

Lotion Preparations of Nutmeg Essential oil (*Myristica fragrans* Houtt) and Repellent Activity Test against *Aedes aegypti* Mosquitoes

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Abstract

Dengue Hemorrhagic Fever (DHF) is a disease caused by the bite of *Aedes aegypti* mosquitoes infected with the Dengue virus. One natural ingredient that can be used as a repellent to prevent mosquito bites is essential oil. Therefore, this research aims to obtain an optimal formulation for nutmeg essential oil lotion, evaluate its influence on lotion properties, assess its skin irritation potential, and determine the repellent activity against female *Aedes aegypti* mosquitoes. Nutmeg fruit seed essential oil (*Myristica fragrans* Houtt) was obtained by distillation. The obtained sample was qualitatively tested first using GC-MS to determine its components. Lotions were formulated using concentrations of nutmeg essential oil at 1%, 3%, and 6%. The prepared lotions were tested for their physical properties and repellent activity using female *Aedes aegypti* mosquitoes as test animals and irritation test. The test result data were statistically analyzed using one-way ANOVA with a confidence level of 95%. The research results obtained standardization of clear nutmeg seed essential oil, yellow in color, with a distinctive nutmeg scent with a refractive index value of (1.475 ± 0.001) and a relative compound content of alpha-pinene of 19.40%. Increasing the concentration resulted in decreased adhesive force with a value of $(p < 0.05)$, increased spreadability $(p < 0.05)$, and increased repellent power $(p < 0.05)$. All lotions have a pH of 7, are stable for 6 storage cycles, and have an irritation index in the very mild to mild irritation category. The conclusion of this research is that nutmeg seed essential oil lotion formulations with various concentration variations, namely formula 1 (1%), formula 2 (3%), and formula 3 (6%), have good physical properties, cause very mild to mild skin irritation, and have the highest repellent activity, which is in formula 3 (6%) at 1098 seconds.

Keywords: *Aedes aegypti*, essential oil, nutmeg, repellent

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Introduction

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus which is transmitted to humans through the bite of the *Aedes aegypti* mosquito. The number of DHF cases is increasing along with climate change in Indonesia. According to data from the Directorate General of Disease Prevention and Control of the Ministry of Health, an increase in cases in March 2024 was found to be 53,131 cases with a death toll of 404 people, while in April 2024 it increased to 60,296 cases with a death toll of 455 people [1].

The use of mosquito repellent applied to the skin is one practical prevention method against mosquito bites. So far, what people have been doing to avoid mosquito bites is using mosquito repellent lotions available in the market, known to contain N, N-diethyl-meta-toluamide (DEET), which can cause skin irritation, pruritus (itching), and erythema (skin redness) [2].

Nutmeg is one of the plants containing essential oil that can be utilized as a botanical pesticide, including as a fruit fly control, mosquito repellent, and antifungal. Nutmeg seeds contain chemical compound components such as α -pinene, eugenol, isoeugenol, isoelemicin, β -pinene, limonene, safrole, and myristicin. Among these compounds, α -pinene, eugenol, and limonene exhibit repellent activity [3].

There have been few studies on using nutmeg seed essential oil as a repellent in terms of activity testing or formulation. Therefore, researchers are interested in investigating the promising repellent ability of nutmeg seed essential oil in lotion form because the liquid consistency of lotion allows for quick and even application on the skin, easy spreading, rapid drying after application, and leaving a thin layer on the skin surface [4].

Nutmeg seed essential oil lotion is prepared with various concentration variations to determine the stable concentration of nutmeg seed essential oil in the lotion during storage, its non-irritating effect on the skin, optimum repellent effect, and good physical properties. Therefore, this study aims to obtain the right formulation of nutmeg seed essential oil lotion, determine the influence of adding nutmeg seed essential oil to the lotion's physical properties, assess the irritant effect of nutmeg seed essential oil lotion on the skin, and determine the repellent activity of nutmeg seed essential oil lotion against female *Aedes aegypti* mosquitoes.

