

Screening seven commercial essential herb oils for larvicidal activity against the mosquito *Aedes aegypti* (Linnaeus), a vector of the dengue virus

Tanawat Chaiphongpachara*, Sedthapong Laojun, Wallapa Wassanasompong

Department of Public Health and Health Promotion, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkhram, Thailand.

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ABSTRACT

Mosquitoes are tiny flying insects of great importance as vectors to many pathogenic organisms, including viruses. *Aedes aegypti* is a primary vector of the dengue virus that causes dengue fever, which is a globally important disease, threatening people in developing countries. In this research, we screened seven commercial herb essential oils, including cassia, cinnamon, East Indian lemongrass, bay, sweet basil, holy basil, and ginger for larvicidal activity against larvae of the dengue virus vector *A. aegypti*. The results revealed the efficacy of seven commercial pure essential oils against mosquito larvae. The cinnamon oil had the highest larvicidal activity ($LC_{50} = 0.03$ ppm and $LC_{90} = 0.04$ ppm), followed by cassia, holy basil, bay, sweet basil, East Indian lemongrass, and ginger essential oils. These results are important from the public health perspective since they relate to a dengue vector that requires alternative organic substances for its control and elimination.

INTRODUCTION

Mosquitoes are tiny flying insects of great importance as vectors to many pathogenic organisms, including the viruses causing dengue fever, yellow fever, chikungunya, West Nile, Japanese encephalitis, and Zika viruses, as well as protozoa, such as *Plasmodium* spp., and filarial nematodes, such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* (Du *et al.*, 2019; Tolle, 2009) responsible for disease and many deaths worldwide (Franklin *et al.*, 2019; Mirzaian *et al.*, 2010). Mosquitoes are considered to be temporary ectoparasites, and the female mosquitoes are obliged to feed on the blood of humans or animals to develop eggs (Killick-Kendrick, 1996). The blood-sucking behavior of the females is a major factor in the transmission of dangerous pathogens to humans (Chaves *et al.*, 2010). *Aedes aegypti* (Linnaeus, 1762), known as “the yellow fever mosquito,” is a diurnal mosquito that is an important vector of the dengue

virus causing dengue fever (Powell *et al.*, 2018). Currently, dengue fever is a globally important disease, threatening people in developing countries, especially in tropical and subtropical regions (Kraemer *et al.*, 2015). *Aedes aegypti* lives in proximity to human habitats (domestication) for taking human blood meals and is commonly found in cities, towns, and villages (Powell and Tabachnick, 2013). Therefore, this common domestic mosquito species can transmit and spread the dengue virus to people easily and quickly. The World Health Organization has reported that the incidence of dengue has increased dramatically worldwide over the past decade and estimates that as many as 390 million cases occur annually in more than 100 countries (World Health Organization, 2020).

Almost all breeding sites of *A. aegypti* are in and around houses, with the female mosquito laying eggs in a wide variety of natural and artificial water-holding containers, such as water tanks, plastic bottles, discarded vehicle tires, and flower vases (Ferede *et al.*, 2018). Control of *A. aegypti* populations, to reduce the risk of dengue infection, focuses on the destruction of larvae and their breeding sites and includes environmental management, source reduction, larvicide, or biological control through the cooperation of people in each area (Roiz *et al.*, 2018). This

*Corresponding Author

Tanawat Chaiphongpachara, College of Allied Health Science, Suan Sunandha Rajabhat University, Samut Songkhram, Thailand.
E-mail: tanawat.ch@ssru.ac.th

contrasts with practices used to control other types of mosquito, such as *Culex*, *Mansonia*, and *Anopheles* species, and the larvae of those are difficult to destroy since breeding sites are abundant and widespread in the natural environment, including rivers, marshes, ponds, and rice fields (Killick-Kendrick, 1996).

