The Repellent Activity Test of Rosemary Leaf (*Rosmarinus officinalis*) Essential Oil Gel Preparations Influence on *Aedes aegypti* Mosquito

Maharani Ayu Manik Pratiwi¹ and Purwati¹,*

¹Faculty of Pharmacy, Universitas 17 Agustus 1945 Jakarta, Indonesia

*putri_nuryadi@yahoo.com

**Abstract.** Rosemary leaf (*Rosmarinus officinalis*) is a plant containing essential oils such as geraniol, linalool, seneol, and borneol. Essential oils used as insect repellents. This research is about repellent test of Rosemary (*Rosmarinus officinalis*) gel for *Aedes aegypti* mosquito which aims to find out whether Rosemary (*Rosmarinus officinalis*) can provide activity as a repellent for *Aedes aegypti* mosquitoes. Rosemary essential oils (*Rosmarinus officinalis*) are formulated in the form of gel preparation with various concentrations namely 6%, 12%, 24% and use control repellent, supply on the market that contains DEET.

Volunteer completed that process over 4 days. The volunteer arms were inserted into the cage containing 50 mosquitoes which had been applied to the gel. The test is performed for 5 minutes every 4 hours, with 5 repetitions. The result shows that Rosemary essential oil (*Rosmarinus officinalis*) gel has a repellent activity against *Aedes aegypti* mosquito on F3 24% concentration with 92.15% protection for 4 hours. The stability of gel preparation that organoleptic do not change the color, taste, smell, and shape. Homogeneous gel preparation, with pH, dissipation, and viscosity are suitable for 8 weeks at different temperatures (4°C, 25°C and 40°C), and do not irritation to the skin.

1. Introduction

Dengue Hemorrhagic Fever (DHF) is one of the acute infectious diseases transmitted by the bite of *Aedes aegypti* mosquitoes. This virus is usually found in subtropical–tropical climates, especially in urban and semi–urban areas that have high levels of population density. In Indonesia, prevention of DHF is carried out by means of 3M that is *mencatup* (closing), *menguras* (draining), and *mengubur* (burying) used goods or water reservoirs that can become *Aedes aegypti* mosquito breeding grounds. One effort to prevent mosquito bites is to use repellents, especially in the morning and evening.

**Repellent** is a material that is applied to the outer surface of the body that can provide personal protection against the bite of mosquitoes or other insects. **Repellent** that is circulating in the market contains DEET (N, N-diethyl-3-methylbenzamide) which has shown satisfactory results in preventing mosquito or other insect bites [1].

One of natural substances that also have the potential to be natural repellents is Rosemary (*Rosmarinus officinalis*). Rosemary leaf (*Rosmarinus officinalis*) is an aromatic plant in which its compounds can cause a distinctive odor or aroma [2;3]. This plant belongs to the family Lamiaceae.
which main components are borneol, linalool, terpineol, caryophyllene, 1,8-cineole, α-pinene, verbenone, these components are found in essential oil which has activity against some types of mosquitoes/insects [4;5;6]. The results of Kardinan’s study (2007) shows that the repellent activity of rosemary leaf extract was found at 12.7% in which could cause flies (Musca domestica) to be knocked down.

In this study, formula was used in the gel preparations. Gel preparations are preferred because they have water content that is cooling, soothing, moisturizing, and easy to use [7]. The base gel used is Carbopol 940. Carbopol is hydrophilic so that its stability is large, it is easy to wash in the water, it has good dispersion on the skin, it can be used on the hairy body parts and it has a good drug release [8].

Based on the description above, the researchers were interested to make a formulation of rosemary (Rosmarinus officinalis L) essential oil gel preparations as a repellent against Aedes aegypti mosquitoes. As there is no reports on the use of rosemary (Rosmarinus officinalis L) essential oil as an anti-mosquito gel, it is hoped that the gel derived from essential oil of rosemary leaf (Rosmarinus officinalis L) can be used as an anti-mosquito alternative ingredient as a substitute on the market.

2. Materials And Methods

2.1. Materials

The ingredients (materials) used in this study were Rosemary Leaves (Rosmarinus officinalis L), carbopol 940, triethanolamine, propylenglycol, methylparaben, ethanol 96%, distilled aqua. In this study the test animals used were female Aedes aegypti mosquitoes obtained from the Insectarial Section of Health Parasitology and Entomology Department of Zoology and Public Health at Faculty of Veterinary Medicine in Institute Pertanian Bogor, West Java.