Materials and Methods

Materials

Fresh nutmeg seeds were collected from Malikrubu Regency, Ternate, North Maluku, Indonesia. Chemical materials such as triethanolamine (TEA), stearic acid, cetaceum, lanolin, liquid paraffin, methylparaben were obtained through PT. Brataco, Indonesia. Commercial lotion products containing DEET (N, N-diethylmeta-toluamide) were obtained from community pharmacy, Ahmad Dahlan University.

Steam distillation apparatus, glassware set, mosquito cage for repellent test. Equipment for spreadability test, adhesivity test, universal pH test, and skin irritation test use male albino rabbits weighing approximately 2 kg, and female *Aedes aegypti* mosquitoes aged 5-10 days collected from the Entomology Laboratory at the Faculty of Public Health, Universitas Ahmad Dahlan (UAD). This research has received ethical feasibility approval from the Ethics Committee of the Faculty of Medicine, Muhammadiyah University of Yogyakarta, Number: 104/EP-FKIK-UMY/III/2016.

Methods

Distillation of nutmeg seeds essential oil

The nutmeg seeds were ground to smaller sizes and then distilled using the steam distillation method to obtain nutmeg seed essential oil. Distillation was carried out for 6 hours. The percentage volume per weight (v/w) of essential oil was calculated. The oil obtained was then clarified by mixing with dried Na_2SO_4 , filtered with filter paper, and ready for characterization [5].

Organoleptic test of nutmeg seeds essential oil

An organoleptic evaluation of the essential oil was performed by observing color, odor, taste, and consistency. The

evaluation of extract consistency was done using a density parameter by pressing the extracted sample between the thumb and the index finger and assigning a subjective density score.

Determination of refractive index of nutmeg seed essential oil

The measurement of the refractive index of nutmeg essential oil aims to determine its purity. This test uses an ABBE ATAGO® refractometer to determine its refractive index which is observed at a temperature of 20°C [6]. The standard data for the refractive index of nutmeg oil is in the range of 1.475 - 1.485 at a temperature of 20°C [7].

Identification of nutmeg seed essential oil components using Gas Chromatography-Mass Spectrometry (GC-MS) method

The obtained essential oil was observed for its compound components using a Shimadzu GC-MS QP110 with a Rastek Rxi-5MS column. The operating conditions of the GC-MS are that it has an ionizing type EI (Electron Impact), column length 30 meters, carrier gas using helium with a flow rate of 1 ml/minute, injection volume 2 microliters, injector temperature 250°C, column temperature 110°C, with increasing temperature 10°C/minute to 200°C, and 5°C/minute to a temperature of 280°C for the next 9 minutes. The mass spectrum was carried out at 70eV with an interval of 0.5 seconds and reading fragments of 45-450 Da. The total GC time was 36 minutes [8]. The results obtained are then compared with the spectral profile of the data in the tool library.

Procedure for formulation nutmeg essential oil lotion

Lotion preparation, is made by dissolving lanolin, stearic acid, cetaceum, and liquid paraffin into a porcelain cup and melting it on a water bath at a temperature of 70°C (mixture 1). Furthermore, Triethanolamine (TEA), and methylparaben are dissolved in hot distilled water (mixture 2). Moreover, mixtures 1 are added to mixture 2 slowly while stirring until a homogeneous mixture is obtained. After the mixture is cold, add nutmeg essential oil according to the formula in Table I, while stirring until homogeneous. The lotion preparation obtained is tested for physical properties, irritation tests, and anti-mosquito activity.

Table 1. Formulas of nutmeg essential oil lotion

Materials	Formulas lotion (g)		
	Formula 1	Formula 2	Formula 3
Nutmeg Essential oil (ml)	0.5	1.5	3
Stearic acid	4	4	4
Triethanolamin	1	1	1
Liquid Paraffin	6	6	6
Lanolin	1	1	1
Cetaceum	3	3	3
Methyl paraben	0.2	0.2	0.2
Distillate water	ad 50	ad 50	ad 50

The adhesivity test of nutmeg essential oil lotion

The adhesive strength test aims to determine the ability of the preparation when it adheres to the skin. Determination of adhesive strength is done by taking 0.5 g of the preparation and then placing it on a glass measuring 76x26 mm, covering it with a glass cover of the same size upside down and then giving a load of 150 grams on top for 5 minutes. Then calculate the time needed for both glasses to come off. The effective adhesive strength requirement is more than 4 seconds [9], [10].