There are many ways to control the immature stages of *Aedes* mosquitoes, including the use of insecticides (Manjarres-Suarez and Olivero-Verbel, 2013) and releasing *Gambusia* mosquitofish into infested water containers as a biological control (Han *et al.*, 2015). However, these popular methods are not always suitable because of certain obstacles, such as the development of resistance to insecticides that are used regularly (Marcombe *et al.*, 2019). Temephos (commercial name Abate), the most popular product for mosquito larva control, is a non-systemic organophosphorus insecticide, which is relatively harmless to humans (Chaiphongpachara and Moolrat, 2017; George *et al.*, 2015). Although this chemical has been highly effective in stopping the spread of dengue virus in many countries, there have been reports of insecticide resistance in mosquito larvae, including Argentina (Albrieu Llinás *et al.*, 2010), Bolivia (Biber *et al.*, 2006), Brazil (Pereira Lima *et al.*, 2003), Cuba (Bisset *et al.*, 2004), El Salvador (Lazcano *et al.*, 2009), Peru, and Venezuela (Rodríguez *et al.*, 2001). The use of larvivorous fish (*Gambusia*) is applicable in containers that are large enough for the fish to live in and survive, but this can be a limitation to their use for controlling the larvae of dengue vectors, and in many countries, this fish is not recommended for use because it is an exotic species that may affect native aquatic fauna if it escapes into the environment (Benelli *et al.*, 2016).

Nowadays, plant-based larvicidal products targeting *Aedes* larvae in breeding sources and containers are gaining increased attention and have been accepted by communities due to their non-toxic effects in the local environment (Chaiphongpachara *et al.*, 2018; Ghosh *et al.*, 2012). However, alternative products derived from plants for killing mosquito larvae in the water are still rare in the marketplace. Essential oils are natural products obtained from the material of a single plant species, including leaves, petals, stems, seeds, and roots (Butnariu and Sarac, 2018). They are popular and have many uses, including medicine for treating microbial skin diseases caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* (Orchard and Vuuren, 2017) and in cosmetic products, such as creams and lotions (Sarkic and Stappen, 2018). Essential oils from some plants have been found to kill insects (Adorjan and

Buchbauer, 2010; Campolo *et al.*, 2018), and it is possible that, based on these, commercial products could be used to control the larval stage of dengue vectors. When considering alternative substances for insect vector control in communities, an important factor for success is that people can easily access and use them (Larson *et al.*, 2017).

Therefore, this laboratory-based research screened seven commercially available herb essential oils reported to kill insects and larvae of some mosquito species for larvicidal activity against larvae of the dengue virus vector *A. aegypti* (L.). The oils were obtained from *Cinnamomum cassia* (Liu *et al.*, 2014), *Cinnamomum zeylanicum* (Jeon *et al.*, 2017), *Cymbopogon flexuosus* (Rahayu *et al.*, 2018), *Pimenta racemosa* (Lee, 2006), *Ocimum basilicum* (Govindarajan *et al.*, 2013), *O. tenuiflorum* (Kamaraj and Rahuman, 2010), and *Zingiber officinale* (Pushpanathan *et al.*, 2008).

MATERIALS AND METHODS

Selection of commercial essential herb oils

Following a literature review for plants with insecticidal activity, seven commercial essential herb oils were selected, including *C. cassia* (cassia), *C. zeylanicum* (cinnamon), *C. flexuosus* (East Indian lemongrass), *P. racemosa* (bay), *O. basilicum* (sweet basil), *O. tenuiflorum* (holy basil), and *Z. officinale* (ginger). The essential oils were purchased from Chemipan Corporation Co., Ltd. (Bangkok, Thailand). The commercial oil products were cosmetic grade, and essential oil (100%) was obtained by steam distillation of herb leaves and packed in amber glass bottles. Detailed oil data and reports of the insecticidal properties of these plants are shown in Table 1. All experiments in this research were conducted from 2014 to 2015 in the laboratory of the College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand.

Rearing of *A. aegypti* larvae

The second-stage larvae of *A. aegypti* were received from the Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand. Bright white plastic larval trays (length 14 × width 11 × depth 7 inches) containing water were used to nurture the larvae under laboratory conditions at 70%–80% relative humidity, 25°C–28°C, and 12:12 light:dark photoperiod. Ground dog biscuits were placed in the trays only once since the larvae only take 1–2 days to develop into late third-stage larvae, which was the stage required for the larvicidal bioassay.

