023). A hand cream that combines emollient and humectant ingredients (lanolin and glycerin) with an antimicrobial active represents one such alternative; the moisturizing function is expected from the base, but moisturizing efficacy was not measured in this study. Natural ingredients are increasingly favored as active substances in topical cosmetic formulations for their comparatively favorable safety profile and added sensory benefits. This popularity, however, should not be equated with an inherently safer profile: essential oils, owing to their complex and variable chemical composition, may also cause skin irritation, sensitization, or allergic contact dermatitis, and should therefore be evaluated cautiously when incorporated into topical products (Taylor & Unakal, 2025). This risk is directly relevant to a leave-on product intended for repeated hand application, and it means that no claim of dermal safety can be made without irritation testing; the present stage of work therefore focuses on formulation, physical characteristics, and in vitro antibacterial activity, and irritation testing is left for subsequent work.

Essential oils in particular have demonstrated antibacterial potential, including nutmeg oil (*Myristica fragrans* Houtt.) (Ashokkumar & Simal-gandara, 2022) (Oo et al., 2021). Nutmeg is an economically valuable spice commodity in Indonesia (Kamelia & Silalahi, 2018), and its essential oil has been characterized as containing bioactive monoterpenes and phenylpropanoids including α-pinene, β-pinene, sabinene, limonene, and myristicin associated with antibacterial, antifungal, and insecticidal properties (Ashokkumar & Simal-gandara, 2022). Because the composition of an essential oil varies with origin, plant part, and extraction method, the activity of a given oil cannot be assumed for other batches. The antibacterial activity of these constituents is generally attributed to their lipophilic character, which allows them to partition into and disrupt the bacterial cell membrane, increasing membrane permeability, causing leakage of intracellular contents, and interfering with membrane associated enzymatic processes, ultimately leading to bacterial cell death (Ashokkumar & Simal-gandara, 2022). This mechanistic basis supports the rationale for exploring nutmeg essential oil as an antibacterial active in topical formulations.

Nutmeg seed essential oil was selected over other natural antibacterial ingredients for three reasons. First, nutmeg is a locally available commodity in Indonesia (Kamelia & Silalahi, 2018), which favors a locally sourced active. Second, the seed oil has been reported to inhibit *S. aureus*, including methicillin-resistant strains, in vitro at low concentrations. Third, antioxidant, antibacterial, and anti-inflammatory activities have been reported for nutmeg seed oil (Matulyte et al., 2020), a combination that may be advantageous for skin that is washed or sanitized frequently. These points justify the selection but do not demonstrate superiority over other essential oils, which was not compared here.

Nutmeg-derived materials have already been formulated into several topical products, but from different plant parts or extract types: a liquid soap containing nutmeg leaf extract (Moningka et al., 2020), an anti-aging cream containing ethanol extract of nutmeg fruit (Luhulima, 2021), an ointment containing ethanol extract of nutmeg seeds (F. Sari et al., 2025), and a rubbing oil containing nutmeg seed oil (Djarami et al., 2020). To the best of the authors' knowledge, a literature search of Google Scholar conducted on July 2025 using the keywords "hand cream nutmeg seed essential oil" found no published study that formulated nutmeg seed essential oil into a hand cream and evaluated its physical characteristics and antibacterial activity againts *S. aureus*. The novelty of this work therefore lies in (i) the use of nutmeg seed essential oil, rather than leaf or fruit extracts, in an O/W hand cream base; (ii) the comparison of three oil concentrations (5.0%, 7.5%, and 10.0%) with a base control and a gentamicin cream control within one design; and (iii) the combined evaluation of physical properties, freeze-thaw stability, and in vitro antibacterial activity. Accordingly, this study was conducted to formulate a hand cream containing nutmeg (*Myristica fragrans* Houtt.) seed essential oil and to evaluate its physical characteristics, freeze-thaw stability, and in vitro antibacterial activity against *S. aureus*, as a first step toward a hand cream with antibacterial properties. Skin irritation and moisturizing efficacy were not evaluated and require further study.

Methods

Materials and Tools

The materials used in this study were nutmeg essential oil (*Myristica fragrans* Houtt.), stearic acid, lanolin, triethanolamine (TEA), glycerin, methyl paraben, distilled water, Mueller-Hinton Agar (MHA), Nutrient Agar (NA), gentamicin sulfate 0.1% cream (PT. Bernofarm, No. Reg: GKL2002362029A1) as the positive control, and *Staphylococcus aureus* as the test microorganism. All pharmaceutical ingredients used in the formulation complied with their respective Certificates of Analysis (CoA). The nutmeg seed essential oil was obtained from Taman Teknologi Pertanian Cigombong, which had previously had its chemical composition

analyzed by gas chromatography-mass spectrometry (GC-MS) at the Regional Health Laboratory (Laboratorium Kesehatan Daerah) of DKI Jakarta Province. The equipment used included an analytical balance, hot plate, beaker glasses, measuring cylinders, glass stirring rods, porcelain evaporating dish, spatulas, thermometer, pH meter, Brookfield viscometer, microscope slides, Petri dishes, caliper, micropipettes, autoclave, incubator, laminar air flow cabinet, and other standard laboratory glassware required for the preparation, evaluation, and antibacterial testing of the hand cream formulations.

