Toxicity of essential oils from orange (*Citrus sinensis* L. Obbeck) and lemongrass (*Cymbopogon nardus* L. Rendle) on *Aedes aegypti* a vector of Dengue Hemorrhagic Fever (DHF)

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Abstract. *Aedes aegypti* mosquito is one of common vectors for the pathogens of different diseases like dengue hemorrhagic fever. The most common approach to vector-borne disease is by chemical control, mostly through the use of insecticides. As compared to synthetic pesticides, essential oils (EOs) are ecologically safe, have no mammalian toxicity or the chances of development of resistance are reasonable and highly popular with the organic growers. This research has studied the larvicidal activity of essential oil from orange (*Citrus sinensis* L. Obbeck) and lemongrass (*Cymbopogon nardus* L. Rendle). Orange and lemongrass cleaned and cut into small pieces, dried and then blended to obtain crude drug samples. Samples distilled with the Stahl distillation method for 4 hours. Furthermore, terpenoids phytochemical test and test against larvae of *Aedes aegypti* larvicides to obtain the LC50 value. The yield of essential oil of orange (*Citrus sinensis* L Obbeck) obtained is 6.84%, while the essential oil of lemongrass (*Cymbopogon nardus* L. Rendle) was 0.96%. In the phytochemical test essential oils either of orange peel and lemongrass showed terpenoids. From the GCMS data, an essential oil from orange and lemongrass contain citronellal and limonene. Citronellal compound acts as an insecticide ingredient that works as an antifeedant and repellent. An essential oil from orange and lemongrass are toxic, with LC50 values obtained from lemongrass 35.133 ppm is smaller than the orange is 790.935 ppm. The essential oil of lemongrass more toxic than the essential oil of orange. Both of essential oils can be used as a green pesticide candidates.

1. Introduction

*Aedes, Anopheles* and *Culex* are common vectors for the pathogens of different diseases like polyarthritis, dengue, chikungunya, filariasis malaria, and yellow fever [1-3]. The major vector to transmit diseases is female mosquitoes. Chemical control like insecticides is the most common approach to vector-borne disease [4]. An effective tool to decrease mosquito populations before they emerge as adults is larviciding [5,6]. The chemical method is sufficient for the control of a large population of mosquitoes, just need small quantities, an effective weapon for quick and easy. One of chemical method is use synthetic organic insecticides. Although it very effective, they are have many bad impacts, resulting in ecological hazards [7]. Synthetic organic insecticides would be highly dangerous for the
environment and there are higher chances of developing resistance in insects and affecting the nontarget organisms [8,9].

Natural products, such as essential oils, are explore to the mosquito larvicidal and adulticidal activity [10,11]. The essential oils are composed of various potent bioactive compounds that are having larvicidal activities against various pests and mosquitoes [12-14]. Essential oils contain phytochemicals that are biodegradable, safe for the non-target organism. It would be an alternative to synthetic insecticides by under of their eco-friendly, inexpensive, easily biodegradable effectiveness and have been also used as pesticides for many years to manage the agricultural pests [15-17]. Phytochemicals of essential oil have previously shown different properties such as bactericial, antiviral, insecticidal, fungicidal or insect growth inhibitor and are known to cause toxic effects on various insects [18,19]. This present investigation aimed was to evaluate the toxicity of essential oils from orange (Citrus sinensis L Obbeck) and lemongrass (Cymbopogon nardus L. Rendle) against Aedes aegypti mosquitoes.

2. Materials and methods

2.1. Chemicals ad reagent
Orange (Citrus sinensis L. Obbeck) and lemongrass (Cymbopogon nardus L. Rendle) taken from Cibiru, Bandung. The 3rd instar larvae of Aedes aegypti mosquitoes from entomology laboratory Institute Technology Bandung. Liebermann-Burchard reagent and ethanol were purchased from Merck.

2.2. Methods

2.2.1. Distillation of essential oils. Orange and lemongrass cleaned and cut into small pieces, dried and then blended to obtain crude drug samples. Both samples (262.45 g orange and 200.15 g lemongrass) distilled with Stahl distillation method for 4 hours.

2.2.2. Phytochemistry assay and mass spectrometric identification. The Liebermann-Burchard reaction (acetic anhydride-conc. H₂SO₄) is used in this test, which produces a blue-green colour with most triterpenes and sterols. Mass spectrometric identification with GCMS – QP2010 Ultra in chemistry laboratory of Universitas Pendidikan Indonesia.

