International Journal Of Health, Engineering And Technology (IJHET) *E-ISSN 2829 – 8683*
Volume 4, Number 4, November 2025, Pages 946 – 956
Email :editorijhess@gmail.com

Formulation and Physical Stability Test of Anti-Mosquito Lotion Preparations Combining Lemongrass (Cymbopogon citratus) and Cosmos caudatus Kunth. Extracts

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Abstract

Dengue hemorrhagic fever (DHF) remains a major public health issue in Indonesia, with 210,644 confirmed cases and 1,239 deaths reported by week 43 of 2024. The frequent use of DEET-based mosquito repellents raises health and environmental concerns, prompting the need for safer natural alternatives. This study aimed to formulate and evaluate a mosquito repellent lotion containing lemongrass (Cymbopogon citratus) and kenikir (Cosmos caudatus Kunth.) leaf extracts. Extracts were obtained through 96% ethanol maceration for five days, and six formulations (F0–F5) were developed using a full factorial experimental design. Physical quality tests included organoleptic, homogeneity, pH, spreadability, adhesiveness, and accelerated stability evaluations, while repellency effectiveness was assessed against Aedes aegypti mosquitoes. Results showed all formulations met pharmaceutical standards, exhibiting stable texture, skin-compatible pH (5.40–5.70), optimal spreadability (6.5–7.0 cm), and good adhesiveness (>4 seconds). Among them, formulation F3 (5% lemongrass + 5% kenikir extracts) demonstrated the highest repellent effectiveness (88%), comparable to a commercial DEET-based product, with no significant physical changes during stability testing. In conclusion, the lemongrass–kenikir lotion provides effective, stable, and safe mosquito repellent activity, offering a promising natural alternative for DHF vector control.

Keywords: Accelerated Stability Testing, Cosmos Caudatus Kunth, Cymbopogon Citratus, Mosquito Repellent Lotion, Natural Insecticides

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) cases in Indonesia have continued to increase significantly in recent years and pose a serious public health threat. According to WHO data, Indonesia experienced a surge in dengue incidents in 2024, with 88,593 confirmed cases and 621 deaths as of April 30, 2024, an approximately threefold increase compared to the same period in 2023 (WHO, 2024). The latest data from the Ministry of Health shows that as of the 43rd week of 2024, 210,644 cases with 1,239 deaths due to DHF were recorded in 259 districts/cities across 32 provinces, while the number of reported dengue suspects reached 624,194 (Ministry of Health, 2024). This increase in cases is not only occurring in endemic areas but also extending to areas previously free of DHF, indicating a worrying geographic expansion (Kasman et al., 2025).

Indonesia, as a tropical country, has ideal climatic conditions for the development of Aedes aegypti and Aedes albopictus mosquitoes, the primary vectors for dengue virus transmission. Its geographical location, being the world's largest archipelago with 29 identified species of malaria-carrying mosquitoes, creates complexities in vector control (WHO Indonesia, 2023). Climate change and the El Niño phenomenon further exacerbate the situation, with previously malaria-free mountainous areas now becoming hotspots for mosquito-borne diseases due to rising temperatures (Ministry of Health, 2024). Urbanization, population density, and high mobility also contribute to the spread of dengue, with Indonesia recording the highest number of dengue cases in the ASEAN region, with approximately 219,000 cases and 774 deaths (WHO Indonesia, 2024).

Various commercial mosquito repellents, such as Autan, Soffel, and Calladine Mosquito, commonly used by the public, contain DEET (diethyltoluamide) as the main active ingredient. While

effective as a repellent, DEET causes a variety of concerning side effects, including severe skin reactions such as large blisters, burning sensations, mucous membrane irritation, seizures, ataxia, encephalopathy, and even coma upon excessive exposure (Roy, 2017). Recent research has shown that DEET can inhibit cholinesterase activity in the central nervous system of both insects and mammals, and its toxic effects are increased when combined with other pesticides such as carbamates (Diptera Journal, 2024). Experimental studies have also shown that exposure to a combination of DEET and permethrin in pregnant rats can cause kidney disease, prostate disease, and various other diseases in subsequent generations (Peng et al., 2022).