Essential Oil Composition (GC-MS)

The nutmeg seed essential oil used in this study was obtained from Taman Teknologi Pertanian Cigombong. Its chemical composition was analyzed by gas chromatography-mass spectrometry (GC-MS) at the Regional Health Laboratory (Laboratorium Kesehatan Daerah) of DKI Jakarta Province (report no. 2,3 / 5644, dated 02 December 2024). By comparing the collected data with the database library of the instrument, the chemicals were identified. Retention times, quality ratings, and the stated % content of the detected substances were all included in the laboratory report. The study lacked specific instrumental operating conditions and identification criteria. As a result, the information supplied by the testing facility was used to interpret the GC-MS data.

Formulation of Hand Cream

A blank formulation without nutmeg essential oil (F0) and three formulations with nutmeg essential oil (*Myristica fragrans* Houtt.) at quantities of 5.0% (F1), 7.5% (F2), adn 10.0% (F3) comprised the four hand cream formulations. The study by Sari et al. (2025), which examined nutmeg seed extract in an oinment formulation, was taken into consideration when choosing the essential oil concentrations. Preliminary formulation testing were used to develop and modify the hand cream base. Table 2 lists each formulation's composition.

Preparation of Hand Cream

The hand cream was prepared by first preparing the required equipment and ingredients according to the formulation. The oil phase, consisting of stearic acid and lanolin, was melted separately in different containers using a water bath. Meanwhile, glycerin was mixed with methyl paraben, triethanolamine (TEA), and distilled water to prepare the aqueous phase. The aqueous phase was heated at 70℃ until the ingredients were completely dissolved. The mortar and pestle were also preheated before the mixing process. After the stearic acid and lanolin had completely melted, both were transferred into the preheated mortar and triturated until a homogeneous oil phase was obtained. The aqueous phase was then added gradually to the oil phase while continuously triturating until a homogeneous cream base was formed. Once the cream base had formed and reached the desired consistency, nutmeg seed essential oil (*Myristica fragrans* Houtt.) was added

according to the concentration specified for each formulation. The mixture was then triturated until the essential oil was uniformly incorporated throughout the cream. The same preparation procedure was applied to all formulations with different concentrations of nutmeg seed essential oil (Loyd V. Allen, Jr. et al., 2011).

Evaluation of Hand Cream

Organoleptic Test

The organoleptic properties of each formulation were evaluated by direct visual observation, including appearance, color, odor, and consistency. The observations were performed before and after the stability test to identify any physical changes. A good hand cream should exhibit a uniform appearance, characteristic odor, appropriate color, and semi-solid consistency without phase separation (Loyd V. Allen, Jr. et al., 2011).

Homogeneity Test

The homogeneity test was carried out by spreading a small amount of cream on a glass slide and observing the presence of coarse particles or phase separation. A homogeneous preparation was indicated by a smooth texture with no visible coarse particles or agglomerates (Estefania et al., 2022).

Emulsion Type Test

The emulsion type was determined by the methylene blue dilution method. A small amount of cream was placed on a glass slide, followed by the addition of 1-2 drops of methylene blue solution, a water-soluble dye. The sample was then mixed and observed to determine the emulsion type. Uniform blue coloration indicated an oil-in-water (O/W) emulsion, whereas blue droplets dispered in an uncolored continuous phase indicated a water-in-oil (W/O) emulsion (Surini & Negoro, 2020).

pH Measurement

The pH of each formulation was measured using a calibrated digital pH meter at room temperature. Measurements were performed in triplicate, and the average value was recorded. According to the acceptable standard for topical cream preparations, the pH should be within the range of 4.5–6.5 to minimize the risk of skin irritation (Estefania et al., 2022).

Viscosity Test

The viscosity of the formulations was determined using a Brookfield viscometer equipped with spindle No. 4. Measurements were carried out at room temperature, and the viscosity values were expressed in centipoise (cPs). According to the Indonesian National Standard (SNI), the acceptable viscosity of cream preparations ranges from 2,000 to 50,000 cPs (Estefania et al., 2022).

Spreadability Test

Approximately 0.5 g of each formulation was placed between two glass plates. A specified load was applied, and the diameter of the spread was measured after

one minute. An acceptable spreadability value for topical cream preparations ranges from 5 to 7 cm (Estefania et al., 2022).

