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Chemical compositions and repellent activity of *Eucalyptus tereticornis* and *Eucalyptus deglupta* essential oils against *Culex quinquefasciatus* mosquito

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ABSTRACT

Objective: This research was to evaluate chemical compositions and repellent activity of *Eucalyptus tereticornis* and *Eucalyptus deglupta* essential oils against *Culex quinquefasciatus* mosquito. **Methods:** Essential oils of *E. tereticornis* and *E. deglupta* were extracted by hidrodistillation method. Chemical compositions of essential oils were analyzed by GC-MS, and mosquito repellent assay were evaluated with World Health Organization Pesticide method with modifications. **Result:** GC-MS analysis indentified that *E. tereticornis* and *E. deglupta* had 24 and 35 compounds with β-pinene and nerolidol as the major compounds, respectively. The IC_{50} and IC_{90} value of *E. tereticornis* and *E. deglupta* against *C. quinquefasciatus* were IC_{50}: 0.08%, IC_{90}: 6.37% and IC_{50}: 0.04%, IC_{90}: 7.23%, respectively. *E. tereticornis* oil showed 91.91% repellency while *E. deglupta* showed 94.05% repellency average at 8% concentration for 4 h exposure times. These two oils were effective as repellent substances when compare with commercial NN-diethyl-m-toluamide 15% with repellency average was 96.23%. **Conclusion:** The result shows that *E. tereticornis* and *E. deglupta* oils could be considered as potent natural repellent agents against *C. quinquefasciatus* mosquito.

INTRODUCTION

Mosquitoes are the cause of several fatal diseases in humans and animals.[1] House mosquito species that are commonly found in the tropics and responsible for some of the diseases spread is *Culex quinquefasciatus* Say. *C. quinquefasciatus* mosquito is the most important vector of filarial parasite of *Wuchereria bancrofti* that causes the filariasis disease in humans.[2] This species is also a vector of diseases of West Nile virus, Japanese encephalitis, and avian malaria. *C. quinquefasciatus* as a vector of lymphatic filariasis has spread widely in the world, especially in the tropical world with about 120 million people infected and around 44 million people having common chronic manifestation.[3] In Indonesia, there are more than 120 million people have been infected with the filariasis with 40 million of them have experienced a serious decline in capability. Infection in humans can occur without symptoms or mild symptoms to result in severe pain such as damage to the central nervous system that is permanent, in some cases fatal to death.[4]

Synthetic pesticides are generally used for against mosquitoes; however, the pesticide has many disadvantages such as the development of mosquitoes resistance, residue problems, an adverse impact on the environment and humans and various ecological problems.[5] The use of synthetic chemicals such as NN-diethyl-m-toluamide (DEET) as mosquitoes repellent have toxic effects on human body and the environment as well as the effects of erythema and pruritus until fatal effects such as seizures, depression, respiratory tract, and comma.[6] Therefore, it is necessary to substitute safer and environmentally friendly mosquito repellent.

One alternative material that can be used is essential oil. Essential oils have been used as antimicrobial, antiviral, antifungal, antiallergy, anti-inflammatory, antioxidant, and insect control.[7-12] Several studies also reported on the effectiveness of essential oils as ingredients of natural mosquitoes repellant.[13-19] The essential oil chemical compounds such as terpenoid have activity against mosquitoes. Chemical compounds such as monoterpenes, sesquiterpenes, diterpenes, and triterpenes on essential oils also have insect activities.[20] Bioactivity of essential oils against insects such as repellent, attractant, poisonous, fumigant, antifeedant, oviposition deterrent, inhibit growth, reducing fertility, and anti-insect vectors.[21]

The eucalyptus leaves are natural sources that can be used to produce essential oils. Usefulness of eucalyptus oil such as perfumes ingredients, disinfectants, liniment, fungicides, cleaning agents, insecticides, medicines, and other medical purposes.[22] Several studies reported that eucalyptus oils had repellent activity against mosquitoes such as *Eucalyptus citriodora* oil and *Eucalyptus globulus*.[23,24] However, a lack of information on the activities of *Eucalyptus tereticornis* and *Eucalyptus deglupta* essential oils from Indonesia as mosquito repellent against *C. quinquefasciatus*.

