

Potential topical natural repellent against *Ae. aegypti*, *Culex* sp. and *Anopheles* sp. mosquitoes

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Abstrak

Latar belakang: Minyak atsiri daun sirih diketahui mempunyai daya proteksi. Dibuatkan losion berdasarkan pengantar sediaan farmasi yang ditambahkan minyak atsiri daun nilam. Sediaan losion dipilih agar dapat menempel lebih lama di permukaan kulit. Tujuan penelitian ini untuk membandingkan daya proteksi antara losion dengan penambahan minyak nilam dan losion tanpa penambahan minyak nilam dibandingkan daya proteksi dengan DEET.

Metode: Penelitian ini merupakan penelitian eksperimental laboratorium. Semua nyamuk uji berasal dari insektarium laboratorium penelitian kesehatan Loka litbang P2B2 Ciamis. Konsentrasi minyak atsiri daun sirih dalam losion adalah 4%; konsentrasi minyak nilam sebagai zat pengikat adalah 0,4%. Formula yang digunakan yaitu formula dasar yang ada pada pengantar sediaan farmasi. Uji repelensi dilakukan dengan menggunakan metoda yang direkomendasikan oleh Komisi pestisida.

Hasil: Diharapkan formulasi losion yang stabil dan masih memenuhi standar formulasi sediaan. Berdasarkan hasil, diperoleh data bahwa DEET dan losion hasil modifikasi memiliki rata-rata daya proteksi di atas 90% selama 6 jam terhadap nyamuk *Ae.aegypti* dan *Culex* sp.

Kesimpulan: Penambahan minyak nilam pada losion sirih dapat meningkatkan daya proteksi terhadap hinggapan nyamuk *Ae. aegypti* dan *Culex* sp. (*Health Science Indones* 2014;1:44-8)

Kata kunci: repelen alamiah, minyak atsiri, daun sirih, daun nilam, *Ae. aegypti*, *Culex* sp.

Abstract

Background: Betel leaf essential oil lotion has been known to have insect repellent properties. A lotion was made based on a pharmaceutical formula from a monograph where patchouli leaf essential oil was added. A lotion preparation was intended to enhance adherence of the formula on the surface of the skin. The purpose of this study was to compare protection percentage of lotion with patchouli oil and without patchouli oil lotion compared to DEET.

Methods: This study is an experimental laboratory-based research. All mosquitoes were acquired from *Loka Litbang P2B2* Ciamis. The lotion was made up of 4% betel leaf oil with 0.4% Patchouli leaf oil which acted as fixative. Repellency test was performed using methods recommended by the Commission on Pesticides.

Results: The lotion made was stable and met the standard of formulations. The results showed that DEET and the lotion had an average protection of above 90% for 6 hours against *Ae.aegypti* and *Culex* sp. mosquitoes.

Conclusion: The addition of patchouli oil on betel lotions can increase protection against *Ae.aegypti* and *Culex* sp. bites. (*Health Science Indones* 2014;1:44-8)

Key words: Natural repellent, betel leaf essential oil, patchouli leaf essential oil, *Ae.aegypti*, *Culex* sp.

Repellent lotion is an alternative insecticide that can be used to avoid mosquito bites. Mosquitoes are vectors of diseases, such as dengue fever and chikungunya from *Ae.aegypti*, Filariasis, Javanese encephalitis, chikungunya from *Culex* sp., and Malaria from *Anopheles* sp.

Repellent lotion is a form of insecticide that is not harmful to the respiratory system compared to insecticides in other forms (spray, fuel and electricity), because it is used directly on the skin and not inhaled.¹ Repellent lotions available in the market, however, generally contain the active chemical ingredient, diethyl toluamide (DEET). This substance is only allowed in Indonesia at a concentration of 15%.² As with other synthetic substances, DEET also have negative effects, including skin irritation, urticaria, and even encephalopathy.³

Utilization of natural ingredients as an active ingredient for repellent lotions has been widely used in research to prevent the spread of disease by mosquito vectors. The use of essential oils as mosquito repellent directly, is less effective because it easily evaporates, so the precise formulations should be developed to strengthen the protective ability. In this study, the essential oil will be formulated as lotions with patchouli oil added as fixatives.⁴ Lotion was in liquid form, which will facilitate distribution of the active ingredients in the lotion and provide better protection to users.