Adhesiveness Test

The adhesiveness test was performed by placing the cream sample between two glass slides under a standardized load. The time required for the slides to separate was recorded in seconds. A good cream preparation should exhibit an adhesion time between 2 and 300 seconds (Estefania et al., 2022).

Stability Test

Physical stability was evaluated using a freeze-thaw cycling test of three cycles (6 days in total). In each cycle, about 50 g of each formulation in a plastic cream jar was stored at 4 ± 2 °C for 24 h and then at 40 ± 2 °C for 24 h. After the third cycle, the formulations were compared with their initial state for changes in appearance, color, odor, consistency, and phase separation. A stable formulation should maintain its physical characteristics without evidence of instability (Lachman, Leon et al., 1994).

Hedonic Test

The hedonic test was conducted among 30 untrained volunteer panelists (aged 19-22 years, male or female, woth ability to detect, recognize, compare, and distinguish organoleptic characteristics, as well as an interest in organoleptic evalution) who met the inclusion criteria. Samples were coded and applied to te back of the hand. Each panelist rated texture, color, odor, and comfort of use on a 7 point hedonic scale, where 1 = strongly dislike, 2 = dislike, 3 = slightly dislike, 4 = neutral, 5 = slightly like, 6 = like, and 7 = strongly like. Scores were analyzed descriptively using Microsoft Excel. All volunteers provided written informed consent, and the protocol was approved by Health Research Ethics Committee Universitas 17 Agustus 1945 Jakarta approval no. 210/KEPK-UTA45JKT/EC/EXP/4./2026, dated 22 April 2026. The assesment used a structured hedonic questionnaire (Efendi et al., 2025).

Antibacterial Activity Test

The antibacterial activity of the hand cream formulations against *Staphylococcus aureus* ATCC 6538P PK/5 was evaluated using the agar well diffusion method (Balouri et al., 2016). A bacterial suspension was prepared and adjusted to a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, and inoculated onto the test medium. Wells with a diameter of 13 mm were prepared aseptically in the agar medium. Approximately 0.1 g of each hand cream formulation was directly placed into the respective wells without dilution. Gentamicin 0.1% cream was used as the positive control because it is a commercially available topical antibiotic with activity against *S. aureus* in the same semisolid dosage form, giving a familiar comparator; it is not a dose equivalent comparator for the essential oil, so the comparison describes relative activity only. The blank

formulation (F0) was used as the negative control. The plates were incubated at 37°C for 24 hours. After incubation, the clear zones were measured using a caliper. The inhibition zone values reported here are net values, calculated by subtracting the 13 mm well diameter from the total clear zone diameter. Each formulation was tested in three replicates prepared from the same formulation batch; these are technical replicates (n = 3) that estimate assay repeatability, not batch-to-batch variability. The mean inhibition zone diameter was calculated, and antibacterial activity was classified as weak (≤ 5 mm), moderate (5–10 mm), strong (10–20 mm), or very strong (> 20 mm) (Davis & Stout, 1971) (Ernawati & Jannah, 2021).

Statistical Analysis

Data are presented as mean ± SD. The antibacterial data comprised five groups (F0, F1, F2, F3, and positive control) with three replicates each (N = 15). Data normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk test, followed by Levene's test to evaluate the homogeneity of variances. Differences among formulations were analyzed using One-Way Analysis of Variance (ANOVA). When statistically significant differences were observed ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post hoc test was performed (Field, 2018). pH, viscosity, spreadability, and adhesiveness (n = 3 per formulation) were compared among F0–F3 with the same procedure. Analyses were performed using IBM SPSS Statistics version 27.

Results and Discussion

Chemical Composition of the Essential Oil

The major compounds reported in the laboratory GC-MS analysis of the nutmeg seed essential oil are listed in Table 1. The oil was dominated by α-pinene (19.66% relative area), consistent with the monoterpene and phenylpropanoid rich profile described for nutmeg seed oil (Ashokkumar & Simal-gandara, 2022) (Oo et al., 2021). The antibacterial results below apply to this oil batch.

Table 1. Major Compounds in Nutmeg Seed Essential Oil (GC-MS)

No.	Compound	Retention time (min)	Relative area (%)
1	α-pinene	3.727	19.66
2	β-pinene	5.122	14.01
3	D-limonene	7.304	3.69
4	Myristicin	50.890	16.68

Hand Cream Formulation

Four hand cream formulations were successfully prepared, consisting of a blank formulation without nutmeg seed essential oil (F0) and three formulations

containing nutmeg seed essential oil (*Myristica fragrans* Houtt.) at concentrations of 5.0% (F1), 7.5% (F2), and 10.0% (F3). The formulations were prepared using stearic acid and lanolin as the oil-phase components, while glycerin, triethanolamine (TEA), methyl paraben, and distilled water constituted the aqueous phase. The formulations were successfully formed into semisolid hand creams suitable for subsequent physical evaluation and antibacterial testing (Skhreli et al., 2022).

