The effectiveness of fixative addition on Zodia (*Evodia suaveolens S.*) and rosemary (*Rosmarinus officinalis l.*) gel against *Aedes aegypti*

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Received: September 8, 2013; Revised: November 7, 2013; Accepted: November 12, 2013.

Abstrak

Latar belakang: *Nyamuk Aedes aegypti merupakan salah satu penyebab penyakit Demam Berdarah Dengue (DBD). Sebagai salah satu upaya pencegahannya, bahan tanaman sering dijadikan sebagai bahan penolak nyamuk, di antaranya Zodia (Evodia suaveolens Scheff) dan Rosemary (Rosmarinus officinalis L.). Salah satu pengembangan yang banyak dilakukan adalah modifikasi sediaan yang mudah dipakai agar lebih tahan lama, misalnya formulasi gel minyak atsiri. Tujuan dari penelitian ini yaitu mengetahui efek penambahan zat fiksatif (minyak nilam) terhadap daya proteksi repelan gel Rosemary dan Zodia.*

Metode: *Penelitian ini bersifat ekperimen yang menggunakan minyak atsiri dari bunga Rosemary dan daun Zodia dengan konsentrasi masing-masing 0,1%, 0,5%, 1%, 2%, 4%. Kontrol (+) menggunakan N,N-diethyl-3-methylbenzamide (DEET) dan kontrol (-) menggunakan lengan tanpa perlakuan. Uji repelan menggunakan lengan sukarelawan yang sudah dilatih. Pengamatan dilakukan tiap jam selama enam jam. Daya proteksi baik jika bernilai 90% atau lebih.*

Hasil: *Data menunjukkan bahwa penambahan zat fiksatif meningkatkan daya proteksi repelan mulai dari konsentrasi 2% untuk Rosemary dan konsentrasi 4% untuk Zodia. Daya proteksi di atas 90% selama 6 jam.*

Kesimpulan: *Penambahan zat fiksatif gel Rosseamary dan Zodia terbuti efektif untuk meningkatkan daya proteksi repelen terhadap nyamuk Aedes aegypti. (**Health Science Indones 2013;2:103-6)***

Kata kunci: *Aedes, Efektivitas repellent; Evodia suaveolens S., Rosmarinus officinalis L.*

Abstract

Background: *Aedes aegypti* is a mosquito is one of the vectors for dengue. One method of preventing dengue is to use bio insecticides from plants. A plant that is often used as a mosquito repellent is Zodia (*Evodia suaveolens s*cheff) and Rosemary (*Rosmarinus officinalis l.*). Some studies have modified the dosage of bio insecticides to achieve more durable repellent, including developing a gel form. The aim of this study is to measure the protective effect of additional fixative material (patchouli oil) on Rosemary and Zodia repellent.

Methods: This was an experimental study using gel made from essential oil of Rosemary flowers and Zodia leaves with concentrations of 0.1%, 0.5 %, 1 %, 2 %, 4 %, respectively. Control (+) group used N, N-diethyl-3 (methylbenzamide (DEET) on the arms, while control (-) group were arms with no treatment.

The repellent was tested on the arms of trained volunteers, which were then observed every hour for six hours. The protective effectiveness was satisfactory if ≧ 90%.

Results: Addition of fixative material increased the durability of repellent, starting from concentration of 2% for Rosemary and concentration of 4% for Zodia. The protective effectiveness was 90% for more than 6 hours.

Conclusion: The modified gel of Zodia and Rosemary proved to be a potential repellent against *Aedes aegypti* mosquitos. ***(Health Science Indones 2013;2:103-6)***

Key words: *Aedes aegypti, Evodia suaveolens S.,* repellent effectiveness, *Rosmarinus officinalis* L.

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Currently, the Indonesian government as well as the community has attempted to decrease the incidence of Dengue Hemorrhagic Fever (DHF) cases, including its morbidity and mortality. Several factors cause dengue cases to remain high, including behavioral changes of mosquito towards community efforts.

