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Repellent Activity of Lotion of Essential Oils from *Piper betle* Linn and *Cymbopogon nardus* [L.] Rendle

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ABSTRACT

Long term use of N,N-diethyl-meta-toluamide (DEET) in insect repellents can cause some effects such as mild irritation, itching, rash, urticaria, contact dermatitis, swelling and seizures. In addition, DEET can also contaminate aquatic environments. Because of these side effects, it is necessary to look for alternative repellents. Betel leaves (*Piper betle* Linn.) and Citronella grass (*Cymbopogon nardus* [L.] Rendle) contain essential oils that repel insects. This study aimed to develop an effective, non-toxic, environmentally friendly, and biodegradable natural insect repellent by formulating a mosquito-repellent lotion from betel leaves and citronella grass essential oils. The betel leaves and citronella grass essential oils were formulated into a lotion with three concentrations (1, 5, 10% w/w). Evaluation of lotions stored at 1% (F1), 5% (F2), and 10% (F3) concentration was conducted in 7 cycles of freeze-thaw. Lotion quality was analyzed for organoleptic, pH, viscosity, homogeneity, stability and skin sensitivity. The evaluation results showed that the four lotion formulae were stable, did not cause skin irritation, were homogeneous, and had a pH of 7. The results of the centrifugation test showed that 1% lotion was stable, while 5% and 10% had phase separation. The protection against *Culex quinquefasciatus* mosquitoes at 1hr, 2hrs and 3hrs was 68.73%, 95.83% and 100%, respectively. The repellent activity of lotions with betel leaves and citronella grass oils was concentration-dependent.

***Keywords*:** *Piper betle* Linn, *Cymbopogon nardus* [L.] Rendle, Essential oil, Repellent, Lotion, *Culex quinquefasciatus*.

Introduction

Insect repellent is a substance that deters insects from biting human skin. Insect repellent products that are available on the market generally contain N,N-diethyl-meta-toluamide (DEET).[1] A DEET is the active ingredient that has long been used in insect-repellent products and it is considered to be the most effective to repel insects.[1] N,N-diethyl-meta-toluamide protects skin from mosquito bites for 8 hours but long term use can cause side effects such as mild irritation, itching, rash, urticaria, contact dermatitis, swelling and convulsions in children under 2 years of age.[2] In addition, DEET can also contaminate aquatic environments and is one of the most frequently detected organic contaminants in wastewaters.[3] Due to these side effects, it is necessary to reconsider the use of this chemical in daily life, including its use as a repellent. One of the alternative ways to reduce the use of DEET is by using natural products extracted from plants in repellents. Natural products are considered safer than synthetic products.[4] There are two medicinal plants that have the potential to be used in repellent are betel leaves (*Piper betle* Linn.) and citronella (*Cymbopogon nardus* [L.] Rendle).[5] Betel leaf contains chavicol acetate, eugenol, *b*-caryophyllene, *g*-muurolene, velence, eucalyptol, chavicol, caryophyllene oxide trans-isoeugenol, acetyl eugenol, and 4-allyl-1,2-diacetoxybenzene.[6,7]

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The essential oil of betel leaf can be used as larvicide, adulticide, and oviposition-deterrent for the *Aedes aegypti* mosquito.[8]
It has also been reported that the volatile oil from *Piper betle* leaves possessed significant larvicidal, ovipositional, and repellency effects against *Aedes aegypti*.[9] Another plant that is rich in essential oil is citronella grass.[10] Citronella essential oil contain constituents like citronella, cadinene, methyl isoeugenol, caryophyllene, geranyl acetate, citronellyl propionate, eugenol, linalool, citral, and limonene. The major component is citronella.[10,11,12] Citronella essential oil is effective for protecting the skin from bites of *Aedes aegypti* (L.) and *Culex* sp. mosquitoes.[13,14,15] Other studies demonstrated the use of these two plants in mosquitoes-repellent formulations. For instance mosquito-repellent lotions made from citronella essential oil protect from *Aedes aegypti* bites at an optimum 5% concentration by 100% for two hours and 99% for four hours.[1] It has been reported that the essential oils of betel leaves and citronella grass can be active as insect repellent against *Aedes aegypti* mosquito for six hours.[6] The aim of the present study was to find an effective, non-toxic, environmentally friendly, and biodegradable natural insect repellent by formulating a mosquito repellent lotion from betel leaf and citronella essential oils.