Table 2. Formulations of Nutmeg Essential Oil Hand Cream

Material (%)	F0	F1	F2	F3	Function
Essential Oil Nutmeg Seed (*Myristica fragrans* Houtt.)	-	5.0	7.5	10.0	Active substance
Stearic acid	18.0	18.0	18.0	18.0	Emulsifying agent
Glycerin	3.0	3.0	3.0	3.0	Humectant
Lanolin	2.0	2.0	2.0	2.0	Emulsifying agent
Triethanolamine (TEA)	1.0	1.0	1.0	1.0	Surfactant
Methyl Paraben	0.1	0.1	0.1	0.1	Preservative
Demineralized water	ad 100	ad 100	ad 100	ad 100	Solvent

Physical Evaluation of Hand Cream

Organoleptic Test

The organoleptic evaluation was performed to observe the physical appearance, color, and odor of the hand cream formulations. The results are presented in Table 3. All formulations (F0, F1, F2, and F3) exhibited a semisolid consistency and a white color. Differences were observed in the odor characteristics. F0, which did not contain nutmeg seed essential oil, exhibited the characteristic odor of lanolin, whereas F1, F2, and F3 exhibited the characteristic odor of nutmeg seed essential oil. The consistent semisolid consistency and white color across all formulations indicate that the addition of nutmeg seed essential oil at concentrations of 5.0–10.0% did not cause observable changes in the physical appearance of the cream. Based on the GC-MS analysis report from the Regional Health Laboratory of DKI Jakarta Province, the essential oil contained volatile compounds belonging to the monoterpene and phenylpropanoid groups, including α-pinene, β-pinene, D-limonene, and myristicin, which contribute to the characteristic aroma of nutmeg essential oil. Overall, the organoleptic evaluation indicated that the addition of nutmeg seed essential oil at concentrations of 5.0%, 7.5%, and 10.0% resulted in hand cream formulations with consistent physical appearance while imparting the characteristic odor of the essential oil (Surini & Negoro, 2020).

Table 3. Organoleptic Characteristics of the Hand Cream Formulations

Formulation	Shape	Color	Odor
F0	Semi Solid	White	Specific lanolin odor
F1	Semi Solid	White	Specific nutmeg seed essential oil odor
F2	Semi Solid	White	Specific nutmeg seed essential oil odor
F3	Semi Solid	White	Specific nutmeg seed essential oil odor

Homogeneity Test

The homogeneity test was conducted to evaluate the uniformity of the hand cream formulations. As shown in Table 4, all formulations (F0, F1, F2, and F3) were homogeneous, with no coarse particles or lumps observed on the glass slide. This result may be related to the use of stearic acid and lanolin in the formulation, which contribute to the formation and stabilization of the emulsion system. Thus, all formulations met the homogeneity requirement for a semisolid topical preparation (Estefania et al., 2022).

Table 4. Homogeneity of the Hand Cream Formulations

Formulation	Homogeneity Test
F0	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous

Emulsion Type Test

The emulsion type test was conducted to determine whether the hand cream formulations were oil-in-water (O/W) or water-in-oil (W/O) emulsions using the methylene blue dilution method. As shown in Table 5, all formulations (F0, F1, F2, and F3) exhibited an oil-in-water (O/W) emulsion type, attributable to the emulsifying system consisting of stearic acid and triethanolamine (TEA). The O/W emulsion type is suitable for topical hand cream preparations because water constitutes the external phase, resulting in a formulation that is relatively easy to spread and provides a lighter sensation during application (Surini & Negoro, 2020).

Table 5. Emulsion Type of the Hand Cream Formulations

Formulation	Emulsion Type
F0	O/W
F1	O/W
F2	O/W
F3	O/W

pH Measurement

The pH measurement was conducted to evaluate the acidity or alkalinity of the hand cream formulations and their compatibility with the skin. The results are presented in Table 6. The pH values of F0, F1, F2, and F3 were 7.64 ± 0.04, 7.61 ± 0.04, 6.61 ± 0.04, and 6.56 ± 0.03, respectively, which were lower at the higher essential oil concentrations. Based on the skin pH range of 4.5–6.5 used as the acceptance criterion, none of the formulations completely met the recommended range; F2 and F3 approached the upper limit. One-way ANOVA showed a significant effect of formulation on pH ($F(3,8) = 760.2$; $p < 0.001$); F2 and F3 were significantly lower than F0 and F1 ($p < 0.001$), whereas F0 vs F1 and F2 vs F3 did not differ. The pH decrease was therefore step-like rather than proportional to oil concentration, and its cause cannot be resolved from these data; additional concentrations should be tested. The relatively high pH may be related to the presence of triethanolamine (TEA), an alkaline component that participates in formation of the stearate emulsifying system. Further formulation optimization is therefore required to adjust the pH closer to the physiological pH range of the skin (Estefania et al., 2022).