The spreadability test of nutmeg essential oil lotion

The spreadability test aims to determine the ability of a lotion preparation to spread on the skin surface when applied. Determination of spreadability is done by taking 0.5 g of the preparation and then placing it on a 20 cm diameter glass plate, covering it with a glass plate cover of the same size upside down and then giving it a load of 150 grams on top for 1 minute and measuring the spread diameter. Spreadability can be seen from the wider the spread distance, the better the penetration power on the skin [11], [12].

Figure 1. Data of macroscopic and microscopic identification of nutmeg seeds
(a). Macroscopic figure, (b). Mikroskopik figure.

Data of distillation of nutmeg essential oil

Distillation of nutmeg essential oil is carried out by steam distillation. According to [18], the yield of nutmeg essential oil ranges from 2-15%. The yield of essential oils in this study was 4.02% v/w. These results are to the theory stated by [18].

Data from organoleptic test results

The results of distillation using the steam-water distillation method obtained essential oil that was clear and light yellow with a distinctive nutmeg aroma by the quality standards for nutmeg oil according to the National Standardization Agency [7]. The organoleptic test results can be seen in Table 3.

Table 3. Organoleptic test results for nutmeg essential oil

No	Test Components	Result
1	Color	Clear – light yellow
2	Odor	Typical nutmeg smell

Data of measuring the refractive index of nutmeg essential oil

The results of the research showed that the refractive index value of nutmeg essential oil (*Myristica fragrans* Houtt) obtained was 1.475 ± 0.001 . Where in theory the quality standard for the refractive index of nutmeg oil is in the range of 1.475 ± 1.485 at a temperature of 20°C [7]. This shows that the refractive index of nutmeg essential oil is by the quality standards of nutmeg seeds.

Compound identification data in nutmeg essential oil

Analysis of nutmeg essential oil components using GC-MS resulted in 35 peaks as shown in Figure 2.

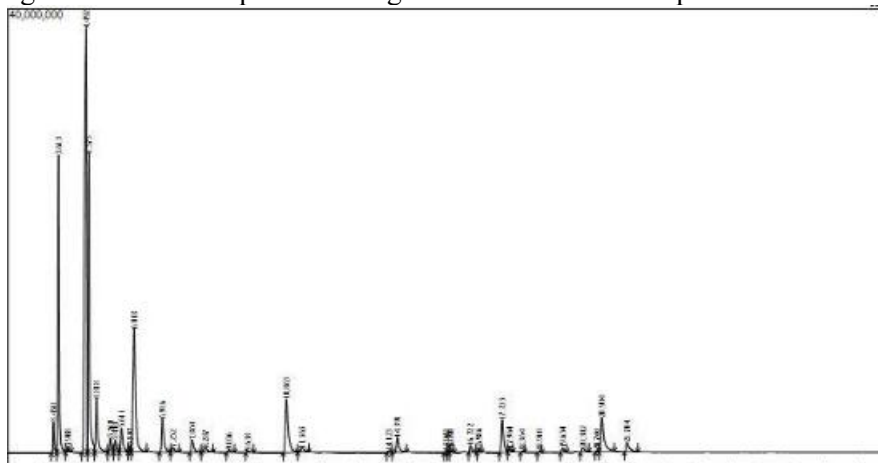


Figure 2. Data from nutmeg essential oil chromatogram using GC-MS

Data from the 35 chromatogram peaks identified 4 compounds that had the highest peaks compared to other compounds. The number of peaks indicates the number of components contained in nutmeg essential oil while the peak area indicates the concentration of the components [19]. The results of the gas chromatography analysis of nutmeg essential oil can be seen in Table 4.