The need for safe and effective natural alternatives to synthetic repellents is becoming increasingly urgent as public awareness of environmentally friendly products increases. Plant-based biopesticides and repellents offer significant advantages because they are eco-friendly, non-phytotoxic, and safe for agro-ecosystems and the environment (Ayilara et al., 2023). Botanical pesticides contain bioactive compounds such as steroids, alkaloids, tannins, terpenes, phenols, flavonoids, and resins that exhibit antifungal, antibacterial, antioxidant, or insecticidal properties with various mechanisms of action, including repellency, inhibition of protein denaturation, and other effects (Horizone Publishing, 2024). Indonesia, with its abundant biodiversity, has great potential to develop natural repellents, in line with the growing consumer demand for a back-to-nature approach, perceived as healthier than synthetic chemicals (Lopez et al., 2025).

Lemongrass (Cymbopogon citratus) and Cosmos caudatus (Cosmos caudatus Kunth.) plants show great potential as natural active ingredients for mosquito repellents based on various recent studies. Lemongrass contains the main components citronellal, citronellol, and geraniol, which have been proven effective as repellents with protection levels of up to 82.45-95% at various concentrations (Luker & Zhai, 2023; Arlitasari et al., 2024). Research shows that lemongrass essential oil at concentrations of 4% and 6% is effective in repelling and killing mosquitoes, and has been registered with the US-EPA as an effective and safe repellent with low toxicity in animal tests (UNNES, 2023). Meanwhile, Cosmos caudatus contains saponin compounds, polyphenols, and essential oils that not only function as mosquito repellents but also have potential as natural insecticides (Suprianto et al., 2021). Recent research shows that kenikir leaf extract at a concentration of 15% can provide optimal protection as a mosquito repellent with an LC50 of 28% against Aedes aegypti larvae in 24 hours (Juriskes, 2024).

This study aims to formulate and evaluate a mosquito repellent lotion containing a combination of lemongrass and kenikir extracts obtained through a maceration method, as well as to test its physical stability and effectiveness as a natural repellent. The urgency of this study lies in the urgent need to develop safe and effective repellent alternatives to reduce dependence on potentially dangerous synthetic chemicals, especially considering the drastic increase in dengue fever cases in Indonesia. The novelty of this study lies in the synergistic combination of lemongrass and kenikir extracts in a lotion formulation that has not been widely explored, as well as the use of accelerated stability testing using a climatic chamber to ensure product quality and safety within an optimal storage period.

RESEARCH METHODS

Types and Methods of Research

This study used a quantitative approach with a true experimental design aimed at testing the effectiveness of a mosquito repellent lotion formulation containing a combination of lemongrass and kenikir extracts. According to Creswell (2018), experimental research is the most appropriate approach to test cause-and-effect relationships by controlling independent variables and measuring their effects on dependent variables. Sugiyono (2023) stated that an experimental design allows researchers to observe the effect of treatments on dependent variables with strict control over confounding variables. This study adopted a full factorial design with six formulation variations (F0–F5) to evaluate the effect of lemongrass and kenikir extract concentrations on the physical characteristics and effectiveness of the repellent lotion.

The research method used was a laboratory experiment with a descriptive and inferential analysis approach to evaluate the lotion formulation. Creswell and Plano Clark (2022) explained that experimental pharmaceutical research requires a systematic approach starting from material preparation, formulation, and final product evaluation. This study used the maceration extraction method in accordance with the

recommendations of Emzir (2021), who stated that maceration is an effective extraction method for thermolabile compounds using 96% ethanol as a solvent. The lotion formulation used an oil-in-water (o/w) type emulsification technique with a two-phase system stabilized using an emulsifier, following the principles of modern pharmaceutical technology (González-González et al., 2022).