Table 1. Detailed data of the seven commercial essential herb oils used in this experiment with brief literature reviews of their insecticidal efficacy.

Essential herb oils (commercial oil country of origin)	Killing arthropods and references
<i>Cinnamomum cassia</i> (France)	Booklice (Liu <i>et al.</i> , 2014) and rice weevil (Lee <i>et al.</i> , 2008)
<i>Cinnamomum zeylanicum</i> (China)	Dust mite, storage mite, and black planthopper (Jeon <i>et al.</i> , 2017)
<i>Cymbopogon flexuosus</i> (India)	German cockroach (Rahayu <i>et al.</i> , 2018)
<i>Pimenta racemosa</i> (China)	Gall midge (Kim <i>et al.</i> , 2012) and <i>Culex</i> mosquito (Leyva <i>et al.</i> , 2012)
<i>Ocimum Basilicum</i> (France)	Fall armyworm (Silva <i>et al.</i> , 2017), gypsy moth (Popović <i>et al.</i> , 2013), vine mealybug (Karamaouna <i>et al.</i> , 2013), <i>Aedes</i> mosquito (Kumar <i>et al.</i> , 2017), and blowfly (Chil-Núñez <i>et al.</i> , 2018)
<i>Ocimum tenuiflorum</i> (China)	Bean weevil (Rodríguez-González <i>et al.</i> , 2019)
<i>Zingiber Officinale</i> (France)	Cotton leafworm (Hamada <i>et al.</i> , 2018) and <i>Culex</i> mosquito (Madreseh-Ghahfarokhi <i>et al.</i> , 2018)

Larvicidal bioassay

The larvicidal test used in this research was carried out according to the procedures of the World Health Organization for laboratory testing of mosquito larvicides (World Health Organization, 2005). For water preparation for testing, 1 ml of absolute methanol (solvent) was mixed with deionized water to dilute each concentration of the oils. The seven commercial essential herb oils were prepared in 250-ml beakers by serial dilution to 0.025, 0.050, 0.075, 0.100, 0.125, 0.150, and 0.175 ppm using deionized water. Following the WHO recommendations, the range of concentrations used was determined by first evaluating a wide range of concentrations until a narrow range was found, which yielded between 10% and 95% larval mortality (World Health Organization, 2005). A total of 25 late third-stage larvae were put into the beaker containing the prepared test herb oils. The mortality of mosquito larvae was recorded after 24 hours. Alive larvae were monitored for normal behavior and movement, whereas dead larvae exhibited no signs of movement. Four replicates per concentration were tested for each oil. A control treatment was also tested using deionized water mixed with 1 ml of methanol.

Statistical analyses

The mean larval mortality of *A. aegypti* larvae and standard error of the mean (SE) were calculated. SE was used to estimate the

uncertainty due to random errors in the mean values of the data, which was calculated from the standard deviation (SD) by the square root of values in the dataset (Altman and Bland, 2005). A statistical comparison of larval mortality among different herb oils was performed using the analysis of variance, followed by the Duncan test in R software. A *p*-value < 0.05 was considered to be statistically significant. The probit analysis was used to calculate LC₅₀ and LC₉₀ (lethal concentration) values for toxicity and activity assessments. The median lethal concentration value is the lowest concentration that kills 50% of the tested mosquito larvae, whereas LC₉₀ value is the lowest concentration that kills 90% of the tested larvae. In this study, the calculations of LC₅₀ and LC₉₀ values use a graphical method based on the Log concentration of essential oils on the X-axis and percentage of larval mortality on the Y-axis. The probit analysis calculated the slope of the probit mortality with the SE of the slope, Chi-squared values, and 95% confidence intervals of the upper and lower limits. The probit analysis operations were conducted by the LdP Line software (<http://www.ehabsoft.com/ldpline/>).

RESULT AND DISCUSSION

The results for the larvicidal activity of seven commercial essential oils against *A. aegypti* larvae at the concentrations ranging from 0.025 to 0.175 ppm evaluated after 24 hours of exposure are shown in Table 2. Mortality increased with an increase in the

Table 2. Probit analysis of larvicidal efficacy of seven essential oils against third-instar larvae of *A. aegypti*.