Table 4. Identification of chemical compounds in nutmeg essential oil

No	Peaks	R. Time	%Area	Compounding
1	2	3.613	12.61	<i>Alpha-Pinene</i>
2	4	4.492	32.30	<i>Sabinene</i>
3	5	4.575	14.07	<i>Beta-pinene</i>
4	11	6.018	10.23	<i>Beta-phellandrene</i>

Data of adhesivity time test

The bond time test results of the nutmeg essential oil lotion showed that formula 1, formula 2, and formula 3 did not meet the bond time requirements. Still, the lotion base met the bond time requirements, which in theory according to [17] has a good attachment time of no less than 4 seconds. This decrease in adhesive power could be caused by the addition of essential oils to each lotion formula ($p < 0.05$). The results of the adhesive test for the lotion preparation are shown in Figure 3.

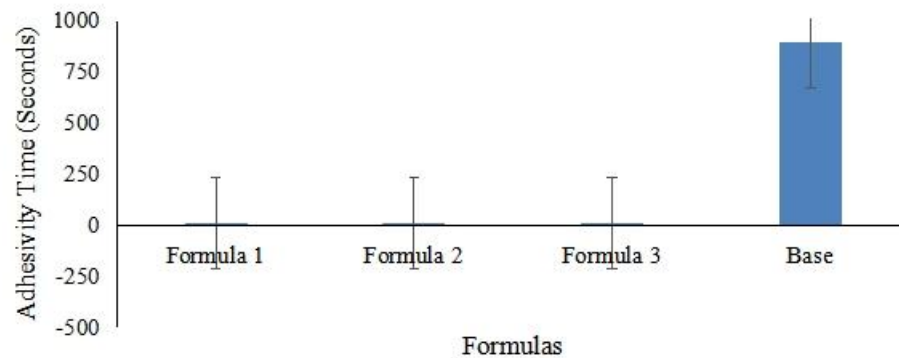


Figure 3. Data of adhesivity test lotion nutmeg seed essential oil

The statistical test results showed that the significance was $0.007 < 0.05$, it was concluded that each formula had no significant differences, where the addition of nutmeg essential oil affected the adhesive time because the lotion viscosity decreased.

Data of spreadability test of nutmeg essential oil lotion

The results show that the distribution diameter is as follows, the base is 6.30 ± 0.48 , formula 1 is 7.30 ± 0.06 cm, formula 2 is 7.76 ± 0.13 , and formula 3 is 7.88 ± 0.12 . According to [20], the spreadability requirement for topical preparations is around 5-7 cm. Based on the results obtained in formula 1, formula 2, formula 3, and the base meets the requirements for good spreadability. The more essential oils added, the wider the distribution capacity. This is because adding more essential oils can give the lotion a softer consistency so that it spreads more widely. The spreadability test results curve can be seen in Figure 4.

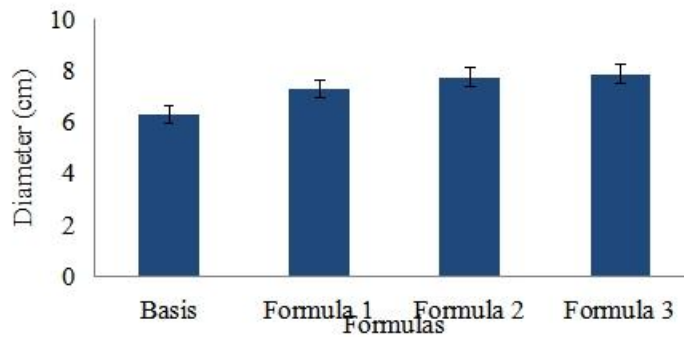


Figure 4. Data of spreadability test of lotion nutmeg seeds essential oil

The results of the statistical test show a p-value = 0.001 so the significance value is <0.05, this shows that each test group gives different results for their inherent ability, except between formula 2 and formula 3 which shows an insignificant difference. These results show that the addition of nutmeg essential oil to each formula affects the distribution diameter, this can happen because the consistency of the lotion becomes softer. This shows the influence of adding essential oils on the spreadability of the lotion.

