

Chemical Compositions and Insecticidal Activity of *Eucalyptus urophylla* Essential oil Against *Culex quinquefasciatus* Mosquito¹

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ABSTRACT

Eucalyptus oils are widely used as spices, perfume industrial materials, food flavorings, and medicines. Several types of *Eucalyptus* oils also have insecticidal activity and as carminative. This study investigated the chemical composition, insecticidal (larvicidal and repellent) activity of *E. urophylla* oil against filarial mosquito *Culex quinquefasciatus*. *E. urophylla* oil was obtained from fresh leaves by water-steam distillation with oil yield 1.08%. *E. urophylla* oil in this study had no color (clear), has odor (typical eucalyptus), with specific gravity 0.941; refractive index 1.465; miscibility in 70% ethanol 1 : 3; and optical rotation (-) 5.83°. The major compounds of the oil were α -pinene (11.73%), 1,8-cineole (49.86%), β -ocimene (6.25%), γ -terpinene (9.11%), and α -terpinyl acetate (7.63%). The result showed the excellent insecticide activity against *C. quinquefasciatus*. The oil provided larvicidal activity with LC₅₀: 80.21 ppm and LC₉₀: 210.18 ppm, and repellent activity with IC₅₀: 0.82% and IC₉₀: 4.88%. The present study showed the effectiveness of *E. urophylla* as natural insecticide against *C. quinquefasciatus*, the mosquito vector of filariasis.

Keywords: *Eucalyptus urophylla* oil, insecticidal, larvicidal, repellent, *Culex quinquefasciatus*

1. INTRODUCTION

Eucalyptus is important plant which widely planted in the world. It consists of more than 700 species and it is in the family of *Myrtaceae*. The leaf of *Eucalyptus* can produce essential oil by distillation process. Eucalyptus oils have main advantage as medicine substance in many pharmacological aids of asthma, reducing inflammation, wound healing, burns, ul-

cers, acne, diabetes, fever, arthritis pain, and pulmonary disease Tuberculosis (Agarwal and Lakshmi, 2013; Song *et al.*, 2009). Eucalyptus is broadly used in traditional medicine for recovering many diseases. Leaves extract of eucalyptus are traditionally used as analgesic, anti-inflammatory, and medicine of antipyretic for the symptoms of respiratory infection such as cold, flu, and sinusitis (Silva *et al.*, 2003).

One of eucalyptus species which easily found

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and originally from Indonesia is *Eucalyptus urophylla* (Joker, 2004). Essential oil of this plant contains bioactive compound which can be used as anti-bacterial, antifungal, analgesic effect, anti-inflammatory, antioxidants, and anti mosquito (Cheng *et al.*, 2009). Terpenoid compounds in eucalyptus oil can function as stomach and breath poison in mosquito larva (Cheng *et al.*, 2009) and obstruct the mosquito olfactory receptors (Nerio *et al.*, 2010; Park *et al.*, 2001).

Culex sp mosquito is biological vector from disease of *Filariasis*, *Chikungunya*, and *Japanese Encephalitis*. *Filariasis* or *elephantiasis* is caused by nematoda worm that attacks lymph of vessels transmitted through the bite of *Culex quinquefasciatus* mosquito. *Filariasis* is one of diseases which causes disablement, stigma, psychosocial and diminishing productivity of patient and the environment. *Filariasis* is widely spread in Indonesia particularly outside of Java island. According to WHO (2013), currently it has been predicted that the larvae has infected more than 700 million people throughout the world, in which 60 million people of them are in South East Asia. In South East Asia, there are 11 countries which are endemic to *filariasis*, one of them is Indonesia.

