Journal of Tropical Pharmacy and Chemistry

Journal homepage: https://jtpc.farmasi.unmul.ac.id

Formulation of Essential Oils as Mosquito Repellent

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Abstract

Essential oil has complex chemical substances with a lot of benefit, one of them is as mosquito or insect repellent. This research aims to find out the best essential oil formula for mosquito repellent. Essential oils that used in this research are citronella, lemongrass, cajeput, and lavender oil. Essential oils are characterized by its color, density, refractive index, and solubility in alcohol. The essential oil colors met the SNI which was pale yellow to clear yellow, the density was 0.8125 - 0.9114 g/ml, the refractive index was 1.4568-1.4665 and soluble in alcohol. The essential oil mixture resulted in 7 series of formula, namely series A1-A5, B1-B5, C1-C5, D1-D5, E1-E5, F1-F5, and G1-G5. The organoleptic test performed on all formula showed that the preferred formula was the formula E3, G3, and F3. All preferred formula was carried out repellent tests against mosquitoes to determine the effectiveness of the oil mixture in Virgin Coconut Oil (VCO) carrier. The result showed that E3 was more effective repellent compare to F3 and G3 formulas, because it has protection value more than 90% until fourth-hour.

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

Submitted: 26 November 2019 **Accepted**: 16 December 2019 **DOI**: https://doi.org/10.25026/jtpc.v5i2.231

■ Introduction

Essential oils are volatile oils produced from plants by distillation process. Indonesia is the major producer for essential oils from clove, citronella, patchouli, and nutmeg, that grow in considerable amounts in Indonesia and are well known in international trade. Essential oils can be obtained from many parts of plants such as leaves, stems, roots, seeds, flowers, fruit and skin. The nature of essential oils is volatile at room temperature, has authentic odor as the original plant, has a bitter taste and is generally soluble in organic solvents. The composition of essential oils consists of

complex chemical substances, which promote the properties as antibacterial, antiviral, pest control and insect repellent [1].

Mosquitoes can make human suffer from dengue fever which transmitted through the bite of an infected mosquito, especially the *Aedes aegypti* and *Ae albopictus*. Dengue fever caused by dengue virus is a contagious disease that poses a serious threat to public health [2]. Cases of dengue fever from January 2018 were 6167 cases, and then increased to 13683 cases in January 2019 (Kemenkes RI).

An effort to prevent dengue fever is application of insect repellent. Insect repellent is a material that has the ability to prevent anthropods (including mosquitoes) from biting on the surface of human skin. Mosquito repellents generally contain diethyl toluamide (DEET), dimethyl phalat and iridine [3]. To avoid those synthetic chemical insecticides, we can use natural repellent to be healthier and environmentally friendly. Natural repellent can be made from plants or commonly called natural insecticides.

Plants produce secondary metabolites as active compounds to protect plants from pests and diseases. Eessential oils that can be used as insects repellent are citronella oil, lemongrass oil, lavender oil and cajuput oil. Citronella oil is obtained from citronella plants which contain 32-45% citronellal as active compounds to repel mosquitoes [4]. Lemongrass can be used as a mosquito repellent because they contain active substances citronellal and geraniol which have the ability to repel mosquitoes [5]. Lavender plants produce lavender essential oil distilled from flowers and the compound linalool and linalyl acetate can be used as a mosquito repellent. Cajuput oil comes from distillation of cajuputti leaves that are produced in Indonesia and have a distinctive odor from its 1,8-cineol compound [6].

In this research, the best formula of essential oils is selected according to organoleptic testing. Hedonic test was carried out to know the preference of each oil mixture with the same oils type and different compositions, then compare among the series of formula to know the highest preference. The purpose of this study was to determine the best mixture of essential oil formulas for mosquito repellent preparations and to determine the protective value of the best formula resulted.

■ Experimental

Materials

The materials used were essential oils of citronella (*Cymbopogon nardus* L), lemongrass (*Cymbopogon citratus*), cajuput (*Meulaleuca leucadendra*), obtained from CV. Pavettia Atsiri and lavender (*Lavandula angustifolia*) obtained from PT Xoso Essential Oil. Another materials were VCO, 95% neutral alcohol, distilled water, fish pellets and ground water to grow mosquitoes. The test box was specially designed to test the protection against mosquito bites.

Method

This research consisted of 3 steps. Step 1 was to determine the characteristics of essential oils. Step 2 was to determine the best formulation of various oil mixtures with organoleptic test. Step 3 was to test the repellent activity against mosquitoes.

Characterization of essential oils

Characterization was done to find out the physical characteristics of the essential oils. The characterization was carried out to determine conformity with established standards and find out the authenticity of essential oils. The parameters analysed include color, specific gravity, solubility in alcohol, and refractive index.