Data from the stability test lotion of nutmeg essential oil

The data of the stability test of nutmeg essential oil lotion after 6 cycles of testing showed that the lotion was stable when stored at temperatures of 4°C and 40°C, stable conditions, no cream was formed and no color changes occurred, and the odor was stable, typical of nutmeg. The data of the lotion stability test results are shown in Table 5 and Figure 5.

Table 5. Data of stability test of nutmeg essential oil lotion

Group	Observation Time						Annotation
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	
Formula 1	-	-	-	-	-	-	Stable
Formula 2	-	-	-	-	-	-	Stable
Formula 3	-	-	-	-	-	-	Stable
Base lotion	-	-	-	-	-	-	Stable

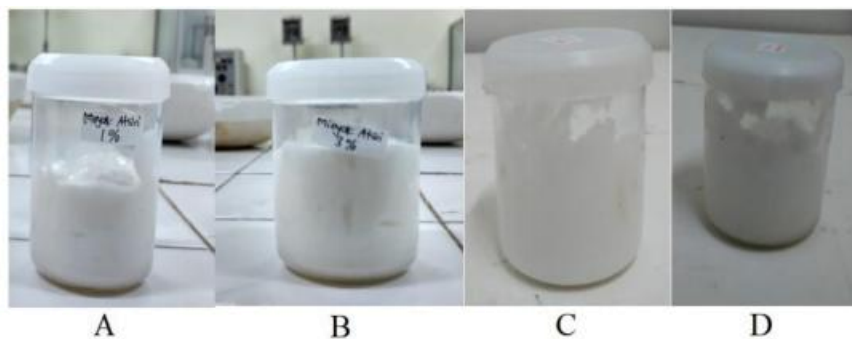


Figure 5. Stability test of nutmeg essential oil lotion (A). Formula 1; (B). Formula 2; (C). Formula 3; (D). Base of lotion

Data of pH lotion of nutmeg seeds essential oil

The results of measuring the pH of the lotion preparations showed that during storage, the pH did not change for formulas 1, 2, 3, and base, where the pH of all formulas showed a pH value of 7. In theory, the pH of the lotion is like that of normal skin, namely pH 5 -7 [21]. The pH test results are shown in Figure 6.

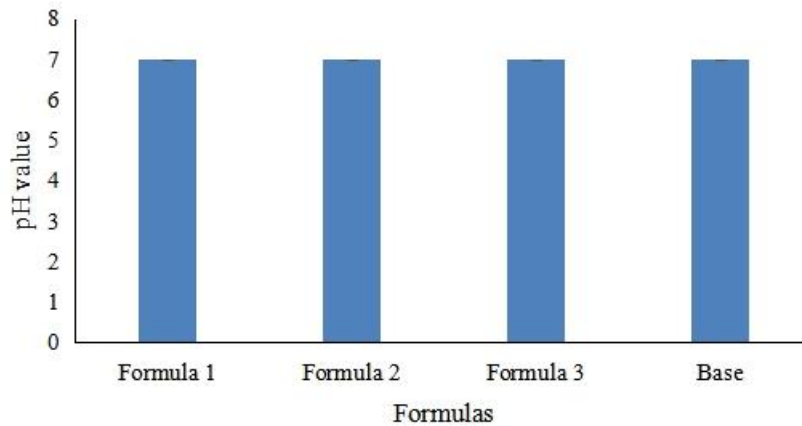


Figure 6. Data of pH value of nutmeg seeds essential oil lotion

Skin irritation test data for nutmeg essential oil lotion

The results of the irritation index calculation can be seen in Table 4. The irritation index data of nutmeg essential oil lotion in formulas 1, 2, and 3 show a score range of 0.00 - 1.9, where the lotion can cause mild irritation to the skin. The test results show that the irritation power of nutmeg essential oil lotion is in the range of very light to mild irritant, where the addition of nutmeg essential oil will increase the risk of skin irritation. Primary irritation test data on nutmeg seed oil emulsion preparations show that essential oil emulsion preparations have very mild irritation properties [22].