Essential oils	Concentrations (ppm)	Percentage of larval mortality (Means ± S.E.)	24-hour exposure		Slope ± SE	χ ²
			LC ₅₀ (UL-LL) (ppm)	LC ₉₀ (UL-LL) (ppm)		
Cassia (<i>C. cassia</i>)	0.025	28.00 ± 6.93	0.03 (0.03–0.03)	0.05 (0.04–0.06)	6.36 ± 0.60	2.28
	0.050	90.00 ± 4.76				
	0.075	100.00 ± 0.00				
	0.100	100.00 ± 0.00				
	0.125	100.00 ± 0.00				
	0.150	100.00 ± 0.00				
	0.175	100.00 ± 0.00				
	Control	0				
Cinnamon (<i>C. zeylanicum</i>)	0.025	44.00 ± 5.89	0.03 (0.01–0.03)	0.04 (0.04–0.05)	7.23 ± 0.97	0.06
	0.050	98.00 ± 1.15				
	0.075	100.00 ± 0.00				
	0.100	100.00 ± 0.00				
	0.125	100.00 ± 0.00				
	0.150	100.00 ± 0.00				
	0.175	100.00 ± 0.00				
	Control	0				
East Indian lemongrass (<i>C. flexuosus</i>)	0.025	0	0.08 (0.07–0.09)	0.12 (0.11–0.14)	7.74 ± 0.53	19.61
	0.050	5.00 ± 5.00				
	0.075	32.00 ± 15.32				
	0.100	90.00 ± 3.46				
	0.125	90.00 ± 4.76				
	0.150	95.00 ± 1.91				
	0.175	100.00 ± 0.00				
	Control	0				

Continued

Essential oils	Concentrations (ppm)	Percentage of larval mortality (Means \pm S.E.)	24-hour exposure		Slope \pm SE	χ^2
			LC ₅₀ (UL-LL) (ppm)	LC ₉₀ (UL-LL) (ppm)		
Bay (<i>P. racemosa</i>)	0.025	0	0.07 (0.06–0.08)	0.12 (0.11–0.15)	6.02 \pm 0.41	22.04
	0.050	9.00 \pm 1.00				
	0.075	71.00 \pm 5.00				
	0.100	78.00 \pm 2.58				
	0.125	87.00 \pm 3.42				
	0.150	96.00 \pm 2.31				
	0.175	100.00 \pm 0.00				
	Control	0				
Sweet basil (<i>O. basilium</i>)	0.025	1.00 \pm 1.00	0.08 (0.06–0.09)	0.12 (0.11–0.17)	7.09 \pm 0.48	51.10
	0.050	8.00 \pm 1.63				
	0.075	39.00 \pm 5.00				
	0.100	78.00 \pm 6.83				
	0.125	91.00 \pm 1.91				
	0.150	100.00 \pm 0				
	0.175	100.00 \pm 0				
	Control	0				
Holy basil (<i>O. tenuiflorum</i>)	0.025	0	0.07 (0.05–0.09)	0.12 (0.11–0.22)	5.62 \pm 0.37	65.69
	0.050	0				
	0.075	81.00 \pm 5.97				
	0.100	83.00 \pm 2.52				
	0.125	87.00 \pm 3.42				
	0.150	90.00 \pm 4.16				
	0.175	97.00 \pm 1.00				
	Control	0				
Ginger (<i>Z. officinale</i>)	0.025	0	0.13 (0.12–0.14)	0.20 (0.18–0.24)	6.60 \pm 0.51	15.91
	0.050	1.00 \pm 1.00				
	0.075	11.00 \pm 6.40				
	0.100	14.00 \pm 3.83				
	0.125	57.00 \pm 13.99				
	0.150	64.00 \pm 4.90				
	0.175	88.00 \pm 3.65				
	Control	0				

ppm = parts per million, LC₅₀ = concentration that killed 50% of the exposed mosquito larvae; LC₉₀ = concentration that killed 90% of the exposed mosquito larvae; UL = upper limit; LL = lower limit; S.E. = standard error; χ^2 = Chi-square. Four replicates per concentration were tested.

concentration of all seven essential oils, with no larval mortality found in the control group. Chi-squared values, which were $p > 0.05$, showed that the models were consistent with the datasets (Table 2).

