



## Plant essential oils enhance the percent mortality of gravid *Aedes aegypti* (Diptera: Culicidae) Mosquitoes, Dengue Vector

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### Abstract

The aim of this study was to investigate plant essential oils enhance the percent mortality of gravid *Aedes aegypti* mosquitoes. Essential oils, the methanol extract of *Curcuma longa* rhizome and *Zingiber officinale* rhizome, and non-essential oils, *Garcinia mangostana* pericarp were tested mortality of gravid *Ae. aegypti* mosquitoes. GC-MS technique was used to detect essential oil compounds in the extract. The concentrations of three extracts were 250, 500, 1000, and 2000 ppm. ar-Tumerone and Zingiberane were mainly essential oil compounds in *C. longa* and *Z. officinale*, while 2-Furancarboxaldehyde in *G. mangostana*. At 24 h exposure, essential oils enhanced the percent mortality of gravid *Ae. aegypti* mosquitoes. The extract of *C. longa* showed the highest toxicity among the other extract with 100 % mortality of gravid *Ae. aegypti* and the LC<sub>50</sub> value of 121.36 ppm. The *C. longa* and *Z. officinale* possessed numerous essential oils could be used as plant insecticides, as alternative to synthetic insecticides.

**Keywords:** Gravid *Aedes aegypti*, *Curcuma longa*, *Zingiber officinale*, *Garcinia mangostana* GC-MS

### 1. Introduction

Dengue hemorrhagic fever is arthropod-borne viral disease transmitted by vector, mainly *Aedes aegypti* known as yellow fever mosquito<sup>[1]</sup>. Globally, the incidence of dengue has increased dramatically in recent decades. As many as 390 million people were infected by dengue virus per year (95% credible interval 284–528 million) worldwide. In addition, 96 million (67–136 million) people of 390 million people manifest clinically (with any severity of disease). It was estimated that 3.9 billion people in 128 countries are at risk of infection with dengue viruses<sup>[2, 3]</sup>.

Currently, there are no the best vaccines or specific treatment for dengue hemorrhagic fever reported. The main key to control or prevent the transmission of dengue virus is to combat dengue vector mosquitoes<sup>[2, 4]</sup>. In the vector control program is used synthetic insecticides. It was reported that the excessive use of synthetic insecticides emerges the development of the mosquito resistance to many synthetic insecticides and result in environment hazards<sup>[5, 6]</sup>. In addition, synthetic insecticides may cause accumulating their residues in food, milk, water and soil and also result in negative health effects to human and ecosystems<sup>[7, 8]</sup>. Therefore, applications of plant insecticides using plant bioactive compounds have been increased as alternative to synthetic insecticides<sup>[8]</sup>.

Medicinal plants abound with natural bioactive compounds. Some of them are known as essential oils. There were 361 essential oils from 269 plant species showed larvicidal activity<sup>[9]</sup>. The essential oils are complex mixtures of volatile compounds contained monoterpenes and sesquiterpenes which are found to be the most bioactive<sup>[9, 10]</sup>. Recently, the essential oils attract considerable interest to investigate in entomology research because they have numerous biological activities such as larvicidal, adulticidal,

and repellent activities against different mosquito species including *Ae. aegypti*<sup>[7, 9]</sup>. The larvicidal, adulticidal, and repellent activities play an important role in vector control program<sup>[10, 11]</sup>. Also, the plant essential oils showed antibacterial, anti-inflammatory, antioxidant, and anti-proliferative properties<sup>[12]</sup>.

Tumeric (*Curcuma longa*, Linn) and Ginger (*Zingiber officinale*, Roscoe) belong to Zingiberaceae family produced the essential oils. The essential oils of *C. longa* rhizome contained ar-Tumeron and derivatives (monoterpenes and sesquiterpenes), demonstrating the highest toxicity to larval stage of *Ae. aegypti*, *Culex sp* and *Anopheles gambiae*<sup>[13]</sup>. Similarly, the larvicidal activity of gingerol, paradol and shogol (monoterpenes), the bioactive essential oils of *Z. officinale*, was reported against *Ae. aegypti*, *Cx. quinquefasciatus*, and *Angiostrongylus cantonesis*<sup>[14]</sup>. Another non-plant essential oils, *Garcinia mangostana* L (Clusiaceae family) products xanthone and derivatives ( $\alpha$ -,  $\beta$ -,  $\gamma$ -Mangostin), showing larvicidal activity against *Ae. aegypti*, *An. stephensi*, *An. gambiae*, and *Cx. pipiens pipiens*<sup>[15]</sup>. Unfortunately, no essential oils of *C. longa* and *Z. officinale* are reported that they can be killed gravid *Aedes sp*, *Culex sp*, and *Anopheles sp* mosquitoes. Other plant species showed adulticidal activity against female *Ae. aegypti* such as *Spondias mombin*<sup>[16]</sup>, *Eclipta alba* and *Andrographis paniculata* against *An. stephensi*<sup>[17]</sup>, *Ocimum gratissimum* and *Datura stramonium* against *An. gambiae*<sup>[18]</sup>.