Table 4. Data of score skin irritation of lotion nutmeg seeds essential oil

Group Test	Observation Time (hours)		
	24	48	72
Formula 1	0.33	0.67	0.00
Formula 2	0.00	0.67	0.33
Formula 3	-0.33	0.33	0.33

Repellent activity test data for nutmeg seed essential oil lotion preparations

The results of the nutmeg essential oil repellent lotion test showed that the greater the concentration of nutmeg essential oil added, the longer the repellent time, where the repellent time is as follows: formula 1 for 49 seconds, formula 2 for 385 seconds, and formula 1098 seconds, while the base for 17 seconds and positive control for 10800 seconds. The positive control is a lotion containing 15% DEET which is more effective than the nutmeg essential oil lotion preparation, and it is necessary to identify the levels of nutmeg essential oil in the lotion during storage, where the storage time will also affect the decrease in the levels of its essential oil. The repellent time data for nutmeg essential oil lotion can be seen in Figure 7.

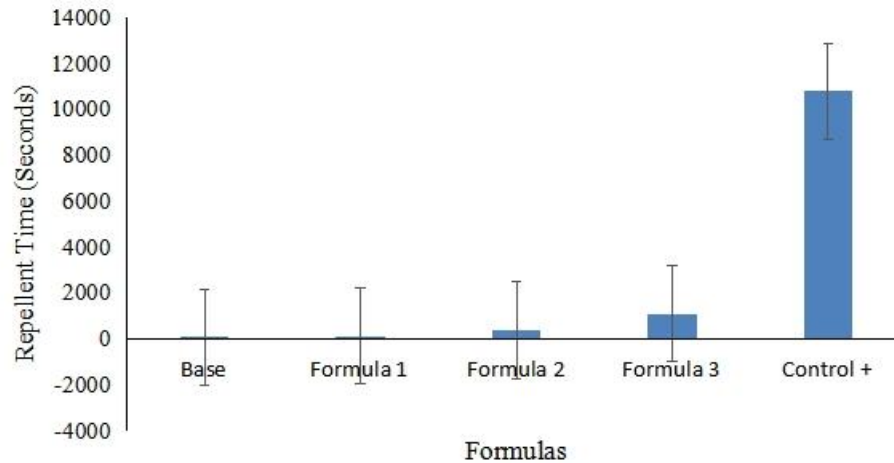


Figure 7. Data of repellent activity lotion of nutmeg seeds essential oil

The statistical test data shows that the significance value of the p-value is 0.000 so the p-value is <0.05 , so it can be concluded that there is a significant difference between the length of time for rejection of formulas 1, 2, 3, base, and positive control. Furthermore, the results of statistical tests between groups showed that the p-value was 0.009, meaning that increasing the concentration of nutmeg essential oil would increase repellent activity.

Conclusion

Nutmeg essential oil lotion has mosquito bite repellent activity, where the skin irritation effect is needed but further study is needed related to the identification of essential oil levels during storage because the anti-mosquito activity can decrease the essential oil levels in the preparation during storage. And it is necessary to develop products that are able to bind essential oils so that they last a long time in the preparation.

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Declaration

- Author contribution** : Azis Ikhsanudin proposing the topic and research methodologies, Satriyo Dwi Raharjo drafting the proposal and performing analysis, Lolita Lolita presenting the data and discussion.
- Funding statement** : No funding is available for this research.
- Conflict of interest** : We declare that there is no competing interests.
- Ethics Declaration** : As the authors, we confirm that this work has been written based on ethical research principles in compliance with our university's regulations and that the necessary permission was obtained from the relevant institution during data collection. We fully support CliPs commitment to upholding high standards of professional conduct and practicing honesty in all academic and professional

activities.

Additional information : No additional information is available for this paper.

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