2.2. Methods

2.2.1. Isolation Distillation Of Rosemary Leaf Essential Oil. Distillation of Rosemary leaf (Rosmarinus officinalis L) essential oil was carried out by using the steam-water distillation method. By employing simplicia, ± 5 kg of Rosemary leaves (Rosmarinus officinalis L) were put into a round bottom flask and distilled aqua was added so that the simplicia was submerged. Distillation process of Rosemary leaves (Rosmarinus officinalis L) was carried out for ± 3 hours at a temperature of 100 °C, above a water bath. When the water boiled, water vapor carried the essential oil particles of Rosemary leaves (Rosmarinus officinalis L) into the condenser part of the distillator. Then, the components of oil and water vapor were shared into a separating funnel, in order to separate the essential oil and water [9]. From the separation, the formation of two layers, oil layer and water layer, was obtained. Oil layer was taken and freed from the rest of water layer, to get pure essential oil by adding Na₂SO₄ Anhydrous.
2.2.2.4. The Determination of Specific Gravity. This specific gravity was determined by using a pycnometer. The pycnometer was washed in water then rinsed with alcohol. The pycnometer was weighed with the lid inserted. Filled the distilled water to the boundary mark, then weighed and noted. After that, weighed back the weight of the pycnometer that had been filled with the distilled water to the tera mark. Next, the weight of distilled water was calculated by reducing the weight of the filled pycnometer with the empty weight of the pycnometer. The procedures for the pycnometer filled with oil was then carried out like the procedures on distilled water. Oil specific gravity is calculated by comparing the weight of oil with the weight of water in the same pycnometer and the same temperature [11].

2.2.2.5. Analyzing Essential Oil. Essential oil that were tested for their compounds were matched with the data contained in GC-MS memory (Agilent Technologies 7890®). The working principle of this method was to identify compounds based on the molecular weight of each compound.

2.2.3. Gel Formulation. Tools and ingredients were prepared for the production of Rosemary leaf (Rosmarinus officinalis L) essential oil gel preparations weighed each gel producing/making ingredients. Measured the volume of ethanol 96%, triethanolamine, and rosemary leaf essential oil according to the required concentration formulation.

As much as ± 10 ml of distilled aqua was heated in a water bath, then put Carbopol 940 into the distilled aqua until it was fully fluffy, then put crushed triethanolamine until the mix became homogeneous and transparent gel mass (gel base) was formed.

Then, dissolved methylparaben with 96% ethanol plus propylene glycol; put the crushed gel base until it became homogeneous. After that, add rosemary leaf essential oil into the gel preparations, finally distilled aqua was added until the weight of 100g was crushed and became homogeneous put it into a prepared container, then the evaluation test of the preparations was carried out.

Table 1. Rosemary Leaf (Rosmarinus officinalis L) Essential Oil Gel Formulation

| Ingredients                  | Concentration (%) | Use                  |
|------------------------------|-------------------|----------------------|
| Rosemary leaf essential oil  | NC 0, F1 6, F2 12, F3 24 | Active substance     |
| Carbopol 940                 | 2 g               | Gel base             |
| Triethanolamine              | 2 ml              | Base stabilizer      |
| Propylene glycol             | 15 g              | Humectant            |
| Ethanol 96%                  | 1 ml              | Solvent              |
| Methyl paraben               | 0.1 g             | Preservative         |
| Distilled Aqua               | ad 100            | Solvent              |

Note: NC = Negative Control
      F = Formula

2.2.4. The Physical Evaluation of The Gel Preparations. The gel preparations stability test that includes checking the physical stability of the preparations, checking for homogeneity, checking for viscosity, spreadability, and irritation test – was carried out for 8 weeks of storage. The preparation is declared stable if the color, odor, and appearance do not change visually during storage and also visually not overgrown with mold. Observations were made at different temperatures, namely 4°C, 25°C, and 40°C and were carried out from the 0th week until the 8th week [12].

