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

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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 (Franklinois 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
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 (Rodriguez 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 (kassia), 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 oil species (country of origin) | Killing arthropods and references |
|-----------------------------------------|----------------------------------|
| Cinnamomum cassia (France)              | Booklice (Liu et al., 2014) and rice weevil (Lee et al., 2008) |
| Cinnamomum zeylanicum (China)           | Dust mite, storage mite, and black planthopper (Jeon et al., 2017) |
| Cymbopogon flexuosus (India)            | Germancockroach (Rahayu et al., 2018) |
| Pimenta racemosa (China)                | Gall midge (Kim et al., 2012) and Culex mosquito (Leyva et al., 2012) |
| Ocimum Basilicum (France)               | Fall armyworm (Silva et al., 2017), gypsy moth (Popović et al., 2013), vine mealybug (Karamaoua et al., 2013), Aedes mosquito (Kumar et al., 2017), and blowfly (Chil-Núñez et al., 2018) |
| Ocimum tenuiflorum (China)              | Bean weevil (Rodriguez-González et al., 2019) |
| Zingiber Officinale (France)            | Cotton leafworm (Hamada et al., 2018) and Culex mosquito (Madreseh-Giahfarokhi et al., 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 LC$_{50}$ and LC$_{90}$ (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 LC$_{90}$ value is the lowest concentration that kills 90% of the tested larvae. In this study, the calculations of LC$_{50}$ and LC$_{90}$ 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

| Essential oils                  | Concentrations (ppm) | Percentage of larval mortality (Means ± S.E.) | 24-hour exposure |
|--------------------------------|----------------------|---------------------------------------------|------------------|
|                                |                      |                                             | LC$_{50}$ (UL-LL) | LC$_{90}$ (UL-LL) | Slope ± SE | $\chi^2$ |
| Cassia (C. cassia)             | 0.025                | 28.00 ± 6.93                                | 0.03             | 0.05             | 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 (C. zeylanicum)       | 0.025                | 44.00 ± 5.89                                | 0.03             | 0.04             | 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 (C. flexuosus) | 0.025            | 0                                           | 0.08             | 0.12             | 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
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 ($LC_{50} = 0.03$ ppm and $LC_{90} = 0.04$ ppm), followed by the essential oils of cassia ($LC_{50} = 0.03$ ppm and $LC_{90} = 0.05$ ppm), holy basil ($LC_{50} = 0.07$ ppm and $LC_{90} = 0.12$ ppm), bay ($LC_{50} = 0.07$ ppm and $LC_{90} = 0.12$ ppm), sweet basil ($LC_{50} = 0.08$ ppm and $LC_{90} = 0.12$ ppm), East Indian lemongrass ($LC_{50} = 0.08$ ppm and $LC_{90} = 0.12$ ppm), and ginger ($LC_{50} = 0.13$ ppm and $LC_{90} = 0.20$ 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 $LC_{50}$ values are shown in Figure 1, whereas $LC_{90}$ 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 environmentally-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 $LC_{50} < 1$ ppm or < 1 ml/l) according to the criteria of Cheng *et al.* (2003), who stated that an LC<sub>90</sub> 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

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

ppm = parts per million, $LC_{50}$ = concentration that killed 50% of the exposed mosquito larvae; $LC_{90}$ = concentration that killed 90% of the exposed mosquito larvae; UL= upper limit; LL= lower limit; S.E.= standard error; $\chi^2$= Chi-square. Four replicates per concentration were tested.
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 \( LC_{50} = 0.03 \text{ ppm} \) and \( LC_{90} = 0.04 \text{ ppm} \).

This result is consistent with the previous research, demonstrating that this oil could eliminate the larvae of \textit{A. aegypti} (Luis, 2010), as well as control larvae of \textit{Culex tritaeniorhynchus} and \textit{Anopheles subpictus} (Govindarajan, 2011). Knauth \textit{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., 2010). 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 (Alitouou et al., 2012), linalool (44.18%) in sweet basil (Ismael, 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 (Cavalcenti 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 larvicideal 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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