Data Analysis Instruments and Techniques

The research instruments used included extraction equipment, formulation, and evaluation of lotion preparations in accordance with pharmacopoeial standards and ICH (International Council for Harmonization) guidelines. Extraction equipment included beakers, measuring cylinders, mortars, and analytical balances for the preparation of simple drugs and the maceration process. For lotion formulation, porcelain dishes, water baths, and mortars were used for emulsification of both phases. Evaluation of the preparations used a pH meter for pH measurement, a climatic chamber for accelerated stability testing, and organoleptic testing equipment, homogeneity, spreadability, and adhesion in accordance with pharmaceutical quality control standards (Bruker, 2025). The validity and reliability of the instruments were verified using pharmaceutical standard validation methods that meet the criteria of accuracy, precision, specificity, linearity, and robustness (Sugiarta et al., 2023).

The data analysis technique used a mixed methods approach with descriptive analysis for the physical characteristics of the preparations and inferential analysis for the repellent effectiveness test. Emzir (2021) emphasized that quantitative pharmaceutical data analysis requires a systematic approach starting from data collection, data cleaning, and interpretation of results. Data from the physical evaluation of the preparations were analyzed descriptively and presented in tables and narratives to describe the organoleptic characteristics, homogeneity, pH, spreadability, and adhesiveness. Inferential statistical analysis used SPSS (Statistical Package for the Social Sciences) software to test data normality with the Shapiro-Wilk test, homogeneity of variance, and one-way analysis of variance (ANOVA) to compare effectiveness between formulations (Harianja et al., 2023). Further analysis used a post-hoc test to identify significant differences between treatment groups, with a significance level of $\alpha = 0.05$ in accordance with pharmaceutical research standards (Fiandini et al., 2023).

Population and Sample

The population in this study was all mosquito repellent lotion formulations that can be made with a combination of lemongrass (Cymbopogon citratus) and cosmos caudatus (Cosmos caudatus Kunth.) extracts at various concentrations. Sugiyono (2023) defines a population as a generalization area consisting of objects or subjects that have certain qualities and characteristics determined by researchers to be studied, and then conclusions are drawn. The research sample consisted of six lotion formulations (F0, F1, F2, F3, F4, F5) representing variations in lemongrass and cosmos caudatus extract concentrations, with F0 as a negative control without active extracts. Sample selection used a purposive sampling technique based on theoretical considerations of effective extract concentrations according to previous scientific literature (Creswell, 2018).

The raw materials, lemongrass and kenikir leaves, were obtained from Banaran Village, Karanganyar, Central Java, with inclusion criteria of fresh, intact plants, without holes, and free from contamination. Sudaryono (2023) stated that the selection of raw material samples must meet strict quality criteria to ensure the validity of the research results. For the repellent effectiveness test, 20 Aedes aegypti mosquitoes were used per test cage that had undergone a screening process to ensure normal health and activity conditions. The sample size was determined based on statistical calculations with a 95% confidence level and an 80% power test, following adequate pharmaceutical experimental design guidelines to detect significant differences between treatments (Chakraborty, 2023).

Research Procedures

The research procedure begins with the raw material preparation stage, which includes sorting, washing, chopping, and drying lemongrass and kenikir simplicia using a shade drying method with a black cloth to prevent degradation of active compounds. Mungwari et al. (2024) stated that the correct drying method is very important to maintain the content of bioactive compounds in the simplicia. The dried simplicia was then ground using a blender and sieved with a number 40 sieve to obtain a homogeneous particle size to increase extraction efficiency. The extraction process was carried out by the maceration method using 96% ethanol solvent for 5 days in a brown bottle to protect from light, then the filtrate was concentrated using a water bath until a thick extract was obtained (Tropical Journal of Natural Product Research, 2024).

The lotion formulation stage uses an oil-in-water (o/w) emulsion system by dividing the ingredients into two phases: an oil phase consisting of stearic acid, cetyl alcohol, liquid paraffin, and methyl paraben; and an aqueous phase containing propylene glycol, glycerin, TEA, distilled water, and active extracts. Both phases are heated separately at 70°C using a water bath, then mixed in a warm mortar with constant stirring until a homogeneous lotion mass is formed. Dehariya et al. (2023) explained that proper emulsification techniques are very important to produce stable and homogeneous preparations. Evaluation of the preparation includes organoleptic tests to observe color, odor, and shape; homogeneity tests using glass slides; pH measurements with a pH meter; spreadability and adhesion tests using standard pharmacopoeial methods; and repellent effectiveness tests using test cages with Aedes aegypti mosquitoes.