Formulations of essential oil

Preparations to make essential oil formulations were done by mixing some oils including citronella, lemongrass, lavender, and eucalyptus into a mixture of 2 mL each. The formulation of essential oils can be seen in Table 1. Then, the formulas were tested by hedonic test. Formulas were made in 7 series with each seri containing 5 samples. The first hedonic test was to determine the most preferred formula from each sample in one seri, and the second hedonic test used selected samples from the first hedonic test to determine the most preferred formula from all series.

Table 1. Formulas of essential oil mixture for hedonic test

Formula	Citronella (mL)	Lemongrass (mL)	Lavender (mL)	Cajuput (mL)
A1	0.4	-	1.6	-
A2	0.5	-	1.5	-
A3	1.0	-	1.0	-
A4	1.5	-	0.5	-
A5	1.6	-	0.4	-
B1	0.7	-	-	1.3
B2	0.8	-	-	1.2
B3	1.0	-	-	1.0
B4	1.4	-	-	0.6
B5	1.5	-	-	0.5
C1	-	-	0.3	1.7
C2	-	-	0.4	1.6
C3	-	-	1.0	1.0
C4	-	-	1.7	0.3
C5	-	-	1.8	0.2
D1	1.8	-	0.1	0.1
D2	1.3	-	0.1	0.6
D3	0.9	-	0.1	1.0
D4	0.5	-	0.1	1.4
D5	0.1	-	0.1	1.8
E1	0.7	1.0	0.3	-
E2	0.5	1.2	0.3	-
E3	0.4	1.3	0.3	-
E4	1.0	0.7	0.3	-
E5	1.2	0.5	0.3	-
F1	0.3	1.7	-	-
F2	1.4	0.6	-	-
F3	0.9	1.1	-	-
F4	1.0	1.0	-	-
F5	1.2	0.8	-	-
G1	-	1.9	0.1	-
G2	-	1.5	0.5	-
G3	-	1.2	0.8	-
G4	-	1.0	1.0	-
G5	-	1.7	0.3	-

Mosquitoes repellent activity of selected formulas

Testing the selected formula was carried out with mosquitoes repellent test method. The test refers to the research of [7] which was carried out by inserting the hands into the mosquito cage for 5 minutes, every hour during 6 hours. Two hands were entered in the same time, the right hand as the control and the left hand as the treatment. The repellent tests required an amount of 25 mosquitoes at every observation and change it if look limp or died. *A. aegypti* was farmed from the egg phase to adult mosquitoes in laboratory. *A. aegypti* adult mosquitoes were 3-5 days old mosquitoes that have not been sucking blood. Testing the protection against mosquitoes need to dilute a 5% of essensial oil formula in 40 mL Virgin Coconut Oil (VCO) as carrier oil, before a 2 mL of this blend applied in one arm hand.

■ Results and Discussion

Characterization of Essential Oils

Essential oils are volatile and have a clear color, but during storage it will turn brown or yellowish. The aroma developed is characteristic of the plant source. It has a bitter taste and easy to evaporate. Essential oils can be obtained from various parts of plants such as stems, leaves, flowers, seeds, roots, fruit and skin from a plant tissue. Essential oils used in this study were citronella, lemongrass, lavender, and cajuput oils.

One of the active compounds that can repel mosquitoes in citronella oil is citronellal (31-38%). The use of citronella oil as mosquito repellent has existed in the 20th century and is still used today [8]. Lemongrass thrives in Indonesia [9]. The distillation of lemongrass leaves is limited because most of them are used directly in food and beverages. The main component of lemongrass is citral with the concentration of 75-85% [10]. Lemongrass can be used as mosquito repellent because it contains geraniol as the active substance to repel the mosquitoes [5]. Lavender essential oil is distilled from lavender flowers with the yield of 1-3% essential oils, which contains linalyl acetate (26.32%), linalool (26.12%), and others [11;12]. According to the research [13], lavender, geranium and citronella plants can be used to repel mosquitoes. But lavender oil can repel not only mosquitoes, but also other insetcs. Cajuput oil comes from cajuputti plants that are thrive in Indonesia. It has a distinctive and pleasant odor. Cajuput oil has the potential to repel mosquitoes [14]. Efficacy of cajuput oil is to treat toothache, headache, anti-mosquito, anti-microbial [15], and to reduce cough in patients with acute bronchitis [16]. The ingredients of cajuput oil are 1,8-cineol (26.28%), α-pinene (1.23%), α-terpineol (9.77%), caryophyllene (3.38%), α-caryophyllene (2.76%), ledol (2.27%), and elemol (3.14%) [17]. According to [1], the part of trees from cajuputti that produce oil is leaves and small branch.