The essential oils are rich monoterpenes and sesquiterpenes produced from *C. longa* and *Z. officinale*, so that they can kill gravid *Ae. aegypti* mosquitoes. The advantages of the study were to know mortality rate of *C. longa* and *Z. officinale* against gravid *Ae. aegypti* mosquitoes, to destroy life cycle of *Ae. aegypti*, and to create herbal insecticides

from the two plants in the future. Therefore, in this study carried out a study on effects of the crude extracts of *C. longa* and *Z.officinale* resulted in enhanced the percent mortality of female *Ae.aegypti* mosquitoes compared with the non- essential oils, *G. mangostana*. The aim of this study was to investigate plant essential oils produced from *C. longa* and *Z. officinale* enhanced the percent mortality of gravid *Aedes aegypti* mosquitoes.

## 2. Materials and Methods

### 2.1 Plant extraction

The *C.longa*, *Z.officinale* and *G. mangostana* extracts were obtained from BALITTRO (The Indonesian Spice and Medicinal Crops Institute) Bogor, West Java. Indonesia. Identification of species from this plant was done by BALITTRO, Bogor, West Java, Indonesia. Absolute methanol was used as solvent. Briefly, *C.longa* rhizome, *Z.officinale* rhizome and *G. mangostana* pericarp were cleaned by tap water and cut a small piece and dried for 3 weeks at room temperature.. Afterwards, they were blended and filtered separately each other so that they were to be powders. 50 g of filtered powder of *C.longa* rhizome, *Z.officinale* rhizome and *G. mangostana* pericarp was put in Erlenmeyer tube (500 ml) and was added 300 ml absolute methanol, keeping them at room temperature for 3 days. Next, each Erlenmeyer contained methanol extracts was filtered using filter papers and sediments were discarded. Finally, the supernatant was be evaporated, to remove methanol, using vacuum evaporator, to obtain the crude extracts. The concentrations of the crude methanol extracts of *C. longa*, *G.mangostana*, and *Z.officinale* were used 250, 500, 1000 and 2000 ppm.

### 2.2 Gas chromatography-mass spectrometry (GC-MS) analysis

A phytochemical screening was conducted to determine the chemical compounds presenting in the crude methanol extract of the *C.longa* rhizome, *Z.officinale* rhizome (essential oils) and *G. mangostana* pericarp (non-essential oils) using Gas Chromatography-Mass Spectrometry (GCMS), Model of GCMS, Agilent Technologies, 6890N Network GC System, made in USA. The procedure and interpretation of GC-MS analysis were conducted in accordance with Agilent Technologies [19].The GCMS analysis was done at Forensic Laboratory Center of Indonesia National Police Headquarter, Jakarta, Indonesia.

### 2.3 Rearing of gravid *Ae.aegypti* mosquito

*Ae. aegypti* eggs were reared in the Entomology Laboratory of Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta. The eggs were put in the basin containing tap water, at  $28\pm 2$  °C, and a photoperiod of 12 h light followed by 12 h dark. In addition, fish feed (grower) were added to increase the growth of the larvae. Tap water was used for rearing larval process. Emerged adults were fed a 10% sucrose solution ad libitum. Mosquitoes were maintained at  $26 \pm 3$ °C,  $80 \pm 4$ % RH and under 12:12 h (light : dark) photoperiod cycles. Only female *Ae.aegypti* mosquitoes were used in this bioassay [20, 21].