This study was conducted to investigate the chemical compounds of *E. tereticornis* and *E. deglupta* oils and evaluated its effectiveness as a repellent against *C. quinquefasciatus* mosquito.

MATERIALS AND METHODS

Plant Material and Distillation of Essential Oils

Fresh leaves of *E. tereticornis* and *E. deglupta* were collected from Forest Research and Education of Wanagama I - Gadjah Mada University in Gunung Kidul District - Yogyakarta, Indonesia. For each sample, 5 kg of fresh leaves of *E. tereticornis* and *E. deglupta* were extracted by hydrodistillation for 6 h. Extraction of oil from eucalyptus leaves was carried out by a hydrodistillation (water-steam distillation) using a 5 kg capacity stainless steel distillation tank apparatus which was specifically designed in a local workshop equipped with the spiral glass condenser cooling system and glass separatory funnel. The oils were obtained kept in labeled bottles, and oils were stored at −20°C until used.

Essential Oil Yield

The 1 g fresh leaves of eucalyptus were oven-dried at 103 ± 2°C until its weight constant and water content were calculated. The oil yields were determined based on dried weight of leaves (w/w). Yields were calculated by the following equation:

Yield (%) = ([weight total oil × oil specific gravity)/weight dry leaves)] × 100.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

The chemical composition of essential oils was analyzed by gas chromatography-mass spectrometry (GC-MS) QP2010S (Shimadzu Co. Ltd., Kyoto, Japan) with a fused-silica capillary column Agilent HP 5 MS (0.25 mm id × 30 m length). Helium gas was used as mobile phase with the gas in the flow rate of 60 ml/min with a split injection, injection volume of 1.0 mL and injection temperature of 240°C. Oven temperature programed 60°C (5 min hold) to 200°C (37 min hold). Ionization was performed by electron impact ionization at 70 eV. Chemical compositions of the oils were calculated based on the peak areas of GC chromatograms. Identification of oil compounds by analyzing chromatogram was achieved through comparison of retention time with Wiley 229 library database and several references.

Repellent Assay

The repellent test used World Health Organization Pesticide Evaluation[25] method with slight modifications. This test uses adult female of *C. quinquefasciatus* mosquitoes. The experiments used a test box cage of 20 cm × 20 cm × 20 cm with 35 female mosquitoes inside. Essential oils of *E. tereticornis* and *E. deglupta* produced were applied to the volunteer's forearm. Before the trial, arms were washed with soap, rinsed clean, and dried. After that, the arm oiled with oil solution of *E. tereticornis* and *E deglupta* for each concentration (2%, 4%, 6%, and 8%). The oil concentrations obtained by dissolving the oil in 70% alcohol. The oiled arm with 1 ml solutions was entered in a test box and let it be fed for 5 min for exposure times of 0-4 h. The number of mosquitoes that landed on the arm during the 60 min was recorded. During the test, arm settled for not move. Repellent tests conducted with 3 replications, where the second and third tests performed on different days, the next day at the same test using different mosquito. Positive and negative controls were used in this study. Negative control was the arms which only spread with 1 ml of 70% alcohol, while the positive control using repellent containing DEET 15%. Percentage repellent of oils with various concentrations against *C. quinquefasciatus* mosquitoes that land and or suck the blood of the arm can be calculated % repellent by the following formula:[26]

Repellent (%) = ([K-P]/K) × 100.

Where K is the number of mosquitoes in the control, *P* is the number of mosquitoes in the treatment arm. The inhibitory concentration 50% (IC_{50}) 90% (IC_{90}) of *E. tereticornis* and *E. deglupta* oils were determined by probit analysis.

Statistical Analysis

All tests and analyses were run in triplicate and average. The results were tested by two-way ANOVA. Significant differences between means were determined by honestly significant different test. $P < 0.05$ considered was statistically significant.