The results obtained from the characterization of essential oils can be seen in Table 2, Table 3, Table 4, and Table 5. The color parameter was in accordance with the standard. The specific gravity of the samples was within the specified range. The refractive index at working temperature of 25°C was also in the

specified range. Refractive index determined by refractometer with the principle of the refraction of the light beam when enters through two types of medium with different densities. The value can be influenced by factors such as the oxidation process and temperature [18]. A low refractive index can be obtained from working at high temperatures. The solubility in alcohol results were in accordance with the Indonesian National Standard (SNI) and the Essential Oil Association (EOA).

Table 2 Characteristics of Citronella Essential Oil

Parameter	Results	SNI (1995) [19]
Color	pale yellow	pale yellow to brownish yellow
Specific gravity	0.812	0.880-0.922
Refractive index	1.466	1.466-1.475
Solubility in 80% alcohol	1:2	1:2 clear

Table 3 Characteristics of Lemongrass Essential Oil

Parameter	Results	EOA (1975)
Color	dark yellow	dark yellow to red
Specific gravity	0.892	0.8-0.9
Refractive index	1.486	1.483-1.489
Solubility in 70% alcohol	1:2	1:2 - 1:4 little cloudy

Tablel 4 Characteristics of Cajuput Essential Oil

Parameter	Results	SNI (2006) [20]
Color	clear yellow	clear to greenish yellow
Specific gravity	0.911	0.900-0.930
Refractive index	1.469	1.450-1.470
Solubility in 80% alcohol	1:3	1:1 - 1:10 clear

Table 5 Characteristics of Lavender Essential Oil

Parameter	Results	CV. Xoso Essential
Color	pale	no color/pale
Specific gravity	0.894	0.880-0.895
Refractive index	1.456	1.450-1.461
Solubility in 96% alcohol	1:3	Soluble

Essential Oil Formulation

The essential oil formulation was made by mixing one essential oil with another essential oil. The mixture was made in a combination of 2 or 3 types of essential oils in 2 mL total volume of each mixture. Initiation of the essential oil formula was based on the subjective assessment of the researchers by considering the aroma produced from the oil mixture. Formulas that have been made are carried out hedonic tests with aroma parameters to obtain objective data from the panelists and determine the most preferred formula.

The hedonic test is an organoleptic test to determine the level of panelists' preference to products. The number of panelists used for hedonic test was 30 people. Panelists were got an assessment of the aroma parameter. There were 5 levels of liking in this test, 5: very like, 4: like, 3: neutral, 2: dislike and 1: very dislike. Formulation was aimed to mask the unpleasant aroma of one of the essential oils which could be pungent and rarely liked by most panelists and to increase the efficacy of the formula itself as a mosquito repellent. This hedonic test was done before repellent test because panelist preference was an important parameter, if it got high repellen activity but the odor was very unpleasant, then the panelist doesn't want to use the final products.

The results obtained from the hedonic test in formula A which was a mixture of citronella oil and lavender oil, can be seen in Figure 1. It showed that formula A1 was the most preferred compared to other formulas. Formula A1 was a formula using a concentration of 0.4 mL of citronella oil and 1.6 mL of lavender oil. Anova test results from formula A showed that formulas A1 to A5 have a significant difference in hedonic score so that Duncan test was need for further test.

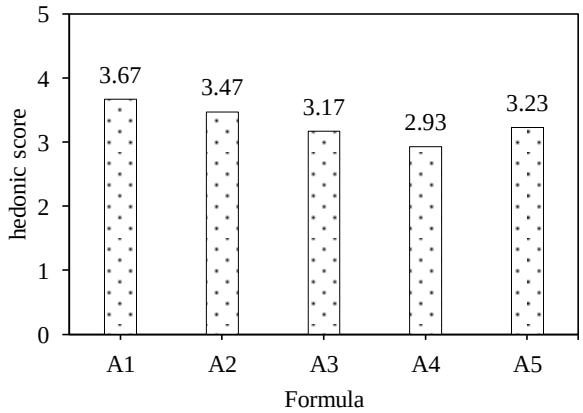

Figure 1. Hedonic test results of formula A. (A1) 0.4 mL of citronella and 1.6 mL of lavender, (A2) 0.5 mL of citronella and 1.5 mL of lavender, (A3) 1.0 mL of citronella and 1.0 mL of lavender, (A4) 1.5 mL of citronella and 0.5 mL lavender and (A5) 1.6 mL citronella and 0.4 mL lavender.