### 2.4. Procedure of cleaning, drying and coating the CDC bottles

This procedure was done as described previously [20]. Briefly, the 250 ml Wheaton bottles were washed with warm soapy water and rinsed thoroughly with tap water at least 3 times. Then, the bottles were placed in an oven (50°C) for 15–20 min until they were thoroughly dry before using them. To assure that the cleaning procedure was adequate, some susceptible mosquitoes were introduced into a sample of recently washed and dried bottles. Afterwards, the caps were removed from the bottles and then, 1 ml of the crude methanol extracts of *C. longa*, *Z.officinale* and *G.mangostana* (2500, 5000, 10 000 and 20 000 ppm) was added in the bottles. Only 1 ml of acetone was added to the control bottle. The content of each bottle was swirled and inverted by gently rotating so that all the sides were coated. After that, the caps were removed and bottles were rolled continuously on their side until all visible signs of the liquid were gone from inside and the bottles were completely dry. The bottles were left for 24 h horizontally (on their sides) and covered with aluminum foil to keep them protected from light.

### 2.5 Bioassay procedure

In this study, bioassay procedure was conducted as describe previously [20, 21]. The bioassay was conducted using 250 ml bottles lying in horizontal position. Using a mouth aspirator, 20 female *Ae.aegypti* (2–5 days-old glucose fed, blood starved) were introduced into each tested bottle including the control bottle. The number of mosquitoes dead or alive was recorded after 24 h exposure. Mosquitoes were considered dead if they could no longer stand. All bioassays were performed at room temperature of  $26 \pm 2$ °C and relative humidity of  $80 \pm 2$ %. All bioassay were conducted in 3 replicates along with control.

### 2.6 Statistical analysis

The percentage of mortality of female *Ae.aegypti* data was calculated by descriptive statistic using Statistical Package for Social Sciences (SPSS 20.0). Probit analysis were employed to determine lethal dosages causing 50% (LC<sub>50</sub>) and 90% (LC<sub>90</sub>) mortality of adult females 24 h post-exposure, and other statistics included 95% upper confidence limit and lower confidence limit, slope and chi-square. The corrected mortality was determined using Abbott's formula whenever required as described previously [21].

### 2.7 Ethical approval

This study has approved by the Health Research Ethics Committee of Faculty Medicine (No. 0848/UN2F1/ETIK/2018), University of Indonesia, Jakarta, Indonesia,

## 3. Results

### 3.1. GC-MS Analysis of *C.longa*, *Z.officinale*, and *G.mangostana*

The results of GC-MS analysis, chemical compositions of the three extracts were summarized in Table 1-3. The total

number of the *C. longa*, *Z.officinale*, and *G.mangostana* compounds was 4, 8 and 19 respectively. Mainly essential oils of the *C. longa* rhizome extracts were ar-Tumeron (39.73 %). *Z. officinale* rhizome extract showed zingiberane (11.11 %) as major essential oils followed by  $\beta$ -Sesquiphelladrene (3.99 %) and ar-Curcumene (2.7 %),  $\gamma$ -

Muurolene (2.62 %),  $\alpha$ -Farnesene (1.55 %), and Paradol (1.42 %). Furthermore, *G. mangostana* pericarp extract contained major compounds, namely 2-Furancarboxaldehyde (0.77 %).

**Table 1:** GC-MS analysis of the methanol extract of *C.longa* rhizome

Compounds	Methanol extract of <i>C.longa</i> rhizome			
	Reference	Retention Time	Peak Area	Quality
ar-Curcumene	186197	7.052	0.66%	95
Zingiberane	191351	7.052	0.60%	90
ar-Tumerone	229959	8.539	39.73%	97
$\alpha$ -Tumerone	229965	8.796	15.87%	97

**Table 2:** GC-MS analysis of the methanol extract of *Z. officinale* rhizome

Compounds	Methanol extract of <i>Z.officinale</i> rhizome			
	Reference	Retention Time	Peak Area	Quality
1,1 Cineol	75871	3.3	0.10%	98
Borneol	75929	4.566	0.21%	97
$\beta$ -Citronellol	80031	4.976	0.05%	97
2-methoxy-4-vinylphenol	66050	5.788	0.03%	94
(+)-cycloisotativene	191890	6.139	0.06%	94
$\alpha$ -Copaene	191910	6.198	0.10%	98
$\beta$ -Elemene	192020	6.327	0.13%	97
$\gamma$ -Elemene	182226	6.669	0.14%	93
$\beta$ -Farnesene	191313	6.78	0.07%	97
Allomadendrene	191831	6.951	0.10%	99
ar-Curcumene	186280	7.053	2.71%	98
Zingiberene	191349	7.147	11.16%	94
$\alpha$ -Farnesene	191302	7.199	1.55%	98
$\gamma$ -Muurolene	191725	7.25	2.62%	98
$\beta$ -Sesquiphelladrene	191355	7.387	3.99%	98
Sesquisabinene hydrate	242135	8.259	0.39%	95
Paradol	163841	8.515	1.42%	91
Hexadecanoic acid	377170	10.199	0.48%	99
9,12-Octadecanoic acid	442587	11.302	0.28%	99