Accelerated stability testing was conducted using a climatic chamber with cyclic temperature conditions of 4°C and 40°C for 6 days per cycle for a total of 3 cycles, following the ICH guidelines for the evaluation of pharmaceutical stability. González-González et al. (2022) stated that accelerated stability testing using stress temperature and humidity conditions is an effective method for predicting the long-term stability of pharmaceutical products in a relatively short time. Parameters evaluated during the stability test included organoleptic characteristics, homogeneity, and pH to ensure no significant changes that could affect product quality and safety. Data obtained from all evaluation stages were then analyzed using SPSS software with appropriate statistical tests to answer the research hypothesis and determine the optimal formulation that meets quality standards and effectiveness as a mosquito repellent.

RESULTS AND DISCUSSION

Organoleptic Test

Based on the research results, organoleptic observations of the mosquito repellent lotion preparation combining lemongrass (Cymbopogon citratus) and cosmos caudatus (Cosmos caudatus Kunth.) extracts showed that each formula had a different color and aroma. The purpose of the organoleptic test was to determine the color, odor, and texture of the lotion preparation. The results of the organoleptic test can be seen in Table 1.

Table 1. Organoleptic Test Results

Formulation	Color	Smell	Form
F0	White	Odorless	Semi-solid
F1	Yellowish white	Distinctive smell	Semi-solid
F2	Brownish yellow	Weak distinctive odor	Semi-solid
F3	White bone	Weak distinctive odor	Semi-solid
F4	White	Distinctive smell	Semi-solid
F5	Yellowish white	Weak distinctive odor	Semi-solid

Information :
F0: Formulation with 0% concentration
F1: Formulation with 10% lemongrass concentration
F2: Formulation with 10% kenikir concentration
F3: Formulation with a concentration of 5% lemongrass and 5% kenikir
F4: Formulation with a concentration of 3% lemongrass and 7% kenikir
F5: Formulation with a concentration of 7% lemongrass and 3% kenikir

Homogeneity Test

This homogeneity test was carried out to determine whether the lotion preparation of lemongrass (Cymbopogon citratus) and kenikir (Cosmos caudatus Kunth.) extracts had been mixed well or were homogeneous.(Yuliansi et al., 2020). The results of the homogeneity test showed that all formulations mixed well. The results of the homogeneity test can be seen in Table 3.

Table 2. Results of Homogeneity Test

Formulation	Results
F0	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous
F4	Homogeneous
F5	Homogeneous

pH test

A pH test was conducted to determine whether the lemongrass (Cymbopogon citratus) and cosmos caudatus (Kunth.) extract lotion preparations met the skin's pH requirements. The pH of topical preparations ranges from 4.5 to 8.(Karim et al., 2022). The results of the pH test can be seen in Table 4.

Table 3. pH Test Results

Formulation	Mark
F0	5.40
F1	5.41
F2	5.67
F3	5.42
F4	5.68
F5	5.70

From the pH results obtained, it can be concluded that the anti-mosquito lotion preparation of lemongrass (Cymbopogon citratus) and cosmos caudatus Kunth. Extract meets the skin pH requirements for topical preparations with a value range of 5.40-5.70.

Spread Power Test

The spreadability test measures the amount of force required to spread the lotion when applied to the skin. A good spreadability for a lotion is between 5 and 7 cm.(Karim et al., 2022). The results of the spread power can be seen in Table 4.