The results obtained from the hedonic test of formula B which was a mixture of citronella oil and cajuput oil can be seen in Figure 2. It showed that formula B1 was the most preferred compared to other formulas. Formula B1 was a formula using 0.7 mL of citronella oil and 1.3 mL of cajuput oil. Anova test results from formula B showed that formula B1 to B5 didn't have a significant difference in hedonic score so there was no need for further tests.

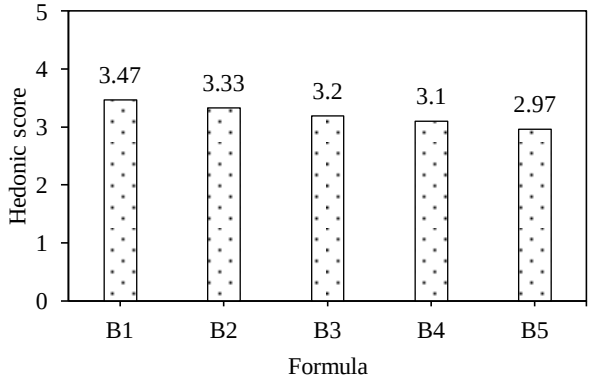

Figure 2. Hedonic test results of formula B. (B1) 0.7 mL of citronella and 1.3 mL of cajuput, (B2) 0.8 mL of citronella and 1.2 mL of cajuput, (B3) 1.0 mL of citronella and 1.0 mL of cajuput, (B4) 1.4 mL of citronella and 0.6 mL of cajuput, and (B5) 1.5 mL of citronella and 0.5 mL of cajuput.

The results obtained from the hedonic test of formula C which was a mixture of lavender oil and cajuput can be seen in Figure 3. It showed that the formula C1 was the most preferred compared to other formulas. Formula C1 was a formula using 0.3 mL lavender oil and 1.7 mL cajuput oil. Anova test results from formula C showed that formulas C1 to C5 didn't have a significant difference so there was no need for further tests.

The results obtained from the hedonic test of formula D which was a mixture of citronella, lavender, and cajuput oil can be seen in Figure 4. It showed that the formula D4 was the most preferred compared to other formulas. Formula D4 was a formula using 1.4 mL cajuput oil, 0.5 mL citronella oil and 0.1 mL lavender oil. Anova test results from formula D showed that formula D1 to D5 have a significant difference in hedonic score so that Duncan test was need for further test.

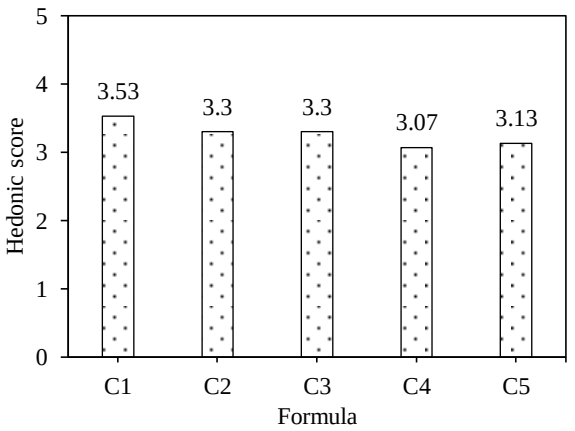

Figure 3. Hedonic test results for formula C. (C1) 0.3 mL lavender and 1.7 mL cajuput, (C2) 0.4 mL lavender and 1.6 mL cajuput, (C3) 1.0 mL lavender and 1.0 mL cajuput, (C4) 1.7 mL lavender and 0.3 mL of cajuput and (C5) 1.8 mL of lavender and 0.2 mL of cajuput.

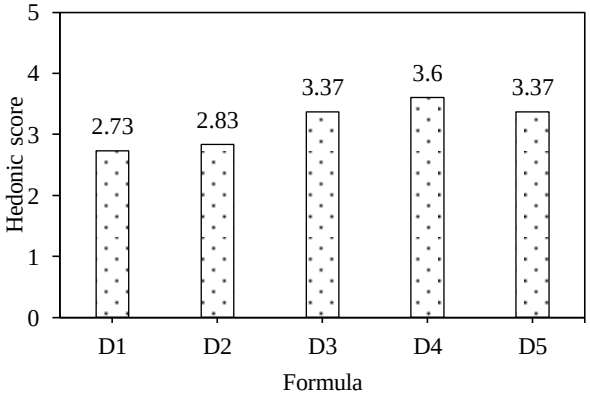

Figure 4. Hedonic test results of formula D. (D1) 1.8 mL of citronella, 0.1 mL of lavender and 0.1 mL of cajuput, (D2) 1.3 mL of citronella, 0.1 mL of lavender and 0.6 mL of cajuput, (D3) 0.9 mL of citronella, 0.1 mL of lavender and 1.0 mL of cajuput, (D4) 0.5 mL of citronella, 0.1 mL of lavender and 1.4 mL of cajuput, and (D5) 0.1 mL of citronella, 0.1 lavender and 1.8 mL of cajuput.