Materials and Methods

Plant material and mosquitoes
The betel leaf (*Piper betle* L.) used in this study was taken in January 2021 from Lambaro, Aceh Besar and the citronella grass (*Cymbopogon nardus* [L.] Rendle) was taken from the garden of the distillation plant in Blangkejeren, Aceh, Indonesia. Betel leaf and citronella grass were identified and confirmed by Dr. Saida Rasnovi, M.Si (70/UN11.1.28.1/DT and 80/UN11.1.28.1/DT) at Laboratory of Biosistematika Tumbuhan, Faculty of Math and Science, Syiah Kuala University, Aceh, Indonesia. *Culex quinquefasciatus* mosquitoes were sourced from the Faculty of Veterinary Medicine at Syiah Kuala University.

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Equipment and chemicals
The materials used were cera alba (Brataco), stearic acid (Brataco), nipasol (Brataco), nipagin (Brataco), propylene glycol (Brataco), trietanolamine (Merck, Germany), lanolin (Brataco), pH stick (brand: MColorpHast™ Merck KGaA Darmstadt Germany), aquadest. The equipment used included an analytical balance (Sartorius cubis series MSE model, Germany), centrifuge (Sartorius Centrisart A-14, Germany), hot plate (Solid), mortar, stamper, waterbath (Nuve NB5-NB9-NB20, Turkey), viscometer Brookfield (Grace M-3600-1-0537, USA).

Distillation of betel leaf and citronella grass essential oils
Betel leaves and citronella grass were washed under running water, then cut into small pieces and aerated under the sunlight and covered with black cloth. The dried leaf pieces were then placed in a vessel of distiller that was already filled with water. The essential oil components are collected into a separatory funnel to separate the essential oil from water.[5]

Formulation of betel leaf and citronella grass essential oils lotion
The mortar was heated using hot water. Lanolin, cera alba, stearic acid and nipasol were melted in a porcelain dish on a water bath (named M1). Nipagin, propylene glycol and triethanolamine were melted in a porcelain dish on a water bath (named M2). The M1 and M2 content were then mixed in a water bath. The mixture mass was then placed in hot mortar and ground quickly to form a lotion. Then, aquadest was added gradually into the mortar. After that, the mixture was stirred until smooth and evenly mixed. The essential oil was added gradually to the mixture in the mortar then mixed until homogenous. Then, the mixture was placed in a calibrated lotion container, and was made up 100 mL with aquadest (Table 1).[16]

Quality analysis of lotions
All the formulated lotion were analyzed for colour, odour, consistency, and sensitivity while the quality was measured for homogeneity, pH, viscosity, and stability. Evaluation of lotions was conducted within 14 days (7 freeze-thaw cycles) which include organoleptic observations by taking a lotion sample for physical examination of the shape, colour and odour.[17,18]

Irritation skin test
The irritation test was conducted by applying the lotion to the back of right hand and left hand of 9 volunteers for 15 minutes. Irritation occurred if the volunteers reported experiencing skin itchiness and redness.[19] Ethical clearance was obtained from the Aceh Health Polytechnic ethical committee (LB.02.03/018/2021) before commencing the study.

Table 1: Formulation of lotion of betel leaf (*Piper betle* Linn.) and citronella grass (*Cymbopogon nardus* [L] Rendle) essential oils

Formula Lotion	F0	F1	F2	F3
Betel leaf and citronella grass essential oils (%)	-	1	5	10
Cera Alba (g)	2.5	2.5	2.5	2.5
Stearic Acid (g)	4	4	4	4
Methyl Paraben (g)	0.5	0.5	0.5	0.5
Propyl Paraben (g)	0.05	0.05	0.05	0.05
Propylene glycol (mL)	5	5	5	5
Triethanolamine (mL)	1	1	1	1
Lanolin (g)	3	3	3	3
Aquadest ad (mL)	100	100	100	100

F0: control lotion, F1: lotion with essential oils 1%, F2: lotion with essential oils 5%, F3: lotion with essential oils 10%

Homogeneity
A test of homogeneity was conducted by applying the lotion to a piece of glass or other transparent material. To pass the test, the lotion should indicate a homogenous structure and no coarse particles were found on the surface of the glass.[1]

pH measurement
The pH measurement was conducted by using a universal pH stick which was dipped in 1 gram of lotion. The ideal pH value is 4.5–7 which is the pH value of normal human skin.[18]