Table 6. pH of the Hand Cream Formulations (mean ± SD)

Formulation	pH
F0	7.64 ± 0.04
F1	7.61 ± 0.04
F2	6.61 ± 0.04
F3	6.56 ± 0.03

Viscosity Test

Viscosity was measured using a Brookfield viscometer with spindle No. 4 at 1.5 rpm. The results (Table 7) showed a non-linear variation with increasing essential oil concentration – increasing from F0 to F1, decreasing in F2, and increasing again to the highest value in F3 – suggesting the viscosity was influenced by several interacting formulation factors rather than essential oil concentration alone. All values ranged from 17,333.33 to 34,000.00 cPs, within the acceptable range

of 2,000–50,000 cPs (Estefania et al., 2022). ANOVA indicated a significant difference among formulations ($F(3,8) = 8.92$; $p = 0.006$), but only F3 differed significantly from F0 and F2 ($p = 0.017$ and 0.007); the other pairs did not differ. Essential oil concentration therefore had no consistent effect on viscosity, and the large SDs (up to ± 6,000 cPs) limit interpretation.

Table 7. Viscosity of the Hand Cream Formulations (mean ± SD)

Formulation	Viscosity (cPs)
F0	20,000.00 ± 6,000.00
F1	26,666.67 ± 4,163.33
F2	17,333.33 ± 3,055.05
F3	34,000.00 ± 3,464.10

Spreadability Test

Spreadability values increased consistently with increasing nutmeg seed essential oil concentration, from 6.25 ± 0.05 cm (F0) to 6.90 ± 0.10 cm (F3) (Table 8), all within the recommended range of 5–7 cm. ANOVA showed a significant effect ($F(3,8) = 49.6$; $p < 0.001$); all oil-containing formulations spread significantly more than F0 ($p \leq 0.002$), and F3 more than F1 and F2 ($p \leq 0.007$), while F1 and F2 did not differ ($p = 0.319$). The increasing concentration of essential oil may have reduced cohesive forces within the cream matrix, allowing the formulation to spread more readily and improving application comfort.

Table 8. Spreadability of the Hand Cream Formulations (mean ± SD)

Formulation	Spreadability (cm)
F0	6.25 ± 0.05
F1	6.55 ± 0.05
F2	6.65 ± 0.05
F3	6.90 ± 0.10

Adhesiveness Test

Adhesiveness values decreased consistently with increasing essential oil concentration (Table 9), from 4.72 ± 0.05 s (F0) to 3.27 ± 0.05 s (F3), remaining within the established requirement of 2–300 seconds. ANOVA showed a significant effect ($F(3,8) = 543.7$; $p < 0.001$), and all pairwise differences were significant ($p \leq 0.013$). This trend is consistent with the spreadability results, in which formulations with higher essential oil concentrations exhibited greater spreading and a shorter adhesion time.

Table 9. Adhesiveness of the Hand Cream Formulations (mean ± SD)

Formulation	Adhesiveness (seconds)
F0	4.72 ± 0.05
F1	4.55 ± 0.05
F2	3.81 ± 0.05
F3	3.27 ± 0.05

Stability Test

Physical stability was evaluated using a freeze-thaw cycling test over three cycles (6 days total). All formulations (F0, F1, F2, and F3) remained physically stable, with no phase separation observed (Table 10). F0 maintained its white color, while F1, F2, and F3 maintained a slightly yellowish-white appearance. These results indicate that increasing the essential oil concentration from 5.0% to 10.0% did not cause observable physical instability under the short freeze-thaw conditions tested; long-term and real-time stability were not evaluated.

Table 10. Freeze-Thaw Stability of the Hand Cream Formulations

Formulation	Initial Color	3rd Cycle Color	Phase Separation
F0	White	White	No separation occurs
F1	White, slight yellowish tinge	White, slight yellowish tinge	No separation occurs
F2	White, slight yellowish tinge	White, slight yellowish tinge	No separation occurs
F3	White, slight yellowish tinge	White, slight yellowish tinge	No separation occurs

Hedonic Test

Based on the hedonic test results for texture, color, aroma, and ease of application, all hand cream formulations (F0, F1, F2, and F3) were generally accepted by the panelists 30 panelists. Formula F0 showed predominantly neutral responses; F1 showed better acceptance than F0, particularly for color and ease of application; F2 received the greatest number of "like" responses for color; and F3 was identified as the most preferred formulation, achieving the highest acceptance scores for texture, aroma, and, ease of application.