**Table 3:** GC-MS analysis of the methanol extract of *G.mangostana* pericarp.

Compounds	Methanol extract of <i>G.mangostana</i> pericarp			
	Reference	Retention Time	Peak Area	Quality
2,5 Furandione	17327	3.574	0.01%	90
(S)-(-)-4-Hydroxy-2-methyl-2-cyclo	17626	3.788	0.06%	90
Benzoic acid	26809	4.617	0.04%	93
2-Furancarboxaldehyde	30439	5.13	0.77%	93
$\alpha$ -Copaene	191910	6.173	0.06%	93
Pentadecanoic acid	377241	10.191	0.07%	96
n-Hexadecanoic acid	337724	10.464	0.03%	99
9,12-Octadecadienoic acid (Z,Z)-methyl ester	442620	11.294	0.12%	99

### 3.2. Adulticidal activity of *C.longa*, *Z.officinale*, and *G.mangostana*

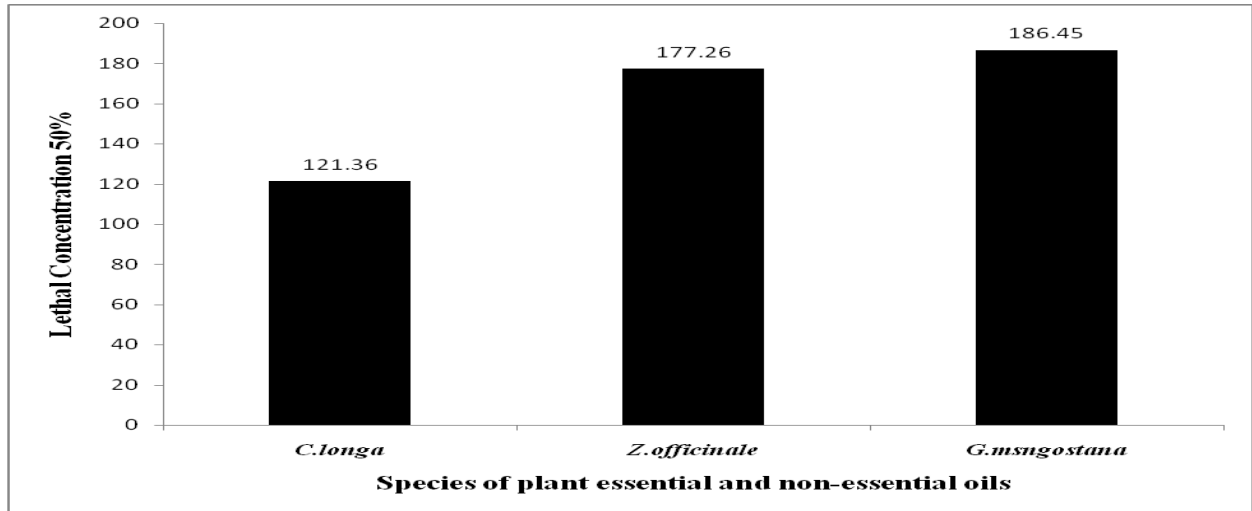
Adulticidal activity of the *C.longa* rhizomes, *Z.officinale* rhizomes, and *G.mangostana* pericarp against adult *Ae.aegypti* females was summarized in Table 4. At 24 h post-exposure, the percent mortality of gravid *Ae.aegypti* mosquitoes induced by the *C.longa* rhizome extract ranged 83.3% - 100% at concentrations of 250 – 2000 ppm, while the *Z.officinale* rhizomes ranged 70.0% -100 % at 250-2000 ppm. In addition, the *G.mangostana* pericarp extract showed the percent mortality of gravid *Ae.aegypti* mosquitoes ranged 63.3% - 100 % at 2500 – 2000 ppm. It was indicated

that the plant essential oils enhanced the percent mortality of gravid *Ae.aegypti* mosquitoes.