Efforts to control Dengue vector include eradication of mosquito's nests, fumigation, and developing mosquito repellent. The use of mosquito repellent has increased because of its simplicity. People only apply the repellant on their skin and then they don't have to apply it again for the next several hours. The repellent commonly contains N, N - diethyl - m - toluamide (DEET) as its active ingredient.[1]

Substances from plants, such as Zodia (*Evodia suaveolens s*chiff) and Rosemary (*Rosmarinus officinalis L.*), can be used as alternative mosquito repellent instead of synthetic chemicals. Zodia compounds containing linalool (46%) and α - pinene (13.26%). Linalool is well recognized as a mosquito repellent.[2] Rosemary flower essential oil contains euginol and sesquiterpenes as its main substances. Euginol and sesquiterpenes compounds are potential anti-malarial and mosquito repellents.[3]

Essential oil repellent applied directly to skin is less effective because it is volatile. Essential oil should be delivered in an appropriate form and dosage for practical and durable application. A gel is an example of appropriate essential oil formulation which is a clear preparation and does not leave stains on the wearer. In addition, a liquid gel also makes the active ingredient gel easier and faster to spread as protection to the wearer. As an addition to optimize the repellent gel, the addition of fixative agents such as patchouli oil will make the scent last longer.[4] The aim of this study was to identify the effect of fixative addition (patchouli oil) on Rosemary and Zodia gel repellent protective effectiveness.

METHODS

This was a laboratory experimental study. The repellent in this study was tested at the Laboratory of Experimental Research in West Java, Indonesia from March to May 2012.

Aedes aegypti mosquitoes which were reared at the Research Workshop insectarium of *Loka Litbang P2B2 Ciamis*. Repellent activity of gel was tested on six human volunteers.

The gel repellent activity referred to a repellent testing method described in literature by the Pesticides commission.[5] Three to four day-old blood starved female adult mosquitoes were used as a mosquitos sample, These were taken randomly from the rearing cages and kept in a test net cage, the tests were conducted in a well-ventilated room with a temperature of 26-30 °C and humidity of 60-80 %. The cages were 50 cm in length, 35 cm in width, and 40 cm in height. They were made of nylon netting with iron wire frames. A hole was made on the front of the cage for a place to insert hands. Based on the Pesticides commission, each cage contained 100 mosquitoes for each treatment.[5] Determination of replications numbers for each treatment group was based on the Federer formula.[6]

Initially, Carbopol Ultrez 10® was mixed with water and TEA to make the repellent gel. Glycerin, ethanol 95% and methylparaben were added. Then Zodia or Rosemary extracts were added to the mixture with the desired concentration.[7]

The area from the fingertips to the elbow of the tested person was cleaned with water and then air-dried. The hands were protected by gloves. DEET served as positive control. DEET and concentrated gel at 0%, 0.5%, 1%, 2% and 4% were applied on the dorsal side of the skin of left arm. The right hand was not smeared by repellent and acted as negative control. The control and treated arms were introduced simultaneously into the cage. The number of bites was counted for 5 minutes every 60 minutes, from 08:00 to 14:00. The procedure was conducted five times. The protection effectiveness value was calculated by using the following formula:

$$\text{Protection (DP)} = ((K - R) / K) \times 100\ \%.$$

K is number of landing mosquitoes on the control group and R is number of landing mosquitoes on the treatment group. The repellent was considered as effective if protection for 6 hours was ≥ 90%.

RESULTS

Table 1 showed a preliminary test of Rosemary and Zodia treatment. On average, all observations up to 2 hours were effective as repellent. However, it became ineffective within 6-hours observation. At a concentration of 4% within 4 hour observation, the repellent remained effective, but its effectiveness declined at 5th and 6th hours of observation.

Table 2 presented Rosemary and Zodia gel with additional fixative agent. The additional fixative materials/agent on repellent increased durability and the repellent remained effective up to 6 hours. With

the addition of patchouli oil fixatives, a concentration of 2 % of Rosemary was a potential repellent. However, the effectiveness of Zodia was seen at a concentration of 4%.