The ways to prevent the transmission of the disease is by eradicating the wiggler and denying the bite of *C. quinquefasciatus* mosquito. Generally, the controlling of this mosquito uses synthetic insecticides. The use of synthetic substance can cause environmental pollution and leads to resistance against the insects eradicated

(Makhaik *et al.*, 2005). Whereas the fatal side effect of synthetic substance of anti mosquito lubricated on the skin is the skin disorders such as skin irritation, depression of respiratory tract, and coma (Briassoulis *et al.*, 2001). Therefore, an anti mosquito from natural substance with minimum side effects is needed, such as essential oil. Essential oil is an active substance which is able to eradicate larvae and to repel mosquito attack to human body.

The scientific study on insecticides activity of *E. urophylla* oil against *C. quinquefasciatus* mosquito from Indonesia has not been done. Therefore, in this study, the chemical composition and the insecticidal (larvacide and repellent) activity of *E. urophylla* oil were evaluated. This study evaluated the effectivity of *E. urophylla* oil as natural larvacide (LC₅₀ and LC₉₀) and repellent (IC₅₀ and IC₉₀) against the *C. quinquefasciatus* mosquito.

2. MATERIALS and METHODS

2.1. Plant material and extraction

The sample of *E. urophylla* leaves were collected from Forest Research and Education Wanagama I, Gunung Kidul, Yogyakarta, Indonesia. The oil was obtained by water-steam distillation from 5 kg fresh leaves of *E. urophylla*. Oil yields were determined based on dried weight of leaves (w/w). The oils produced were stored at approximately 0°C prior to used as test sample.

2.2. GC-MS analysis

The chemical composition of *E. urophylla* oil was analyzed using Gas Chromatography - Mass Spectrometry (GC-MS) QP2010S (Shimadzu Co. Ltd, Kyoto, Japan). The test used capillary column of Agilent HP 5 MS with 30 meters long. Helium gas was used as mobile phase with gas flow rate in 60 ml /minutes with injection split, injection volume of 1.0 μ l, injection temperature of 310°C. The spectrometer ionization used EI (electron-impact ionization) on 70 eV. The relative amounts of individual componens were based on peak obtained. The identification of chemical components was confirmed by comparing retention time to Wiley 229 data base library in the instrument and was then compared with several literatures.

2.3. Quality test

The quality test provided an overview of actual *E. urophylla* oil condition at the time of testing. Quality test was applied as supporting data through organoleptic test and physico-chemical properties of *E. urophylla* oil including the determination of color, odor, specific grafitly, refractive index, optical rotation, and the miscibility in ethanol based on the method of Standar Nasional Indonesia (SNI) 06-3954-2006. These properties can frequently be attributed to essential oil constituent. Oil quality also correlated with heavy fraction and chemical contents in oil which affect its bio-activity such as an insecticidal. Organoleptic

test of color and odor was evaluated by using sight and odor sense directly. The specific grafitly was measured using pycnometer 2 ml and then was converted with correction factor adjusted with the space temperature when the measurement was done, the measurement of optical rotation was done by using polarimeter, the measurement of oil refractive index was done by using hand-refractrometer N-3000e, and the miscibility in ethanol used 70% ethanol.

2.4. Larvae and adult mosquitoes rearing

Mosquito eggs of *C. quinquefasciatus* which had hatched were treated until being larvae and adult mosquitoes. The mosquitoes were bred in mosquito's cage. In their development, the moquitoes were bred until the age of 5-14 days. Along those days, the mosquitoes were fed sugar solvent, dog biscuit, and were fed mice.

2.5. Larvicidal analysis

The larvicidal assay used Mohtar *et al.* (1999) method with slight modification. The sample of this research was larva of *C. quinquefasciatus* on stadium III-IV. The total of larva in each group was 20. Each replicate containing 200 ml of the oil solution was placed in a 250 ml plastic cup. Control of this study was distilled water without *E. urophylla* oil. The sample of *E. urophylla* oil and pure commercial compound of 1,8-cineole (as a comparison) with 4 different concentrations (L1: 40 ppm, L2: 80 ppm, L3: 160 ppm, L4: 240 ppm) was prepared and a test with three replicates.

After 24 hours, the total died larvas in each samples were calculated. Percentage of larva mortality was determined by the following equation:

$$\text{Percentage of Mortality (\%)} = (M/C) \times 100$$

where M is total of died larva and C is total of larva.