RESULTS AND DISCUSSION

Essential Oil Yields

The yield of *E. tereticornis* and *E. deglupta* oils were 0.94% and 0.75%, respectively. The yield of the oils this study were better compared with the yield of essential oil of *Eucalyptus urophylla* in the amount of 0.15-0.19%.[27] Another study reported that yield of 3 types of eucalyptus essential oils were 0.043% for

E. pellita essential oil, while essential oils of *E. grandis* had yield of 0.161%, and *E. urophylla* had yield of 0.143%.[28] Compared with the previous studies, essential oils in this study were more higher. The difference amount of *E. tereticornis* and *E. deglupta* oils with other eucalyptus oils are probably due to the different plant species, distillation methods, plant site, and yield calculation. Each eucalyptus species have varying morphology different to others mainly on the leaves, which produce the most essential oil, such as shape, size, and color of the leaves. The type of essential oils plant which has characteristics taper-shaped leaves, thin leaf surface, and green can produce a high yield. This is consistent with this study that *E. tereticornis* leaf which has characteristics taper-shaped leaves, thin and green produce higher oil yield of 0.94% than *E. deglupta* leaf which has characteristic roundish leaves, thicker leaf surface, and green leaves produce a yield of 0.75%.[29]

Chemical Constituents

Chemical compounds of *E. tereticornis* and *E. deglupta* oils in this study can be categorized as monoterpenes because it only has two isoprene units (C_5). Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) are the main component of essential oils. Monoterpenes have the properties in the form of a colorless liquid, insoluble in water, smell fragrant, and can be distilled with water vapor. Several monoterpene compounds are limonene, α-pinene, and also terpene alcohols as well as sitronellol, linalool, nerol and geraniol.[30]

Chemical compounds of *E. tereticornis* and *E. deglupta* essential oils show in Table 1. The GC-MS analysis showed that 24 compounds were identified in *E. tereticornis* oil with the main compound was β-pinene (32.06%). The major compounds (>5%) of *E. tereticornis* oil are β-pinene (32.06%), benzene (15.58%), limonene (14.61%), α-pinene (14.04%), and aromadendrene (5.46%). In addition, there are also other compounds such as β-myrcrene, 1.8-cineole, β-phellandrene, γ-terpinene, pinocarvone, fenchyl alcohol, trans-pinocarveol, geraniol, α-terpineol, linalyl propionate, borneol, myrtenol, benzenemethanol, caryophyllene oxide, globulol, nerolidol, α-eudesmol, guaiol, and β-eudesmol.

GC-MS analysis identified 35 compounds in *E. deglupta* oil with the main compound was nerolidol (31.61%). The major compounds (>5%) of *E. deglupta* oil are nerolidol (31.61%), α-phellandrene (10.98%), benzene (10.49%), and aromadendrene (5.61%). In addition, there is also a chemical compound with a small amount such as α-pinene, β-pinene α-terpinene, limonene, 1,8-cineole, β-phellandrene, ocimene, γ-terpinene, terpinolene, α-gurjunene, cyclohexen, caryophyllene, alloaromadendrene, piperitone, α-humulene, α-terpineol, α-terpinyl acetate, ladene, α-selinene, β-selinene, geranyl acetate, δ-cadinene, sabinyl acetate, ascaridole, caryophyllene oxide, acetonyl cyclohexanone, globulol, viridiflorol, α-bisabolol, β-eudesmol, and juniper camphor.

The results show that chemical compounds in *E. tereticornis* and *E. deglupta* oils mostly consist of group of monoterpenes ($C_{10}H_{16}$, $C_{10}H_{16}O$, $C_{10}H_{18}O$), and sesquiterpene ($C_{15}H_{24}$, $C_{15}H_{24}O$, $C_{15}H_{26}O$). The chemical compounds of *E. tereticornis* and *E. deglupta* oils have compatibility compounds with the results of the *E. pellita* oil, which has compounds such as β-pinene, 1,8-cineole, α-pinene, limonene, globulol, α-terpineol, and