Table 4. Results of the Spreadability Test

Formulation	Mark
F0	6.6 cm
F1	6.4 cm
F2	7.0 cm
F3	6.9 cm
F4	6.5 cm
F5	6.9 cm

From the results of the spreadability test above, it can be concluded that the anti-mosquito lotion preparation of lemongrass (Cymbopogon citratus) and cosmos caudatus Kunth. The extract has met the spreadability requirements with a spreadability value of around 6.5-7.0 cm.

Adhesion Test

This adhesion test aims to determine how quickly a lotion preparation adheres to the skin surface after application. This test is performed by placing the preparation in the center of a watch glass and covering it with another glass. For 5 minutes, a 50 g weight is placed on the cover glass. After that, the weight is removed, and the adhesion time is recorded as the amount of time it takes for the two glasses to separate. For topical preparations, the adhesion time should be at least 4 seconds.(Karim et al., 2022)The results of the adhesive strength test can be seen in Table 5.

Table 5. Adhesion Test Results

Formulation	Mark
F0	4.46 seconds
F1	4.68 seconds
F2	4.39 seconds
F3	4.16 seconds
F4	4.72 seconds
F5	5.59 seconds

From the results of the adhesive power obtained, it shows that the anti-mosquito lotion preparation with lemongrass (Cymbopogon citratus) and kenikir (Cosmos caudatus Kunth.) extract is in line with research.*(Karim et al*., 2022)This says that good adhesion time is at least 4 seconds.

Repellent Test

The repellent test was conducted in a test cage containing 20 antiviral-treated Aedes aegypti mosquitoes. Four formulas, one positive control, and one negative control (without treatment), were tested for 5 minutes to observe the effectiveness of each formula against mosquitoes. The repellent test was replicated three times to determine which lotion was most effective. The results of the repellent test can be seen in Table 6.

Table 6. Mosquito Repellent Test

	Replication 1	Replication 2	Replication 3
K-	171	167	141
F0	124	121	127
F1	47	50	51
F2	96	71	67
F3	19	23	21
K+	5	8	17

Information :
K-: Negative control (no treatment)
F0: Formulation with 0% concentration
F1: Formulation with 10% lemongrass concentration
F2: Formulation with 10% kenikir concentration
F3: Formulation with a concentration of 5% lemongrass and 5% kenikir
K+ : Positive control ((autan)
From the table above, the percentage can be calculated in the following way, to get the results that can be seen in Table 7.$\frac{((k-)-p)}{k-}$ x 100 %

Table 7. Percentage of Mosquito Repellent Test

	K-	Treatment	Results %
F0 R1	160	124	22%
F0 R2	167	121	27%
F0 R3	155	127	18%
F1 R1	160	47	70%
F1 R2	167	50	70%
F1 R3	155	51	67%
F2 R1	160	69	56%
F2 R2	167	71	57%
F2 R3	155	67	56%
F3 R1	160	19	88%
F3 R2	167	23	87%
F3 R3	155	21	86%

Accelerated Stability Test

Accelerated stability testing was conducted by storing the lotion preparation (packaged in a container) in a climatic chamber with a cold temperature of 4 °C and a hot temperature of 40 °C, where the two temperatures are considered one cycle. The experiment lasted for 6 days with three cycles. The stability test was conducted with the aim of determining whether a product is suitable for use or not. The stability test included organoleptic testing, homogeneity testing, and pH testing for three cycles. The results of the accelerated stability test using a climatic chamber.

1. **Organoleptic test**

Table 8. Organoleptic Test Results

Formula	Before accelerated stability testing			After accelerated stability testing		
	Form	Color	Smell	Form	Color	Smell
F0	Semi-solid	White	Odorless	Semi-solid	White	Odorless
F1	Semi-solid	Yellowish white	Distinctive smell	Semi-solid	Yellowish white	Distinctive smell
F2	Semi-solid	Brownish yellow	Weak distinctive odor	Semi-solid	Brownish yellow	Weak distinctive odor
F3	Semi-solid	White bone	Weak distinctive odor	Semi-solid	White bone	Weak distinctive odor
F4	Semi-solid	White	Distinctive smell	Semi-solid	White	Distinctive smell
F5	Semi-solid	Yellowish white	Weak distinctive odor	Semi-solid	Yellowish white	Weak distinctive odor