2.2.5. The Repellent Activity Test Of Rosemary Leaf (Rosmarinus Officinalis L) Essensial Oil Against Aedes Aegypti Mosquitos

2.2.5.1. Probandus Volunteer Preparation. Testing repellent activity was done according to WHOPES and Environmental Protection Agency (EPA) literature. This repellent testing was conditioned as in the original environment. Volunteers or Probandus from this test have certain requirements that must be
met, namely the age limit of volunteers must be around 18-55 y.o, volunteers are not sensitive or have no allergies to mosquito bites, volunteers must not use fragrances [perfumes] for 12 hours, from before the start of the test or during the test and do not have a distinctive odor that can interfere with the testing, volunteers are non-smokers or should not be smoking or exposed to cigarette exposure for 12 hours before testing and during testing, volunteers have signed an agreement sheet with the researchers, that volunteers are ready to do the testing, this testing is not for pregnant and breastfeeding women. The Preparation Phase for Testing the Repellent Activity of Rosemary Leaf (Rosmarinus officinalis L) Essential Oil required 100 female Aedes aegypti mosquitoes and a mosquito cage. Mosquitoes were fasted for 3-5 days before treatment—the testing was carried out in a cool room of 24°C, humidity ± 60%, with not too bright light—adjusted to the habitat of Aedes aegypti mosquitoes. Each treatment used 50 mosquitoes for 1 mosquito cage, with 5 times repetition every 5 minutes for 4 hours. This test required 5 probandus volunteers for all formulas.

2.2.5.2. Repellent Testing Of Rosemary Leaf Essential Oil. The testing phase of this repellent activity followed the standard method of pesticide efficacy by Pesticide Commission of Agriculture Department in 1995. This test began by inserting the left arm without applying the active ingredients of gel preparations (control arms), into a cage containing 50 Aedes aegypti mosquitoes that have been fasted, after that, the number of mosquitoes that perched (attacked) on the left hand was counted. Next, the volunteers’ right arm was applied with the gel preparations with active ingredients (treatment arm) the application of the gel preparations was carried out evenly from the wrist to the tip of the elbow. Then, the right arm was put into mosquito cages every 5 minutes for 4 hours starting from the 0th hour. Then the number of mosquitoes that perched (attacked) was counted each mosquito perched was counted on each interruption for 10 seconds. The repellent testing was repeated 5 times per hour and each repetition was carried out for 5 minutes to 4 hours with different probandus [volunteers]. The distance from each test is 5 minutes and likewise for the positive control.

2.2.5.3. The Efficacy Criteria. At the end of the testing, the protective power was assessed (measured) as a proportion of the number of mosquitoes perched on the treatment arm and the number of mosquitoes perched on the control arm calculated using the following formula:

\[
\text{Protection Power (PP)} = \left( \frac{K-R}{K} \right) \times 100\%
\]

Note:
K: the number of perched mosquitoes on the control arm
R: the number of perched mosquitoes on the treatment arm

After obtaining a protective power percentage, each concentration was assessed to find out an effective concentration for obtaining a protective power percentage of 50% and 90% until the fourth hour [13].

2.3. Data Analysis
Data analysis was done with One-Way ANOVA test using SPSS software and continued with Post Hoc Test (LSD). The evaluation results of the gel preparations test were analyzed descriptively [14].

3. Results and Discussion

3.1. The Identification Of Rosemary Leaf Essential Oil Determination
The distillation process was carried out for 3 hours by adding simplicia rosemary leaves that have been aerated under the sun for not too long and produced essential oils as much as 26 ml and the yield produced is 1.3% v/w. The results obtained from the physical and chemical properties identification and determination of Rosemary leaf essential oil were the specific gravity of rosemary essential oil was 0.90718 g/cm³ and the value of the bias (refractive) index was 1.463.
3.2. Analysis Of Essential Oil Component

The principle of the GC-MS method works with two combined methods of gas chromatography (GC) which function as a separator for various mixed components in the sample, while mass spectrometry (MS) functions to detect each molecule that has been separated in the gas chromatography system (Agusta, 2000). The results of the analysis of essential oil components using the GC-MS method show that there are some chemical compounds contained in rosemary oil (Rosmarinus officinalis L).