2.6. Repellent analysis

The repellent test used World Health Organization Pesticides Evaluation Scheme (WHOPES, 2009) method with slight modification. This test used adult female mosquitoes of *C. quinquefasciatus*. The experiment used cages in the size of 20 cm × 20 cm × 20 cm in which there were 35 mosquitoes inside. *E. urophylla* oil and pure commercial compound of 1,8-cineole (as a comparison) applied in the forearm of a volunteer. Before experiment, the forearm was washed with soap and rinsed until it was clear, and then it was dried for sure. After that, the arm was oiled with *E. urophylla* oil and 1,8-cineole for each concentration (2%, 4%, 6%, and 8%). The oil concentration was required by dissolving the oil in ethanol 70% each with 1 ml volume. The oiled arm was entered in a test cage and let it be fed for five minutes for each duration of 0 hour, 1 to 4 hours. During the experiment, the arm was tried not to move. The test used three replicates for each dose and used different mosquitoes. This study used two controls which were negative and positive controls. The negative control

was the arm oiled with 1 ml of ethanol 70%, while the positive control was the arm oiled with anti mosquito lotion of N,N-diethyl-metoluamide (DEET) 15%.

Percentage of repellent (inhibitory concentration) was assessed as the proportion of mosquitoes perching on the arm of experiment with all mosquitoes perching on the arm of control was determined with the following equation:

$$\text{Percentage of Repellent (\%)} = \frac{R_C - R_P}{R_C} \times 100$$

where R_C is all mosquitoes perching on the arm of control (volunteer) and R_P is all mosquitoes perching on the arm of experiment (volunteer) after treatment.

2.7. Statistical analysis

The larvicidal activity of *E. urophylla* oil for lethal concentration 50% (LC_{50}) and 90% (LC_{90}) and mosquito repellent activity for inhibition concentration 50% (IC_{50}) and 90% (IC_{90}) were determined by probit regression. The analysis of variance (ANOVA) was used for analyzing the larvicidal activity and the repellent activity. Data were compared using Scheffe's test, results with $p < 0.05$ were considered to be statistically significant.

3. RESULTS and DISCUSSION

3.1. Oil yield

The oil extracted by water-steam distillation

from the leaves of *E. urophylla* provided oil yield of 1.08%. When compared with others research for the same species of *eucalyptus*, oil yield value in this study was quite high. The yield obtained for the same species *E. urophylla* reported by Coffi *et al.* (2012) was 0.4%. As for the other species as *Eucalyptus camadulensis*, *E. deglupta*, *E. grandis*, and *E. torelliana* were 0.70%; 0.7%; 0.54%, and 0.20%, respectively (Coffi *et al.*, 2012) and for *E. pellita* was 0.89% (Astiani, 2014). However, when compared with studies Cheng *et al.* (2009) on *E. urophylla* with a yield of 2.19%, the *E. urophylla* oil produced in this study was lower. The yield difference in this study compared to other studies probably due to the differences in distillation methods, the basis for calculating the yield, different species, the difference plants site, and other factors.

3.2. Chemical composition of *E. urophylla* oil

GC-MS analysis identified 17 chemical compounds in *E. urophylla* oil. These chemical compounds were included in the group of monoterpene hydrocarbons, oxygenated monoterpene, monoterpenes, esters, and sesquiterpenes (Table 1).