The results obtained from the hedonic test of formula E which was a mixture of citronella, lemongrass and lavender oil can be seen in Figure 5. It showed that the formula E3 was the most preferred compared to other formulas. Formula E3 was a formula using 1.3 mL of citronella oil, 0.4 mL of lemongrass and lavender of 0.3 mL. Anova test results from formula E showed that formulas E1 to E5 didn't not have a significant difference so there was no need for further tests.

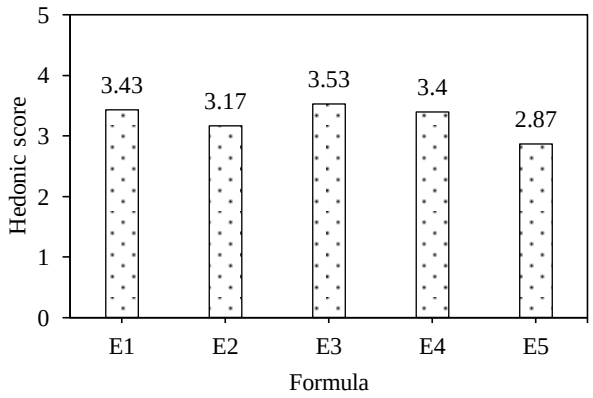

Figure 5. Hedonic test results of formula E. (E1) 0.7 mL of citronella, 1.0 mL of lemongrass and 0.3 mL of lavender, (E2) 0.5 mL of citronella, 1.2 mL of lemongrass and 0.3 mL of lavender, (E3) 0.4 mL of citronella, 1.3 mL of lemongrass and 0.3 mL of lavender, (E4) 1.0 mL of citronella, 0.7 mL of lemongrass and 0.3 mL of lavender, and (E5) 1.2 mL of citronella, 0.5 mL of lemongrass and 0.3 mL of lavender.

The results obtained from the hedonic test of formula F which was a mixture of citronella and lemongrass oils can be seen in Figure 6. It showed that the F3 formula was the most preferred compared to others. F3 was a formula using a concentration of 1.1 mL of citronella oil and 0.9 mL of lemongrass oil. Anova test results from the formula F showed that the formula F1 to F5 didn't have a significant difference so no need for further test.

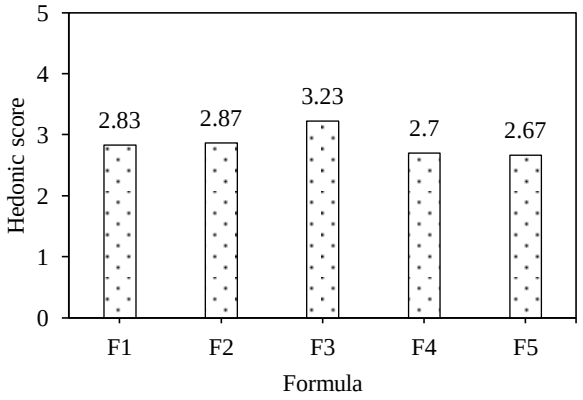

Figure 6. Hedonic test results for formula F. (F1) 0.3 mL of citronella and 1.7 mL of lemongrass, (F2) 1.4 mL of citronella and 0.4 mL of lemongrass, (F3) 0.9 mL of citronella and 1.1 mL of lemongrass, (F4) 1.0 mL of citronella and 1.0 mL of lemongrass, and (F5) 1.2 mL of citronella and 0.8 mL of lemongrass.

The results obtained from the organoleptic test of formula G which was a mixture of citronella and lavender oil can be seen in Figure 7. It showed that the formula G3 was the most preferred compared to other formulas. Formula G3 was a formula using 1.2 mL of citronella oil and 0.8 mL of lavender oil. Anova test results from the formula G showed that the formula G1 to G5 didn't have a significant difference in hedonic score.