Table 2. Components Analysis Results of Rosemary Leaf (Rosmarinus officinalis L) Essential Oil with GC-MS (Gas Chromatography Mass Spectrometry) Method

| No. | Sample Type                      | Compound      | Content   |
|-----|----------------------------------|---------------|-----------|
| 1   | Rosemary Leaf Essential Oil      | Alpha - Pinene| 27.75 %   |
|     |                                  | 1,8-Cineole   | 23.18 %   |
|     |                                  | Camphene      | 2.85 %    |
|     |                                  | beta-myrene   | 1.26 %    |
|     |                                  | 4-Terpineol   | 1.10 %    |
|     | (Rosmarinus officinalis L)       | 1-Verbenon    | 12.76 %   |
|     |                                  | Borneol       | 6.40 %    |
|     |                                  | Geraniol      | 5.01 %    |
|     |                                  | beta-Linalool | 3.76 %    |
|     |                                  | Camphor       | 2.93 %    |
|     |                                  | trans-caryophyllene | 2.35 % |

3.3. Physical Evaluation Of The Gel Preparations

3.3.1. Organoleptic Test. In this test the addition of the essential oil concentration was done to test/check whether the gel preparations experiencing color changes in the 8 weeks storage at different temperatures (4°C, 25°C, 40°C) and the test revealed that it did not experience any change in each formula.

Table 3. Organoleptic Test

| Formula   | Shape | Color | Rasa   | Smell               |
|-----------|-------|-------|--------|---------------------|
| Negative Control | Thick | Clear | Tasteless | Odorless          |
| FI        | Thick | White | Tasteless | Aromatic Smell +   |
| F II      | Thick | White | Tasteless | Aromatic Smell ++  |
| F III     | Thick | White | Tasteless | Aromatic Smell +++ |

Information :  
+: Mild aromatic  
++: Medium aromatic  
+++: Strong aromatic

3.3.2. The Homogeneity Test. The results reveal that each formula shows good homogeneity at 3 different temperatures in which there are no coarse grains on the glass plate.

3.3.3. pH Test. pH test was done by using a pH meter. The pH results of Rosemary Leaf essential oil gel preparations still meet the required requirements within 8 weeks and within 3 different temperatures as the pH value is still in the normal pH range for human skin, which is 4.50 - 6.5.
### Table 4. pH Test

| Formula | Storage Time (Week) | pH Test 4°C±2°C | pH Test 27°C±2°C | pH Test 40°C±2°C |
|---------|---------------------|-----------------|-----------------|-----------------|
| F I     | 0                   | 6.08            | 6.02            | 5.98            |
|         | 2                   | 6.05            | 5.98            | 5.92            |
|         | 4                   | 6.02            | 5.92            | 5.87            |
|         | 6                   | 6.00            | 5.87            | 5.80            |
|         | 8                   | 5.94            | 5.82            | 5.72            |
| F II    | 0                   | 5.95            | 5.90            | 5.85            |
|         | 2                   | 5.90            | 5.86            | 5.75            |
|         | 4                   | 5.87            | 5.87            | 5.80            |
|         | 6                   | 5.83            | 5.80            | 5.74            |
|         | 8                   | 5.80            | 5.72            | 5.72            |
| F III   | 0                   | 5.85            | 5.75            | 5.70            |
|         | 2                   | 5.80            | 5.78            | 5.65            |
|         | 4                   | 5.84            | 5.73            | 5.60            |
|         | 6                   | 5.71            | 5.63            | 5.58            |
|         | 8                   | 5.62            | 5.58            | 5.53            |

### 3.3.4. Viscosity Test

The determination results of the viscosity of the gel preparations showed high viscosity at the beginning of production/making and tend to continue to decline. This declining viscosity happened because of several factors including the concentration of the essential oil formulation, storage time and temperature used. The higher the concentration of rosemary essential oil formulation used in the gel preparations formulation, the lower the resulting viscosity is. The results of the data obtained were still in the range between 5,000-100,000 centipoise and optimal 20,000 cp.