Table 1: Chemical composition of *E. tereticornis* and *E. deglupta* oil

No	Components*	Retention time (min)	Area percentage (%)	
			E. tereticornis	*E. deglupta*
1	α-pinene	3.641	14.04	3.91
2	β-pinene	5.298	32.06	0.76
3	β-myrcrene	6.898	0.88	-
4	α-phellandrene	7.041	-	10.98
5	α-terpinene	7.332	-	0.32
6	Limonene	7.899	14.61	1.40
7	1,8-cineole	8.072	1.32	3.62
8	β-phellandrene	8.158	0.21	1.79
9	Ocimene	9.113	-	1.80
10	γ-terpinene	9.367	0.87	0.67
11	Benzene	10.197	15.58	10.49
12	Terpinolene	10.499	-	0.56
13	α-gurjunene	18.014	-	2.51
14	Pinocarvone	18.376	0.67	-
15	Fenchyl alcohol	18.900	0.61	-
16	Cyclohexen	19.356	-	0.77
17	Caryophyllene	19.751	-	4.97
18	Aromadendrene	20.097	5.46	5.61
19	Trans-pinocarveol	20.671	0.98	-
20	Alloaromadendrene	20.951	-	0.75
21	Piperitone	21.200	-	0.34
22	Geraniol	21.468	0.46	-
23	α-humulene	21.483	-	0.81
24	α-terpineol	21.708	0.92	0.34
25	Linalyl propionate	21.720	2.23	-
26	Borneol	21.835	0.47	-
27	α-Terpinyl acetate	21.963	-	0.64
28	Ledene	22.242	-	0.81
29	β-Selinene	22.775	-	2.21
30	α-Selinene	22.914	-	2.12
31	Geranyl acetate	23.467	-	0.47
32	δ-cadinene	23.757	-	1.84
33	Myrtenol	24.603	0.99	-
34	Sabinyl acetate	24.349	-	0.49
35	Ascaridole	24.675	-	0.28
36	Benzene methanol	25.242	0.32	-
37	Caryophyllene oxide	28.444	2.13	1.45
38	Acetonyl cyclohexanone	28.810	-	0.58
39	Globulol	29.094	1.39	2.98
40	Nerolidol	30.013	0.42	31.61
41	Viridiflorol	30.661	-	0.45
42	α-eudesmol	31.047	1.02	-
43	Guaiol	32.355	0.64	-
44	α-bisabolol	33.175	-	0.35
45	β-eudesmol	33.347	1.72	0.64
46	Juniper camphor	33.819	-	0.68

*Identification by Wiley 229 library. *E. tereticornis: Eucalyptus tereticornis, E. deglupta: Eucalyptus deglupta* γ-terpinene.[31] Likewise, there is a compatibility of another study from *E. citriodora* oil which has compounds such as benzene, globulol, cadinene, caryophyllene, cyclohexen, and terpenyl acetate.[32]

Mostly, eucalyptus oils have 1,8-cineole as the main compound. However, in this study, 1,8-cineole content of *E. tereticornis* and *E. deglupta* are relatively small with value of 1.32% and 3.62%, respectively. Several studies also reported a small 1,8-cineole content in some species of eucalyptus oils such as in *E. urophylla* oil with 1.57%[33] and *E. citriodora* oil with 1,8-cineole content of 0.14%,[34] 0.1-0.8%,[35] and 0.03-0.42%.[36] Even though eucalyptus oils in this study contains small amounts of 1,8-cineole, but these oils have distinctive odor due to present of other compounds such as benzene, nerolidol, α-pinene, and β-pinene. α-Pinene and β-pinene compounds have aromatic elements commonly used as an ingredient in the perfume industry, disinfectants, insecticides, and pharmaceuticals.

Repellent Activity

Repellent assay of *E. tereticornis* and *E. deglupta* oils in this study against *C. quinquefasciatus* used female mosquitoes on several levels of concentration (2%, 4%, 6%, and 8%) and compared with DEET 15% as the positive control and the untreated as negative control were observed at exposure times 0-4 h. The repellent activity of *E. tereticornis* and *E. deglupta* oils against *C. quinquefasciatus* mosquito presented in Table 2.