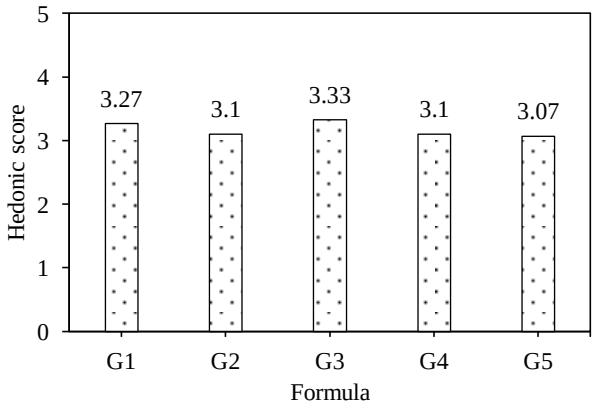

Figure 7. Hedonic test results of formula G. (G1) 1.9 mL of lemongrass and 0.1 mL of lavender, (G2) 1.5 mL of lemongrass and 0.5 mL of lavender, (G3) 1.2 mL of lemongrass and 0.8 mL of lavender, (G4) 1.0 mL of lemongrass and 1.0 mL lavender and (G5) 1.7 mL lemongrass and 0.3 mL lavender.

The hedonic test resulted in formulas which have the highest score from each series, so it was 7 samples altogether. Those 7 formulas were carried out by second hedonic test to produce the best 3 samples. The 7 highest score were formulas A1, B1, C1, D4, E3, F3, and G3. The highest score formula obtained from further hedonic test can be seen in Figure 8, namely the formulas E3, G3, and F3. Determination of the best sample was based on the highest average score of the panelists' preference and visual appearance of the oil mixture produced. The resulting oil mixture consists of citronella oil, lemongrass oil and lavender oil. The three oils were most widely used in repelling mosquitoes [13]. The best formula has concentrations of lemongrass 1.3 mL, 1.2 mL, and 1.1 mL and it was more than the concentration of citronella and lavender. This indicated that the panelists prefer

the lemony aroma of lemongrass oil. The content of lemongrass oil was high in citral content that of 75%-85% [10]. Anova test results from the selected formulas showed that the seven formulas have no significant difference in hedonic score.

The 3 highest hedonic score formula was then tested a mosquitoes repellent test. Repellen is a substance that repel insect or pest from the object being treated [21]. Repellent test was carried out using *A. aegypti* mosquitoes obtained from breeding in laboratory to ensure it free from dengue virus. *A.aegypti* mosquitoes like dark places and avoid direct exposure to sunlight [22]. Selected formula for repellent test was mixed with VCO as carrier oil. A 5% of essential oil formula was added to 40 mL VCO and applied at amount of 2 mL in one hand and another hand was covered with 2 mL control VCO without essential oil. VCO is an edible vegetable oil extracted from coconut kernel enzymatically in low temperature to get clear oil and free from radicals [23]. According to [24], VCO contains fatty acids which can soften the skin, especially lauric and oleic acids.

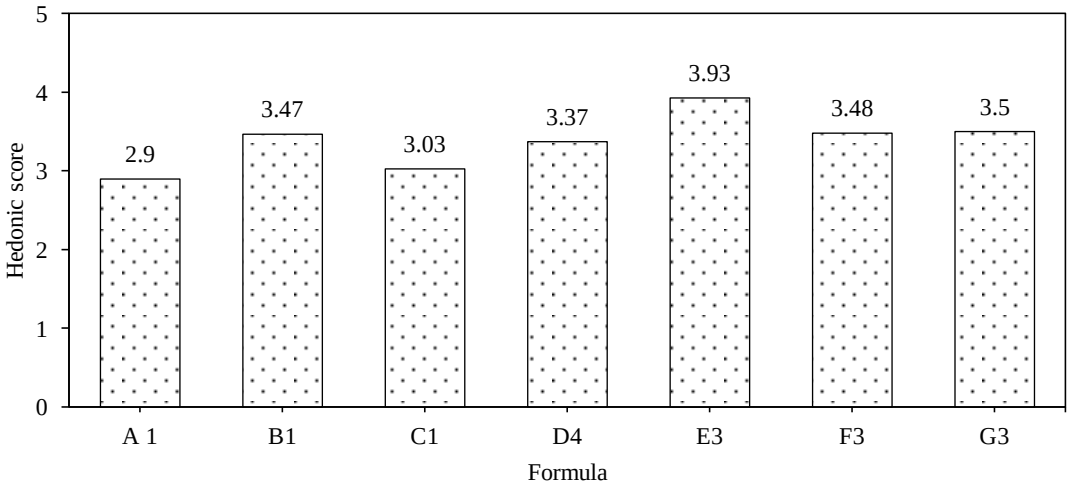

Figure 8. Hedonic score from 7 selected formulas

Tabel 7 Average protection power of 3 selected formula

Essential oil formula	Average protection value (%) at every hours						
	0	1	2	3	4	5	6
Formula E3	100	98.31	95.93	94.24	92.16	82.98	83.91
Formula F3	99.75	98.30	98.65	88.36	86.48	81.23	87.75
Formula G3	99.23	96.95	94.29	75.29	72.22	60.00	63.18