Based on Table 1, *E. urophylla* oil in this study had five main compounds (percentage of concentration > 5.00%) namely α -pinene (11.73%), 1,8-cineole (49.86%), β -ocimene (6.25%), γ -terpinene (9.11%), and α -terpinyl acetate (7.63%). The previous researchs of

some species of eucalyptus found that the main compound of eucalyptus was 1,8-cineole. Compared with the other researches on chemical compound in *E. urophylla* oil, it was found that there were some differences in the main compounds and compounds percentage. The main compounds in *E. urophylla* oil species derived from Congo were α -pinene (10.1%), 1,8-cineole (57.7%), and limonene (6.4%) (Cimanga *et al.*, 2002). Other researches found that the main compound of *E. urophylla* from Brazil were α -pinene (13.3%), 1,8-cineole (44.7%), and α -terpinyl acetate (11.7%) (Coffi *et al.*, 2012), and from Ethiopia were α -pinene (15.6%), β -pinene (9.1%), 1,8-cineole (34.5%), and α -terpinyl acetate (11.6%) (Kambu *et al.*, 1982). Singh *et al.* (1988) also found that there were differences in the main compounds of *E. urophylla* oil from India with the main compounds namely p-cymene (75.0%) and α -pinene (7.0%). These different main compounds were probably due to the differences in chemotype (Elaiissi *et al.*, 2010), species (Elaiissi *et al.*, 2011), site, rainfall and soil nutrition that influenced the plants metabolism (Baser and Buchbauer, 2010). Those differences then effected the composition of chemical compounds in the oil produced.

3.3. Quality of *E. urophylla* oil

The quality analysis shows the current condition of *E. urophylla* oil used during the test and associated with its chemical compounds. Quality analysis of organoleptic and phys-

Table 1. Chemical Composition of *E. urophylla* Oil

No.	R.T ^a	Compound	Group	Molecular Formula	Percentage (%)
1.	8.069	α -Pinene	Monoterpene hydrocarbons	C ₁₀ H ₁₆	11.73
2.	9.506	β -Pinene	Monoterpene hydrocarbons	C ₁₀ H ₁₆	1.75
3.	10.001	β -Myrcene	Monoterpene hydrocarbons	C ₁₀ H ₁₆	0.52
4.	10.478	α -Phellandrene	Monoterpene hydrocarbons	C ₁₀ H ₁₆	1.36
5.	11.242	ρ -Cymene	Monoterpene hydrocarbons	C ₁₀ H ₁₆	3.73
6.	11.560	1,8-Cineole	Oxygenated monoterpene	C ₁₀ H ₁₈ O	49.86
7.	11.706	β -Ocimene	Monoterpene	C ₁₀ H ₁₆	6.25
8.	12.040	(E)-Ocimene	Monoterpene	C ₁₀ H ₁₆	0.51
9.	12.448	γ -Terpinene	Monoterpene	C ₁₀ H ₁₆	9.11
10.	13.443	Terpinolene	Monoterpene	C ₁₀ H ₁₆	1.57
11.	14.817	Neo-Allo-Ocimene	Monoterpene	C ₁₀ H ₁₆	0.40
12.	16.447	Terpineol-4	Oxygenated monoterpene	C ₁₀ H ₁₈ O	0.85
13.	16.889	α -Terpineol	Oxygenated monoterpene	C ₁₀ H ₁₈ O	1.32
14.	21.737	α -Terpinyl acetate	Ester	C ₁₂ H ₂₀ O ₂	7.63
15.	23.808	β -Caryophyllene	Sesquiterpene	C ₁₅ H ₂₄	2.37
16.	25.842	Bycyclo Germacrene	Sesquiterpene	C ₁₅ H ₂₄	0.45
17.	26.502	Δ -Cadinene	Sesquiterpene	C ₁₅ H ₂₄	0.58
Total					100.00

^a R.T: Retention time

ico-chemical properties of *E. urophylla* oil were showed on Table 2. The test results on color, odor, and specific gravity, miscibility in 70% ethanol, refractive index, and optical rotation were used to determine the quality of oil produced. In this study, the results were compared with the standard SNI No. 06-3954-2006, and also compared with pure commercial compounds of 1,8-cineole. *E. urophylla* oil produced in this research was clear (no color) with typical odor of eucalyptus. Odor of *E. urophylla* oil was produced in this study probably due to the presence of terpenoid compounds that have activity as insecticidal (Cheng *et al.*, 2009).