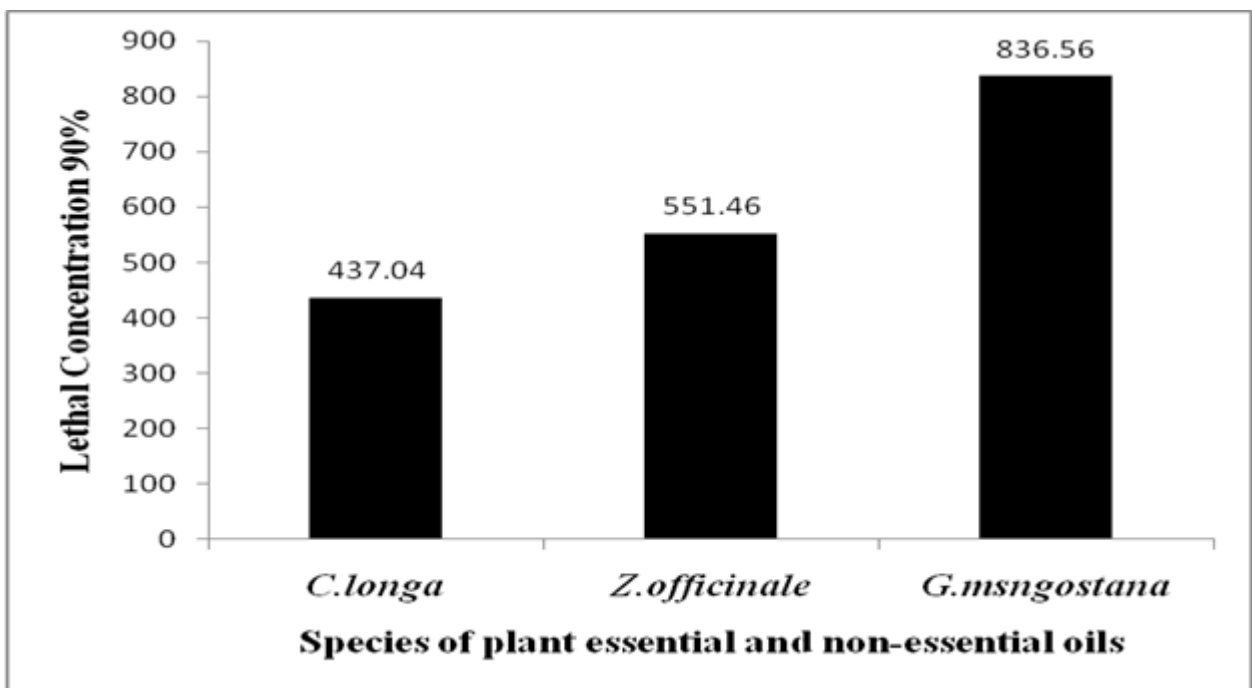
Figure 1 and 2 shows the LC<sub>50</sub> and LC<sub>90</sub> of the all tested extracts. The *C.longa* rhizomes extract showed LC<sub>50</sub> value of 121.36 ppm (95% CI = 8.16 – 210.61) and LC<sub>90</sub> value of 437.04 ppm (95% CI = 287.30 – 852.94). The *Z.officinale* rhizomes extract exhibited LC<sub>50</sub> value of 2499.34 ppm (95% CI = 1230.96 – 3606, 85) and LC<sub>90</sub> value of 22693.61 ppm (95% CI = 14380.01 – 59191.85). In addition, the *G.mangostana* pericarp extract yielded LC<sub>50</sub> value of 186.45 ppm (95% CI = 60.28 – 286.82) and LC<sub>90</sub> value of 836.56 (95% CI= 586.56 -1802.46).

**Table 4:** Mortality percentage of gravid *Ae.egypti* mosquitoes induced by *C.longa*, *Z.officinale*, and *G.mangostana*

Concentrations (ppm)	Mortality percentage of gravid <i>Ae.egypti</i> mosquitoes		
	<i>C.longa</i>	<i>Z.officinale</i>	<i>G.mangostana</i>
250	83.3%	70.0%	63.3%
500	86.7%	80.0%	76.7%
1000	100.0%	96.0%	90.0%
2000	100.0%	100.0%	100.0%