Centrifugation test
The centrifugation test was carried out by placing a centrifugation tube containing 10 g of emulsion in a centrifuge to rotate at 3800 rpm for 30 minute.[17]

Viscosity
A viscosity measurement of the lotion was made using a Brookfield viscometer. A container with 100mL lotion is placed in a viscometer tube. Measurement was conducted three using spindle number 03 at the speed of 100 rpm.[1]

Repellency testing
Mosquito repellent testing was carried out on 9 volunteers by putting their hands in a mosquito cage of 40 x 50 x 60cm that was containing 20 *Culex quinquefasciatus* mosquitoes. The skin of the volunteers' hands was washed first to remove dirt and odours. Then, lotion was applied to one of their hands evenly from the elbow to the fingers. Hand rubbing and washing are not allowed after the lotion has been applied. The test was carried out in three parts, starting from the lotion with a concentration of 1%, then a test of 5%, and finally 10%. Control formulations (lotion base containing no essential oils) were applied on the other hand. Subsequently, both of the volunteers' hands were put into a cage containing 20 mosquitoes and were observed for 3hrs. This was conducted in triplicable for the different concentrations of repellent. Mosquitoes that landed on their skin were observed and counted.[19]

$$\%\ Protective\ Power = \frac{C - T}{C} \times 100\%$$

Where:
C = Number of mosquitoes on the control arm
T = Number of mosquitoes on the treatment arm

Data analysis
The yield of essential oils and quality of formulated lotions were expressed as the mean and SD. The protective power of the lotions against *Culex quinquefasciatus* were analyzed using One-Way ANOVA to see the differences among treatments. If there were any significant differences, it was continued with Post Hoc analysis using Duncan.

Results and Discussion

The distillation of 47 kg of fresh betel leaf yielded 55 mL of essential oil. Organoleptically, the essential oil of betel leaf is blackish green and has a distinctive smell of betel leaf with a yield of 0.11%. Meanwhile, the distillation of 33 kg citronella grass yielded 50 mL of citronella grass essential oil with yellow colour and distinctive smell of citronella, which is a yield of 0.15%. The lotions were made using 4 formulae: the lotion-based/negative control (F0), and then betel leaf and citronella grass essential oils at a concentration of 1% (F1), 5% (F2), and 10% (F3). The process of lotion-making involves a water phase and an oil phase. Evaluation of the formulation was conducted in 14 days (7 cycles of freeze-thaw). On day 1, the lotions were stored at 4ºC and then on day 2 at 27ºC. Evaluation of mosquito-repellent formulation conducted on day 1 and day 14 was an organoleptic and skin sensitivity analysis shown in Table 2. Quality analysis of the lotion (pH, viscosity, homogeneity, and stability) is shown in Table 3. The efficacy test of the mosquito lotion to repel *Culex quenquifasciatus* at different hours after application on hands is presented in Figure 1.

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Table 2: Organoleptic and skin sensitivity analysis of the formulated lotions

Lotion Formula	Colour		Odour		Consistency		Skin Sensitivity		
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Skin rash	Itch	Swollen
F0	White	White	No odour	No odour	Thick	Thick	Nil	Nil	Nil
F1	White	White	Very mild betel oil and citronella oil	Very mild betel oil and citronella oil	Thick	Thick	Nil	Nil	Nil
F2	White	Brownish white	Mild betel oil and citronella oil	Mild betel oil and citronella oil	Thick	Thick	Nil	Nil	Nil
F3	Brownish white	Brownish white	Mild betel oil and citronella oil	Mild betel oil and citronella oil	Thick	Thick	Nil	Nil	Nil

F0: control lotion, F1: lotion with essential oils 1%, F2: lotion with essential oils 5%, F3: lotion with essential oils 10%.

Table 3: Quality analysis of the lotions

Characteristic	Lotion Formula			
	F0	F1	F2	F3
pH	7	7	7	7
Viscosity (cP)	671 ± 3.60	378 ± 2.64	176 ± 3.46	82 ± 2.64
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
Stability	Stable	Stable	Undergo phase separation	Undergo phase separation

F0: control lotion, F1: lotion with essential oils 1%, F2: lotion with essential oils 5%, F3: lotion with essential oils 10%. Values for the pH are the means of 3 replications. The Observation was conducted on day 14.