E3 : E3: 0.4 mL of citronella, 1.3 mL of lemongrass and 0.3 mL of lavender
F3 : (F3) 0.9 mL of citronella and 1.1 mL of lemongrass,
G3 : (G3) 1.2 mL of lemongrass and 0.8 mL of lavender,

This repellent test was carried out by inserting a hand into the mosquito cage and counting how many mosquitoes are attached to the control arm and test arm for 5 minutes within 6 hours. From the repellen test, formula E3 (citronella, lemongrass and lavender) yielded an average protection capacity of above 90% until the 4th hour, while formula F3 (citronella and lemongrass) and G3 (lemongrass and lavender) yielded an average of 90% protection only until the 2nd hour. According to [25], repellent protection should be reaching an average of 90% until the 6th hour. From Table 7, it can be seen that average protection capacity decreased every hour. The decrease was caused by the volatility of essential oil that evaporated from the test arm or being

swept away by objects that come in contact with the arm during testing.

The mosquito repellent test resulted that the formula E3 was the performance as a repellent because it has a long protective capacity of 90% up to the 4th hour compared to formulas F3 and G3. Formula E3 was a formula that consists of three types of essential oils, each of which has the ability to resist insects such as mosquitoes. Based on research conducted by [26] the effectiveness of citronellal in citronella oil is high as a mosquito repellent. Citronellal in lemongrass oil has contact poison properties against mosquitoes. The nature of contact poison is that it can enter the mosquito's body through the skin which causes the mosquito to feel dehydrated. Dehydration or continued loss of fluid can cause mosquitoes to die [27]. Research [5] said that lemongrass can be used as a mosquito repellent because it contains the active substance geraniol which has the ability to resist mosquitoes. According to the research of Mirawati et al. (2018) [13] lavender, geranium and citronella plants can be used to repel mosquitoes.

According to research [28], the handling of dengue fever vectors using natural repellent from natural materials is one of the ways to prevent its spreading. The quality and amount of essential oils have influenced by the environment, such as air oxygen, sunlight and heat. According to [29], in a native state of volatile oil, average protection power will be decreased every hour. Anova test results from the three best formulas didn't give significant differences.

Conclusion

Formula E3 from a mixture of citronella, lemongrass, and lavender oil have the highest hedonic score compare with formula G3 (lemongrass and lavender) and F3 (citronella and lemongrass) with the hedonic score of .3.93 (like). It also has the highest potency as mosquito repellent because its protective power more than 90% for 4 hours, while, F3 and G3 formulas have protective power above 90% only until the 2nd hour. E3 formula was quite effective as mosquito repellent but the use of this repellent is recommended repeatedly or when the insect effectively bites.

References

[1] Widiyanto A, Siarudin M. 2013. Karakteristik daun dan rendemen minyak atsiri lima jenis tumbuhan kayu putih. *Jurnal Penelitian Hasil Hutan*. 31(4): 235-241.

[2] Widoyono. 2005. *Penyakit Tropis, Epidemiologi, Penularan, Pencegahan dan Pemberantasannya*. Jakarta (ID): Penerbit Erlangga.

[3] Raina. 2011. *Ensiklopedi Tanaman Obat untuk Kesehatan*. Yogyakarta (ID): Absolute Jogja.

[4] Yulvianti M, Sari RM, Amaliah ER. 2014. Pengaruh perbandingan campuran pelarut N-Heksana-Etanol terhadap kandungan sitronelal hasil ekstraksi sereh wangi (*Cymbopogon nardus L*). *Jurnal Integrasi Proses*. 5(1): 8-14.

[5] Yulianis, Dachriyanus, Putra AD. 2018. Uji aktifitas anti nyamuk minyak atsiri sereh dapur dalam bentuk semprot. *Jurnal Ipteks Terapan*. 12(1): 78-83.

[6] Kardinan A. 2003. *Tanaman Pengusir dan Pembasmi Nyamuk Vol I*. Jakarta (ID): Agromedia Pustaka.

[7] Boesri H, Heriyanto B, Susanti L, Handayani SW. 2015. Uji repelen (daya tolak) beberapa ekstrak tumbuhan terhadap gigitan nyamuk *Aedes Aegypti* vector demam berdarah dengue. *Jurnal Vektor*. 7(2):79-84.

[8] Maia MF, Moore SJ. 2011. Plant-based insect repellents: a review of their efficacy, development dan testing. *Malaria Jurnal*. 10(1): 1-14.

[9] Mulyani S, Mulyaningsih B, Lestari AW, Ana FM, Anna DSS. 2013. Lemongrass, cloves, orange leaves as insence combustible for *Aedes aegypti* repellent. *Traditional Medicine Journal*. 18(3): 195-200.