Patchouli oil has 100% protection against *Ae.aegypti* for two hours at laboratory conditions.⁵ Betel leaf, in Latin *Piper betel* L., is one of many treatment plants are grown in Indonesia. The leaves contain various chemicals including saponins, phenolics, alkaloids and essential oils that can be used as repellent.⁶ Betel leaves also contain eugenol, although in much lower concentrations than clove leaf (30% vs. 71%)^{7,8} Eugenol is essential oil of clove leaf, which has been proven to control mosquito larvae and adult mosquitoes⁹, or reject the mosquitoes.⁷

The aim of this study was to compare the protective properties of betel leaf oil in lotion modified by the addition of patchouli oil to lotion without modification, and to the repellent effect of DEET.

METHODS

Type of research

This study was an laboratory-based experimental study. Characterization and repellency testings were

done in research laboratory facilities of 'Loka Litbang P2B2 Ciamis' from May to July 2013.

Species of mosquito

All test mosquitoes (*Ae.aegypti*, *Culex* sp., and *Anopheles* sp.) came from the insectarium at *Loka Litbang P2B2 Ciamis*. Samples were obtained by purposive sampling method, males were separated from female mosquitoes, and followed by random sampling where female mosquitoes in cages taken randomly. Samples were obtained by separating the male and female mosquitoes, male mosquitoes were removed from the cage by using an aspirator. Males can be distinguished by their bushy antennae. Female mosquitoes were taken from the population and used in the treatments. They were 3-5 days old (counting from emergence from pupae), and sugar fed only. One hundred female mosquitoes were used for all treatment, with replications performed four times for four consecutive days. There was a total of 1600 mosquitoes species.¹⁰ Full blood and dead mosquitoes were not included in this study.

Lotion preparation

The concentration of betel leaf essential oils in the lotion was 4%, while the concentration of patchouli leaf essential oils was 0.4%. This formula was obtained from a formula in the *Pengantar Sediaan Farmasi* monograph. Weighted base lotion was: cetyl alcohol, stearic acid, lanolin put into a porcelain cup (part I) and melted in a water bath at a temperature of 75°C. Methyl paraben was dissolved into hot distilled water, and then glycerin and triethanolamine (TEA) were added (part II). Then, part I was put into a heat porcelain mortar, and added the second part, stirred until homogeneous. For formulation, betel oil was weighed and then part II added to the base until it weighed 100 g. The mixture was then put into an appropriate container. The pH of the mixture was checked. After all was homogenous, distilled water was added until the concentration was 100 % (w/w).²

Repellency test procedure

Repellency test was performed using methods recommended by the Commission on Pesticides.¹⁰ This test uses the right and left hands of the volunteers. At the time of testing, the volunteer's body temperature, ambient temperature, humidity and the environment were as identical as possible. The right hand was the negative control and left

hand was the treatment. Each arm was inserted into the testing cage for five minutes and repeated every 60 minutes for 6 hours. Testing was also done using DEET and without application as comparison. Protection percentage of each treatment was calculated by the formula:¹⁰

$$DP = [(K - R) / K] \times 100\%$$

Description:

DP = Protection percentage

K = number of mosquitoes that landed on the right arm (control)

R = number of mosquitoes that landed on the left arm (treatment)

Before the main test, preliminary tests were done to determine the amount of lotion applied to the hand. The optimal amount was 1 mg. This was applied from the fingertips to the elbow, and 1 mg of lotion without essential oils applied as a control. DEET lotion was used for positive control. Right arm and left were alternately inserted into the test cage. Tests were carried out for 5 minutes per arm and done on the hour for 6 hours.¹⁰

The number of mosquitoes that landed in the treatment and control arms on an hourly basis was the dependent variable. This was used to acquire the value of protection.

RESULTS

Body temperature, ambient temperature, and humidity chamber test were confounding variables because they affect the mosquitoes as well as the volunteers. The

values of these variables were difficult to control. But body temperature of volunteers smeared with DEET and test lotion did not vary much and was relatively stable during the 6 hours and therefore did not affect the analysis of the data obtained.