All seven commercial essential herb oils showed high toxicity to *A. aegypti* larvae (Table 2). Cinnamon essential oil had the highest larvicidal activity (LC₅₀ = 0.03 ppm and LC₉₀ = 0.04 ppm), followed by the essential oils of cassia (LC₅₀ = 0.03 ppm and LC₉₀ = 0.05 ppm), holy basil (LC₅₀ = 0.07 ppm and LC₉₀ = 0.12 ppm), bay (LC₅₀ = 0.07 ppm and LC₉₀ = 0.12 ppm), sweet basil (LC₅₀ = 0.08 ppm and LC₉₀ = 0.12 ppm), East Indian lemongrass (LC₅₀ = 0.08 ppm and LC₉₀ = 0.12 ppm), and ginger (LC₅₀ = 0.13 ppm and LC₉₀ = 0.20 ppm) (Table 2).

The statistical analysis ranked the essential oils as follows for efficacy: (cinnamon = cassia) > (holy basil = bay =

sweet basil = East Indian lemongrass) > (ginger). The LC₅₀ values are shown in Figure 1, whereas LC₉₀ values are shown in Figure 2.

The results of this study revealed the efficacy against *Aedes* mosquito larvae of seven commercial pure essential oils, from cassia, cinnamon, East Indian lemongrass, bay, sweet basil, holy basil, and ginger. These products have the advantage of being easily accessible, relatively inexpensive, and environmental-friendly (Massebo *et al.*, 2009). The previous studies have indicated that many essential oils have the potential to eliminate the larvae of *A. aegypti* (Cheng *et al.*, 2003; Dias and Moraes, 2014). The seven essential oils were highly toxic to mosquito larvae (all with LC₅₀ < 1 ppm or < 1 ml/l) according to the criteria of Cheng *et al.* (2003), who stated that an LC₅₀ value < 50 ml/l equated to “highly active.” The larvicidal bioactivity of essential oils is mainly attributed to the major plant components but is also