[10] Zaituni, Khathir R, Agustina R. 2016. Penyulingan minyak atsiri sereh dapur (*Cymbopogon citratus*) dengan metode penyulingan air-uap. *Jurnal Ilmiah Mahasiswa Pertanian Unsyiah*. 1(1): 1009-1016.

[11] Nindatu M, Tuhumury NL, Kaihena M. 2011. Pengembangan ekstrak etanol daun lavender *(Lavandula angustifolia)* sebagai antinyamuk vector filarisasi *Culex sp*. *Jurnal Kedokteran dan Kesehatan Program Studi Pendidikan Dokter Universitas Pattimura*. 4(1): 19-27.

[12] McLain DE. 2009. Chronic Health Effects Assessment of Spike Lavender Oil. Walker Donet and Associates.

[13] Mirawati P, Simaremare ES, Pratiwi RD. 2018. Test of repellent efeentiveness of lotion containing the combination of essential oils of zodiac leaf oil (*Evodiia suaveolens Scheff*) and Lemongrass (*Cymbopogon citratus*) on *Aedes*

Aegypti. *Pharmaceutical Journal of Indonesia*: 15(1): 1-15.
[14] Kardinan A. 2007. *Tanaman Pengusir dan Pembasmi Nyamuk Vol II*. Jakarta (ID): Agromedia Pustaka.
[15] Aligiannis N, Kalpoutzakis EC, Chinou IB, Mitakou S, Gikas E, Tsarbopoulos A. 2001. Composition and antimicrobial activity of the esssential oils of five taxa of *sideritis* from Greece. *Journal Agricultural and Food Chemistry*. 49(2): 811-815.
[16] Fischer J, Dethlefsen U. 2013. Efficacy of cineole in patients suffering from acute bronchitis: a placebo-controlled double-blind trial. *Journal Cough*. 9:25.
[17] Helfiansyah R, Sastrohamidjojo H, Riyanto. 2013. Isolasi, identifikasi dan pemurnian senyawa 1,8 sineol minyak kayu putih (*Melaleuca Leucadendron*). *ASEAN Journal of Systems Engineering*. 1(1): 19-24.
[18] Ketaren S. 1984. *Pengantar Teknologi Minyak atsiri*. Jakarta (ID): PN Balai Pustaka.
[19] [BSN] Badan Standarisasi Nasional. 1995. Standar Nasional Indonesia (SNI). SNI 06-3953-1995. Minyak Sereh. Jakarta (ID): Dewan Standarisasi Indonesia.
[20] [BSN] Badan Standarisasi Nasional. 2006. Standar Nasional Indonesia (SNI). SNI 06-3954-2006. Minyak Kayu Putih. Jakarta (ID): Dewan Standarisasi Indonesia.
[21] Nerio LS, Olivero-Verbel J, Stashenko E. 2010. Repellent activity of essential oils: A Review. *Bioresource Technology*. 101(1): 372-378.
[22] Novasari AM, Sasongkowati R. 2017. Efektivitas larutan biji srikaya (*Annona squmosa L.*) sebagai insektisida terhadap kematian nyamuk *Aedes aegypti* dengan metode liquid elektrik. *Jurnal Kesehatan Lingungan*. 9(2): 200-208.
[23] Handayani RS, Lalagiy D, Panjaitan RU. 2011. Pencegahan luka tekan mellaui pijat menggunakan *virgin coconut oil*. *Jurnal Keperawatan Indonesia*. 14(3): 141-148.
[24] Lucida H, Salman, Hervian MS. 2008. Uji daya peningkat penetrasi *virgin coconut oil* (VCO) dalam basis krim. *Jurnal Sains dan Teknologi Farmasi*. 13(1): 23-30.
[25] Utomo PP, Nana S. 2014. Perbandingan daya proteksi losion anti nyamuk dari beberapa jenis minyak atsiri tanaman pengusir nyamuk. *Biopropal Industri*. 5(2): 79-84.
[26] Kim JK, Kang CS, Lee JK, Kim YR, Han HY, Yun HK. 2005. Evaluation of repellency effect of two natural aroma mosquito repellent compounds, citronella and citronellal. *Entomological Research*. 35(2): 117-120.
[27] Djojosumarto P. 2008. *Pestisida dan Aplikasinya. Cet 1*. Jakarta (ID): AgroMedia Pustaka.
[28] Widawati M. 2014. Sediaan losion minyak atsiri *piper betle L*. dengan penambahan minyak nilam sebagai repelan nyamuk *Aedes aegypti*. *Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara*. 10(2): 77-82.
[29] Gunawan D, Mulyani S. 2004. *Ilmu Obat Alam*. Jakarta (ID): Penebar Swadaya.