Antibacterial Activity Against Staphylococcus aureus

The antibacterial activity of the hand cream formulations against Staphylococcus aureus ATCC 6538P PK/5 was evaluated using the agar well diffusion method (Table 11); the values are net inhibition-zone diameters (excluding

the 13 mm well). The negative control (F0) showed no inhibition zone (0.00 ± 0.00 mm), confirming that the cream base did not exhibit antibacterial activity. All formulations containing nutmeg seed essential oil exhibited antibacterial activity: F1 (5.0%) produced a mean inhibition zone of 4.33 ± 2.25 mm (weak), F2 (7.5%) produced 10.00 ± 1.32 mm (moderate), and F3 (10.0%) produced the largest inhibition zone of 10.83 ± 1.53 mm (strong), approaching the positive control, gentamicin (12.00 ± 1.32 mm). This activity may be associated with the bioactive constituents of nutmeg essential oil identified in the GC-MS analysis report from the Regional Health Laboratory of DKI Jakarta Province, including α-pinene, β-pinene, D-limonene, and myristicin.

One-Way ANOVA showed a statistically significant difference in mean inhibition zone diameters among the groups ($F(4,10) = 35.679$; $p < 0.001$). The Tukey HSD post hoc test showed that F0 differed significantly from the other groups ($p < 0.05$), confirming that the cream base without essential oil did not exhibit antibacterial activity. The positive control differed significantly from F1, whereas no statistically significant differences were observed between the positive control and F2 ($p = 0.498$) or F3 ($p = 0.864$), and F2 and F3 did not differ significantly from each other ($p = 0.954$). These findings indicate that although F3 produced the highest mean inhibition zone, its antibacterial activity was statistically comparable to F2 and the positive control. Therefore, increasing the essential oil concentration from 7.5% to 10.0% did not result in a statistically significant improvement in antibacterial activity. With $n = 3$ per group, the absence of a significant difference between F2/F3 and the positive control indicates insufficient evidence of a difference, not demonstrated equivalence. Levene's test did not reject homogeneity of variances ($p = 0.171$) and Shapiro-Wilk p-values were > 0.05 in groups with variance (F0 was constant), but these checks have little power at this sample size. A Kruskal–Wallis test ($H = 12.06$; $p = 0.017$) and an ANOVA excluding F0 ($F(3,8) = 12.75$; $p = 0.002$) supported an overall difference, whereas Welch's ANOVA excluding F0 was borderline ($p = 0.041$). Considering the comparable antibacterial effectiveness and the lower amount of essential oil required, F2 (7.5%) can be considered the more efficient concentration among the tested formulations, in terms of antibacterial effectiveness and efficiency of active ingredient use rather than as a formal optimization result.

Table 11. Inhibition Zone Diameters (net of 13 mm well) against *S. aureus* ATCC 6538P PK/5

Formula	R1 (mm)	R2 (mm)	R3 (mm)	Mean ± SD (mm)	Inhibitory Activity
K+ (Gentamicin)	13	12.5	10.5	12.00 ± 1.32	Strong
F0	0	0	0	0.00 ± 0.00	-
F1 (5.0%)	6.5	2	4.5	4.33 ± 2.25	Weak
F2 (7.5%)	11.5	9.5	9	10.00 ± 1.32	Moderate
F3 (10.0%)	12.5	10.5	9.5	10.83 ± 1.53	Strong

Note: K+ = gentamicin 0.1% cream (positive control); F0 = cream base (negative control). Mean ± SD of three technical replicates from one formulation batch.



Figure 1. Hand Cream Formulations Containing Nutmeg Essential Oil (*Myristica fragrans* Houtt.)

Limitations of the Study

This study has several limitations. The antibacterial test used one reference strain (*S. aureus* ATCC 6538P PK/5) and three replicates taken from a single formulation batch; these are technical rather than independent replicates, so batch-to-batch variability is not captured and the ANOVA may overstate certainty (pseudoreplication). Agar well diffusion is semi-quantitative, and the diffusion of a lipophilic oil through agar may underestimate its activity; no MIC or MBC was determined. The positive control differs from the test samples in chemical class and dose, so comparisons with it are descriptive. The pH of all formulations was outside the recommended skin range. Stability was assessed only by a short freeze-thaw test, the hedonic test was descriptive, and skin irritation, moisturizing efficacy, and

in vivo antibacterial efficacy were not evaluated. Finally, the GC-MS analysis was performed externally. These limitations mean that the findings are preliminary.

Conclusion

Nutmeg seed essential oil (*Myristica fragrans* Houtt.) at 5.0–10.0% could be incorporated into homogeneous, semisolid O/W hand cream formulations that remained physically stable after three freeze-thaw cycles. Viscosity (17,333.33–34,000.00 cPs), spreadability (6.25–6.90 cm), and adhesiveness met the reference requirements, whereas the pH (6.56–7.64) exceeded the recommended skin range of 4.5–6.5 and requires optimization. In vitro, the formulations showed antibacterial activity against *S. aureus* ATCC 6538P PK/5 that increased with oil concentration (4.33, 10.00, and 10.83 mm for F1, F2, and F3; positive control 12.00 mm). F2 and F3 did not differ significantly from each other or from the 0.1% gentamicin cream under the conditions tested, so F2 (7.5%) was the most efficient concentration among those tested. These findings are preliminary: they rest on one bacterial strain and technical replicates from one batch, and they do not establish equivalence to gentamicin, in vivo efficacy, skin safety, or moisturizing efficacy.