From Table 2 can be seen that negative control has 0% repellent activity and positive control (DEET 15%) has 96.23% repellent activity. The results showed tendency between the increasing repellent activities with increasing of concentration. At concentrations 2%, 4%, 6%, and 8% *E. tereticornis* oil has repellent activity of 53.96%, 70.87%, 76.46%, and 91.91%, respectively. While for *E. deglupta* oil at concentrations 2%, 4%, 6%, and 8% have repellent activity of 63.43%, 73.21%, 85.88% and 94.05%, respectively. This study also showed repellent activity decrease related with the length of exposure time (Table 2). Repellent activity of *E. tereticornis* and *E. deglupta* oils at concentration of 8% shows great potential at 0 h, which reaching 100%. Moreover, repellent activity value for concentration 8% at 4 h for *E. tereticornis* and *E. deglupta* were 91.91% and 94.05%, respectively. The effectiveness of essential oils at 0 h provide the highest value possibly due to the aromatic compounds such as cineole, benzene, nerolidol, limonene, α-pinene, and β-pinene contained in the essential oil of eucalyptus and a given concentration is still sufficient to prevent mosquito attack. However, on the exposure time 1-3 h, the potential of eucalyptus essential oil has begun to decrease. This can be seen from repellent percentage value begins to decline between 50% and 90%. At time exposure 4 h, the potential of eucalyptus oils against repellent percentage continues to decline ranged between 40% and 80%. The decreasing of oils repellent activity probably due evaporate of some volatile oils and aromatic compounds in oils such as 1,8-cineole, benzene, nerolidol, limonene, α-pinene, and β-pinene. It caused the distinctive smell of eucalyptus oil is reduced, thus lowering ability as a mosquito repellent.

Repellent activity percentage obtained from two essential oils in this study was not much different from the percentage of the positive control repellent ranged between 95% and 100%, while the negative control repellent has percentage of 0% (100% or more mosquito landed on the arm). *E. deglupta* oil had the most influence effectively, in concentration 8% had percentage repellent activity 100% at 0 h, 96-86% at 1-3 h, and at 4 h had the repellent activity 83%. ANOVA analysis showed that concentration of *E. tereticornis* and *E. deglpta* oils had significant value as repellent against *C. quinquefasciatus* mosquito. The higher concentration had the higher repellent activity. It also showed that the higher concentration of *E. tereticornis* and *E. deglupta* oils, the fewer the number of mosquitoes that land and suck the blood of the arm (Table 2). Compared to our previous study for *E. urophylla* with same concentration,[37] this study showed similar result as mosquito repellent, but this study showed stronger activity as mosquito repellent compared with *E. camaldulensis.*[38]

IC_{50} and IC_{90} values in each of eucalyptus oil, in this study, are presented in Figure 1. IC_{50} and IC_{90} on *E. tereticornis* oil

Table 2: Repellent activity of *E. tereticornis* and *E. deglupta* oils against *C. quinquefasciatus* mosquito

Sample	Repellent (%)					Average
	0 h	1 h	2 h	3 h	4 h	
(-) control*	0.00	0.00	0.00	0.00	0.00	0.00±0.00
(+) control**	100.00	100.00	97.91	92.98	90.28	96.23±1.98
E. tereticornis (%)						
2	58.58	55.61	54.38	52.12	49.11	53.96±4.15a
4	78.00	74.78	71.33	66.47	63.79	70.87±2.98b
6	84.69	78.53	76.82	72.60	69.68	76.46±5.66c
8	100.00	96.51	92.70	86.92	83.41	91.91±3.50d
E. deglupta (%)						
2	70.30	66.52	64.03	60.33	55.97	63.43±5.30[a]
4	83.62	76.52	73.77	68.03	64.12	73.21±4.38[b]
6	96.25	90.55	85.23	80.79	76.57	85.88±4.73[c]
8	100.00	100.00	96.90	90.90	82.46	94.05±2.21[d]

*Without treatment, **DEET 15%. *E. tereticornis: Eucalyptus tereticornis, E. deglupta: Eucalyptus deglupta, C. quinquefasciatus: Culex quinquefasciatus*
The value in the column followed by the same letter is not significantly different at $P<0.05$.