### Table 5. Viscosity Test

| Formula | Storage Time (Week) | Viscosity Test 4°C±2°C | Viscosity Test 27°C±2°C | Viscosity Test 40°C±2°C |
|---------|---------------------|------------------------|------------------------|------------------------|
| F I     | 0                   | 17200 cps              | 17000 cps              | 16800 cps              |
|         | 2                   | 17500 cps              | 17200 cps              | 17000 cps              |
|         | 4                   | 17900 cps              | 17500 cps              | 17300 cps              |
|         | 6                   | 18200 cps              | 17900 cps              | 17500 cps              |
|         | 8                   | 18400 cps              | 18100 cps              | 17800 cps              |
| F II    | 0                   | 16400 cps              | 16000 cps              | 15200 cps              |
|         | 2                   | 16700 cps              | 16200 cps              | 15500 cps              |
|         | 4                   | 16900 cps              | 16500 cps              | 15800 cps              |
|         | 6                   | 17200 cps              | 16800 cps              | 16000 cps              |
|         | 8                   | 17400 cps              | 17000 cps              | 16400 cps              |
| F III   | 0                   | 14900 cps              | 14000 cps              | 13500 cps              |
|         | 2                   | 15100 cps              | 14200 cps              | 13800 cps              |
|         | 4                   | 15400 cps              | 14500 cps              | 14100 cps              |
|         | 6                   | 15800 cps              | 14800 cps              | 14500 cps              |
|         | 8                   | 16000 cps              | 15300 cps              | 14900 cps              |

### 3.3.5. Spreadability Test

The results of the data obtained show that the spreadability of the gel preparations is still in the range of 5-7cm. This results meet the spreadibility requirements of nice and convenient spread for topical use.
3.3.6. Irritation Test. The irritation test was observed in order to check the sensitivity of the skin to an ingredient. The irritation test was carried out with a closed patch test method on 3 probandus [volunteers] in each formula. This test was done by applying the gel preparations to the arms of the volunteers – then the application area was observed to find out whether an irritation reaction occurred after 24 hours of the gel preparations application. The results of irritation test obtained in this test after 24 hours of the gel preparations application give a negative result in which the gel preparations do not show a negative effect on the skin; there is no occurrence of rough scaly skin, redness or itching around the skin of the probandus.

3.4. The Repellent Activity Test Of Rosemary Leaf Essential Oil Gel

Table 6. The Test Results of Average Protection Power of Rosemary (Rosmarinus officinalis L) Essential Oil Gel Preparations and the Positive Control

| Formula        | 0th hour | 1st hour | 2nd hour | 3rd hour | 4th hour | Average Protection Power |
|----------------|----------|----------|----------|----------|----------|--------------------------|
| FI (6%)        | 91.49    | 91.19    | 89.76    | 87.88    | 85.24    | 89.11                    |
| F II (12%)     | 92.93    | 91.57    | 90.18    | 89.72    | 86.69    | 90.21                    |
| F III (24%)    | 94.44    | 93.26    | 92.08    | 91.07    | 89.19    | 92.15                    |
| Positive Control | 97.82  | 97.14    | 96.47    | 96.67    | 95.56    | 96.73                    |

![The Chart of Average Protection Power of Rosemary (Rosmarinus officinalis L) Essential Oil Gel Preparations](image)

From the results of the observations shown above, the protective power of rosemary leaf essential oil gel preparations which was calculated for 4 hours shows that all concentrations of the gel preparations provide protection power above 50% until the 4th hour. The 6% concentration had a protection power of 91.49% at the 0th hour and decreased at the 4th hour to 85.24%. The 12% concentration had a protective power of 92.93% at the 0th hour and decreased at the 4th hour to 86.69% and the 24% concentration of rosemary leaf essential oil gel preparations had a protective power of 94.44% at the 0th and decreased at the 4th hour to 89.19% while the positive control using the repellent preparations that contains DEET which is circulating in the market had protective power of 97.82% at the 0th hour and decreased at the 4th hour to 95.56%. From these results it is concluded that the change in protective power from hour to hour on the concentrations of FI, FII, and FIII happened because the essential oil contained in the gel evaporated, so that over time, the odor (fragrance) from the essential oil would disappear and resulted
in decreased repellent potential, compared to synthetic repellent DEET. The results of the testing of the protective power for 4 hours of rosemary leaf essential oil gel preparations have an average protective power of 89.11%; 90.21%; 92.15% at each concentration of 6%; 12%; 24%, while the average protective power on positive control is 96.73%. This shows that the rosemary leaf essential oil gel preparations has activity as a repellent against *Aedes aegypti* mosquitoes, although it is still below the average protective power of repellent preparations circulating in the market.