2.2.3. Larval bioassay. The third instar larvae of Aedes aegypti mosquito was used for the larvicidal property of the EOs, according to the WHO protocol [20]. In every 100 ml of the glass beaker, fifteen 3rd instar larvae were transferred. The essential oils were tested at 9 different concentrations as follows 0, 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 ppm respectively. Oils were dissolved in ethanol as a stock solution and added to the beakers to produce the desired concentration. The test was replicated three times for each test concentration. A total number of dead larvae in each beaker were counted after 24 h, and percentage of larval mortality was calculated.

2.2.4. Statistical analysis. The average larval mortality data were subjected to regression analysis using the probit table.

3. Results and discussion
The yield of essential oil of orange (Citrus sinensis L. Obbeck) obtained is 6.84%, while the essential oils of lemongrass (Cymbopogon nardus L. Rendle) were 0.96%.
Figure 1. Stahl distillation to isolated the essential oils from orange and lemongrass.

In the phytochemical test essential oils either of orange peel and lemongrass showed terpenoids. From the GC-MS data, α-pinena, methyl salicylate, δ-carene, citronellal and limonene were found as the major aromatic compounds in lemongrass oil (Cymbopogon nardus L. Rendle). Limonene, citronellal, citronellol, geraniol, linalool, α-pinene, myrcene, sabinene, linalyl acetate, geraniol and α-terpineol were found in orange oil (Citrus sinensis L. Obbeck).

Figure 2. GCMS spectrum of essential oil from lemongrass oil (Cymbopogon nardus L. Rendle).

The five major compounds and their fragmentation of essential oil from lemongrass (Cymbopogon nardus L. Rendle) showed in Table 1.

Table 1. Identification of major chemical compositions by GC-MS of essential oil from lemongrass (Cymbopogon nardus L. Rendle).

| No | R.time | % Area | Mol. weight | Fragmentation (m/z) | Compound name   |
|----|--------|--------|-------------|---------------------|-----------------|
| 1  | 4.403  | 30.42  | 136         | 37, 39, 53, 67, 77, 93, 121, 136 | α-Pinene        |
| 2  | 10.402 | 13.74  | 152         | 39, 53, 65, 92, 120, 152, 154 | Methyl Salicylate |
| 3  | 5.830  | 11.66  | 136         | 38, 41, 67, 79, 93, 121, 136 | δ-carene        |
| 4  | 9.228  | 9.71   | 154         | 41, 55, 69, 83, 95, 111, 121, 139, 154 | Citronellal |
| 5  | 6.215  | 3.39   | 136         | 41, 53, 68, 79, 93, 107, 121, 136 | Limonene       |
A safe environment due to mosquito control is the main reason for using essential oils compared to synthetic pesticides. Essential oils from orange (*Citrus sinensis* L Obbeck) and lemongrass (*Cymbopogon nardus* L. Rendle) have the power to kill mosquito larvae that is quite promising.

**Table 2.** Relative toxicity of essential oils from orange (*Citrus sinensis* L. Obbeck) and lemongrass (*Cymbopogon nardus* L. Rendle) against 3rd instar larvae of *Aedes aegypti* mosquitoes after 24 h of treatment.

| Essential oils | LC$_{50}$ (mg/l) | 95% confidence limits (mg/l) | Fit of probit line |
|---------------|-----------------|-----------------------------|-------------------|
| orange        | 790.935         | 1.933 – 3.102               | 15.154            |
| lemongrass    | 35.133          | 0.708 - 1.431               | 4.261             |

LC$_{50}$ (lethal concentration required to kill 50% of the population) values was highly toxic if LC$_{50} \leq$ 30 ppm, moderately toxic if LC$_{50} \leq$ 1000 ppm, and nontoxic if LC$_{50} >$ 1000 ppm. An essential oil from orange (*Citrus sinensis* L Obbeck) and lemongrass (*Cymbopogon nardus* L. Rendle) are toxic, with LC$_{50}$ values obtained from lemongrass 35.133 ppm is smaller than the orange is 790.935 ppm. The essential oil of lemongrass more toxic than the essential oil of orange.

4. **Conclusion**

Both of essential oil from orange (*Citrus sinensis* L. Obbeck) and lemongrass (*Cymbopogon nardus* L. Rendle) contain citronellal and limonene. Citronellal compound acts as an insecticide ingredient that works as an antifeedant and repellent. The findings of the present investigation revealed that orange and lemongrass oil have a larvicidal effect which is concentration-dependent for the *Aedes aegypti* larvae. Based on research results essential oil can kill mosquito larvae, so they can be used as a green pesticide candidates.

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