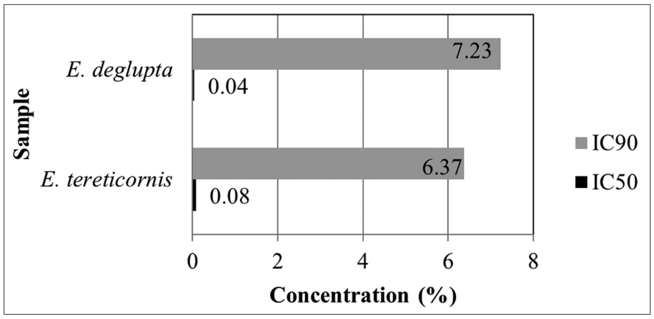

Figure 1: IC_{50} and IC_{90} of *Eucalyptus deglupta* and *Eucalyptus tereticornis* oils against *Culex quinquefasciatus* mosquito

were 0.08% and 6.37%, while for *E. deglupta* oil, the value for IC_{50} and IC_{90} were 0.04% and 7.23%, respectively. IC_{50} and IC_{90} values of these eucalyptus oils are both almost the same. Figure 1 shows that eucalyptus oils of *E. tereticornis* and *E. deglupta,* in this study, have repellant activity with the ability to repel mosquitoes as much as 50% at a concentration of oil 0.08% and 0.04%, and were able to repel mosquitoes as much as 90% at a concentration of 6.37% and 7.23%, respectively.

Antimosquito activity contained in *E. tereticornis* and *E. deglupta* essential oils apparantly due to terpenoid compounds of each species. Terpenoids compounds are natural compounds that have the ability as repellant. Terpenoid compounds in *E. tereticornis* oil such as 1,8-cineole, α-pinene, β-pinene, limonene, and benzene. The effectiveness of *E. tereticornis* oil probably due to the presence of β -pinene which is the main compound. It confirmed by previous research on essential oil of *Artemisia vulgaris* which effective as an insect repellent against *Aedes aegypti* due to the myrcene, beta-pinene, limonene, and 1,8-cineole contents.[39] While for *E. deglupta* oil, the terpenoids compounds such as 1,8-cineole, limonene, nerolidol, α-pinene, and β-pinene. The presence of nerolidol as the main compound of *E. deglupta* alleged influence the effectiveness of this oil as mosquito repellent. Several previous studies also explained that nerolidol have effect as insect repellent.[40,41]

The highest concentrations (8%) were used in this study it safe for the skin. Based on the BC center for disease control,[42] the eucalyptus oil with a concentration of 30% is still safe to skin and respiratory tracts. Several studies of eucalyptus oils also confirmed that eucalyptus oils with a certain concentration are still safe for the skin. Some studies also used eucalyptus oils at a dose >10% for the ointment.[43,44] However, terpenoids compounds also can cause toxic and poisons activity for mosquito's respiration.[23] Terpenoids content in essential oils can be toxic to mosquitoes, such as limonene, which is a contact poison for insect control. Based on the way of entry into the mosquito's body, the terpenoid compounds such as 1,8-cineole and limonene can be divided into three groups, which is contact poison, stomach poison, and respiratory poison.[45] Contact poison gets into the body of the mosquito through the skin, trachea or directly from the insect's mouth. Mosquitoes will die if it has direct contact with the essential oils due to continuous body fluid loss that causes mosquito dehydrated. Stomach poison can kill mosquitoes by going to digestion through food in a meal. Terpenoids compounds found in eucalyptus oils will enter the digestive organs of insects and absorb by the intestinal wall and then translocated to the deadly target area, for example, leading to the nerve center of mosquitoes, respiration organs, and poisoning the stomach cells. Respiratory toxins derived from the distinctive smell of eucalyptus oils that go through the trachea of mosquitoes in the form of microparticles suspended in air. Mosquitoes will die when inhaling the smell of microparticles in sufficient quantity.[28] Therefore, mosquito refused (repellent) to perch on the arm that had been oiled with *E. tereticornis* and *E. deglupta* essential oils due to terpenoids content that can be toxic for mosquitoes.

CONCLUSION

This study showed that the essential oil of *E. tereticornis* and *E. deglupta* has strong repellant activities against *C. quinquefasciatus* mosquitoes. The repellant activity of eucalyptus oils due to its terpenoid compounds combined with the smell disliked by mosquitoes such as 1,8-cineole, α-pinene, β-pinene, limonene, benzene, and nerolidol. However, this study was not evaluating the effectiveness of authentic compounds of terpenoids. Therefore, further studies are needed to determine the effectiveness of each compound, as well as its effectiveness against other mosquito species. This study indicates that essential oils of *E. tereticornis* and *E. deglupta* can be used as environmentally friendly alternative repellent materials against *C. quinquefasciatus* mosquito.

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