Based on the results of statistical analysis using One Way ANOVA between the treatment of increased concentration of essential oil on the obtained protection power against *Aedes aegypti* mosquitoes, show a significance value of 0,000 (p <0.05) both in the repellent activity test against *Aedes aegypti* mosquitoes, and its formulation in the gel preparations. This means that the increased concentration of rosemary leaf essential oil gel preparations makes a significant difference to the protection ability against *Aedes aegypti* mosquitoes.

4. Conclusion

Based on the results of the study conducted, it can be concluded that: Rosemary leaf (*Rosmarinus officinalis L*) essential oil can be made in the form of *Aedes aegypti* mosquito repellent gel as seen from good and stable results of the physical evaluation of the entire preparations formula from homogeneity test, pH test, determination of viscosity, spreadability, and irritation test.

Rosemary leaf (*Rosmarinus officinalis L*) essential oil gel preparations has activity as a repellent against *Aedes aegypti* mosquitoes with an average protective power of 92.15% for 4 hours in formula III with a concentration of 24%.

The higher concentration of essential oils of rosemary leaf (*Rosmarinus officinalis L*) used in gel preparations, the higher the produced repellent activity against *Aedes aegypti* mosquitoes. However, this activity is still lower than the positive control.

5. References

[1] Katsambas, A. Lotti, T., Dessinioti, and C., D’Erme, A.M., 2015. *European Handbook Of Dermatological Treatments*. 3th Ed. Berlin Heudelberg: Springer-Verlag.

[2] Grainge, M. Dan S. Ahmed. 1988. *Handbook of Plants with Pest Control Properties*. New York : J Wiley Publisher.

[3] Maia, M.F., and Moore, S.J., 2011. *Plants Based Insect Repellents: A Review Of Their Efficacy, Development and Testing*. Malar J., 10 (Suppl 1): S11.

[4] Kardinan, Agus. 2007. *Daya Tolak Ekstrak Tanaman Rosemary (Rosmarinus officinalis) terhadap Lalat. Bul. Litro. Vol. XVIII No. 2*

[5] Simon, J.E., A.F. Chadwick and L.E. Craker, 1984. *The Scientific Literature On Selected Herbs And Aromatic And Medicinal Plants Of Temperate Zone*. Archon Books, 770

[6] Wibowo, Ardianto. 2012. Minyak Atsiri dari Daun Rosemary (*Rosmarinus officinalis*) sebagai Insektisida Alami Melalui Metode Hidrodestilasi. *Jurnal Sains dan Seni*. Vol. 1 (1). hal 1-4

[7] Ansel, H.C. 2005. *Pengantar Bentuk Sediaan Farmasi*. Edisi Keempat. Penerjemah: Farida Ibrahim. Jakarta: UI-Pres. Halaman 390-391.

[8] Voight. 1984. *Buku Ajar Teknologi Farmasi*. Yogyakarta. Gadjah Mada University Press, Yogyakarta, 337-400 hlm.

[9] Koensoemardiyah. 2010. *Minyak Atsiri untuk Industri Makanan, Kosmetik, dan Aromaterapi*. Yogyakarta.

[10] Depkes RI. 2000. *Parameter Standart Umum Ekstak Tumbuhan Obat*. Departemen Kesehatan Republik Indonesia. Jakarta.

[11] Guenther, E. 1988. *Minyak Atsiri Jilid I*. Terjemahan S. Ketaren. Jakarta: UI Press. 103-297 hlm.

[12] Abdassah, M., Rusdiana, T., Subghan, A., Hidayati, G. (2009). Formulasi Gel Pengelupas Kulit Mati yang Mengandung Etil Vitamin C dalam Sistem Penghantaran Macrobead. *Jurnal Ilmu Kefarmastian Indonesia*. 107.
[13]. Komisi Pestisida Nabati. 1995. Pestisida untuk Pertanian dan Kehutanan. Direktorat Pupuk dan Pestisida. Direktorat Jenderal Bina Sarana Pertanian. Departemen Pertanian. Jakarta.

[14]. Subana. 2000. Statistik Pendidikan. Bandung : Penerbit Pustaka Setia.