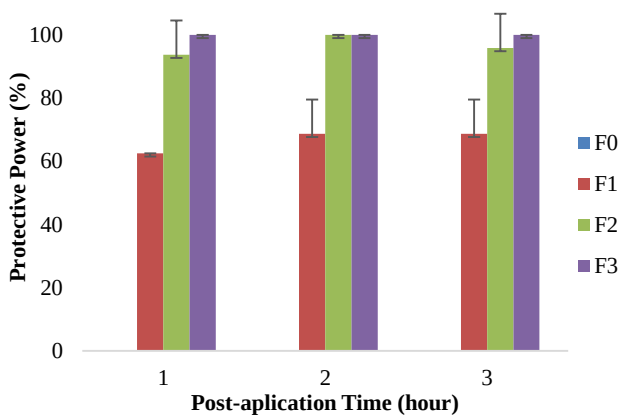

Figure 1: Protective power (%) of formulated lotions against *Culex quinquefasciatus* mosquitoes at different hours after application to the hands. F0: control lotion, F1: lotion with essential oils 1%, F2: lotion with essential oils 5%, F3: lotion with essential oils 10%. Values shown are the mean of 3 replications.

Table 4: Protective power test of betel leaf and citronella grass essential oil lotions

Formula	Protective Power (%)
F0	0^{a}
F1	68.73^{b}
F2	95.83^{c}
F3	100^{c}

F0: control lotion, F1: lotion with essential oils 1%, F2: lotion with essential oils 5%, F3: lotion with essential oils 10%. Values shown are the means of 3 replications. Different letters above each bar were significantly different (P<0.05) in the 3hrs after application.

As it is shown, the negative control provided no protection, meanwhile, F1, F2, and F3 (at 1%, 5%, and 10% concentration) provided protected for the entire three hours (Figure 1). The protective power test of lotion that combines betel leaf and citronella grass essential oils after three hours is presented in Table 4.

This study aims to develop an effective formula of essential oils from betel leaf and citronella grass to be used as a mosquito repellent. The essential oil extraction was carried out using a steam and water distillation method. The essential oils extracted were used to make repellent lotions that contained various amounts of essential oil. Essential oil was used in the lotion because lotion has a liquid consistency that allows it to be applied quickly and evenly on the skin and then dries after application. Observation that was conducted on day 1 and day 14 was an organoleptic analysis of lotion to identify changes in colour, odour, consistency, and skin sensitivity that may occur during storage. The results showed that the distinctive smell of citronella grass and betel leaf essential oils and the consistency in the lotion did not change, while the colour changed in the lotion that hasa concentration of 10% (F3). An irritation test was performed to identify the side effects of the lotion applied to the skin. There were no side effects reported nor observed on the volunteers' skin after using the lotion (Table 2).

A test of homogeneity was conducted to determine if the active substances were completely incorporated into the lotion base. The homogeneity observations conducted for 14 consecutive days showed that the lotions had a homogeneous composition and met the desired criteria.[1] Data collection of the pH of the lotions for 14 days showed that no pH change was found. All formulas had a pH of 7 and did not change during storage (Table 3). The addition of the essential oils did not affect the pH of the lotions. This indicates that the lotion is chemically stable; no chemical reaction or interaction occurs either between the substance and the container or between the substances and the lotion. Indonesian National Standard (SNI) number 16-4399-1996 proposed that the pH of lotions should range from 4.5 to 8. Thus, the lotion complies with the SNI and is safe for human skin. If the pH value of the lotion is too low (<4.5), it can cause irritation and itching. If the pH value is too high (>8), it can cause the skin to become slippery, dry, or affect the skin elasticity.[20] The lotion viscosity was measured using a Brookfield viscometer with spindle Number L3 that