The specific gravity of *E. urophylla* oil produced in this study was higher than standard of SNI with value 0.941. This is probably due to presence of oxygenated terpenes in the *E. urophylla* oil such as 1,8-cineole, terpineol-4, and α -terpineol. Refractive index of *E. urophylla* oil in this study prescribed with the SNI No. 06-3954-2006 standard with value 1,465. Miscibility in 70% ethanol of *E. urophylla* oil in this study was 1 : 3. If compared with the standard of SNI No. 06-3954-2006, this value was accordance with the standard. Optical rotation value of *E. urophylla* oil in this study was lower than the standard, that was (-) 5.83°. This optical rotation value probably

Table 2. Organoleptic and Physico Properties of *E. urophylla* Oil

Sample	Color	Odor	Specific Gravity (20°C)	Refractive Index (20°C)	Miscibility in 70% Ethanol	Optical Rotation (°)
<i>E.urophylla</i>	colorless	Typical Eucalyptus Oil	0.941	1.465	1:3	(-) 5.83
SNI Standard ^a	Colorless to yellow green	Typical Cajuput Oil	0.900-0.930	1.450-1.470	1:1-1:10	(-) 4-0
1,8-Cineole ^b	Colorless	Typical Cajuput Oil	0.905-0.925	1.458-1.462	1:1	(-) 0.5- (+) 0.5

^a Standard based on Indonesian National Standard (SNI) number 06-3954-2006.

^b Data source: Pujiarti *et al.*, 2011.

Table 3. Larvicidal Percentage (%) of *E. urophylla* Oil Against *C. quinquefasciatus* Mosquito

Sample	Concentration (ppm) ^a			
	40	80	160	240
<i>E. urophylla</i>	23.33 a	45.00 b	81.67 c	95.00 c
1,8-cineole	35.00 ab	51.67 b	95.00 c	100.00 cd

^a Value of larvicidal percentage in the column followed by the same letter is not significantly different at $p < 0.05$

due to the present of α -terpineol and β -caryophyllene on the oil. The previous study (Pujiarti *et al.*, 2011) showed that α -terpineol had optical rotation (-) 35°-(-) 33° and β -caryophyllene had optical rotation (-) 10°-(-) 5°.

3.4. Larvicidal activity

The exposition of *E. urophylla* oil solvent on instar larva III-IV *C. quinquefasciatus* for 24 hours produces data of cumulative percentage to larva death as seen in Table 3. Percentages of larva mortality to concentration of 40 ppm, 80 ppm, 160 ppm, and 240 ppm were 23.33%, 45%, 81.67%, and 95%, respectively. The lowest percentage of mortality on concentration of 40 ppm was 23.33% and the highest percentage of mortality on concentration of 240 ppm was 95%. While in the control, there was no dead larva. The mortality average of larvae were sig-

nificant different with statistical significance ($p < 0.05$). Concentration of *E. urophylla* oil solvent with concentration on 40 ppm and 80 ppm gave significant percentage value to concentration of 160 ppm and 240 ppm, meanwhile the concentration of 160 ppm and 240 ppm was not apparently different. Probit analysis to percentage of larva's mortality (after presented for 24 hours) had LC_{50} value of 80.21 ppm and LC_{90} value of 210.18 ppm.

E. urophylla oil in this study was very effective as larvicidal. Compared with the Monzon *et al.* (1994) study by using extract of *E. globosum* with LC_{90} 280,000 ppm, it was found that in this study *E. urophylla* oil was very effective as larvicidal. This was possibly caused by the different active substances in its plant and the consequence of contact poison or stomach poison. It was assumed that contact poison affected the rate of oxygen consumption bring-

Table 4. LC₅₀ and LC₉₀ of *E. urophylla* Oil Against *C. quinquefasciatus* Larvae

Concentration	Lethal Average (%)	LC ₅₀ (ppm) ^a	LC ₉₀ (ppm) ^b
<i>E.urophylla</i>			
40 ppm	23.33		
80 ppm	45.00	80.21	210.18
160 ppm	81.67		
240 ppm	95.00		
1,8-cineole			
40 ppm	35.00		
80 ppm	51.67	62.93	177.94
160 ppm	95.00		
240 ppm	100.00		

^a LC₅₀: Lethal Concentration 50%^b LC₉₀: Lethal Concentration 90%

ing about the disturbance of metabolism process in larva body, which is inflicted larva's mortality (Manaf, 1988).