**Fig 1:** Lethal concentration 50% (LC<sub>50</sub>) of the *C. longa*, *Z. officinale* and *G.mangostana* Extracts



**Fig 2:** Lethal concentration 90% (LC<sub>90</sub>) of the *C.longa*, *Z.officinale* and *G.mangostana* extracts.

**4. Discussions**

The presence of essential oils, bioactive compounds, in medicinal plants is very important to be used as plant insecticides in vector control program [22]. The essential oils showed larvicidal, adulticidal, and repellent activities against different mosquito species are needed to lower density of mosquito and transmission vector borne diseases. The findings of the present study showed that the essential oils of *C. longa* and *Z. officinale* increased the percent mortality against female *Ae.aegypti* mosquitoes compared with the extract of *G.mangostana* due to bioactive essential

oils. Therefore, it is suggested that the plant essential oils could be involved in the vector control program. GC-MS analysis of *C. longa* showed that 4 essential oils were found in rhizome and ar-Tumerone (39.73 %) was mainly compounds followed by  $\alpha$ -Tumerone, ar-Curcumine, and Zingiberane. A study by Naz *et al.* [23] reported that 25.3% ar-Tumerone, major compounds, was identified in *C.longa* methalonic rhizome extract by GC-MS. Furthermore, the purified ar-Tumerone showed higher larvicidal activity against *Ae.aegypti* larvae (LC<sub>50</sub> = 2.5

ppm) compared with the unpurified essential oils contained ar-Tumerone and others <sup>[24]</sup>. In the present study showed that crude essential oils increased the percent mortality against female *Ae.aegypti* mosquitoes. It was suggested that *C.longa* exhibited both larvicidal and adulticidal activities against *Ae.aegypti* and ar-Tumerone was a major key to regulator biological activity.

In this study, there were 19 essential oils obtained from *Z. officinale* rhizome extract identified by GC-MS and of which zingiberane (11.1%) were major compounds followed by  $\beta$ -Sesquiphelladrene (4.0%) and ar-Curcumene (2.7 %),  $\gamma$ -Muurolene (2.6 %),  $\alpha$ -Farnesene (1.6 %), and Paradol (1.4 %). Sharma *et al.*<sup>[25]</sup> reported that 46.71% Zingiberane (sesquiterpene) were mainly compounds in *Z.officinale* rhizome extract analyzed by GC-MS. Isolate compounds from *Z.officinale* rhizome especially 4-gingerol showed the most effective compounds to kill *Ae.aegypti* larvae (LC<sub>50</sub> = 4.25 ppm) compared with 6-dehydrogingeridione and 6-dihydrogingeridione <sup>[26]</sup>. In this study, the essential oils of *Z. officinale* crude extract induced higher mortality of female *Ae.aegypti* mosquitoes compared with the *G.mangostana* extract.

The essential oils have many benefits in medicine aspects because they have numerous biological activities; antioxidant, anti-inflammatory, cancer chemoprotective, cytotoxicity, allelopathic <sup>[10]</sup>. Gingerol has radioprotective effects in laboratory animals and in cultured cells in vitro <sup>[27]</sup>. Furthermore, the essential oils are used for cosmetics, perfume and related household products due to their pleasant odour <sup>[28]</sup>.

Few studies have investigated adulticidal mechanisms of the essential oils. The essential oils from *Chamaecyparis nookatensis* and *Calocedrus decurrens* have adulticidal mechanisms. The different target sites of point mutation consisted of sodium channel para locus mutation (L1014FKDR), ACE-1 gene, and  $\gamma$ -aminobutyric acid receptor mutation of Rdl locus of *An.gambiae*. The mode of action was different with currently synthetic insecticides, permethrin, organophosphate, carbamate, and dieldrin <sup>[29]</sup>. Up to date, adulticidal mechanisms of the essential oils of *C.longa* and *Z.officinale* are unknown.

engue viruses enter to human through the bite of an infective female *Ae.aegypti* mosquito. They acquire dengue viruses during feeding on the blood of an infected person. Within female mosquito, the dengue virus infects the mosquito mid-gut and then distributes to the salivary gland for 8-12 days. After the incubation time, the virus may be transmitted to human during subsequent probing or feeding <sup>[30]</sup>. Dead female *Ae.aegypti* could not transmit dengue virus. Therefore, mortality of female *Ae.aegypti* mosquitoes is very important in epidemiology of *Ae.aegypti* especially to decrease dengue transmission in an area. In the present study showed the essential oils of *C. longa* and *Z.officinale* increased the percent mortality of gravid *Ae.aegypti* mosquitoes in laboratory scale, so that they could be employed as a natural insecticide in the vector control program.

## Conclusion

Tumeric (*C.longa*) and ginger (*Z.officinale*) possessed numerous essential oils especially ar-Tumerone and Zingiberane in the rhizomes detected by GC-MS. They enhanced the percent mortality of gravid *Ae.aegypti* mosquitoes compared with the non-essential oils, *G.mangostana*. As a result, *C. longa* and *Z.officinale* have adulticidal activity, so that they could be a strong candidate of plant adulticide as alternative to synthetic insecticides.

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