2. **Homogeneity Test**

Table 9. Results of Homogeneity Test

Formulation	Before accelerated stability testing	After accelerated stability testing	Results
F0	Homogeneous	Homogeneous	As per the requirements
F1	Homogeneous	Homogeneous	As per the requirements
F2	Homogeneous	Homogeneous	As per the requirements
F3	Homogeneous	Homogeneous	As per the requirements
F4	Homogeneous	Homogeneous	As per the requirements
F5	Homogeneous	Homogeneous	As per the requirements

3. pH test

Table 10. pH Test

Cycle	Day observation	F0	F1	F2	F3	F4	F5
1.	Day 1 (40 °C)	5.42	5.41	5.55	5.60	5.65	5.64
	Day 2 (4 °C)	5.38	5.43	5.67	5.47	5.62	5.65
2.	Day 3 (40 °C)	5.45	5.43	5.66	5.62	5.72	5.63
	Day 4 (4 °C)	5.55	5.45	5.67	5.65	5.69	5.68
3.	Day 5 (40 °C)	5.60	5.53	5.70	5.59	5.70	5.74
	Day 6 (4 °C)	5.60	5.65	5.72	5.60	5.74	5.68

Repellent Test

The results showed that the F3 formula (a combination of 5 g lemongrass extract and 5 g kenikir) had an 88% mosquito repellent effectiveness, which was considered good as a repellent. Data analysis using SPSS included normality tests (Shapiro-Wilk), homogeneity, and one-way ANOVA. The normality test showed that all data were normally distributed (significance > 0.05), and the homogeneity test showed homogeneous data (significance = 0.266). The ANOVA results showed a significant difference between groups (significance = 0.000). Further tests showed that only F3 and K+ had no significant difference, meaning that F3 had a repellent power equivalent to the positive control (K+). Meanwhile, F0, F1, F2, and K- had significant differences when compared to F3 and K+, indicating that the repellent power of the formulas was not comparable.

pH test

The pH test data of the combination lotion of lemongrass and kenikir extracts were analyzed using SPSS through normality, homogeneity, and One-Way ANOVA tests. The Shapiro-Wilk normality test showed that all formulas had a significance value >0.05 (F0–F5), indicating that the data were normally distributed. The homogeneity test also showed homogeneous data (significance = 0.079). However, the ANOVA results showed a significance value of 0.000 (<0.05), which means there was a significant difference in pH between the formulas.

CONCLUSION

This study successfully formulated a mosquito repellent lotion containing a combination of lemongrass (Cymbopogon citratus) and cosmos caudatus (Cosmos caudatus Kunth.) extracts that met physical quality standards with acceptable organoleptic characteristics, good homogeneity, skin-friendly pH (5.40-5.70), optimal spreadability (6.5-7.0 cm), and adequate adhesion (>4 seconds). The main findings showed that formula F3, with a combination of 5% lemongrass extract and 5% cosmos caudatus extract, provided the highest mosquito repellent effectiveness of 88%, equivalent to a commercial positive control. Accelerated stability tests using a climatic chamber for three cycles showed no significant changes in organoleptic characteristics, homogeneity, and pH, indicating good physical stability. Statistical analysis confirmed significant differences between formulations, with F3 as the optimal formulation that provided a synergistic effect between the two extracts in providing protection against Aedes aegypti mosquitoes.

The limitations of this study include the stability evaluation, which only focused on physical aspects without long-term chemical stability analysis, and the repellent effectiveness test conducted under controlled laboratory conditions for a short exposure period (5 minutes). Future studies are recommended to conduct long-term stability tests with real-time storage

conditions, evaluation of repellent activity with longer exposure durations, and dermal safety tests to ensure human skin tolerance. The practical implications of this study indicate the great potential for developing natural repellents based on local Indonesian plant extracts as a safe alternative to synthetic products containing DEET, which can contribute to the national dengue vector control program and support the environmentally friendly, natural ingredient-based cosmetics industry.

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