The effectiveness of *E. urophylla* oil as larvicidal was also likely due to the present of active compounds in oil such as 1,8-cineole and α -terpineol. In tis study, 1,8-cineole have larvicidal activity slightly higher than *E. urophylla* oil with LC₅₀ value of 62.93 ppm and LC₉₀ value of 177.94 ppm (Table 4). Some previous studies also reported the activity of 1,8-cineole as strong insecticides (Alzogaray *et al.*, 2011; Ukeh *et al.*, 2011). Other study reported the effectiveness of α -terpineol as anti insect and louse (Chu and Jiang, 2011). Chemical compounds in essential oil as toxic terpenoid also could be functioned as stomach and breath poisons in larva (Cheng *et al.*, 2009).

3.5. Repellent activity

Repellents activities of *E. urophylla* oil against *C. quinquefasciatus* mosquito were

shown in Table 5. The statistical analysis showed significant difference between oil concentrations ($p < 0.05$), while for duration time were not significant different. This result shows that the oil concentrations has significantly effect as mosquito repellent. The table showed the increasing average of repellent percentage to *C. quinquefasciatus* mosquito in accordance with the increasing of concentration percentage used, meanwhile the duration time (4 hours) not effected to the effectiveness of *E. urophylla* oil as repellent. The average percentages of repellents on concentration 2%, 4%, 6%, and 8% were 68.88%, 88.08%, 94.10%, and 100%, respectively. The lowest repellent percentage was 68.88% on concentration 2% and the highest repellent percentage was 100% on concentration of 8%.

Probit analysis gave an estimation of the concentration of *E. urophylla* oil having protection to contact with *C. quinquefasciatus* mosquito in IC₅₀ with average value 0.82% and IC₉₀ with average value 4.88% (Table 6). Compared with

Table 5. Repellent Percentage of *E. urophylla* Oil Against *C. quinquefasciatus* Mosquito

Concentration	Repellent (%)					Average (%) ^a
	0 min ^b	60 min ^b	120 min ^b	180 min ^b	240 min ^b	
<i>E. urophylla</i>						
2%	62.81	67.19	70.02	73.64	70.72	68.88 a
4%	85.04	87.41	86.39	89.26	92.31	88.08 b
6%	95.28	91.61	95.92	93.29	94.41	94.10 c
8%	100.00	100.00	100.00	100.00	100.00	100.00 d
1,8-cineole						
2%	64.67	69.93	70.75	75.17	71.33	70.37 a
4%	87.41	90.48	89.26	89.51	92.24	89.78 b
6%	97.20	96.60	96.64	97.90	97.18	97.10 c
8%	100.00	100.00	100.00	100.00	100.00	100.00 d
Control (+) ^c	100.00	100.00	100.00	100.00	100.00	100.00 d
Control (-) ^d	0.00	0.00	0.00	0.00	0.00	0.00 e

^a The value of repellent percentage in the column followed by the different letter is significantly different at $p < 0.05$

^b min: minute, ^c Control (+): DEET 15%, ^d Control (-): 70% ethanol.