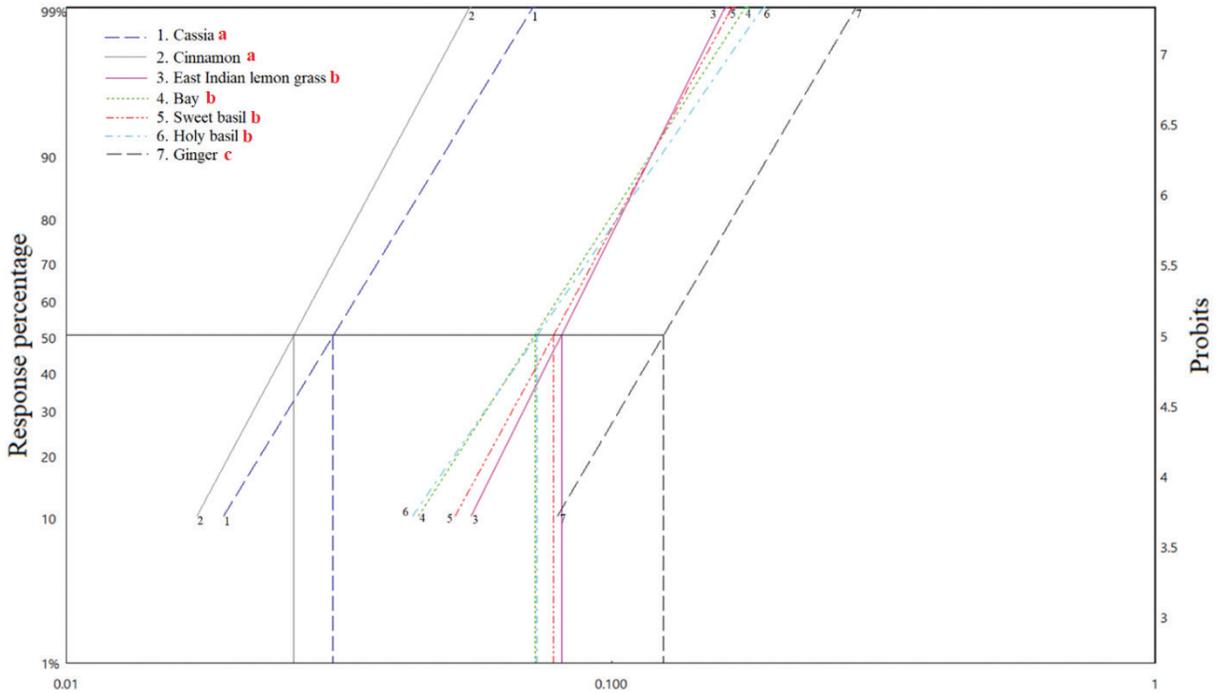


Figure 1. Graph showing LC_{50} values of seven essential oils against *A. aegypti* larvae after 24-hour exposure. Statistically significant ($p < 0.05$) differences are indicated by different red lowercase letters after the names of the oils in the inset key, top left.

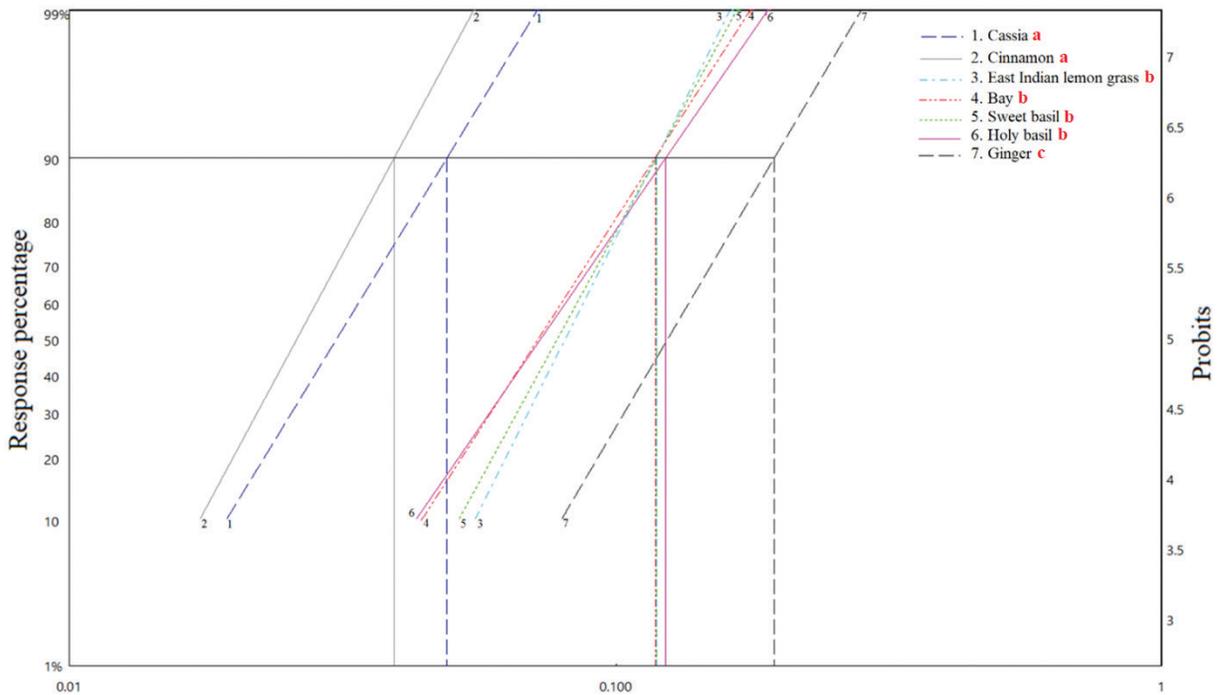


Figure 2. Graph showing the LC_{90} values of seven essential oils against *A. aegypti* larvae after 24-hour exposure. Statistically significant ($p < 0.05$) differences are indicated by different red lowercase letters after the names of the oils in the inset key, top right.

related to secondary substances, in which the former may work synergistically to enhance activity (Dias and Moraes, 2014).