Figure 1 shows protection from the betel mixed with patchouli lotion, which had the highest value compared to other treatments. In the *Ae.aegypti* mosquito test, the lotion had greater protection power compared to DEET. The protection power was 100% in hour-0 and hour-1, and decreased slowly, but still reached 90%, up to hour-6. This protection power was greater compared to DEET in hour-1 to hour-5, only in hour-6 was the power equal at 90%. It was noted, that without the addition of patchouli oil, the protection power decreased to less than 90% from hour-4 onwards.

In figure 2 shows the protection power of betel mixed with patchouli lotion against *Culex* sp. Mosquito. With DEET, protection was 100% at hour-0 and continued to decline, where it became more 90% in hour-4. In the subsequent hours, it continued to decline to less than 90% in the next hours and at hour-6 it was only 87%. DEET, on the other hand, only declined to 96% up to hour-6. The betel lotion only protected for more than 90% in the first two hours and continued to decrease to only 77% at hour-6.

Figure 3 shows that the protection power of DEET was more than 90% until hour-6. The modified betel lotion protected for more than 90% only until hour-3, declined to 80% in hour-4, continued to decline to 61% in hour-5, and was only 23% at hour-6. At hour-0 to 5, the betel lotion was still high, and at hour-6, protection was higher compared to the modified betel lotion.

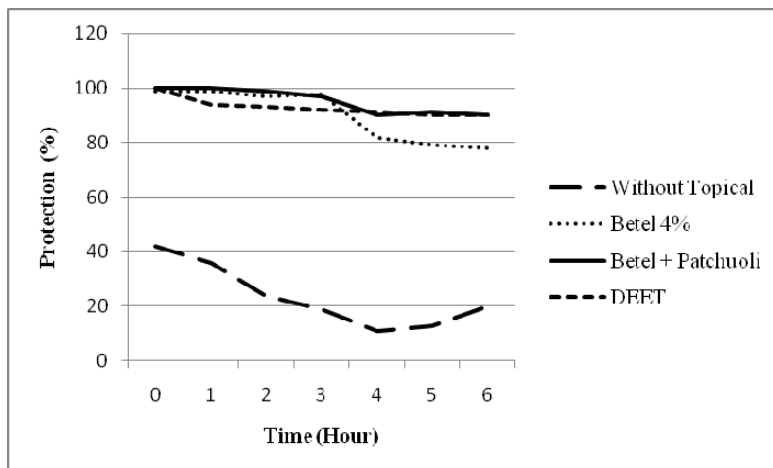


Figure 1. Trends in protection power against *Ae. aegypti* mosquito

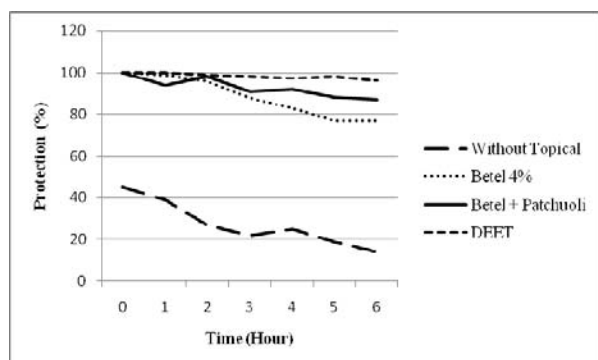


Figure 2. Trends in protection power against *Culex sp.* mosquito

Of the three observations, the modified betel lotion protection against *Ae. aegypti* mosquito was still at 90%, equal to DEET, until hour-6 which was higher than the betel lotion. While for *Culex sp.*, although protection at hour-6 was reduced to less than 90%, it was still higher compared to the betel lotion. For *Anopheles sp.*, protection by modified betel lotion declined rapidly at hour-6, quite unlike DEET which remained stable, and was even lower compared to the betel lotion.

DISCUSSION

This study showed that the addition of patchouli oil as fixative can enhance the protection power of betel oil against *Ae. aegypti* and *Culex sp.* But it did not enhance protection against *Anopheles Sp.* mosquitoes.