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rotated at 100 rpm. Viscosity measurements aim to determine the ease of lotion when applied to the skin. Lotions are emulsions, which are mixtures of immiscible liquids in which one is scattered in the other, and this is stabilized by emulsifiers. Emulsifiers work by reducing the surface tension of the two phases and creating a thin film around the dispersed phase droplets, to make possible the formation of the emulsion and its long-term stability.[21] There are two types of emulsions, namely water in oil (W/O) and oil in water emulsions (O/W).[22] In this study, lotions with betel oil and citronella oil were a type of oil in water emulsion (O/W) in which the oil droplets were finely dispersed in the aqueous phase. Lotions of this type are easily spread on the surface of the skin, feel less oily, and are easy to wash off with water.[23] The result showed that the viscosity values of F0 and F1 were 671 cP and 378 cP which was higher than F2 and F3 (176 cP and 82 cP) (Table 3). From this result, it can be concluded that the higher the essential oil concentration, the lower the viscosity will be. This may be because the essential oil is consistently more runny than the lotion base, so it will decrease the viscosity if it is added to the lotion.[16] Centrifugation testing was conducted to determine the stability of the lotion of all formulas. The observation of the stability of the lotion stored in 7 cycles of freeze-thaw (at 27°C and 4°C) showed that the control lotion and lotion containing 1% essential oil showed no phase separation such as creaming or cracking occurred and therefore was fairly stable. The 5% and 10% lotions underwent phase separation as was seen from their lack of clarity and the presence of oil components that floated on the surface. Therefore, the level of stability was poor (Table 3). This was due to the higher concentration of betel leaf and citronella grass essential oils added.[24]

The efficacy test of the lotion indicated that the higher the essential oil concentration in the lotion, the higher protective power against mosquito bites. In addition to being an effective repellent, betel leaf and citronella grass essential oil lotion can also be used as a larvicide. This has been proven because at 5% concentration the lotion can kill two mosquitoes and at 10% concentration, the lotion can kill six mosquitos. The main content of citronella grass essential oil includes monoterpenes such as citronellal, citronellol, geraniol, geranial, and citral. These are the compounds that could be responsible for the repellent efficacy.[11,12] Citronella grass is known for its toxic effects on mosquito larvae. This effect relates to the citronella content of leaves and stalks.[25] Mosquitoes lose body fluids and die if they come into direct contact with citronella.[26] *Piper betle* L. contains phenol compounds and phenol derivatives from propenyl, allylpyrocatechol, allylpyrocatechol acetate, cineol, carvacrol, chavicol, chavibetol, chavibetol acetate, caryophyllene, cadin, cretin, eugenol, estragole, chavibetol, chavibetol methyl ether, and *p*-cymene. These compounds have a neurotoxic effect, causing rapid damage and death in insects.[27,28] Phenolics are compounds that provide efficacy in betel leaf essential oil.[5] Other compounds in *Piper betle* L. leaves that can also kill mosquitos are chavicol and eugenol. Both of these compounds have antimicrobial and synergistic effects as pesticides, especially as larvicides.[29,30] From the ANOVA test, it was found that the mosquito repellent lotion with betel leaf and citronella grass essential oils was very effective at repelling mosquitoes (P=0.00). After Duncan's test, it was seen that there was a significant difference between the control lotion and the lotion with added essential oils at concentrations of 1%, 5%, and 10%. However, there was no significant difference found between concentrations of 5% and 10% (P> 0.05) over the three hours of the repellent activity test (Table 4). According to Afify (2019), mosquito repellent lotion will target the palpi and antennae of mosquitoes because these two parts are highly sensitive to the aroma of the modified lotion. The aroma of the essential oils masks the human body odour, which interferes with the ability of mosquitoes to detect the presence of humans.[31] The essential oils evaporate into the air, carrying a scent that is detected by chemical receptors on the mosquito antennas and transmitted to nerve impulses. The scent causes mosquitoes to avoid the source of the smell.[32] From the data presented above, it can be concluded that betel leaf and citronella grass essential oils incorporated into lotions at 1%, 5%, and 10% concentrations can be used as a mosquito repellent. This complies with World Health Organization Pesticides Evaluation Scheme (WHOPES) stating that the concentration starts can be considered effective when it has 50% to 100% protective power.[33]

Conclusion

The repellent activity of lotions formulated with betel leaves (*Piper betel* Linn) and citronella grass (*Cymbopogon nardus* [L.] Rendle) oils at concentrations of 1%, 5%, and 10% were effective as repellents because they had protection power >50%. The repellent activity was concentration-dependent, where a concentration of 10% displayed the highest protection. This research is important to the effort of developing repellents from natural materials that are environmentally friendly so that people can use them as an alternative to synthetic repellents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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