Suggestions

Further formulation optimization is recommended to adjust the pH of the hand cream closer to the physiological skin pH range of 4.5–6.5, for example by re-evaluating the concentration of triethanolamine (TEA) or incorporating a pH-adjusting agent. Future studies are also suggested to evaluate the long-term stability, safety, and skin irritation potential of the formulation through dermal irritation testing, as well as to explore the 7.5% (F2) concentration further as the most efficient formulation, given its antibacterial activity that is statistically comparable to the 10.0% formulation and the positive control while requiring a lower amount of active ingredient. Further studies should (i) repeat the antibacterial test with at least three independently prepared formulation batches; (ii) include additional strains (including methicillin-resistant *S. aureus* and other skin flora) and determine MIC/MBC; (iii) evaluate moisturizing efficacy and skin irritation; and (iv) involve a larger hedonic panel. Such evidence is needed before this hand cream can be proposed as a topical antibacterial and moisturizing product.

References

Ariyadi, R., Maulani, P. A., Ruhimat, U., & Hidana, R. (2023). Identification Of Staphylococcus Aureus Bacteria On The Palms Of Visitors To Panumbangan Health Center. *Mukhtabar : Journal Of Medical Laboratory Technology*, *1*(2), 57–64. Https://Doi.Org/10.52221/Mjmlt.V1i2.376

Ashokkumar, K., & Simal-Gandara, J. (2022). *Nutmeg (Myristica Fragrans Houtt .)*

Essential Oil : A Review On Its Composition , Biological , And Pharmacological Activities. *December 2021*, 2839–2851. Https://Doi.Org/10.1002/Ptr.7491

Balouri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods For In Vitro Evaluating Antimicrobial Activity: A Review. *Journal Of Pharmaceutical Analysisi*, *6*(2), 71–79. Https://Doi.Org/Https://Doi.Org/10.1016/J.Jpha.2015.11.005

Davis, W. W., & Stout, T. R. (1971). Disc Plate Method Of Microbiological Antibiotic Assay. *American Society For Microbiology*, *22*(4), 659–665. Https://Doi.Org/Https://Doi.Org/10.1128/Am.22.4.659-665.1971

Djarami, J., Pelu, A. D., & Bugis, S. (2020). Formulasi Minyak Gosok Dari Biji Pala (Myristica Fragrans) Dan Uji Antibakteri Terhadap Bakteri Staphylococcus Aureus. *Jurnal Fisioterapi Dan Ilmu Kesehatan Sisthana (JUFDIKES)*. Https://Doi.Org/Https://Doi.Org/10.55606/Jufdikes.V2i1.152

Efendi, Y. N., Sambodo, D. K., & Pratiwi, Dyah Anggraeni Budhi. (2025). *Evaluasi Uji Klinik Fase Satu Dan Uji Hedonik Krim Kombinasi Ekstrak Rumput Laut Merah Sumbawa Dan Ekstrak Kulit Buah Jeruk Lemon*. *9*(1), 40–46. Https://Journal.Stikessuryaglobal.Ac.Id/Index.Php/Hspj/Article/View/1001

Ernawati, E., & Jannah, N. (2021). Aktivitas Antimikroba Perasan Daun Kirinyuh (Chromolaena Odorata L.) Terhadap Candida Albicans Dan Pseudomonas Aeruginosa. *Jurnal Kedokteran Dan Kesehatan*, *17*(2), 137. Https://Doi.Org/10.24853/Jkk.17.2.137-144

Estefania, K. V., Silalahi, J., Sumaiyah, S., & Satria, D. (2022). Formulation And Evaluation Of Cream Turmeric Extract Preparations From Turmeric Rhizomes (Curcuma Domestica Val.). *Indonesian Journal Of Pharmaceutical And Clinical Research*, *5*(1), 01–09. Https://Doi.Org/10.32734/Idjpcr.V5i1.6479

Field, A. (2018). *Discoverng Statistics Using IBM SPSS Statistics*. SAGE Publications.

Husna, C. A., Hanina, Humaryanto, Gading, P. W., Aurora, W. I. D., & Harahap, H. (2018). Nurul Iman Tentang Infeksi Staphylococcus Aureus Di Kulit Dengan Metode Penyuluhan. *AVERROUS: Jurnal Kedokteran Dan Kesehatan Malikussaleh*, *5*(2), 99. Https://Doi.Org/10.29103/Averrous.V4i2.1041

Kamelia, L. P. L., & Silalahi, P. Y. (2018). *BUAH PALA*. *11*(April), 96–101.