The lotion obtained met the Pesticide Committee standards. Protection against *Ae. aegypti* still reached 90% at hour-6. The result was similar to a study using rosemary (*Rosmarinus officinalis L.*) essential oil gel formula and Zodia (*Evodia suaveolens S.*) with modifications by applying patchouli oil as fixative.¹¹ Repellency studies against *Ae. aegypti* showed some effect, but without the addition of a fixative, protection was lower. This occurred a study using Fennel (*Foeniculum vulgare*), that the highest concentration (10%) showed protection at hour-0 at was only 86% and declined to 8.2% at hour-6.⁴ Also in a study using neem seed oil repellent lotion (*Azadirachta indica A. Juss*) of 1.5% concentration, protection was 88.67% at hour-0, and continued to decline to 51.24% at hour-6.¹²

Protection for *Culex sp.* reach 90% until hour-2, declined and reached 87% at hour-6. This protection was similar to the results using Sembung pahit (*Blumea lacera*) extract of 6% concentration against

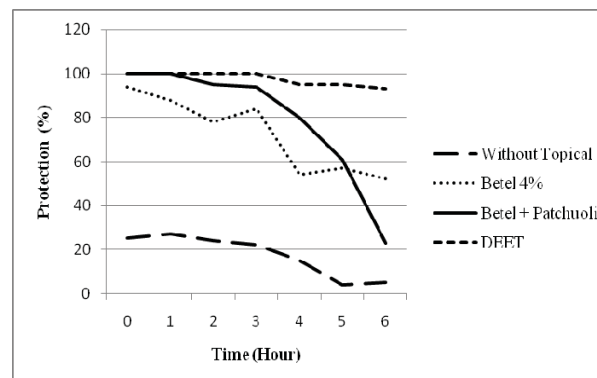


Figure 3. Trends in protection power against *Anopheles sp.* mosquito

Culex quinquefasciatus, while a 4% concentration of Sembung pahit provided 90% protection at hour-1.¹³

Protection against *Anopheles sp.* mosquito was more than 90% until hour-3. In the following hour, it plummeted to 23%, which was even lower than the value of the modified betel lotion of 52%. The results of this study differ from a repellency test using Sembung pahit (*Blumea lacera*) extract which showed a higher protection power against *Anopheles stephensi* than against *Culex quinquefasciatus*.¹³ Other studies to assess the protection time using essential oils such as, clove (*Syzygium aromaticum*), lemon grass (*Cymbopogon citratus*), and citronella (*Cymbopogon nardus*) showed protection time against *Anopheles dirus* was higher than against *Ae. aegypti* and *Culex quinquefasciatus*.¹⁴ The differences in these results may be due to differences in the condition of mosquitoes each region and the sensitivity to certain active substances of mosquito each species.

In this study, the addition of fixative in betel lotion enhanced protection at hour-6 by an average of 4.3% - 6.5%. Although there was no significant difference, but it could be used as a basis for further modifications either by using different essential oils or by differing concentrations of the fixative added.

DEET is a synthetic chemical compound capable of providing better protection and longer lasting, against three types of test mosquitoes. Protection provided by DEET at hour-6 was still 90%. Modified lotions provided the same protection as DEET against *Ae. aegypti* only, and not against *Culex sp.* or *Anopheles sp.* The addition of a fixative made the betel lotion as effective as DEET against the *Ae. aegypti*. But nevertheless, the use of synthetic chemicals, such as DEET, may be a health risk that result in some side effects.¹⁵ So the use of a lotion with natural active

substances would be better as an alternative to DEET. Products with natural ingredients are generally safer than synthetic repellent.¹⁶ The results of this study indicate that the use of natural materials as active ingredients in the repellent lotion is potentially useful.

The main drawback of a natural mosquito repellent is its shorter durability compared to DEET. This study proves that the modification which is the addition of a fixative agent like patchouli oil can increase the potency of natural ingredients to be used as repellent. Mosquito repellent made from betel lotion made in this study focused on Palpi and antennae of mosquitoes, because mosquitoes Palpi and antennae are very sensitive to the scent of the eugenol compound. Then the scent of plants extracts can mask the smell of the human body that disrupts the ability of mosquitoes to detect humans.¹⁷

Control of mosquitoes that are vectors of various diseases by using modified natural repellent is one alternative that is often used. But it would be better if it was done together with environmental, biological, physical and chemical controls. If ways to control mosquitoes is successful, the expected incidence of mosquito-borne diseases will be reduced.

In conclusion, betel lotion modification proved to give higher protection compared to the betel lotion without modification on *Ae. aegypti* and *Culex sp.* Further studies should be made on the concentration of betel oil used, and the fixative to be added.

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