Table 6. IC₅₀ and IC₉₀ of *E. urophylla* Oil Against *C. quinquefasciatus* Mosquito

IC ^a	Concentration (%)					Average (%)
	0 min ^b	60 min ^b	120 min ^b	180 min ^b	240 min ^b	
<i>E. urophylla</i>						
IC ₅₀	1.20	0.90	0.79	0.53	0.66	0.82
IC ₉₀	5.17	5.15	4.83	4.67	4.57	4.88
1,8-cineole						
IC ₅₀	0.82	0.50	0.61	0.37	0.45	0.55
IC ₉₀	4.38	4.06	4.04	4.52	4.07	4.21

^a IC: Inhibitory Concentration (IC₅₀: Inhibitory Concentration 50%, IC₉₀: Inhibitory Concentration 90%)

^b min.: minute.

negative control and positive control (DEET 15%), the *E. urophylla* oil used in this research was more effective. With the oil concentration 8% provided 100% repellent, its mean that *E. urophylla* oil was more effective and safer to use as the material of anti repellent against *C. quinquefasciatus* mosquito.

Maia and Moore (2011) state that insects detected odor brought when the compounds odor

out in connection with odor receptor protein in ciliated dendrites of specialized odor receptor presented with the surrounding outside. Odor receptors responding to the DEET also responded to 1,8-cineole and linalool in *C. quinquefasciatus* mosquito. Insecticide activity of *E. urophylla* oil as repellent was related to active compound in this oil including pinene, 1,8-cineole, and caryophyllene. In this study, major

compound of 1,8-cineole shows mosquito repellent activity with IC_{50} value of 0.55% and IC_{90} value of 4.21% (Table 6). Maia and Moore (2011) also reported that active compounds as α -pinene, β -pinene, 1,8-cineole, carophyllene, and saponins were plant components having repellent activity because they influenced odor receptor proteins to insects.

4. CONCLUSION

The main compounds of *E. urophylla* oil in this study were α -pinene (11.73%), 1,8-cineole (49.86%), β -ocimene (6.25%), γ -terpinene (9.11%), and α -terpinyl acetate (7.63%). *E. urophylla* oil in this study was very effective as larvicidal (LC_{50} : 80.21 ppm and LC_{90} : 210.18 ppm) and repellents (IC_{50} : 0.82% and IC_{90} : 4.88%) in preventing transmission of filariasis brought by vector of *C. quinquefasciatus* mosquito. The effectiveness of *E. urophylla* oil as larvicidal and mosquito *C. quinquefasciatus* repellent probably due to the presence of major compound of 1,8-cineole. Several compounds and its combination probably also have insecticidal activity, but they were not investigated this time. Therefore, further investigations of synergic effect of compounds as insecticidal activity against *C. quinquefasciatus* and field evaluation are necessary.

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REFERENCES

- Agarwal, R., Lakshmi, T. 2013. Eucalyptus oil in dentistry: A mini review. *International Journal of Drug Development & Research* 5(4): 58~61.
- Alzogaray, R.A., Lucia, A., Zerba, E.N., Masuh, H.M. 2011. Insecticidal activity of essential oils from eleven *Eucalyptus spp.* and two hybrids: Lethal and sublethal effects of their major components on *Blattella germanica*. *Journal of Economic Entomology* 104(2): 595~600.
- Anonymous. 2006. Cajuput oil. Indonesia National Standardization, SNI 06-3954-2006. National Standardization Agency of Indonesia, Indonesia.
- Anonymous. 2013. Lymphatic Filariasis: A Handbook of Practical Entomology for National Lymphatic Filariasis Elimination Programmes. World Health Organization. Avenue Appia, Geneva.
- Astiani, D.P., Jayuska, A., Arreneuz, S. 2014. Antibacterial activity of *Eucalyptus pellita* essential oil *Escherichia coli* and *Staphylococcus aureus*. *Equator Journal Chemical* 3(3): 44~48.
- Baser, K.C.B., Buchbauer, G. 2010. Handbook of Essential Oil, Science, Technology and Application. CRC Press, London, New York.
- Brissoulis, G., Narlioglou, M., Hatzis, T. 2001. Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. *Human & Experimental Toxicology* 20: 8~14.
- Cheng, S.S., Huang, G.G., Chen, Y.J., Jane Yu, D., Chen, W.J., Chang, S.T. 2009. Chemical compositions and larvicidal activities of leaf essential oils from two *Eucalyptus* species. *Bioresource Technology* 100: 452~456.
- Chu, S.S., Jiang, G.H. 2011. Insecticidal activity and composition of essential oil of *Ostericum sieboldii* (Apiaceae) against *Sitophilus zeamais* and