The commercial cinnamon essential oil had the highest larvicidal activity, with a $LC_{50} = 0.03$ ppm and $LC_{90} = 0.04$ ppm.

This result is consistent with the previous research, demonstrating that this oil could eliminate the larvae of *A. aegypti* (Luis, 2010), as well as control larvae of *Culex tritaeniorhynchus* and *Anopheles subpictus* (Govindarajan, 2011). Knauth *et al.* (2018)

studied cinnamon essential oil (*C. zeylanicum*) and found that cinnamaldehyde (65%–80%) and eugenol (5%–10%) were the primary constituents. Cinnamaldehyde is an organic aromatic compound commonly found in cinnamon essential oil (Kaskatepe *et al.*, 2016). Cheng *et al.* (2004) revealed that cinnamaldehyde had the effect of killing larvae of *A. aegypti* based on a laboratory experiment, whereas eugenol is a natural phenylpropanoid, formally derived from guaiacol, and is found in many aromatic and medicinal plants such as cinnamon, clove, and bay leaves (Carvalho *et al.*, 2015). The previous research has studied the activity of eugenol derivatives against *A. aegypti* larvae and found that they were associated with the death of larvae (Barbosa *et al.*, 2012).

Although pure cinnamon essential oil was the most effective, the other six essential oils (from cassia, holy basil, bay, sweet basil, East Indian lemongrass, and ginger) also exhibited strong effects against *A. aegypti* larvae, all with LC₅₀ and LC₉₀ values < 1 ppm. The cassia essential oil contains terpenoids as the major components (Zhang *et al.*, 2019), which have reported toxicity to insects (Castilhos *et al.*, 2018), whereas other essential oils have different major compounds including β-caryophyllene (38.90%) in holy basil (Sharma *et al.*, 2016), eugenol (45.2%–52.7%) in the bay (Alitonou *et al.*, 2012), linalool (44.18%) in sweet basil (Ismail, 2006), citral-a (33.1%) in East Indian lemongrass (Chowdhury *et al.*, 2010), and zingiberene (23.69%) in ginger (Choudhari and Kareppa, 2013). All of these major compounds are toxic to insects (Tabari *et al.*, 2017; Tak and Isman, 2016). These results are consistent with the previous research reporting the toxicity of cassia (Zhu *et al.*, 2008), holy basil (Chokechaijaroenporn *et al.*, 1994), bay (Leyva *et al.*, 2009), sweet basil (Kumar *et al.*, 2017), East Indian lemongrass (Cavalcanti *et al.*, 2004), and ginger essential oils (Kalaivani *et al.*, 2012) to mosquito larvae.

In this study, differences in the efficacy of the seven essential oils against mosquito larvae allow them to be placed into three groups according to their strength: group 1—cinnamon and cassia, group 2—holy basil, bay, sweet basil, and East Indian lemongrass, and group 3—ginger. This information could be important when selecting different essential plant oils to control larvae within a community. The differences in the efficacy of the different types of oil arise from several factors, primarily active components in the plants and the extraction method (Dias and Moraes, 2014). The larvicidal test used in this study has shown that the efficacy of commercial pure essential oils to kill mosquito larvae in water is very high compared to results from the previous research (Dias and Moraes, 2014). The high efficacy on mosquito larvae may be because essential oils, which are commercially available, are cosmetic grade pure oils which are not diluted or affected by solvents or other additives.

CONCLUSION

The results from this research are important from the public health perspective since they relate to a dengue vector (mosquito) that requires alternative organic substances for its control and elimination. It is clear that commercial essential oils of cassia, cinnamon, East Indian lemongrass, bay, sweet basil, holy basil, and ginger are highly effective at killing *Aedes* mosquito

larvae. The important advantage of these oils is that they are easily accessible to the public and their use in the community could be promoted to aid control of *A. aegypti* larvae further.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interests.

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