The protection effectiveness values obtained were test for normality. This meant the data were not normally distributed and homogeneous, the Anova test requirements were not met. Therefore, the Kruskal-Wallis test was done to find the difference between the effects of treatment. There was no potential significant difference within four hours of observations between DEET and gel treatment. Starting from the fifth hour, there was a significant difference between the two treatment groups, because the hours proficiency level has a value of $p < 0.05$.

Table 1. Protection in preliminary test of repellent against Ae.aegypti

Treatments	Protection at each hour (%)						
	0	1	2	3	4	5	6
Rosmarimus officinelis L							
Control (+) DEET	100	100	100	100	100	100	100
Control (-) no treatment	26.32	25.03	25.03	18.62	10.00	12.57	15.44
0.1%	100	100	79.59	63.79	22.22	46.25	27.37
0.5%	100	100	81.13	67.69	30.00	46.93	31.63
1%	100	100	87.03	70.49	74.35	73.09	63.90
2%	100	100	100	100	89.47	88.8	89.05
4%	100	100	100	100	90.47	89.65	89.51
Evodia suaveolens S							
Control (+) DEET	100	100	100	100	100	100	100
Control (-) no treatment	28.63	24.44	24.44	15.67	15.20	15.16	12.00
0.1%	100	100	81.81	75	28.57	55.88	25.71
0.5%	100	100	83.33	76.92	29.76	57.14	27.77
1%	100	100	100	79	29.80	57.89	53.40
2%	100	100	100	100	34.61	58.04	54.32
4%	100	100	100	100	93.50	89.80	87.87

Table 2. Protection in real test of repellent against *Ae.aegypti*

Treatments	Protection at each hour (%)						
	0	1	2	3	4	5	6
Rosmarimus officinelis L							
Kontrol (+) DEET	100	100	100	100	100	100	100
0.1%	100	100	81.81	75	28.57	27.38	28.57
0.5%	100	100	83.33	76.92	29.76	29.83	29.34
1%	100	100	100	79	29.80	57.89	53.40
2%	100	100	100	100	100	90.38	90
4%	100	100	100	100	100	94.92	90.13
Evodia suaveolens S							
Kontrol (+) DEET	100	100	100	100	100	100	100
0.1%	100	100	86.88	63.79	22.22	58.79	25.71
0.5%	100	100	87.32	67.69	30	60.93	27.77
1%	100	100	100	70.49	74.35	73.09	63.90
2%	100	100	100	100	89.47	88.8	89.05
4%	100	100	100	100	100	94.92	94.04

DISCUSSION

A natural substance that was effective as a repellent was not found. Niradita reported that currently DEET remains an effective substance for repellent.[8] It can last for 8 hours. This study found that in the positive control, DEET provided protection for up to six, with 100% protection that lasted for several hours. However, the use of chemicals as repellent is now beginning to be avoided and replaced with bio repellent.

Rosemary and Zodia gel are potential repellents, but their effectiveness is considered low (less than 90 %). This result was similar to a study by Shinta which found that essential oil of Zodia and Rosemary were able to protect against mosquitoes landing, but they were not effective against Aedes aegypti mosquitoes were protection was less than 90 %.[9] This result was also similar to studies by and by Sianipar.[10.11]

Ineffectiveness of Zodia and Rosemary gel as repellent for Aedes aegypti may be caused by several factors, including gel durability and human factor. Physiologically, human sweat have mixed with the applied active substances may change the structure of the active substance leading to less optimal repellent effects.[8-]

This study showed that Rosemary and Zodia gel have repellent effects, but their effectiveness does not meet standards of the Pesticides commission. Modifications by applying patchouli oil as additional fixative agent may increase durability of Zodia and Rosemary gel.

The activity of gel without additional fixative started to decline after four hours, Rosemary gel has added fixative act as repellent at concentrations starting from 2 %, while Zodia gel at a concentration of 4%.

In conclusion, A modified gel of Zodia and Rosemary proved to be a potential repellent against *Aedes aegypti* mosquitos. Further studies should be carried out on repellent properties of its active compounds.

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