Lachman, Leon, E., Lieberman, Herbert A., E., Kanig, Joseph L., E., & Siti Suyatmi, T. (1994). *Teori Dan Praktek Farmasi Industri 3* (H. A. L. Dan J. L. K. Leon Lachman (Ed.); Third Edit). Jakarta: UI-Press, 1994. Https://Lib.Ui.Ac.Id/Detail?Id=137426&Lokasi=Lokal

Loyd V. Allen, Jr., P., Nicholas G. Popovich, P., & Howard C. Ansel, P. (2011). *PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS* (D. B. Troy (Ed.); 9th Editio).

Luhulima, A. M. (2021). *Formulasi Dan Uji Sifat Fisik Sediaan Krim Ekstrak Etanol Buah Pala (Myristica Fragrans) Sebagai Anti Aging*. *5*(2), 46–59.

Matulyte, I., Jekabsone, A., Jankauskaite, L., Zavistanaviciute, P., Sakiene, V., Bartkiene, E., Ruzauskas, M., Kopustinskiene, D. M., Santini, A., & Bernatoniene, J. (2020). *The Essential Oil And Hydrolats From Myristica Fragrans Seeds With Magnesium Aluminometasilicate*.

Moningka, M. V., Pareta, D. N., Hariyadi, H., & Potalangi, N. (2020). Formulasi Dan Uji Aktivitas Antibakteri Sediaan Sabun Cair Ekstrak Daun Pala Myristica Fragrans Houtt. *Biofarmasetikal Tropis*, *3*(2), 17–26. Https://Doi.Org/10.55724/J.Biofar.Trop.V3i2.280

Oo, T., Saiboonjan, B., Srijampa, S., Srisrattakarn, A., Suttahanut, K., Chanawong, A., Lulitanond, A., & Tippayawat, P. (2021). Inhibition Of Bacterial Efflux Pumps By Crude Extracts And Essential Oil From Myristica Fragrans Houtt. (Nutmeg) Seeds Against Methicillin-Resistant Staphylococcus Aureus. *Molecules*. Https://Doi.Org/Https:Doi.Org/10.3390/Molecules26154662

Rahmawati Amalia Yunia. (2020). *Disinfektan Surfaktan*. *July*, 1–23.

Sari, F., Angreni, W., Halimah, N., Meutia, R., Sabrina, N. I., Farmasi, D., Kuala, U. S., Aceh, B., Aceh, B., Aceh, B., Farmasi, D., Kuala, U. S., & Aceh, B. (2025). *FORMULASI DAN UJI ANTIBAKTERI Staphylococcus Aureus EKSTRAK ETANOL BIJI BUAH PALA (Myristica Fragrans Houtt) DALAM SEDIAAN SALEP FORMULATION AND ANTIBACTERIAL TESTING Staphylococcus Aureus ETHANOL EXTRACT OF NUTMEG SEEDS (Myristica Fragrans Houtt) Pub*. *9*(2), 157–163.

Sari, Y. R., & Niken. (2022). Uji Daya Hambat Ekstrak Daun Kirinyuh (Chromolaena Odorata) Terhadap Pertumbuhan Bakteri Staphylococcus Aureus. *Jurnal Kesehatan Saintika Meditory*, *4*(4657), 78–84.

Skhreli, R., Terziu, R., Memushaj, L., & Dhamo, K. (2022). *Formulation And Stability Evaluation Of A Cosmetics Emulsion Loaded With Different Concentrations Of Synthetic And Natural Preservative*. *5*(1). Https://Doi.Org/Https://Doi.Org/10.62400/Jbs.V5i1.6373

SURINI, S., & NEGORO, N. M. (2020). DEVELOPMENT OF MICROEMULSION AND WATER/OIL/WATER MULTIPLE EMULSION CONTAINING BETA-ARBUTIN, LACTIC ACID, AND SODIUM ASCORBYL PHOSPHATE. *International Journal Of Applied Pharmaceutics*, 212–220. Https://Doi.Org/10.22159/Ijap.2020.V12s1.FF048

Suyasa, I. B. O. (2020). Gambaran Methicillin Resistant Staphylococcus Aureus (MRSA) Pada Petugas Kesehatan RSUD Wangaya Kota Denpasar. *Meditory : The Journal Of Medical Laboratory*, *8*(1), 46–52. Https://Doi.Org/10.33992/M.V8i1.1074

Taylor, T. A., & Unakal, C. G. (2025). Staphylococcus Aureus Infection. In *Statpearls*. Http://Www.Ncbi.Nlm.Nih.Gov/Pubmed/9709046

Wulandari, S., Wulan, P., Pane, E. B., Adzki, R. A., & Saputra, D. (2023). *Perilaku*

Hidup Bersih Dan Sehat (Tata Cara Pembuatan Hand Sanitizer). *3*(1), 38–44.