- Tribolium castaneum*. Records of Natural Products 5(2): 74~81.
- Coffi, K., Soleymane, K., Harisololo, R., Bi Balo, T., Claude, C.J., Pierre, C., Gilles, F., Antoine, A.C. 2012. Monoterpene hydrocarbons, major components of the dried leaves essential oils of five species of the genus *Eucalyptus* from Côte d'Ivoire. Natural Science 4 (2): 106~111.
- Elaissi, A., Medini, H., Khouja, M.L., Simmonds, M., Lynene, F., Farhat, F., Chemli, R., Harzallah-Skhiri, F. 2010. Variation in volatile leaf oils of eleven *Eucalyptus* species harvested from Korbus Arboreta (Tunisia). Chemistry & Biodiversity 7(7): 1841~1854.
- Elaissi, A., Medini, H., Simmonds, M., Lynene, F., Farhat, F., Chemli, R., Harzallah-Skhiri, F., Khouja, M.L. 2011. Variation in volatile leaf oils of seven *Eucalyptus* species harvested from Zerniza Arboreta (Tunisia). Chemistry & Biodiversity 8(2): 362~372.
- Joker, D. 2004. *Eucalyptus urophylla* S.T. Blake. Seed Leaflet. Forest and Landscape.
- Maia, M.F., Moore, S.J. 2011. Plant-based insect repellents: A review of their efficacy, development and testing. Malaria Journal 10: 11~15.
- Makhaik, M., Naik, S.N., Tewary, D.K. 2005. Evaluation of anti-mosquito properties of essential oils. Journal of Scientific & Industrial Research 64: 129~133.
- Manaf, S. 1988. Preliminary Study on The Insecticide Effect of Seed Extract *Annona squamosa* Against *Martinius demestoides*. Food and Nutritions Report, Biological Science and Biotechnology. Yogyakarta.
- Mohtar, M., Yarmo, M.A., Kadri, A. 1999. The effects of *Nerium indicum* leaf extract on *Aedes aegypti* larvae. Journal of Tropical Forest Products 5(1): 87~92.
- Monzon, R.B., Alviator, J.P., Luczon, L.L.C., Morales, A.S., Mutuc, F.E.S. 1994. Larvicidal potential of five Philippine plants against *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say.). Southeast Asian Journal of Tropical Medicine Public Health 25: 755~759.
- Nerio, L.S., Olivero-Verbel, J., Stashenko, E. 2010. Repellent activity of essential oils: A review. Bioresource Technology 101: 372~378.
- Park, H.M., Kim, J., Chang, K.S., Kim, B.S., Yang, Y.J., Kim, G.H. 2011. Larvicidal activity of Myrtaceae essential oil and their components against *Aedes aegypti*, acute toxicity on *Daphnia Magna* and Aqueous Residue. Journal of Medical Entomology 48 (2): 405~410.
- Pujiarti, R., Ohtani, Y., Ichiura, H. 2011. Physicochemical properties and chemical compositions of *Melaleuca leucadendron* leaf oils taken from the plantations in Java, Indonesia. Journal of Wood Science 57: 446~451.
- Song, A., Wang, Y., Liu, Y. 2009. Study on the chemical constituents of the essential oil of the leaves of *Eucalyptus globulus* Labill. Asian Journal of Traditional Medicines 4(4): 134~140.
- Ukeh, D.A., Sylvia, B.A., Umoetok. 2011. Repellent effects of five monoterpenoid odours against *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) in Calabar, Nigeria. Crop Protection 30: 1351~1355.
- WHOPES (World Health Organization Pesticides Evaluation Scheme). 2009. Guideline for Efficacy Testing of Mosquito Repellent for Human Skin. Geneva.